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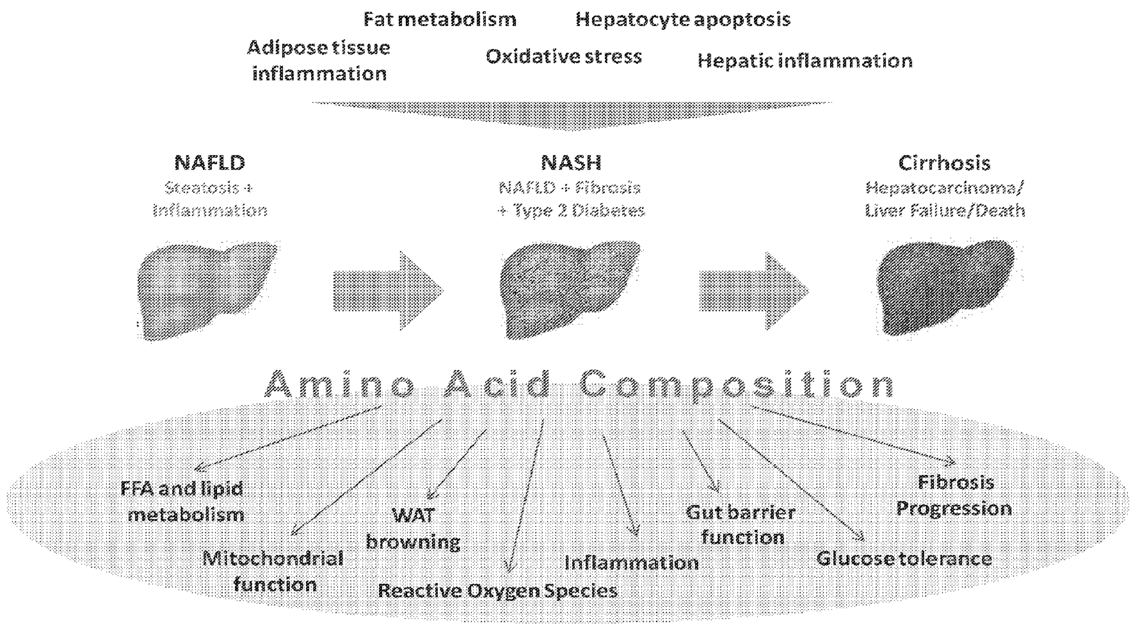

FIG. 2

(57) Abstract: This disclosure provides pharmaceutical compositions comprising amino acid entities and uses thereof. Methods for improving liver function and for treating liver diseases comprising administering an effective amount of the compositions to a subject in need thereof are also disclosed.

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AMINO ACID COMPOSITIONS AND METHODS FOR THE TREATMENT OF LIVER DISEASES

RELATED APPLICATIONS

This application claims priority to U.S. Serial No. 62/436,073 filed December 19, 2016, U.S. Serial No. 62/443,205 filed January 6, 2017, U.S. Serial No. 62/491,773 filed April 28, 2017, U.S. Serial No. 62/545,322 filed August 14, 2017, and U.S. Serial No. 62/576,267 filed October 24, 2017, the contents of which are each incorporated herein by reference in their entireties.

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a disease characterized by fatty deposits in the liver due to causes other than alcohol. NAFLD is the most prevalent liver disease in developed countries and affects close to 25% of the people in the United States. Non-alcoholic steatohepatitis (NASH) is the most severe form of NAFLD, which can lead to inflammation of the liver, fibrosis, cirrhosis, chronic liver failure, and hepatocellular carcinoma (HCC).

Currently, there are no approved therapies for treating NASH or NAFLD. Accordingly, there is an unmet need for new treatments in NAFLD and NASH.

SUMMARY

Disclosed herein, at least in part, is a composition including at least four different amino acid entities.

In some embodiments, the composition is capable of one, two, three, four, five, or six or all of:

a) decreasing or preventing liver fibrosis;
b) decreasing or preventing liver injury;
c) decreasing or preventing hepatocyte inflammation;
d) improving, e.g., increasing, glucose tolerance;
e) decreasing or preventing steatosis;
f) decreasing or preventing hepatocyte ballooning; or
g) improving gut function.

In some embodiments, the composition comprises a leucine (L)-amino acid entity, an arginine (R)-amino acid entity, a glutamine (Q)-amino acid entity; and an antioxidant or reactive oxygen species (ROS) scavenger (e.g., a N-acetylcysteine (NAC) entity, e.g., NAC). In some embodiments, at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length.

In some embodiments:

(i) an amino acid entity (e.g., at least one, two, or three of the amino acid entities) of (a) is selected from Table 2; and/or

(ii) (A) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or (B) the composition further comprises a serine (S)-amino acid entity.

In any of the aspects and embodiments disclosed herein, the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 0.5 to 3 : 0.5 to 4 : 1 to 4 : 0.1 to 2.5, e.g., the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 1 : 1.5 : 2 : 0.15 or about 1 : 1.5 : 2 : 0.3. In certain embodiments, the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 1 +/- 15% : 1.5 +/- 15% : 2 +/- 15% : 0.15 +/- 15% or about 1 +/- 15% : 1.5 +/- 15% : 2 +/- 15% : 0.3 +/- 15%. In any of the aforesaid embodiments in this paragraph, the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 1 : 0.75 : 2 : 0.15 or about 1 : 0.75 : 2 : 0.3. In any of the aforesaid embodiments in this paragraph, the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 1 +/- 15% : 0.75 +/- 15%: 2 +/- 15%: 0.15 +/- 15% or about 1 +/- 15%: 0.75 +/- 15% : 2 +/- 15% : 0.3 +/- 15%.

In any of the aspects and embodiments disclosed herein, the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the L-glutamine or salt thereof, and the NAC or salt thereof is about 1 : 0.5: 0.5: 1.5 : 2 : 0.15 or about 1 : 0.5: 0.5: 1.5 : 2 : 0.3.

In any of the aspects and embodiments disclosed herein, the composition further comprises one or both of L-glycine and L-serine. In some embodiments, the composition comprises an L-amino acid entity, an I-amino acid entity, an V-amino acid entity, an R-amino acid entity, an L-glutamine or a salt thereof, an NAC or a salt thereof, and an L-glycine. In certain embodiments, the composition comprises an L-amino acid entity, an I-amino acid entity, a V-amino acid entity, an R-amino acid entity, an L-glutamine or a salt thereof, an NAC or a salt thereof, and an L-serine. In certain embodiments, the composition comprises an L-amino acid entity, an I-amino acid entity, an V-amino acid entity, an R-amino acid entity, an L-glutamine or a salt thereof, an NAC or a salt thereof, an L-glycine, and an L-serine. In any of the aforesaid embodiments in this paragraph, the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the L-glutamine or salt thereof, and the NAC or salt thereof is about 1 : 0.5: 0.5: 1.5 : 2 : 0.15 or about 1 : 0.5: 0.5: 1.5 : 2 : 0.3. In any of the aforesaid embodiments in this paragraph, the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the L-glutamine or salt thereof, and the NAC or salt thereof is about 1 +/- 15% : 0.5 +/- 15% : 0.5 +/- 15% : 1.5 +/- 15% : 2 +/- 15% : 0.15 +/- 15% or about 1 +/- 15% : 0.5 +/- 15%: 0.5 +/- 15%: 1.5+/- 15% : 2 +/- 15%: 0.3+/- 15%.

In any of the aspects and embodiments disclosed herein, the composition comprises about 0.5 g to about 10 g of the L-amino acid entity, about 0.25 g to about 5 g of the I-amino acid entity, about 0.25 g to about 5 g of the V-amino acid entity, about 0.5 g to about 20 g of the R-amino acid entity, about 1 g to about 20 g of the L-glutamine or a salt thereof, and about 0.1 g to about 5 g of the NAC or a salt thereof, e.g., the composition comprises about 1 g of the L-amino acid entity, about 0.5 g of the I-amino acid entity, about 0.5 g of V-amino acid entity, about 1.5 g of R-amino acid entity, about 2 g of L-glutamine or a salt thereof, and about 0.15 g or about 0.3 g of NAC or a salt thereof. In certain embodiments, the composition comprises about 0.15 g of NAC. In certain embodiments, the composition comprises about 0.3 g of NAC. In embodiments, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, and about 0.9 g of NAC or a salt thereof.

In any of the aspects and embodiments disclosed herein,, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, about 0.9 g of NAC or a salt thereof, and about 6 g of L-serine or a salt thereof. In embodiments, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, about 0.9 g of NAC or a salt thereof, and about 6.67 g of L-serine or a salt thereof. In embodiments, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, about 0.9 g of NAC or a salt thereof, about 9 g of L-serine or a salt thereof, and about 9 g of L-glycine or a salt thereof. In embodiments, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, about 0.9 g of NAC or a salt thereof, about 3.33 g of L-serine or a salt thereof, and about 3.33 g of L-glycine or a salt thereof.

In one aspect, the invention features a composition including free amino acids, wherein the amino acids include arginine, glutamine, N-acetylcysteine, and a branched-chain amino acid chosen from one, two, or all of leucine, isoleucine, and valine.

In any of the aspects and embodiments disclosed herein, the branched-chain amino acid is leucine, isoleucine, and valine.

In any of the aspects and embodiments disclosed herein, the wt ratio of leucine, isoleucine, valine, arginine, glutamine, N-acetylcysteine is 1 : 0.5 : 0.5 : 1.5 : 2 : 0.15. In certain embodiments, the wt ratio of leucine, isoleucine, valine, arginine, glutamine, N-acetylcysteine is 1 +/- 15% : 0.5 +/- 15%: 0.5 +/- 15%: 1.5 +/- 15%: 2 +/- 15%: 0.15+/- 15%.

In any of the aspects and embodiments disclosed herein, a total weight (wt) of the amino acids is about 2 g to about 60 g. In some embodiments, the total wt of the amino acids is about 6 g, about 12 g, about 18 g, about 24 g, or about 48 g.

In any of the aspects and embodiments disclosed herein, the composition includes about 0.5 g to about 10 g of leucine, about 0.25 g to about 5 g of isoleucine, about 0.25 g to about 5 g of valine, about 1 g to about 20 g of arginine, about 1 g to about 20 g of glutamine, and about 0.1 g to about 5 g of N-acetylcysteine.

In any of the aspects and embodiments disclosed herein, the composition includes about 1 g of leucine, about 0.5 g of isoleucine, about 0.5 g of valine, about 1.5 g of arginine, about 2 g of glutamine, and about 0.15 g of N-acetylcysteine.

In any of the aspects and embodiments disclosed herein, the composition includes about 2 g of leucine, about 1 g of isoleucine, about 1 g of valine, about 3.0 g of arginine, about 4 g of glutamine, and about 0.3 g of N-acetylcysteine.

In any of the aspects and embodiments disclosed herein, the composition includes about 4 g of leucine, about 2 g of isoleucine, about 2 g of valine, about 6.0 g of arginine, about 8 g of glutamine, and about 0.6 g of N-acetylcysteine.

In any of the aspects and embodiments disclosed herein, the amino acids include about 10 wt % to about 30 wt % leucine, about 5 wt % to about 15 wt % isoleucine, about 5 wt % to about 15 wt % valine, about 15 wt % to about 40 wt % arginine, about 20 wt % to about 50 wt % glutamine, and about 1 wt % to about 8 wt % n-acetylcysteine.

In any of the aspects and embodiments disclosed herein, the amino acids include about 16 wt % to about 18 wt % leucine, about 7 wt % to about 9 wt % isoleucine, about 7 wt % to about 9 wt % valine, about 28 wt % to about 32 wt % arginine, about 31 wt % to about 34 wt % glutamine, and about 1 wt % to about 5 wt % n-acetylcysteine.

In any of the aspects and embodiments disclosed herein, the amino acids include about 16.8 wt % leucine, about 8.4 wt % isoleucine, about 8.4 wt % valine, about 30.4 wt % arginine, about 33.6 wt % glutamine, and about 2.5 wt % n-acetylcysteine.

In some embodiments of any of the compositions or methods disclosed herein (wherein the ratios discussed in (1)-(26) below are weight ratios):

1) the ratio of the L-amino acid entity to the I-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 2:1;

2) the ratio of L-amino acid entity to V-amino acid entity is at least 2:1, at least 3:1, at least 3.5:1, at least 4:1, or at least 5:1, and not more than 6: 1, e.g., the ratio of L-amino acid entity to V-amino acid entity is about 4:1;

3) the ratio of the L-amino acid entity to the R-amino acid entity is at least 1:1, at least 3.5:3, at least 4:3, or at least 2:1, and not more than 5:2, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 4:3;

4) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is at least 0.5:1, or at least 0.75:1, and not more than 1.5 to 1 or not more than 2:1, e.g., the ratio of the L-amino acid entity to the L-glutamine or salt thereof is about 1:1;

5) the ratio of the L-amino acid entity to the NAC entity or a salt thereof is at least 2:1, at least 3:1, at least 3.5:1, or at least 4:1, and not more than 5 to 1 or not more than 6:1, e.g., the ratio of the L-amino acid entity to the NAC entity or salt thereof is about 4:1 (e.g., 4:0.9);

6) optionally wherein the ratio of the L-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:3, greater than 1.5:3, about 2:3, or about 3:5, and not more than 2.5:3 or 1:1, e.g., the ratio of the L-amino acid entity to the S-amino acid entity is about 2:3, or the ratio of the L-amino acid entity to the S-amino acid entity is about 3:5; or

7) a combination of two, three, four, five, or six of (1)-(6).

In some embodiments of any of the compositions or methods disclosed herein:

8) the ratio of I-amino acid entity to V-amino acid entity is at least 1:1, at least 1.5:1, or about 2:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of I-amino acid entity to V-amino acid entity is about 2:1;

9) the ratio of the I-amino acid entity to the R-amino acid entity is greater than 1:3, greater than 1.5:3, or about 2:3, and not more than 2.5:3 or not more than 1:1, e.g., the ratio of the I-amino acid entity to the R-amino acid entity is about 2:3;

10) the ratio of the I-amino acid entity to the L-glutamine or a salt thereof is at least 1:4, at least 1:3, or about 1:2, and not more than 1:1 or not more than 2:1, e.g., the ratio of the I-amino acid entity to the L-glutamine or salt thereof is about 1:2;

11) the ratio of the I-amino acid entity to the NAC entity or a salt thereof is at least 1:1, at least 1.5:1, or about 2:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of the I-amino acid entity to the NAC entity or salt thereof is about 2:1 (e.g., 2:0.9);

12) optionally wherein the ratio of the I-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:4, greater than 1.5:4, about 1:3, or about 3:10, and not more than 1.5:3 or 2:3, e.g., the ratio of the I-amino acid entity to the S-amino acid entity is about 1:3, or the ratio of the I-amino acid entity to the S-amino acid entity is about 3:10; or

13) a combination of two, three, four, or five of (8)-(12).

In some embodiments of any of the compositions or methods disclosed herein:

14) the ratio of the V-amino acid entity to the R-amino acid entity is greater than 1:4, greater than 1.5:4, or about 1:3, and not more than 1:2 or not more than 1:1, e.g., the ratio of the V-amino acid entity to the R-amino acid entity is about 1:3;

15) the ratio of the V-amino acid entity to the L-glutamine or a salt thereof is greater than 1:5, or greater than 1.5:5, about 1:4, and not more than 1.5:4 or not more than 1:3, e.g., the ratio of the V-amino acid entity to the L-glutamine or salt thereof is about 1:4;

16) the ratio of the V-amino acid entity to the NAC entity or a salt thereof is at least 1:2, at least 1.5:2, or about 1:1, and not more than 1.5:1 or not more than 2:1, e.g., the ratio of the V-amino acid entity to the NAC entity or salt thereof is about 1:1 (e.g., 1:0.9);

17) optionally wherein the ratio of the V-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:8, greater than 1:7, about 1:6, or about 3:20, and not more than 1.5:6 or 1:3, e.g., the ratio of the V-amino acid entity to the S-amino acid entity is about 1:6, or the ratio of the V-amino acid entity to the S-amino acid entity is about 3:20; or

18) a combination of two, three, or four of (14)-(17).

In some embodiments of any of the compositions or methods disclosed herein:

19) the ratio of the R-amino acid entity to the L-glutamine or a salt thereof is greater than 1:2, greater than 1.25:2, or about 3:4, and not more than 3.5:4 or not more than 1:1, e.g., the ratio of the R-amino acid entity to the L-glutamine or salt thereof is about 3:4;

20) the ratio of the R-amino acid entity to the NAC entity or a salt thereof is at least 4:1, at least 4:1.5, or about 3:1, and not more than 3:1.5 or not more than 3:2, e.g., the ratio of the R-amino acid entity to the NAC entity or salt thereof is about 3:1 (e.g., 3:0.9);

21) optionally wherein the ratio of the R-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:4, greater than 1:3, about 1:2,, or about 9:20, and not more than 1.5:2 or 1:1, e.g., the ratio of the R-amino acid entity to the S-amino acid entity is about 1:2, or the ratio of the R-amino acid entity to the S-amino acid entity is about 9:20; or

22) a combination of two or three of (19)-(21).

In some embodiments of any of the compositions or methods disclosed herein:

23) the ratio of the L-glutamine to the NAC entity or a salt thereof is at least 5:1, at least 5:1.5, or about 4:1, and not more than 4:1.5 or not more than 3:1, e.g., the ratio of the L-glutamine to the NAC entity or salt thereof is about 4:1 (e.g., 4:0.9);

24) optionally wherein the ratio of the L-glutamine to the S-amino acid entity or a salt thereof is greater than 1:3, greater than 1.25:3, about 2:3, or about 3:5, and not more than 2.5:3 or 1:1, e.g., the ratio of the L-glutamine to the S-amino acid entity is about 2:3, or the ratio of the L-glutamine to the S-amino acid entity is about 3:5; or

25) a combination of (23) and (24).

In some embodiments of any of the compositions or methods disclosed herein:

26) the ratio of the NAC entity to the S-amino acid entity or a salt thereof is greater than 1:8, greater than 1:7, or about 1:6, and not more than 1:5 or not more than 1.5:5, e.g., the ratio of the NAC entity to the S-amino acid entity is about 1:6 (e.g., 0.9:6 or 2.7:20).

In an embodiment, the composition satisfies the properties of (1)-(7) defined above.

In certain embodiments, the composition satisfies the properties of at least 2, 3, 4, 5, 6, or 7 of any of properties (1)-(26) defined above.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 : 6 : 3 : 9 : 12 : 2.7.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 : 6 : 3 : 9 : 12 : 2.7 : 18.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 : 6 : 3 : 9 : 12 : 2.7 : 20.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15%.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 18 +/- 15%.

In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 : 6 : 3 : 9 : 12 : 2.7 : 9: 9. In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 : 6 : 3 : 9 : 12 : 2.7 : 10: 10.

In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 9 +/- 15% : 9 +/- 15%. In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 10 +/- 15% : 10 +/- 15%.

In any of the aspects and embodiments disclosed herein, the composition further includes one or more pharmaceutically acceptable excipients.

In some embodiments, the excipients are selected from the group consisting of citric acid, lecithin, a sweetener, a dispersion enhancer, a flavoring, a bitterness masking agent, and a natural or artificial coloring.

In some embodiments, the composition is in the form of a solid, powder, solution, or gel.

In some embodiments, the amino acids consist of leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine.

Another aspect of the invention features a dietary composition including the composition of any one of the foregoing aspects or embodiments, e.g., wherein the dietary composition is chosen from a medical food, a functional food, or a supplement.

In some embodiments, the dietary composition is chosen from a medical food, a functional food, or a supplement.

In some embodiments, the subject has type 2 diabetes and/or a relatively high BMI.

In some embodiments, the subject has non-alcoholic fatty liver disease (NAFLD).

In some embodiments, the subject has non-alcoholic fatty liver (NAFL).

In some embodiments, the subject has pediatric NAFLD.

In some embodiments, the patient has steatosis.

In some embodiments, the subject has non-alcoholic steatohepatitis (NASH).

In some embodiments, the subject has fibrosis.

In some embodiments, the subject has cirrhosis.

In some embodiments, the subject has AFLD.

In some embodiments, the subject has ASH.

In some embodiments, the subject has hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

In some embodiments, the subject has type 2 diabetes.

In some embodiments, the composition promotes weight loss in the subject.

In some embodiments of the method or the dietary composition for use, the composition is administered at a dose of about 15 g/d to about 90 g/d.

In some embodiments of the method or the dietary composition for use, the composition is administered at a dose of about 18 g/d, about 24 g/d, about 36/d, about 54 g/d, or about 72 g/d.

In some embodiments of the method or the dietary composition for use, the composition is administered one, two, to three times per day.

In some embodiments of the method or the dietary composition for use, the composition is administered at a dose of about 6 g, about 8 g, about 12 g, about 16 g, about 18 g, or about 24 g three times per day.

One embodiment provides a nutritional supplement, dietary formulation, functional food, medical food, food, or beverage comprising a composition described herein. Another embodiment provides a nutritional supplement, dietary formulation, functional food, medical food, food, or beverage comprising a composition described herein for use in the management of any of the diseases or disorders described herein. The composition disclosed herein can be used to improve liver function in a subject with fatty liver disease, such as non-alcoholic fatty liver disease (NAFLD; e.g. NAFL or non-alcoholic steatohepatitis (NASH)) or alcoholic fatty liver disease (AFLD; e.g., alcoholic steatohepatitis (ASH)). Thus, a method, including a dosage regimen, for treating (e.g., inhibiting, reducing, ameliorating, or preventing) various liver disorders, diseases, or symptoms thereof using the amino acid entity compositions is disclosed herein. The composition can also be used as a dietary composition, e.g., a medical food, a functional food, or a supplement.

Another aspect of the invention features a method for treating one or more symptoms selected from the group consisting of decreased fat metabolism, hepatocyte apoptosis, hepatocyte ballooning, inflammation of adipose tissue, inflammation of hepatic tissue, fibrosis, and oxidative stress, wherein the method includes administering to a subject in need thereof an effective amount of the composition of any one of aspects or embodiments disclosed herein.

In some embodiments, the subject has non-alcoholic fatty liver disease (NAFLD).

In some embodiments, the subject has non-alcoholic fatty liver (NAFL).

In some embodiments, the subject has pediatric NAFLD.

In some embodiments, the patient has steatosis.

In some embodiments, the subject has non-alcoholic steatohepatitis (NASH).

In some embodiments, the subject has alcoholic fatty liver disease (AFLD).

In some embodiments, the subject has alcoholic steatohepatitis (ASH)).

In some embodiments, the subject has fibrosis.

In some embodiments, the subject has cirrhosis.

In some embodiments, the subject has one, two, or more (e.g., all) of hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

In some embodiments, the subject has type 2 diabetes.

Another aspect of the invention features a method for treating non-alcoholic fatty liver disease (NAFLD) including administering to a subject in need thereof an effective amount of the composition of any one of the aspects or embodiments disclosed herein.

In some embodiments, the subject has NAFL.

In some embodiments, the subject has pediatric NAFLD.

In some embodiments, the patient has steatosis.

Another aspect of the invention features a method for treating non-alcoholic steatohepatitis (NASH) including administering to a subject in need thereof an effective amount of the composition of any one of the aspects or embodiments disclosed herein.

In some embodiments, the subject has fibrosis.

Another aspect of the invention features a method for treating AFLD including administering to a subject in need thereof an effective amount of the composition of any one of the aspects or embodiments disclosed herein.

In some embodiments, the subject has ASH.

Another aspect of the invention features a method for treating cirrhosis including administering to a subject in need thereof an effective amount of the composition of any one of the aspects or embodiments disclosed herein.

In some embodiments, the subject has hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

In some embodiments, administering the composition results in an improvement in one or more metabolic symptoms in the subject. In some embodiments, the improvement in one or more metabolic symptoms is selected from the following: increased free fatty acid and lipid metabolism, improved mitochondrial function, white adipose tissue (WAT) browning, decreased reactive oxygen species (ROS), increased levels of glutathione (GSH), decreased hepatic inflammation, decreased hepatocyte ballooning, improved gut barrier function, increased insulin secretion, or improved glucose tolerance.

In some embodiments, the increased free fatty acid and lipid metabolism occurs in the liver.

In some embodiments, administration of the composition results in an improvement in one or more metabolic symptoms after a treatment period of 24 hours.

In some embodiments, the method further includes determining the level of one, two, three, four, five, six, seven, eight, nine, ten, or more (*e.g.,* all) of the following:

a) alanine aminotransferase (ALT);

b) aspartate aminotransferase (AST);

c) adiponectin;

d) N-terminal fragment of type III collagen (proC3);

e) caspase-cleaved keratin 18 fragments (M30 and M65);

f) IL-1 beta;

g) C-reactive protein;

h) PIIINP;

i) a tissue inhibitor of metalloproteinase (TIMP); e.g., TIMP1 or TIMP2;

j) MCP-1;

k) FGF-21;

l) Col1a1;

m) Acta2;

n) a matrix metalloproteinase (MMP), e.g., MMP-13, MMP-2, MMP-9, MT1-MMP, MMP-3, or MMP-10;

o) ACOX1;

p) IL-10; or

q) NF-kB.

In some embodiments, administration of the composition results in an improvement in one or more of a)-q) after a treatment period of 24 hours.

In some embodiments, the composition is administered prior to a meal.

In some embodiments, the composition is administered concurrent with a meal.

In some embodiments, the composition is administered following a meal.

In some embodiments, the composition is administered with a second agent.

In some embodiments, the second agent is selected from the group consisting of a farnesoid X receptor (FXR) agonist, a stearoyl CoA desaturase inhibitor, a CCR2 and CCR5 chemokine antagonist, a PPAR alpha and delta agonist, a caspase inhibitor, a galectin-3 inhibitor, an acetyl CoA carboxylase inhibitor, or an ileal sodium bile acid co-transporter inhibitor.Another aspect of the invention provides a method of maintaining or improving liver health comprising administering to a subject an effective amount of any of the compositions described herein. Another embodiment provides a method of providing nutritional support or supplementation to a subject suffering from NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH) comprising administering to the subject an effective amount of a composition described herein. Yet another embodiment provides a method of providing nutritional supplementation that aids in the management of NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH) to a subject comprising administering to the subject in need thereof an effective amount of a composition described herein.

Additional features and embodiments of the present invention include one or more of the following.

Another aspect of the invention features a composition comprising:

a) a L-amino acid entity chosen from L-leucine or a salt thereof, or β-hydroxy-β-methybutyrate (HMB) or a salt thereof, or a combination of L-leucine or a salt thereof and HMB or a salt thereof;

b) an R-amino acid entity chosen from:L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof, or a combination of two or three of L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof;

c) L-glutamine or a salt thereof; and

d) N-acetylcysteine (NAC) or a salt thereof.

In an embodiment, L-leucine is provided as part of a dipeptide comprising L-leucine, or a salt thereof, or a tripeptide comprising L-leucine, or a salt thereof.

In an embodiment, L-arginine is provided as part of a dipeptide comprising L-arginine, or a salt thereof, or a tripeptide comprising L-arginine, or a salt thereof.

In an embodiment L-glutamine is provided as part of a dipeptide comprising L-glutamine, or a salt thereof, or a tripeptide comprising L- glutamine, or a salt thereof.

In an embodiment NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

In some embodiments of any of the compositions or methods disclosed herein, one, two, three, or four of methionine (M), tryptophan (W), valine (V), or cysteine (C) is absent, or if present, is present at a percentage of the composition by weight (wt. %) of less than 10%. In some embodiments, the total wt. % of (a)-(d) is greater than the total wt. % of any other amino acid entity in the composition.

In some embodiments of any of the compositions or methods disclosed herein, one, two, three, or four of the amino acids in (a)-(d) is provided as part of a dipeptide or tripeptide, e.g., in an amount of at least 10 wt. % of the composition. In certain embodiments, the dipeptide is a homodipeptide or heterodipeptide of any of the amino acids in (a)-(d), e.g., one, two, three, or four of the amino acids in (a)-(d) is a homodipeptide or heterodipeptide. In certain embodiments, the tripeptide is a homotripeptide or heterotripeptide of any of (a)-(d), e.g., one, two, three, or four of (a)-(d) is a homotripeptide or heterotripeptide.

In some embodiments of any of the compositions or methods disclosed herein, (a) is a L-amino acid entity dipeptide or a salt thereof (e.g., a L-leucine dipeptide or a salt thereof). In some embodiments, (a) is a homodipeptide. In some embodiments, (a) is a heterodipeptide, e.g., Ala-Leu.

In some embodiments of any of the compositions or methods disclosed herein, (b) is a L-arginine dipeptide or a salt thereof. In some embodiments, (b) is a homodipeptide. In some embodiments, (b) is a heterodipeptide, e.g., Ala-Arg.

In some embodiments of any of the compositions or methods disclosed herein, (c) is a L-glutamine dipeptide or a salt thereof. In some embodiments, (c) is a homodipeptide, e.g., Gln-Gln. In some embodiments, (c) is a heterodipeptide, e.g., Ala-Gln.

In some embodiments of any of the compositions or methods disclosed herein:

f) a wt. % of the L-glutamine or a salt thereof in the composition is greater than the wt. % of the R-amino acid entity;

g) the wt. % of the L-glutamine or a salt thereof in the composition is greater than the wt. % of the L-amino acid entity;

h) the wt. % of the R-amino acid entity in the composition is greater than the wt. % of the L-amino acid entity; or

i) a combination of two or three of (f)-(h).

In some embodiments of any of the compositions or methods disclosed herein,the wt. % of the L-glutamine or a salt thereof in the composition is at least 5% greater than the wt. % of the R-amino acid entity, e.g., the wt. % of the L-glutamine or a salt thereof is at least 10%, 15%, 20%, or 25% greater than the wt. % of the R-amino acid entity.

In some embodiments of any of the compositions or methods disclosed herein,the wt. % of the L-glutamine or a salt thereof in the composition is at least 20% greater than the wt. % of the L-amino acid entity, e.g., the wt. % of the L-glutamine or a salt thereof in the composition is at least 25%, 30%, 35%, 40%, 45%, or 50% greater than the wt. % of the L-amino acid entity.

In some embodiments of any of the compositions or methods disclosed herein,the wt. % of the R-amino acid entity in the composition is at least 10% greater than the wt. % of the L-amino acid entity, e.g., the wt. % of the R-amino acid entity in the composition is at least 15%, 20%, 25%, or 30% greater than the wt. % of the L-amino acid entity.

In some embodiments of any of the compositions or methods disclosed herein:

j) the ratio of the L-amino acid entity to the R-amino acid entity is at least 1:4, or at least 2:5, and not more than 3:4, e.g., the ratio of L-amino acid entity to R-amino acid entity is about 2:3;

k) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is at least 1:4, or at least 1:3, and not more than 3:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:2;

l) the ratio of the R-amino acid entity to the L-glutamine or a salt thereof is at least 1:4, or at least 1:2, and not more than 6:7, e.g., the ratio of the R-amino acid entity to the L-glutamine or a salt thereof is about 3:4; or

m) a combination of two or three of (j)-(l).

In an embodiment, the composition satisfies the properties of (j)-(l) defined above.

In certain embodiments, the composition satisfies the properties of at least 2, 3, or 4 of any of properties (j)-(m) defined above.

In some embodiments of any of the compositions or methods disclosed herein, the composition further comprises one or both of an isoleucine (I)-amino acid-entity and a valine (V)-amino acid-entity, e.g., both the I-amino acid-entity and the V-amino acid-entity are present.

In some embodiments of any of the compositions or methods disclosed herein:

n) the wt. % of the L-amino acid-entity in the composition is greater than or equal to the wt. % of the I-amino acid-entity and the V-amino acid-entity in combination;

o) the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination in the composition is greater than or equal to the wt. % of the L-glutamine or a salt thereof;

p) the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination in the composition is less than the wt. % of the R-amino acid entity;

q) the wt. % of the R-amino acid entity and the L-glutamine or a salt thereof in the composition is greater than the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination; or

r) a combination of two, three, or four of (n)-(q).

In some embodiments of any of the compositions or methods disclosed herein:

s) the wt. % of the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or a salt thereof is at least 50% of the composition, or at least 70% of the composition, but not more than 90% of the composition;

t) the wt. % of the NAC or a salt thereof is at least 1%, or at least 2%, but not more than 10% of the composition;

u) the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination is at least 15%, or at least 20%, but not more than 50% of the composition;

v) the wt. % of the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or a salt thereof is at least 40%, or at least 50%, but not more than 80% of the composition; or

w) a combination of two, three, or four of (s)-(v).

In some embodiments of any of the compositions or methods disclosed herein:

x) the ratio of the L-amino acid entity to the I-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5 to 1 or not more than 3:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 2:1;

y) the ratio of L-amino acid entity to V-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5 to 1 or not more than 3:1, e.g., the ratio of L to V is about 2:1;

z) the ratio of the L-amino acid entity to the R-amino acid entity is greater than 1:3, greater than 1.5 to 3, and less than 3:3, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 2:3;

aa) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is greater than 1:4, greater than 1.5 to 4 and less than 4:4, or less than 3:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:2; or

bb) a combination of two, three, or four of (x)-(aa).

In an embodiment, the composition satisfies the properties of (x)-(aa) defined above.

In certain embodiments, the composition satisfies the properties of at least 2, 3, 4, or 5 of any of properties (x)-(bb) defined above.

In some embodiments of any of the compositions or methods disclosed herein:

cc) the ratio of the I-amino acid entity to the V-amino acid entity is at least 0.5:1, or at least 0.75:1, and not more than 1.5 to 1 or not more than 2:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 1:1;

dd) the ratio of the I-amino acid entity to the R-amino acid entity is at least 0.5:3, or at least 0.75:3, and not more than 2:3, or not more than 1.5:3, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 1:3;

ee) the ratio of the I-amino acid entity to the L-glutamine or a salt thereof is at least 0.5:4, or at least 0.75:4, and not more than 3:4, or not more than 2:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:4; or

ff) or a combination of two or three of (cc)-(ee).

In an embodiment, the composition satisfies the properties of (cc)-(ee) defined above.

In certain embodiments, the composition satisfies the properties of at least 2, 3, or 4 of any of properties (cc)-(ff) defined above.

In some embodiments of any of the compositions or methods disclosed herein:

gg) the ratio of the L-amino acid entity to the V-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5 to 1 or not more than 3:1, e.g., the ratio of the L-amino acid entity to the V-amino acid entity is about 2:1;

hh) the ratio of the L-amino acid entity to the R-amino acid entity is greater than 1:3 or greater than 1.5 to 3, and less than 3:3, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 2:3;

ii) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is greater than 1:4 or greater than 1.5 to 4, and less than 4:4 or less than 3:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:2; or

jj) a combination of two or three of (gg)-(ii).

In an embodiment, the composition satisfies the properties of (gg)-(ii) defined above.

In certain embodiments, the composition satisfies the properties of at least 2, 3, or 4 of any of properties (gg)-(jj) defined above.

In some embodiments of any of the compositions or methods disclosed herein:

kk) the ratio of the V-amino acid entity to the L-glutamine or a salt thereof is at least 0.5:4, or at least 0.75:4, and not more than 3:4, or not more than 2:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:4;

ll) the ratio of the V-amino acid entity to the R-amino acid entity is at least 0.5:3, or at least 0.75:3, and not more than 2:3, or not more than 1.5:3, e.g., the ratio of the V-amino acid entity to the R-amino acid entity is about 1:3;

mm) the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is at least 1:4, or at least 2:3, or not more than 5:7, or not more than 6:7, e.g., the ratio is about 6:11; or

nn) a combination of two or three of (kk)-(mm).

In an embodiment, the composition satisfies the properties of (kk)-(mm) defined above.

In certain embodiments, the composition satisfies the properties of at least 2, 3, or 4 of any of properties (kk)-(nn) defined above.

In some embodiments of any of the compositions or methods disclosed herein, the composition further comprises an S-amino acid entity.

In some embodiments of any of the compositions or methods disclosed herein:

1) the ratio of the L-amino acid entity to the I-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 2:1;

2) the ratio of L-amino acid entity to V-amino acid entity is at least 2:1, at least 3:1, at least 3.5:1, at least 4:1, or at least 5:1, and not more than 6: 1, e.g., the ratio of L-amino acid entity to V-amino acid entity is about 4:1;

3) the ratio of the L-amino acid entity to the R-amino acid entity is at least 1:1, at least 3.5:3, at least 4:3, or at least 2:1, and not more than 5:2, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 4:3;

4) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is at least 0.5:1, or at least 0.75:1, and not more than 1.5 to 1 or not more than 2:1, e.g., the ratio of the L-amino acid entity to the L-glutamine or salt thereof is about 1:1;

5) the ratio of the L-amino acid entity to the NAC entity or a salt thereof is at least 2:1, at least 3:1, at least 3.5:1, or at least 4:1, and not more than 5 to 1 or not more than 6:1, e.g., the ratio of the L-amino acid entity to the NAC entity or salt thereof is about 4:1 (e.g., 4:0.9);

6) optionally wherein the ratio of the L-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:3, greater than 1.5:3, about 2:3, or about 3:5, and not more than 2.5:3 or 1:1, e.g., the ratio of the L-amino acid entity to the S-amino acid entity is about 2:3, or the ratio of the L-amino acid entity to the S-amino acid entity is about 3:5; or

7) a combination of two, three, four, five, or six of (1)-(6).

In some embodiments of any of the compositions or methods disclosed herein:

8) the ratio of I-amino acid entity to V-amino acid entity is at least 1:1, at least 1.5:1, or about 2:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of I-amino acid entity to V-amino acid entity is about 2:1;

9) the ratio of the I-amino acid entity to the R-amino acid entity is greater than 1:3, greater than 1.5:3, or about 2:3, and not more than 2.5:3 or not more than 1:1, e.g., the ratio of the I-amino acid entity to the R-amino acid entity is about 2:3;

10) the ratio of the I-amino acid entity to the L-glutamine or a salt thereof is at least 1:4, at least 1:3, or about 1:2, and not more than 1:1 or not more than 2:1, e.g., the ratio of the I-amino acid entity to the L-glutamine or salt thereof is about 1:2;

11) the ratio of the I-amino acid entity to the NAC entity or a salt thereof is at least 1:1, at least 1.5:1, or about 2:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of the I-amino acid entity to the NAC entity or salt thereof is about 2:1 (e.g., 2:0.9);

12) optionally wherein the ratio of the I-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:4, greater than 1.5:4, about 1:3, or about 3:10, and not more than 1.5:3 or 2:3, e.g., the ratio of the I-amino acid entity to the S-amino acid entity is about 1:3, or the ratio of the I-amino acid entity to the S-amino acid entity is about 3:10; or

13) a combination of two, three, four, or five of (8)-(12).

In some embodiments of any of the compositions or methods disclosed herein:

14) the ratio of the V-amino acid entity to the R-amino acid entity is greater than 1:4, greater than 1.5:4, or about 1:3, and not more than 1:2 or not more than 1:1, e.g., the ratio of the V-amino acid entity to the R-amino acid entity is about 1:3;

15) the ratio of the V-amino acid entity to the L-glutamine or a salt thereof is greater than 1:5, or greater than 1.5:5, about 1:4, and not more than 1.5:4 or not more than 1:3, e.g., the ratio of the V-amino acid entity to the L-glutamine or salt thereof is about 1:4;

16) the ratio of the V-amino acid entity to the NAC entity or a salt thereof is at least 1:2, at least 1.5:2, or about 1:1, and not more than 1.5:1 or not more than 2:1, e.g., the ratio of the V-amino acid entity to the NAC entity or salt thereof is about 1:1 (e.g., 1:0.9);

17) optionally wherein the ratio of the V-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:8, greater than 1:7, about 1:6, or about 3:20, and not more than 1.5:6 or 1:3, e.g., the ratio of the V-amino acid entity to the S-amino acid entity is about 1:6, or the ratio of the V-amino acid entity to the S-amino acid entity is about 3:20; or

18) a combination of two, three, or four of (14)-(17).

In some embodiments of any of the compositions or methods disclosed herein:

19) the ratio of the R-amino acid entity to the L-glutamine or a salt thereof is greater than 1:2, greater than 1.25:2, or about 3:4, and not more than 3.5:4 or not more than 1:1, e.g., the ratio of the R-amino acid entity to the L-glutamine or salt thereof is about 3:4;

20) the ratio of the R-amino acid entity to the NAC entity or a salt thereof is at least 4:1, at least 4:1.5, or about 3:1, and not more than 3:1.5 or not more than 3:2, e.g., the ratio of the R-amino acid entity to the NAC entity or salt thereof is about 3:1 (e.g., 3:0.9);

21) optionally wherein the ratio of the R-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:4, greater than 1:3, about 1:2, or about 9:20, and not more than 1.5:2 or 1:1, e.g., the ratio of the R-amino acid entity to the S-amino acid entity is about 1:2, or the ratio of the R-amino acid entity to the S-amino acid entity is about 9:20; or

22) a combination of two or three of (19)-(21).

In some embodiments of any of the compositions or methods disclosed herein:

23) the ratio of the L-glutamine to the NAC entity or a salt thereof is at least 5:1, at least 5:1.5, or about 4:1, and not more than 4:1.5 or not more than 3:1, e.g., the ratio of the L-glutamine to the NAC entity or salt thereof is about 4:1 (e.g., 4:0.9);

24) optionally wherein the ratio of the L-glutamine to the S-amino acid entity or a salt thereof is greater than 1:3, greater than 1.25:3, about 2:3, or about 3:5, and not more than 2.5:3 or 1:1, e.g., the ratio of the L-glutamine to the S-amino acid entity is about 2:3, or the ratio of the L-glutamine to the S-amino acid entity is about 3:5; or

25) a combination of (23) and (24).

In some embodiments of any of the compositions or methods disclosed herein:

26) the ratio of the NAC entity to the S-amino acid entity or a salt thereof is greater than 1:8, greater than 1:7, or about 1:6, and not more than 1:5 or not more than 1.5:5, e.g., the ratio of the NAC entity to the S-amino acid entity is about 1:6 (e.g., 0.9:6 or 2.7:20).

In an embodiment, the composition satisfies the properties of (1)-(7) defined above.

In certain embodiments, the composition satisfies the properties of at least 2, 3, 4, 5, 6, or 7 of any of properties (1)-(26) defined above.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 : 6 : 3 : 9 : 12 : 2.7.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 : 6 : 3 : 9 : 12 : 2.7 : 18.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 : 6 : 3 : 9 : 12 : 2.7 : 20.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15%.

In an embodiment, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 18 +/- 15%.

In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 : 6 : 3 : 9 : 12 : 2.7 : 9: 9. In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 : 6 : 3 : 9 : 12 : 2.7 : 10: 10.

In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 9 +/- 15% : 9 +/- 15%. In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 10 +/- 15% : 10 +/- 15%.

In some embodiments of any of the compositions or methods disclosed herein:

oo) a wt. % of the L-amino acid entity in the composition is greater than the wt. % of the NAC or a salt thereof;

pp) a wt. % of the R-amino acid entity in the composition is greater than the wt. % of the NAC or a salt thereof;

qq) a wt. % of the L-glutamine or a salt thereof in the composition is greater than the wt. % of the NAC or a salt thereof; or

rr) a combination of two or three of (oo)-(qq).

In some embodiments of any of the compositions or methods disclosed herein, at least one of (a)-(d) is a free amino acid, e.g., two, three, or four of (a)-(d) are a free amino acid, e.g., at least 50 wt. % of the total wt. of the composition is one or more amino acid entities in free form.

In some embodiments of any of the compositions or methods disclosed herein, at least one of (a)-(d) is in a salt form, e.g., one, two, three, or four of (a)-(d) is in a salt form, e.g., at least 10 wt. % of the total wt. of the composition is one or more amino acid entities in salt form.

In some embodiments of any of the compositions or methods disclosed herein, the composition is capable of one, two, three, four, five, or all of:

a) decreasing or preventing liver fibrosis;
b) decreasing or preventing liver injury;
c) decreasing or preventing hepatocyte inflammation;
d) improving, e.g., increasing, glucose tolerance;
e) decreasing or preventing steatosis;
f) decreasing or preventing hepatocyte ballooning; or
g) improving gut function.

In some embodiments of any of the compositions or methods disclosed herein, the composition further comprises one or both of L-glycine and L-serine. In certain embodiments, the composition comprises an L-amino acid entity, an I-amino acid entity, an V-amino acid entity, an R-amino acid entity, an L-glutamine or a salt thereof, an NAC or a salt thereof, and an L-glycine. In certain embodiments, the composition comprises an L-amino acid entity, an I-amino acid entity, an V-amino acid entity, an R-amino acid entity, an L-glutamine or a salt thereof, an NAC or a salt thereof, and an L-serine. In certain embodiments, the composition comprises an L-amino acid entity, an I-amino acid entity, an V-amino acid entity, an R-amino acid entity, an L-glutamine or a salt thereof, an NAC or a salt thereof, an L-glycine, and an L-serine. In any of the aforesaid embodiments in this paragraph, the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the L-glutamine or salt thereof, and the NAC or salt thereof is about 1 : 0.5: 0.5: 1.5 : 2 : 0.15 or about 1 : 0.5: 0.5: 1.5 : 2 : 0.3.

In some embodiments of any of the compositions or methods disclosed herein, the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 0.5 to 3 : 0.5 to 4 : 1 to 4 : 0.1 to 2.5, e.g., the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 1 : 1.5 : 2 : 0.15, about 1 : 1.5 : 2 : 0.225, about 1 : 1.5 : 2 : 0.3, or about 1 : 1.5 : 2 : 0.5. In any of the aforesaid embodiments in this paragraph, the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 1 : 0.75 : 2 : 0.15, about 1 : 0.75 : 2 : 0.225, about 1 : 0.75 : 2 : 0.3, or about 1 : 0.75 : 2 : 0.5.

In some embodiments, the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the L-glutamine or salt thereof, and the NAC or salt thereof is about 1 : 0.5: 0.5: 1.5 : 2 : 0.15, about 1 : 0.5: 0.5: 1.5 : 2 : 0.225, about 1 : 0.5: 0.5: 1.5 : 2 : 0.3, or about 1 : 0.5: 0.5: 1.5 : 2 : 0.5.

In some embodiments of any of the compositions or methods disclosed herein, the composition comprises about 0.5 g to about 10 g of the L-amino acid entity, about 0.25 g to about 5 g of the I-amino acid entity, about 0.25 g to about 5 g of the V-amino acid entity, about 0.5 g to about 20 g of the R-amino acid entity, about 1 g to about 20 g of the L-glutamine or a salt thereof, and about 0.1 g to about 5 g of the NAC or a salt thereof, e.g., the composition comprises about 1 g of the L-amino acid entity, about 0.5 g of the I-amino acid entity, about 0.5 g of V-amino acid entity, about 1.5 g of R-amino acid entity, about 2 g of L-glutamine or a salt thereof, and about 0.15 g, about 0.225 g, about 0.3 g, or about 0.5 g of NAC or a salt thereof. In certain embodiments, the composition comprises about 0.15 g of NAC. In certain embodiments, the composition comprises about 0.3 g of NAC. In embodiments, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, and about 0.9 g of NAC or a salt thereof. In embodiments, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, about 0.9 g of NAC or a salt thereof, and about 6 g of L-serine or a salt thereof. In embodiments, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, about 0.9 g of NAC or a salt thereof, and about 6.67 g of L-serine or a salt thereof. In embodiments, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, about 0.9 g of NAC or a salt thereof, about 3 g of L-serine or a salt thereof, and about 3 g of L-glycine or a salt thereof. In embodiments, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of

V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, about 0.9 g of NAC or a salt thereof, about 3.33 g of L-serine or a salt thereof, and about 3.33 g of L-glycine or a salt thereof.

In some embodiments of any of the compositions or methods disclosed herein, the composition comprises:

a) L-Leucine or a salt thereof;

b) L-Isoleucine or a salt thereof;

c) L-Valine or a salt thereof;

d) L-Arginine or a salt thereof;

e) L-Glutamine or a salt thereof; and

f) NAC or a salt thereof.

In an embodiment, L-Leucine is provided as part of a dipeptide comprising L-Leucine, or a salt thereof, or a tripeptide comprising L-Leucine, or a salt thereof.

In an embodiment, L-Isoleucine is provided as part of a dipeptide comprising L-Isoleucine, or a salt thereof, or a tripeptide comprising L- Isoleucine, or a salt thereof.

In an embodiment, L-Valine is provided as part of a dipeptide comprising L- Valine, or a salt thereof, or a tripeptide comprising L- Valine, or a salt thereof.

In an embodiment, L-Arginine is provided as part of a dipeptide comprising L-Arginine, or a salt thereof, or a tripeptide comprising L-Arginine, or a salt thereof.

In an embodiment L-Glutamine is provided as part of a dipeptide comprising L-Glutamine, or a salt thereof, or a tripeptide comprising L- Glutamine, or a salt thereof.

In an embodiment NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

In some embodiments of any of the compositions or methods disclosed herein, the composition comprises a combination of 4 to 20 different amino acid entities, e.g., a combination of 5 to 15 different amino acid entities.

In some embodiments of any of the compositions or methods disclosed herein, at least two, three, four, or more amino acid entities are not comprised in a peptide of more than 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues in length.

Another aspect of the invention features a method for improving liver function, wherein the method comprises administering to a subject in need thereof an effective amount of a composition comprising:

a) a L-amino acid entity chosen from L-leucine or a salt thereof, or β-hydroxy-β-methybutyrate (HMB) or a salt thereof, or a combination of L-leucine or a salt thereof and HMB or a salt thereof;

b) an R-amino acid entity chosen from:L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof, or a combination of two or three of L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof;

c) L-glutamine or a salt thereof; and

d) N-acetylcysteine (NAC) or a salt thereof.

In an embodiment, L-Leucine is provided as part of a dipeptide comprising L-Leucine, or a salt thereof, or a tripeptide comprising L-Leucine, or a salt thereof.

In an embodiment, L-Arginine is provided as part of a dipeptide comprising L-Arginine, or a salt thereof, or a tripeptide comprising L-Arginine, or a salt thereof.

In an embodiment L-Glutamine is provided as part of a dipeptide comprising L-Glutamine, or a salt thereof, or a tripeptide comprising L- Glutamine, or a salt thereof.

In an embodiment NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

Another aspect of the invention features a method for treating one or more symptoms selected from the group consisting of decreased fat metabolism, hepatocyte apoptosis, hepatocyte ballooning, inflammation of adipose tissue, inflammation of hepatic tissue, fibrosis, liver injury, steatosis, glucose tolerance, and oxidative stress, wherein the method comprises administering to a subject in need thereof an effective amount of a composition comprising:

a) a L-amino acid entity chosen from L-leucine or a salt thereof, or β-hydroxy-β-methybutyrate (HMB) or a salt thereof, or a combination of L-leucine or a salt thereof and HMB or a salt thereof;

b) an R-amino acid entity chosen from:L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof, or a combination of two or three of L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof;

c) L-glutamine or a salt thereof; and

d) N-acetylcysteine (NAC) or a salt thereof.

In an embodiment, L-Leucine is provided as part of a dipeptide comprising L-Leucine, or a salt thereof, or a tripeptide comprising L-Leucine, or a salt thereof.

In an embodiment, L-Arginine is provided as part of a dipeptide comprising L-Arginine, or a salt thereof, or a tripeptide comprising L-Arginine, or a salt thereof.

In an embodiment L-Glutamine is provided as part of a dipeptide comprising L-Glutamine, or a salt thereof, or a tripeptide comprising L- Glutamine, or a salt thereof.

In an embodiment NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

Another aspect of the invention features a method for treating fatty liver disease, wherein the method comprises administering to a subject in need thereof an effective amount of a composition comprising:

a) a L-amino acid entity chosen from L-leucine or a salt thereof, or β-hydroxy-β-methybutyrate (HMB) or a salt thereof, or a combination of L-leucine or a salt thereof and HMB or a salt thereof;

b) an R-amino acid entity chosen from:L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof, or a combination of two or three of L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof;

c) L-glutamine or a salt thereof; and

d) N-acetylcysteine (NAC) or a salt thereof.

In an embodiment, L-Leucine is provided as part of a dipeptide comprising L-Leucine, or a salt thereof, or a tripeptide comprising L-Leucine, or a salt thereof.

In an embodiment, L-Arginine is provided as part of a dipeptide comprising L-Arginine, or a salt thereof, or a tripeptide comprising L-Arginine, or a salt thereof.

In an embodiment L-Glutamine is provided as part of a dipeptide comprising L-Glutamine, or a salt thereof, or a tripeptide comprising L- Glutamine, or a salt thereof.

In an embodiment NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

In some embodiments of any of the compositions or methods disclosed herein, e.g., of any of the methods described herein, the subject has a disease or disorder selected from the group consisting of non-alcoholic fatty liver (NAFL), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic fatty liver disease (AFLD), and alcoholic steatohepatitis (ASH). In certain embodiments, the subject has pediatric NAFLD.

In some embodiments of any of the compositions or methods disclosed herein, e.g., of any of the methods described herein, the subject has a high BMI, obesity, gut leakiness, gut dysbiosis, or gut microbiome disturbance.

In some embodiments of any of the compositions or methods disclosed herein, e.g., of any of the methods described herein, the subject has cirrhosis, hepatocarcinoma, an increased risk of liver failure, an increased risk of death, metabolic syndrome, or type 2 diabetes.

In some embodiments of any of the compositions or methods disclosed herein, e.g., of any of the methods described herein, the subject has increased levels of inflammatory cytokines relative to a normal subject, e.g., the subject has increased levels of TNFα relative to a normal subject e.g., without the one or more symptoms or without the fatty liver disease.

In some embodiments, e.g., of any of the methods described herein, the subject exhibits muscle atrophy or has a decreased ratio of muscle tissue to adipose tissue relative to a normal subject, e.g., without the one or more symptoms or without a fatty liver disease, e.g., the subject exhibits muscle atrophy without one or both of fibrosis or cirrhosis.

In some embodiments, e.g., of any of the methods described herein, the subject exhibits reverse lipid transport from adipose tissue to liver tissue.

In some embodiments, e.g., of any of the methods described herein, the subject is treated with a composition, e.g., any composition as described herein.In some embodiments of any of the aspects described herein:

(i) an amino acid entity (e.g., at least one, two, or three of the amino acid entities) of (a) is selected from Table 2; and/or

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1B are graphs showing the effect of treatment with an amino acid composition (Amino Acid Composition A-1) on the NAFLD activity score, ballooning, and fibrosis in the STAM mouse model (FIG. 1A) and in the FATZO mouse model (FIG. 1B).

FIG. 2 is a schematic showing the metabolic symptoms of patients with non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and cirrhosis prior to administration of a composition comprising amino acid entities described herein (top) and the improvement in patients with NAFLD, NASH, and cirrhosis after administration of the composition (bottom).

FIG. 3 is a schematic showing treatment regimens for administration of an amino acid composition to STAM and FATZO mice.

FIGS. 4A-4E are a series of graphs and images showing the effect of treating STAM and FATZO mice with an amino acid composition on the NAFLD activity score (NAS), steatosis, inflammation, and liver fibrosis as determined with histology.

FIGS. 5A-5B are images showing the levels of liver unsaturated fatty acids and acylcarnitines of STAM mice treated with the amino acid composition.

FIG. 6 is an image of a gene map of the liver gene expression pattern following treatment with the amino acid composition in STAM mice showing activation of ACOX1.

FIGS. 7A-7D are images of gene maps of the liver gene expression pattern following treatment with the amino acid composition in STAM mice showing upstream regulator activation of anti-inflammatory IL-10 (FIG. 7A); inhibition of pro-inflammatory NF-kB (FIG. 7B), interferons, IL-1b, and IL-2 (FIG. 7C); and suppression of the fibrogenic TGF-b signaling pathway.

FIG. 8 is a series of graphs showing MCP-1 and MIP-1 protein levels, which are the ligands of C-C chemokine receptor types 2 (CCR2) and 5 (CCR5), following treatment with the amino acid composition.

FIGS. 9A-9L are a series of microscopy images shown lipid accumulation in primary human hepatocytes following treatment with vehicle control (FIGS. 9A-9D), a LIVRQNAC amino acid composition (FIGS. 9E-9H), or free fatty acids and TNFα (FF+TNF; FIGS. 9I-9L).

FIG. 10 is a series of microscopy images showing liver histology (H&E stain or Sirius Red stain for collagen deposition) from FATZO mice after administration of the indicated amino acid compositions.

FIG. 11 is a series of microscopy images showing liver histology from FATZO mice after administration of the indicated amino acid compositions.

FIG. 12 is a series of graphs showing NAFLD activity scores (top left panel), Sirius Red staining (top right panel), steatosis levels (bottom left panel), inflammation levels (bottom middle panel), and ballooning (bottom right panel) observed in fixed liver tissues from FATZO mice after administration of the indicated amino acid compositions.

DETAILED DESCRIPTION

The present invention provides, at least in part, methods and compositions comprising at least four different amino acid entities. In some embodiments, the composition is capable of one, two, three, four, five, or six or all of:

a) decreasing or preventing liver fibrosis;
b) decreasing or preventing liver injury;
c) decreasing or preventing hepatocyte inflammation;
d) improving, e.g., increasing, glucose tolerance;
e) decreasing or preventing steatosis;
f) decreasing or preventing hepatocyte ballooning; or
g) improving gut function.

In some embodiments, at least one amino acid entity in the compositions is not a peptide of more than 20 amino acid residues in length.

In some embodiments, the composition comprises a leucine (L)-amino acid entity, an arginine (R)-amino acid entity, a glutamine (Q)-amino acid entity; and an antioxidant or reactive oxygen species (ROS) scavenger (e.g., a N-acetylcysteine (NAC) entity, e.g., NAC). In some embodiments, at least one amino acid entity is not a peptide of more than 20 amino acid residues in length. In some embodiments, the composition is capable of improving gut barrier function.

The composition described herein can be administered to a subject to provide a beneficial effect in one or both of improving liver function or treating (e.g., revering, reducing, ameliorating, or preventing) a liver disease (e.g., a fatty liver disease). A subject that may be treated with the composition include a subject having non-alcoholic fatty liver disease (NAFLD; e.g., pediatric NAFLD), such as a subject with non-alcoholic steatohepatitis (NASH) or NAFL, or subjects with alcoholic fatty liver disease (AFLD), such as alcoholic steatohepatitis (ASH). In particular, the subject may have one, two, or more (e.g., all) of a high BMI, obesity, fibrosis, or cirrhosis. The subject may also have one, two, or more (e.g., all) of gut leakiness, gut dysbiosis, or gut microbiome disturbance.

The subject may exhibit an improvement in liver function or liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)) after administration of a composition comprising a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity; and an antioxidant or ROS scavenger, e.g., a NAC entity, e.g., NAC. For example, the amino acid entity composition may be administered to the subject for a treatment period of, e.g., two weeks, three weeks, four weeks, five weeks, six weeks, seven weeks, eight weeks, nine weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, or longer at a dose of about 15 total grams per day to about 90 total grams per day (e.g., a total of about 48 g or a total of about 72 g per day).

Treatment with the amino acid entity composition can result in improved liver function in a subject, e.g., by one, two, three, four, five or more (e.g., all) of increasing free fatty acid and lipid metabolism, improving mitochondrial function, browning of white adipose tissue (WAT), decreasing reactive oxygen species (ROS), increasing levels of glutathione (GSH), decreasing hepatic inflammation, improving gut barrier function, increasing insulin secretion, or improving glucose tolerance.

In some embodiments, the composition is for use as a medicament in improving liver function in a subject (e.g., a subject with a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)). In some embodiments, the composition including amino acid entities is for use as a medicament in treating (e.g., reversing, reducing, ameliorating, or preventing) a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)) in a subject.

In some embodiments, the composition is for use in the manufacture of a medicament for improving liver function in a subject (e.g., a subject with a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)). In some embodiments, the composition including amino acid entities is for use in the manufacture of a medicament for treating (e.g., reversing, reducing, ameliorating, or preventing) a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)) in a subject.

Additionally, the compositions can be used in methods of dietary management of a subject (e.g., a subject without a liver disease or with a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)).

One embodiment provides a nutritional supplement, dietary formulation, functional food, medical food, food, or beverage comprising a composition described herein. Another embodiment provides a nutritional supplement, dietary formulation, functional food, medical food, food, or beverage comprising a composition described herein for use in the management of any of the diseases or disorders described herein.

One embodiment provides a method of maintaining or improving liver health comprising administering to a subject an effective amount of a composition described herein. Another embodiment provides a method of providing nutritional support or supplementation to a subject with a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)) comprising administering to the subject an effective amount of a composition described herein. Yet another embodiment provides a method of providing nutritional supplementation that aids in the management of liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)) comprising administering to the subject in need thereof an effective amount of a composition described herein.

Definitions

Terms used in the claims and specification are defined as set forth below unless otherwise specified.

It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

As used herein, the term “amino acid entity” refers to an amino acid in one or both of free form or salt form, an amino acid residue of a peptide (e.g., of a dipeptide, oligopeptide, or polypeptide), a derivative of an amino acid, a precursor of an amino acid, or a metabolite of an amino acid .

As used herein the term “XXX amino acid entity” refers to an amino acid entity that if a free amino acid, comprises free XXX or XXX in salt form; if a peptide, refers to a peptide comprising an XXX residue; if a derivative, refers to a derivative of XXX; if a precursor, refers to a precursor of XXX; and if a metabolite, refers to a XXX metabolite. For example, where XXX is leucine (L), then L-amino acid entity refers to free L or L in salt form, a peptide comprising a L residue, a L derivative, a L precursor, or a metabolite of L; where XXX is arginine (R), then R-amino acid entity refers to free R or R in salt form, a peptide comprising a R residue, a R derivative, a R precursor, or a metabolite of R; where XXX is glutamine (Q), then Q-amino acid entity refers to free Q or Q in salt form, a peptide comprising a Q residue, a Q derivative, a Q precursor, or a metabolite of Q; and where XXX is N-acetylcysteine (NAC), then NAC-amino acid entity refers to free NAC or NAC in salt form, a peptide comprising a NAC residue, a NAC derivative, a NAC precursor, or a metabolite of NAC.

"About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values.

An **"**amino acid**"** refers to an organic compound having an amino group (-NH_2), a carboxylic acid group (-C(=O)OH), and a side chain bonded through a central carbon atom, and includes essential and non-essential amino acids, as well as natural and unnatural amino acids.

The proteogenic amino acids, shown below, are known by three- and one-letter abbreviations in addition to their full names. For a given amino acid, these abbreviations are used interchangeably herein. For example, Leu, L or leucine all refer to the amino acid leucine; Ile, I or isoleucine all refer to the amino acid isoleucine; Val, V or valine all refer to the amino acid valine; Arg, R or arginine all refer to the amino acid arginine; and Gln, Q or glutamine all refer to the amino acid glutamine.

Likewise, the non-natural amino acid derivative N-acetylcysteine may be referred to interchangeably by "NAC" or "N-acetylcysteine."

Amino acids may be present as D- or L- isomers. Unless otherwise indicated, amino acids referred to herein are L-isomers of amino acids.

Table 1. Amino acid names and abbreviations.

Amino acid	Three-letter	One-letter
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E

Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

The term “effective amount” as used herein means an amount of an amino acid, or pharmaceutical composition which is sufficient enough to significantly and positively modify the symptoms and/or conditions to be treated (e.g., provide a positive clinical response). The effective amount of an active ingredient for use in a pharmaceutical composition will vary with the particular condition being treated, the severity of the condition, the duration of treatment, the nature of concurrent therapy, the particular active ingredient(s) being employed, the particular pharmaceutically-acceptable excipient(s) and/or carrier(s) utilized, and like factors with the knowledge and expertise of the attending physician.

A “pharmaceutical composition” described herein comprises at least one amino acid and a pharmaceutically acceptable carrier or excipient. In some embodiments, the pharmaceutical composition is used as a therapeutic, a nutraceutical, a medical food, or as a supplement.

The term “pharmaceutically acceptable” as used herein, refers to amino acids, materials, excipients, compositions and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

A composition, formulation or product is "therapeutic" if it provides a beneficial clinical effect. A beneficial clinical effect can be shown by lessening the progression of a disease and/or alleviating one or more symptoms of the disease.

A "unit dose" or "unit dosage" as used herein means an amount or dose of medicine prepared in an individual packet or container for convenience, safety, or monitoring. A "unit dose" or "unit dosage" comprises the drug product or drug products in the form in which they are marketed for use, with a specific mixture of active ingredients and inactive components (excipients), in a particular configuration (such as a capsule shell, for example), and apportioned into a particular dose.

As used herein, the terms "treat," "treating," or "treatment" of a liver disease refer in one embodiment, to ameliorating, e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH) , (i.e., slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another embodiment, "treat," "treating," or "treatment" refers to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. In yet another embodiment, "treat," "treating," or "treatment" refers to modulating a symptom of a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)), either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In yet another embodiment, "treat," "treating," or "treatment" refers to preventing or delaying the onset or development or progression of a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)).

Determination of amino acid weight percent and amino acid ratios in a composition

The weight ratio of a particular amino acid or particular amino acids in a composition or mixture of amino acids is the ratio of the weight of the particular amino acid or amino acids in the composition or mixture compared to the total weight of amino acids present in the composition or mixture. This value is calculated by dividing the weight of the particular amino acid or of the particular amino acids in the composition or mixture by the weight of all amino acids present in the composition or mixture.

Compositions comprising Amino Acid Entities

The present disclosure provides compositions, e.g., pharmaceutical compositions, comprising amino acid entities. These pharmaceutical compositions are made up of amino acid entities including amino acids in one or both of free form or salt form, amino acid residues of a peptide (e.g., of a dipeptide, oligopeptide, or polypeptide), derivatives of an amino acid, precursors of an amino acid, or metabolites of an amino acid. For example, the compositions can include a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, a glutamine (Q)-amino acid entity; and an antioxidant or reactive oxygen species (ROS) scavenger, e.g., a N-acetylcysteine (NAC) entity, e.g., NAC (Table 2). In particular, at least one amino acid entity is not a peptide of more than 20 amino acid residues in length.

Table 2. Amino acid entities include amino acids, precursors, metabolites, and derivatives of the compositions described herein.

	Exemplary Amino Acid	Precursors	Metabolites	Derivatives
L	L-Leucine	Oxo-leucine	HMB (beta-hydroxy-beta-methybutyrate); Oxo-leucine; Isovaleryl-CoA	D-Leucine; N-Acetyl-Leucine
I	L-Isoleucine	2-Oxo-3-methyl-valerate; Threonine	2-Oxo-3-methyl-valerate; Methylbutyrl-CoA	D-Isoleucine; N-Acetyl-Isoleucine
V	L-Valine	2-Oxo-valerate	Isobutryl-CoA; 3-HIB-CoA; 3-HIB	D-Valine; N-Acetyl-Valine
R	L-Arginine	Argininosuccinate; Citrulline; Aspartate; Glutamate	Ornithine; Citrulline; Agmatine; Creatine	D-Arginine; N-Acetyl-Arginine;
Q	L-Glutamine	Glutamate	Carbamoyl-P; Glutamate	D-Glutamine; N-Acetyl-Glutamine;

NAC	N-Acetylcysteine	Serine; Acetylserine; Cystathionine;	Glutathione; Cystathionine; Homocysteine; Methionine	D-Cysteine; L-Cysteine; Cystine; Cysteamine
S	L-Serine	Phosphoserine, P-hydroxypyruvate, L-Glycine	Glycine, Tryptophan, Acetylserine, Cystathionine, Phosphatidylserine	

It is contemplated that alternatives to serine that can be an S-amino acid entity include, for example, glycine, threonine, or a combination of serine and glycine (e.g., a 1:1 ratio of serine and glycine).

In some embodiments, the total weight of the L-amino acid entity, R-amino acid entity, Q-amino acid entity; and ROS scavenger, e.g., a NAC entity, e.g., NAC, is greater than the total wt. of other amino acid entities in the composition. In certain embodiments, two, three, or more (e.g., all) of methionine (M), trytophan (W), or valine (V) may be absent from the amino acid entity composition, or if present, are present at less than 2 weight (wt.) %.

In some embodiments, one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity. The R-amino acid entity can be present, e.g., at an amount of at least 2 wt. %, at least 3 wt. %, at least 4 wt. %, at least 5 wt. %, at least 6 wt. %, at least 7 wt. %, or at least 8 wt. % greater than the L-amino acid entity. The Q-amino acid entity can be present, e.g., at an amount of at least 2 wt. %, at least 3 wt. %, at least 4 wt. %, or at least 5 wt. % greater than the L-amino acid entity.

In some embodiments, the L-amino acid entity is selected from the group consisting of a precursor, a metabolite, and a derivative. In certain embodiments, the L-amino acid entity is selected from the group consisting of L-leucine, β-hydroxy-β-methybutyrate (HMB), oxo-leucine, isovaleryl-CoA, D-leucine, and n-acetylleucine. In one embodiment, the L-amino acid entity is L-leucine. In another embodiment, the L-amino acid entity is HMB.

In some embodiments, the R-amino acid entity is selected from the group consisting of a precursor, a metabolite, and a derivative. In certain embodiments, the R-amino acid entity is selected from the group consisting of L-arginine, D-arginine, ornithine, argininosuccinate, citrulline, aspartate, glutamate, agmatine, and N-acetyl-arginine. In one embodiment, the R-amino acid entity is L-arginine. In one embodiment, the R-amino acid entity is creatine. In another embodiment, the R-amino acid entity is ornithine.

In some embodiments, the Q-amino acid entity is selected from the group consisting of a precursor, a metabolite, and a derivative. In certain embodiments, the Q-amino acid entity is selected from the group consisting of L-glutamine, glutamate, carbamoyl-P, glutamate, D-glutamine, and n-acetylglutamine. In one embodiment, the Q-amino acid entity is L-glutamine.

In some embodiments, the NAC-amino acid entity is selected from the group consisting of a precursor, a metabolite, and a derivative. In certain embodiments, the NAC-amino acid entity is selected from the group consisting NAC, serine, acetylserine, cystathionine, cystathionine, homocysteine, methionine, glutathione, D-cysteine, and L-cysteine. In one embodiment, the NAC entity is NAC. In one embodiment, the NAC entity is glutathione.

In various embodiments, the composition further comprises one or two additional branched-chain amino acid (BCAA)-entities, e.g., one or both of an isoleucine (I)-amino acid-entity and a valine (V)-amino acid-entity. In some embodiments, both the I-amino acid-entity and the V-amino acid-entity are present. In certain embodiments, the L-entity is present at a higher amount (% by weight) than one or both of the I-amino acid-entity and the V-amino acid-entity (e.g., the L-entity is present at an amount of at least 10 wt. %, at least 15 wt. %, at least 20 wt. %, at least 25 wt. %, at least 30 wt. %, at least 35 wt. %, at least 40 wt. %, at least 45 wt. %, or at least 50 wt. % greater than one or both of the I-amino acid-entity and the V-amino acid-entity).

In some embodiments, the I-amino acid entity is selected from the group consisting of a salt, a precursor, a metabolite, and a derivative. In certain embodiments, the I-amino acid entity is selected from the group consisting of L-isoleucine, 2-oxo-3-methyl-valerate, threonine, 2-oxo-3-methyl-valerate, methylbutyrl-CoA, D-isoleucine, and N-acetyl-isoleucine. In one embodiment, the I-amino acid entity is L-isoleucine.

In some embodiments, the V-amino acid entity is selected from the group consisting of a precursor, a metabolite, and a derivative. In certain embodiments, the V-amino acid entity is selected from the group consisting of L-valine, 2-oxo-valerate, isobutryl-CoA, 3-HIB-CoA, 3-HIB, D-valine, and N-acetyl-valine. In one embodiment, the I-amino acid entity is L-valine.

In some embodiments, the composition comprises L-leucine or a leucine metabolite (e.g., HMB), L-arginine or an L-arginine metabolite (e.g., creatine or ornithine), L-glutamine, and NAC or a NAC metabolite, e.g., glutathione. In one embodiment, the composition comprises L-leucine, L-arginine, L-glutamine, and NAC. In one embodiment, the composition comprises HMB, creatine, L-glutamine, and glutathione. In one embodiment, the composition comprises HMB, ornithine, L-glutamine, and glutathione. In one embodiment, the composition comprises HMB, L-arginine, L-glutamine, and NAC. In one embodiment, the composition comprises L-leucine, creatine, L-glutamine, and NAC. In one embodiment, the composition comprises L-leucine, ornithine, L-glutamine, and NAC. In one embodiment, the composition comprises L-leucine, L-arginine, L-glutamine, and glutathione.

In some embodiments, the weight (wt.) ratio of the L-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 0.5 to 3 : 0.5 to 4 : 1 to 4 : 0.1 to 2.5. In one embodiment, the wt. ratio of the L-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 1 : 1.5 : 2 : 0.15.

In some embodiments, the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 0.5 to 2 : 0.1 to 1 : 0.1 to 1 : 0.5 to 3 : 0.5 to 4 : 0.1 to 0.5. In an embodiment, the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 1 : 0.5 : 0.5 : 1.5 : 2 : 0.15.

In various embodiments, the total wt. of amino acids present is about 2 g to about 60 g. In certain embodiments, the total wt. of amino acids present is about 6 g, about 12 g, about 18 g, about 24 g, or about 48 g. In one embodiment, the total wt. of amino acids present is about 6 g. In one embodiment, the total wt. of amino acids present is about 12 g. In one embodiment, the total wt. of amino acids present is about 18 g. In an embodiment, the total wt. of amino acids present is about 24 g. In one embodiment, the total wt. of amino acids present is about 48 g.

In some embodiments, the composition comprises about 0.5 g to about 10 g of the L-amino acid entity, about 0.25 g to about 5 g of the I-amino acid entity, about 0.25 g to about 5 g of the V-amino acid entity, about 1 g to about 20 g of the R-amino acid entity, about 1 g to about 20 g of the Q-amino acid entity, and about 0.1 g to about 5 g of the NAC-amino acid entity. In an embodiment, the composition comprises about 1 g of the L-amino acid entity, about 0.5 g of the I-amino acid entity, about 0.5 g of V-amino acid entity, about 1.5 g of R-amino acid entity, about 2 g of Q-amino acid entity, and about 0.15 g of NAC-amino acid entity. In an embodiment, the composition comprises about 2 g of the L-amino acid entity, about 1 g of the I-amino acid entity, about 1 g of the V-amino acid entity, about 3 g of the R-amino acid entity, about 4 g of the Q-amino acid entity, and about 0.3 g of the NAC-amino acid entity. In an embodiment, the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 2 g of the V-amino acid entity, about 6 g of the R-amino acid entity, about 8 g of the Q-amino acid entity, and about 0.6 g of the NAC-amino acid entity.

In some embodiments, the the amino acids comprise about 10 wt % to about 30 wt % leucine, about 5 wt % to about 15 wt % isoleucine, about 5 wt % to about 15 wt % valine, about 15 wt % to about 40 wt % arginine, about 20 wt % to about 50 wt % glutamine, and about 1 wt % to about 8 wt % n-acetylcysteine. In certain embodiments, the amino acids comprise about 16 wt % to about 18 wt % leucine, about 7 wt % to about 9 wt % isoleucine, about 7 wt % to about 9 wt % valine, about 28 wt % to about 32 wt % arginine, about 31 wt % to about 34 wt % glutamine, and about 1 wt % to about 5 wt % n-acetylcysteine. In an embodiment, the amino acids comprise about 16.8 wt % leucine, about 8.4 wt % isoleucine, about 8.4 wt % valine, about 30.4 wt % arginine, about 33.6 wt % glutamine, and about 2.5 wt % n-acetylcysteine.

In any of the foregoing embodiments, at least one amino acid entity is a free amino acid, e.g., one, two, three, or more (e.g., all) amino acid entities are a free amino acid. In some embodiments, the L-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is a free amino acid entity. In certain embodiment, the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity a free amino acid.

In any of the foregoing embodiments, at least one amino acid entity is in a salt form, e.g., one, two, three, or more (e.g., all) of the amino acid entities is in a salt form. In some embodiments, wherein the L-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is in a salt form. In certain embodiments, the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is in a salt form.

In any of the foregoing embodiments, the composition comprises a combination of 2 to 20 different amino acid entities, e.g., 5 to 15 different amino acid entities.

In some embodiments, the NAC entity is more stable than cysteine. In certain embodiments, the NAC entity does not comprise cysteine.

In some embodiments, the composition further comprises one, two, three, four, five, six, seven, eight, nine, ten, or more (e.g., all) or more of serine, glycine, glutamine, HMB, arginine, L-leucine, citrulline, glutamine, ornithine, L-cysteine, cystine, or glutathione.

In some embodiments, the composition further comprises serine.

In some embodiments, the composition further comprises glycine.

In some embodiments, the composition further comprises carnitine.

In some embodiments, the composition includes arginine, glutamine, N-acetylcysteine, and a branched-chain amino acid (BCAA) chosen from one, two, or all of leucine, isoleucine, and valine.

In some embodiments, the BCAA is leucine.

In some embodiments, the BCAA is isoleucine.

In some embodiments, the BCAA is valine.

In some embodiments, the BCAA is leucine and isoleucine.

In some embodiments, the BCAA is leucine and valine.

In some embodiments, the BCAA is isoleucine and valine.

In some embodiments, the BCAA is leucine, isoleucine, and valine.

In particular, the composition may consist of leucine, isoleucine, valine, arginine, glutamine, and N-acetylcysteine.

In some embodiments, the amino acids leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5: 2 : 0.1-0.3. In some embodiments, the amino acids leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5: 2 : 0.15. In some embodiments, the amino acids leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5: 2 : 0.25.

In some embodiments, the amino acids leucine, isoleucine, valine, arginine (e.g., arginine HCl), glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5-1.81 : 2 : 0.1-0.3. In some embodiments, the amino acids leucine, isoleucine, valine, arginine (e.g., arginine HCl), glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5-1.81 : 2 : 0.15. In some embodiments, the amino acids leucine, isoleucine, valine, arginine (e.g., arginine HCl), glutamine, and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5-1.81 : 2 : 0.25.

In some embodiments, the amino acids leucine, isoleucine, valine, arginine (e.g., arginine HCl), glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.81 : 2 : 0.1-0.3. In some embodiments, the amino acids leucine, isoleucine, valine, arginine (e.g., arginine HCl), glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.81 : 2 : 0.15. In some embodiments, the amino acids leucine, isoleucine, valine, arginine (e.g., arginine HCl), glutamine, and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.81 : 2 : 0.25.

In some embodiments, the amino acids leucine, isoleucine, valine, arginine HCl, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5 : 2 : 0.1 to 0.3. In some embodiments, the amino acids leucine, isoleucine, valine, arginine HCl, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5 : 2 : 0.25. In some embodiments, the amino acids leucine, isoleucine, valine, arginine HCl, glutamine and N-acetylcysteine are present in a weight ratio of 1 : 0.5 : 0.5 : 1.5 : 2 : 0.15.

In some embodiments, the amino acids leucine, isoleucine, valine, arginine HCl, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.81 : 2 : 0.1 to 0.3. In some embodiments, the amino acids leucine, isoleucine, valine, arginine HCl, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.81 : 2 : 0.25. In some embodiments, the amino acids leucine, isoleucine, valine, arginine HCl, glutamine and N-acetylcysteine are present in a weight ratio of 1 : 0.5 : 0.5 : 1.81 : 2 : 0.15.

In some embodiments, the amino acids leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.81 : 2 : 0.1-0.3. In some embodiments, the amino acids leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.81 : 2 : 0.15. In some embodiments, the amino acids leucine, isoleucine, valine, arginine, glutamine, and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.81 : 2 : 0.25.

In some embodiments, the amino acids leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5 : 2 : 0.1 to 0.3. In some embodiments, the amino acids leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine are present in a weight ratio of about 1 : 0.5 : 0.5 : 1.5 : 2 : 0.25. In some embodiments, the amino acids leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine are present in a weight ratio of 1 : 0.5 : 0.5 : 1.5 : 2 : 0.15.

In some embodiments, a total weight (wt) of the amino acids is about 2 g to about 60 g.

In some embodiments, the total weight of amino acids present is about 5 g, about 6 g, about 7 g, about 11 g, about 12g, about 13 g, about 14 g, about 15 g, about 16 g, about 17 g, about 18 g, about 19 g, about 20 g, about 21 g, about 22 g, about 23 g, about 24 g, about 25 g, about 26 g, about 27 g, about 28 g, about 29 g, about 30 g, about 31 g, about 32 g, about 33 g, about 34 g, about 35 g, about 36 g, about 37 g, about 38 g, about 39 g, about 40 g, about 41 g, about 42 g, about 43 g, about 44 g, about 45 g, about 46 g, about 47 g, about 48 g, about 49 g, or about 50 g.

In certain embodiments, the total wt of the amino acids is about 6 g.

In certain embodiments, the total wt of the amino acids is about 12 g.

In certain embodiments, the total wt of the amino acids is about 18 g.

In certain embodiments, the total wt of the amino acids is about 24 g.

In certain embodiments, the total wt of the amino acids is about 48 g.

In some embodiments, the composition includes about 0.5 g to about 10 g of leucine, about 0.25 g to about 5 g of isoleucine, about 0.25 g to about 5 g of valine, about 1 g to about 20 g of arginine, about 1 g to about 20 g of glutamine, and about 0.1 g to about 5 g of N-acetylcysteine.

In some embodiments, the composition includes at least 1 g of leucine, at least 0.5 g of isoleucine, at least 0.5 g of valine, at least 1.5 g of arginine (or 1.81 g of arginine HCl), at least 2 g of glutamine, and at least 0.15 g of N-acetylcysteine.

In some embodiments, the composition includes about 1 g of leucine, about 0.5 g of isoleucine, about 0.5 g of valine, about 1.5 g of arginine (or 1.81 g of arginine HCl), about 2 g of glutamine, and about 0.15 g of N-acetylcysteine.

In some embodiments, the composition includes at least 2 g of leucine, at least 1 g of isoleucine, at least 1 g of valine, at least 3.0 g of arginine (or 3.62 g of arginine HCl), at least 4 g of glutamine, and at least 0.3 g of N-acetylcysteine.

In some embodiments, the composition includes about 2 g of leucine, about 1 g of isoleucine, about 1 g of valine, about 3.0 g of arginine (or 3.62 g of arginine HCl), about 4 g of glutamine, and about 0.3 g of N-acetylcysteine.

In some embodiments, the composition includes at least 4 g of leucine, at least 2 g of isoleucine, at least 2 g of valine, at least 6.0 g or arginine (or 7.24 g of arginine HCl), at least 8 g of glutamine, and at least 0.6 g of N-acetylcysteine.

In some embodiments, the composition includes about 4 g of leucine, about 2 g of isoleucine, about 2 g of valine, about 6.0 g or arginine (or 7.24 g of arginine HCl), about 8 g of glutamine, and about 0.6 g of N-acetylcysteine.

In some embodiments, the composition includes at least 1.0 g of leucine, at least 0.5 g of isoleucine, at least 0.5 g of valine, at least 1.5 g of arginine, at least 2.0 g of glutamine, or at least 0.15 g of N-acetylcysteine. In some embodiments, the composition includes about 1.0 g of leucine, about 0.5 g of isoleucine, about 0.5 g of valine, about 1.5 g of arginine, about 2.0 g of glutamine, or about 0.15 g of N-acetylcysteine.

In some embodiments, the composition includes at least 1.0 g of leucine, at least 0.5 g of isoleucine, at least 0.5 g of valine, at least 1.5 g of arginine, at least 2.0 g of glutamine, and at least 0.25 g of N-acetylcysteine. In some embodiments, the composition includes about 1.0 g of leucine, about 0.5 g of isoleucine, about 0.5 g of valine, about 1.5 g of arginine, about 2.0 g of glutamine, and about 0.25 g of N-acetylcysteine.

In some embodiments, the amino acids of the composition include about 10 wt % to about 30 wt % leucine, about 5 wt % to about 15 wt % isoleucine, about 5 wt % to about 15 wt % valine, about 15 wt % to about 40 wt % arginine, about 20 wt % to about 50 wt % glutamine, and about 1 wt % to about 8 wt % n-acetylcysteine.

In some embodiments, the amino acids of the composition include about 16 wt % to about 18 wt % leucine, about 7 wt % to about 9 wt % isoleucine, about 7 wt % to about 9 wt % valine, about 28 wt % to about 32 wt % arginine, about 31 wt % to about 34 wt % glutamine, and about 1 wt % to about 5 wt % n-acetylcysteine.

In some embodiments, the amino acids of the composition include about 16.8 wt % leucine, about 8.4 wt % isoleucine, about 8.4 wt % valine, about 30.4 wt % arginine, about 33.6 wt % glutamine, and about 2.5 wt % n-acetylcysteine.

In some embodiments, the composition comprises one or more excipients selected from the group consisting of: citric acid, lecithin, a sweetener, a dispersion enhancer, a flavoring, a bitterness masking agent, and a natural or artificial coloring.

In some embodiments, the composition comprises citric acid.

In some embodiments, the composition is in the form of a solid, powder, solution, or gel. In certain embodiments, the composition is in the form of a powder (e.g. in a packet)

In some embodiments, the composition includes one or more pharmaceutically acceptable excipients, wherein the amino acids comprise leucine, arginine, glutamine, and N-acetylcysteine. An aspect of the present disclosure provides a composition comprising free amino acids and one or more pharmaceutically acceptable excipients, wherein the amino acids consist of leucine, arginine, glutamine, and N-acetylcysteine. In some embodiments, the amino acids leucine, arginine, glutamine, N-acetylcysteine and glycine are present in a weight ratio of 1 : 1.5 : 2 : 0.15. In some embodiments, the composition comprises at least 1.0 g of leucine, at least 1.5 g of arginine, at least 2.0 g of glutamine, or at least 0.15 g of N-acetylcysteine. In some embodiments, the composition comprises at least 1.5 g of arginine and at least 2.0 g of glutamine. In some embodiments, the amino acids leucine, arginine, glutamine, and N-acetylcysteine are present in weight % of each compared to total amino acid weight of 20.4 to 22.6%, 30.6 to 33.9%, 40.9 to 45.2%, and 3.1 to 3.4%, respectively. In some embodiments, the amino acids leucine, arginine, glutamine, and N-acetylcysteine, are present in weight % of each compared to total amino acid weight of 21.5%, 32.3%, 43.0%, and 3.2%, respectively.

In some embodiments, the composition further includes a farnesoid X receptor (FXR) agonist, a stearoyl CoA desaturase inhibitor, a CCR2 and CCR5 chemokine antagonist, a PPAR alpha and delta agonist, a caspase inhibitor, a galectin-3 inhibitor, an acetyl CoA carboxylase inhibitor, or an ileal sodium bile acid co-transporter inhibitor. In some embodiments, the composition further comprises an FXR agonist. In certain embodiments, the FXR agonist is obeticholic acid. In some embodiments, the composition further includes one or more of: LMB-763, LJN-452, emricasan, and cenicriviroc.

An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine HCl, glutamine, and N-acetylcysteine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.5 : 1.81 : 2 : 0.15 (Table 3). An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine, glutamine, and N-acetylcysteine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.5 : 1.5 : 2 : 0.15 (Table 4).

Table 3. Exemplary amino acid components of the composition including Arginine HCl.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2

Leucine	1	16.78	1.00 g	2 g	4 g
Isoleucine	0.5	8.39	0.50 g	1 g	2 g
Valine	0.5	8.39	0.50 g	1 g	2 g
Arginine HCl	1.81	30.37	1.81 g	3.62 g	7.24 g
Glutamine	2	33.56	2.00 g	4 g	8 g
N-acetylcysteine	0.15	2.52	0.15 g	0.3 g	0.6 g
Total amino acids			5.96 g	~12 g	~24 g

Table 4. Exemplary amino acid components of the composition including Arginine.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	17.70	1.00 g	2	4
Isoleucine	0.5	8.85	0.50 g	1	2
Valine	0.5	8.85	0.50 g	1	2
Arginine	1.5	26.55	1.5 g	3	6
Glutamine	2	35.4	2.00 g	4	8
N-acetylcysteine	0.15	2.65	0.15 g	0.3	0.6
Total amino acids			5.65 g	11.3 g	22.6 g

An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine HCl, glutamine, and N-acetylcysteine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.5 : 0.905 : 2 : 0.15 (Table 5). An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine, glutamine, and N-acetylcysteine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.5 : 0.75 : 2 : 0.15 (Table 6).

Table 5. Exemplary amino acid components of the composition including Arginine HCl.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	19.78	1.00 g	2 g	4 g
Isoleucine	0.5	9.89	0.50 g	1 g	2 g
Valine	0.5	9.89	0.50 g	1 g	2 g
Arginine HCl	0.905	17.90	0.905 g	1.81 g	3.62 g

Glutamine	2	39.56	2.00 g	4 g	8 g
N-acetylcysteine	0.15	2.97	0.15 g	0.3 g	0.6 g
Total amino acids			5.06 g	~10 g	~20 g

Table 6. Exemplary amino acid components of the composition including Arginine.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	20.41	1.00 g	2	4
Isoleucine	0.5	10.20	0.50 g	1	2
Valine	0.5	10.20	0.50 g	1	2
Arginine	0.75	15.31	0.75 g	1.5	3
Glutamine	2	40.82	2.00 g	4	8
N-acetylcysteine	0.15	3.06	0.15 g	0.3	0.6
Total amino acids			4.9 g	9.8 g	19.6 g

An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine HCl, glutamine, and N-acetylcysteine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.25 : 0.905 : 1 : 0.225 (Table 7). An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine, glutamine, and N-acetylcysteine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.25 : 0.75 : 1 : 0.225 (Table 8).

Table 7. Exemplary amino acid components of the composition including Arginine HCl.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	25.77	1.00 g	2 g	4 g
Isoleucine	0.5	12.89	0.50 g	1 g	2 g
Valine	0.25	6.44	0.25 g	0.50 g	1 g
Arginine HCl	0.905	23.32	0.905 g	1.81 g	3.62 g
Glutamine	1	25.77	1.00 g	2 g	4 g
N-acetylcysteine	0.225	5.80	0.225 g	0.45 g	0.9 g
Total amino acids			3.88 g	7.76 g	15.52 g

Table 8. Exemplary amino acid components of the composition including Arginine.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	26.85	1.00 g	2	4
Isoleucine	0.5	13.42	0.50 g	1	2
Valine	0.25	6.71	0.25 g	0.5	1
Arginine	0.75	20.13	0.75 g	1.5	3
Glutamine	1	26.85	1.00 g	2	4
N-acetylcysteine	0.225	6.04	0.225 g	0.45	0.9
Total amino acids			3.725 g	7.45 g	14.9 g

An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine HCl, glutamine, N-acetylcysteine, and serine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.25 : 0.905 : 1 : 0.225 : 0.667 (Table 9). An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine, glutamine, N-acetylcysteine, and serine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.25 : 0.75 : 1 : 0.225 : 1.5 (Table 10).

Table 9. Exemplary amino acid components of the composition including Arginine HCl.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	18.59	1.00 g	2 g	4 g
Isoleucine	0.5	9.29	0.50 g	1 g	2 g
Valine	0.25	4.65	0.25 g	0.50 g	1 g
Arginine HCl	0.905	16.82	0.905 g	1.81 g	3.62 g
Glutamine	1	18.59	1.00 g	2 g	4 g
N-acetylcysteine	0.225	4.18	0.225 g	0.45 g	0.9 g
Serine	1.5	27.88	1.5	3	6
Total amino acids			5.38 g	10.76 g	21.52 g

Table 10. Exemplary amino acid components of the composition including Arginine.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	19.14	1.00 g	2	4

Isoleucine	0.5	9.57	0.50 g	1	2
Valine	0.25	4.78	0.25 g	0.5	1
Arginine	0.75	14.35	0.75 g	1.5	3
Glutamine	1	19.14	1.00 g	2	4
N-acetylcysteine	0.225	4.31	0.225 g	0.45	0.9
Serine	1.5	28.71	1.5	3	6
Total amino acids			5.225	10.45	20.9

An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine HCl, glutamine, N-acetylcysteine, and serine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.25 : 0.905 : 1 : 0.225 : 0.667 (Table 11). An exemplary Amino Acid Composition includes leucine, isoleucine, valine, arginine, glutamine, N-acetylcysteine, and serine as its amino acid entities in a wt. ratio of 1 : 0.5 : 0.25 : 0.75 : 1 : 0.225 : 1.667 (Table 12).

Table 11. Exemplary amino acid components of the composition including Arginine HCl.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	18.02	1.00 g	2 g	4 g
Isoleucine	0.5	9.01	0.50 g	1 g	2 g
Valine	0.25	4.50	0.25 g	0.50 g	1 g
Arginine HCl	0.905	16.31	0.905 g	1.81 g	3.62 g
Glutamine	1	18.02	1.00 g	2 g	4 g
N-acetylcysteine	0.225	4.05	0.225 g	0.45 g	0.9 g
Serine	1.667	30.09	1.67 g	3.33 g	6.67 g
Total amino acids			5.55 g	11.09 g	22.19 g

Table 12. Exemplary amino acid components of the composition including Arginine.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	18.54	1.00 g	2	4
Isoleucine	0.5	9.27	0.50 g	1	2
Valine	0.25	4.64	0.25 g	0.5	1

Arginine	0.75	13.91	0.75 g	1.5	3
Glutamine	1	18.54	1.00 g	2	4
N-acetylcysteine	0.225	4.17	0.225 g	0.45	0.9
Serine	1.667	30.92	1.67 g	3.33 g	6.67 g
Total amino acids			5.395 g	10.78 g	21.57 g

The disclosure also provides a composition including at least four different amino acid entities (e.g., four, five, six, or more different amino acid entitites), in which the composition is capable of one, two, three, four, five, or all of:

a) one or both of decreasing or preventing one or both of liver fibrosis or liver injury;

b) one or both of decreasing or preventing hepatocyte inflammation;

c) improving, e.g., increasing, glucose tolerance;

d) one or both of decreasing or preventing steatosis; or

e) one or both of decreasing or preventing hepatocyte ballooning,

provided that at least one amino acid entity is not a peptide of more than 20 amino acid residues in length.

In some embodiments, the composition includes at least four different amino acid entities (e.g., four, five, six, or more different amino acid entities) that decreases or prevents one or both of liver fibrosis or liver injury. For instance, the reducing and/or inhibiting liver fibrosis and/or liver injury comprises can include reducing a level of one or both of collagen, e.g., type I and III collagen or α-smooth muscle actin (αSMA).

In some embodiments, the composition includes at least four different amino acid entities (e.g., four, five, six, or more different amino acid entities) that decreases or prevents hepatocyte inflammation. In some embodiments, the reducing and/or inhibiting liver fibrosis and/or liver injury includes reducing a level or activity of one, two, three, four, or more (e.g., all) of a matrix metalloproteinase (MMP) (e.g., MMP-13, MMP-2, MMP-9, MT1-MMP, MMP-3, or MMP-10), a tissue inhibitor of metalloproteinase (TIMP) (e.g., TIMP1), aspartate transaminase (AST), alanine transaminase (ALT), or N-terminal fragment of type III collagen (proC3).

In some embodiments, the decreasing or preventing hepatocyte inflammation comprises reducing a level or activity of one, two, three, four, five, six, seven or more (e.g., all) of NF-kB, interferons, IL-1b, IL-2, MCP-1, MIP-1, a caspase-cleaved keratin 18 fragments (e.g., one or both of M30 or M65), or C-reactive protein. In an embodiment, the decreasing or preventing hepatocyte inflammation comprises increasing a level or activity of IL-10.

In an embodiment, the improving, e.g., increasing, glucose tolerance, comprises increasing a level or activity of adiponectin. In an embodiment, the improving, e.g., increasing, glucose tolerance, comprises decreasing a level or activity of FGF-21.

In certain embodiments, the hepatocyte inflammation comprises LPS induced hepatocyte inflammation.

In some embodiments, the composition is capable of enhancing fatty acid oxidation, e.g., one or both of reducing levels of unsaturated fatty acids or increasing levels of acylcarnitine (e.g., in a STAM mouse model or a FATZO mouse model). In certain embodiments, the reduction in levels of unsaturated fatty acids is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the level of change shown in Table 53, e.g., measured as described in Example 9. In certain embodiments, the increase in levels of acylcarnitine is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the level of change shown in Table 53, e.g., measured as described in Example 9.

In certain embodiments, the composition is capable of reducing, or reduces, liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of alanine transaminase (ALT), e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

In certain embodiments, the composition is capable of reducing, or reduces, liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of aspartate transaminase (AST), e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

In certain embodiments, the composition is capable of reducing, or reduces, alanine transaminase (ALT) by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of ALT, e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

In certain embodiments, the composition is capable of reducing, or reduces, aspartate transaminase (AST) by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of AST, e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

In certain embodiments, the composition is capable of reducing, or reduces, liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of hydroxyproline, e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

In certain embodiments, the composition is capable of reducing, or reduces, hydroxyproline levels by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of hydroxyproline, e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

In certain embodiments, the composition is capable of reducing, or reduces, liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using LX-2 cells, e.g., levels of Col1a1, Acta2, and/or TIMP2 in LX-2 cells, e.g., as assessed using a nucleic acid amplification method, e.g., PCR or qRT-PCR, e.g., as described in Example 7, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; NAC; or an amino acid composition comprising L-arginine, L-glutamine, and NAC).

In certain embodiments, the composition is capable of reducing, or reduces, expression of one or more collagen biomarkers (e.g., Col1a1, Acta2, and/or TIMP2) by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using LX-2 cells, e.g., levels of Col1a1, Acta2, and/or TIMP2 in LX-2 cells, e.g., as assessed using a nucleic acid amplification method, e.g., PCR or qRT-PCR, e.g., as described in Example 7, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; or NAC).

In certain embodiments, the composition is capable of reducing, or reduces, expression of one or more collagen biomarkers (e.g., Col1a1) by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using primary hepatic stellate cells, e.g., levels of Col1a1 in primary hepatic stellate cells, e.g., as assessed using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 12, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

In certain embodiments, the composition is capable of increasing, or increases, expression of one or more collagen biomarkers (e.g., procollagen 1α1) by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using primary hepatic stellate cells, e.g., levels of procollagen 1α1in primary hepatic stellate cells, e.g., as assessed using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 12, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

In certain embodiments, the composition is capable of reducing, or reduces, hepatocyte inflammation by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using HepG2 cells, e.g., decreased activity, e.g., decreased TNFα-induced activity of NF-kB in a reporter assay in HepG2 cells, as described in Example 8, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; or NAC).

In certain embodiments, the composition is capable of reducing, or reduces, TNFα-induced activity of NF-kB in HepG2 cells by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using HepG2 cells, e.g., decreased activity, e.g., decreased TNFα-induced activity of NF-kB in a reporter assay in HepG2 cells, as described in Example 8, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; or NAC).

In certain embodiments, the composition is capable of increasing, or increases, glucose tolerance, e.g., in a STAM mouse model or in a FATZO mouse model, by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of glucose levels, e.g., using glucose oxidase, e.g., using a glucometer, e.g., as described in Example 5, e.g., relative to a reference composition (e.g., a vehicle control or a positive control, e.g., metformin).

In certain embodiments, the composition is capable of increasing, or increases, blood glucose metabolism, e.g., in a STAM mouse model or in a FATZO mouse model, by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of glucose levels, e.g., using glucose oxidase, e.g., using a glucometer, e.g., as described in Example 5, e.g., relative to a reference composition (e.g., a vehicle control or a positive control, e.g., metformin).

In certain embodiments, the composition is capable of decreasing, or decreases, steatosis and/or inflammation by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of MCP1/CCL2, e.g., in primary hepatocytes, e.g., using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 10, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

In certain embodiments, the composition is capable of decreasing, or decreases, MCP1/CCL2 levels by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of MCP1/CCL2, e.g., in primary hepatocytes, e.g., using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 10, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).In certain embodiments, the composition is capable of decreasing, or decreases, TNFα inflammatory response by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of MCP1/CCL2 or an assay of IL-6, e.g., in primary hepatic stellate cells, e.g., using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 11, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

In certain embodiments, the composition is capable of decreasing, or decreases, MCP1/CCL2 levels and/or IL-6 levels by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of MCP1/CCL2 or an assay of IL-6, e.g., in primary hepatic stellate cells, e.g., using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 11, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

In any of the foregoing embodiments, the reference composition comprises a single amino acid entity, e.g., a L-amino acid entity, an I-amino acid entity, a V-amino acid entity, a R-amino acid entity, a Q-amino acid entity, or a NAC-amino acid entity, each assayed separately as a free amino acid, or a combination of amino acid entities (e.g., a L-amino acid entity, an I-amino acid entity, and a V-amino acid entity; a R-amino acid entity, a Q-amino acid entity, and a NAC-amino acid entity; a L-amino acid entity, an I-amino acid entity, V-amino acid entity, a R-amino acid entity, and a Q-amino acid entity). In certain embodiments, the reference composition comprises vehicle (e.g., PBS or saline).

In some embodiments, the composition that decreases and/or prevents liver fibrosis and/or liver injury comprises one or more branched-chain amino acid (BCAAs), one or more conditionally essential amino acid (CEAAs), and an antioxidant or reactive oxygen species (ROS) scavenger.

In some embodiments, the composition that decreases and/or prevents hepatocyte inflammation comprises one or more BCAAs, one or more CEAAs, and an antioxidant or ROS scavenger.

In some embodiments, the composition that increases glucose tolerance comprises one or more BCAAs, one or more CEAAs, and an antioxidant or ROS scavenger.

In some embodiments, the composition that decreases and/or prevents steatosis comprises one or more BCAAs, one or more CEAAs, and an antioxidant or ROS scavenger.

In some embodiments, the composition that decreases and/or prevents hepatocyte ballooning comprises one or more BCAAs, one or more CEAAs, and an antioxidant or ROS scavenger.

In an embodiment, the BCAA comprises a L-amino acid entity. In an embodiment, the BCAAs comprise a L-amino acid entity and an I-amino acid entity. In an embodiment, the BCAAs comprise a L-amino acid entity and a V-amino acid entity. In an embodiment, the BCAAs comprise a L-amino acid entity, a V-amino acid entity, and an I-amino acid entity. In an embodiment, the CEAA comprises a R-amino acid entity. In an embodiment, the CEAA comprises a Q-amino acid entity. In an embodiment, the CEAA comprises a R-amino acid entity and a Q-amino acid entity. In an embodiment, the antioxidant or ROS scavenger comprises a NAC entity, e.g., NAC.

In some embodiments, the composition comprises a) a L-amino acid entity, an R-amino acid entity, and a Q-amino acid entity; and b) an antioxidant or ROS scavenger, e.g., a NAC entity, e.g., NAC.

In some embodiments, the composition further comprises an I-amino acid-entity or a V-amino acid-entity. In other embodiments, the composition further comprises an I-amino acid-entity and a V-amino acid-entity.

<u>Production of the Amino Acid Compositions</u>

Amino acids used to make the compositions may be agglomerated, and/or instantized to aid in dispersal and/or solubilization.

The amino acid compositions of the present disclosure may be made using amino acids and amino acid derivatives from the following sources, or other sources may used: FUSI-BCAA™ Instantized Blend (L-Leucine, L-Isoleucine and L-Valine in 2:1:1 weight ratio), FUSIL™ Instantized L-Leucine, L-Arginine HCl, and L-Glutamine may be obtained from Ajinomoto Co., Inc; N-acetyl-cysteine may be obtained from Spectrum Chemical.

To produce the amino acid compositions of the instant disclosure, the following general steps may be used: the starting materials (individual amino acids and excipients) may be blended in a blending unit, followed by verification of blend uniformity and amino acid content, and filling of the blended powder into stick packs or other unit dosage form. The content of stick packs or other unit dosage forms may be dispersed in water at time of use for oral administration.

Formulations

The pharmaceutical compositions of the present disclosure may be in a form suitable for oral use (for example as tablets, lozenges, hard or soft capsules, aqueous or oily suspensions, emulsions, dispersible powders or granules, syrups or elixirs, medical food products, nutraceuticals), for topical use (for example as creams, ointments, gels, or aqueous or oily solutions or suspensions), for administration by inhalation (for example as finely divided powder) or for parental administration (for example as a sterile aqueous or oily solution for intravenous, subcutaneous, intramuscular dosing or as a suppository for rectal dosing).

Excipients

The amino acid compositions of the present disclosure may be compounded or formulated with one or more excipients. Non-limiting examples of suitable excipients include a tastant, a flavorant, a buffering agent, a preservative, a stabilizer, a binder, a compaction agent, a lubricant, a dispersion enhancer, a disintegration agent, a flavoring agent, a sweetener, and a coloring agent.

In some embodiments, the excipient comprises a buffering agent. Non-limiting examples of suitable buffering agents include citric acid, sodium citrate, magnesium carbonate, magnesium bicarbonate, calcium carbonate, and calcium bicarbonate.

In some embodiments, the excipient comprises a preservative. Non-limiting examples of suitable preservatives include antioxidants, such as alpha-tocopherol and ascorbate, and antimicrobials, such as parabens, chlorobutanol, and phenol.

In some embodiments, the composition comprises a binder as an excipient. Non-limiting examples of suitable binders include starches, pregelatinized starches, gelatin, polyvinylpyrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinyloxoazolidone, polyvinylalcohols, C12-C18 fatty acid alcohol, polyethylene glycol, polyols, saccharides, oligosaccharides, and combinations thereof.

In some embodiments, the composition comprises a lubricant as an excipient. Non-limiting examples of suitable lubricants include magnesium stearate, calcium stearate, zinc stearate, hydrogenated vegetable oils, sterotex, polyoxyethylene monostearate, talc, polyethyleneglycol, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, and light mineral oil.

In some embodiments, the composition comprises a dispersion enhancer as an excipient. Non-limiting examples of suitable dispersants include starch, alginic acid, polyvinylpyrrolidones, guar gum, kaolin, xanthan gum, bentonite, purified wood cellulose, sodium starch glycolate, isoamorphous silicate, and microcrystalline cellulose as high HLB emulsifier surfactants.

In some embodiments, the composition comprises a disintegrant as an excipient. In some embodiments, the disintegrant is a non-effervescent disintegrant. Non-limiting examples of suitable non-effervescent disintegrants include starches such as corn starch, potato starch, pregelatinized and modified starches thereof, sweeteners, clays, such as bentonite, micro-crystalline cellulose, alginates, sodium starch glycolate, gums such as agar, guar, locust bean, karaya, pecitin, and tragacanth. In some embodiments, the disintegrant is an effervescent disintegrant. Non-limiting examples of suitable effervescent disintegrants include sodium bicarbonate in combination with citric acid, and sodium bicarbonate in combination with tartaric acid.

In some embodiments, the excipient comprises a flavoring agent. Flavoring agents can be chosen from synthetic flavor oils and flavoring aromatics; natural oils; extracts from plants, leaves, flowers, and fruits; and combinations thereof. In some embodiments, the flavoring agent is selected from cinnamon oils; oil of wintergreen; peppermint oils; clover oil; hay oil; anise oil; eucalyptus; vanilla; citrus oil such as lemon oil, orange oil, grape and grapefruit oil; and fruit essences including apple, peach, pear, strawberry, raspberry, cherry, plum, pineapple, and apricot.

In some embodiments, the excipient comprises a sweetener. Non-limiting examples of suitable sweeteners include glucose (corn syrup), dextrose, invert sugar, fructose, and mixtures thereof (when not used as a carrier); saccharin and its various salts such as the sodium salt;

dipeptide sweeteners such as aspartame; dihydrochalcone compounds, glycyrrhizin; Stevia Rebaudiana (Stevioside); chloro derivatives of sucrose such as sucralose; and sugar alcohols such as sorbitol, mannitol, sylitol, and the like. Also contemplated are hydrogenated starch hydrolysates and the synthetic sweetener 3,6-dihydro-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide, particularly the potassium salt (acesulfame-K), and sodium and calcium salts thereof.

In some embodiments, the composition comprises a coloring agent. Non-limiting examples of suitable color agents include food, drug and cosmetic colors (FD&C), drug and cosmetic colors (D&C), and external drug and cosmetic colors (Ext. D&C). The coloring agents can be used as dyes or their corresponding lakes.

Particular excipients may include one or more of: citric acid, lecithin, (e.g. Alcolec F100), sweeteners (e.g. sucralose, sucralose micronized NF, acesulfame potassium (e.g. Ace-K)), a dispersion enhancer (e.g. xanthan gum (e.g. Ticaxan Rapid-3)), flavorings (e.g. vanilla custard #4306, Nat Orange WONF #1326, lime 865.0032U, and lemon 862.2169U), a bitterness masking agent (e.g. 936.2160U), and natural or artificial colorings (e.g. FD&C Yellow 6).

Methods of Treatment

The composition as described herein can be administered to improve liver function, e.g., in a patient with a liver disease. The composition as described herein can also be administered to treat (e.g., reverse, reduce, ameliorate, or prevent) a disorder, e.g., a liver disease in a subject. The present disclosure provides methods of treating a liver disease selected from fatty liver disease (steatohepatitis), alcoholic steatohepatitis (ASH), non-alcoholic fatty liver disease (NAFLD), non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), liver fibrosis, and cirrhosis. In particular, an effective amount of the composition can be administered (e.g., according to a dosage regimen described herein) to treat a subject with non-alcoholic fatty liver disease (NAFLD), non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), or cirrhosis.

Patients with Liver Disease

In some embodiments, a subject has fatty liver disease selected from NAFLD and AFLD. In some embodiments, the subject has pediatric NAFLD. In some embodiments, the subject with NAFLD has NASH or NAFL. In some embodiments, the subject with AFLD has ASH.

In certain embodiments, the subject exhibits symptoms of gut leakiness. In certain embodiments, the subject has gut dysbiosis. In certain embodiments, the subject has gut microbiome disturbance. The subject may have increased levels of inflammatory cytokines, e.g., increased TNFα, relative to a normal subject without a fatty liver disease.

In certain embodiments, the subject exhibits muscle atrophy, e.g., has a decreased ratio of muscle tissue to adipose tissue, e.g., relative to a normal subject without a fatty liver disease. For example, the subject exhibits muscle atrophy without fibrosis and/or cirrhosis.

In certain embodiments, the subject exhibits reverse lipid transport from adipose tissue to liver tissue.

In some embodiments, the subject has fibrosis. The subject may have cirrhosis. The subject may also have a metabolic syndrome.

In certain embodiments, the subject has one, two, or more (e.g., all) of hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

In some embodiments, the subject has type 2 diabetes.

In some embodiments, the subject with a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)) is a mammal (e.g., a human). In some embodiments, the subject has been diagnosed with NAFLD, NASH or cirrhosis. In some embodiments, the subject has not received prior treatment with a composition as described herein (e.g., the subject is a naïve subject). In some embodiments, the subject with NAFLD, NASH or cirrhosis has diabetes (e.g., type 2 diabetes).

In some embodiments, the subject has NAFLD. In some embodiments, the subject has NAFL. In certain embodiments, the subject (e.g., a child or an adolescent) has pediatric NAFLD. In some embodiments, the subject has hepatic steatosis. In some embodiments, a subject with pediatric NAFLD has steatosis.

In some embodiments, the subject has non-alcoholic steatohepatitis (NASH). In some embodiments, the subject with NASH has fibrosis.

In some embodiments, the subject has cirrhosis. In some embodiments, the subject with cirrhosis has fibrosis. In some embodiments, the subject with cirrhosis has hepatocarcinoma. In some embodiments, the subject with cirrhosis has an increased risk of liver failure. In some embodiments, the subject with cirrhosis has hepatocarcinoma, an increased risk of liver failure, and an increased risk of death.

In some embodiments, a subject exhibits a symptom of liver disease (e.g. NAFLD, NASH, or cirrhosis), e.g., a metabolic symptom, prior to administration of the composition. In some embodiments, a subject exhibits a metabolic symptom of liver disease (e.g. NAFLD, NASH, or cirrhosis) selected from one, two, three, four, five, six, or more (e.g., all) of decreased fat metabolism, hepatocyte apoptosis, hepatocyte ballooning, inflammation of adipose tissue, inflammation of hepatic tissue, hepatocyte ballooning, oxidative stress (e.g., reactive oxygen species (ROS), decreased gut barrier function, decreased insulin secretion, or decreased glucose tolerance (e.g., relative to a healthy subject without a liver disease).

In some embodiments, a subject exhibits modulated (e.g., increased) levels of a biomarker prior to administration of the composition. In some embodiments, a subject exhibits modulated levels of a biomarker selected from one, two, three, four, five, six, seven, eight, nine, or more (*e.g.,* all) of ACOX1; IL-10; NF-kB, an interferon, IL-2; glutathione (GSH); alanine aminotransferase (ALT); aspartate aminotransferase (AST); adiponectin; N-terminal fragment of type III collagen (proC3); caspase-cleaved keratin 18 fragments (M30 and M65); IL-1β; C-reactive protein; PIIINP; TIMP1; MCP-1; or FGF-21 (e.g., relative to a healthy subject without a liver disease).

In some embodiments, the subject exhibits increased levels of ALT, e.g., relative to a healthy subject without a liver disease.

In some embodiments, the subject exhibits increased levels of AST, e.g., relative to a healthy subject without a liver disease.

Improvement in Symptoms of Liver Disease

The composition as described herein can be administered to treat (e.g., reverse, reduce, ameliorate, or prevent) a subject (e.g., a human) with a liver disease, thereby improving a symptom of a liver disease in the patient. In some embodiments, the composition is administered to a subject with NAFLD. In some embodiments, the composition is administered to a subject with NAFL. In some embodiments, the composition is administered to a subject with NASH. In some embodiments, the composition is administered to a subject with cirrhosis of the liver.

In some embodiments, administration of a composition (e.g., at a dosage regimen described herein) results in an improvement in one or more symptoms of NAFLD, e.g., a metabolic symptom of NAFLD, in a subject.

In some embodiments, administration of the composition results in increased free fatty acid and lipid metabolism in a subject with NAFLD (e.g., a subject with pediatric NAFLD). In some embodiments, administration of the composition results in improved mitochondrial function in a subject with NAFLD (e.g., a subject with pediatric NAFLD). In some embodiments, administration of the composition results in white adipose tissue (WAT) browning in a subject with NAFLD (e.g., a subject with pediatric NAFLD).

In some embodiments, administration of the composition results in decreased reactive oxygen species (ROS) in a subject with NAFLD (e.g., a subject with pediatric NAFLD). In some embodiments, administration of the composition results in increased levels of glutathione (GSH) in a subject with NAFLD (e.g., a subject with pediatric NAFLD).

In some embodiments, administration of the composition results in decreased hepatic inflammation in a subject with NAFLD (e.g., a subject with pediatric NAFLD). In some embodiments, administration of the composition results in decreased hepatocyte ballooning in a subject with NAFLD (e.g., a subject with pediatric NAFLD).

In some embodiments, administration of the composition results in improved gut barrier function in a subject with NAFLD (e.g., a subject with pediatric NAFLD).

In some embodiments, administration of the composition results in increased insulin secretion in a subject with NAFLD (e.g., a subject with pediatric NAFLD). In some embodiments, administration of the composition results in improved glucose tolerance in a subject with NAFLD (e.g., a subject with pediatric NAFLD).

In some embodiments, the composition reduces or inhibits liver fibrosis in a subject with NAFLD (e.g., a subject with pediatric NAFLD). In some embodiments, the composition reduces or inhibits liver fibrosis in a subject with NAFLD (e.g., a subject with pediatric NAFLD).

In some embodiments, the composition reduces liver fat in a subject with NAFLD (e.g., a subject with pediatric NAFLD). In some embodiments, the composition reduces liver enzyme levels (e.g., ALT or AST) in blood or plasma from a subject with NAFLD (e.g., a subject with pediatric NAFLD).

In some embodiments, administration of a composition (e.g., at a dosage regimen described herein) including amino acid entities results in an improvement in one or more symptoms of NASH, e.g., a metabolic symptom of NASH, in a subject.

In some embodiments, administration of the composition results in increased free fatty acid and lipid metabolism in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes). In some embodiments, administration of the composition results in improved mitochondrial function in a subject with NASH. In some embodiments, administration of the composition results in white adipose tissue (WAT) browning in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes).

In some embodiments, administration of the composition results in decreased reactive oxygen species (ROS) in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes). In some embodiments, administration of the composition results in increased levels of glutathione (GSH) in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes).

In some embodiments, administration of the composition results in decreased hepatic inflammation in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes). In some embodiments, administration of the composition results in decreased hepatocyte ballooning in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes).

In some embodiments, administration of the composition results in improved gut barrier function in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes).

In some embodiments, administration of the composition results in increased insulin secretion in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes). In some embodiments, administration of the composition results in improved glucose tolerance in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes).

In some embodiments, the composition reduces or inhibits liver fibrosis in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes). In some embodiments, the composition reduces or inhibits liver fibrosis in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes).

In some embodiments, the composition reduces liver fat in a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes). In some embodiments, the composition reduces liver enzyme levels (e.g., ALT or AST) in blood or plasma from a subject with NASH (e.g., a subject with NAFLD, fibrosis, and type 2 diabetes).

In some embodiments, administration of a composition (e.g., at a dosage regimen described herein) including amino acid entities results in an improvement in one or more symptoms of cirrhosis, e.g., a metabolic symptom of cirrhosis, in a subject.

In some embodiments, administration of the composition results in decreased reactive oxygen species (ROS) in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death). In some embodiments, administration of the composition results in increased levels of glutathione (GSH) in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death).

In some embodiments, administration of the composition results in decreased hepatic inflammation in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death). In some embodiments, administration of the composition results in decreased hepatocyte ballooning in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death).

In some embodiments, administration of the composition results in improved gut barrier function in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death).

In some embodiments, administration of the composition results in increased insulin secretion in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death). In some embodiments, administration of the composition results in improved glucose tolerance in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death).

In some embodiments, the composition reduces or inhibits liver fibrosis in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death). In some embodiments, the composition reduces or inhibits liver fibrosis in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death).

In some embodiments, the composition reduces liver fat in a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death). In some embodiments, the composition reduces liver enzyme levels (e.g., ALT or AST) in blood or plasma from a subject with cirrhosis (e.g., a subject with hepatocarcinoma, increased risk of liver failure, and increased risk of death).

Dosage Regimens

The composition can be administered according to a dosage regimen described herein to treat (*e.g.*, inhibit, reduce, ameliorate, or prevent) a disorder, *e.g.*, a liver disease in a subject (e.g., a human). In some embodiments, the subject has NAFLD. In some embodiments, the subject has NAFL. In some embodiments, the subject has NASH. In some embodiments, the subject has cirrhosis.

The composition can be provided to a patient with a liver disease (e.g., NAFL, NASH, or cirrhosis) in either a single or multiple dosage regimens. In some embodiments, doses are administered, e.g., twice daily, three times daily, four times daily, five times daily, six times daily, seven times daily, or more. In some embodiments, the composition is administered for at least 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, or 2 weeks. In some embodiments, the composition is administered for at least 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, or longer. In some embodiments, the composition is administered chronically, e.g., more than 30 days, e.g., 31 days, 40 days, 50 days, 60 days, 3 months, 6 months, 9 months, one year, two years, or three years).

In some embodiments, the composition is administered at a dose of about 2 g to about 60 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In some embodiments, the composition is administered at a dose of about 5 g to about 15 g, about 10 g to about 20 g, about 20 g to about 40 g, or about 30 g to about 50 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day).

In some embodiments, the composition is administered at a dose of about 5 g to about 10 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In some embodiments, the composition is administered at a dose of about 6 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In an embodiment, the composition is administered at a dose of about 6 g total amino acids three times per day.

In some embodiments, the composition is administered at a dose of about 10 g to about 20 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In some embodiments, the composition is administered at a dose of about 12 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In an embodiment, the composition is administered at a dose of about 12 g total amino acids three times per day.

In some embodiments, the composition is administered at a dose of about 20 g to about 40 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In some embodiments, the composition is administered at a dose of about 18 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In an embodiment, the composition is administered at a dose of about 18 g total amino acids three times per day.

In some embodiments, the composition is administered at a dose of about 20 g to about 40 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In some embodiments, the composition is administered at a dose of about 24 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In an embodiment, the composition is administered at a dose of about 24 g total amino acids three times per day.

In some embodiments, the composition is administered at a dose of about 30 g to about 50 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In some embodiments, the composition is administered at a dose of about 48 g total amino acids, e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day). In an embodiment, the composition is administered at a dose of about 48 g total amino acids three times per day.

In some embodiments, the composition is administered at a dose of about 5 grams, about 8 grams, about 9 grams, about 10 grams, about 11 grams, about 12 grams, about 13 grams, about 14 grams, about 15 grams, about 16 grams, about 17 grams, about 18 grams, about 19 about grams, about 20 grams, about 21 grams, about 22 grams, about 24 grams, about 25 grams, about 26 grams, about 27 grams, about 28 grams, about 29 grams, or about 30 grams total amino acids (e.g., about 12 g or about 24 g) , e.g., once per day, twice per day, three times per day, four times per day, five times per day, or six times per day (e.g., three times per day).

In some embodiments, the composition is administered every 2 hours, every 3 hours, every 4 hours, every 5 hours, every 6 hours, every 7 hours, every 8 hours, every 9 hours, or every 10 hours to a subject with a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)).

In an embodiment, the composition is administered to a subject with NAFLD prior to a meal. In an embodiment, the composition is administered to a subject with NAFLD concurrent with a meal. In an embodiment, the composition is administered to a subject with NAFLD following a meal.

In an embodiment, the composition is administered to a subject with NAFL prior to a meal. In an embodiment, the composition is administered to a subject with NAFL concurrent with a meal. In an embodiment, the composition is administered to a subject with NAFL following a meal.

In an embodiment, the composition is administered to a subject with NASH prior to a meal. In an embodiment, the composition is administered to a subject with NASH concurrent with a meal. In an embodiment, the composition is administered to a subject with NASH following a meal.

In an embodiment, the composition is administered to the subject with cirrhosis prior to a meal. In an embodiment, the composition is administered to a subject with cirrhosis concurrent with a meal. In an embodiment, the composition is administered to a subject with cirrhosis following a meal.

In an embodiment, the composition includes at least 1 g of leucine, at least 0.5 g of isoleucine, at least 0.5 g of valine, at least 1.5 g of arginine (or 1.81 g of arginine HCl), at least 2 g of glutamine, and at least 0.15 g of N-acetylcysteine for administration three times per day (e.g., for a total of at least 18 g per day).

In an embodiment, the composition includes about 1 g of leucine, about 0.5 g of isoleucine, about 0.5 g of valine, about 1.5 g of arginine (or 1.81 g of arginine HCl), about 2 g of glutamine, and about 0.15 g of N-acetylcysteine for administration three times per day (e.g., for a total of about 18 g per day).

In an embodiment, the composition includes at least 2 g of leucine, at least 1 g of isoleucine, at least 1 g of valine, at least 3.0 g of arginine (or 3.62 g of arginine HCl), at least 4 g of glutamine, and at least 0.3 g of N-acetylcysteine for administration three times per day (e.g., a total of at least 36 g per day).

In an embodiment, the composition includes about 2 g of leucine, about 1 g of isoleucine, about 1 g of valine, about 3.0 g or arginine (or 3.62 g of arginine HCl), about 4 g of glutamine, and about 0.3 g of N-acetylcysteine for administration three times per day (e.g., a total of about 36 g per day).

In an embodiment, the composition includes at least 4 g of leucine, at least 2 g of isoleucine, at least 2 g of valine, at least 6.0 g of arginine (or 7.24 g of arginine HCl), at least 8 g of glutamine, and at least 0.6 g of N-acetylcysteine for administration three times per day (e.g., a total of at least 72 g per day).

In an embodiment, the composition includes about 4 g of leucine, about 2 g of isoleucine, about 2 g of valine, about 6.0 g of arginine (or 7.24 g of arginine HCl), about 8 g of glutamine, and about 0.6 g of N-acetylcysteine for administration three times per day (e.g., a total of about 72 g per day).

In an embodiment, the composition includes at least 1 g of leucine, at least 0.5 g of isoleucine, at least 0.5 g of valine, at least 0.75 g of arginine (or 0.905 g of arginine HCl), at least 2 g of glutamine, and at least 0.15 g of N-acetylcysteine for administration three times per day (e.g., for a total of at least 18 g per day).

In an embodiment, the composition includes about 1 g of leucine, about 0.5 g of isoleucine, about 0.5 g of valine, about 0.75 g of arginine (or 0.905 g of arginine HCl), about 2 g of glutamine, and about 0.15 g of N-acetylcysteine for administration three times per day (e.g., for a total of about 18 g per day).

In an embodiment, the composition includes at least 2 g of leucine, at least 1 g of isoleucine, at least 1 g of valine, at least 1.5 g of arginine (or 1.81 g of arginine HCl), at least 4 g of glutamine, and at least 0.3 g of N-acetylcysteine for administration three times per day (e.g., a total of at least 36 g per day).

In an embodiment, the composition includes about 2 g of leucine, about 1 g of isoleucine, about 1 g of valine, about 1.5 g or arginine (or 1.81 g of arginine HCl), about 4 g of glutamine, and about 0.3 g of N-acetylcysteine for administration three times per day (e.g., a total of about 36 g per day).

In an embodiment, the composition includes at least 4 g of leucine, at least 2 g of isoleucine, at least 2 g of valine, at least 3.0 g of arginine (or 3.62 g of arginine HCl), at least 8 g of glutamine, and at least 0.6 g of N-acetylcysteine for administration three times per day (e.g., a total of at least 72 g per day).

In an embodiment, the composition includes about 4 g of leucine, about 2 g of isoleucine, about 2 g of valine, about 3.0 g of arginine (or 3.62 g of arginine HCl), about 8 g of glutamine, and about 0.6 g of N-acetylcysteine for administration three times per day (e.g., a total of about 72 g per day).

In an embodiment, the composition includes at least 1 g of leucine, at least 0.5 g of isoleucine, at least 0.25 g of valine, at least 0.75 g of arginine (or 0.905 g of arginine HCl), at least 1 g of glutamine, and at least 0.225 g of N-acetylcysteine for administration three times per day (e.g., for a total of at least 18 g per day).

In an embodiment, the composition includes about 1 g of leucine, about 0.5 g of isoleucine, about 0.25 g of valine, about 0.75 g of arginine (or 0.905 g of arginine HCl), about 1 g of glutamine, and about 0.225 g of N-acetylcysteine for administration three times per day (e.g., for a total of about 18 g per day).

In an embodiment, the composition includes at least 2 g of leucine, at least 1 g of isoleucine, at least 0.5 g of valine, at least 1.5 g of arginine (or 1.81 g of arginine HCl), at least 2 g of glutamine, and at least 0.45 g of N-acetylcysteine for administration three times per day (e.g., a total of at least 36 g per day).

In an embodiment, the composition includes about 2 g of leucine, about 1 g of isoleucine, about 0.5 g of valine, about 1.5 g or arginine (or 1.81 g of arginine HCl), about 2 g of glutamine, and about 0.45 g of N-acetylcysteine for administration three times per day (e.g., a total of about 36 g per day).

In an embodiment, the composition includes at least 4 g of leucine, at least 2 g of isoleucine, at least 1 g of valine, at least 3 g of arginine (or 3.62 g of arginine HCl), at least 4 g of glutamine, and at least 0.9 g of N-acetylcysteine for administration three times per day (e.g., a total of at least 72 g per day).

In an embodiment, the composition includes about 4 g of leucine, about 2 g of isoleucine, about 1 g of valine, about 3 g of arginine (or 3.62 g of arginine HCl), about 4 g of glutamine, and about 0.9 g of N-acetylcysteine for administration three times per day (e.g., a total of about 72 g per day).

In an embodiment, the composition includes at least 1 g of leucine, at least 0.5 g of isoleucine, at least 0.25 g of valine, at least 0.75 g of arginine (or 0.905 g of arginine HCl), at least 1 g of glutamine, at least 0.225 g of N-acetylcysteine, and at least 1.5g or about 1.67 g of serine for administration three times per day (e.g., for a total of at least 18 g per day or for a total of at least 20 g per day).

In an embodiment, the composition includes about 1 g of leucine, about 0.5 g of isoleucine, about 0.25 g of valine, about 0.75 g of arginine (or 0.905 g of arginine HCl), about 1 g of glutamine, about 0.225 g of N-acetylcysteine, and about 1.5 g or about 1.67 g of serine for administration three times per day (e.g., for a total of about 18 g per day or for a total of at least 20 g per day).

In an embodiment, the composition includes at least 2 g of leucine, at least 1 g of isoleucine, at least 0.5 g of valine, at least 1.5 g of arginine (or 1.81 g of arginine HCl), at least 2 g of glutamine, at least 0.45 g of N-acetylcysteine, and at least 3 g or about 3.33 g of serine for administration three times per day (e.g., a total of at least 36 g per day or for a total of at least 40 g per day).

In an embodiment, the composition includes about 2 g of leucine, about 1 g of isoleucine, about 0.5 g of valine, about 1.5 g or arginine (or 1.81 g of arginine HCl), about 2 g of glutamine, about 0.45 g of N-acetylcysteine, and about 3 g or about 3.33 g of serine for administration three times per day (e.g., a total of about 36 g per day or for a total of at least 40 g per day).

In an embodiment, the composition includes at least 4 g of leucine, at least 2 g of isoleucine, at least 1 g of valine, at least 3 g of arginine (or 3.62 g of arginine HCl), at least 4 g of glutamine, at least 0.9 g of N-acetylcysteine, and at least 6 g or about 6.67 g of serine for administration three times per day (e.g., a total of at least 90 g per day).

In an embodiment, the composition includes about 4 g of leucine, about 2 g of isoleucine, about 1 g of valine, about 3 g of arginine (or 3.62 g of arginine HCl), about 4 g of glutamine, about 0.9 g of N-acetylcysteine, and about 6 g or about 6.67 g of serine for administration three times per day (e.g., a total of about 90 g per day).In some embodiments, the composition comprises four stick packs, each stick pack comprising 25% of the quantity of each amino acid included in the composition (e.g., as described herein).

Secondary Agents

In some embodiments, the method further comprises administering a farnesoid X receptor (FXR) agonist, a stearoyl CoA desaturase inhibitor, a CCR2 and CCR5 chemokine antagonist, a PPAR alpha and delta agonist, a caspase inhibitor, a galectin-3 inhibitor, an acetyl CoA carboxylase inhibitor, or an ileal sodium bile acid co-transporter inhibitor prior to, concurrently with, or after administration of the amino acid composition.

In some embodiments, the method further includes administering an FXR agonist. In some embodiments, the FXR agonist is obeticholic acid. In some embodiments, the method further includes administering one or more of: LMB-763, LJN-452, emricasan, and cenicriviroc.

Dietary Compositions

The composition including amino acid entities can be dietary compositions, e.g., chosen from a medical food, a functional food, or a supplement.

The composition including amino acid entities can be for use as a dietary composition, e.g., chosen from a medical food, a functional food, or a supplement. In some embodiments, the dietary composition is for use in a method comprising adminstering the composition to a subject.

In some embodiments, the subject has one or both of type 2 diabetes or a relatively high BMI.

In some embodiments, the subject has fatty liver disease.

In some embodiments, the subject has NAFLD (e.g., pediatric NAFLD). In an embodiment, the subject has NASH. In an embodiment, the subject has NAFL.

In some embodiments, the subject has AFLD. In an embodiment, the subject has ASH.

In some embodiments, the subject has one, two, three, four, or more (e.g., all) of fibrosis, cirrhosis, hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

In some embodiments, the composition promotes weight loss in the subject.

In some embodiments, administration of the dietary composition results in an improvement in one or more metabolic symptoms in the subject, e.g., one or more metabolic symptoms is selected from the following: increased free fatty acid and lipid metabolism (e.g., in the liver), improved mitochondrial function, white adipose tissue (WAT) browning, decreased reactive oxygen species (ROS), increased levels of glutathione (GSH), decreased hepatic inflammation, decreased hepatocyte ballooning, improved gut barrier function, increased insulin secretion, or glucose tolerance. In certain embodiments, administration of the composition results in an improvement in one or more metabolic symptoms after a treatment period of 24 hours.

The method can further include determining the level of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more (e.g., all) of the following:

a) alanine aminotransferase (ALT);

b) aspartate aminotransferase (AST);

c) adiponectin;

d) N-terminal fragment of type III collagen (proC3);

e) caspase-cleaved keratin 18 fragments (M30 and M65);

f) IL-1 beta;

g) C-reactive protein;

h) PIIINP;

i) a tissue inhibitor of metalloproteinase (TIMP); e.g., TIMP1 or TIMP2;

j) MCP-1;

k) FGF-21;

l) Col1a1;

m) Acta2;

n) a matrix metalloproteinase (MMP), e.g., MMP-13, MMP-2, MMP-9, MT1-MMP, MMP-3, or MMP-10;

o) ACOX1;

p) IL-10; or

q) NF-kB.

In certain embodiments, administration of the composition results in an improvement in one or more of a)-q) after a treatment period of 24 hours.

In some embodiments, the subject exhibits increased levels of one or both of ALT or AST prior to administraton of the composition, e.g., relative to a healthy subject without a liver disease. In some embodiments, administration of the composition results in a decrease in levels of one or both of ALT or AST.

Methods of Providing an Amino Acid to a Subject

The present disclosure features a method of providing amino acid entities to a subject comprising administering to the subject an effective amount of a composition described herein, e.g., a composition comprising a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, a glutamine (Q)-amino acid entity; and an antioxidant or reactive oxygen species (ROS) scavenger, e.g., a N-acetylcysteine (NAC) entity, e.g., NAC. In some embodiments, at least one amino acid entity is not a peptide of more than 20 amino acid residues in length.

The present disclosure also features a method of increasing one, two, three, or more (e.g., all) amino acid entities in a subject comprising administering to the subject an effective amount of the composition described herein. In some embodiments, administration of the composition results in an increase in the amino acid entities in one, two, or more (e.g., all) of blood, plasma, or serum of the subject, e.g., in a blood, plasma, or serum sample from the subject.

Biomarkers

Any of the methods disclosed herein can include evaluating or monitoring the effectiveness of administering a composition including amino acid entities to a subject with a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)).

In embodiments, the value of effectiveness to the composition in treating a subject with a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)) comprises a measure of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, or more (e.g., all) of the following:

a) alanine aminotransferase (ALT);

b) aspartate aminotransferase (AST);

c) adiponectin;

d) N-terminal fragment of type III collagen (proC3);

e) caspase-cleaved keratin 18 fragments (M30 and M65);

f) IL-1 beta;

g) C-reactive protein;

h) PIIINP;

i) a tissue inhibitor of metalloproteinase (TIMP); e.g., TIMP1 or TIMP2;

j) MCP-1;

k) FGF-21;

l) Col1a1;

m) Acta2;

n) a matrix metalloproteinase (MMP), e.g., MMP-13, MMP-2, MMP-9, MT1-MMP, MMP-3, or MMP-10;

o) ACOX1;

p) IL-10; or

q) NF-kB.

In some embodiments of any of the methods disclosed herein, the measure of one or more of a)-q) is obtained from a sample acquired from the subject with a liver disease (e.g., NAFLD (e.g., NASH or NAFL) or AFLD (e.g., ASH)). In some embodiments, the sample is chosen from a blood sample (*e.g.,* a plasma sample) or a liver sample.

In some embodiments, the subject is evaluated prior to receiving, during, or after receiving, a composition including amino acid entities.

In some embodiments, administration of the composition including amino acid entities (e.g., at a dose of about 2 g to about 60 g total amino acids, e.g., about 6 g, about 12 g, about 18 g, about 24 g, or about 48 g), results in an improvement in one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, or more (e.g., all) of the following:

a) alanine aminotransferase (ALT);

b) aspartate aminotransferase (AST);

c) adiponectin;

d) N-terminal fragment of type III collagen (proC3);

e) caspase-cleaved keratin 18 fragments (M30 and M65);

f) IL-1 beta;

g) C-reactive protein;

h) PIIINP;

i) a tissue inhibitor of metalloproteinase (TIMP); e.g., TIMP1 or TIMP2;

j) MCP-1;

k) FGF-21;

l) Col1a1;

m) Acta2;

n) a matrix metalloproteinase (MMP), e.g., MMP-13, MMP-2, MMP-9, MT1-MMP, MMP-3, or MMP-10;

o) ACOX1;

p) IL-10; or

q) NF-kB.

In some embodiments, administration of the composition including amino acid entities (e.g., at a dose of about 2 g to about 60 g total amino acids, e.g., about 6 g, about 12 g, about 18 g, about 24 g, or about 48 g three times daily), results in an improvement in one, two, three, four, five, six, seven, eight, nine, ten, or more (*e.g.,* all) of a)-k) after a treatment period of, about 24 hours, about 72 hours, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, or 12 weeks. In certain embodiments, administration of the composition results in an improvement in one, two, three, four, five, or more (*e.g.,* all) of a)-k) after a treatment period of about 2 weeks.

NUMBERED EMBODIMENTS

The invention is further described with reference to the following numbered embodiments.

1. A composition comprising:

a) a leucine (L)-amino acid entity, an arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) an antioxidant or reactive oxygen species (ROS) scavenger, e.g., a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

optionally wherein:

(i) an amino acid entity (e.g., at least one, two, or three of the amino acid entities) of (a) is selected from Table 2; or

(ii) (A) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or (B) the composition further comprises a serine (S)-amino acid entity.

1A. The composition of embodiment 1, wherein the composition satisfies the property of (i).

1B. The composition of any of the preceding embodiments, wherein the composition satisfies the property of (ii)(A).

1C. The composition of any of the preceding embodiments, wherein the composition satisfies the property of (ii)(B).

1D. The composition of any of the preceding embodiments, wherein the composition further comprises an S-amino acid entity, and wherein the S-amino acid entity is present at a higher amount than any other amino acid entity.

2. The composition of any of embodiments 1-1D, wherein the composition comprises an amino acid and three amino acid entities.

3. The composition of any of embodiments 1-1D, wherein the composition comprises an amino acid precursor and three amino acid entities.

4. The composition of any of embodiments 1-1D, wherein the composition comprises an amino acid metabolite and three amino acid entities.

5. The composition of any of embodiments 1-1D, wherein the composition comprises an amino acid derivative and three amino acid entities.

6. The composition of any of embodiments 1-1D, wherein the composition comprises two amino acids and two amino acid entities.

7. The composition of any of embodiments 1-1D, wherein the composition comprises two amino acid precursors and two amino acid entities.

8. The composition of any of embodiments 1-1D, wherein the composition comprises two amino acid metabolites and two amino acid entities.

9. The composition of any of embodiments 1-1D, wherein the composition comprises two amino acid derivatives and two amino acid entities.

10. The composition of any of embodiments 1-1D, wherein the composition comprises three amino acids and one amino acid entity.

11. The composition of any of embodiments 1-1D, wherein the composition comprises three amino acid precursors and one amino acid entity.

12. The composition of any of embodiments 1-1D, wherein the composition comprises three amino acid metabolites and one amino acid entity.

13. The composition of any of embodiments 1-1D, wherein the composition comprises three amino acid derivatives and one amino acid entity.

14. The composition of any of embodiments 1-2, wherein the composition comprises L-leucine, a R-amino acid entity, and a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

15. The composition of any of embodiments 1-2, 2, 14, or 380, wherein the composition comprises L-leucine, R-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

16. The composition of any of embodiments 1-2, 14, or 381, wherein the composition comprises L-leucine, argininosuccinate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

17. The composition of any of embodiments 1-2, 14, or 382, wherein the composition comprises L-leucine, citrulline, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

18. The composition of any of embodiments 1-2, 14, or 383, wherein the composition comprises L-leucine, aspartate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

19. The composition of any of embodiments 1-2, 14, or 384, wherein the composition comprises L-leucine, L-glutamate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

20. The composition of any of embodiments 1-2, 14, or 385, wherein the composition comprises L-leucine, ornithine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

21. The composition of any of embodiments 1-2, 14, or 386, wherein the composition comprises a L-leucine, agmatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

22. The composition of any of embodiments 1-2, 14, or 387, wherein the composition comprises a L-leucine, creatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

23. The composition of any of embodiments 1-2, 14, or 388, wherein the composition comprises L-leucine, D-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

24. The composition of any of embodiments 1-2, 14, or 389, wherein the composition comprises L-leucine, N-acetyl-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

25. The composition of any of embodiments 1-2, 14, or 428, wherein the composition comprises L-leucine, a R-amino acid entity, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

26. The composition of any of embodiments 1-2, 14, or 429, wherein the composition comprises L-leucine, a R-amino acid entity, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

27. The composition of any of embodiments 1-2, 14, or 430, wherein the composition comprises L-leucine, a R-amino acid entity, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., a NAC entity.

28. The composition of any of embodiments 1-2, 14, or 431, wherein the composition comprises L-leucine, a R-amino acid entity, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

29. The composition of any of embodiments 1-2, 14, or 432, wherein the composition comprises L-leucine, a R-amino acid entity, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

30. The composition of any of embodiments 1-2, 14, or 445, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and NAC.

31. The composition of any of embodiments 1-2, 14, or 446, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and serine.

32. The composition of any of embodiments 1-2, 14, or 447, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and acetylserine.

33. The composition of any of embodiments 1-2, 14, or 448, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and cystathionine.

34. The composition of any of embodiments 1-2, 14, or 449, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and glutathione.

35. The composition of any of embodiments 1-2, 14, or 450, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and homocysteine.

36. The composition of any of embodiments 1-2, 14, or 451, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and methionine.

37. The composition of any of embodiments 1-2, 14, or 452, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and D-cysteine.

38. The composition of any of embodiments 1-2, 14, or 453, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and L-cysteine.

39. The composition of any of embodiments 1-2, 14, or 454, wherein the composition comprises L-leucine, a R-amino acid entity, a Q-amino acid entity, and cystine.

40. The composition of any of embodiments 1-2, 14, 380, or 428, wherein the composition comprises L-leucine, L-arginine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

41. The composition of any of embodiments 1-2, 14, 381, or 429, wherein the composition comprises L-leucine, argininosuccinate, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

42. The composition of any of embodiments 1-2, 14, 382, or 431, wherein the composition comprises L-leucine, citrulline, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

43. The composition of any of embodiments 1-2, 14, or 383, wherein the composition comprises L-leucine, aspartate, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

44. The composition of any of embodiments 1-2, 14, 380, or 445, wherein the composition comprises L-leucine, L-arginine, a Q-amino acid entity, and NAC.

45. The composition of any of embodiments 1-2, 14, 381, or 446, wherein the composition comprises L-leucine, argininosuccinate, a Q-amino acid entity, and serine.

46. The composition of any of embodiments 1-2, 14, 382, or 447, wherein the composition comprises L-leucine, citrulline, a Q-amino acid entity, and acetylserine.

47. The composition of any of embodiments 1-2, 14, 383, or 448, wherein the composition comprises L-leucine, aspartate, a Q-amino acid entity, and cystathionine.

48. The composition of any of embodiments 1-2, 14, 384, or 449, wherein the composition comprises L-leucine, glutamate, a Q-amino acid entity, and glutathione.

49. The composition of any of embodiments 1-2, 14, 385, or 450, wherein the composition comprises L-leucine, ornithine, a Q-amino acid entity, and homocysteine.

50. The composition of any of embodiments 1-2, 14, 386, or 451, wherein the composition comprises L-leucine, agmatine, a Q-amino acid entity, and methionine.

51. The composition of any of embodiments 1-2, 14, 387, or 452, wherein the composition comprises L-leucine, creatine, a Q-amino acid entity, and D-cysteine.

52. The composition of any of embodiments 1-2, 14, 388, or 453, wherein the composition comprises L-leucine, D-arginine, a Q-amino acid entity, and L-cysteine.

53. The composition of any of embodiments 1-2, 14, 389, or 454, wherein the composition comprises L-leucine, N-acetyl-arginine, a Q-amino acid entity, and cystine.

54. The composition of any of embodiments 1-2, 14, 428, or 445, wherein the composition comprises L-leucine, a R-amino acid entity, L-glutamine, and NAC.

55. The composition of any of embodiments 1-2, 14, 429, or 446, wherein the composition comprises L-leucine, a R-amino acid entity, glutamate, and serine.

56. The composition of any of embodiments 1-2, 14, 430, or 447, wherein the composition comprises L-leucine, a R-amino acid entity, carbamoyl-P, and acetylserine.

57. The composition of any of embodiments 1-2, 14, 432, or 448, wherein the composition comprises L-leucine, a R-amino acid entity, N-acetyl-glutamine, and cystathionine.

58. The composition of any of embodiments 1-2, 14, 433, or 449, wherein the composition comprises L-leucine, a R-amino acid entity, L-glutamine, and glutathione.

59. The composition of any of embodiments 1-2, 14, or 450, wherein the composition comprises L-leucine, a R-amino acid entity, glutamate, and homocysteine.

60. The composition of any of embodiments 1-2, 14, or 451, wherein the composition comprises L-leucine, a R-amino acid entity, carbamoyl-P, and methionine.

61. The composition of any of embodiments 1-2, 14, or 452, wherein the composition comprises L-leucine, a R-amino acid entity, N-acetyl-glutamine, and D-cysteine.

62. The composition of any of embodiments 1-2, 14, or 453, wherein the composition comprises L-leucine, a R-amino acid entity, L-glutamine, and L-cysteine.

63. The composition of any of embodiments 1-2, 14, or 454, wherein the composition comprises L-leucine, a R-amino acid entity, a glutamate, and cystine.

64. The composition of any of embodiments 1-2, 14, 380, or 445, wherein the composition comprises L-leucine, L-arginine, L-glutamine, and NAC.

65. The composition of any of embodiments 1-2, 14, 381, or 446, wherein the composition comprises L-leucine, argininosuccinate, glutamate, and serine.

66. The composition of any of embodiments 1-2, 14, 382, or 447, wherein the composition comprises L-leucine, citrulline, carbamoyl-P, and acetylserine.

67. The composition of any of embodiments 1-2, 14, 383, or 448, wherein the composition comprises L-leucine, aspartate, D-glutamine, and cystathionine.

68. The composition of any of embodiments 1-2, 14, 384, or 449, wherein the composition comprises L-leucine, glutamate, L-glutamine, and glutathione.

69. The composition of any of embodiments 1-2, 14, 385, or 450, wherein the composition comprises L-leucine, ornithine, glutamate, and homocysteine.

70. The composition of any of embodiments 1-2, 14, 386, or 451, wherein the composition comprises L-leucine, agmatine, carbamoyl-P, and methionine.

71. The composition of any of embodiments 1-2, 14, 387, or 452, wherein the composition comprises L-leucine, creatine, D-glutamine and D-cysteine.

72. The composition of any of embodiments 1-2, 14, 388, or 453, wherein the composition comprises L-leucine, D-arginine, a Q-amino acid entity, and L-cysteine.

73. The composition of any of embodiments 1-2, 14, 389, or 454, wherein the composition comprises L-leucine, N-acetyl-arginine, argininosuccinate, and cystine.

74. The composition of embodiment 1 or 3, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

75. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, and a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

76. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, L-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

77. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, argininosuccinate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

78. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, citrulline, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

79. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, aspartate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

80. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, glutamate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

81. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, ornithine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

82. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, agmatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

83. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, creatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

84. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, D-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenge, e.g., a NAC entity.

85. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, N-acetyl-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

86. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

87. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

88. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., a NAC entity.

89. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

90. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

91. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and NAC.

92. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and serine.

93. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and acetylserine.

94. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and cystathionine.

95. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and glutathione.

96. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and homocysteine.

97. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and methionine.

98. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and D-cysteine.

99. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and L-cysteine.

100. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and a NAC entity.

101. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a Q-amino acid entity, and cystine.

102. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, L-arginine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

103. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, argininosuccinate, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

104. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, citrulline, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

105. The composition of embodiment 1-1D, 3, or 74,wherein the composition comprises oxo-leucine, aspartate, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

106. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, L-arginine, a Q-amino acid entity, and NAC.

107. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, argininosuccinate, a Q-amino acid entity, and serine.

108. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, citrulline, a Q-amino acid entity, and acetylserine.

109. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, aspartate, a Q-amino acid entity, and cystathionine.

110. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, glutamate, a Q-amino acid entity, and glutathione.

111. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, ornithine, a Q-amino acid entity, and homocysteine.

112. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, agmatine, a Q-amino acid entity, and methionine.

113. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, creatine, a Q-amino acid entity, and D-cysteine.

114. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, D-arginine, a Q-amino acid entity, and L-cysteine.

115. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, N-acetyl-arginine, a Q-amino acid entity, and cystine.

116. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, L-glutamine, and NAC.

117. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, glutamate, and serine.

118. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, carbamoyl-P, and acetylserine.

119. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, N-acetyl-glutamine, and cystathionine.

120. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, L-glutamine, and glutathione.

121. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, glutamate, and homocysteine.

122. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, carbamoyl-P, and methionine.

123. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, N-acetyl-glutamine, and D-cysteine.

124. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, L-glutamine, and L-cysteine.

125. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, a R-amino acid entity, a glutamate, and cystine.

126. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, L-arginine, L-glutamine, and NAC.

127. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, argininosuccinate, glutamate, and serine.

128. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, citrulline, carbamoyl-P, and acetylserine.

129. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, aspartate, D-glutamine, and cystathionine.

130. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, N-acetyl-glutamine, L-glutamine, and glutathione.

131. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, ornithine, glutamate, and homocysteine.

132. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, agmatine, carbamoyl-P, and methionine.

133. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, creatine, D-glutamine and D-cysteine.

134. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, D-arginine, a Q-amino acid entity, and L-cysteine.

135. The composition of embodiment 1-1D, 3, or 74, wherein the composition comprises oxo-leucine, N-acetyl-arginine, argininosuccinate, and cystine.

136. The composition of embodiment 1-1D or 4, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

137. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, L-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

138. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, argininosuccinate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

139. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, citrulline, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

140. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, aspartate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

141. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, glutamate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

142. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, ornithine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

143. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, agmatine, a Q-amino acid entity, and an antioxidant or ROS scavenger e.g., a NAC entity.

144. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, creatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

145. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, D-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenge e.g., a NAC entity.

146. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, N-acetyl-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

147. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, L-glutamine, and an antioxidant or ROS scavenger, e.g., an antioxidant, e.g., a NAC entity.

148. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, glutamate, and an antioxidant or ROS scavenger, e.g., an antioxidant, e.g., a NAC entity.

149. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., an antioxidant, e.g., a NAC entity.

150. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, D-glutamine, and an antioxidant or ROS scavenger, e.g., an antioxidant, e.g., a NAC entity.

151. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., an antioxidant, e.g., a NAC entity.

152. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and NAC.

153. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and serine.

154. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and acetylserine.

155. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and cystathionine.

156. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and glutathione.

157. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and homocysteine.

158. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and methionine.

159. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and D-cysteine.

160. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and L-cysteine.

161. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and cysteine.

162. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a Q-amino acid entity, and cystine.

163. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, L-arginine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

164. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, argininosuccinate, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

165. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, citrulline, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

166. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, aspartate, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

167. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, L-arginine, a Q-amino acid entity, and NAC.

168. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, argininosuccinate, a Q-amino acid entity, and serine.

169. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, citrulline, a Q-amino acid entity, and acetylserine.

170. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, aspartate, a Q-amino acid entity, and cystathionine.

171. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, glutamate, a Q-amino acid entity, and glutathione.

172. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, ornithine, a Q-amino acid entity, and homocysteine.

173. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, agmatine, a Q-amino acid entity, and methionine.

174. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, creatine, a Q-amino acid entity, and D-cysteine.

175. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, D-arginine, a Q-amino acid entity, and L-cysteine.

176. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, N-acetyl-arginine, a Q-amino acid entity, and cystine.

177. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, L-glutamine, and NAC.

178. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, glutamate, and serine.

179. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, carbamoyl-P, and acetylserine.

180. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, N-acetyl-glutamine, and cystathionine.

181. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, L-glutamine, and glutathione.

182. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, glutamate, and homocysteine.

183. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, carbamoyl-P, and methionine.

184. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, N-acetyl-glutamine, and D-cysteine.

185. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, L-glutamine, and L-cysteine.

186. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, a R-amino acid entity, a glutamate, and cystine.

187. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, L-arginine, L-glutamine, and NAC.

188. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, argininosuccinate, glutamate, and serine.

189. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, citrulline, carbamoyl-P, and acetylserine.

190. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, aspartate, D-glutamine, and cystathionine.

191. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, N-acetyl-glutamine, L-glutamine, and glutathione.

192. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, ornithine, glutamate, and homocysteine.

193. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, agmatine, carbamoyl-P, and methionine.

194. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, creatine, D-glutamine and D-cysteine.

195. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, D-arginine, a Q-amino acid entity, and L-cysteine.

196. The composition of embodiment 1-1D, 4, or 136, wherein the composition comprises HMB, N-acetyl-arginine, argininosuccinate, and cystine.

197. The composition of embodiment 1-1D, or 4, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

198. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, L-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

199. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, argininosuccinate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

200. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, citrulline, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

201. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, aspartate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

202. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, glutamate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

203. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, ornithine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

204. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, agmatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

205. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, creatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

206. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, D-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

207. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, N-acetyl-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

208. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

209. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

210. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., a NAC entity.

211. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

212. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

213. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and NAC.

214. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and serine.

215. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and acetylserine.

216. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and cystathionine.

217. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and glutathione.

218. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and homocysteine.

219. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and methionine.

220. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and D-cysteine.

221. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and L-cysteine.

222. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and cysteine.

223. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a Q-amino acid entity, and cystine.

224. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, L-arginine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

225. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, argininosuccinate, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

226. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, citrulline, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

227. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, aspartate, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

228. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, L-arginine, a Q-amino acid entity, and NAC.

229. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, argininosuccinate, a Q-amino acid entity, and serine.

230. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, citrulline, a Q-amino acid entity, and acetylserine.

231. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, aspartate, a Q-amino acid entity, and cystathionine.

232. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, glutamate, a Q-amino acid entity, and glutathione.

233. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, ornithine, a Q-amino acid entity, and homocysteine.

234. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, agmatine, a Q-amino acid entity, and methionine.

235. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, creatine, a Q-amino acid entity, and D-cysteine.

236. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, D-arginine, a Q-amino acid entity, and L-cysteine.

237. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, N-acetyl-arginine, a Q-amino acid entity, and cystine.

238. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, L-glutamine, and NAC.

239. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, glutamate, and serine.

240. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, carbamoyl-P, and acetylserine.

241. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, N-acetyl-glutamine, and cystathionine.

242. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, L-glutamine, and glutathione.

243. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, glutamate, and homocysteine.

244. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, carbamoyl-P, and methionine.

245. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, N-acetyl-glutamine, and D-cysteine.

246. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, L-glutamine, and L-cysteine.

247. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, a R-amino acid entity, a glutamate, and cystine.

248. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, L-arginine, L-glutamine, and NAC.

249. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, argininosuccinate, glutamate, and serine.

250. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, citrulline, carbamoyl-P, and acetylserine.

251. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, aspartate, D-glutamine, and cystathionine.

252. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, N-acetyl-glutamine, L-glutamine, and glutathione.

253. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, ornithine, glutamate, and homocysteine.

254. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, agmatine, carbamoyl-P, and methionine.

255. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, creatine, D-glutamine and D-cysteine.

256. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, D-arginine, a Q-amino acid entity, and L-cysteine.

257. The composition of embodiment 1-1D, 4, or 197, wherein the composition comprises isovaleryl-CoA, N-acetyl-arginine, argininosuccinate, and cystine.

258. The composition of embodiment 1-1D or 5, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

259. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, L-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

260. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, argininosuccinate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

261. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, citrulline, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

262. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, aspartate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

263. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, glutamate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

264. The composition of embodiment 1-1D, 5, or 258, wherein composition comprises D-leucine, ornithine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

265. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, agmatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

266. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, creatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

267. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, D-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

268. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, N-acetyl-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

269. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

270. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

271. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., a NAC entity.

272. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

273. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, N-acetyl-glutamine, and an antioxidant or ROS scavenger, ee.g., a NAC entity.

274. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and NAC.

275. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and serine.

276. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and acetylserine.

277. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and cystathionine.

278. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and glutathione.

279. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and homocysteine.

280. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and methionine.

281. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and D-cysteine.

282. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and L-cysteine.

283. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and cysteine.

284. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a Q-amino acid entity, and cystine.

285. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, L-arginine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

286. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, argininosuccinate, glutamate, and an antioxidant or ROS scavengere.g., a NAC entity.

287. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, citrulline, D-glutamine, and an antioxidant or ROS scavengere.g., a NAC entity.

288. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, aspartate, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

289. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, L-arginine, a Q-amino acid entity, and NAC.

290. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, argininosuccinate, a Q-amino acid entity, and serine.

291. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, citrulline, a Q-amino acid entity, and acetylserine.

292. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, aspartate, a Q-amino acid entity, and cystathionine.

293. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, glutamate, a Q-amino acid entity, and glutathione.

294. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, ornithine, a Q-amino acid entity, and homocysteine.

295. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, agmatine, a Q-amino acid entity, and methionine.

296. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, creatine, a Q-amino acid entity, and D-cysteine.

297. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, D-arginine, a Q-amino acid entity, and L-cysteine.

298. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, N-acetyl-arginine, a Q-amino acid entity, and cystine.

299. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, L-glutamine, and NAC.

300. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, glutamate, and serine.

301. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, carbamoyl-P, and acetylserine.

302. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, N-acetyl-glutamine, and cystathionine.

303. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, L-glutamine, and glutathione.

304. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, glutamate, and homocysteine.

305. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, carbamoyl-P, and methionine.

306. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, N-acetyl-glutamine, and D-cysteine.

307. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, L-glutamine, and L-cysteine.

308. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, a R-amino acid entity, a glutamate, and cystine.

309. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, L-arginine, L-glutamine, and NAC.

310. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, argininosuccinate, glutamate, and serine.

311. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, citrulline, carbamoyl-P, and acetylserine.

312. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, aspartate, D-glutamine, and cystathionine.

313. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, N-acetyl-glutamine, L-glutamine, and glutathione.

314. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, ornithine, glutamate, and homocysteine.

315. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, agmatine, carbamoyl-P, and methionine.

316. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, creatine, D-glutamine and D-cysteine.

317. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, D-arginine, a Q-amino acid entity, and L-cysteine.

318. The composition of embodiment 1-1D, 5, or 258, wherein the composition comprises D-leucine, N-acetyl-arginine, argininosuccinate, and cystine.

319. The composition of embodiment 1-1D or 5, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

320. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, L-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

321. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, argininosuccinate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

322. The composition of embodiment 1-1D, 5, or 319,, wherein the composition comprises N-acetyl-leucine, citrulline, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

323. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, aspartate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

324. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, glutamate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

325. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, ornithine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

326. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, agmatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

327. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, creatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

328. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, D-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

329. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, N-acetyl-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

330. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

331. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

332. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., a NAC entity.

333. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

334. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

335. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and NAC.

336. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and serine.

337. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and acetylserine.

338. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and cystathionine.

339. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and glutathione.

340. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and homocysteine.

341. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and methionine.

342. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and D-cysteine.

343. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and L-cysteine.

344. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and cysteine.

345. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a Q-amino acid entity, and cystine.

346. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, L-arginine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

347. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, argininosuccinate, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

348. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, citrulline, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

349. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, aspartate, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

350. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, L-arginine, a Q-amino acid entity, and NAC.

351. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, argininosuccinate, a Q-amino acid entity, and serine.

352. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, citrulline, a Q-amino acid entity, and acetylserine.

353. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, aspartate, a Q-amino acid entity, and cystathionine.

354. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, glutamate, a Q-amino acid entity, and glutathione.

355. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, ornithine, a Q-amino acid entity, and homocysteine.

356. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, agmatine, a Q-amino acid entity, and methionine.

357. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, creatine, a Q-amino acid entity, and D-cysteine.

358. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, D-arginine, a Q-amino acid entity, and L-cysteine.

359. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, N-acetyl-arginine, a Q-amino acid entity, and cystine.

360. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, L-glutamine, and NAC.

361. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, glutamate, and serine.

362. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, carbamoyl-P, and acetylserine.

363. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, N-acetyl-glutamine, and cystathionine.

364. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, L-glutamine, and glutathione.

365. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, glutamate, and homocysteine.

366. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, carbamoyl-P, and methionine.

367. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, N-acetyl-glutamine, and D-cysteine.

368. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, L-glutamine, and L-cysteine.

369. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, a R-amino acid entity, a glutamate, and cystine.

370. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, L-arginine, L-glutamine, and NAC.

371. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, argininosuccinate, glutamate, and serine.

372. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, citrulline, carbamoyl-P, and acetylserine.

373. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, aspartate, D-glutamine, and cystathionine.

374. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, N-acetyl-glutamine, L-glutamine, and glutathione.

375. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, ornithine, glutamate, and homocysteine.

376. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, agmatine, carbamoyl-P, and methionine.

377. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, creatine, D-glutamine and D-cysteine.

378. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, D-arginine, a Q-amino acid entity, and L-cysteine.

379. The composition of embodiment 1-1D, 5, or 319, wherein the composition comprises N-acetyl-leucine, N-acetyl-arginine, argininosuccinate, and cystine.

380. The composition of embodiment 1-1D or 2, wherein the composition comprises a L-amino acid entity, L-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

381. The composition of embodiment 1-1D or 2, wherein the composition comprises a L-amino acid entity, argininosuccinate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

382. The composition of embodiment 1-1D, 3, or 4, wherein the composition comprises a L-amino acid entity, citrulline, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

383. The composition of embodiment 1-1D or 3, wherein the composition comprises a L-amino acid entity, aspartate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

384. The composition of embodiment 1-1D or 3, wherein the composition comprises a L-amino acid entity, glutamate, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

385. The composition of embodiment 1-1D or 4, wherein the composition comprises a L-amino acid entity, ornithine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

386. The composition of embodiment 1-1D or 4, wherein the composition comprises a L-amino acid entity, agmatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

387. The composition of embodiment 1-1D or 4, wherein the composition comprises a L-amino acid entity, creatine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

388. The composition of embodiment 1-1D or 5, wherein the composition comprises a L-amino acid entity, D-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

389. The composition of embodiment 1-1D or 5, wherein the composition comprises a L-amino acid entity, N-acetyl-arginine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

390. The composition of embodiment 1-1D, 3, or 384, wherein the composition comprises L-leucine, glutamate, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

391. The composition of embodiment 1-1D, 4, or 385, wherein the composition comprises L-leucine, ornithine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

392. The composition of embodiment 1-1D, 4, or 386, wherein the composition comprises a L-amino acid entity, agmatine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

393. The composition of embodiment 1-1D, 4, or 387, wherein the composition comprises a L-amino acid entity, creatine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

394. The composition of embodiment 1-1D, 4, or 388, wherein the composition comprises a L-amino acid entity, D-arginine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

395. The composition of embodiment 1-1D, 4, or 389, wherein the composition comprises a L-amino acid entity, D-arginine, N-acetyl-arginine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

396. The composition of embodiment 1-1D or 380, wherein the composition comprises a L-amino acid entity, L-arginine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

397. The composition of any of embodiments 1-2, or 381, wherein the composition comprises a L-amino acid entity, argininosuccinate, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

398. The composition of embodiment 1-1D, 3, 4, or 382, wherein the composition comprises a L-amino acid entity, citrulline, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., a NAC entity.

399. The composition of embodiment 1-1D, 3, or 383, wherein the composition comprises a L-amino acid entity, aspartate, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

400. The composition of embodiment 1-1D, 3, or 384, wherein the composition comprises a L-amino acid entity, glutamate, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

401. The composition of embodiment 1-1D, 4, or 385, wherein the composition comprises a L-amino acid entity, ornithine, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

402. The composition of embodiment 1-1D, 4, or 386, wherein the composition comprises a L-amino acid entity, agmatine, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

403. The composition of embodiment 1-1D, 4, or 387, wherein the composition comprises a L-amino acid entity, creatine, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

404. The composition of embodiment 1-1D, 5, or 388, wherein the composition comprises a L-amino acid entity, D-arginine, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., a NAC entity.

405. The composition of embodiment 1-1D, 5, or 389, wherein the composition comprises a L-amino acid entity, N-acetyl-arginine, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

406. The composition of embodiment 1-1D, 3, 80, or 445, wherein the composition comprises a L-amino acid entity, L-arginine, L-glutamine, and NAC.

407. The composition of any of embodiments 1-2, 381, or 446, wherein the composition comprises a L-amino acid entity, argininosuccinate, glutamate, and serine.

408. The composition of embodiment 1-1D, 3, 4, 382, or 447, wherein the composition comprises a L-amino acid entity, citrulline, carbamoyl-P, and acetylserine.

409. The composition of embodiment 1-1D, 3, 383, or 448, wherein the composition comprises a L-amino acid entity, aspartate, glutamate, and cystathionine.

410. The composition of embodiment 1-1D, 3, 384, or 449, wherein the composition comprises a L-amino acid entity, glutamate, D-glutamine, and glutathione.

411. The composition of embodiment 1-1D, 4, 385, or 448, wherein the composition comprises a L-amino acid entity, ornithine, N-acetyl-glutamine, and cystathionine.

412. The composition of embodiment 1-1D, 4, 386, or 450, wherein the composition comprises a L-amino acid entity, agmatine, L-glutamine, and homocysteine.

413. The composition of embodiment 1-1D, 4, 387, or 451, wherein the composition comprises a L-amino acid entity, creatine, glutamate, and methionine.

414. The composition of embodiment 1-1D, 5, 388, or 454, wherein the composition comprises a L-amino acid entity, D-arginine, carbamoyl-P, and D-cysteine.

415. The composition of embodiment 1-1D, 5, 389, or 453, wherein the composition comprises a L-amino acid entity, N-acetyl-arginine, glutamate, and L-cysteine.

416. The composition of embodiment 1-1D, 380, or 454, wherein the composition comprises a L-amino acid entity, L-arginine, L-glutamine, and cystine.

417. The composition of embodiment 1-1D, 6, or 445, wherein the composition comprises a L-amino acid entity, L-arginine, a Q-amino acid, and NAC.

418. The composition of any of embodiments 1-2, or 446, wherein the composition comprises a L-amino acid entity, argininosuccinate, a Q-amino acid, and serine.

419. The composition of embodiment 1-1D, 3, or 447, wherein the composition comprises a L-amino acid entity, citrulline, a Q-amino acid, and acetylserine.

420. The composition of embodiment 1-1D, 4, or 448, wherein the composition comprises a L-amino acid entity, aspartate, a Q-amino acid, and cystathionine.

421. The composition of embodiment 1-1D, 3, or 449, wherein the composition comprises a L-amino acid entity, glutamate, a Q-amino acid, and glutathione.

422. The composition of embodiment 1-1D, 4, or 448, wherein the composition comprises a L-amino acid entity, ornithine, a Q-amino acid, and cystathionine.

423. The composition of embodiment 1-1D, 4, or 450, wherein the composition comprises a L-amino acid entity, agmatine, a Q-amino acid, and homocysteine.

424. The composition of embodiment 1-1D, 4, or 451, wherein the composition comprises a L-amino acid entity, creatine, a Q-amino acid, and methionine.

425. The composition of embodiment 1-1D, 5, or 452, wherein the composition comprises a L-amino acid entity, D-arginine, a Q-amino acid, and D-cysteine.

426. The composition of embodiment 1-1D, 5, or 453, wherein the composition comprises a L-amino acid entity, N-acetyl-arginine, a Q-amino acid, and L-cysteine.

427. The composition of embodiment 1-1D, 5, or 454, wherein the composition comprises a L-amino acid entity, L-arginine, a Q-amino acid, and cystine.

428. The composition of embodiment 1-1D or 2, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, L-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

429. The composition of embodiment 1-1D, 3, or 4, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, glutamate, and an antioxidant or ROS scavenger, e.g., a NAC entity.

430. The composition of embodiment 1-1D or 4, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., a NAC entity.

431. The composition of embodiment 1-1D or 5, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

432. The composition of embodiment 1-1D or 5, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, N-acetyl-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

433. The composition of embodiment 1-1D, 5, or 431, wherein the composition comprises a L-leucine, a R-amino acid entity, D-glutamine, and an antioxidant or ROS scavenger, e.g., a NAC entity.

434. The composition of embodiment 1-1D, 4 or 430, wherein the composition comprises a L-leucine, L-arginine, carbamoyl-P, and an antioxidant or ROS scavenger, e.g., a NAC entity.

435. The composition of any of embodiments 1-2, 428, or 445, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, L-glutamine, and NAC.

436. The composition of embodiment 1-1D, 4, 429, or 446, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, glutamate, and serine.

437. The composition of embodiment 1-1D, 4, 430, or 447, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, carbamoyl-P, and acetylserine.

438. The composition of embodiment 1-1D, 5, 431, or 448, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, D-glutamine, and cystathionine.

439. The composition of embodiment 1-1D, 5, 432, or 449, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, N-acetyl-glutamine, and glutathione.

440. The composition of any of embodiments 1-2, 428, or 450, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, L-glutamine, and homocysteine.

441. The composition of embodiment 1-1D, 3, 4, 429, or 451, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, glutamate, and methionine.

442. The composition of embodiment 1-1D, 4, 430, or 452, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, carbamoyl-P, and D-cysteine

443. The composition of embodiment 1-1D, 5, 431, or 453, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, D-glutamine, and L-cysteine.

444. The composition of embodiment 1-1D, 5, 432, or 454, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, N-acetyl-glutamine, and cystine.

445. The composition of embodiment 1-1D or 5, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and NAC.

446. The composition of embodiment 1-1D or 3, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and serine.

447. The composition of embodiment 1-1D or 3, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and acetylserine.

448. The composition of embodiment 1-1D or 3, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and cystathionine.

449. The composition of embodiment 1-1D or 4, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and glutathione.

450. The composition of embodiment 1-1D or 4, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and homocysteine.

451. The composition of embodiment 1-1D or 4, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and methionine.

452. The composition of embodiment 1-1D or 5, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and D-cysteine.

453. The composition of embodiment 1-1D or 5, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and L-cysteine.

454. The composition of embodiment 1-1D or 5, wherein the composition comprises a L-amino acid entity, a R-amino acid entity, a Q-amino acid entity, and cystine.

455. The composition of embodiment 1-1D or 2, wherein the composition comprises a L-amino acid, ornithine, a Q-amino acid entity, and an antioxidant or ROS scavenger, e.g., a NAC entity.

456. The composition of embodiment 1-1D or 455, wherein the composition comprises L-leucine, ornithine, l-glutamine, and NAC.

457. The composition of embodiment 1-1D or 455, wherein the composition comprises HMB, ornithine, l-glutamine, and NAC.

458. The composition of any of the foregoing embodiments, wherein the composition comprises L-leucine or a leucine metabolite (e.g., HMB), L-arginine or an L-arginine metabolite (e.g., creatine), l-glutamine, and NAC or a NAC metabolite, e.g., glutathione.

459. The composition of any of the foregoing embodiments, wherein the composition comprises L-leucine or a leucine metabolite (e.g., HMB), L-arginine or an L-arginine metabolite (e.g., creatine), L-glutamine, and NAC or a NAC metabolite, e.g., glutathione.

460. The composition of any of the previous embodiments, further comprising an isoleucine (I)-amino acid entity.

461. The composition of embodiment 460, wherein the I-amino acid entity is an amino acid.

462. The composition of embodiment 460 or 461, wherein the amino acid entity is L-isoleucine.

463. The composition of embodiment 460, wherein the I-amino acid entity is an amino acid precursor.

464. The composition of embodiment 460 or 463, wherein the I-amino acid entity is 2-oxo-3-methyl-valerate.

465. The composition of embodiment 460 or 463, wherein the I-amino acid entity is threonine.

466. The composition of embodiment 460, wherein the I-amino acid entity is an amino acid metabolite.

467. The composition of embodiment 460 or 466, wherein the I-amino acid entity is 2-oxo-3-methyl-valerate

468. The composition of embodiment 460 or 466, wherein the I-amino acid entity is methylbutyrl-CoA.

469. The composition of embodiment 460, wherein the I-amino acid entity is an amino acid derivative.

470. The composition of embodiment 460 or 469, wherein the I-amino acid entity is D-isoleucine.

471. The composition of embodiment 460 or 469, wherein the I-amino acid entity is N-acetyl-isoleucine.

472. The composition of any of the previous embodiments, further comprising a valine (V)-amino acid entity.

473. The composition of embodiment 472, wherein the V-amino acid entity is an amino acid.

474. The composition of embodiment 472 or 473, wherein the V-amino acid entity is L-valine.

475. The composition of embodiment 472, wherein the V-amino acid entity is an amino acid precursor.

476. The composition of embodiment 472 or 475, wherein the V-amino acid entity is 2-oxo-valerate.

477. The composition of embodiment 472, wherein the V-amino acid entity is an amino acid metabolite.

478. The composition of embodiment 472 or 477, wherein the V-amino acid entity is isobutryl-CoA.

479. The composition of embodiment 472 or 477, wherein the V-amino acid entity is 3-HIB-CoA.

480. The composition of embodiment 472 or 477, wherein the V-amino acid entity is 3-HIB.

481. The composition of embodiment 472, wherein the V-amino acid entity is an amino acid derivative.

482. The composition of embodiment 472 or 481, wherein the V-amino acid entity is D-valine.

483. The composition of embodiment 472 or 481, wherein the V-amino acid entity is N-acetyl-valine.

484. The composition of any of the preceding embodiments, further comprising L-glycine.

485. The composition of any of the preceding embodiments, further comprising an S-amino acid entity (e.g., L-serine, phosphoserine, P-hydroxypyruvate, L-glycine, tryptophan, acetylserine, cystathionine, phosphatidylserine, or any combination thereof, e.g., L-serine and L-glycine).

486. The composition of any of the preceding embodiments, further comprising carnitine.

487. The composition of any of the preceding embodiments, comprising:

a) a L-amino acid entity chosen from L-leucine or a salt thereof, or β-hydroxy-β-methybutyrate (HMB) or a salt thereof or a combination of L-leucine or a salt thereof and HMB ora salt thereof;

b) an R-amino acid entity chosen from L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof or a combination of two or three of L-arginine or a salt thereof, ornithine or a salt thereof, or creatine or a salt thereof;

c) L-glutamine or a salt thereof; and

d) N-acetylcysteine (NAC) or a salt thereof.

488. The composition of any of the preceding embodiments, wherein the L-Leucine is provided as part of a dipeptide comprising L-Leucine, or a salt thereof, or a tripeptide comprising L-Leucine, or a salt thereof.

489. The composition of any of the preceding embodiments, wherein the L-Arginine is provided as part of a dipeptide comprising L-Arginine, or a salt thereof, or a tripeptide comprising L-Arginine, or a salt thereof.

490. The composition of any of the preceding embodiments, wherein the L-Glutamine is provided as part of a dipeptide comprising L-Glutamine, or a salt thereof, or a tripeptide comprising L-Glutamine, or a salt thereof

491. The composition of any of the preceding embodiments, wherein the NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

492. The composition of any of the preceding embodiments, wherein one, two, three, or four of methionine (M), tryptophan (W), valine (V), or cysteine (C) is absent, or if present, is present at less than 10 weight (wt.) % of the composition.

493. The composition of any of the preceding embodiments, wherein the total wt. % of (a)-(d) is greater than the total wt. % of any other amino acid entity in the composition.

494. The composition of any of the preceding embodiments, wherein one, two, three, or four of the amino acids in (a)-(d) is provided as part of a dipeptide or tripeptide, e.g., in an amount of at least 10 wt. % of the composition.

495. The composition of embodiment 494, wherein the dipeptide is a homodipeptide or heterodipeptide of any of the amino acids in (a)-(d), e.g., one, two, three, or four of the amino acids in (a)-(d) is a homodipeptide or heterodipeptide.

496. The composition of embodiment 494, wherein the tripeptide is a homotripeptide or heterotripeptide of any of the amino acids in (a)-(d), e.g., one, two, three, or four of the amino acids in (a)-(d) is a homotripeptide or heterotripeptide.

497. The composition of any of the preceding embodiments, wherein (a) is a L-amino acid entity dipeptide or a salt thereof (e.g., a L-leucine dipeptide or a salt thereof)

498. The composition of embodiment 497, wherein (a) is a homodipeptide or a heterodipeptide, e.g., Ala-Leu.

499. The composition of any of the preceding embodiments, wherein (b) is a L-arginine dipeptide or a salt thereof.

500. The composition of embodiment 499, wherein (b) is a homodipeptide or a heterodipeptide, e.g., Ala-Arg.

501. The composition of any of the preceding embodiments, wherein (c) is a L-glutamine dipeptide or a salt thereof.

502. The composition of embodiment 501, wherein (c) is a homodipeptide, e.g., Gln-Gln, or wherein (c) is a heterodipeptide, e.g., Ala-Gln.

503. The composition of any of the preceding embodiments, wherein:

f) a wt. % of the L-glutamine or a salt thereof in the composition is greater than the wt. % of the R-amino acid entity;

g) the wt. % of the L-glutamine or a salt thereof in the composition is greater than the wt. % of the L-amino acid entity;

h) the wt. % of the R-amino acid entity in the composition is greater than the wt. % of the L-amino acid entity; or

i) a combination of two or three of (f)-(h).

504. The composition of any of the preceding embodiments, wherein the wt. % of the L-glutamine or a salt thereof in the composition is at least 5% greater than the wt. % of the R-amino acid entity, e.g., the wt. % of the L-glutamine or a salt thereof is at least 10%, 15%, 20%, or 25% greater than the wt. % of the R-amino acid entity

505. The composition of any of the preceding embodiments, wherein the wt. % of the L-glutamine or a salt thereof in the composition is at least 20% greater than the wt. % of the L-amino acid entity, e.g., the wt. % of the L-glutamine or a salt thereof in the composition is at least 25%, 30%, 35%, 40%, 45%, or 50% greater than the wt. % of the L-amino acid entity.

506. The composition of any of the preceding embodiments, wherein the wt. % of the R-amino acid entity in the composition is at least 10% greater than the wt. % of the L-amino acid entity, e.g., the wt. % of the R-amino acid entity in the composition is at least 15%, 20%, 25%, or 30% greater than the wt. % of the L-amino acid entity.

507. The composition of any of the preceding embodiments, wherein:

j) the ratio of the L-amino acid entity to the R-amino acid entity is at least 1:4, or at least 2:5, and not more than 3:4, e.g., the ratio of L-amino acid entity to R-amino acid entity is about 2:3;

k) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is at least 1:4, or least 1:3, and not more than 3:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:2;

l) the ratio of the R-amino acid entity to the L-glutamine or a salt thereof is at least 1:4, or least 1:2, and not more than 6:7, e.g., the ratio of the R-amino acid entity to the L-glutamine or a salt thereof is about 3:4; or

m) a combination of two or three of (j)-(l).

508. The composition of any of the preceding embodiments, further comprising one or both of an isoleucine (I)-amino acid-entity and a valine (V)-amino acid-entity, e.g., both the I-amino acid-entity and the V-amino acid-entity are present.

509. The composition of embodiment 508, wherein:

n) the wt. % of the L-amino acid-entity in the composition is greater than or equal to the wt. % of the I-amino acid-entity and the V-amino acid-entity in combination;

o) the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination in the composition is greater than or equal to the wt. % of the L-glutamine or a salt thereof;

p) the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination in the composition is less than the wt. % of the R-amino acid entity;

q) the wt. % of the R-amino acid entity and the L-glutamine or a salt thereof in the composition is greater than the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination; or

r) a combination of two, three, or four of (n)-(q).

510. The composition of embodiment 508 or 509, wherein:

s) the wt. % of the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or a salt thereof is at least 50% of the composition, or at least 70% of the composition, but not more than 90% of the composition;

t) the wt. % of the NAC or a salt thereof is at least 1%, or at least 2%, but not more than 10% of the composition;

u) the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination is at least 15%, or at least 20%, but not more than 50% of the composition;

v) the wt. % of the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or a salt thereof is at least 40%, or at least 50%, but not more than 80% of the composition; or

w) a combination of two, three, or four of (s)-(v).

511. The composition of any of embodiments 508-510, wherein:

x) the ratio of the L-amino acid entity to the I-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5 to 1 or not more than 3:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 2:1;

y) the ratio of L-amino acid entity to V-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5 to 1 or not more than 3:1, e.g., the ratio of L to V is about 2:1;

z) the ratio of the L-amino acid entity to the R-amino acid entity is greater than 1:3, greater than 1.5 to 3, and less than 3:3, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 2:3;

aa) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is greater than 1:4, greater than 1.5 to 4 and less than 4:4, or less than 3:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:2; or

bb) a combination of two, three, or four of (x)-(aa).

512. The composition of any of embodiments 508-511, wherein:

cc) the ratio of the I-amino acid entity to the V-amino acid entity is at least .5:1, or at least .75:1, and not more than 1.5 to 1 or not more than 2:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 1:1;

dd) the ratio of the I-amino acid entity to the R-amino acid entity is at least .5:3, or at least .75:3, and not more than 2:3, or not more than 1.5:3, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 1:3;

ee) the ratio of the I-amino acid entity to the L-glutamine or a salt thereof is at least .5:4, or at least .75:4, and not more than 3:4, or not more than 2:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:4; or

ff) or a combination of two or three of (cc)-(ee).

513. The composition of any of embodiments 508-512, wherein:

gg) the ratio of the L-amino acid entity to the V-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5 to 1 or not more than 3:1, e.g., is the ratio of the L-amino acid entity to the V-amino acid entity is about 2:1;

hh) the ratio of the L-amino acid entity to the R-amino acid entity is greater than 1:3, greater than 1.5 to 3, and less than 3:3, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 2:3;

ii) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is greater than 1:4, greater than 1.5 to 4 and less than 4:4, or less than 3:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:2; or

jj) a combination of two or three of (gg)-(ii).

514. The composition of any of embodiments 508-513, wherein:

kk) the ratio of the V-amino acid entity to the L-glutamine or a salt thereof is at least .5:4, or at least .75:4, and not more than 3:4, or not more than 2:4, e.g., the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is about 1:4;

ll) the ratio of the V-amino acid entity to the R-amino acid entity is at least .5:3, or at least .75:3, and not more than 2:3, or not more than 1.5:3, e.g., the ratio of the V-amino acid entity to the R-amino acid entity is about 1:3;

mm) the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is at least 1:4, or at least 2:3, or not more than 5:7, or not more than 6:7, e.g., the ratio is about 6:11; or

nn) a combination of two or three of (kk)-(mm).

515. The composition of any of the preceding embodiments, wherein:

oo) a wt. % of the L-amino acid entity in the composition is greater than the wt. % of the NAC or a salt thereof;

pp) a wt. % of the R-amino acid entity in the composition is greater than the wt. % of the NAC or a salt thereof;

qq) a wt. % of the L-glutamine or a salt thereof in the composition is greater than the wt. % of the NAC or a salt thereof; or rr) a combination of two or three of (oo)-(qq).

516. The composition of any of the preceding embodiments, wherein at least one of the amino acids of (a)-(d) is a free amino acid, e.g., two, three, or four of the amino acids of (a)-(d) are a free amino acid, e.g., at least 50 wt. % of the total wt. of the composition is one or more amino acid entities in free form.

517. The composition of any of the preceding embodiments, wherein at least one of the amino acids of (a)-(d) is in a salt form, e.g., one, two, three, or four of the amino acids of (a)-(d) is in a salt form, e.g., at least 10 wt. % of the total wt. of the composition is one or more amino acid entities in salt form.

518. The composition of embodiment 517, wherein at least 10 wt. % of the total wt. of the composition is one or more amino acid entities in salt form.

519. The composition of any of the preceding embodiments, wherein the composition is capable of one, two, three, four, five, or all of:

a) decreasing or preventing liver fibrosis;
b) decreasing or preventing liver injury;
c) decreasing or preventing hepatocyte inflammation;
d) improving, e.g., increasing, glucose tolerance;
e) decreasing or preventing steatosis;
f) decreasing or preventing hepatocyte ballooning; or
g) improving gut function.

520. The composition of any of the preceding embodiments, wherein the composition further comprises a serine (S)-amino acid entity, e.g., a S-amino acid entity chosen from L-serine, phosphoserine, P-hydroxypyruvate, L-glycine, tryptophan, acetylserine, cystathionine, cysteine, phosphatidylserine, and D-serine or a combination thereof, e.g., a combination of L-serine and L-glycine.

521. The composition of embodiment 520, wherein the composition comprises an L-amino acid entity, an I-amino acid entity, an V-amino acid entity, an R-amino acid entity, an L-glutamine or a salt thereof, an NAC or a salt thereof, and an L-glycine.

522. The composition of embodiment 520, wherein the composition comprises an L-amino acid entity, an I-amino acid entity, an V-amino acid entity, an R-amino acid entity, an L-glutamine or a salt thereof, an NAC or a salt thereof, and an L-serine.

523. The composition of embodiment 520, wherein the composition comprises an L-amino acid entity, an I-amino acid entity, an V-amino acid entity, an R-amino acid entity, an L-glutamine or a salt thereof, an NAC or a salt thereof, an L-glycine, and an L-serine.

524. The composition of any of embodiments 520-523, wherein the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the L-glutamine or salt thereof, and the NAC or salt thereof is about 1 : 0.5: 0.5: 1.5 : 2 : 0.15 or about 1 : 0.5: 0.5: 1.5 : 2 : 0.3.

525. The composition of any of the preceding embodiments, wherein the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 0.5 to 3 : 0.5 to 4 : 1 to 4 : 0.1 to 2.5, e.g., the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 1 : 1.5 : 2 : 0.15 or about 1 : 1.5 : 2 : 0.3.

526. The composition of embodiment 525, wherein the wt. ratio of the L-amino acid entity, the R-amino acid entity, the L-glutamine or a salt thereof, and the NAC or salt thereof is about 1 : 0.75 : 2 : 0.15 or about 1 : 0.75 : 2 : 0.3.

527. The composition of any of the preceding embodiments, wherein the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity,

the L-glutamine or salt thereof, and the NAC or salt thereof is about 1 : 0.5: 0.5: 1.5 : 2 : 0.15 or about 1 : 0.5: 0.5: 1.5 : 2 : 0.3.

528. The composition of any of embodiments 13-33, wherein the composition comprises about 0.5 g to about 10 g of the L-amino acid entity, about 0.25 g to about 5 g of the I-amino acid entity, about 0.25 g to about 5 g of the V-amino acid entity, about 0.5 g to about 20 g of the R-amino acid entity, about 1 g to about 20 g of the L-glutamine or a salt thereof, and about 0.1 g to about 5 g of the NAC or salt thereof, e.g., the composition comprises about 1 g of the L-amino acid entity, about 0.5 g of the I-amino acid entity, about 0.5 g of V-amino acid entity, about 1.5 g of R-amino acid entity, about 2 g of L-glutamine or a salt thereof, and about 0.15 g or about 0.3 g of NAC or salt thereof.

529. The composition of embodiment 528, wherein the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 1 g of V-amino acid entity, about 3 g of R-amino acid entity, about 4 g of L-glutamine or a salt thereof, and about 0.9 g of NAC or a salt thereof.

530. The composition of any of the preceding embodiments, wherein the composition comprises:

a) L-leucine or a salt thereof;
b) L-isoleucine or a salt thereof;
c) L-valine or a salt thereof;
b) L-arginine or a salt thereof;
e) L-glutamine or a salt thereof; and
f) NAC or a salt thereof.

531. The composition of embodiment 530, wherein the L-Leucine is provided as part of a dipeptide comprising L-Leucine, or a salt thereof, or a tripeptide comprising L-Leucine, or a salt thereof.

532. The composition of embodiment 530 or 531, wherein the L-Isoleucine is provided as part of a dipeptide comprising L-Isoleucine, or a salt thereof, or a tripeptide comprising L-Isoleucine, or a salt thereof.

533. The composition of any of embodiments 530-532, wherein the L-Valine is provided as part of a dipeptide comprising L-Valine, or a salt thereof, or a tripeptide comprising L-Valine, or a salt thereof.

534. The composition of any of embodiments 530-533, wherein the L-Arginine is provided as part of a dipeptide comprising L-Arginine, or a salt thereof, or a tripeptide comprising L-Arginine, or a salt thereof.

535. The composition of any of embodiments 530-534, wherein the L-Glutamine is provided as part of a dipeptide comprising L-Glutamine, or a salt thereof, or a tripeptide comprising L-Glutamine, or a salt thereof.

536. The composition of any of embodiments 530-535, wherein the NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

537. The composition of any of the preceding embodiments, wherein the composition comprises a combination of 4 to 20 different amino acid entities, e.g., a combination of 5 to 15 different amino acid entities.

538. The composition of any of the preceding embodiments, wherein at least two, three, four, or more amino acid entities is not a peptide of more than 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues in length.

539. A method for improving liver function, wherein the method comprises administering to a subject in need thereof an effective amount of a composition of any of the preceding embodiments.

540. The method of embodiment 539, wherein the L-Leucine is provided as part of a dipeptide comprising L-Leucine, or a salt thereof, or a tripeptide comprising L-Leucine, or a salt thereof.

541. The method of embodiment 539 or 540, wherein the L-Arginine is provided as part of a dipeptide comprising L-Arginine, or a salt thereof, or a tripeptide comprising L-Arginine, or a salt thereof.

542. The method of any of embodiments 539-541, wherein the L-Glutamine is provided as part of a dipeptide comprising L- Glutamine, or a salt thereof, or a tripeptide comprising L-Glutamine, or a salt thereof.

543. The method of any of embodiments 539-542, wherein the NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

544. A method for treating one or more symptoms selected from the group consisting of decreased fat metabolism, hepatocyte apoptosis, hepatocyte ballooning, inflammation of adipose tissue, inflammation of hepatic tissue, fibrosis, liver injury, steatosis, glucose tolerance, and oxidative stress, wherein the method comprises administering to a subject in need thereof an effective amount of a composition of any of the preceding embodiments.

545. The method of embodiment 544, wherein the L-Leucine is provided as part of a dipeptide comprising L-Leucine, or a salt thereof, or a tripeptide comprising L-Leucine, or a salt thereof.

546. The method of embodiment 544 or 545, wherein the L-Arginine is provided as part of a dipeptide comprising L-Arginine, or a salt thereof, or a tripeptide comprising L-Arginine, or a salt thereof.

547. The method of any of embodiments 544-546, wherein the L-Glutamine is provided as part of a dipeptide comprising L- Glutamine, or a salt thereof, or a tripeptide comprising L-Glutamine, or a salt thereof.

548. The method of any of embodiments 544-547, wherein the NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

549. A method for treating fatty liver disease, wherein the method comprises administering to a subject in need thereof an effective amount of a compositionof any of the preceding embodiments.

550. The method of embodiment 549, wherein the L-Leucine is provided as part of a dipeptide comprising L-Leucine, or a salt thereof, or a tripeptide comprising L-Leucine, or a salt thereof.

551. The method of embodiment 549 or 550, wherein the L-Arginine is provided as part of a dipeptide comprising L-Arginine, or a salt thereof, or a tripeptide comprising L-Arginine, or a salt thereof.

552. The method of any of embodiments 549-551, wherein the L-Glutamine is provided as part of a dipeptide comprising L- Glutamine, or a salt thereof, or a tripeptide comprising L-Glutamine, or a salt thereof.

553. The method of any of embodiments 549-552, wherein the NAC is provided as a part of a dipeptide comprising NAC, or a salt thereof, or a tripeptide comprising NAC, or a salt thereof.

554. The method of any of the preceding embodiments, wherein the subject has a disease or disorder selected from the group consisting of non-alcoholic fatty liver (NAFL), non-alcoholic

fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic fatty liver disease (AFLD), and alcoholic steatohepatitis (ASH).

555. The method of embodiment 554, wherein the subject has pediatric NAFLD.

556. The method of any of the preceding embodiments, wherein the subject has a high BMI, obesity, gut leakiness, gut dysbiosis or gut microbiome disturbance.

557. The method of any of the preceding embodiments, wherein the subject has cirrhosis, hepatocarcinoma, an increased risk of liver failure, an increased risk of death, metabolic syndrome, or type 2 diabetes.

558. The method of any of the preceding embodiments, wherein the subject has increased levels of inflammatory cytokines relative to a normal subject, e.g., the subject has increased levels of TNFα relative to a normal subject e.g., without the one or more symptoms or without the fatty liver disease.

559. The method of any of the preceding embodiments, wherein the patient exhibits muscle atrophy or has a decreased ratio of muscle tissue to adipose tissue relative to a normal subject, e.g., without the one or more symptoms or without a fatty liver disease, e.g., the patient exhibits muscle atrophy without one or both of fibrosis or cirrhosis.

560. The method of any of the preceding embodiments, wherein the subject exhibits reverse lipid transport from adipose tissue to liver tissue.

561. The composition of any of the preceding embodiments, wherein the composition comprises free amino acids, wherein the amino acids comprise arginine, glutamine, N-acetylcysteine, and a branched-chain amino acid chosen from one, two, or all of leucine, isoleucine, and valine.

562. The composition of embodiment 561, wherein the branched-chain amino acid is leucine, isoleucine, and valine.

563. The composition of embodiment 561 or 562, wherein the wt ratio of leucine, isoleucine, valine, arginine, glutamine, N-acetylcysteine is 1 : 0.5 : 0.5 : 1.5 : 2 : 0.15.

564. The composition of any of embodiments 561-563, wherein a total weight (wt) of the amino acids is about 2 g to about 60 g.

565. The composition of embodiment 564, wherein the total wt of the amino acids is about 6 g, about 12 g, about 18 g, about 24 g, or about 48 g.

566. The composition of any of embodiments 561-565, wherein the composition comprises about 0.5 g to about 10 g of leucine, about 0.25 g to about 5 g of isoleucine, about 0.25 g to about 5 g of valine, about 1 g to about 20 g of arginine, about 1 g to about 20 g of glutamine, and about 0.1 g to about 5 g of N-acetylcysteine.

567. The composition of embodiment 566, wherein the composition comprises about 1 g of leucine, about 0.5 g of isoleucine, about 0.5 g of valine, about 1.5 g of arginine, about 2 g of glutamine, and about 0.15 g of N-acetylcysteine.

568. The composition of embodiment 566, wherein the composition comprises about 2 g of leucine, about 1 g of isoleucine, about 1 g of valine, about 3.0 g of arginine, about 4 g of glutamine, and about 0.3 g of N-acetylcysteine.

569. The composition of embodiment 566, wherein the composition comprises about 4 g of leucine, about 2 g of isoleucine, about 2 g of valine, about 6.0 g of arginine, about 8 g of glutamine, and about 0.6 g of N-acetylcysteine.

570. The composition of any of embodiments 561-566, wherein the amino acids comprise about 10 wt % to about 30 wt % leucine, about 5 wt % to about 15 wt % isoleucine,

about 5 wt % to about 15 wt % valine, about 15 wt % to about 40 wt % arginine, about 20 wt % to about 50 wt % glutamine, and about 1 wt % to about 8 wt % n-acetylcysteine.

571. The composition of embodiment 570, wherein the amino acids comprise about 16 wt % to about 18 wt % leucine, about 7 wt % to about 9 wt % isoleucine, about 7 wt % to about 9 wt % valine, about 28 wt % to about 32 wt % arginine, about 31 wt % to about 34 wt % glutamine, and about 1 wt % to about 5 wt % n-acetylcysteine.

572. The composition of embodiment 571, wherein the amino acids comprise about 16.8 wt % leucine, about 8.4 wt % isoleucine, about 8.4 wt % valine, about 30.4 wt % arginine, about 33.6 wt % glutamine, and about 2.5 wt % n-acetylcysteine.

573. The composition of any of the preceding embodiments, wherein the composition further comprises one or more pharmaceutically acceptable excipients.

574. The composition of embodiment 573, wherein the excipients are selected from the group consisting of citric acid, lecithin, a sweetener, a dispersion enhancer, a flavoring, a bitterness masking agent, and a natural or artificial coloring.

575. The composition of any of the preceding embodiments, wherein the composition is in the form of a solid, powder, solution, or gel.

576. The composition of any of the preceding embodiments, wherein the amino acids consist of leucine, isoleucine, valine, arginine, glutamine and N-acetylcysteine.

577. A method for treating one or more symptoms selected from the group consisting of decreased fat metabolism, hepatocyte apoptosis, hepatocyte ballooning, inflammation of adipose tissue, inflammation of hepatic tissue, fibrosis, and oxidative stress, wherein the method comprises administering to a subject in need thereof an effective amount of the composition of any one of embodiments 561-576.

578. The method of embodiment 577, wherein the subject has non-alcoholic fatty liver disease (NAFLD).

579. The method of embodiment 577 or 578, wherein the subject has pediatric NAFLD.

580. The method of embodiment 578 or 579, wherein the patient has steatosis.

581. The method of embodiment 577, wherein the subject has non-alcoholic steatohepatitis (NASH).

582. The method of embodiment 581, wherein the subject has fibrosis.

583. The method of embodiment 577, wherein the subject has cirrhosis.

584. The method of embodiment 583, wherein the subject has hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

585. The method of any of embodiments 577-584, wherein the subject has type 2 diabetes.

586. A method for treating non-alcoholic fatty liver disease (NAFLD) comprising administering to a subject in need thereof an effective amount of the composition of any of embodiments 561-576.

587. The method of embodiment 586, wherein the subject has pediatric NAFLD.

588. The method of embodiment 586 or 587, wherein the patient has steatosis.

589. A method for treating non-alcoholic steatohepatitis (NASH) comprising administering to a subject in need thereof an effective amount of the composition of any of embodiments 561-576.

590. The method of embodiment 589, wherein the subject has fibrosis.

591. A method for treating cirrhosis comprising administering to a subject in need thereof an effective amount of the composition of any of embodiments 561-576.

592. The method of embodiment 591, wherein the subject has hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

593. The method of any of embodiments 577-592, wherein administering the composition results in an improvement in one or more metabolic symptoms in the subject.

594. The method of embodiment 593, wherein the improvement in one or more metabolic symptoms is selected from the following: increased free fatty acid and lipid metabolism, improved mitochondrial function, white adipose tissue (WAT) browning, decreased reactive oxygen species (ROS), increased levels of glutathione (GSH), decreased hepatic inflammation, decreased hepatocyte ballooning, improved gut barrier function, increased insulin secretion, or glucose tolerance.

595. The method of embodiment 594, wherein the increased free fatty acid and lipid metabolism occurs in the liver.

596. The method of embodiment 594 or 595, wherein administration of the composition results in an improvement in one or more metabolic symptoms after a treatment period of 24 hours.

597. The method of any of embodiments 577-596, wherein the method further comprises determining the level of one, two, three, four, five, six, seven, eight, nine, ten, or more (*e.g.,* all) of the following:

a) alanine aminotransferase (ALT);

b) aspartate aminotransferase (AST);

c) adiponectin;

d) N-terminal fragment of type III collagen (proC3);

e) caspase-cleaved keratin 18 fragments (M30 and M65);

f) IL-1 beta;

g) C-reactive protein;

h) PIIINP;

i) TIMP1;

j) MCP-1; or

k) FGF-21.

598. The method of embodiment 597, wherein administration of the composition results in an improvement in one or more of a)-k) after a treatment period of 24 hours.

599. The method of any of embodiments 577-598, wherein the composition is administered prior to a meal.

600. The method of any of embodiments 577-598, wherein the composition is administered concurrent with a meal.

601. The method of any of embodiments 577-598, wherein the composition is administered following a meal.

602. The method of any of embodiments 577-601, wherein the composition is administered with a second agent.

603. The method of embodiment 602, wherein the second agent is selected from the group consisting of a farnesoid X receptor (FXR) agonist, a stearoyl CoA desaturase inhibitor, a CCR2 and CCR5 chemokine antagonist, a PPAR alpha and delta agonist, a caspase inhibitor, a galectin-3 inhibitor, an acetyl CoA carboxylase inhibitor, or an ileal sodium bile acid co-transporter inhibitor.

604. A dietary composition comprising the composition of any of embodiments 561-576, e.g., wherein the dietary composition is chosen from a medical food, a functional food, or a supplement.

605. The composition of any of embodiments 561-576 for use as a dietary composition, e.g., wherein the dietary composition is chosen from a medical food, a functional food, or a supplement.

606. The dietary composition of embodiment 605, wherein the subject has type 2 diabetes and/or a relatively high BMI.

607. The dietary composition of any of embodiments 605 or 606, wherein the subject has non-alcoholic fatty liver disease (NAFLD).

608. The dietary composition of any of embodiments 605-607, wherein the subject has pediatric NAFLD.

609. The dietary composition of any of embodiments 605-608, wherein the patient has steatosis.

610. The dietary composition of any of embodiments 605-609, wherein the subject has non-alcoholic steatohepatitis (NASH).

611. The dietary composition of embodiment 610, wherein the subject has fibrosis.

612. The dietary composition of any of embodiments 604-606, wherein the subject has cirrhosis.

613. The dietary composition of embodiment 612, wherein the subject has hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

614. The dietary composition of any of embodiments 604-613, wherein the subject has type 2 diabetes.

615. The dietary composition of any of embodiments 604-614, wherein the composition promotes weight loss in the subject.

616. The method or dietary composition of any of the preceding embodiments, wherein the composition is administered at a dose of about 15 g/d to about 90 g/d.

617. The method or dietary composition of embodiment 616, wherein the composition is administered at a dose of about 18 g/d, about 24 g/d, about 36/d, about 54 g/d, or about 72 g/d.

618. The method or dietary composition of any of the preceding embodiments, wherein the composition is administered one, two, to three times per day.

619. The method or dietary composition of any of the preceding embodiments, wherein the composition is administered at a dose of about 6 g, about 8 g, about 12 g, about 16 g, about 18 g, or about 24 g three times per day.

620. The composition of any of the preceding embodiments, wherein:

1) the ratio of the L-amino acid entity to the I-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 2:1;

2) the ratio of L-amino acid entity to V-amino acid entity is at least 2:1, at least 3:1, at least 3.5:1, at least 4:1, or at least 5:1, and not more than 6: 1, e.g., the ratio of L-amino acid entity to V-amino acid entity is about 4:1;

3) the ratio of the L-amino acid entity to the R-amino acid entity is at least 1:1, at least 3.5:3, at least 4:3, or at least 2:1, and not more than 5:2, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 4:3;

4) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is at least 0.5:1, or at least 0.75:1, and not more than 1.5 to 1 or not more than 2:1, e.g., the ratio of the L-amino acid entity to the L-glutamine or salt thereof is about 1:1;

5) the ratio of the L-amino acid entity to the NAC entity or a salt thereof is at least 2:1, at least 3:1, at least 3.5:1, or at least 4:1, and not more than 5 to 1 or not more than 6:1, e.g., the ratio of the L-amino acid entity to the NAC entity or salt thereof is about 4:1 (e.g., 4:0.9);

6) optionally wherein the ratio of the L-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:3, greater than 1.5:3, about 2:3, or about 3:5, and not more than 2.5:3 or 1:1, e.g., the ratio of the L-amino acid entity to the S-amino acid entity is about 2:3, or the ratio of the L-amino acid entity to the S-amino acid entity is about 3:5; or

7) a combination of two, three, four, five, or six of (1)-(6).

621. The composition of embodiment 620, wherein:

8) the ratio of I-amino acid entity to V-amino acid entity is at least 1:1, at least 1.5:1, or about 2:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of I-amino acid entity to V-amino acid entity is about 2:1;

9) the ratio of the I-amino acid entity to the R-amino acid entity is greater than 1:3, greater than 1.5:3, or about 2:3, and not more than 2.5:3 or not more than 1:1, e.g., the ratio of the I-amino acid entity to the R-amino acid entity is about 2:3;

10) the ratio of the I-amino acid entity to the L-glutamine or a salt thereof is at least 1:4, at least 1:3, or about 1:2, and not more than 1:1 or not more than 2:1, e.g., the ratio of the I-amino acid entity to the L-glutamine or salt thereof is about 1:2;

11) the ratio of the I-amino acid entity to the NAC entity or a salt thereof is at least 1:1, at least 1.5:1, or about 2:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of the I-amino acid entity to the NAC entity or salt thereof is about 2:1 (e.g., 2:0.9);

12) optionally wherein the ratio of the I-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:4, greater than 1.5:4, about 1:3, or about 3:10, and not more than 1.5:3 or 2:3, e.g., the ratio of the I-amino acid entity to the S-amino acid entity is about 1:3, or the ratio of the I-amino acid entity to the S-amino acid entity is about 3:10; or

13) a combination of two, three, four, or five of (8)-(12).

622. The composition of embodiment 620 or 621, wherein:

14) the ratio of the V-amino acid entity to the R-amino acid entity is greater than 1:4, greater than 1.5:4, or about 1:3, and not more than 1:2 or not more than 1:1, e.g., the ratio of the V-amino acid entity to the R-amino acid entity is about 1:3;

15) the ratio of the V-amino acid entity to the L-glutamine or a salt thereof is greater than 1:5, or greater than 1.5:5, about 1:4, and not more than 1.5:4 or not more than 1:3, e.g., the ratio of the V-amino acid entity to the L-glutamine or salt thereof is about 1:4;

16) the ratio of the V-amino acid entity to the NAC entity or a salt thereof is at least 1:2, at least 1.5:2, or about 1:1, and not more than 1.5:1 or not more than 2:1, e.g., the ratio of the V-amino acid entity to the NAC entity or salt thereof is about 1:1 (e.g., 1:0.9);

17) optionally wherein the ratio of the V-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:8, greater than 1:7, about 1:6, or about 3:20, and not more than 1.5:6 or 1:3, e.g., the ratio of the V-amino acid entity to the S-amino acid entity is about 1:6, or the ratio of the V-amino acid entity to the S-amino acid entity is about 3:20; or

18) a combination of two, three, or four of (14)-(17).

623. The composition of any of embodiments 620-622, wherein:

19) the ratio of the R-amino acid entity to the L-glutamine or a salt thereof is greater than 1:2, greater than 1.25:2, or about 3:4, and not more than 3.5:4 or not more than 1:1, e.g., the ratio of the R-amino acid entity to the L-glutamine or salt thereof is about 3:4;

20) the ratio of the R-amino acid entity to the NAC entity or a salt thereof is at least 4:1, at least 4:1.5, or about 3:1, and not more than 3:1.5 or not more than 3:2, e.g., the ratio of the R-amino acid entity to the NAC entity or salt thereof is about 3:1 (e.g., 3:0.9);

21) optionally wherein the ratio of the R-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:4, greater than 1:3, about 1:2,, or about 9:20, and not more than 1.5:2 or 1:1, e.g., the ratio of the R-amino acid entity to the S-amino acid entity is about 1:2, or the ratio of the R-amino acid entity to the S-amino acid entity is about 9:20; or

22) a combination of two or three of (19)-(21).

624. The composition of any of embodiments 620-623, wherein:

23) the ratio of the L-glutamine to the NAC entity or a salt thereof is at least 5:1, at least 5:1.5, or about 4:1, and not more than 4:1.5 or not more than 3:1, e.g., the ratio of the L-glutamine to the NAC entity or salt thereof is about 4:1 (e.g., 4:0.9);

24) optionally wherein the ratio of the L-glutamine to the S-amino acid entity or a salt thereof is greater than 1:3, greater than 1.25:3, about 2:3, or about 3:5, and not more than 2.5:3 or 1:1, e.g., the ratio of the L-glutamine to the S-amino acid entity is about 2:3, or the ratio of the L-glutamine to the S-amino acid entity is about 3:5; or

25) a combination of (23) and (24).

625. The composition of any of embodiments 620-624, wherein:

26) the ratio of the NAC entity to the S-amino acid entity or a salt thereof is greater than 1:8, greater than 1:7, or about 1:6, and not more than 1:5 or not more than 1.5:5, e.g., the ratio of the NAC entity to the S-amino acid entity is about 1:6 (e.g., 0.9:6 or 2.7:20).

626. The composition of any of embodiments 620-625, wherein the composition satisfies the properties of (1)-(7) defined above.

627. The composition of any of embodiments 620-626, wherein the composition satisfies the properties of at least 2, 3, 4, 5, 6, or 7 of any of properties (1)-(26) defined above.

628. The composition of any of embodiments 620-627, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 : 6 : 3 : 9 : 12 : 2.7.

629. The composition of any of embodiments 620-628, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 : 6 : 3 : 9 : 12 : 2.7 : 18.

630. The composition of any of embodiments 620-629, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino

acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 : 6 : 3 : 9 : 12 : 2.7 : 20.

631. The composition of any of embodiments 620-630, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15%.

632. The composition of any of embodiments 620-631, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 18 +/- 15%.

633. The composition of any of embodiments 620-632, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 : 6 : 3 : 9 : 12 : 2.7 : 9: 9. In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 : 6 : 3 : 9 : 12 : 2.7 : 10: 10.

634. The composition of any of embodiments 620-633, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 9 +/- 15% : 9 +/- 15%. In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 10 +/- 15% : 10 +/- 15%.

635. A pharmaceutical composition comprising the composition of any of the preceding embodiments.

636. The composition of any of the preceding embodiments, wherein the L-amino acid entity is chosen from the group consisting of L-leucine, β-hydroxy-β-methybutyrate (HMB), oxo-leucine, isovaleryl-CoA, D-leucine, and n-acetyl-leucine, or a combination thereof.

637. The composition of any of the preceding embodiments, wherein the R-amino acid entity is chosen from the group consisting of L-arginine, ornithine, argininosuccinate, citrulline, aspartate, glutamate, agmatine, creatine, D-arginine, and N-acetyl-arginine, or a combination thereof.

638. The composition of any of the preceding embodiments, wherein the Q-amino acid entity is chosen from the group consisting of L-glutamine, glutamate, carbamoyl-P, glutamate, D-glutamine, and n-acetylglutamine, or a combination thereof.

639. The composition of any of the preceding embodiments, wherein the NAC-amino acid entity is chosen from the group consisting of NAC, serine, acetylserine, cystathionine, glutathione, homocysteine, methionine, D-cysteine, L-cysteine, cysteamine, and cystine, or a combination thereof.

640. The composition of any of the preceding embodiments, wherein the S-amino acid entity is chosen from the group consisting of L-serine, phosphoserine, P-hydroxypyruvate, L-glycine, tryptophan, acetylserine, cystathionine, and phosphatidylserine.

641. A dietary composition comprising the composition of any of the preceding embodiments, wherein the dietary compositions is chosen from a medical food, a functional food, or a supplement.

642. A method of providing amino acid entities to a subject comprising administering to the subject an effective amount of the composition of any of the preceding embodiments.

643. A method of manufacturing or making a composition comprising forming a composition comprising the following:

a) a L-amino acid entity,

b) an R-amino acid entity,

c) a Q-amino acid entity;

d) a NAC entity, e.g., NAC; and

optionally, e) an S-amino acid entity;

provided that:

f) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, wherein:

(i) the amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity.

644. The method of any of the preceding embodiments, wherein:

1) the ratio of the L-amino acid entity to the I-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 2:1;

2) the ratio of L-amino acid entity to V-amino acid entity is at least 2:1, at least 3:1, at least 3.5:1, at least 4:1, or at least 5:1, and not more than 6: 1, e.g., the ratio of L-amino acid entity to V-amino acid entity is about 4:1;

3) the ratio of the L-amino acid entity to the R-amino acid entity is at least 1:1, at least 3.5:3, at least 4:3, or at least 2:1, and not more than 5:2, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 4:3;

4) the ratio of the L-amino acid entity to the L-glutamine or a salt thereof is at least 0.5:1, or at least 0.75:1, and not more than 1.5 to 1 or not more than 2:1, e.g., the ratio of the L-amino acid entity to the L-glutamine or salt thereof is about 1:1;

5) the ratio of the L-amino acid entity to the NAC entity or a salt thereof is at least 2:1, at least 3:1, at least 3.5:1, or at least 4:1, and not more than 5 to 1 or not more than 6:1, e.g., the ratio of the L-amino acid entity to the NAC entity or salt thereof is about 4:1 (e.g., 4:0.9);

6) optionally wherein the ratio of the L-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:3, greater than 1.5:3, about 2:3, or about 3:5, and not more than

2.5:3 or 1:1, e.g., the ratio of the L-amino acid entity to the S-amino acid entity is about 2:3, or the ratio of the L-amino acid entity to the S-amino acid entity is about 3:5; or

7) a combination of two, three, four, five, or six of (1)-(6).

645. The method of embodiment 644, wherein:

8) the ratio of I-amino acid entity to V-amino acid entity is at least 1:1, at least 1.5:1, or about 2:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of I-amino acid entity to V-amino acid entity is about 2:1;

9) the ratio of the I-amino acid entity to the R-amino acid entity is greater than 1:3, greater than 1.5:3, or about 2:3, and not more than 2.5:3 or not more than 1:1, e.g., the ratio of the I-amino acid entity to the R-amino acid entity is about 2:3;

10) the ratio of the I-amino acid entity to the L-glutamine or a salt thereof is at least 1:4, at least 1:3, or about 1:2, and not more than 1:1 or not more than 2:1, e.g., the ratio of the I-amino acid entity to the L-glutamine or salt thereof is about 1:2;

11) the ratio of the I-amino acid entity to the NAC entity or a salt thereof is at least 1:1, at least 1.5:1, or about 2:1, and not more than 2.5:1 or not more than 3:1, e.g., the ratio of the I-amino acid entity to the NAC entity or salt thereof is about 2:1 (e.g., 2:0.9);

12) optionally wherein the ratio of the I-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:4, greater than 1.5:4, about 1:3, or about 3:10, and not more than 1.5:3 or 2:3, e.g., the ratio of the I-amino acid entity to the S-amino acid entity is about 1:3, or the ratio of the I-amino acid entity to the S-amino acid entity is about 3:10; or

13) a combination of two, three, four, or five of (8)-(12).

646. The method of embodiment 644 or 645, wherein:

14) the ratio of the V-amino acid entity to the R-amino acid entity is greater than 1:4, greater than 1.5:4, or about 1:3, and not more than 1:2 or not more than 1:1, e.g., the ratio of the V-amino acid entity to the R-amino acid entity is about 1:3;

15) the ratio of the V-amino acid entity to the L-glutamine or a salt thereof is greater than 1:5, or greater than 1.5:5, about 1:4, and not more than 1.5:4 or not more than 1:3, e.g., the ratio of the V-amino acid entity to the L-glutamine or salt thereof is about 1:4;

16) the ratio of the V-amino acid entity to the NAC entity or a salt thereof is at least 1:2, at least 1.5:2, or about 1:1, and not more than 1.5:1 or not more than 2:1, e.g., the ratio of the V-amino acid entity to the NAC entity or salt thereof is about 1:1 (e.g., 1:0.9);

17) optionally wherein the ratio of the V-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:8, greater than 1:7, about 1:6, or about 3:20, and not more than 1.5:6 or 1:3, e.g., the ratio of the V-amino acid entity to the S-amino acid entity is about 1:6, or the ratio of the V-amino acid entity to the S-amino acid entity is about 3:20; or

18) a combination of two, three, or four of (14)-(17).

647. The method of any of embodiments 644-646, wherein:

19) the ratio of the R-amino acid entity to the L-glutamine or a salt thereof is greater than 1:2, greater than 1.25:2, or about 3:4, and not more than 3.5:4 or not more than 1:1, e.g., the ratio of the R-amino acid entity to the L-glutamine or salt thereof is about 3:4;

20) the ratio of the R-amino acid entity to the NAC entity or a salt thereof is at least 4:1, at least 4:1.5, or about 3:1, and not more than 3:1.5 or not more than 3:2, e.g., the ratio of the R-amino acid entity to the NAC entity or salt thereof is about 3:1 (e.g., 3:0.9);

21) optionally wherein the ratio of the R-amino acid entity to the S-amino acid entity or a salt thereof is greater than 1:4, greater than 1:3, about 1:2,, or about 9:20, and not more than 1.5:2 or 1:1, e.g., the ratio of the R-amino acid entity to the S-amino acid entity is about 1:2, or the ratio of the R-amino acid entity to the S-amino acid entity is about 9:20; or

22) a combination of two or three of (19)-(21).

648. The method of any of embodiments 644-647, wherein:

23) the ratio of the L-glutamine to the NAC entity or a salt thereof is at least 5:1, at least 5:1.5, or about 4:1, and not more than 4:1.5 or not more than 3:1, e.g., the ratio of the L-glutamine to the NAC entity or salt thereof is about 4:1 (e.g., 4:0.9);

24) optionally wherein the ratio of the L-glutamine to the S-amino acid entity or a salt thereof is greater than 1:3, greater than 1.25:3, about 2:3, or about 3:5, and not more than 2.5:3 or 1:1, e.g., the ratio of the L-glutamine to the S-amino acid entity is about 2:3, or the ratio of the L-glutamine to the S-amino acid entity is about 3:5; or

25) a combination of (23) and (24).

649. The method of any of embodiments 644-648, wherein:

26) the ratio of the NAC entity to the S-amino acid entity or a salt thereof is greater than 1:8, greater than 1:7, or about 1:6, and not more than 1:5 or not more than 1.5:5, e.g., the ratio of the NAC entity to the S-amino acid entity is about 1:6 (e.g., 0.9:6 or 2.7:20).

650. The method of any of embodiments 644-649, wherein the composition satisfies the properties of (1)-(7) defined above.

651. The method of any of embodiments 644-650, wherein the composition satisfies the properties of at least 2, 3, 4, 5, 6, or 7 of any of properties (1)-(26) defined above.

652. The method of any of embodiments 644-651, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 : 6 : 3 : 9 : 12 : 2.7.

653. The method of any of embodiments 644-652, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 : 6 : 3 : 9 : 12 : 2.7 : 18.

654. The method of any of embodiments 644-653, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 : 6 : 3 : 9 : 12 : 2.7 : 20.

655. The method of any of embodiments 644-654, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15%.

656. The method of any of embodiments 644-655, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 18 +/- 15%.

657. The method of any of embodiments 644-656, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 : 6 : 3 : 9 : 12 : 2.7 : 9: 9. In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 : 6 : 3 : 9 : 12 : 2.7 : 10: 10.

658. The method of any of embodiments 644-657, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 9 +/- 15% : 9 +/- 15%. In certain embodiments, the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, the S-amino acid entity, and the L-glycine is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 10 +/- 15% : 10 +/- 15%.

659. The composition or method of any of the preceding embodiments, wherein the composition is capable of enhancing fatty acid oxidation, e.g., one or both of reducing levels of unsaturated fatty acids or increasing levels of acylcarnitine (e.g., in a STAM mouse model or a FATZO mouse model). In certain embodiments, the reduction in levels of unsaturated fatty acids is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the level of change shown in Table 53, e.g., measured as described in Example 9. In certain embodiments, the increase in levels of acylcarnitine is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the level of change shown in Table 53, e.g., measured as described in Example 9.

660. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of alanine transaminase (ALT), e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

661. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of aspartate transaminase (AST), e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

662. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, alanine transaminase (ALT) by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of ALT, e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

663. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, aspartate transaminase (AST) by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of AST, e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

664. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of hydroxyproline, e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

665. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, hydroxyproline levels by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of hydroxyproline, e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition (e.g., a vehicle control).

666. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using LX-2 cells, e.g., levels of Col1a1, Acta2, and/or TIMP2 in LX-2 cells, e.g., as assessed using a nucleic acid amplification method, e.g., PCR or qRT-PCR, e.g., as described in Example 7, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; NAC; or an amino acid composition comprising L-arginine, L-glutamine, and NAC).

667. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, expression of one or more collagen biomarkers (e.g., Col1a1, Acta2, and/or TIMP2) by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using LX-2 cells, e.g., levels of Col1a1, Acta2, and/or TIMP2 in LX-2 cells, e.g., as assessed using a nucleic acid amplification method, e.g., PCR or qRT-PCR, e.g., as described in Example 7, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; or NAC).

668. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, expression of one or more collagen biomarkers (e.g., Col1a1) by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using primary hepatic stellate cells, e.g., levels of Col1a1 in primary hepatic stellate cells, e.g., as assessed using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 12, e.g., relative to a reference composition

(e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

669. The composition or method of any of the preceding embodiments, wherein the composition is capable of increasing, or increases, expression of one or more collagen biomarkers (e.g., procollagen 1α1) by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using primary hepatic stellate cells, e.g., levels of procollagen 1α1in primary hepatic stellate cells, e.g., as assessed using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 12, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

670. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, hepatocyte inflammation by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using HepG2 cells, e.g., decreased activity, e.g., decreased TNFα-induced activity of NF-kB in a reporter assay in HepG2 cells, as described in Example 8, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; or NAC).

671. The composition or method of any of the preceding embodiments, wherein the composition is capable of reducing, or reduces, TNFα-induced activity of NF-kB in HepG2 cells by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using HepG2 cells, e.g., decreased activity, e.g., decreased TNFα-induced activity of NF-kB in a reporter assay in HepG2 cells, as described in Example 8, e.g.,

relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; or NAC).

672. The composition or method of any of the preceding embodiments, wherein the composition is capable of increasing, or increases, glucose tolerance, e.g., in a STAM mouse model or in a FATZO mouse model, by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of glucose levels, e.g., using glucose oxidase, e.g., using a glucometer, e.g., as described in Example 5, e.g., relative to a reference composition (e.g., a vehicle control or a positive control, e.g., metformin).

673. The composition or method of any of the preceding embodiments, wherein the composition is capable of increasing, or increases, blood glucose metabolism, e.g., in a STAM mouse model or in a FATZO mouse model, by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of glucose levels, e.g., using glucose oxidase, e.g., using a glucometer, e.g., as described in Example 5, e.g., relative to a reference composition (e.g., a vehicle control or a positive control, e.g., metformin).

674. The composition or method of any of the preceding embodiments, wherein the composition is capable of decreasing, or decreases, steatosis and/or inflammation by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of MCP1/CCL2, e.g., in primary hepatocytes, e.g., using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 10, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

675. The composition or method of any of the preceding embodiments, wherein the composition is capable of decreasing, or decreases, MCP1/CCL2 levels by at least 1%, 2%, 3%,

4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of MCP1/CCL2, e.g., in primary hepatocytes, e.g., using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 10, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

676. The composition or method of any of the preceding embodiments, wherein the composition is capable of decreasing, or decreases, TNFα inflammatory response by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of MCP1/CCL2 or an assay of IL-6, e.g., in primary hepatic stellate cells, e.g., using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 11, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

677. The composition or method of any of the preceding embodiments, wherein the composition is capable of decreasing, or decreases, MCP1/CCL2 levels and/or IL-6 levels by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of MCP1/CCL2 or an assay of IL-6, e.g., in primary hepatic stellate cells, e.g., using an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 11, e.g., relative to a reference composition (e.g., a vehicle control; an amino acid composition comprising L-leucine, L-isoleucine, L-valine; an amino acid composition comprising L-arginine, L-glutamine, and NAC; an amino acid composition comprising L-leucine, L-isoleucine, L-valine, L-arginine, and L-glutamine; valine; glutamine; arginine; isoleucine; leucine; or NAC).

678. The composition of any of the preceding embodiments for use as a medicament.

679. The composition of any of the preceding embodiments for use in a method as disclosed herein.

680. The use of a composition of any of the preceding embodiments in the manufacture of a medicament.

681. The use of a composition of any of the preceding embodiments in the manufacture of a medicament for treating any of the disorders or conditions disclosed herein.

Although many of the above embodiments are shown in dependent form, it is contemplated that any of the embodiments or combinations thereof may be in independent form.

EXAMPLES

The Example below is set forth to aid in the understanding of the inventions, but is not intended to, and should not be construed to, limit its scope in any way.

Example 1: Method of producing the amino acid compositions

The amino acid compositions of the instant disclosure and formulations thereof may be made according to methods known in the art. They may also be made by the methods described below.

The starting materials (individual amino acids and excipients) are blended and sieved to generate a powder blend, which is filled into stick packs. The contents of the stick packs are dispersed in water at time of use for oral administration. An example of the mixing and reconstitution protocols, and stick pack formulations made thereby, are provided below.

Mixing protocol

1. Ingredients were weighed into a container.
2. The container was sealed and placed in a Turbula mixer and contents mixed on low setting for 2 minutes.
3. The blended powder was sieved using a No. 14 screen and any clumps not passing through the sieve were broken apart.
4. The blended and sieved powder was transferred back to the container and mixed in a Turbula mixer on low for 10 minutes.

Table 13. Stick pack formulations

	Placebo	**Formulation of Amino Acid Composition A-1**	**Formulation of Amino Acid Composition A-2**
Ingredient	**Amount per stick pack (g)**		
FUSI-BCAA™ Instantized Blend (2:1:1 L:I:V)	0.00	2.00	2.00
(contains L-Leucine)	*N/A*	*(1.00)*	*(1.00)*
(contains L-Isoleucine)	*N/A*	*(0.50)*	*(0.50)*
(contains L-Valine)	*N/A*	*(0.50)*	*(0.50)*
L-Arginine HCl	0.00	1.50	1.81
L-Glutamine	0.00	2.00	2.00

N-Acetylcysteine	0.00	0.25	0.15
Citric Acid	0.98	0.67	0.67
Lecithin (Alcolec F100)	0.59	0.83	0.83
Acesulfame Potassium	0.04	0.05	0.05
Sucralose micronized NF	0.02	0.03	0.03
Xanthan Gum (Ticaxan Rapid-3)	0.24	0.24	0.24
Vanilla Custard #4306	0.06	0.06	0.06
Maltrin QD M500 maltodextrin NF	5.75	0.00	0.00
Nat Orange WONF #1326	0.36	0.36	0.36
Lime 865.0032U	0.05	0.05	0.05
Lemon 862.2169U	0.05	0.05	0.05
Bitterness Masking 936.2160U	0.12	0.12	0.12
FD&C Yellow 6	0.01	0.01	0.01
FD&C Red 40 (1:100 in M500)	0.0667	0.00	0.0000
Total (g)	**8.33**	**8.22**	**8.42**

Reconstitution protocol

Stick pack formulations were reconstituted according to the following protocol:

1. A total (g) "amount per stick pack" of powder blend was weighed.

2. About 118.3 g (4 oz) of cold filtered water was weighed into a sealable container.

3. The "amount per stick pack" of the powder blend was transferred to the sealable container and the container was sealed.

4. The container was shaken vigorously for 20 to 30 seconds.

Example 2: Analytical characterization of the amino acid compositions

Described below are methods used to characterize some of the physicochemical properties in formulations of the amino acid compositions prepared according to Example 1.

Identification and Assay. The identification and assay of % label claim of each amino acid present in **Formulations of Amino Acid Composition A-1 and Amino Acid Composition A-2** was evaluated by reversed phase HPLC.

Amino acid analysis

Briefly, an amino acid analysis method using reversed-phase high pressure liquid chromatography (HPLC) was developed to measure free amino acid content (except for N-acetylcysteine) in formulations of amino acid compositions described herein following resuspension. Column and chromatographic conditions were modified from Agilent Technical Note: "Automated Amino Acid Analysis Using an Agilent Poroshell HPH-C18 Column (Agilent Application Note 5991-5571EN)". Primary amino acids in the sample are derivatized online using the Agilent 1260 or 1290 UPLC well-plate autosampler using o-phthaldialdehyde (OPA). Separation is achieved using an Agilent ZORBAX Eclipse Plus column (4.6 mm ID x 100 mm, 3.5 μm). The OPA-derivatives of primary amino acids are detected using fluorescence (FLD) at 340 nm emission/450 nm excitation wavelengths and UV detection at 338 nm. Individual amino acids are expected to elute according to known representative chromatograms of amino acid standards. Concentrations of amino acids in samples are determined by fitting a sample peak area to a standard curve. Alternatively, amino acid analysis may be performed using derivatization with AccQ-Tag chemistry and standards (Waters).

Amino acid analysis: N-acetylcysteine

For N-acetylcysteine (NAC), an HPLC test method was developed based on the United States Pharmacopeia Monograph Chapter 39 (USP <39>) for "Acetylcysteine" to determine the content of N-acetylcysteine of reconstituted powder of formulations described herein. This HPLC method involves the use of a reversed-phase column without any derivatization step. The separation was done using a column of C-18 backbone as the stationary phase, and 0.05 M KH_2PO_4 as the mobile phase. UV detection was performed at 214 nm. The column was then flushed with 5% acetonitrile to remove any residual sample components at the end of each injection. At the end of the sequence, a low flow "system flush" procedure involving stronger organic solvents is used to preserve the column for storage. N-Acetylcysteine is expected to elute according to known representative chromatograms of standards. Concentrations of N-acetylcysteine in samples are determined by fitting a sample peak area to a standard curve.

Results for **Formulation of Amino Acid Composition A-1** (compared to theoretical g per serving) are shown in **Table 14**. For glutamine, a mean mass per serving of 1.84 g was observed; compared to 2.00 g theoretical per serving, this gives a % agreement (or % label claim value) of 92%. For arginine, a mean mass per serving of 1.69 g was observed; compared to 1.50 g theoretical per serving, this gives a % agreement (or % label claim value) of 113%. For valine, a mean mass per serving of 0.51 g was observed; compared to 0.50 g theoretical per serving, this gives a % agreement (or % label claim value) of 101%. For isoleucine, a mean mass per serving of 0.52 g was observed; compared to 0.50 g theoretical per serving, this gives a % agreement (or % label claim value) of 104%. For leucine, a mean mass per serving of 1.04 g was observed; compared to 1.00 g theoretical per serving, this gives a % agreement (or % label claim value) of 104%. For N-acetylcysteine (NAC), a mean mass per serving of 0.28 g was observed; compared to 0.25 g theoretical per serving, this gives a % agreement (or % label claim value) of 111%. Overall, the amino acids and amino acid derivatives in the **Formulation of Amino Acid Composition A-1** had a range of % label claims of 92-113%.

Table 14. % Label Claim Results: Formulation of Amino Acid Composition A-1

	g of Individual Amino Acid/ per serving of Amino Acid Composition A-1					
Sample Name	**GLN**	**ARG**	**VAL**	**ILE**	**LEU**	**NAC**
AA Comp A-1	1.84	1.70	0.51	0.52	1.05	0.28
AA Comp A-1	1.82	1.68	0.50	0.52	1.04	0.28
AA Comp A-1	1.84	1.70	0.51	0.52	1.05	0.28
Mean	1.84	1.69	0.51	0.52	1.04	0.28
Theoretical g/serving for each AA	2.00	1.50	0.50	0.50	1.00	0.25
% Agreement Observed/ Theoretical*100	**92**	**113**	**101**	**104**	**104**	**111**

Results for **Formulation of Amino Acid Composition A-2** (compared to theoretical g per serving) are shown in **Table 15**. For glutamine, a mean mass per serving of 2.102 g was observed; compared to 2.00 g theoretical per serving, this gives a % agreement (or % label claim value) of 105.1%. For arginine, a mean mass per serving of 1.922 g was observed; compared to 1.5 g theoretical per serving, this gives a % agreement (or % label claim value) of 107.5%. For valine, a mean mass per serving of 0.536 g was observed; compared to 0.50 g theoretical per serving, this gives a % agreement (or % label claim value) of 107.5%. For isoleucine, a mean mass per serving of 0.531 g was observed; compared to 0.50 g theoretical per serving, this gives a % agreement (or % label claim value) of 106.2%. For leucine, a mean mass per serving of 1.058 g was observed; compared to 1.00 g theoretical per serving, this gives a % agreement (or % label claim value) of 105.8%. For N-acetylcysteine (NAC), a mean mass per serving of 0.153 g was observed; compared to 0.15 g theoretical per serving, this gives a % agreement (or % label claim value) of 101.7%. Overall, the amino acids and amino acid derivatives in the **Formulation of Amino Acid Composition A-2** had a range of mean % label claims of 101-107%. Individual samples had a range of % label claims of 98.3-108.8%.

Table 15. % Label Claim Results: Formulation of Amino Acid Composition A-2

	g of Individual Amino Acid/ per serving of Amino Acid Composition A-2					
Sample Name	**GLN**	**ARG**	**VAL**	**ILE**	**LEU**	**NAC**
AA Comp A-2	2.09	1.92	0.54	0.53	1.05	0.16
AA Comp A-2	2.10	1.90	0.53	0.53	1.06	0.15
AA Comp A-2	2.11	1.96	0.54	0.53	1.06	0.15
AA Comp A-2	2.11	1.90	0.54	0.53	1.06	0.15
Mean	2.102	1.922	0.536	0.531	1.058	0.153
Theoretical g/serving for each AA	2.00	1.50	0.50	0.50	1.00	0.15
% Agreement Observed/ Theoretical*100	**105.1**	**106.6**	**107.5**	**106.2**	**105.8**	**101.7**

Example 3: Pharmacokinetic characterization of the amino acid compositions

The amino acid compositions of the present disclosure were characterized in rodent and human subjects for their pharmacokinetic effects on amino acid concentrations in response to ingestion of the compositions.

Rat Pharmacokinetics

The pharmacokinetic effects of a formulation of **Amino Acid Composition A-1** were tested in rats. After an overnight fast, rats were given the formulation by oral gavage. Portal vein and jugular vein blood was collected just before the dose, and at 5, 15, 30, 60, 120, 240 and 360 minutes thereafter. Plasma concentration of amino acid levels were measured, and maximum concentration (C_{max}), time of maximum concentration (T_{max}), and half-life ($T_{1/2}$) were determined. Maximum concentration values are corrected for baseline endogenous amino acid levels. Results for rat PK studies are shown below in Tables 16-21.

Table 16. Leucine Rat PK - Formulation of Amino Acid Composition A-1

Dose (mg/kg)	C_{max} (μM)	T_{max} (h)	$T_{½}$ (h)
266	421	1.0	1.2

Table 17. Isoleucine Rat PK - Formulation of Amino Acid Composition A-1

Dose (mg/kg)	C_{max} (μM)	T_{max} (h)	$T_{½}$ (h)
133	176	0.6	1.0

Table 18. Valine Rat PK - Formulation of Amino Acid Composition A-1

Dose (mg/kg)	C_{max} (μM)	T_{max} (h)	$T_{½}$ (h)
133	323	0.9	1.5

Table 19. Arginine Rat PK - Formulation of Amino Acid Composition A-1

Dose (mg/kg)	C_{max} (μM)	T_{max} (h)	$T_{½}$ (h)

399	896	1.0	1. 1

Table 20. Glutamine Rat PK - Formulation of Amino Acid Composition A-1

Dose (mg/kg)	C_{max} (µM)	T_{max} (h)	$T_{½}$ (h)
531	300	1.8	4.1

Table 21. N-acetylcysteine Rat PK - Formulation of Amino Acid Composition A-1

Dose (mg/kg)	C_{max} (µM)	T_{max} (h)	$T_{½}$ (h)
66	34	0.9	0.8

Allometric scaling was assumed to convert rat mg/kg doses into human equivalent mg/kg doses. A comparison of these equivalent mg/kg doses and human gram doses gram (assuming bodyweight of 70 kg) is shown in **Table 22**.

Table 22. Amino Acid Doses: Comparison of Rat and Human

Dose	Leu	Ile	Val	Arg	Gln	NAC
Rat (mg/kg)	266	133	133	399	531	66
Human (mg/kg)	43	21	21	64	86	11
Human (g)	3	1.5	1.5	4.5	6	0.75

Human Pharmacokinetics

The impacts of orally administered **Formulation of Amino Acid Composition A-1** prepared according to Example 1 on amino acid pharmacokinetics was evaluated in six apparently healthy human subjects between the ages of 18 and 40. Changes in plasma concentrations of amino acids in response to ingestion of the **Formulation of Amino Acid Composition A-1** at two doses (High: 3 stick packs, ~18 g of amino acids; vs. Low: 1 stick pack, ~6 g of amino acids) were determined. Blood samples (3 mL) were collected after an initial baseline and in specific intervals thereafter [i.e., 0 (pre-administration), 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes]. Plasma concentration of amino acid levels were measured, and maximum concentration (C_{max}), time of maximum concentration (T_{max}), half-life ($T_{1/2}$) and total exposure (area under curve for plasma concentration time-courses of leucine, isoleucine, valine, arginine and glutamine) were determined. Maximum concentration and total exposure values are corrected for baseline endogenous amino acid levels. These results are shown in Table 23-27.

Table 23. Leucine Human PK - Formulation of Amino Acid Composition A-1

Dose	Dose (g)	C_{max} (μM)	T_{max} (h)	$T_{½}$ (h)	AUC_{last} (μM-h)
HIGH	3.0	294	0.8	1.1	471
LOW	1.0	117	0.8	1.3	153

Table 24. Isoleucine Human PK - Formulation of Amino Acid Composition A-1

Dose	Dose (g)	C_{max} (μM)	T_{max} (h)	$T_{½}$ (h)	AUC_{last} (μM-h)
HIGH	1.5	141	0.7	0.8	194
LOW	0.5	52	0.8	0.6	54

Table 25. Valine Human PK - Formulation of Amino Acid Composition A-1

Dose	Dose (g)	C_{max} (μM)	T_{max} (h)	$T_{½}$ (h)	AUC_{last} (μM-h)

HIGH	1.5	238	0.8	1.3	400
LOW	0.5	89	0.8	1.7	101

Table 26. Arginine Human PK - Formulation of Amino Acid Composition A-1

Dose	**Dose (g)**	**C_{max} (µM)**	**T_{max} (h)**	**$T_{½}$ (h)**	**AUC_{last} (µM-h)**
HIGH	4.5	177	0.8	1.6	311
LOW	1.5	69	0.8	1.3	111

Table 27. Glutamine Human PK - Formulation of Amino Acid Composition A-1

Dose	**Dose (g)**	**C_{max} (µM)**	**T_{max} (h)**	**$T_{½}$ (h)**	**AUC_{last} (µM-h)**
HIGH	6.0	190	0.9	2.9	332
LOW	2.0	103	1.1	3.0	186

Example 4: Therapeutic Amino Acid Composition A-1 treatment improves liver fibrosis in an animal model of chemically induced fibrosis

Amino Acid Composition A-1 was tested for its ability to affect liver fibrosis in a model of chemically induced liver fibrosis. A commonly used model of experimental hepatic fibrosis is induced chemically in mice using carbon tetrachloride; CCl_4 (Gideon Smith, Animal Models of Cutaneous and Hepatic Fibrosis; Progress in Molecular Biology and Translational Science, Vol. 105, pp. 371- 408). CCl_4 causes inflammation, hepatocyte damage, necrosis and fibrosis after 4 weeks of treatment and cirrhosis after 8 weeks. Liver fibrosis induced in mice by carbon tetrachloride (CCl_4) resembles important properties of human liver fibrosis including inflammation, regeneration and fiber formation.

Animals

Male BALB/c mice 7 to 8 weeks of age were used for this study. Animals were housed four per cage, kept on a standard 12 hr light cycle and given free access to water and standard mouse chow. Food and water were available ad libitum.

Procedure

Animals were dosed with 5% CCl_4 or vehicle intraperitoneally (IP) typically 3 days a week for 4 weeks. CCl4 was formulated weekly. 10 ml/kg of **Amino Acid Composition A-1** at 23 mg/ml, 76 mg/ml or 153 mg/ml was dosed by oral gavage twice daily. Animals were weighed twice weekly and blood was collected via retro-orbital sinus once per week for serum. After four weeks, blood was collected for serum isolation and mice were euthanized via cervical dislocation. Two lobes of liver were removed – the left lobe was placed in a tube containing 10% formalin for histopathology, while the right lobe was weighed and placed in a beadbeater tube containing 2.3 mm zirconia beads and 2x volume of 1:100 protease inhibitor (Sigma Aldrich, #P8340). Tissue samples were homogenized for 2 minutes in a beadbeater machine and immediately spun down at 3,000 rpm for 15 minutes at 4°C. Serum was analyzed for ALT/AST levels at weeks 2 and 4. Homogenized liver samples were further evaluated for Hydroxyproline (Hyp) content to identify formation of liver fibrosis.

Hydroxyproline (week 4)

Hydroxyproline (4-hydroxyproline, Hyp) is a common nonproteinogenic amino acid and is used as an indirect measure of the amount of collagen present, indicative of fibrosis. Hepatic Hyp content levels in CCl_4-treated animals were significantly higher than vehicle treated animals. Data are mean ± standard deviation (stdev); "Comp A-1": Amino Acid Composition A-1; *p<0.05 compared to vehicle control by unpaired T test. Raw data are shown in Table 28.

Table 28. Hepatic Hyp content level results

	Hydroxyproline				
	Vehicle/Sham	**Vehicle/CCL4**	**Comp A-1, 23mg/ml**	**Comp A-1, 76mg/ml**	**Comp A-1, 153mg/ml**
mean	**0.160**	**0.263***	**0.280**	**0.228**	**0.201**
stdev	0.067	0.107	0.104	0.124	0.057

AST levels and ALT levels

Aspartate transaminase (AST) and alanine transaminase (ALT) are commonly measured clinical biomarkers of liver health. Both AST and ALT levels were significantly elevated in CCl_4 administered animals for the entire duration of the study, suggesting that liver damage has occurred. Data are mean ± standard deviation (stdev); "Comp A-1": **Amino Acid Composition A-1;** p values are compared to vehicle/CCl4 control; by one-tailed T test; n.s. not significant. Raw data are shown in Tables 29 and 30.

Table 29. ALT level results

	Liver ALT				
	Vehicle/Sham	**Vehicle/CCL4**	**Comp A-1, 23mg/ml**	**Comp A-1, 76mg/ml**	**Comp A-1, 153mg/ml**
mean	**1608.4**	**4153.4**	**3694.9**	**3023.4**	**2992.7**
stdev	1099.5	1427.4	2106.4	1343.8	1674.2
			n.s.	p<0.05	p=0.0371

Table 30. AST level results

	Liver AST				
	Vehicle/Sham	**Vehicle/CCL4**	**Comp A-1, 23mg/ml**	**Comp A-1, 76mg/ml**	**Comp A-1, 153mg/ml**
mean	**155.8**	**933.6**	**879.2**	**554.7**	**680.4**
stdev	69.7	237.0	527.3	336.6	431.2
			n.s.	p<0.01	p=0.0394

SUMMARY

Treatment with **Amino Acid Composition A-1** resulted in reduction of chemically-induced fibrosis as indicated by reduced levels of hydroxyproline, a marker for collagen production, and in improvement of clinical biomarkers of liver damage as indicated by reduction in levels of liver enzymes ALT and AST (Tables 31-33).

Table 31. Hepatic Hyp content level results: raw data

Hydroxyproline				
Vehicle/Sham	Vehicle/CCL4	Comp A-1, 23mg/ml	Comp A-1, 76mg/ml	Comp A-1, 153mg/ml
0.122	0.241	0.246154	0.190323	0.248649
0.277	0.318	0.529578	0.174684	0.24
0.152	0.298	0.234783	0.226549	0.18
0.108	0.493	0.216393	0.169128	0.174233
0.123	0.2	0.294737	0.175887	0.133333
0.108	0.196	0.22439	0.107692	0.135758
0.232	0.183	0.305512	0.212389	0.210219
	0.177	0.393064	0.316191	0.150265
		0.272897	0.612174	0.231293
		0.192683	0.18018	0.308824
		0.164341	0.218803	
			0.203279	
			0.17971	

Table 32. ALT level results: raw data

Liver ALT				
Vehicle/Sham	Vehicle/CCL4	Comp A-1, 23mg/ml	Comp A-1, 76mg/ml	Comp A-1, 153mg/ml
685.0737	4963.448	1299.647	4325.237	2611.524
2623.343	578.7053	5069.816	4325.237	2150.594
1606.933	5235.278	5566.202	2304.237	1866.945
3805.214	2115.138	5188.003	1051.454	696.8924
779.6234	4384.331	3828.851	1488.746	1725.121
637.7988	4207.05	330.5123	4313.419	3722.483
1417.834	5471.652	649.6176	4112.501	5211.641
1311.466	5105.273	1441.471	2859.717	4797.986
	3462.471	5495.29	2564.249	1216.916
	4147.957	4892.536	5318.009	1796.033
	5436.196	5329.828	2836.079	5069.816
	3852.489	5247.097	2457.881	5046.179
	5034.36		1346.922	

Table 33. AST level results: raw data

Liver AST				
Vehicle/Sham	**Vehicle/CCL4**	**Comp A-1, 23mg/ml**	**Comp A-1, 76mg/ml**	**Comp A-1, 153mg/ml**
95.37346	908.3081	315.7015	703.1751	508.1721
57.38585	1050.129	928.5682	720.9027	335.9616
239.7263	877.918	1389.484	371.4167	379.0142
194.1412	660.1224	1047.596	262.5189	211.8688
123.231	599.3423	589.2123	267.5839	510.7046
102.971	675.3175	181.4787	819.6704	885.5156
237.1938	1470.525	285.3115	629.7324	1214.742
196.6737	1070.389	305.5715	414.4693	941.2307
	733.5651	1690.853	505.6396	252.3889
	976.6858	1100.779	1485.72	297.974
	1088.116	1232.469	356.2217	1437.602
	918.4382	1483.187	406.8718	1189.416
	1108.376		267.5839	

Example 5: Therapeutic treatment with Amino Acid Composition A-1 improves oral glucose tolerance in a pre-clinical animal model

Amino Acid Composition A-1 and metformin were tested for their ability to affect glucose tolerance in a genetically obese B6.Cg-Lep^{ob}/J (ob/ob) mouse model (Maida A, *et al.*, 2010, PMID: 20972533).

Model description

B6.Cg-Lep^{ob}/J (ob/ob) mice harbor a spontaneous mutation of leptin (Lep) gene. ob/ob mice exhibit hyperphagia, obesity, and metabolic syndrome/T2DM-like symptoms, *e.g.* hyperglycemia, hyperinsulinemia, and insulin resistance. ob/ob mice have impaired intestinal barrier function, gut microbial translocation, and an inflammatory, fibrogenic phenotype of hepatic stellate cells (Brun P *et al.*, 2004, PMID: 17023554). ob/ob mice develop skeletal muscle hypoplasia in quadriceps femoris, similar to the effect of aging in humans (Hamrick MW *et al.*, 2004, PMID: 15003785). ob/ob mice exhibit intolerance to glucose and insulin. Metformin lowers plasma glucose (Cool B, *et al.*, Cell Metab 2006, PMID: 16753576), liver triglyceride, and reverses NAFLD in ob/ob mice (Lin HZ *et al.*, 2000, PMID: 10973319; Cool B, *et al.*, Cell Metab 2006, PMID: 16753576). A single dose of metformin treatment reduces blood glucose and improves glucose tolerance (OGTT) in C57/BL6.

Experimental design

Eight-week-old male ob/ob mice were subjected to treatment of test articles (**Amino Acid Composition A-1** and metformin) followed by oral glucose tolerance test (OGTT) on Day 3. Mice were randomized by body weight and unfasted blood glucose on Day -1. Body weight was recorded daily in the morning before AM dosing on Day 1, Day 2, and Day 3. Test articles were dosed by oral gavage at 10 ml/kg. Dosage of a test article was calculated based on daily body weight. Treatment schedule and dose are listed in the following section (Table 34). AM doses were administered at 0700, and PM doses were administered at 1800. Oral glucose tolerance test (OGTT) was performed after 6-hour fasting on Day 3.

Table 34. Treatment schedule

Group	Test article	#	Dosing Schedule
Group 1	Vehicle	N= 5	Vehicle dosed on Day 1 and Day 2 at 0700 and 1800, and Day 3 at 0700 and 30 min before OGTT for a total of 6 doses.
Group 2	Metformin	N= 5	Metformin hydrochloride (450 mg/kg, QD PO at the beginning of dark cycle) dosed on Day -1, Day 1, and Day 2 at 1800, and at 30 min before OGTT on Day 3 for a total of 4 doses.
Group 3	**Amino Acid Composition A-1**	N= 5	**Amino Acid Composition A-1** (1500 mg/kg, BID PO at 0700 and 1800) dosed on Day 1 and Day 2 at 0700 and 1800, and Day 3 at 0700 and 30 min before OGTT for a total of 6 doses.
Group 4	**Amino Acid Composition A-1**	N= 5	**Amino Acid Composition A-1** (3000 mg/kg, BID PO at 0700 and 1800) dosed on Day 1 and Day 2 at 0700 and 1800, and Day 3 at 0700 and 30 min before OGTT for a total of 6 doses.

Baseline glucose and biochemistry (insulin, triglyceride and cholesterol)

Mice were fasted for 6 hours prior to OGTT test. Food was removed at 0700 hours on Day 3; water was provided during fasting. Blood samples were collected from tail snip or facial puncture at -30 min (relative to OGTT) into K_2EDTA tubes for baseline glucose and blood biochemistry (insulin, triglyceride, and cholesterol). Blood glucose was measured by a glucometer (SDI StatStrip Xpress or equivalent). Plasma was collected in K_2EDTA and saved at -80°C.

Oral glucose tolerance test (OGTT)

Mice were bled for baseline glucose and plasma at -30 min. Test articles were then dosed by oral gavage at -30 min. Glucose was administered *per os* (P.O.) at a dosage of 2.0 g/kg body weight. Blood glucose levels were measured at 0 min immediately prior to glucose injection and then at 15, 30, 60, 120 and 240 minutes thereafter (shown as 0.25, 0.5, 1, 2, and 4 hours in Table 35 below).

Results are shown in Table 35. Data are mean ± standard deviation (stdev). (p values by Dunnett's multiple comparisons: **p<0.005 compared to vehicle control; ***p<0.001 compared to vehicle control; ****p<0.0005 compared to vehicle control.)

Results

Table 35. OGTT results: Mean Blood glucose levels (mg/dl) and standard deviations (stdev)

	Vehicle		**Amino Acid Comp A-1, 1500 mg/kg**		**Amino Acid Comp A-1, 3000 mg/kg**		**Metformin**	
Timepoint (hours)	mean blood glucose level (mg/dl)	stdev	mean blood glucose level (mg/dl)	stdev	mean blood glucose level (mg/dl)	stdev	mean blood glucose level (mg/dl)	stdev
-1	**241.8**	108.3	**245.6**	89.4	**229.6**	78.2	**196.4**	59.8
0	**282.6**	47.0	**374.6**	97.6	**303.0**	77.1	**199.4**	62.6
0.25	**655.0**	107.2	**575.6**	73.8	**456.2****	36.6	**353.6******	73.6
0.5	**640.6**	92.6	**555.2**	84.0	**513.0**	47.9	**390.2*****	99.5
1	**378.0**	111.1	**386.6**	27.5	**316.4**	86.1	**317.6**	116.9
2	**236.6**	54.8	**243.5**	18.4	**230.0**	101.1	**158.2**	44.0
4	**197.8**	53.3	**214.8**	56.8	**179.8**	81.3	**109.4**	29.0

SUMMARY

Treatment with **Amino Acid Composition A-1** resulted in improvement of oral glucose tolerance, as indicated by improved blood glucose clearance upon oral glucose loading. In addition, 3-day treatment with **Amino Acid Composition A-1** did not alter baseline blood glucose in ob/ob mice (Table 36).

Table 36. OGTT results: Blood glucose levels (mg/dl) raw data

Timepoint (hours)	**Vehicle**				
-1	211	141	211	219	427
0	239	256	273	284	361
0.25	741	676	514	578	766
0.5	551	621	604	630	797
1	305	317	327	369	572
2	182	243	230	203	325
4	146	203	167	188	285

Timepoint (hours)	**Amino Acid Comp A-1, 1500 mg/kg**				
-1	224	220	190	191	403
0	331	548	347	316	331
0.25	526	702	580	531	539
0.5	532	419	621	587	617
1	431	365	367	395	375
2	246		243	220	265
4	192		193	175	299

Timepoint (hours)	**Amino Acid Comp A-1, 3000 mg/kg**				
-1	137	294	179	214	324
0	242	359	203	329	382
0.25	412	490	438	443	498
0.5	513	482	467	512	591
1	240	351	220	342	429
2	161	334	148	160	347
4	120	235	110	139	295

Timepoint (hours)	**Metformin**				
-1	265	248	183	120	166
0	274	234	220	129	140

0.25	387	397	427	310	247
0.5	462	439	482	307	261
1	365	399	431	239	154
2	154	183	219	124	111
4	118	93	155	101	80

Example 6: Therapeutic treatment of NAFLD, NASH, and HCC with Amino Acid Composition A-1 in a pre-clinical animal model

Amino Acid Composition A-1 and Obeticholic acid (6α-ethyl-chenodeoxycholic acid; "OCA") were tested for their ability to treat NASH in the STAM™ model (Stelic Institute & Co., Tokyo, Japan; Saito K. *et al.*, 2015 Sci Rep 5: 12466). Two additional groups of normal C57BL/6 mice fed standard chow and vehicle treated STAM™ mice were included as controls. All animals receiving treatment or vehicle were treated starting at 6 weeks until 9 weeks of age. Compounds were administered via oral gavage, with a dose volume of 10 ml/kg. **Amino Acid Composition A-1** was administered twice daily at a dose of 1500 mg/kg, and OCA was administered once daily at a dose of 30 mg/kg.

STAM™ mouse model description

STAM™ is a model for non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC), developed by SMC Laboratories, Inc. and created by the combination of chemical and dietary interventions using C57BL/6 mice (Saito K. *et al.*, 2015 Sci Rep 5: 12466). Mice are treated with a low dose of streptozotocin at birth and fed a high fat diet starting at 4 weeks. Evidence of fatty liver is present by 5 weeks, followed by NASH by 7 weeks and fibrosis by 9 weeks.

Induction of NASH

NASH was induced in 53 male mice by a single subcutaneous injection of 200 μg streptozotocin (STZ, Sigma-Aldrich, USA) solution 2 days after birth and feeding with high fat diet (HFD, 57 kcal% fat, Cat# HFD32, CLEA Japan, Japan) after 4 weeks of age.

Route of drug administration, preparation of dosing solutions and treatment doses

Amino Acid Composition A-1, OCA and Vehicle (described below) were administered by oral route in a volume of 10 mL/kg. **Amino Acid Composition A-1** was solubilized in deionized water to 150 mg/ml (10X). OCA (Advanced ChemBlocks Inc.) was resuspended in 0.5% methycellulose in water to 3 mg/ml (10X). **Amino Acid Composition A-1** was administered at a dose of 1500 mg/kg twice daily (9 am and 7 pm). OCA was administered at a dose of 30 mg/kg once daily (9 am).

Histological analyses

Liver samples from mice in Group 2 (Vehicle), 3 (**Amino Acid Composition A-1**) and 4 (OCA) were used for the following assays. For HE staining, sections were cut from paraffin blocks of liver tissue prefixed in Bouin's solution and stained with Lillie-Mayer's Hematoxylin (Muto Pure Chemicals Co., Ltd., Japan) and eosin solution (Wako Pure Chemical Industries). NAFLD Activity score (NAS) was calculated according to the criteria of Kleiner (Kleiner D.E. *et al.*, *Hepatology*, 2005;41:1313).

EXPERIMENTAL DESIGN

Study groups

Group 1: STZ: Ten neonatal STZ-primed mice were fed with a normal diet *ad libitum* without any treatment until 9 weeks of age.

Group 2: Vehicle: Ten NASH mice were orally administered vehicle (10% phosphate buffered saline, pH 7.2) in a volume of 10 mL/kg twice daily (9 am and 7 pm) from 6 to 9 weeks of age.

Group 3: **Amino Acid Composition A-1**: Ten NASH mice were orally administered water for irrigation supplemented with **Amino Acid Composition A-1** at a dose of 1500 mg/kg twice daily (9 am and 7 pm) from 6 to 9 weeks of age.

Group 4: OCA: Ten NASH mice were orally administered 0.5% methylcellulose supplemented with OCA at a dose of 30 mg/kg once daily (9 am) from 6 to 9 weeks of age.

Group 5: Normal: Ten normal mice were fed with a normal diet *ad libitum* without any treatment until 9 weeks of age.

Group 6: HFD: Ten normal mice were fed with a high fat diet *ad libitum* without any treatment until 9 weeks of age.

Histological analysis results: HE staining, NAFLD activity score and α-smooth muscle actin staining

Non-alcoholic fatty liver disease activity score results

The non-alcoholic fatty liver disease (NAFLD) activity score was assessed via histological analysis and grading of H&E stained liver sections from each animal. This score is the sum of three individual scores that grade the degree of steatosis (0-3), inflammation (0-2), and hepatocyte ballooning (0-2). All tissues were graded using the scoring criteria of Kleiner *et al.* (Kleiner *et al.* Hepatology. 2005; 41(6): 1313-21). Results are shown in **Table 37**. Data are mean ± standard deviation (stdev). Normal C57BL/6 mice fed standard chow had a mean score of 0 +/- 0. Vehicle treated STAM™ mice had a mean score of 4.7 +/- 0.67. **Amino Acid Composition A-1** treated mice had a mean score of 3.1 +/- 0.74. OCA treated mice had a mean score of 2.9 +/- 0.74. Both **Amino Acid Composition A-1** and OCA were statistically different from vehicle for NAFLD Activity Score when compared using Dunnett's multiple comparisons test (**Amino Acid Composition A-1** p=0.0001, OCA p=0.0001).

Similarly, **Amino Acid Composition A-1** treated mice showed a mean ballooning score of 0.4 +/- 0.52, compared to a mean ballooning score for vehicle treated STAM™ mice of 1.6 +/- 0.52, and a mean ballooning score for OCA treated mice of 0.3 +/- 0.48. Both **Amino Acid Composition A-1** and OCA were statistically different from vehicle for ballooning score when compared using Dunnett's multiple comparisons test (**Amino Acid Composition A-1** p=0.0001, OCA p=0.0001). Raw data are shown in Tables 37-40.

Table 37. NAFLD Activity Score

NAFLD Activity Score (NAS)				
Condition	Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
Mean	**0**	**4.7**	**3.1**	**2.9**
stdev	0	0.67	0.74	0.74

Table 38. NAFLD Activity: Steatosis Score

	Steatosis			
	Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
Mean	**0**	**1**	**0.9**	**0.8**
stdev	0	0.00	0.32	0.42

Table 39. NAFLD Activity: Inflammation Score

	Inflammation			
	Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
Mean	**0**	**2.1**	**1.8**	**1.8**
stdev	0	0.32	0.63	0.79

Table 40. NAFLD Activity: Ballooning Score

	Ballooning			
	Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
Mean	**0**	**1.6**	**0.4**	**0.3**
stdev	0	0.52	0.52	0.48

Fibrosis: Sirius Red staining results

Fibrosis was assessed by analysis of Sirius red positively stained cell area from stained liver sections from each animal. Images were quantified using the percent of positively stained area was used as a measure of fibrosis. Results of this analysis are shown in **Table 39.** Data are mean ± standard deviation (stdev). Normal C57BL/6 mice fed standard chow had a mean positive area of 0.286 +/- 0.09. Vehicle treated STAM™ mice had a mean positive area of 1.1 +/- 0.26. **Amino Acid Composition A-1** treated mice had a mean positive area of 0.828 +/- 0.33. OCA treated mice had a mean score of 0.776 +/- 0.25. **Amino Acid Composition A-1** and OCA were statistically different from vehicle when compared using Dunnett's multiple comparisons test (**Amino Acid Composition A-1** p=0.00494, OCA p<0.016). Raw data are shown in Table 41.

Table 41. Fibrosis (mean positively stained area, Sirius red)

Condition	Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
Mean	**0.286**	**1.1**	**0.828**	**0.776**
stdev	0.09	0.26	0.33	0.25

Similarly to the statistically significant improvement in the NAFLD activity score, ballooning, and fibrosis in the STAM mouse model after treatment with **Amino Acid Composition A-1** (FIG. 1A), a statistically significant improvement in the NAFLD activity score, ballooning, and fibrosis was determined in the high-fat, high fructose and cholesterol diet (HFFC) mouse model after treatment with **Amino Acid Composition A-1** (FIG. 1B).

α-Smooth muscle actin (α-SMA) staining results

Liver sections of all mice were stained for the marker α-smooth muscle actin (αSMA) to identify activated hepatic stellate cells. Images were quantified using the percent of positively stained area was used as a measure of stellate cell activation. Results are shown in **Table 42**. Data are mean ± standard deviation (stdev); p values are compared to vehicle-treated STAM mice control; by one-tailed T test.

Normal C57BL/6 mice fed standard chow had a mean positive area of 0.682 +/- 0.26. Vehicle treated STAM™ mice had a mean positive area of 2.128 +/- 0.50. **Amino Acid Composition A-1** treated mice had a mean positive area of 1.657 +/- 0.84. OCA treated mice had a mean score of 1.562 +/- 0.31.

Table 42. Activated hepatic stellate cells (mean positively stained area, α-smooth muscle actin)

Condition	Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
Mean	**0.682**	**2.128**	**1.657**	**1.562**
stdev	0.26	0.50	0.84	0.31
			p=0.073	p<0.05

SUMMARY

Treatment with **Amino Acid Composition A-1** significantly reduced NASH severity to levels equivalent to Farnesoid X Receptor (FXR) inhibition by OCA (which is currently under clinical investigation by Intercept Pharmaceuticals, Inc. for treatment of NASH), as indicated by significant reduction in NAFLD Activity Score (NAS) (mean NAS: 3.1 +/- 0.74 for **Amino Acid Composition A-1** vs. vehicle treated STAM™ mice mean score of 4.7 +/- 0.67, compared to OCA treated mice mean score of 2.9 +/- 0.74), and development of fibrosis as indicated by the downregulation of hepatic stellate cell activation (mean αSMA positively stained area: 1.657 +/- 0.84 for **Amino Acid Composition A-1** vs. vehicle treated STAM™ mice mean area of 2.128 +/- 0.50, compared to OCA treated mice mean area of 1.562 +/- 0.31).

Table 43. NAFLD Activity Score: raw data

Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
0	6	3	4
0	5	4	2
0	5	4	2
0	4	3	4
0	5	2	3
0	5	2	3
0	4	3	2
0	4	3	3
0	4	3	3
0	5	4	3

Table 44. NAFLD Activity: Steatosis Score: raw data

Steatosis			
Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
0	1	1	1
0	1	1	1
0	1	1	1
0	1	1	1
0	1	0	1
0	1	1	0

0	1	1	1
0	1	1	0
0	1	1	1
0	1	1	1

Table 45. NAFLD Activity: Inflammation Score: raw data

Inflammation			
Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
0	3	1	2
0	2	2	1
0	2	2	1
0	2	2	2
0	2	1	2
0	2	1	3
0	2	2	1
0	2	2	3
0	2	2	2
0	2	3	1

Table 46. NAFLD Activity: Ballooning Score: raw data

Ballooning			
Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
0	2	1	1
0	2	1	0
0	2	1	0
0	1	0	1
0	2	1	0
0	2	0	0
0	1	0	0
0	1	0	0
0	1	0	0
0	2	0	1

Table 47. Fibrosis (mean positively stained area, Sirius red): raw data

Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
0.26	0.79	1.07	0.36
0.35	1.43	0.58	0.56
0.19	1.44	0.48	1.1
0.31	1.36	0.58	1.19
0.19	1.04	1.07	0.89
0.36	0.75	0.34	0.91
0.24	1.07	0.86	0.66
0.37	1.13	1.43	0.72
0.18	0.83	0.96	0.68
0.41	1.16	0.91	0.69

Table 48. Activated hepatic stellate cells (mean positively stained area, α-smooth muscle actin): raw data

Normal C57BL/6 mice	Vehicle-treated STAM mice	**Amino Acid Composition A-1** treated STAM mice	OCA treated STAM mice
0.47	2.16	0.81	1.46
0.59	2.77	1.35	1.51
1.13	2.21	1.3	1.49
0.52	1.5	3.03	1.17
0.75	2.87	2.04	1.49
0.46	1.93	0.97	1.5
0.37	1.6	3.08	1.13
0.85	1.46	1.91	2.03
0.62	2.36	1.15	1.87
1.06	2.42	0.93	1.97

Example 7: Reduction of fibrogenic gene expression in hepatic stellate cells treated with an amino acid composition

Hepatic stellate cells in a healthy liver are in the space of Disse, between the hepatocytes and liver sinusoidal endothelial cells. In response to liver injury hepatic stellate cells become activated, proliferative and contractile, increase production of αSMA, secretion of type I and III collagens and specific MMP and TIMP proteins. LX-2 cells were selected as a model of activated hepatic stellate cells and used to test whether specific amino acid compositions would reduce fibrogenic gene expression induced with TGFβ1.

LX-2 hepatic stellate cells (Millipore) were seeded on day 0 at 1.67E4 cells per well in collagen I coated 96-well microplates (ThermoFisher) in Dulbecco's Modified Eagle Medium (DMEM, Corning) supplemented with 2% heat inactivated fetal bovine serum (HI-FBS, HyClone) and 0.2% Primocin (InVivoGen) and incubated overnight at 37°C, 5% CO2. On day 1, cells were washed twice with 150 µL per well DPBS (Gibco) and replaced with amino acid free DMEM (US Biologicals) containing a defined custom amino acid concentration based on the mean physiological concentrations in blood based on values published in the Human Metabolome Database (1,2,3), with 25 mM Glucose, 1 mM Sodium Pyruvate and a dose curve of defined amino acid compositions LIVRQ+N-Acetylcysteine, LIVRQ, RQ+N-Acetylcysteine, N-acetylcysteine, LIV at 40X the concentration present in the basal HMDB (Human Metabolome Database (Wishart DS, Tzur D, Knox C, et al., *HMDB: the Human Metabolome Database.* Nucleic Acids Res. 2007 Jan;35(Database issue):D521-6. 17202168)) derived amino acid concentrations or individually with leucine, isoleucine, valine, arginine, glutamine or cysteine at 50X the HMDB derived concentrations. Combinations containing N-acetylcysteine were dosed with 10 mM. Cells were pretreated for 6 hours at 37°C, 5% CO2. After pretreatment, TGFβ1 (R&D Systems) or vehicle was spiked into each well for a final concentration of 5 ng/mL and cells were incubated under this stimulus for a further 12 hours at 37°C, 5%CO2.

After 12 hour incubation, RNA was prepared as described using the FastLane Cell Multiplex Kit (Qiagen) as described in the manufacturers protocol. Two microliters of cell lysate was utilized in subsequent qRT-PCR experiments using the FastLane Cell Multiplex Kit at a reduced final qPCR reaction volume of 20 µL. Quantitative PCR was conducted on lysates to determine collagen-1a1 expression normalized to β-actin housekeeping expression using the ΔΔCt method using TaqMan primer probes (Integrated DNA Technologies: Col1A1, Hs.PT.58.15517795; Actb, Hs.PT.39a.22214847; Acta2, Hs.PT.56a.24853961; Timp2, Hs.PT.58.14780594).

Results:

Table 49 shows the Col1a1, Acta2, and Timp2 gene expression in LX-2 cells treated with amino acid combinations compared to vehicle with or without TGFβ1 stimulus. LIVRQ+N-Acetylcysteine, LIVRQ, RQ+N-Acetylcysteine, and N-acetylcysteine reduced Col1a1 expression and Timp2 expression. LIVRQ+N-acetylcysteine shows the largest reduction of Col1a1, Acta2, and Timp2 gene expression. LIVRQ-N-acetylcysteine reduces Acta2 expression significantly greater than N-Acetylcysteine alone, RQ+N-acetylcysteine, and LIV. LIVRQ+N-acetylcysteine reduces Timp2 expression significantly greater than any of the other combinations (Table 49).

Table 49.

		Col1a1			Acta2			Timp2		
TGFβ1	Amino Acid Supplement	Mean	Std. Deviation	Number of values	Mean	Std. Deviation	Number of values	Mean	Std. Deviation	Number of values
Yes	Vehicle	2.861	0.3151	4	0.801	0.1149	4	1.658	0.2791	4
No	Vehicle	1.042	0.3102	4	1.006	0.1190	4	1.022	0.2400	4
Yes	LIVRQNAC	1.267	0.4106	4	0.292	0.0969	4	0.535	0.0306	4
Yes	LIVRQ	1.787	0.2926	4	0.267	0.0637	4	0.975	0.2006	4
Yes	RQNAC	1.664	0.3320	4	0.487	0.1042	4	0.897	0.1932	4
Yes	NAC	1.659	0.4695	4	0.647	0.1097	4	1.076	0.0681	4
Yes	LIV	2.831	0.3404	3	0.793	0.0812	4	1.927	0.0944	4

Table 50 shows the Col1a1 expression of individual amino acids with or without TGFβ1 stimulus at 1X or 50X the HMDB derived amino acid concentration. Individually, only cysteine showed a significant decrease in Col1a1 expression at 50X.

Table 50.

		Col1a1		
TGFβ1	Amino Acid Supplement	Mean	Std. Deviation	Number of values
No	Vehicle	1.015	0.1832	8
Yes	1X CYS	2.491	0.1588	4
Yes	50X CYS	1.695	0.3310	4
Yes	1X ILE	2.020	0.1451	4
Yes	50X ILE	2.028	0.3667	4
Yes	1X LEU	1.901	0.3360	4

Yes	50X LEU	2.372	0.4153	4
Yes	1X VAL	2.093	0.2157	4
Yes	50X VAL	2.203	0.5762	4

		Col1a1		
TGFβ1	Amino Acid Supplement	Mean	Std. Deviation	Number of values
No	Vehicle	1.010	0.1510	8
Yes	1X ARG	1.620	0.6691	4
Yes	50X ARG	1.970	0.7740	4

		Col1a1		
TGFβ1	Amino Acid Supplement	Mean	Std. Deviation	Number of values
No	Vehicle	1.012	0.1681	8
Yes	1X GLN	2.340	0.7069	4
Yes	50X GLN	2.194	0.3359	4

Example 8: Reduction in hepatocyte inflammation after treatment with an amino acid composition

The ability of amino acids to influence hepatocyte inflammation was assessed using HepG2 Hepatocellular Carcinoma cells stably expressing NF-kB luciferase reporter system (Signosis, Inc.). HepG2 cells were seeded on day 0 in 4.5e4 in a 96-well microplates (ThermoFisher) in Dulbecco's Modified Eagle Medium (DMEM, Corning) supplemented with 0.1% heat inactivated fetal bovine serum (HI-FBS, HyClone) and 0.2% Primocin (InVivoGen) and incubated overnight at 37°C, 5% CO_2. On day 1, cells were washed once with 150 µL per well DPBS (Gibco) and replaced with amino acid free DMEM (US Biologicals) containing a defined custom amino acid concentration based on the mean physiological concentrations in blood based on values published in the Human Metabolome Database (Wishart DS, Tzur D, Knox C, et al., *HMDB: the Human Metabolome Database.* Nucleic Acids Res. 2007 Jan;35(Database issue):D521-6. 17202168), with 25 mM Glucose, 1 mM Sodium Pyruvate and a dose curve of defined amino acid compositions (i.e. vehicle, LIVRQ+N-acetylcysteine, LIVRQ, RQ+N-acetylcysteine, N-acetylcysteine alone, LIV or individually with Leucine, Isoleucine, Valine, Arginine, Glutamine, and Cysteine) at 50X (Table 51). Cells were pretreated in the defined media for 12 hours at 37°C, 5% CO_2. After pretreatment, TNFα (R&DSystems) or vehicle was spiked into each well for a final concentration of 100 pM and cells were incubated under this stimulus for an additional 6 hours at 37°C, 5%CO_2. After 12-hour incubation, cells were washed 1x in 150ul cold PBS and lysed using Passive Lysis Buffer and luciferase assay was performed according to manufacturer's protocol (Signosis). Firefly luciferase activity was assessed using a Bio-Tek SynergyH4 plater reader and luminometer (Sitcheran R*, Comb WC, Cogswell PC, Baldwin AS*. Essential role for epidermal growth factor receptor in glutamate receptor signaling to NF-kappaB. *Mol Cell Biol.* (2008) Aug;28(16):5061-70. Epub 2008 Jun 9).

TNFα -stimulated NF-kB activity was unaffected by treating cells in 50x Leucine, Isoleucine, Valine, Arginine, and Glutamine, relative to the 1x Plasma amino acid baseline media. Pretreating cells in 50x Cysteine did result in a significant blunting of TNFα-induced NF-kB activity. Combinatorial treatments with the single amino acids did have varying effects on the NF-kB reporter activity, but importantly, the combination of all 6 amino acids together (LIVRQNAC) resulted in the most significant inhibition of TNFα induced NF-kB activity in liver cells (Table 51).

Table 51.

TNFα	Amino Acid Supplement	NF-kB Reporter Activity		
		Mean	Std. Deviation	Number of values
100pM	Vehicle (1x AA)	8865.50	333.05	2
100pM	LIVRQNAC	3960.50	678.12	2
100pM	LIVRQ	5685.00	1453.81	2
100pM	RQNAC	5618.00	926.31	2
100pM	NAC	6852.00	1023.89	2
100pM	LIV	5911.00	422.85	2
100pM	1x L	5811.00	134.35	2
100pM	50x L	6070.50	58.69	2
100pM	1x I	8129.50	713.47	2
100pM	50x I	8937.50	17.68	2
100pM	1x V	7255.50	557.91	2
100pM	50x V	5992.00	644.88	2
100pM	1x R	10170.50	140.71	2
100pM	50xR	9760.00	1083.29	2

100pM	1x Glu	8201.00	2091.62	2
100pM	50x Glu	7313.50	1054.30	2
100pM	1x Cys	9968.50	1614.33	2
100pM	50x Cys	6820.50	23.34	2

Example 9: Treatment with an Amino Acid Composition ameliorates NASH progression in two rodent models by impacting lipid metabolism, inflammation, and fibrosis

The amino acid composition is formulated to simultaneously target multiple mechanisms of disease pathology to safely and effectively treat NASH (Table 52). As described herein, the efficacy of the amino acid composition was studied in two established mouse models of NASH to determine the effect of the amino acid composition on signs and symptoms associated with NASH and related disorders (FIG. 2).

Table 52. Exemplary amino acid components of the amino acid composition.

Amino acid	wt. ratio	wt. %	g / packet	g dose #1	g dose #2
Leucine	1	16.78	1.00 g	2 g	4 g
Isoleucine	0.5	8.39	0.50 g	1 g	2 g
Valine	0.5	8.39	0.50 g	1 g	2 g
Arginine HCl	1.81	30.37	1.81 g	3.62 g	7.24 g
Glutamine	2	33.56	2.00 g	4 g	8 g
N-acetylcysteine	0.15	2.52	0.15 g	0.3 g	0.6 g
Total amino acids			5.96 g	~12 g	~24 g

Animal studies

The STAM^{TM} mouse is a model for non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC), developed by SMC Laboratories, Inc. Evidence of fatty liver is present by 5 weeks of age, followed by NASH by 7 weeks of age, and fibrosis by 9 weeks of age. Male STAM mice were generated in C57BL/6 mice, which received a low dose streptozotocin 2 days after birth and were fed a high fat diet (57% kcal fat, HFD32, CLEA Japan, Inc.) starting at 4 weeks old (Saito K. et al., 2015 Sci Rep 5: 12466; hereby incorporated by reference in its entirety). The amino acid composition was administered to STAM mice at a dose of 1.6 m/kg twice daily for 3 weeks starting at 6 weeks of age. One group of vehicle treated

STAM mice was included as a control. Unfasted mice were euthanized at 9 weeks old. Plasma and liver samples were harvested for further analysis (FIG. 3).

The FATZO™ mouse is an inbred, polygenic model of obesity, metabolic syndrome, and NASH, developed by Crown Bioscience, Inc (Peterson RG. Et al., 2017 PLoS One; hereby incorporated by reference in its entirety). Male FATZO mice were fed a high fat, fructose, and cholesterol (HFFC) diet (40% kcal fat, D12079B, Research Diets, Inc. and 5% fructose in drinking water) starting at 6 weeks old to induce NAFLD and NASH. Evidence of fatty liver is present by 4 weeks post induction, followed by NASH by 16 weeks post induction and fibrosis by 20 weeks of induction. The designed amino acid composition was administered at a dose of 3.0 g/kg twice daily for 4 weeks starting at 16 weeks post induction (FIG. 3). One group of vehicle treated FATZO mice was included as control. Unfasted mice were euthanized at 20 weeks post-induction. Plasma and liver samples were harvested for further analysis.

Histological Analysis

The Aperio ScanScope CS whole slide digital imaging system (Vista, CA) was used for imaging in H&E, Picric Sirius Red, SMA, F4/80. Images were captured from whole slides.

The livers were evaluated by veterinary pathologists blind to sample ID using the NASH Clinical Research Network (CRN) liver histological scoring system (Kleiner DE, et al., 2015, hereby incorporated by reference in its entirety). The NASH CRN Scoring System assesses progression of steatosis, lobular inflammation, hepatocyte ballooning, degeneration, and fibrosis. One cross section of liver for each case was analyzed with the NASH score system. Steatosis, lobular inflammation, and fibrosis progression was assessed on a 0-3 scale. Ballooning degeneration was assessed on a 0-2 scale.

The Positive Pixel Count algorithm of the Aperio Automatic Image Quantitation was used to quantify the percentage of a specific stain present in a scanned slide image. A range of color (range of hues and saturation) and three intensity ranges (weak, positive, and strong) were masked and evaluated. The algorithm counted the number and intensity-sum in each intensity range, along with three additional quantities: average intensity, ratio of strong/total number, and average intensity of weak positive pixels.

A specific positive pixel algorithm was used for imaging the Sirius Red and Oil Red O liver sections. The positive pixel algorithm was modified to distinguish between the orange and blue colors. Alterations from the normal “hue value” (0.1 to 0.96) and “color saturation” (0.04 to 0.29), were made for the Sirius Red evaluation. Vasculature and artifacts were excluded from analysis.

Liver Triglyceride and Cholesterol Measurement

Liver total lipid-extracts were obtained by Folch’s method (Folch J. et al., J. Biol. Chem.1957;226: 497; hereby incorporated by reference in its entirety). Liver samples were homogenized in chloroform-methanol (2:1, v/v) and incubated overnight at room temperature. After washing with chloroform-methanol-water (8:4:3, v/v/v), the extracts were evaporated to dryness, and dissolved in isopropanol. Liver triglyceride and cholesterol contents were measured by the Triglyceride E-test and Cholesterol E-test, respectively.

Gene Expression Analysis

Liver RNA samples were converted into cDNA libraries using the Illumina TruSeq Stranded mRNA sample preparation kit (Illumina # RS-122-2103). Transcriptome were analyzed at Q2 Solutions (Morrisville, NC). RNA Seq data were normalized and analyzed using Ingenuity Pathway Analysis (QIAGEN Bioinformatics). Mouse liver gene expression at the pathway level was focused on because it is translatable to human NAFLD (Teufel A, et al., Gastroenterology, 2016, hereby incorporated by reference in its entirety).

Metabolite Analysis

Metabolic profiling based on both capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) and LC-TOFMS platforms was performed at Human Metabolome Technologies (Yamagata, Japan). Metabolites in the samples were identified by comparing the migration time and m/z ratio with authentic standards and quantified by comparing their peak areas with those of authentic standards.

Liver Cytokine/Chemokine Measurement

The levels of IL-1b, MCP-1, and MIP-1 protein in liver were quantified using the multiplex ELISA Assay (Meso Scale Discovery, Rockville, Maryland).

The Amino Acid Composition Improves Ballooning and Fibrosis in Both STAM and FATZO mice

Treatment with the amino acid composition significantly reduced NAFLD activity scores (NAS) in both STAM and FATZO mice (FIG. 4A). Treatment with the amino acid composition also significantly decreased hepatocyte ballooning in STAM mice (FIG. 4B). Scores of steatosis and inflammation were not changed according to histological measures by treatment of STAM mice with the amino acid composition. The Sirius Red-positive, fibrosis area was significantly lowered by treating the STAM mice with the amino acid composition, while the Oil Red O area was not changed by treating the STAM mice with the amino acid composition (FIG. 4C). Liver triglyceride and cholesterol levels were not changed.

Treatment with the amino acid composition also significantly decreased hepatocyte ballooning in FATZO mice (FIG. 4D). Scores of steatosis and inflammation as well as liver triglyceride and cholesterol levels were not changed in the FATZO mice treated with the amino acid composition treatment. The Sirius Red-positive, fibrosis area was significantly lowered by treatment of the FATZO mice with the amino acid composition, while the Oil Red O area was not changed by treatment of the FATZO mice with the amino acid composition treatment (FIG. 4E).

The Amino Acid Composition Enhances Fatty Acid Oxidation

NAFLD is characterized by hepatic lipid accumulation. Liver triglyceride is attributable to a precise balance between acquisition by de novo lipogenesis and uptake of non-esterified fatty acids from the plasma, versus disposal by fatty acid oxidation and by the secretion of triglyceride-rich lipoproteins (Kawano Y, Cohen DE, J Gastroenterol. 2013, hereby incorporated by reference in its entirety). Compared to control mice, STAM mice had higher liver unsaturated fatty acids, which were reduced by treatment with the amino acid composition (FIG. 5A and Table 53). Liver acylcarnitines in STAM mice were increased by treatment with the amino acid composition, suggesting enhanced fatty acid beta-oxidation (FIG. 5B and Table 53).

Table 53. P-values and fold changes for liver acylcarnitine and unsaturated fatty acids following treatment of STAM mice with the amino acid composition (treated) compared to control.

Lipid	KEGG ID	HMDB ID	Control p-val	Control fold change	Treated p-val	Treated fold change
AC(13:1)	No ID	No ID		-1.61	7.94E-02	1.32
FA(14:3)	No ID	No ID	5.17E-03	1.69	4.83E-01	-1.24
FA(20:3)	No ID	No ID	6.97E-06	18.29	1.35E-01	-2.17
FA(22:4)	No ID	No ID	7.12E-07	34.79	3.15E-01	-1.79
FA(22:5)-1	No ID	No ID	2.39E-02	3.31	1.19E-01	-1.80
FA(22:5)-2	No ID	No ID	2.11E-04	3.03	1.69E-02	-1.92
Linoleic acid	C01595	HMDB00673	9.90E-04	3.18	1.68E-02	-1.77
Linolenic acid	C06427	HMDB01388	3.57E-05	35.88	4.08E-02	-1.99
Oleic acid	C00712	HMDB00207	1.95E-04	18.05	3.67E-02	-1.88
Palmitoleic acid	C08362	HMDB03229	9.84E-05	2.69	2.70E-02	-1.48

Differential gene expression patterns in the liver impacted by treatment with the amino acid composition were interpreted in the context of the upstream regulator systems biology knowledgebase framework developed by Ingenuity Pathway Analysis. Computed z-scores indicated that the gene expression patterns are consistent with activation of ACOX1, which encodes peroxisomal fatty acid oxidation, as an upstream regulator (FIG. 6 and Table 54).

Table 54. P-values and fold changes for gene expression associated with the ACOX1 pathway following treatment of STAM mice with the amino acid composition (treated) compared to control.

gene	IPA_upstream _regulator	ACOX1 _path	IPA_gene _name	Control fold change	Control p-val	Treated fold change	Treated p-val
Akr1c6	ACOX1	ACOX1	AKR1C4	-1.68	1.88E-06	1.207	8.606E-02
C9	ACOX1	ACOX1		-3.10	7.81E-07	1.370	1.678E-02
Ces3a	ACOX1	ACOX1		-2.10	2.69E-06	1.379	3.900E-02
Ces3b	ACOX1	ACOX1		-3.16	1.05E-07	1.476	8.274E-02
Cyp2c50	ACOX1	ACOX1	Cyp2c54	-1.72	1.24E-04	1.243	6.999E-02
Cyp4a12a	ACOX1	ACOX1		-1.59	4.60E-03	1.293	8.589E-02
Cyp7b1	ACOX1	ACOX1		-4.45	4.29E-04	1.408	7.877E-02
Egfr	NFKB;ACOX1	ACOX1		-1.98	1.31E-04	1.348	1.187E-02
Gstp1	ACOX1	ACOX1		-2.31	2.56E-06	1.281	2.924E-02

Mup1	ACOX1	ACOX1		-7.69	1.47E-03	1.781	7.683E-02
Mup11	ACOX1	ACOX1		-2.47	9.01E-03	1.703	5.779E-02
Mup14	ACOX1	ACOX1		-2.05	1.27E-02	1.395	4.890E-02
Mup16	ACOX1	ACOX1		-6.27	4.38E-03	1.465	7.558E-02
Mup6	ACOX1	ACOX1		-1.73	2.27E-02	1.330	5.784E-02
Selenbp2	TGFB;IL10; ACOX1	ACOX1	Selenbp1	-15.77	3.73E-05	3.015	2.916E-02
Serpina1 c	TGFB;ACOX1	ACOX1		-2.25	7.22E-09	1.290	5.612E-02
Serpina1 e	TGFB;ACOX1	ACOX1		-43.20	3.93E-08	2.361	1.852E-02
Slc4a4	ACOX1	ACOX1		1.55	7.00E-06	-1.209	4.682E-03
Trib3	IL2;NFKB;AC OX1	ACOX1		2.40	3.14E-04	-1.472	1.987E-02

The Amino Acid Composition Tempers Inflammation Pathways

Inflammation is a "second-hit" of NASH. The differential gene expression patterns in the liver as a result of treatment with the amino acid composition yielded z-scores within IPA analysis associated with upstream regulator activation of anti-inflammatory IL-10 (FIG. 7A) and inhibition of pro-inflammatory NF-kB (FIG. 7B and Table 55), interferons, IL-1b, and IL-2 (FIG. 7C and Table 55). At the protein level, treatment with the amino acid composition significantly down-regulated hepatic MCP-1 and MIP-1, which are the ligands of C-C chemokine receptor types 2 (CCR2) and 5 (CCR5), respectively (FIG. 8). Thus, treatment with the amino acid composition tempered the immune system toward an anti-inflammatory state, which may dampen NASH progression.

Table 55. P-values and fold changes for gene expression associated with the ACOX1 pathway following treatment of STAM mice with the amino acid composition (treated) compared to control.

gene	IPA upstream regulator	IPA gene name	IL10_ path	IL1b_ path	IL2 path	NFKB _path	TGFB _path	Control fold change	Control p-val	Treated fold change	Treated p-val
Abcb1a	NFKB; IL10		IL10			NFKB		3.02	1.55E-06	-1.239	1.047E-01
Abcb1b	NFKB; IL10		IL10			NFKB		-2.24	4.90E-03	1.272	9.745E-02
Acta1	TGFB						TGFB	7.96	5.87E-03	-1.849	1.044E-01
Adora1	TGFB						TGFB	1.94	1.99E-05	-1.222	3.262E-02

AK007436	NFKB	ADAMTS9				NFKB		2.32	6.38E-03	-1.788	6.451E-02
AK043676	IL1b	PFKP		IL1b				1.68	3.12E-03	-1.462	1.586E-02
AK154184	IL1b; TGFB	CYBA		IL1b			TGFB	1.99	1.02E-03	-1.222	9.247E-02
AK158038	IL2	NAV1			IL2			1.62	2.63E-02	-1.659	1.587E-02
Atf5	IL1b; IL2			IL1b	IL2			1.71	3.00E-04	-1.223	9.622E-02
Bcl2a1d	IL1b; IL2; NFKB			IL1b	IL2	NFKB		4.00	1.74E-04	-1.733	3.064E-02
Capn5	IL2				IL2			1.51	2.05E-05	-1.235	5.908E-03
Ccr1	IL1b;IL2;TGFB			IL1b	IL2		TGFB	2.82	1.28E-03	-1.352	7.496E-02
Cd274	IL1b;IL2;NFKB			IL1b	IL2	NFKB		2.37	1.71E-07	-1.282	1.506E-02
Cd83	IL1b;IL2;NFKB;TGFB			IL1b	IL2	NFKB	TGFB	2.41	5.36E-07	-1.434	6.661E-03
Chst11	TGFB						TGFB	2.91	7.87E-06	-1.308	1.881E-02
Clec2i	TGFB						TGFB	1.80	1.01E-03	-1.226	5.963E-02
Egfr	NFKB					NFKB		-1.98	1.31E-04	1.348	1.187E-02
Entpd1	IL2				IL2			1.87	1.84E-04	-1.218	8.413E-02
Fgf21	TGFB						TGFB	49.56	1.03E-03	-1.478	4.472E-02
Gabrd	TGFB						TGFB	5.82	1.48E-04	-1.511	5.978E-02
Gbp4	IL1b;IL10	Gbp6	IL10	IL1b				1.60	1.01E-03	-1.234	5.234E-02
Gbp5	IL10		IL10					1.81	7.82E-05	-1.217	6.120E-02
Gm8909	IL1b;NFKB;IL10	HLA-A	IL10	IL1b		NFKB		4.03	8.19E-04	-1.943	2.569E-02
Gpr85	TGFB						TGFB	2.46	1.77E-02	-1.685	6.628E-02
Gucy2c	TGFB						TGFB	2.35	8.97E-03	-1.395	1.080E-01
Hk2	IL1b;IL2			IL1b	IL2			2.00	2.01E-04	-1.301	9.834E-02
Hsd17b6	TGFB						TGFB	2.89	4.20E-05	-1.244	1.036E-02
Il1rn	IL1b;NFKB;TGFB;IL10		IL10	IL1b		NFKB	TGFB	4.43	5.94E-09	-1.273	1.951E-02
Lama3	IL1b			IL1b				-3.09	1.90E-05	1.650	5.542E-02
Lck	IL2				IL2			1.92	8.87E-04	-1.246	3.027E-02
Lifr	IL1b;IL2;TGFB			IL1b	IL2		TGFB	-4.43	2.43E-05	1.406	2.099E-02
Msr1	TGFB						TGFB	1.58	1.13E-03	-1.226	5.836E-02
Mst1r	TGFB						TGFB	2.08	4.17E-03	-1.523	1.147E-02
Nlrp3	TGFB						TGFB	1.93	1.30E-03	-1.506	2.456E-02
P2ry14	TGFB						TGFB	3.29	4.67E-05	-1.268	4.039E-02
Pcsk1	IL1b			IL1b				2.07	9.00E-03	-2.070	8.150E-02
Pla2g4a	IL1b;TGFB			IL1b			TGFB	2.10	7.94E-05	-1.265	1.064E-01
Plb1	IL1b			IL1b				2.01	2.09E-02	-1.696	2.324E-02
Rgs16	IL1b;IL2;NFKB			IL1b	IL2	NFKB		7.68	2.47E-05	-1.604	4.319E-02
Saa4	IL1b			IL1b				-1.72	4.76E-02	1.283	9.771E-02
Selenbp2	TGFB;IL10	Selenbp1	IL10				TGFB	-15.77	3.73E-05	3.015	2.916E-02
Sema3b	TGFB						TGFB	4.12	4.78E-05	-1.285	9.461E-02
Serpina1c	TGFB						TGFB	-2.25	7.22E-09	1.290	5.612E-02

Serpina1e	TGFB						TGFB	-43.20	3.93E-08	2.361	1.852E-02
Serpina3k	IL1b;NFKB;TGFB			IL1b		NFKB	TGFB	-2.95	1.85E-08	1.500	1.125E-02
Serpinb2	IL1b;NFKB			IL1b		NFKB		1.94	4.98E-02	-1.935	1.865E-02
Slc23a2	TGFB						TGFB	2.00	1.02E-05	-1.258	2.229E-02
Slc2a6	NFKB;IL10		IL10			NFKB		1.79	2.68E-02	-1.281	9.775E-02
Slc7a1	NFKB;TGFB					NFKB	TGFB	1.64	3.96E-03	-1.324	8.657E-02
Slc7a11	IL1b;IL10		IL10	IL1b				65.45	1.35E-03	-1.869	1.068E-01
Tk1	IL1b			IL1b				-2.40	4.99E-06	1.295	4.036E-02
Tlr11	IL10		IL10					1.66	6.11E-03	-1.368	6.005E-02
Tlr2	IL1b;IL2;NFKB;TGFB;IL10		IL10	IL1b	IL2	NFKB	TGFB	2.12	5.32E-05	-1.300	5.430E-02
Trib3	IL2;NFKB				IL2	NFKB		2.40	3.14E-04	-1.472	1.987E-02
Xcl1	IL2				IL2			2.52	1.22E-03	-1.796	6.279E-02

The Amino Acid Composition Prevents Fibrogenesis Pathways

Fibrosis is at the nexus of several biologic processes, such as metabolic dysregulation, inflammation, and cell death. Lipid accumulation in hepatocytes and chronic inflammation induce fibrogenic activation of hepatic stellate cells (Wobser H, et al., Cell Res. 2009, which is hereby incorporated by reference in its entirety). The liver gene expression pattern resulting from treatment with the amino acid composition was consistent with the suppression of the fibrogenic TGF-b signaling pathway (Fig. 7D).

Increasing evidence implicates that CCR2/CCR5 and their ligands, including MCP-1/MIP-1, promote macrophage recruitment and hepatic stellate cell activation which contribute to fibrosis following liver tissue damage (Lefebvre E, et al., PLoS One 2016, which is hereby incorporated by reference in its entirety). The amino acid composition displayed a potent antifibrotic activity in the STAM model of NASH via reducing hepatic TGF-b signaling and MCP-1 and MIP-1 proteins (FIG. 8).

Conclusion

The amino acid composition demonstrated consistent disease modifying activity in both STAM and FATZO mouse models of NASH including improvement in NAS and amelioration of ballooning and fibrosis. The activity of the amino acid composition appears to be driven, at least in part, via increase in fatty acid oxidation, reduction in levels of key cytokines and transcription pathways associated with liver inflammation and fibrosis.

Example 10: Hepatocyte model for steatosis and inflammation

Hepatocyte lipotoxicity appears to be a central driver of hepatic cellular injury via oxidative stress and endoplasmic reticulum (ER) stress. The ability of amino acids to influence steatosis (lipid accumulation) and inflammation in hepatocytes was assessed using human primary hepatocytes (Lonza, TRL).

Cell seeding and maintenance

Primary hepatocytes lot nos. from two healthy human donors were seeded on day 0 at density of 6e04 cells in 96 well optical microplates (Thermofisher) in hepatocyte plating media (William's E medium (Gibco) supplemented with 10% heat-inactivated FBS (Atlanta Bio), 2mM Glutamax (Gibco), 1x ITS plus (R&D systems), and 0.2% Primocin (InVivoGen) and incubated for 6 hours at 37°C, 5% CO_2. After 6 hours, cells were washed twice with 150 ul William's E medium and incubated overnight at 37°C, 5% CO_2 with serum-free hepatocytes culture media (Hepatocytes defined medium (Corning)) supplemented with 5 ug human recombinant EGF (Corning), 2mM Glutamax (Gibco), and 1x Penicillin/Streptomycin. On day 1, cells were washed twice with 150 μL per well William's E medium (Gibco) and incubated for 24h in the hepatocyte culture media in the same conditions described above.

Amino acids pre-treatment

On day 2, cells were washed twice with 150 ul DPBS 1X (Gibco) and maintained in amino acid-free WEM (US Biologicals) containing a defined custom amino acid concentration based on the mean physiological concentrations in blood. The values are published in the Human Metabolome Database (Wishart DS, Tzur D, Knox C, et al., *HMDB: the Human Metabolome Database.* Nucleic Acids Res. 2007 Jan; 35(Database issue):D521-6. 17202168; which is hereby incorporated by reference in its entirety). This custom media is supplemented with 11 mM Glucose, 0.272 mM Sodium Pyruvate, and a dose curve of defined amino acid compositions (i.e., vehicle, LIVRQ+N-acetylcysteine, LIVRQ, RQ+N-acetylcysteine, N-acetylcysteine alone, LIV, or individually with L-Leucine, L-Isoleucine, L-Valine, L-Arginine, L-Glutamine, and L-Cysteine) at various ranges of concentrations. Cells were maintained in this defined media for 24 hours at 37°C, 5% CO_2.

Co-treatment with free fatty acids and different amino acids combination

After pre-treatment, cells were exposed to free fatty acids (FFA) at 250 uM with a ratio of 2:1 (Oleate:Palmitate) supplemented with TNF-α (Thermofisher) at 1 ng/ml or vehicle. Cells were incubated with the FFAs mixture and the different amino acids combinations for 24 hours at 37°C, 5%CO_2. After 24 hours incubation, media was removed for cytokine analysis and replaced by fresh media containing the same stimulus conditions and amino acid concentrations. Cells were incubated for an additional 48 hours for a total of 72 hours of FFA and TNFα stimulation.

Cytokine Analysis after 24h by ELISA

Human CCL2 (MCP-1) was measured by ELISA (Human CCK2/MCP-1 DuoSet ELISA, R&D Systems) at 1/5 or 1/10 dilution in 1X Reagent Diluent (Reagent Ancillary Kit 2, R&D Systems). Data were normalized to the specific per well cell density determined by nuclei count stained by Hoechst 3342 (Life technologies) in the fluorescence microscopy described below.

Intracellular lipid accumulation analysis after 72h by fluorescence microscopy

After 72 hours, cells were washed twice in 100ul PBS 1x (Gibco), fixed with 4% Paraformaldehyde, and washed twice with PBS 1x (100 ul). After fixation, lipids were stained with HCS LipidTOX Red Neutral (Thermofisher Scientific) diluted 1000x and nuclei were stained with Hoechst 3342 (Life Technologies) diluted to 4ug/ml. The LipidTOX™ neutral lipid stain has an extremely high affinity for neutral lipid droplets that was detected by fluorescence microscopy using a high content imager (Molecular Devices).

Results

Lipid accumulation and steatosis phenotypes

Primary human hepatocytes from healthy donors were found to have low levels of lipid accumulation (FIG. 9A-9D). Treatment of the cells with free fatty acids (FF) + TNFα induced lipid accumulation (FIG. 9I-9L) with a macro-steatosis phenotype. Treatment with LIVRQNAC changed the hepatocyte phenotypes from macro-steatosis to micro-steatosis (FIG. 9E-9H).

MCP1/CCL2 secretion

Tables 56-59 show the baseline subtracted secretion of MCP1/CCL2 in primary human hepatocytes cells from two healthy donors (donor 1 for Tables 56 and 57, and donor 2 for Tables 58 and 59). LIVRQNAC, LIVRQNAC+G, LIVRQNAC+S, LIVRQ and RQNAC significantly decreased MCP1/CCL2 secretion in both donors. The combination LIV, however, significantly increased MCP1/CCL2 secretion only in one of the donors. The addition of arginine (R) and glutamine (Q) to a combination of LIV decreased the secretion of MCP1/CCL2 in both donors compared to LIV alone. Individually, N-acetyl cysteine and glutamine are shown to significantly decrease MCP1/CCL2 secretion, while arginine increased MCP1 secretion. Isoleucine, Leucine and Valine did not have an effect on MCP1/CCL2 secretion.

Table 56. Changes in MCP1 expression for donor 1 upon administration of amino acid compositions

Amino Acid Supplement	Conc. (X)	MCP1 expression relative to Control - Donor 1				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	-24.1616	0.032252	3	0.0001	****
LIVRQNAC	30	-22.2916	2.119583	3	0.0001	****
LIVRQNAC	20	-18.4363	0.850597	3	0.0005	***
LIVRQNAC	10	-14.3383	1.854977	3	0.0074	**
LIVRQNAC	1	0	1.048045	3		
LIVRQNAC+G	40	-22.0824	0.873105	3	0.0001	****
LIVRQNAC+G	30	-19.2605	1.611788	3	0.0003	***
LIVRQNAC+G	20	-17.5807	2.893835	3	0.0009	***
LIVRQNAC+G	10	-13.7521	3.068991	3	0.0106	*
LIVRQNAC+G	1	0	1.682719	3		
LIVRQNAC+S	40	-32.4703	0.340537	3	0.0001	****
LIVRQNAC+S	30	-30.768	1.339048	3	0.0001	****
LIVRQNAC+S	20	-25.5964	1.854519	3	0.0001	****
LIVRQNAC+S	10	-17.8326	1.974033	3	0.0008	***
LIVRQNAC+S	1	2.37E-15	18.41384	3		
LIV	40	15.52052	6.323205	3	0.0094	**
LIV	30	12.3111	10.02706	3	0.0475	*
LIV	20	12.6686	4.109608	3	0.0401	*
LIV	10	-5.18869	1.579468	3	0.6477	ns

LIV	1	-1.2E-15	8.178943	3		
LIVRQ	40	-25.9576	0.484283	3	0.0028	**
LIVRQ	30	-23.6562	2.599721	3	0.0099	**
LIVRQ	20	-13.4723	3.427666	3	0.6401	ns
LIVRQ	10	-9.22141	7.599407	3	0.9986	ns
LIVRQ	1	-8.23198	5.80889	3		
RQNAC	40	-21.4681	2.903892	3	0.0003	***
RQNAC	30	-17.1873	5.202568	3	0.0038	**
RQNAC	20	-12.1782	2.907484	3	0.0506	ns
RQNAC	10	-8.89378	4.748653	3	0.206	ns
RQNAC	1	1.18E-15	10.02527	3		
N-Acetyl Cysteine	40	-17.6065	1.211739	3	0.0009	***
N-Acetyl Cysteine	20	-10.8919	2.27818	3	0.0545	ns
N-Acetyl Cysteine	10	-2.49755	8.795693	3	0.9424	ns
N-Acetyl Cysteine	5	-0.76286	7.457085	3	0.9991	ns
N-Acetyl Cysteine	0	0	6.716428	3		

Table 57. Changes in MCP1 expression for donor 1 upon administration of single amino acid compositions

Amino Acid Supplement	Conc. (µM)	MCP1 expression relative to Control- Donor 1				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	14.16805	19.23365	3	0.6777	ns
Valine	11710	77.73396	137.82	3	0.9998	ns
Valine	4684	23.6867	46.48697	3	0.2502	ns
Valine	234	-2.4E-15	13.86902	3		
Arginine	5440	10.9386	4.79774	3	0.0057	**
Arginine	2720	6.526801	4.266971	3	0.1517	ns
Arginine	1088	5.114414	4.685563	3	0.3321	ns
Arginine	109	2.37E-15	0.666016	3		
Glutamine	22484	-21.8392	1.113443	3	0.0004	***
Glutamine	11242	-9.00139	1.68951	3	0.2459	ns
Glutamine	3747	-0.89805	6.374471	3	0.9991	ns
Glutamine	749	0	9.549143	3		
Isoleucine	6639	-0.205	2.292188	3	0.9998	ns
Isoleucine	3320	-2.41722	2.382379	3	0.4907	ns
Isoleucine	1328	-0.30729	2.409691	3	0.9992	ns
Isoleucine	66	-1.2E-15	3.163838	3		

Leucine	15270	-1.36762	3.37035	3	0.8675	ns
Leucine	7635	1.895506	3.757642	3	0.6872	ns
Leucine	3054	3.340489	3.016641	3	0.2201	ns
Leucine	153	5.92E-16	3.132507	3		
N-Acetyl Cysteine	10000	-17.6065	1.211739	3	0.0009	***
N-Acetyl Cysteine	5000	-10.8919	2.27818	3	0.0545	ns
N-Acetyl Cysteine	2500	-2.49755	8.795693	3	0.9424	ns
N-Acetyl Cysteine	1000	-0.76286	7.457085	3	0.9991	ns
N-Acetyl Cysteine	0	0	6.716428	3		

Table 58. Changes in MCP1 expression for donor 2 upon administration of amino acid compositions

Amino Acid Supplement	Conc. (X)	MCP1 expression relative to Control - Donor 2				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	-24.5376	1.632923	3	0.0001	****
LIVRQNAC	30	-13.6824	2.562571	3	0.0001	****
LIVRQNAC	20	-8.42053	1.545343	3	0.0001	****
LIVRQNAC	10	2.126223	0.453924	3	0.0007	***
LIVRQNAC	1	-4.7E-15	0.412226	3		
LIVRQNAC+G	40	-35.3651	2.08381	3	0.0007	***
LIVRQNAC+G	30	-30.3247	5.225183	3	0.001	***
LIVRQNAC+G	20	-17.0719	4.522244	3	0.0119	*
LIVRQNAC+G	10	-14.2586	2.767898	3	0.049	*
LIVRQNAC+G	1	-7.1E-15	7.613666	3		
LIVRQNAC+S	40	-35.8381	1.404782	3	0.0001	****
LIVRQNAC+S	30	-30.9946	2.372062	3	0.0001	****
LIVRQNAC+S	20	-16.8831	3.223007	3	0.0004	***
LIVRQNAC+S	10	-5.60595	10.2119	3	0.1887	
LIVRQNAC+S	1	2.37E-15	4.4168	3		
LIV	40	-46.7898	8.664441	3	0.3692	ns
LIV	30	-34.5953	16.84743	3	0.6246	ns
LIV	20	-28.0851	31.84348	3	0.7684	ns
LIV	10	-11.0006	72.74556	3	0.9889	ns
LIV	1	9.47E-15	60.93638	3		
LIVRQ	40	-129.802	7.067989	3	0.0008	***
LIVRQ	30	-110.034	4.53852	3	0.0042	**
LIVRQ	20	-33.3611	31.87706	3	0.6524	

LIVRQ	10	-3.30904	71.03267	3	0.9999	
LIVRQ	1	-4.7E-15	46.12987	3		
RQNAC	40	-133.48	1.908424	3	0.0006	***
RQNAC	30	-123.712	1.043889	3	0.0013	**
RQNAC	20	-109.575	5.533323	3	0.0044	**
RQNAC	10	-55.8583	22.72309	3	0.2273	
RQNAC	1	1.42E-14	43.79031	3		
N-Acetyl Cysteine	10000	-28.4419	1.694	3	0.0001	***
N-Acetyl Cysteine	5000	-10.5725	4.362178	3	0.0012	**
N-Acetyl Cysteine	2500	-4.0591	5.600773	3	0.0572	ns
N-Acetyl Cysteine	1000	1.602474	3.423109	3	0.0001	****
N-Acetyl Cysteine	0	0	2.068861	3		

Table 59. Changes in MCP1 expression for donor 2 upon administration of single amino acid compositions

Amino Acid Supplement	Conc. (μM)	MCP1 expression relative to Control- Donor 2				
		Mean	**Std. Deviation**	**Number of values**	**P-value***	**Significance**
Valine	23420	-30.7921	22.55378	3	0.6118	ns
Valine	11710	38.24762	28.44112	3	0.4268	ns
Valine	4684	10.79011	51.87642	3	0.9835	ns
Valine	234	-1.4E-14	30.91388	3		
Arginine	5440	8.493664	22.98385	3	0.9913	ns
Arginine	2720	24.06261	63.49489	3	0.7429	ns
Arginine	1088	24.95224	52.94171	3	0.7192	ns
Arginine	109	-4.7E-15	11.27976	3		
Glutamine	22484	-138.873	10.74317	3	0.0001	****
Glutamine	11242	-90.6558	15.43989	3	0.0037	**
Glutamine	3747	-45.0574	41.63249	3	0.2474	ns
Glutamine	749	2.84E-14	59.86955	3	0.7631	
Isoleucine	6639	18.62132	26.01824	3	0.5663	ns
Isoleucine	3320	-5.64461	7.719105	3	0.9882	ns
Isoleucine	1328	26.62309	5.65413	3	0.2613	ns
Isoleucine	66	0	4.245462	3		
Leucine	15270	-26.6436	10.08177	3	0.2607	ns
Leucine	7635	-2.98815	21.00205	3	0.9989	ns

Leucine	3054	16.11014	8.662188	3	0.68	ns
Leucine	153	-4.7E-15	7.63396	3		
N-Acetyl Cysteine	10000	-28.4419	1.694	3	0.0001	***
N-Acetyl Cysteine	5000	-10.5725	4.362178	3	0.0012	**
N-Acetyl Cysteine	2500	-4.0591	5.600773	3	0.0572	ns
N-Acetyl Cysteine	1000	1.602474	3.423109	3	0.0001	****
N-Acetyl Cysteine	0	0	2.068861	3		

Example 11: Hepatic Stellate Cell – TNFα Inflammatory Response

Methods

Primary human hepatic stellate cells were obtained from Samsara Sciences based on the following criteria for selecting donors: adult age (between 18 and 50 years), normal BMI (>18.5 and <25), and absence of confounding liver disease. Primary human hepatic stellate cells grown in Complete HSC Medium to ~80% confluence in T75 or T150 flasks below passage 10 were seeded into sterile, collagen I coated, 96-well optical plastic microplates (ThermoScientific, 152036) at 4000 cells per well (~1250 cells per cm^2) and incubated for 6 hours at 37°C, 5% CO_2 in a humidified incubator.

After 6 hours, plates were removed from the incubator and the medium gently pipetted off and washed once with 150 μL per well DPBS. The DPBS was removed and the pretreatment medium (± single amino acid dropout, 1XHMDB DMEM + 3% dialyzed FBS + 0.2% Primocin, ± supplemental amino acid dose; see experiment for medium composition) was applied to the cells at 150 μL per well. Plates were returned to the incubator overnight, ~14-15 hours.

After overnight pretreatment, the medium was removed from the cells, and the same pretreatment medium, now supplemented with 3 ng/mL TNFα is applied. Each plate contained 3 ng/mL TNFα in 1X human plasma amino acid (HMDB or PAA) concentration medium, 0 ng/mL in 1XHMDB, and 3 ng/mL TNFα + 50 nM Bengamide in 1XHMDB to serve as controls. Plates were incubated for 12 hours at 37°C, 5% CO_2.

After 12 hour stimulus with TNFα, supernatant was removed and frozen at -80°C in two separate aliquots. Plates were washed gently once with DPBS and 100 μL per well of 1XHMDB

DMEM + 3% dialyzed FBS + 0.2% Primocin + 10% CCK-8 viability reagent (Dojindo). Plates were incubated for 1 hour at 37°C, 5% CO_2.

After 1 hour of incubation, viability was measured on the Synergy plate reader (Absorbance at 977 (test), 900 (reference), and 450 (CCK8) nm). Immediately, the medium was removed and the plates were fixed with 70 µL per well 4% paraformaldehyde in PBS at room temperature for 20 minutes, followed by two 150 µL PBS washes, and stored with 100 µL per well PBS at 4°C until immunofluorescence staining.

Human CCL2/MCP1 and Human IL-6 were measured by ELISA (Human CCK2/MCP-1 DuoSet ELISA, R&D Systems; Human IL-6 DuoSet ELISA, R&D Systems) at 1/5 and 1/20 dilution in 1X Reagent Diluent (Reagent Ancillary Kit 2, R&D Systems). Data were normalized to the specific per well cell density determined by Hoechst stained nuclei count.

Results

Pro-inflammatory MCP-1 Chemokine Secretion

Tables 60-63 show per-cell normalized MCP-1 chemokine secretion in primary human hepatic stellate cells from two donors as a fold change from the plasma amino acid background. Statistical significance calculated by one-way ANOVA with Dunnett's multiple comparison test within each treatment group. LIVRQNAC+G and RQNAC singificantly decrease MCP-1 secretion in both donors. LIVRQNAC, LIVRQNAC+S reduced MCP1 secretion and was statistically significant in one of two donors. Individually, each of valine, arginine, and leucine had no significant impact on MCP-1 secretion. Glutamine reduced MCP1 secretion in both donors but was only statistically significant in one of two donors. N-acetyl cysteine significantly reduced MCP-1 secretion in both donors.

Table 60. Changes in MCP1 secretion for donor 3 upon administration of amino acid compositions

Amino Acid Supplement	Conc. (X)	Fold Change MCP1 Secretion Normalized Per Cell				
		Mean	Std. Deviation	Number of values	Significance	P-value
LIVRQNAC	40	0.6237	0.2500	3	ns	0.2763
LIVRQNAC	30	0.6180	0.2436	3	ns	0.2657
LIVRQNAC	20	0.5679	0.1728	3	ns	0.1863

LIVRQNAC	10	0.5548	0.2139	3	ns	0.1694
LIVRQNAC	1	1.0000	0.3619	3		
LIVRQNAC+G	40	0.6216	0.0903	3	**	0.0036
LIVRQNAC+G	30	0.6742	0.0549	3	**	0.0095
LIVRQNAC+G	20	0.6373	0.0888	3	**	0.0047
LIVRQNAC+G	10	0.7075	0.0610	3	*	0.0179
LIVRQNAC+G	1	1.0000	0.1704	3		
LIVRQNAC+S	40	0.5911	0.1451	3	ns	0.2045
LIVRQNAC+S	30	0.5932	0.1943	3	ns	0.2077
LIVRQNAC+S	20	0.5760	0.1681	3	ns	0.1828
LIVRQNAC+S	10	0.6820	0.2396	3	ns	0.3845
LIVRQNAC+S	1	1.0000	0.4098	3		
LIV	40	1.2677	0.5786	3	ns	0.7802
LIV	30	1.3632	0.5837	3	ns	0.8368
LIV	20	1.3336	0.4754	3	ns	0.7964
LIV	10	1.3745	0.5427	3	ns	0.9132
LIV	1	1.0000	0.3186	3		
LIVRQ	40	1.3042	0.4140	3	ns	0.7695
LIVRQ	30	1.2208	0.4403	3	ns	0.9036
LIVRQ	20	0.9915	0.3521	3	ns	0.9999
LIVRQ	10	0.9968	0.3907	3	ns	0.9999
LIVRQ	1	1.0000	0.4257	3		
RQNAC	40	0.3220	0.0282	3	****	0.0001
RQNAC	30	0.4353	0.0941	3	****	0.0001
RQNAC	20	0.4629	0.0998	3	***	0.0001
RQNAC	10	0.6513	0.0925	3	**	0.0028
RQNAC	1	1.0000	0.1132	3		
N-Acetyl Cysteine	40	0.4485	0.0587	3	***	0.0002
N-Acetyl Cysteine	20	0.5413	0.1018	3	***	0.0009
N-Acetyl Cysteine	10	0.6565	0.0502	3	**	0.007
N-Acetyl Cysteine	5	0.8492	0.1515	3	ns	0.2738
N-Acetyl Cysteine	0	1.0000	0.1142	3		

Table 61. Changes in MCP1 secretion for donor 3 upon administration of single amino acid compositions

Amino Acid Supplement	Conc. (μM)	Fold Change MCP1 Secretion Normalized Per Cell				
		Mean	Std. Deviation	Number of values	Significance	P-value
Valine	23420	1.2651	0.1295	3	ns	0.1126
Valine	11710	1.0204	0.1126	3	ns	0.9956

Valine	4684	1.0630	0.0878	3	ns	0.8999
Valine	234	1.0000	0.2008	3		
Arginine	5440	0.7840	0.2753	3	ns	0.7069
Arginine	2720	0.8821	0.2249	3	ns	0.9264
Arginine	1088	0.9435	0.3221	3	ns	0.9903
Arginine	109	1.0000	0.3404	3		
Glutamine	22484	0.6212	0.1952	3	ns	0.2465
Glutamine	11242	0.6106	0.2085	3	ns	0.226
Glutamine	3747	0.6036	0.2596	3	ns	0.2135
Glutamine	749	0.7048	0.2473	3	ns	0.4593
Glutamine	562	1.0000	0.2185	3		
Isoleucine	6639	1.2084	0.1334	3	ns	0.284
Isoleucine	3320	1.2169	0.0589	3	ns	0.2565
Isoleucine	1328	1.5550	0.2070	3	**	0.0038
Isoleucine	66	1.0000	0.1188	3		
Leucine	15270	1.1808	0.2601	3	ns	0.5156
Leucine	7635	1.3054	0.1748	3	ns	0.1491
Leucine	3054	1.1479	0.0605	3	ns	0.6605
Leucine	153	1.0000	0.0784	3		
N-Acetyl Cysteine	10000	0.4485	0.0587	3	***	0.0002
N-Acetyl Cysteine	5000	0.5413	0.1018	3	***	0.0009
N-Acetyl Cysteine	2500	0.6565	0.0502	3	**	0.007
N-Acetyl Cysteine	1000	0.8492	0.1515	3	ns	0.2738
N-Acetyl Cysteine	0	1.0000	0.1142	3		

Table 62. Changes in MCP1 secretion for donor 4 upon administration of amino acid compositions

Amino Acid Supplement	Conc. (X)	Fold Change MCP1 Secretion Normalized Per Cell				
		Mean	Std. Deviation	Number of values	Significance	P-value
LIVRQNAC	40	0.7791	0.0740	3	ns	0.1328
LIVRQNAC	30	0.6333	0.1114	3	*	0.0116
LIVRQNAC	20	0.6997	0.1013	3	*	0.0352
LIVRQNAC	10	0.8114	0.1271	3	ns	0.2216
LIVRQNAC	1	1.0000	0.1607	3		
LIVRQNAC+G	40	0.6738	0.0979	3	*	0.0454
LIVRQNAC+G	30	0.7117	0.0783	3	ns	0.0794
LIVRQNAC+G	20	0.6735	0.1127	3	*	0.0452
LIVRQNAC+G	10	0.7682	0.0563	3	ns	0.1778
LIVRQNAC+G	1	1.0000	0.2452	3		

LIVRQNAC+S	40	0.5780	0.0781	3	**	0.0025
LIVRQNAC+S	30	0.5393	0.1185	3	**	0.0013
LIVRQNAC+S	20	0.6487	0.0732	3	**	0.0085
LIVRQNAC+S	10	0.6872	0.0118	3	*	0.017
LIVRQNAC+S	1	1.0000	0.1803	3		
LIV	40	0.7010	0.1399	3	**	0.0059
LIV	30	0.8883	0.0530	3	ns	0.3745
LIV	20	0.9284	0.0579	3	ns	0.7114
LIV	10	0.8663	0.0569	3	ns	0.2428
LIV	1	1.0000	0.0928	3		
LIVRQ	40	1.2235	0.0592	3	ns	0.4365
LIVRQ	30	1.1653	0.0558	3	ns	0.6679
LIVRQ	20	0.8845	0.2698	3	ns	0.862
LIVRQ	10	1.0110	0.0738	3	ns	0.9999
LIVRQ	1	1.0000	0.3016	3		
RQNAC	40	0.4312	0.0994	3	***	0.0006
RQNAC	30	0.3910	0.0649	3	***	0.0003
RQNAC	20	0.5579	0.2079	3	**	0.0037
RQNAC	10	0.5545	0.0663	3	**	0.0035
RQNAC	1	1.0000	0.0987	3		
N-Acetyl Cysteine	40	0.5011	0.0756	3	***	0.0001
N-Acetyl Cysteine	20	0.6728	0.1024	3	**	0.003
N-Acetyl Cysteine	10	0.8033	0.1101	3	ns	0.058
N-Acetyl Cysteine	5	0.6437	0.0648	3	**	0.0017
N-Acetyl Cysteine	0	1.0000	0.0673	3		

Table 63. Changes in MCP1 secretion for donor 4 upon administration of single amino acid compositions

Amino Acid Supplement	Conc. (μM)	Fold Change MCP1 Secretion Normalized Per Cell				
		Mean	Std. Deviation	Number of values	Significance	P-value
Valine	23420	1.1525	0.0406	3	ns	0.9999
Valine	11710	1.1544	0.1743	3	ns	0.8877
Valine	4684	1.0942	0.0846	3	ns	0.3545
Valine	234	1.0000	0.1464	3		
Arginine	5440	0.9456	0.0639	3	ns	0.9076
Arginine	2720	1.0446	0.0741	3	ns	0.9449
Arginine	1088	1.0453	0.1733	3	ns	0.9423
Arginine	109	1.0000	0.1486	3		
Glutamine	22484	0.7039	0.0544	3	**	0.0065
Glutamine	11242	0.7129	0.2237	3	**	0.0077

Glutamine	3747	0.6639	0.0467	3	**	0.0027
Glutamine	749	0.7782	0.0860	3	*	0.0452
Glutamine	562	1.0000	0.0709	6		
Isoleucine	6639	0.9103	0.0536	3	ns	0.5597
Isoleucine	3320	0.8830	0.0872	3	ns	0.3538
Isoleucine	1328	1.3338	0.1099	3	**	0.0044
Isoleucine	66	1.0000	0.0853	3		
Leucine	15270	1.5745	0.0844	3	ns	0.1886
Leucine	7635	1.7129	0.6026	3	ns	0.0885
Leucine	3054	1.5342	0.1746	3	ns	0.2332
Leucine	153	1.0000	0.2040	3		
N-Acetyl Cysteine	10000	0.5011	0.0756	3	***	0.0001
N-Acetyl Cysteine	5000	0.6728	0.1024	3	**	0.003
N-Acetyl Cysteine	2500	0.8033	0.1101	3	ns	0.058
N-Acetyl Cysteine	1000	0.6437	0.0648	3	**	0.0017
N-Acetyl Cysteine	0	1.0000	0.0673	3		

IL-6 Cytokine Secretion

Tables 64-67 show per-cell normalized IL-6 cytokine secretion in primary human hepatic stellate cells from two donors as a fold change from the plasma amino acid background. Statistical significance calculated by one-way ANOVA with Dunnett's multiple comparison test within each treatment group. LIVRQNAC, LIVRQNAC+S and RQNAC significantly reduced IL-6 secretion in one of two donors. LIVRQNAC+G, LIVRQNAC+S and RQNAC decreased IL-6 secretion in both donors. LIV and LIVRQ did not have a significant impact on IL-6 secretion in either donor. Individually, valine, arginine, isoleucine, and leucine had no significant effect on IL-6 secretion. N-acetyl cysteine reduced IL-6 secretion in both donors but was only statistically significant in one of two donors. Glutamine significantly reduced IL-6 secretion in both donors.

Table 64. Changes in IL-6 cytokine secretion for donor 1 upon administration of amino acid compositions

Amino Acid Supplement	Conc. (X)	Fold Change IL-6 Secretion Normalized Per Cell				
		Mean	Std. Deviation	Number of values	Significance	P-value
LIVRQNAC	40	0.4857	0.0915	3	***	0.0004
LIVRQNAC	30	0.5667	0.0941	3	**	0.0014
LIVRQNAC	20	0.6671	0.0431	3	**	0.0088
LIVRQNAC	10	0.6579	0.1231	3	**	0.0074

LIVRQNAC	1	1.0000	0.1361	3		
LIVRQNAC+G	40	0.4995	0.1427	3	ns	0.0949
LIVRQNAC+G	30	0.5722	0.2185	3	ns	0.1679
LIVRQNAC+G	20	0.6185	0.1769	3	ns	0.2376
LIVRQNAC+G	10	0.7040	0.2809	3	ns	0.4276
LIVRQNAC+G	1	1.0000	0.3513	3		
LIVRQNAC+S	40	0.5397	0.1569	3	*	0.0105
LIVRQNAC+S	30	0.5513	0.1190	3	*	0.0122
LIVRQNAC+S	20	0.6264	0.1593	3	*	0.0338
LIVRQNAC+S	10	0.6799	0.1218	3	ns	0.0703
LIVRQNAC+S	1	1.0000	0.1671	3		
LIV	40	1.3536	0.4767	3	ns	0.6216
LIV	30	1.2423	0.3135	3	ns	0.8437
LIV	20	1.2321	0.4818	3	ns	0.8611
LIV	10	1.1421	0.3489	3	ns	0.9704
LIV	1	1.0000	0.1647	3		
LIVRQ	40	0.8274	0.2003	3	ns	0.7863
LIVRQ	30	0.8880	0.2175	3	ns	0.938
LIVRQ	20	0.8468	0.1100	3	ns	0.8431
LIVRQ	10	0.9247	0.2696	3	ns	0.984
LIVRQ	1	1.0000	0.3311	3		
RQNAC	40	0.3958	0.0947	3	*	0.0109
RQNAC	30	0.4433	0.1317	3	*	0.0177
RQNAC	20	0.4936	0.1079	3	*	0.0297
RQNAC	10	0.5729	0.1741	3	ns	0.0674
RQNAC	1	1.0000	0.3440	3		
N-Acetyl Cysteine	40	0.5716	0.2306	3	ns	0.2067
N-Acetyl Cysteine	20	0.6121	0.1718	3	ns	0.2729
N-Acetyl Cysteine	10	0.7354	0.2816	3	ns	0.5703
N-Acetyl Cysteine	5	0.7141	0.2509	3	ns	0.5098
N-Acetyl Cysteine	0	1.0000	0.3472	3		

Table 65. Changes in IL-6 cytokine secretion for donor 1 upon administration of single amino acid compositions

Amino Acid Supplement	Conc. (μM)	Fold Change IL-6 Secretion Normalized Per Cell				
		Mean	Std. Deviation	Number of values	Significance	P-value
Valine	23420	1.0404	0.2175	3	ns	0.9949
Valine	11710	0.9562	0.3332	3	ns	0.9935
Valine	4684	0.9790	0.1777	3	ns	0.9993
Valine	234	1.0000	0.2868	3		

Arginine	5440	0.7776	0.1994	3	ns	0.6927
Arginine	2720	1.0231	0.4381	3	ns	0.9993
Arginine	1088	0.9828	0.2957	3	ns	0.9997
Arginine	109	1.0000	0.1728	3		
Glutamine	22484	0.5138	0.0818	3	**	0.0046
Glutamine	11242	0.5136	0.1189	3	**	0.0046
Glutamine	3747	0.5460	0.0891	3	**	0.0072
Glutamine	749	0.6320	0.1181	3	*	0.0249
Glutamine	562	1.0000	0.2226	3		
Isoleucine	6639	1.0859	0.1489	3	ns	0.764
Isoleucine	3320	1.1156	0.0776	3	ns	0.5903
Isoleucine	1328	1.0233	0.1536	3	ns	0.9922
Isoleucine	66	1.0000	0.1276	3		
Leucine	15270	1.0767	0.0246	3	ns	0.853
Leucine	7635	1.1215	0.0872	3	ns	0.6249
Leucine	3054	1.1762	0.2273	3	ns	0.3655
Leucine	153	1.0000	0.1535	3		
N-Acetyl Cysteine	10000	0.5716	0.2306	3	ns	0.2067
N-Acetyl Cysteine	5000	0.6121	0.1718	3	ns	0.2729
N-Acetyl Cysteine	2500	0.7354	0.2816	3	ns	0.5703
N-Acetyl Cysteine	1000	0.7141	0.2509	3	ns	0.5098
N-Acetyl Cysteine	0	1.0000	0.3472	3		

Table 66. Changes in IL-6 cytokine secretion for donor 2 upon administration of amino acid compositions

Amino Acid Supplement	Conc. (X)	Fold Change IL-6 Secretion Normalized Per Cell				
		Mean	Std. Deviation	Number of values	Significance	P-value
LIVRQNAC	40	0.9911	0.1150	3	ns	0.9998
LIVRQNAC	30	0.9560	0.0473	3	ns	0.9404
LIVRQNAC	20	1.0008	0.1450	3	ns	0.9999
LIVRQNAC	10	1.0845	0.0707	3	ns	0.6567
LIVRQNAC	1	1.0000	0.0553	3		
LIVRQNAC+G	40	0.8055	0.1705	3	ns	0.4153
LIVRQNAC+G	30	0.8218	0.1567	3	ns	0.4855
LIVRQNAC+G	20	0.9236	0.1642	3	ns	0.9342
LIVRQNAC+G	10	1.1076	0.2097	3	ns	0.8216
LIVRQNAC+G	1	1.0000	0.0416	3		
LIVRQNAC+S	40	0.9508	0.0933	3	ns	0.967
LIVRQNAC+S	30	0.8581	0.0364	3	ns	0.4836
LIVRQNAC+S	20	0.8289	0.0765	3	ns	0.3356

LIVRQNAC+S	10	0.8487	0.1018	3	ns	0.432
LIVRQNAC+S	1	1.0000	0.2312	3		
LIV	40	0.9122	0.0773	3	ns	0.8233
LIV	30	1.0994	0.0987	3	ns	0.7586
LIV	20	1.0400	0.2330	3	ns	0.9857
LIV	10	0.9579	0.1077	3	ns	0.9828
LIV	1	1.0000	0.0540	3		
LIVRQ	40	0.9327	0.0639	3	ns	0.8313
LIVRQ	30	0.8421	0.1125	3	ns	0.2361
LIVRQ	20	0.7871	0.0932	3	ns	0.0841
LIVRQ	10	0.8693	0.0750	3	ns	0.3744
LIVRQ	1	1.0000	0.1428	3		
RQNAC	40	0.8711	0.0816	3	ns	0.5267
RQNAC	30	0.7460	0.1133	3	ns	0.0843
RQNAC	20	0.7838	0.0708	3	ns	0.1544
RQNAC	10	0.8781	0.1566	3	ns	0.5705
RQNAC	1	1.0000	0.1557	3		
N-Acetyl Cysteine	40	0.7064	0.0418	3	ns	0.0508
N-Acetyl Cysteine	20	0.8111	0.1049	3	ns	0.2549
N-Acetyl Cysteine	10	0.9180	0.2230	3	ns	0.8353
N-Acetyl Cysteine	5	0.9161	0.1067	3	ns	0.8252
N-Acetyl Cysteine	0	1.0000	0.0632	3		

Table 67. Changes in IL-6 cytokine secretion for donor 2 upon administration of single amino acid compositions

Amino Acid Supplement	Conc. (μM)	Fold Change IL-6 Secretion Normalized Per Cell				
		Mean	Std. Deviation	Number of values	Significance	P-value
Valine	23420	0.9015	0.0930	3	ns	0.4967
Valine	11710	0.9218	0.1179	3	ns	0.6516
Valine	4684	1.0383	0.1014	3	ns	0.9291
Valine	234	1.0000	0.0696	3		
Arginine	5440	0.8895	0.0897	3	ns	0.547
Arginine	2720	0.9401	0.1611	3	ns	0.8654
Arginine	1088	0.9924	0.0692	3	ns	0.9996
Arginine	109	1.0000	0.1263	3		
Glutamine	22484	0.5993	0.0611	3	****	0.0001
Glutamine	11242	0.6478	0.0371	3	****	0.0001
Glutamine	3747	0.7100	0.0356	3	***	0.0003
Glutamine	749	0.7673	0.0222	3	**	0.0017
Glutamine	562	1.0000	0.1027	6		
Isoleucine	6639	1.1648	0.1125	3	ns	0.1448

Isoleucine	3320	0.9096	0.0916	3	ns	0.5304
Isoleucine	1328	1.1020	0.0987	3	ns	0.4446
Isoleucine	66	1.0000	0.0641	3		
Leucine	15270	1.0183	0.1155	3	ns	0.9795
Leucine	7635	0.9574	0.0590	3	ns	0.8187
Leucine	3054	1.0011	0.0618	3	ns	0.9999
Leucine	153	1.0000	0.0277	3		
N-Acetyl Cysteine	10000	0.7064	0.0418	3	ns	0.0508
N-Acetyl Cysteine	5000	0.8111	0.1049	3	ns	0.2549
N-Acetyl Cysteine	2500	0.9180	0.2230	3	ns	0.8353
N-Acetyl Cysteine	1000	0.9161	0.1067	3	ns	0.8252
N-Acetyl Cysteine	0	1.0000	0.0632	3		

Example 12: TGFβ1 Fibrogenic Gene Expression of Hepatic Stellate Cell

Primary human hepatic stellate cells were obtained from Samsara Sciences based on the following criteria for selecting donors: adult age (between 18 and 50 years), normal BMI (>18.5 and <25), and absence of confounding liver disease. Cells grown in Complete HSC Medium to ~80% confluence in T75 or T150 flasks below passage 10 were seeded into sterile, collagen I coated, 96-well optical plastic microplates (ThermoScientific, 152036) at 6000 cells per well (~1250 cells per cm2) and incubated overnight at 37°C, 5% CO2 in a humidified incubator in DMEM with 2% Fetal Bovine Serum and 1% Antibiotic-Antimycotic.

After the overnight incubation, plates were removed from the incubator and the medium was gently pipetted off and washed twice with 150 µL per well DPBS. The DPBS was removed and the pretreatment medium (± single amino acid dropout, 1XHMDB DMEM + 1% Antibiotic-Antimycotic, 10 mM HEPES, ± supplemental amino acid dose; see experiment for medium composition) was applied to the cells at 150 µL per well. Plates were returned to the incubator for 10.5 hours.

After 10.5 hour pretreatment, the medium was removed from the cells, and the same pretreatment medium, now supplemented with 3 ng/mL TGFβ1, was applied. Each plate contained 3 ng/mL TGFβ1 in 1X human plasma amino acid (HMDB or PAA) concentration medium, 0 ng/mL in 1XHMDB, and 3 ng/mL TGFβ1 + 20 µM Silybin in 1XHMDB to serve as controls. Plates were then incubated for 24 hours at 37°C, 5% CO2.

After 24 hour stimulus, supernatant was removed and frozen at -80°C in two separate aliquots. The cells were then washed with 125 µL per well Buffer FCW (FastLane Cell

Multiplex NR Kit, Qiagen, 216713). The wash buffer was immediately removed and 50 μL of Cell Processing Mix (containing genomic DNA Wipeout buffer) was applied to lyse cells, incubating for 10 minutes at room temperature. RNA lysate was then transferred to 96-well qPCR plates, sealed, and gDNA was digested on thermal cycler at 75°C for 5 minutes. RNA lysate was frozen at -80°C.

Each 20 μL one-step RT-qPCR reaction contained 4 μL of RNA lysate. Gene expression of Col1a1, Timp2, and Gapdh were multiplexed using the HEX, Cy5, and FAM fluorescent channels, respectively, with commercially available primer-probe mixes (the Human Col1a1 Primer-Probe Set, HEX; the Human Timp2 Primer-Probe Set, Cy5; and the Human Gapdh Primer-Probe Set, FAM from IDT). Gene expression was evaluated using the $\Delta\Delta$Cq method within each single amino acid dropout and supplementation by normalizing to its own 1X HMDB concentration.

Human Procollagen Iα1 was measured from the supernatant by ELISA (Human Pro-Collagen I alpha 1 DuoSet ELISA, R&D Systems) at 1/100 dilution in 1X Reagent Diluent (Reagent Ancillary Kit 2, R&D Systems).

Results

Col1a1 Gene Expression

Tables 68, 69, 69-1, 69-2, 69-3, and 69-4 show the mean fold change in Col1a1 gene expression in primary human hepatic stellate cells from three different healthy donors. LIVRQNAC and LIVRQNAC+S showed significantly decreased Col1a1 gene expression in two of three donors. LIVRQNAC+G and RQNAC showed significantly decreased Col1a1 expression in all three donors. LIVRQ showed a significant change in Col1a1 gene expression in only one donor. LIV alone did not significantly change Col1a1 gene expression.

Each of leucine, isoleucine, valine, and arginine did not significantly change Col1a1 gene expression in any donor when the amino acid was administered alone. Glutamine decreased Col1a1 gene expression in two of three donors. N-acetyl cysteine significantly reduced Col1a1 gene expression in all three donors.

Table 68. Fold change of Col1a1 gene expression after administration of an amino acid composition, normalized to Gapdh expression in a first donor

Amino Acid Supplement	Conc. (X)	Col1a1 Fold Expression Relative to Control				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	0.91	0.08	4	ns	0.401
LIVRQNAC	30	0.87	0.10	4	ns	0.1073
LIVRQNAC	20	0.88	0.04	4	ns	0.1483
LIVRQNAC	10	0.90	0.08	4	ns	0.3035
LIVRQNAC	1	1.00	0.10	4		
LIVRQNAC+G	40	0.73	0.15	4	**	0.0053
LIVRQNAC+G	30	0.79	0.08	4	*	0.0252
LIVRQNAC+G	20	0.84	0.08	4	ns	0.1181
LIVRQNAC+G	10	0.79	0.11	4	*	0.0286
LIVRQNAC+G	1	1.00	0.03	4		
LIVRQNAC+S	40	0.79	0.05	4	*	0.0325
LIVRQNAC+S	30	0.86	0.13	4	ns	0.1848
LIVRQNAC+S	20	0.96	0.10	4	ns	0.9287
LIVRQNAC+S	10	0.85	0.12	4	ns	0.1566
LIVRQNAC+S	1	1.00	0.10	4		
LIV	40	0.93	0.03	4	ns	0.5561
LIV	30	1.04	0.07	4	ns	0.8872
LIV	20	1.04	0.09	4	ns	0.9069
LIV	10	1.05	0.10	4	ns	0.8156
LIV	1	1.00	0.07	4		
LIVRQ	40	0.75	0.03	4	***	0.001
LIVRQ	30	0.73	0.05	4	***	0.0004
LIVRQ	20	0.80	0.03	4	**	0.0054
LIVRQ	10	0.84	0.08	4	*	0.0208
LIVRQ	1	1.01	0.13	4		
RQNAC	40	0.51	0.07	4	****	0.0001
RQNAC	30	0.49	0.02	4	****	0.0001
RQNAC	20	0.59	0.04	4	****	0.0001
RQNAC	10	0.68	0.07	4	****	0.0001
RQNAC	1	1.00	0.11	4		
N-Acetyl Cysteine	40	0.76	0.06	4	**	0.0011
N-Acetyl Cysteine	20	1.02	0.08	4	ns	0.9921
N-Acetyl Cysteine	10	1.07	0.08	4	ns	0.5517
N-Acetyl Cysteine	5	1.00	0.08	4	ns	0.9999
N-Acetyl Cysteine	0	1.00	0.06	4		

Table 69. Fold change of Col1a1 gene expression after administration of a single amino acid composition, normalized to Gapdh expression in the first donor

Amino Acid Supplement	Conc. (μM)	Col1a1 Fold Expression Relative to Control				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	1.00	0.05	4	ns	0.9996
Valine	11710	1.09	0.17	4	ns	0.5528
Valine	4684	1.05	0.11	4	ns	0.8851
Valine	234	1.00	0.08	4		
Arginine	5440	1.12	0.18	4	ns	0.2151
Arginine	2720	1.03	0.03	4	ns	0.9625
Arginine	1088	0.99	0.06	4	ns	0.9989
Arginine	109	1.00	0.03	4		
Glutamine	22484	0.53	0.01	4	****	0.0001
Glutamine	11242	0.62	0.05	4	****	0.0001
Glutamine	3747	0.70	0.03	3	****	0.0001
Glutamine	749	1.00	0.07	4	ns	0.9999
Glutamine	562	1.00	0.07	3		
Isoleucine	6639	1.11	0.07	4	ns	0.7553
Isoleucine	3320	1.10	0.14	4	ns	0.7944
Isoleucine	1328	1.05	0.22	4	ns	0.9831
Isoleucine	66	1.01	0.21	4		
Leucine	15270	0.99	0.10	4	ns	0.994
Leucine	7635	1.12	0.16	4	ns	0.5049
Leucine	3054	1.11	0.15	4	ns	0.5499
Leucine	153	1.00	0.11	4		
N-Acetyl Cysteine	10000	0.76	0.06	4	**	0.0011
N-Acetyl Cysteine	5000	1.02	0.08	4	ns	0.9921
N-Acetyl Cysteine	2500	1.07	0.08	4	ns	0.5517
N-Acetyl Cysteine	1000	1.00	0.08	4	ns	0.9999
N-Acetyl Cysteine	0	1.00	0.06	4		

Table 69-1. Fold change of Col1a1 gene expression after administration of an amino acid composition, normalized to Gapdh expression in second donor.

Amino Acid Supplement	Conc. (X)	Col1a1 Fold Expression Relative to Control				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	0.72	0.05	4	****	0.0001

LIVRQNAC	30	0.72	0.02	4	****	0.0001
LIVRQNAC	20	0.70	0.03	4	****	0.0001
LIVRQNAC	10	0.71	0.08	4	****	0.0001
LIVRQNAC	1	1.00	0.02	4		
LIVRQNAC+G	40	0.60	0.09	4	****	0.0001
LIVRQNAC+G	30	0.68	0.07	4	***	0.0001
LIVRQNAC+G	20	0.71	0.09	4	***	0.0003
LIVRQNAC+G	10	0.69	0.06	4	***	0.0002
LIVRQNAC+G	1	1.00	0.07	4		
LIVRQNAC+S	40	0.66	0.02	4	****	0.0001
LIVRQNAC+S	30	0.69	0.06	4	****	0.0001
LIVRQNAC+S	20	0.76	0.05	4	***	0.0002
LIVRQNAC+S	10	0.77	0.04	4	***	0.0003
LIVRQNAC+S	1	1.00	0.11	4		
LIV	40	1.20	0.21	4	ns	0.1032
LIV	30	1.10	0.09	4	ns	0.6074
LIV	20	1.10	0.04	4	ns	0.6031
LIV	10	1.02	0.08	4	ns	0.9981
LIV	1	1.00	0.11	4		
LIVRQ	40	1.23	0.13	4	ns	0.1945
LIVRQ	30	1.12	0.13	4	ns	0.7176
LIVRQ	20	1.08	0.24	4	ns	0.8874
LIVRQ	10	1.14	0.16	4	ns	0.5632
LIVRQ	1	1.00	0.11	4		
RQNAC	40	0.54	0.03	4	****	0.0001
RQNAC	30	0.55	0.06	4	****	0.0001
RQNAC	20	0.58	0.04	4	****	0.0001
RQNAC	10	0.73	0.04	4	***	0.0007
RQNAC	1	1.01	0.16	4		
N-Acetyl Cysteine	40	0.57	0.06	4	****	0.0001
N-Acetyl Cysteine	20	0.69	0.06	4	****	0.0001
N-Acetyl Cysteine	10	0.69	0.09	4	***	0.0001
N-Acetyl Cysteine	5	0.69	0.05	4	***	0.0001
N-Acetyl Cysteine	0	1.00	0.10	4		

Table 69-2. Fold change of Col1a1 gene expression after administration of a single amino acid composition, normalized to Gapdh expression in second donor.

Amino Acid Supplement	Conc. (μM)	Col1a1 Fold Expression Relative to Control				
		Mean	Std. Deviation	Number of values	P-value*	Significance

Valine	23420	1.05	0.03	4	ns	0.9194
Valine	11710	0.98	0.11	4	ns	0.9827
Valine	4684	1.05	0.18	4	ns	0.8893
Valine	234	1.00	0.11	4		
Arginine	5440	1.15	0.10	4	ns	0.2773
Arginine	2720	1.15	0.14	4	ns	0.2759
Arginine	1088	0.99	0.15	4	ns	0.9938
Arginine	109	1.00	0.12	4		
Glutamine	22484	0.86	0.07	4	ns	0.1411
Glutamine	11242	0.91	0.09	4	ns	0.4365
Glutamine	3747	1.04	0.14	4	ns	0.9811
Glutamine	749	1.02	0.13	4	ns	0.9988
Glutamine	562	1.01	0.12	8		
Isoleucine	6639	1.03	0.07	4	ns	0.8931
Isoleucine	3320	0.99	0.08	4	ns	0.9841
Isoleucine	1328	0.97	0.10	4	ns	0.9157
Isoleucine	66	1.00	0.02	4		
Leucine	15270	1.13	0.14	4	ns	0.0811
Leucine	7635	1.05	0.05	4	ns	0.7277
Leucine	3054	1.06	0.03	4	ns	0.5342
Leucine	153	1.00	0.03	4		
N-Acetyl Cysteine	10000	0.57	0.06	4	****	0.0001
N-Acetyl Cysteine	5000	0.69	0.06	4	****	0.0001
N-Acetyl Cysteine	2500	0.69	0.09	4	***	0.0001
N-Acetyl Cysteine	1000	0.69	0.05	4	***	0.0001
N-Acetyl Cysteine	0	1.00	0.10	4		

Table 69-3. Fold change of Col1a1 gene expression after administration of an amino acid composition, normalized to Gapdh expression in third donor.

Amino Acid Supplement	Conc. (X)	Col1a1 Fold Expression Relative to Control				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	0.81	0.09	4	**	0.008
LIVRQNAC	30	0.70	0.06	4	***	0.0001
LIVRQNAC	20	0.79	0.08	4	**	0.0035
LIVRQNAC	10	0.79	0.07	4	**	0.0039
LIVRQNAC	1	1.00	0.06	4		
LIVRQNAC+G	40	0.63	0.10	4	***	0.0002
LIVRQNAC+G	30	0.64	0.02	4	***	0.0003
LIVRQNAC+G	20	0.75	0.14	4	**	0.005

LIVRQNAC+G	10	0.71	0.11	4	**	0.0017
LIVRQNAC+G	1	1.00	0.03	4		
LIVRQNAC+S	40	0.79	0.11	4	*	0.0316
LIVRQNAC+S	30	0.79	0.04	4	*	0.0309
LIVRQNAC+S	20	0.77	0.09	4	*	0.0208
LIVRQNAC+S	10	0.85	0.09	4	ns	0.1434
LIVRQNAC+S	1	1.01	0.16	4		
LIV	40	1.00	0.16	4	ns	0.9999
LIV	30	0.94	0.16	4	ns	0.8685
LIV	20	1.08	0.08	4	ns	0.6767
LIV	10	0.93	0.04	4	ns	0.7713
LIV	1	1.00	0.05	4		
LIVRQ	40	1.00	0.05	4	ns	0.9999
LIVRQ	30	1.07	0.13	4	ns	0.8753
LIVRQ	20	1.10	0.13	4	ns	0.6983
LIVRQ	10	1.05	0.21	4	ns	0.9641
LIVRQ	1	1.00	0.07	4		
RQNAC	40	0.64	0.05	4	***	0.0003
RQNAC	30	0.70	0.13	4	**	0.0018
RQNAC	20	0.66	0.05	4	***	0.0005
RQNAC	10	0.87	0.15	4	ns	0.2175
RQNAC	1	1.00	0.04	4		
N-Acetyl Cysteine	40	0.62	0.01	4	***	0.0005
N-Acetyl Cysteine	20	0.73	0.10	4	**	0.0083
N-Acetyl Cysteine	10	0.82	0.09	4	ns	0.0909
N-Acetyl Cysteine	5	0.91	0.12	4	ns	0.4954
N-Acetyl Cysteine	0	1.01	0.16	4		

Table 69-4. Fold change of Col1a1 gene expression after administration of a single amino acid composition, normalized to Gapdh expression in second donor.

Amino Acid Supplement	Conc. (μM)	Col1a1 Fold Expression Relative to Control				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	1.13	0.12	4	ns	0.7199
Valine	11710	1.27	0.31	4	ns	0.1735
Valine	4684	1.22	0.16	4	ns	0.3247
Valine	234	1.01	0.13	4		
Arginine	5440	1.02	0.09	4	ns	0.9702
Arginine	2720	0.99	0.09	4	ns	0.9973
Arginine	1088	0.95	0.02	4	ns	0.5384

Arginine	109	1.00	0.05	4		
Glutamine	22484	0.81	0.11	4	*	0.0113
Glutamine	11242	0.81	0.11	4	**	0.0087
Glutamine	3747	1.00	0.03	4	ns	0.9999
Glutamine	749	0.96	0.07	4	ns	0.8697
Glutamine	562	1.00	0.10	8		
Isoleucine	6639	1.03	0.04	4	ns	0.9974
Isoleucine	3320	0.94	0.13	4	ns	0.8329
Isoleucine	1328	0.94	0.17	4	ns	0.7947
Isoleucine	66	1.02	0.20	4		
Leucine	15270	1.07	0.12	4	ns	0.9535
Leucine	7635	1.00	0.16	4	ns	0.998
Leucine	3054	1.08	0.23	4	ns	0.9185
Leucine	153	1.01	0.19	4		
N-Acetyl Cysteine	10000	0.62	0.01	4	***	0.0005
N-Acetyl Cysteine	5000	0.73	0.10	4	**	0.0083
N-Acetyl Cysteine	2500	0.82	0.09	4	ns	0.0909
N-Acetyl Cysteine	1000	0.91	0.12	4	ns	0.4954
N-Acetyl Cysteine	0	1.01	0.16	4		

Procollagen Iα1 Secretion

Tables 70, 71, 71-1, 71-2, 71-3, and 71-4 show the fold change in procollagen Iα1 in primary human hepatic stellate cells from three different healthy donors normalized to their respective baseline amino acid conditions. Statistical significance calculated by one-way ANOVA with Dunnett's multiple comparison test within each treatment group. The combination LIV significantly increased procollagen Iα1 secretion in all three donors. The addition of arginine (R) and glutamine (Q) to a combination of LIV counteracted the profibrogenic effect of LIV alone. LIVRQNAC, LIVRQNAC+G, LIVRQNAC+S and RQNAC significantly decreased procollagen Iα1 secretion in all three donors. Individually, N-acetyl cysteine was shown to significantly decrease procollagen Iα1 secretion in two of the three donors. Valine significantly increased procollagen Iα1 secretion in only one of two donors, while isoleucine and arginine significantly increased procollagen Iα1 secretion in two of three donors. In other words, glutamine administered individually did not have a significant impact on procollagen Iα1 secretion. As such, the reduction of the profibrogenic effect of LIV with arginine and glutamine relative to that of LIV alone would not have been expected based on the effect of individual amino acid treatments.

Table 70. Fold change of procollagen 1α1 secretion after administration of an amino acid composition in a first donor

Amino Acid Supplement	Conc. (X)	Procollagen Iα1 Secretion (Fold Change of 1X)				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	0.6283	0.0585	3	***	0.0001
LIVRQNAC	30	0.5975	0.0709	3	****	0.0001
LIVRQNAC	20	0.6504	0.0622	4	***	0.0001
LIVRQNAC	10	0.8287	0.0936	4	*	0.0277
LIVRQNAC	1	1.0000	0.0908	4		
LIVRQNAC+G	40	0.5288	0.0402	3	***	0.0006
LIVRQNAC+G	30	0.6297	0.0200	3	**	0.0042
LIVRQNAC+G	20	0.5926	0.0634	4	**	0.001
LIVRQNAC+G	10	0.7404	0.0920	4	*	0.0267
LIVRQNAC+G	1	1.0000	0.2151	4		
LIVRQNAC+S	40	0.5900	0.0450	3	***	0.0003
LIVRQNAC+S	30	0.5562	0.1242	3	***	0.0002
LIVRQNAC+S	20	0.6844	0.0638	3	**	0.0022
LIVRQNAC+S	10	0.7003	0.0946	3	**	0.0032
LIVRQNAC+S	1	1.0000	0.0311	3		
LIV	40	1.3017	0.1474	3	ns	0.0518
LIV	30	1.3358	0.1922	3	*	0.0305
LIV	20	1.2592	0.0747	3	ns	0.0997
LIV	10	1.0149	0.1089	3	ns	0.9997
LIV	1	1.0000	0.0828	3		
LIVRQ	40	1.0070	0.1716	3	ns	0.9999
LIVRQ	30	1.0190	0.1103	3	ns	0.9983
LIVRQ	20	1.1403	0.0516	3	ns	0.3875
LIVRQ	10	1.0454	0.0908	3	ns	0.9609
LIVRQ	1	1.0000	0.0935	3		
RQNAC	40	0.3622	0.0166	3	****	0.0001
RQNAC	30	0.4232	0.0819	3	****	0.0001
RQNAC	20	0.5819	0.0574	3	***	0.0001
RQNAC	10	0.8181	0.0703	3	*	0.0313
RQNAC	1	1.0000	0.0967	3		
N-Acetyl Cysteine	40	0.5076	0.0154	3	****	0.0001
N-Acetyl Cysteine	20	0.6593	0.0914	3	***	0.0003

N-Acetyl Cysteine	10	0.7939	0.0715	3	**	0.01
N-Acetyl Cysteine	5	0.9175	0.0519	3	ns	0.3855
N-Acetyl Cysteine	0	1.0000	0.0686	3		

Table 71. Fold change of procollagen 1α1 secretion after administration of a single amino acid composition in the first donor

Amino Acid Supplement	Conc. (μM)	Procollagen Iα1 Secretion (Fold Change of 1X)				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	1.2139	0.0544	3	ns	0.1392
Valine	11710	1.2069	0.0881	3	ns	0.155
Valine	4684	1.1203	0.1908	3	ns	0.5111
Valine	234	1.0000	0.1389	4		
Arginine	5440	1.0646	0.0939	3	ns	0.4155
Arginine	2720	1.1757	0.0466	3	*	0.01
Arginine	1088	1.0291	0.0615	4	ns	0.8428
Arginine	109	1.0000	0.0389	4		
Glutamine	22484	1.0564	0.1293	3	ns	0.8468
Glutamine	11242	1.0888	0.0261	3	ns	0.5648
Glutamine	3747	1.0757	0.1003	4	ns	0.6356
Glutamine	749	0.9790	0.0836	4	ns	0.993
Glutamine	562	1.0000	0.0596	3		
Isoleucine	6639	1.2144	0.1129	3	ns	0.0537
Isoleucine	3320	1.1366	0.0938	3	ns	0.2411
Isoleucine	1328	0.9229	0.0614	3	ns	0.6321
Isoleucine	66	1.0000	0.0953	3		
Leucine	15270	1.1710	0.1043	3	ns	0.094
Leucine	7635	1.0915	0.0832	3	ns	0.4736
Leucine	3054	1.1410	0.1245	4	ns	0.1424
Leucine	153	1.0000	0.0481	4		

Table 71-1. Fold change of procollagen 1α1 secretion after administration of an amino acid composition in the second donor

Amino Acid Supplement	Conc. (X)	Procollagen Iα1 Secretion (Fold Change of 1X)				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	0.7465	0.0551	3	**	0.0041
LIVRQNAC	30	0.6829	0.0991	3	***	0.0007
LIVRQNAC	20	0.6922	0.0281	4	***	0.0004

LIVRQNAC	10	0.7879	0.0748	4	**	0.0085
LIVRQNAC	1	1.0000	0.1141	4		
LIVRQNAC+G	40	0.6372	0.0267	3	****	0.0001
LIVRQNAC+G	30	0.7347	0.0324	3	****	0.0001
LIVRQNAC+G	20	0.6716	0.0552	4	****	0.0001
LIVRQNAC+G	10	0.7823	0.0579	4	***	0.0001
LIVRQNAC+G	1	1.0000	0.0580	4		
LIVRQNAC+S	40	0.8756	0.0372	3	ns	0.1229
LIVRQNAC+S	30	0.7340	0.0432	3	**	0.0019
LIVRQNAC+S	20	0.7405	0.0491	3	**	0.0022
LIVRQNAC+S	10	0.7472	0.0710	3	**	0.0027
LIVRQNAC+S	1	1.0000	0.1031	3		
LIV	40	1.4409	0.0697	3	****	0.0001
LIV	30	1.3679	0.0156	3	***	0.0001
LIV	20	1.3418	0.1090	3	***	0.0002
LIV	10	1.2176	0.0343	3	**	0.0057
LIV	1	1.0000	0.0396	3		
LIVRQ	40	0.9851	0.0534	3	ns	0.9965
LIVRQ	30	1.0185	0.0735	3	ns	0.9921
LIVRQ	20	0.9212	0.0215	3	ns	0.4893
LIVRQ	10	0.9558	0.0580	3	ns	0.8556
LIVRQ	1	1.0000	0.1134	3		
RQNAC	40	0.6363	0.0432	3	***	0.0002
RQNAC	30	0.6154	0.0196	3	***	0.0001
RQNAC	20	0.7060	0.0851	3	***	0.0009
RQNAC	10	0.8385	0.0248	3	*	0.041
RQNAC	1	1.0000	0.1071	3		
N-Acetyl Cysteine	40	0.8383	0.0378	3	ns	0.4053
N-Acetyl Cysteine	20	0.7378	0.1347	3	ns	0.1002
N-Acetyl Cysteine	10	0.8877	0.2282	3	ns	0.6842
N-Acetyl Cysteine	5	0.8387	0.0832	3	ns	0.407
N-Acetyl Cysteine	0	1.0000	0.0808	3		

Table 71-2. Fold change of procollagen 1α1 secretion after administration of a single amino acid composition in the second donor

Amino Acid Supplement	Conc. (μM)	Procollagen Iα1 Secretion (Fold Change of 1X)				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	1.3068	0.0963	3	**	0.0019
Valine	11710	1.2877	0.1122	3	**	0.0029

Valine	4684	1.2865	0.0717	4	**	0.0018
Valine	234	1.0000	0.0589	4		
Arginine	5440	1.1304	0.0187	3	ns	0.0937
Arginine	2720	1.0722	0.0791	3	ns	0.4483
Arginine	1088	1.0126	0.0822	4	ns	0.989
Arginine	109	1.0000	0.0778	4		
Glutamine	22484	0.7143	0.0566	3	**	0.0058
Glutamine	11242	0.7080	0.0246	3	**	0.005
Glutamine	3747	0.7541	0.0860	4	*	0.0102
Glutamine	749	0.9191	0.1171	4	ns	0.5776
Glutamine	562	1.0000	0.1003	3		
Isoleucine	6639	1.5423	0.1489	3	**	0.006
Isoleucine	3320	1.4940	0.0238	3	*	0.0102
Isoleucine	1328	1.4811	0.2307	3	*	0.0117
Isoleucine	66	1.0000	0.1264	3		
Leucine	15270	0.9518	0.0406	3	ns	0.9292
Leucine	7635	1.2628	0.1763	3	ns	0.0607
Leucine	3054	1.0781	0.1735	4	ns	0.7374
Leucine	153	1.0000	0.0681	4		

Table 71-3. Fold change of procollagen 1α1 secretion after administration of an amino acid composition in the third donor

Amino Acid Supplement	Conc. (X)	Procollagen Iα1 Secretion (Fold Change of 1X)				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	0.9052	0.0344	3	ns	0.5685
LIVRQNAC	30	0.7456	0.0895	3	*	0.0192
LIVRQNAC	20	0.7817	0.0680	4	*	0.03
LIVRQNAC	10	0.9774	0.1451	4	ns	0.9927
LIVRQNAC	1	1.0000	0.1116	4		
LIVRQNAC+G	40	0.7040	0.0080	3	**	0.002
LIVRQNAC+G	30	0.6249	0.0819	3	***	0.0003
LIVRQNAC+G	20	0.6863	0.1334	4	***	0.0006
LIVRQNAC+G	10	1.0068	0.0642	4	ns	0.9998
LIVRQNAC+G	1	1.0000	0.0724	4		
LIVRQNAC+S	40	0.9190	0.0772	3	ns	0.3351
LIVRQNAC+S	30	0.8107	0.0596	3	*	0.0101
LIVRQNAC+S	20	0.8878	0.0129	3	ns	0.1296
LIVRQNAC+S	10	0.9814	0.0458	3	ns	0.9852

LIVRQNAC+S	1	1.0000	0.0780	3		
LIV	40	1.3233	0.0667	3	**	0.0024
LIV	30	1.2510	0.1070	3	*	0.0125
LIV	20	1.2702	0.0639	3	**	0.0079
LIV	10	1.1912	0.1049	3	ns	0.0532
LIV	1	1.0000	0.0521	3		
LIVRQ	40	1.2020	0.1119	3	ns	0.1081
LIVRQ	30	1.1380	0.0955	3	ns	0.3407
LIVRQ	20	0.9489	0.1179	3	ns	0.9263
LIVRQ	10	1.0786	0.0764	3	ns	0.7564
LIVRQ	1	1.0000	0.1056	3		
RQNAC	40	0.6590	0.0860	3	**	0.0012
RQNAC	30	0.6708	0.0407	3	**	0.0016
RQNAC	20	0.9135	0.1192	3	ns	0.5063
RQNAC	10	0.8783	0.0515	3	ns	0.245
RQNAC	1	1.0000	0.0740	3		
N-Acetyl Cysteine	40	0.6962	0.0189	3	*	0.0125
N-Acetyl Cysteine	20	0.8521	0.0709	3	ns	0.2666
N-Acetyl Cysteine	10	0.9391	0.1250	3	ns	0.8641
N-Acetyl Cysteine	5	1.0897	0.1245	3	ns	0.6511
N-Acetyl Cysteine	0	1.0000	0.1133	3		

Table 71-4. Fold change of procollagen 1α1 secretion after administration of a single amino acid composition in the third donor

Amino Acid Supplement	Conc. (μM)	Procollagen Iα1 Secretion (Fold Change of 1X)				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	1.1139	0.1077	3	ns	0.5315
Valine	11710	1.0498	0.1773	3	ns	0.918
Valine	4684	1.0428	0.1036	4	ns	0.9323
Valine	234	1.0000	0.1203	4		
Arginine	5440	1.2125	0.0862	3	*	0.0112
Arginine	2720	1.1314	0.0820	3	ns	0.1114
Arginine	1088	1.0623	0.0629	4	ns	0.5378
Arginine	109	1.0000	0.0760	4		
Glutamine	22484	1.0121	0.0730	3	ns	0.9989
Glutamine	11242	1.1204	0.1056	3	ns	0.2356
Glutamine	3747	0.9734	0.0900	4	ns	0.9747
Glutamine	749	1.0317	0.0644	4	ns	0.9538
Glutamine	562	1.0000	0.0447	3		

Isoleucine	6639	1.4465	0.0958	3	**	0.0014
Isoleucine	3320	1.2703	0.0352	3	*	0.024
Isoleucine	1328	1.2687	0.0374	3	*	0.0247
Isoleucine	66	1.0000	0.1629	3		
Leucine	15270	0.9892	0.0260	3	ns	0.9979
Leucine	7635	1.2027	0.0693	3	ns	0.0638
Leucine	3054	1.1399	0.1385	4	ns	0.1844
Leucine	153	1.0000	0.1077	4		

Example 13: Cytokine Secretion in Primary Human Macrophages

Isolation of Peripheral Blood Mononuclear Cell (PBMC)

Unpurified buffy coats (Research Blood Components) were carefully poured into 50mL centrifuge tubes and diluted with room temperature Dulbecco's Phosphate Buffered Saline (dPBS) with Calcium and Magnesium (Gibco). Diluted buffy coats were further divided into four total 50mL centrifuge tubes at 20mL per tube. Lymphocyte Separation Medium (Corning) was carefully pipetted to the bottom of each centrifuge tube. Mixtures were centrifuged at 850 X g for 32 minutes at 20°C with 0 deceleration and acceleration.

The PBMC layer was separated from other components after centrifugation and added to new 50mL centrifuge tube containing 25mL dPBS. Total volume was brought up to 50mL with dPBS and centrifuged at 600 X g for 10 minutes at 20°C with acceleration of 9, deceleration of 5. Supernatant was carefully removed from cell pellets. The cell pellets were resuspended using 10mL dPBS. Total volume was then brought up to 50mL using dPBS and centrifuged at 450 X g for 5 min at 20°C with acceleration of 9, deceleration of 9. The supernatant removal and cell pellet resuspension was repeated again.

The supernatant was then carefully removed from cell pellets. Cell pellets were resuspended in 10mL dPBS without calcium or magnesium and filtered through a 70 uM cell strainer. The total PBMC number was determined using a Cellometer K2 automated cell counter. A total of 5E6 cells were saved for flow cytometric analysis. Remaining cells were centrifuged at 490 X g for 5 minutes at 20°C with acceleration of 9, deceleration of 9.

CD14+ Cell Selection

CD14+ cells were selected using EasySep™ Human CD14 Positive Selection Kit II (STEMCELL Technologies). Cells were resuspended in cold EasySep™ Buffer (STEMCELL Technologies) at 1X10^8 cells/mL. A total of 100 uL/mL EasySep™ Human CD14 Positive Selection Cocktail II was added to the cell suspension, mixed, and incubated at room temperature for 10 minutes. A total of 100 uL/mL RapidSpheres were added to the mixture and incubated at room temperature for 3 minutes after mixing, then RoboSep buffer was added to bring up the total volume to 10mL. The mixture in a 15 mL tube was placed in magnet and incubated at room temperature for 3 minutes. Supernatant was discarded and 10 mL fresh EasySep™ buffer was added to 15mL tube. The addition of RoboSep buffer, mixing, and discarding of supernatant was was repeated two more times.

Negative and positive fractions were centrifuged at 490 X g for 5 minutes at 20°C with acceleration of 9, deceleration of 9, and resuspended in DMEM (Gibco) and 10% Heat Inactivated Fetal Bovine Serum (Atlanta Bio) and Penicillin/Streptomycin. Cells were counted and centrifuged again at 490 X g for 5 minutes at 20°C with acceleration of 9, deceleration of 9. After centrifugation, cell were resuspended in DMEM (Gibco) and 10% Heat Inactivated Fetal Bovine Serum (Atlanta Bio) and Penicillin/Streptomycin containing 500 U/mL GM- and plated at 1-2 X 10^6 cells/mL on 10cm tissue culture plates. Cells were kept in 37°C, 5%CO2 in between feedings/harvest.

CD14+ Cell Feeding

Cells were fed every 3-4 days by removing media and unattached cells, centrifuging at 490 X g for 5 minutes at 20C with acceleration of 9, deceleration of 9, and resuspending in fresh DMEM (Gibco) and 10% Heat Inactivated Fetal Bovine Serum (Atlanta Bio) and Penicillin/Streptomycincontaining 500 U/mL GM-CSF. Resuspended cells were seeded back onto 10cm tissue culture plates and incubated at 37°C, 5%CO2.

Macrophage Harvest

After complete cell attachment, culture supernatant was removed and cultures were washed 1X with 5mL PBS. A total of 3mL room temperature Cellstripper was added and cultures were incubated at 37°C, 5% CO2 for approximately 10 minutes until cells were rounded and beginning to detach. Cell scraper was used to completely detach cells from plate. Collected cell were spun down at 490g for 5 min at room temperature and resuspended in 10% DMSO in Heat Inactivated Fetal Bovine Serum and immediately frozen in -80C.

Screen

Primary human PMBC derived macrophages were seeded on day 0 at 3.0E4 cells per well in 96-well microplates (ThermoFisher) in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with penicillin-streptomycin (Hyclone) and 10% heat inactivated fetal bovine serum (HI-FBS) (Atlanta Bio) and incubated overnight at 37°C, 5% CO2. On day 1, cells were washed once with 150uL per well DPBS (Gibco) and treated with 75uL of:

a. Amino acid free DMEM (US Biologicals) containing a defined custom amino acid concentration based on the mean physiological concentrations in blood based on values published in the Human Metabolome Database (HMDB), with 6mM glucose, 1mM sodium pyruvate, 10 mM HEPES, 0.2% primocin (InVivoGen); or
b. The same medium described above with one amino acid at various concentrations including complete dropout.

On day 2, cells were treated with 75uL of the same mediums described above supplemented with 0.30ng/mL lipopolysaccharide (LPS) (Sigma) for a final concentration of 0.15ng/mL LPS. Control wells were treated with 1uM BX-795 (Tocis), 1uM TAK242 (Sigma), 0.15ng/mL LPS, or phosphate buffered saline (PBS).

On day 3, the supernatant was collected and immediately frozen in -80°C freezer. Cells were washed once with 150uL DPBS and viability was assessed using the WST-8 Cell Proliferation Cytotoxicity Assay (Dojindo). Following the assay, cells were washed twice with 150uL PBS and fixed with 4% paraformaldehyde for 5 min followed by two additional washes with 150uL PBS. Protein levels in supernatant samples were analyzed by ELISA for IL-6 and TNFa using commercially available kits (R&D Systems) according to manufacturer-supplied protocols. Results are shown in Tables 71-5 through 71-10 below.

Table 71-5. IL-6 Measurements: Donor 1

Amino Acid Supplement	Conc. (X)	Donor 1 IL-6 Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	-619.787	114.1592	3	0.0001	****

LIVRQNAC	30	-525.849	63.87122	3	0.0001	****
LIVRQNAC	20	-290.199	33.5584	3	0.0092	**
LIVRQNAC	10	51.81434	183.3933	3	0.9479	ns
LIVRQNAC	1	0	148.7761	3	na	na
LIVRQNAC+G	40	-1099.11	44.1139	3	0.0001	****
LIVRQNAC+G	30	-903.836	107.7113	3	0.0001	****
LIVRQNAC+G	20	-616.626	114.7826	3	0.0001	****
LIVRQNAC+G	10	-367.918	98.44611	3	0.0007	***
LIVRQNAC+G	1	0	172.9553	3	na	na
LIVRQNAC+S	40	-968.997	90.53282	3	0.0001	****
LIVRQNAC+S	30	-798.326	52.89122	3	0.0001	****
LIVRQNAC+S	20	-506.804	63.85224	3	0.0001	****
LIVRQNAC+S	10	-243.259	114.742	3	0.0365	*
LIVRQNAC+S	1	0	259.8506	3	na	na
LIV	40	4.918642	62.7077	3	0.9999	ns
LIV	30	86.01907	128.1151	3	0.7604	ns
LIV	20	112.1501	83.62436	3	0.564	ns
LIV	10	54.22668	63.10515	3	0.9392	ns
LIV	1	0	75.98804	3	na	na
LIVRQ	40	322.0706	73.87715	3	0.0033	**
LIVRQ	30	297.8004	34.60168	3	0.0072	**
LIVRQ	20	604.021	203.8836	3	0.0001	****
LIVRQ	10	289.1798	57.78952	3	0.0095	**
LIVRQ	1	0	93.58494	3	na	na
RQNAC	40	-911.011	12.65475	3	0.0001	****
RQNAC	30	-766.912	26.23659	3	0.0001	****
RQNAC	20	-511.403	32.15983	3	0.0001	****
RQNAC	10	-201.63	6.477522	3	0.1054	ns
RQNAC	1	0	174.9658	3	na	na
N-Acetyl Cysteine	40	-914.194	56.77271	3	0.0001	****
N-Acetyl Cysteine	20	-553.802	85.27013	3	0.0001	****
N-Acetyl Cysteine	10	-121.142	53.05191	3	0.4973	ns
N-Acetyl Cysteine	5	308.1772	263.4651	3	0.0052	**
N-Acetyl Cysteine	0	0	45.08485	3	na	na

Amino Acid Supplement	Conc. (μM)	Donor 1 IL-6 Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	-106.268	155.3559	3	0.7885	ns
Valine	11710	-97.25	77.26313	3	0.8339	ns
Valine	4684	-85.9843	74.99317	3	0.8841	ns

Valine	234	0	124.8497	3	na	na
Arginine	5440	357.4394	154.8508	3	0.0159	*
Arginine	2720	-186.57	85.86105	3	0.3477	ns
Arginine	1088	-181.36	131.6475	3	0.3722	ns
Arginine	109	0	282.0306	3	na	na
Glutamine	22484	440.1437	114.443	3	0.0022	**
Glutamine	11242	397.1745	23.36272	3	0.0064	**
Glutamine	3747	291.5443	81.30853	3	0.0623	ns
Glutamine	749	0	73.06692	3	na	na
Isoleucine	6639	-218.332	146.5098	3	0.221	ns
Isoleucine	3320	-15.8843	89.88616	3	0.9998	ns
Isoleucine	1328	25.98372	323.6109	3	0.9984	ns
Isoleucine	66	0	48.21125	3	na	na
Leucine	15270	84.46122	68.15253	3	0.8902	ns
Leucine	7635	-69.9873	99.00843	3	0.9398	ns
Leucine	3054	244.9743	355.6551	3	0.1442	ns
Leucine	153	0	61.85589	3	na	na

Treatment with LIVRQNAC, LIVRQNAC + G, LIVRQNAC + S, RQNAC, and NAC significantly reduced LPS-induced IL-6 secretion in primary human monocyte-derived macrophages. Treatment with LIVRQ significantly increased IL-6 secretion, while LIV had no effect. Arginine and glutamine administered alone increased IL-6 secretion while other amino acids alone did not effect IL-6 secretion. Two Way ANOVA Dunnett Multiple Comparisons was performed for statistical analysis. Mean values represented as baseline subtracted values.

Table 71-6. IL-6 Measurements: Donor 2

Amino Acid Supplement	Conc. (X)	Donor 2 IL-6 Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	-27.1916	1.853569	3	0.0003	***
LIVRQNAC	30	-21.5766	1.709414	3	0.0045	**
LIVRQNAC	20	-8.20655	8.458638	3	0.5143	ns
LIVRQNAC	10	-1.71581	6.104437	3	0.9965	ns
LIVRQNAC	1	-2.4E-15	11.85079	3		
LIVRQNAC+G	40	-33.2001	3.55425	3	0.0001	****
LIVRQNAC+G	30	-30.8468	0.854995	3	0.0001	****
LIVRQNAC+G	20	-18.4318	4.870421	3	0.0187	*
LIVRQNAC+G	10	14.63551	21.82024	3	0.0824	ns

LIVRQNAC+G	1	2.37E-15	8.607557	3		
LIVRQNAC+S	40	-26.5993	2.963677	3	0.0004	***
LIVRQNAC+S	30	-14.2166	1.460268	3	0.0954	ns
LIVRQNAC+S	20	-8.2522	2.917345	3	0.5095	ns
LIVRQNAC+S	10	8.127841	1.783214	3	0.5227	ns
LIVRQNAC+S	1	0	6.232673	3		
LIV	40	34.10306	1.950493	3	0.0001	****
LIV	30	31.10835	9.757211	3	0.0001	****
LIV	20	20.32684	3.17293	3	0.0081	**
LIV	10	15.10204	9.179111	3	0.0697	ns
LIV	1	-7.1E-15	4.738966	3		
LIVRQ	40	49.62156	17.37012	3	0.0001	****
LIVRQ	30	42.9625	7.798872	3	0.0001	****
LIVRQ	20	48.38603	13.08566	3	0.0001	****
LIVRQ	10	45.99191	15.19687	3	0.0001	****
LIVRQ	1	1.18E-15	6.324379	3		
RQNAC	40	-36.5521	1.877658	3	0.0001	****
RQNAC	30	-26.3768	0.744676	3	0.0004	***
RQNAC	20	-18.7428	1.353649	3	0.0164	*
RQNAC	10	-3.74427	4.74578	3	0.9393	ns
RQNAC	1	2.37E-15	12.26314	3		
N-Acetyl Cysteine	40	-33.7585	0.895842	3	0.0001	****
N-Acetyl Cysteine	20	-24.9999	1.083467	3	0.0008	***
N-Acetyl Cysteine	10	-9.75111	2.381012	3	0.3617	ns
N-Acetyl Cysteine	5	-0.79458	5.988677	3	0.9998	ns
N-Acetyl Cysteine	0	-2.4E-15	1.900091	3		

Amino Acid Supplement	Conc. (µM)	Donor 2 IL-6 Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	4.395899	10.35903	3	0.973	ns
Valine	11710	-1.19605	7.303571	3	0.9998	ns
Valine	4684	-4.52846	4.069907	3	0.97	ns
Valine	234	-4.7E-15	9.361734	3		
Arginine	5440	-12.4164	0.292618	3	0.5017	ns
Arginine	2720	-13.6102	2.1177	3	0.4207	ns
Arginine	1088	-9.70116	9.286942	3	0.6995	ns
Arginine	109	2.37E-15	14.30728	3		
Glutamine	22484	34.38845	7.467725	3	0.0026	**
Glutamine	11242	63.31441	35.02748	3	0.0001	****
Glutamine	3747	22.51543	9.686139	3	0.0721	ns

Glutamine	749	2.37E-15	2.203881	3		
Isoleucine	6639	-1.77438	10.22772	3	0.999	ns
Isoleucine	3320	2.305485	1.328015	3	0.9975	ns
Isoleucine	1328	-2.31776	9.121049	3	0.9974	ns
Isoleucine	66	0	12.3413	3		
Leucine	15270	47.59735	16.64049	3	0.0001	****
Leucine	7635	30.46065	7.144005	3	0.0087	**
Leucine	3054	29.60609	13.39676	3	0.0111	*
Leucine	153	7.11E-15	6.308577	3		

Treatment with LIVRQNAC, LIVRQNAC + G, LIVRQNAC + S, RQNAC, and NAC significantly reduced LPS-induced IL-6 secretion in primary human monocyte-derived macrophages. Treatment with LIVRQ and LIV significantly increased IL-6 secretion. Glutamine and leucine administered alone increased IL-6 secretion, while the other amino acids alone had no effect. Two Way ANOVA Dunnett Multiple Comparisons was performed for statistical analysis. Mean values represented as baseline subtracted values.

Table 71-7. IL-6 Measurements: Donor 3

Amino Acid Supplement	Conc. (X)	Donor 3 IL-6 Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	-18.2445	4.129349	3	0.7529	ns
LIVRQNAC	30	-16.8219	1.366045	3	0.8001	ns
LIVRQNAC	20	-13.4826	12.48206	3	0.8948	ns
LIVRQNAC	10	-34.4539	37.38053	3	0.2356	ns
LIVRQNAC	1	-1.4E-14	14.03982	3		
LIVRQNAC+G	40	-54.4799	5.467815	3	0.0228	*
LIVRQNAC+G	30	-48.3118	1.960574	3	0.0513	ns
LIVRQNAC+G	20	-55.792	7.763897	3	0.019	*
LIVRQNAC+G	10	-44.8309	14.34972	3	0.0783	ns
LIVRQNAC+G	1	0	26.01471	3		
LIVRQNAC+S	40	-14.5337	15.82418	3	0.868	ns
LIVRQNAC+S	30	-25.9127	10.00119	3	0.479	ns
LIVRQNAC+S	20	-25.8862	21.61536	3	0.48	ns
LIVRQNAC+S	10	-11.9742	10.3333	3	0.9277	ns
LIVRQNAC+S	1	-4.3E-14	15.34164	3		
LIV	40	10.21257	37.58938	3	0.9576	ns
LIV	30	-32.6891	24.862	3	0.2771	ns

LIV	20	27.66715	39.40901	3	0.4207	ns
LIV	10	9.44789	71.20002	3	0.9677	ns
LIV	1	-4.7E-14	27.50075	3		
LIVRQ	40	74.9145	12.55033	3	0.001	***
LIVRQ	30	120.1764	20.21514	3	0.0001	****
LIVRQ	20	77.12007	11.45452	3	0.0007	***
LIVRQ	10	67.95483	43.58345	3	0.003	**
LIVRQ	1	-2.4E-14	27.62048	3		
RQNAC	40	-45.9765	5.740028	3	0.0683	ns
RQNAC	30	-53.3845	16.45009	3	0.0265	*
RQNAC	20	-65.6761	3.400465	3	0.0044	**
RQNAC	10	-32.8776	33.99103	3	0.2724	ns
RQNAC	1	-2.8E-14	23.14404	3		
N-Acetyl Cysteine	40	-140.851	4.662272	3	0.0001	****
N-Acetyl Cysteine	20	-122.656	8.219985	3	0.0001	****
N-Acetyl Cysteine	10	-103.586	28.4385	3	0.0001	****
N-Acetyl Cysteine	5	-70.3269	8.563896	3	0.0021	**
N-Acetyl Cysteine	0	-9.5E-15	11.75797	3		

Amino Acid Supplement	Conc. (µM)	Donor 3 IL-6 Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	-29.2004	25.98066	3	0.4329	ns
Valine	11710	-43.8022	8.331697	3	0.1239	ns
Valine	4684	-30.0609	8.478329	3	0.4072	ns
Valine	234	4.26E-14	17.2027	3		
Arginine	5440	-6.80983	0.643932	3	0.9922	ns
Arginine	2720	-7.50318	22.06663	3	0.9888	ns
Arginine	1088	31.5786	70.48311	3	0.3642	ns
Arginine	109	0	17.26952	3		
Glutamine	22484	108.5158	55.59202	3	0.0001	****
Glutamine	11242	98.4903	58.37	3	0.0001	****
Glutamine	3747	25.35457	16.40416	3	0.556	ns
Glutamine	749	3.79E-14	16.54987	3		
Isoleucine	6639	-16.3663	8.09174	3	0.9718	ns
Isoleucine	3320	0	19.80362	3	0.9928	ns
Isoleucine	1328	-28.9897	13.10903	3	0.6593	ns
Isoleucine	66	-6.69039	13.72995	3		
Leucine	15270	#	#	3	#	#
Leucine	7635	#	#	3	#	#
Leucine	3054	#	#	3	#	#

Leucine	153	#	#	3	#	#
#	Leucine was not measured in Exp3 due to technical error					

Treatment with LIVRQNAC + G, LIVRQNAC + S, RQNAC, and NAC significantly reduced LPS-induced IL-6 secretion in primary human monocyte-derived macrophages. Treatment with LIVRQ increased IL-6 secretion, while LIV and LIVRQNAC had no statistically significant effects on IL-6 secretion. Glutamine administered alone significantly increased IL-6 secretion, while other amino acids alone had no effect. Two Way ANOVA Dunnett Multiple Comparisons was performed for statistical analysis. Mean values represented as baseline subtracted values.

Table 71-8. TNFalpha Measurements: Donor 1

Amino Acid Supplement	Conc. (X)	Donor 1 TNFa Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	-422.74	4.347575	3	0.0001	****
LIVRQNAC	30	-389.74	1.004633	3	0.0001	****
LIVRQNAC	20	-336.69	3.007435	3	0.0001	****
LIVRQNAC	10	-246.04	27.61929	3	0.0001	****
LIVRQNAC	1	0	36.31082	3		
LIVRQNAC+G	40	-490.92	4.427614	3	0.0001	****
LIVRQNAC+G	30	-447.73	9.819865	3	0.0001	****
LIVRQNAC+G	20	-377.32	5.837159	3	0.0001	****
LIVRQNAC+G	10	-268.29	9.642365	3	0.0001	****
LIVRQNAC+G	1	0	37.44353	3		
LIVRQNAC+S	40	-415.03	4.800449	3	0.0001	****
LIVRQNAC+S	30	-379.44	4.694868	3	0.0001	****
LIVRQNAC+S	20	-323.77	7.971135	3	0.0001	****
LIVRQNAC+S	10	-209.59	21.15676	3	0.0001	****
LIVRQNAC+S	1	0	30.0492	3		
LIV	40	60.37	20.26331	3	0.0065	**
LIV	30	42.09	22.95664	3	0.0865	ns
LIV	20	63.37	37.24144	3	0.004	**
LIV	10	45.61	44.71078	3	0.0556	ns
LIV	1	0	10.49958	3		
LIVRQ	40	6.38	17.1283	3	0.9909	ns
LIVRQ	30	-6.72	18.9622	3	0.989	ns
LIVRQ	20	38.38	39.85515	3	0.1333	ns
LIVRQ	10	-18.95	10.84371	3	0.6982	ns

LIVRQ	1	0	36.96184	3		
RQNAC	40	-408.44	1.179877	3	0.0001	****
RQNAC	30	-390.41	1.341282	3	0.0001	****
RQNAC	20	-338.2	3.284307	3	0.0001	****
RQNAC	10	-251.35	4.121085	3	0.0001	****
RQNAC	1	0	51.06933	3		
N-Acetyl Cysteine	40	-644.49	2.42197	3	0.0001	****
N-Acetyl Cysteine	20	-561.33	8.435064	3	0.0001	****
N-Acetyl Cysteine	10	-446.88	12.22132	3	0.0001	****
N-Acetyl Cysteine	5	-326.24	11.10173	3	0.0001	****
N-Acetyl Cysteine	0	0	42.00516	3		

Amino Acid Supplement	Conc. (μM)	Donor 1 TNFa Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	-14.98	20.86784	3	0.9928	ns
Valine	11710	-41.77	36.61662	3	0.7784	ns
Valine	4684	-40.37	32.31016	3	0.7974	ns
Valine	234	0	24.8661	3		
Arginine	5440	62.06	48.80326	3	0.4786	ns
Arginine	2720	5.12	15.47951	3	0.9998	ns
Arginine	1088	-24.33	17.74317	3	0.9577	ns
Arginine	109	0	18.5366	3		
Glutamine	22484	-103.07	27.02483	3	0.0985	ns
Glutamine	11242	-65.24	23.02631	3	0.4346	ns
Glutamine	3747	-45.7	28.56445	3	0.7222	ns
Glutamine	749	0	30.75138	3		
Isoleucine	6639	-40.95	78.56369	3	0.7896	ns
Isoleucine	3320	-96.3	45.66981	3	0.1339	ns
Isoleucine	1328	-42.68	21.07739	3	0.7657	ns
Isoleucine	66	0	115.9559	3		
Leucine	15270	-46.21	29.00402	3	0.7148	ns
Leucine	7635	-23.04	40.08864	3	0.965	ns
Leucine	3054	42.04	77.19161	3	0.7746	ns
Leucine	153	0	157.6578	3		

Treatment with LIVRQNAC, LIVRQNAC + G, LIVRQNAC + S, RQNAC, and NAC significantly reduced LPS-induced TNFa secretion in primary human monocyte-derived macrophages. Treatment with LIV increased TNFa secretion, while LIVRQ had no signficiant effects on TNFa secretion. None of the individually administered amino acids had an effect on

TNFa secretion. Two Way ANOVA Dunnett Multiple Comparisons was performed for statistical analysis. Mean values represented as baseline subtracted values.

Table 71-9. TNFalpha Measurements: Donor 2

Amino Acid Supplement	Conc. (X)	Donor 2 TNFa Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
LIVRQNAC	40	-98.1341	2.118962	3	0.0001	****
LIVRQNAC	30	-85.1019	1.385677	3	0.0001	****
LIVRQNAC	20	-64.3364	10.07525	3	0.0001	****
LIVRQNAC	10	-38.3512	5.120689	3	0.0001	****
LIVRQNAC	1	0	5.45587	3		
LIVRQNAC+G	40	-91.3454	5.994009	3	0.0001	****
LIVRQNAC+G	30	-82.4397	4.200763	3	0.0001	****
LIVRQNAC+G	20	-61.247	8.702492	3	0.0001	****
LIVRQNAC+G	10	-23.9913	7.471422	3	0.008	**
LIVRQNAC+G	1	-4.7E-15	4.578295	3		
LIVRQNAC+S	40	-74.1572	4.163823	3	0.0001	****
LIVRQNAC+S	30	-64.0016	5.549308	3	0.0001	****
LIVRQNAC+S	20	-47.5673	3.970363	3	0.0001	****
LIVRQNAC+S	10	-28.635	7.390447	3	0.0012	**
LIVRQNAC+S	1	-4.7E-15	7.564883	3		
LIV	40	49.84155	4.092799	3	****	0.0001
LIV	30	29.1118	14.72509	3	***	0.001
LIV	20	30.17595	5.797518	3	***	0.0006
LIV	10	16.68974	10.85983	3	ns	0.0974
LIV	1	0	10.41523	3		
LIVRQ	40	64.1705	27.82953	3	****	0.0001
LIVRQ	30	50.92104	6.955429	3	****	0.0001
LIVRQ	20	45.65882	19.0128	3	****	0.0001
LIVRQ	10	32.37038	19.44425	3	***	0.0002
LIVRQ	1	-4.7E-15	5.942707	3		
RQNAC	40	-84.147	5.821583	3	****	0.0001
RQNAC	30	-77.9626	1.626776	3	****	0.0001
RQNAC	20	-63.3754	3.494595	3	****	0.0001
RQNAC	10	-37.6072	1.88043	3	****	0.0001
RQNAC	1	-9.5E-15	4.727924	3		
N-Acetyl Cysteine	40	-103.984	0.720962	3	0.0001	****
N-Acetyl Cysteine	20	-88.6528	0.668195	3	0.0001	****
N-Acetyl Cysteine	10	-70.8382	12.08717	3	0.0001	****

N-Acetyl Cysteine	5	-54.1596	11.06287	3	0.0001	****
N-Acetyl Cysteine	0	9.47E-15	2.926881	3		

Amino Acid Supplement	Conc. (μM)	Donor 2 TNFa Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	-1.25079	12.85688	3	0.9991	ns
Valine	11710	-0.83505	8.524018	3	0.9998	ns
Valine	4684	-0.00221	5.127759	3	0.9999	ns
Valine	234	-4.7E-15	8.717375	3		
Arginine	5440	-0.57378	8.672536	3	0.9999	ns
Arginine	2720	-3.76334	2.467885	3	0.9594	ns
Arginine	1088	-12.7222	4.764842	3	0.2488	ns
Arginine	109	1.42E-14	3.511446	3		
Glutamine	22484	11.50181	6.216029	3	0.3311	ns
Glutamine	11242	20.03996	11.90208	3	0.0279	*
Glutamine	3747	9.338214	9.748253	3	0.5134	ns
Glutamine	749	-9.5E-15	7.275868	3		
Isoleucine	6639	19.25756	5.097831	3	0.0365	*
Isoleucine	3320	10.26061	7.861148	3	0.4307	ns
Isoleucine	1328	2.918887	1.921961	3	0.9836	ns
Isoleucine	66	4.74E-15	6.264135	3		
Leucine	15270	46.68507	11.63209	3	0.0001	****
Leucine	7635	41.97528	6.512087	3	0.0001	****
Leucine	3054	31.74019	11.56537	3	0.0002	***
Leucine	153	0	0.482598	3		

Treatment with LIVRQNAC, LIVRQNAC + G, LIVRQNAC + S, RQNAC, and NAC significantly reduced LPS-induced TNFa secretion in primary human monocyte-derived macrophages. Treatment with LIV and LIVRQ increased TNFa secretion. Leucine, isoleucine, and glutamine administered individually increased TNFa secretion, while the other amino acids had no effect. Two Way ANOVA Dunnett Multiple Comparisons was performed for statistical analysis. Mean values represented as baseline subtracted values.

Table 71-10. TNFalpha Measurements: Donor 3

Amino Acid Supplement	Conc. (X)	Donor 3 TNFa Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance

LIVRQNAC	40	-18.7507	2.487301	3	0.0001	****
LIVRQNAC	30	-15.5979	0.932399	3	0.0006	***
LIVRQNAC	20	-10.7042	3.013527	3	0.026	*
LIVRQNAC	10	-8.49034	2.434812	3	0.1029	ns
LIVRQNAC	1	0	4.067982	3		
LIVRQNAC+G	40	-14.6552	3.149813	3	0.0013	**
LIVRQNAC+G	30	-11.6973	2.026588	3	0.0129	*
LIVRQNAC+G	20	-8.0218	0.671662	3	0.1331	ns
LIVRQNAC+G	10	-4.8035	1.658348	3	0.5453	ns
LIVRQNAC+G	1	-2.4E-15	5.625453	3		
LIVRQNAC+S	40	-14.247	1.800575	3	0.0018	**
LIVRQNAC+S	30	-15.1388	1.568817	3	0.0009	***
LIVRQNAC+S	20	-12.4722	3.334857	3	0.0073	**
LIVRQNAC+S	10	-6.72057	1.833554	3	0.2549	ns
LIVRQNAC+S	1	0	4.171555	3		
LIV	40	14.07984	11.14252	3	0.002	**
LIV	30	1.759786	1.102706	3	0.9748	ns
LIV	20	14.51396	10.41503	3	0.0014	**
LIV	10	8.560957	12.86074	3	0.0989	ns
LIV	1	2.37E-15	3.660423	3		
LIVRQ	40	25.84453	0.659584	3	0.0001	****
LIVRQ	30	33.74883	5.974096	3	0.0001	****
LIVRQ	20	20.94481	2.163828	3	0.0001	****
LIVRQ	10	15.45187	3.942596	3	0.0007	***
LIVRQ	1	0	4.575346	3		
RQNAC	40	-21.5102	1.191926	3	0.0001	****
RQNAC	30	-20.8898	2.622446	3	0.0001	****
RQNAC	20	-19.9558	3.302225	3	0.0001	****
RQNAC	10	-9.09425	5.483242	3	0.0725	ns
RQNAC	1	0	6.189505	3		
N-Acetyl Cysteine	40	-55.3093	0.809363	3	0.0001	****
N-Acetyl Cysteine	20	-48.4373	1.563179	3	0.0001	****
N-Acetyl Cysteine	10	-41.7266	3.533914	3	0.0001	****
N-Acetyl Cysteine	5	-33.6246	0.253484	3	0.0001	****
N-Acetyl Cysteine	0	4.74E-15	8.55997	3		

Amino Acid Supplement	Conc. (μM)	Donor 3 TNFa Measurements				
		Mean	Std. Deviation	Number of values	P-value*	Significance
Valine	23420	3.688279	7.532913	3	0.8962	ns
Valine	11710	-2.59866	2.586099	3	0.9674	ns

Valine	4684	0.126	0.903014	3	0.9999	ns
Valine	234	-2.4E-15	2.731283	3		
Arginine	5440	-1.76662	4.067694	3	0.992	ns
Arginine	2720	-0.96691	4.86075	3	0.9991	ns
Arginine	1088	3.131153	10.346	3	0.9384	ns
Arginine	109	3.55E-15	4.325877	3		
Glutamine	22484	29.14034	17.71417	3	0.0001	****
Glutamine	11242	18.00238	14.58602	3	0.0061	**
Glutamine	3747	1.935546	2.127977	3	0.9887	ns
Glutamine	749	0	5.196592	3		
Isoleucine	6639	-1.66019	4.262718	3	0.9938	ns
Isoleucine	3320	3.308901	3.745411	3	0.9262	ns
Isoleucine	1328	-6.22991	0.48195	3	0.5976	ns
Isoleucine	66	-2.4E-15	3.844593	3		
Leucine	15270	#	#	3	#	#
Leucine	7635	#	#	3	#	#
Leucine	3054	#	#	3	#	#
Leucine	153	#	#	3	#	#

Leucine was not measured in Exp3 due to technical error

Treatment with LIVRQNAC, LIVRQNAC + G, LIVRQNAC + S, RQNAC, and NAC significantly reduced LPS-induced TNFa secretion in primary human monocyte-derived macrophages. Treatment with LIV and LIVRQ increased TNFa secretion. Individually administered amino acids had no significant effect on TNFa secretion, except for glutamine which increased TNFa secretion. Two Way ANOVA Dunnett Multiple Comparisons was performed for statistical analysis. Mean values represented as baseline subtracted values.

Example 14: Treatment of NAFLD Patients with an Amino Acid Composition

The study described herein features the administration of a composition including amino acids to treat patients with NAFLD. The composition can include about 2 g of leucine, about 1 g of isoleucine, about 1 g of valine, about 3.62 g of arginine, about 4 g of glutamine, and about 0.3 g of N-acetylcysteine for administration three times per day (e.g., a total of about 36 g per day). The composition can also include about 2 g of leucine, about 1 g of isoleucine, about 1 g of valine, about 3.62 g of arginine, about 4 g of glutamine, and about 0.6 g of N-acetylcysteine for administration three times per day (e.g., a total of about 37 g per day).

Alternatively, the composition can include about 4 g of leucine, about 2 g of isoleucine, about 2 g of valine, about 7.24 g of arginine, about 8 g of glutamine, and about 0.6 g of N-acetylcysteine for administration two or three times per day (e.g., a total of about 48 g or a total of about 72 g per day). The composition can also include about 4 g of leucine, about 2 g of isoleucine, about 2 g of valine, about 7.24 g of arginine, about 8 g of glutamine, and about 1.2 g of N-acetylcysteine for administration two or three times per day (e.g., a total of about 49 g or a total of about 73 g per day).

For each composition, the dose can be administered prior to, concurrently with, or following a meal. Alternatively, the composition is not administered immediately before, with, or after a meal. The amino acid composition can be administered for a period of at least 12 weeks, e.g., for 12 weeks, 13 weeks, 14 weeks, 15 weeks, or 16 weeks. In particular, the amino acid composition is administered for a period of at least 16 weeks, e.g., for 16 weeks. The composition can be administered orally.

Key criteria for selecting NAFLD patients for enrollment in a clinical study of the amino acid composition include:1) a diagnosis of NAFLD; 2) type 2 diabetes; 3) a relatively high BMI; 4) a NAFLD Fibrosis Score of less than 0.6; 5) a liver biopsy; and 7) a MRI and/or CT assessment of the liver. The patients can have type 2 diabetes in addition to NAFLD.

Fatty liver disease can be document by a prior history of steatosis confirmed within 3 months of screening by at least one of the following methods: liver fat by MRI with a PDFF ≥ 8%; fibroscan with Control Attenuation Parameter ≥ 300 dB/m; or liver biopsy indicating non-NASH NAFLD steatosis > Grade I. If the patient does not have this documented prior history of steatosis within 3 months of screening, then a liver fat score of ≥10% must be documented at the time of screening using the following formula:

Predicted percent liver fat = 10^(-0.805 + (0.282 * metabolic syndrome [yes =1/no= 0]) + (0.078 *type 2 diabetes [yes =2/no= 0]) + (0.525 * log10(insulin mU/L)) + (0.521 * log10(AST U/L)) – (0.454 *log10(AST/ALT))[34]

Patients can be on stable exercise, diet and lifestyle routine within 3 months prior to screening, with no major body weight fluctuations, e.g., subjects should be within ±3% of their body weight over the last 3 months at the time of screening. Patients can have a body mass index (BMI) ≥ 32 kg/m2 at screening. For sites whose MRI equipment cannot accommodate a patient with a BMI of ≥ 45 kg/m2, an upper limit between 40 to 45 kg/m2 may be applied.

Patients must be on a stable dose of glucose-lowering medication (which can include metformin, sulfonylureas, dipeptidyl peptidase-4 (DPP-4) inhibitors, sodium-glucose co-transporter 2 (SGLT2) inhibitors, or long-acting basal insulin) for at least 3 months before Screening and plan to remain on the same medication without anticipated dose adjustments of their medications for the duration of the study. Patients may be included in the study if they are concurrently treated with anti-hypertensive medications (e.g., beta blockers, hydrochlorothiazide, ACE inhibitors, angiotensin receptor blockers), medications for dyslipidemia (e.g., statins, fibrates), and medication for hypothyroidism (e.g., levothyroxine), so long as they have been on stable doses and regimen of these medications for at least 3 months before Screening and plan to remain on the same medication without anticipated dose adjustments of their medications for the duration of the study. Patients may be on vitamin supplements (e.g. multivitamins; vitamin E <400 IU/day). However, they must be on stable doses and regimen of these vitamin supplements for at least 3 months before screening without anticipated dose adjustments for the duration of the study.

Primary endpoints in the study include the safety and tolerability of administering the composition including amino acids to patients with NAFLD. Secondary endpoints indicative of an improvement in symptoms of NAFLD include the following: 1) intrahepatic fat reduction by MRI; and 2) assessment of biomarkers involved in liver biochemistry, fibrosis/apoptosis, and meteabolism. The following biomarkers can be assessed in a sample (e.g., a plasma or liver sample) from a NAFLD patient: a) alanine aminotransferase (ALT); b) aspartate aminotransferase (AST); c) adiponectin; d) N-terminal fragment of type III collagen (proC3); e) caspase-cleaved keratin 18 fragments (M30 and M65); f) IL-1 beta; g) C-reactive protein; h) PIIINP; i) TIMP1; j) MCP-1; k) FGF-21; or l) gamma glutamyl transferase (GGT). For example, a subject with NAFLD can exhibit a decrease in levels of one, two, or more (e.g., all) of ALT, AST, or GGT after treatment with the composition.

The patient may exhibit a mean change in plasma glucose, insulin, homeostatic model assessment insulin resistance (HOMA IR), lipid profile, hemoglobin A1c (HbA1c) and other metabolic parameters from, e.g., baseline to Weeks 6 and 12, including changes in plasma glucose and insulin levels in the setting of an oral glucose tolerance test (oGTT) from, e.g., baseline to Weeks 6 and 12. The patient may exhibit a mean change in body weight from, e.g., baseline to Weeks 6 and 12.

Administration of the amino acid composition can result in an improvement in the NAFLD activity score, glucose tolerance, hepatocyte inflammation, liver fibrosis or liver injury, steatosis, or hepatocyte ballooning in the patient.

Example 15. Treatment of NASH in a Mouse Model with an Amino Acid Composition

Induction of NASH in Mice

In one example, the effects of LIVRQNAC and related amino acid compositions in the obesity, metabolism-driven non-alcoholic steatohepatitis (NASH) in FATZO mouse model was examined.

MATERIALS AND METHODS

Induction of NASH in Mice

NASH was induced in 60 male FATZO mice by a western diet (Research Diet # D12079B; fat 40 %kcal, protein 17 %kcal, carbohydrate 43 %kcal) supplemented with 5% fructose in the drinking water (WDF) during a 16 week induction phase. Diets and water were available *ad libitum*. Littermate control male FATZO mice fed with a control diet (n=6, Purina # 5008; fat 17 %kcal, protein 27 %kcal, carbohydrate 56 %kcal) and sterile water were set up for control purpose. Mice were housed in plastic cages with microisolator. Sterilized bedding was replaced once a week. Mice were housed three per cage and maintained on a twelve hour light cycle throughout study duration. Room temperature was monitored daily and maintained at 22-25°C. Body weight was recorded every week during the induction phase.

Study Design

Following 16 weeks diet induction, 6 mice remained on control diet (group 1, Control) while 60 induced mice were randomized on body weight and plasma glucose (fed) for assignment to the following treatments. FATZO mice were administered with test articles starting at 16 weeks post western diet NASH induction for 4 weeks. Test articles were administered by oral gavage. Animals were euthanized at 20 weeks post western diet NASH induction, and tissues were harvested for analysis.

Group	(n)	Treatment (oral)	Diet
1	6	Vehicle	5008 WDF
2	10	Vehicle	D12079B + 5% Fructose
3	10	LIVRQNAC (1500 mg/kg)	D12079B + 5% Fructose
4	10	LIVRQNAC (3000 mg/kg)	D12079B + 5% Fructose
5	10	LIVRQNAC+G (3885 mg/kg)	D12079B + 5% Fructose
6	10	LRQNAC (2469 mg/kg)	D12079B + 5% Fructose
7	10	Obeticholic acid (OCA) 30 mg/kg/day	D12079B + 5% Fructose

Test Articles

LIVRQNAC, LIVRQNAC+G, LRQNAC, and OCA (Advanced ChemBlocks, Inc.), incipient, and water for irrigation were provided by Axcella Health, Inc. 0.5% Methylcellulosewas provided by CrownBio, Inc. Dosing solutions were prepared according to Appendix 1. TA compounds (amino acid compositions) were amino acid blends formulated fresh daily in water for irrigation (Baxter # 27F7114) and the excipients 0.125% Xanthan Gum, 1.5 mM Sodium Lauryl Sulfate and 0.28% Lecithin. Obeticholic acid (OCA) was suspended in 0.5% methylcellulose in water for irrigation. All test articles were stored refrigerated. TA compounds were provided in frozen powder form by the sponsor. Dosing was continued for 4 weeks.

Leucine dosages of LIVRQNAC+G and LRQNAC were matched to that of LIVRQNAC.

Amino Acid Compositions

Ingredient	Grade	Supplier	Supplier Part Number	Lot Number
Fusi-BCAA Unflavored (2:1:1 L-Leu:L-Ile:L-Val)	Instantized (0.3-0.9% Lecithin)	Ajinomoto (AjiPure)	33555	OH704
L-Arginine HCl	USP	Sigma (Ajinomoto)	A4599	CDB0352V
L-Arginine HCl	USP	Sigma (Ajinomoto)	A4599	CDB0352V

L-Glutamine	USP	Ajinomoto	32824	R014A003
Glycine	USP	Ajinomoto	30359	R015T008
Acetylcysteine (NAC)	USP	Spectrum Chemical	AC126	1FI0576

	LIVRQNAC	**LIVRQNAC+G**	**LRQNAC**
Ingredient	**Daily Dose (g)**	**Daily Dose (g)**	**Daily Dose (g)**
Fusi-BCAA Unflavored (2:1:1 L-Leu:L-Ile:L-Val)	24.0	24.0	
Fusil (L-Leucine)			12.0
L-Arginine HCl	18.0	18.0	18.0
L-Glutamine	24.0	24.0	24.0
Glycine		20.0	
Acetylcysteine (NAC)	1.8	1.8	1.8
AMINO ACIDS =	67.8	87.8	55.8

Test Articles Administration

LIVRQNAC, LIVRQNAC+G, LRQNAC, OCA and Vehicle were administered by oral gavage at a volume of 10 mL/kg throughout the study. Dosages were calculated by daily body weight. LIVRQNAC, LIVRQNAC+G, LRQNAC, and Vehicle were administered twice per day (BID), while OCA was administered once a day (QD) in the morning. Mice receiving OCA once per day (QD), and one vehicle QD. Doses were administered by oral gavage at 0700 and 1800 by oral gavage for 4 weeks.

Body Weight and Blood Glucose

The viability, clinical signs and behavior were monitored daily. Body weight was recorded daily during the dosing period. Blood samples were collected weekly in the AM (0700) via tail clip for glucose measurement (StatStrip glucometer).

Necropsy and Sample Harvest

Animals were anesthetized with CO2 inhalation and exsanguinated via cardiac puncture for euthanasia. Terminal blood samples (K2EDTA) were obtained by cardiac puncture in anesthetized animals at termination. Samples were provided frozen to Axcella Health. Organ weights (total liver, gonadal fat pads) were recorded. Pancreas, and small intestine and gonadal fat pads were fixed in 10% Buffered Formalin and prepared as directed in protocol. A section of small intestine, gonadal fat pad and liver were also snap frozen in liquid nitrogen and shipped to the sponsor.

Histological Analyses

The liver tissues were fixed in Bouin's solution at 4°C for 24 hours followed by baths of standard concentrations of alcohol then xylene to prepare the tissues for paraffin embedding. After being embedded in paraffin and cooled, five-micron sections were cut and stained for routine H&E and Picric Sirius Red. A section of both right and left lobes of the livers were frozen in OCT for analysis of lipid content with Oil-Red-) staining. The Aperio whole slide digital imaging system (Scan Scope CS, Vista, CA) was used for imaging. All slides were imaged at 20x. The scan time ranged from 1.5 minutes to a maximum time of 2.25 minutes. The whole images were housed and stored in their Spectrum software system and images were shot from the whole slides.

The livers were evaluated using the NASH liver criteria for scoring. In this mouse study, one cross section of liver for each case was analyzed with the NASH score system. According to the published NASH CRN Scoring System, this scoring system comprises of NAFLD Activity Score (NAS), fibrosis stage and identification of NASH by pattern recognition. The NAS can range from 0 to 8 and is calculated by the sum of scores of steatosis (0-3), lobular inflammation (0-3) and hepatocyte ballooning (0-2) from H&E stained sections. Fibrosis was scored (0-4) from picrosirius red stained slides. The NASH system is used for human liver 18 gauge biopsies. Steatosis, lobular inflammation, hepatocyte. balloon degeneration, fibrosis, NAS and the presence of NASH by pattern recognition were systematically assessed. In this study we evaluated one total cross section of liver per mouse in this study. This is about 15 times the size of an 18 gauge human liver biopsy. The pathology score was determined as 0, +1, +2, or +3. The lesions were scored on location (periportal, centrilobular, and mid zonal) and fat accumulation (focal, periportal, and/ or centrilobular). The other part of the score was distribution of the lesions: focal, multifocal and/or diffuse. Also, mild, moderate and severity of the lesions. These parameters made up the total NASH score.

All immunohistochemical staining steps were performed using the Dako FLEX SYSTEM on an automated immunostainer; incubations were done at room temperature and Tris buffered saline plus 0.05% Tween 20, pH 7.4 (TBS - Dako Corp.) was used for all washes and diluents. Thorough washing was performed after each incubation. Primary antibodies included anti-mouse SMA, F4/80, Mac-2, and Picric Sirius Red. Control sections were treated with an isotype control using the same concentration as primary antibodies to verify the staining specificity.

White adipose tissue (WAT) adipocyte size was analyzed from the H&E stained sections. Using the Aperio Image Scope application, 3 localized regions (edge of tissue, tissue not surrounding vascular area, tissue surrounding vascular area) of each tissue specimen were assessed by measuring the area of 10 largest adipocytes of the region. Within each tissue, 10 hot spots of each regions were quantified (um^2) and averaged.

Pancreatic beta-islet cells were identified by immunohistochemical staining.

Image Analysis

Aperio Automatic Image Quantitation was employed to quantify positive pixels of immunohistochemical staining, Oil-Red O, and Sirius Red staining. The Positive Pixel Count algorithm was used to quantify the percentage of a specific stain present in a scanned slide image. A range of color (range of hues and saturation) and three intensity ranges (weak, positive, and strong) were masked and evaluated. The algorithm counted the number and intensity-sum in each intensity range, along with three additional quantities: average intensity, ratio of strong/total number, and average intensity of weak positive pixels. The positive pixel algorithm was modified to distinguish between the orange and blue colors. Alterations from the normal “hue value” (0.1 to 0.96) and “color saturation” (0.04 to 0.29), were made for the Sirius Red evaluation. Vasculature and artifacts were excluded from analysis.

Liver Gene Expression Analysis

Liver gene expression of MCP-1 and MIP-1a was measured by quantitative PCR.

Liver Cytokine and Chemokine Measurement

Liver IL-1b, MCP-1, and MIP-1 protein levels were quantified using the multiplex ELISA Assay (Meso Scale Discovery, Rockville, Maryland).

Statistical Analysis

Statistical analyses of liver histological scores were performed using Bonferroni Multiple Comparison Test on GraphPad Prism 6 (GraphPad Software Inc., USA). P values < 0.05 were considered statistically significant. Results were expressed as mean ± SEM. Comparisons were made between Group 2 (Vehicle) and the following groups; Group 3 (LIVRQNAC 1,500 mg/kg), Group 4 (LIVRQNAC 3,000 mg/kg), Group 5 (LIVRQNAC+G, 3,885 mg/kg), and (LRQNAC, 2,469 mg/kg).

RESULTS

Body and Liver Weight

Feeding the western diet supplemented with fructose (WDF) for 16 weeks elicited significant effects on body weight compared to control fed animals. Prior to administration of test agent, animals fed the WDF were significantly heavier (47.6 ± 0.45 vs. 43.9 ± 1.03g; $p <$ 0.01) compared to animals fed the control diet.

Body weight decreased compared to baseline values in all treatment groups; there were no significant differences in weight loss compared to vehicle (-7.6 ± 0.9, -6.9 ± 1.3, -6.8 ± 1.4, -5.7 ± 1.2, -6.4 ± 1.0, -4.7 ± 1.6 and -3.9 ± 1.5% for control, vehicle, LIVRQNAC (1500 mg/kg), LIVRQNAC (3000 mg/kg), LIVRQNAC+G, LRQNAC, and OCA, respectively; $p< 0.4992$).

Liver weight (% body weight) was significantly higher in vehicle treated animals fed WDF compared to control diet (7.22 ± 0.3 vs. 5.05 ± 0.24%; $p< 0.0001$); however, in animals fed WDF, no significant effects compared to vehicle were noted in any treatment group (7.22 ± 03, 7.14 ± 0.3, 7.19 ± 0.26, 6.69 ± 0.18, 7.02 ± 0.5 and 6.81 ± 0.2 for vehicle, LIVRQNAC (1500 mg/kg), LIVRQNAC (3000 mg/kg), LIVRQNAC+G, LRQNAC, and OCA, respectively; $p<$ 0.7450).

Blood Glucose

Feeding the western diet supplemented with fructose (WDF) for 16 weeks elicited significant effects on glycemia compared to control fed animals. Prior to administration of test agent, animals fed the WDF had significantly lower glucose (160.0 ± 3.01 vs. 218.3 ± 28.6 mg/dL; p< 0.0001) compared to animals fed the control diet.

Blood glucose, although higher in control animals at baseline, remained relatively stable during 4 weeks of compound administration. When averaged over the dosing period, there were no significant differences in average blood glucose compared to vehicle for any treatment group (166.0 ± 9.7, 157.1 ± 4.6, 154.6 ± 2.3, 159.4 ± 3.8, 155.5 ± 3.8, 153.6 ± 3.0 and 169.7 ± 6.3 mg/dL for control, vehicle, LIVRQNAC (1500 mg/kg), LIVRQNAC (3000 mg/kg), LIVRQNAC+G, LRQNAC, and OCA, respectively; $p< 0.1587$).

Liver Triglyceride and Cholesterol

Liver triglyceride and cholesterol content were similarly elevated after WDF feeding compared to vehicle treated animals fed control diet (liver triglyceride $p<0.0040$; liver cholesterol: p< 0.0001). Among animals fed WDF, there were no significant differences in liver triglyceride ($p<0.1206$) when compared to vehicle for any treatment group. While OCA reduced liver cholesterol content compared to vehicle by 32% (p<0.05), no amino acid composition treatment group affected liver cholesterol as compared to WDF feeding vehicle group.

Liver	**Vehicle**	**LIVRQNAC 1.5 g/kg**	**LIVRQNAC 3.0 g/kg**	**LIVRQNAC+G**	**LRQNAC**	**OCA**
Triglyceride	31.49±5.85	47.63±1.19	47.94±1.37	50.57±1.58	49.47±1.4	49.81±1.63
Cholesterol	8.37±0.065	7.74±0.318	7.48±0.697	6.42±0.648	7.84±0.104	5.63±0.495

Liver Histology

FATZO mice fed with the control diet developed mild steatosis and no inflammation, ballooning, or fibrosis (FIG. 10). FATZO mice fed with the WDF and treated with vehicle developed significant steatosis, mild inflammation, ballooning, and fibrosis. In contrast to predominantly macrovesicular steatosis in the vehicle groups, a mixture of predominantly microvesicular and diminished macrovesicular steatosis was observed in LIVRQNAC, LIVRQNAC+G and LRQNAC groups, as shown in FIG. 11.

The NAFLD activity score is calculated from histological scoring of steatosis (0-3), inflammation (0-3), and ballooning (0-2) in fixed liver tissues. In WDF fed animals, all amino acid composition treatments produced a significant reduction in the NAS compared to the vehicle treatment group (FIG. 12). LIVRQNAC and amino acid composition treatments reduced liver steatosis as compared to vehicle, although only LIVRQNAC+G and LRQNAC reached statistical significance ($p<0.05$), while LIVRQNAC did not (LIVRQNAC 3.0 g/kg, $p=0.12$). All amino acid composition treatments significantly attenuated hepatocyte ballooning, the biomarker of lipotoxicity and cell death. Amino acid composition treatments did not significantly alter liver inflammation. In conclusion, amino acid composition-associated improvement of liver pathology is mainly attributed to attenuation of hepatocyte ballooning.

There was no significant effect of OCA on the NAS score and NAS components compared to vehicle.

Liver Pathology	**Vehicle**	**LIVRQNAC 1.5 g/kg**	**LIVRQNAC 3.0 g/kg**	**LIVRQNAC+G**	**LRQNAC**	**OCA**
NAS	3.65±0.183	2.70±0.213	2.89±0.111	2.83±0.186	2.72±0.147	3.72±0.147
Steatosis	1.8±0.133	1.6±0.163	1.44±0.176	1.33±0.167	1.33±0.167	1.78±0.147
Inflammation	0.9±0.1	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Ballooning	0.95±0.05	0.1±0.1	0.44±0.176	0.50±0.144	0.39±0.111	0.94±0.056

Livers from vehicle treated animals demonstrated a mild fibrosis; score of 0.8±0.1. Only livers from animals treated with LIVRQNAC (1500 mg/kg) demonstrated a significant reduction in fibrosis when compared to the vehicle treated group, (0.2±0.1 versus 0.8±0.1, $p<0.01$), but not with LIVRQNAC (3000 mg/kg), LIVRQNAC+G or LRQNAC. Sirius Red collagen staining demonstrated that all amino acid composition treatments had significantly lower collagen deposition compared to vehicle (LIVRQNAC 1500 mg/kg, $p<0.01$; LIVRQNAC 3000 mg/kg, $p<0.01$; LIVRQNAC+G, $p=0.09$; LRQNAC, $p<0.05$). OCA did not affect liver fibrosis score or Sirius Red collagen staining area.

Fibrosis	**Vehicle**	**LIVRQNAC 1.5 g/kg**	**LIVRQNAC 3.0 g/kg**	**LIVRQNAC+G**	**LRQNAC**	**OCA**
Fibrosis	0.8±0.133	0.2±0.133	0.44±0.176	0.44±0.176	0.33±0.167	0.67±0.167
Sirius Red	1.82±0.279	0.77±0.116	0.72±0.092	0.107±0.218	0.79±0.183	1.59±0.36

Consistent with liver triglyceride levels, amino acid composition treatments did not alter liver Oil Red O staining area compared to vehicle group. OCA reduced Oil Red O staining area ($p<0.05$).

Oil Red O	Vehicle	LIVRQNAC 1.5 g/kg	LIVRQNAC 3.0 g/kg	LIVRQNAC+G	LRQNAC	OCA
Oil Red O	0.32±0.019	0.28±0.022	0.30±0.022	0.26±0.023	0.29±0.018	0.24±0.021
Triglyceride	31.49±5.85	47.63±1.19	47.94±1.37	50.57±1.58	49.47±1.4	49.81±1.63

Liver Gene Expression

MCP-1 (CCL2) and MIP-1a (CCL3) are proinflammatory chemokines that mediate liver inflammation via macrophage and neutrophil recruitment. MCP-1 and MIP-1a are the ligands of CCR2 and CCR5, respectively, which serve the promising therapeutic targets to treat liver fibrosis in NASH. MCP-1 and MIP-1a RNA expression levels in the liver were significantly upregulated in the WDF fed mice as compared to control diet-fed mice, as shown in Tables 74 and 75.

Table 74. Fold change in MCP-1 mRNA levels after administration of amino acid compositions

MCP-1	Ctrl diet	Vehicle	LIVRQNAC (3000 mg/kg)	LIVRQNAC+G (3885 mg/kg)	LRQNAC (2469 mg/kg)
Mean	0.1457	1.079	1.396	0.6102	0.8777
SEM	0.0291	0.1956	0.3414	0.09597	0.2315

Table 75. Fold change in MIP-1a mRNA levels after administration of amino acid compositions

MIP-1a	Ctrl diet	Vehicle	LIVRQNAC (3000 mg/kg)	LIVRQNAC+G (3885 mg/kg)	LRQNAC (2469 mg/kg)
Mean	0.08328	1.194	1.67	0.814	1.514
SEM	0.02141	0.25	0.3366	0.1029	0.525

LIVRQNAC and LRQNAC treatments did not significantly alter liver MCP-1 and MIP-1a RNA expression as compared to vehicle group. LIVRQNAC+G treatment resulted in slightly lower liver MCP-1 RNA expression as compared to vehicle group (p=0.054) and LIVRQNAC group (p<0.05). Similarly, LIVRQNAC+G treatment resulted in slightly lower liver MCP-1 RNA expression as compared to vehicle group although the difference was not significant (p=0.19) and LIVRQNAC group (p<0.05).

Liver Chemokines and Cytokines

Consistent with RNA data (FIG. 25), liver MCP-1 and MIP-1a protein levels were elevated in the WDF fed mice as compared to control diet-fed mice, as shown in Tables 76 and 77.

Table 76. Mean liver MCP-1 protein levels after administration of amino acid compositions

MCP-1	Ctrl diet	Vehicle	LIVRQNAC (3000 mg/kg)	LIVRQNAC+G (3885 mg/kg)	LRQNAC (2469 mg/kg)
Mean	41.47	278.3	392	221.1	247.1
SEM	7.463	61.41	83.97	36.6	75.16

Table 77. Mean liver MIP-1a protein levels after administration of amino acid compositions

MIP-1a	Ctrl diet	Vehicle	LIVRQNAC (3000 mg/kg)	LIVRQNAC+G (3885 mg/kg)	LRQNAC (2469 mg/kg)
Mean	23.16	191.8	282.9	142.8	141.1
SEM	5.429	30.03	58.88	17.04	24.36

Liver MCP-1 and MIP-1a protein levels were also positively correlated with RNA expression levels, as shown in Tables 78 and 79.

Table 78. Correlations between MCP-1 protein and RNA levels after administration of amino acid compositions

Ctrl diet	y=0.0022x+0.0542	R2=0.3202
Vehicle	y=0.0029x+0.3316	R2=0.7986
LIVRQNAC (3000 mg/kg)	y=0.0036x+0.0144	R2=0.7831
LIVRQNAC+G (3885 mg/kg)	y=0.0018x+0.2542	R2=0.3988
LRQNAC (2469 mg/kg)	y=0.0027x+0.2969	R2=0.6857

Table 79. Correlations between MIP-1a protein and RNA levels after administration of amino acid compositions

Ctrl diet	y=0.001x+0.0593	R2=.069
Vehicle	y=0.0057x+0.191	R2=0.4202
LIVRQNAC (3000 mg/kg)	y=0.0051x+0.2334	R2=0.7887
LIVRQNAC+G (3885 mg/kg)	y=0.0045x+0.1817	R2=0.4403
LRQNAC (2469 mg/kg)	y=0.0064x+0.1814	R2=0.4875

LIVRQNAC and LRQNAC treatments did not significantly alter liver MCP-1 and MIP-1a protein levels as compared to vehicle group. LIVRQNAC+G treatment slightly lowered liver MCP-1 (p=0.095) and MIP-1a (p<0.05) protein levels as compared to LIVRQNAC group. Additionally, liver MCP-1 and MIP-1a protein levels positively correlated, as shown in Table 80.

Table 80. Correlations between MCP-1 and MIP-1a protein levels after administration of amino acid compositions

Ctrl diet	y=0.6803x-5.0537	R2=0.8744
Vehicle	y=0.389x+83.574	R2=0.6325
LIVRQNAC (3000 mg/kg)	y=0.6615x+23.609	R2=0.8903
LIVRQNAC+G (3885 mg/kg)	y=0.4437x+44.728	R2=0.9082
LRQNAC (2469 mg/kg)	y=0.3108x+75.901	R2=0.9241

Proinflammatory cytokines IL-1b, IL-6, TNFa, and CXCL1 protein levels in liver were elevated in the WDF fed mice as compared to control diet-fed mice, as shown in Tables 81-84.

Table 81. Mean liver IL-1b protein levels after administration of amino acid compositions

IL-1b	Ctrl diet	Vehicle	LIVRQNAC (3000 mg/kg)	LIVRQNAC+G (3885 mg/kg)	LRQNAC (2469 mg/kg)
Mean	10.53	22.31	22.11	14.42	28.85
SEM	1.248	6.063	5.739	3.299	10.41

Table 82. Mean liver IL-6 protein levels after administration of amino acid compositions

IL-6	Ctrl diet	Vehicle	LIVRQNAC (3000 mg/kg)	LIVRQNAC+G (3885 mg/kg)	LRQNAC (2469 mg/kg)
Mean	5.838	8.452	7.298	5.77	6.71
SEM	0.3536	2.723	2.043	1.06	1.625

Table 83. Mean liver CXCL1 protein levels after administration of amino acid compositions

CXCL1	Ctrl diet	Vehicle	LIVRQNAC (3000 mg/kg)	LIVRQNAC+G (3885 mg/kg)	LRQNAC (2469 mg/kg)
Mean	41.42	105.9	119.6	77.77	84.9
SEM	2.487	13.26	26.15	8.023	10.49

Table 84. Mean liver TNFα protein levels after administration of amino acid compositions

TNFa	Ctrl diet	Vehicle	LIVRQNAC (3000 mg/kg)	LIVRQNAC+G (3885 mg/kg)	LRQNAC (2469 mg/kg)
Mean	1.703	3.71	4.574	2.974	4.119
SEM	0.5641	0.4647	0.5654	0.1513	0.8341

LIVRQNAC, LIVRQNAC+G, and LRQNAC treatments did not significantly alter IL-1b, IL-6, TNFa, and CXCL1 protein levels as compared to vehicle. Liver TNFa levels were lower by LIVRQNAC+G treatment as compared to LIVRQNAC.

SUMMARY

Based on clinical observations, WDF-fed FATZO mice gained more body weight that those fed with a control diet. Fed blood glucose levels were comparable between WDF-fed and control diet-fed mice despite of the difference in body weight change. All treatments were well tolerated in FATZO mice. Both WDF-fed and control diet-fed mice lose body weight during the treatment period, which may be due to the stress associated with administration of test articles or vehicle via oral gavage twice a day.

NAS was significantly attenuated in all amino acid composition treatment groups as compared to vehicle, predominantly attributing to ballooning score. Hepatocyte ballooning was significantly reduced in all the amino acid composition treatment groups. Steatosis was significantly reduced in LIVRQNAC+G and LRQNAC treatment groups. LIVRQNAC also lowered steatosis, although the difference was not significant. Inflammation was not affected by amino acid composition treatments. Despite the histological improvement in steatosis score in LIVRQNAC+G and LRQNAC treatment groups, liver triglyceride, cholesterol, and Oil-Red O staining remained unchanged by amino acid composition treatments. Consistent with the histological and biochemical data, de novo lipogenesis enzymes FASN and ACACA RNA levels were not affected by amino acid composition treatment.

Although liver triglyceride levels were not affected by amino acid composition treatments, the characteristics of hepatocyte steatosis were differed by amino acid composition treatments. Liver of the WDF-fed mice (vehicle group) demonstrated predominantly macrovesicular steatosis. In contrast, macrovesicular steatosis was diminished, and a mixture of microvesicular and macrovesicular steatosis in all amino acid composition treatment groups. The biological meaning and mechanism of amino acid compositions on macro- to microvesicular steatosis phenotypes merit further investigation.

Liver fibrosis score in FATZO model of NAFLD was significantly attenuated by LIVRQNAC treatment at low dose but not at high dose. LIVRQNAC+G and LRQNAC had no effect on fibrosis. Nonetheless, Sirius Red collagen staining demonstrated that LIVRQNAC, LIVRQNAC+G and LRQNAC significantly reduced collagen deposition in the liver.

Consistent with liver inflammation scores, liver RNA and protein levels of the proinflammatory chemokine MCP-1 and MIP-1a and cytokines IL-1b, IL-6, TNFa, and CXCL1 were not significantly affected by amino acid composition treatment. It is of interest to note that LIVRQNAC+G (equivalent to LIVRQNAC plus Glycine) treatment had lower liver MCP-1, MIP-1a, and TNFa as compared to LIVRQNAC.

Increased liver oxidative stress associated with inflammation is observed during NAFLD and NASH. Glutathione (GSH) is a pivotal endogenous anti-oxidant which can counteract reactive oxygen species. Glycine and its direct metabolic precursor, serine, are substrates for GSH biosynthesis. Thus, serine and/or glycine supplementation helps replenish GSH and ameliorates NAFLD and NASH. LIVRQNACG treatment had lower inflammation chemokines and cytokines in the liver, supporting that supplementation of glycine or serine is beneficial in NAFLD and NASH.

In conclusion, all three amino acid compositions (LIVRQNAC, LIVRQNAC+G and LRQNAC) tested in FATZO mice attenuate NAFLD activity scores, hepatocyte ballooning, and fibrosis. These amino acid compositions can be used to treat NASH. Glycine-containing amino acid compositions can further reduce liver inflammation which results in reduced liver fibrosis.

While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

WHAT IS CLAIMED IS:

1. A composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity.

2. The composition of claim 1, wherein one, two, three, or more (e.g., all) of methionine (M), trytophan (W), valine (V), or cysteine (C) is absent, or if present, is present at less than 10 weight (wt.) %, 9 wt. %, 8 wt. %, 7 wt. %, 6 wt. %, 5 wt. %, 4 wt. %, 3 wt. %, 2 wt. %, or 1 wt. %.

3. The composition of claim 1 or 2, wherein the total wt. % of (a)-(d) is greater than the total wt. % of any other amino acid entity in the composition.

4. The composition of any of the preceding claims, wherein:

f) a wt. % of the Q-amino acid entity in the composition is greater than the wt. % of the R-amino acid entity;

g) the wt. % of the Q-amino acid entity in the composition is greater than the wt. % of the L-amino acid entity;

h) the wt. % of the R-amino acid entity in the composition is greater than the wt. % of the L-amino acid entity; or

i) a combination of two or three of (f)-(h).

5. The composition of any of the preceding claims, wherein the wt. % of the Q-amino acid entity in the composition is at least 5% greater than the wt. % of the R-amino acid entity, e.g., the wt. % of the Q-amino acid entity is at least 10%, 15%, 20%, or 25% greater than the wt. % of the R-amino acid entity

6. The composition of any of the preceding claims, wherein the wt. % of the Q-amino acid entity in the composition is at least 20% greater than the wt. % of the L-amino acid entity, e.g., the wt. % of the Q-amino acid entity in the composition is at least 25%, 30%, 35%, 40%, 45%, or 50% greater than the wt. % of the L-amino acid entity.

7. The composition of any of the preceding claims, wherein the wt. % of the R-amino acid entity in the composition is at least 10% greater than the wt. % of the L-amino acid entity, e.g., the wt. % of the R-amino acid entity in the composition is at least 15%, 20%, 25%, or 30% greater than the wt. % of the L-amino acid entity.

8. The composition of any of the preceding claims, wherein:

j) the ratio of the L-amino acid entity to the R-amino acid entity is at least 1:4, or at least 2:5, and not more than 3:4, e.g., the ratio of L-amino acid entity to R-amino acid entity is about 2:3;

k) the ratio of the L-amino acid entity to the Q amino acid entity is at least 1:4, or least 1:3, and not more than 3:4, e.g., the ratio of the L-amino acid entity to the Q-amino acid entity is about 1:2;

l) the ratio of the R-amino acid entity to the Q amino acid entity is at least 1:4, or least 1:2, and not more than 6:7, e.g., the ratio of the R-amino acid entity to the Q-amino acid entity is about 3:4; or

m) a combination of two or three of (j)-(l).

9. The composition of any of the preceding claims, further comprising one or two additional branched-chain amino acid (BCAA)-entities, e.g., one or both of an isoleucine (I)-amino acid-entity and a valine (V)-amino acid-entity.

10. The composition of claim 9, wherein:

n) the wt. % of the L-amino acid-entity in the composition is greater than or equal to the wt. % of the I-amino acid-entity and the V-amino acid-entity in combination;

o) the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination in the composition is greater than or equal to the wt. % of the Q-amino acid entity;

p) the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination in the composition is less than the wt. % of the R-amino acid entity;

q) the wt. % of the R-amino acid entity and the Q-amino acid entity in the composition is greater than the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination; or

r) a combination of two, three, or four of (n)-(q).

11. The composition of claim 9 or 10, wherein:

s) the wt. % of the R-amino acid entity, the Q-amino acid entity, and the NAC or a salt thereof is at least 50% of the composition, or at least 70% of the composition, but not more than 90% of the composition;

t) the wt. % of the NAC or a salt thereof is at least 1%, or at least 2%, but not more than 10% of the composition;

u) the wt. % of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination is at least 15%, or at least 20%, but not more than 50% of the composition;

v) the wt. % of the R-amino acid entity, the Q-amino acid entity, and the NAC or a salt thereof is at least 40%, or at least 50%, but not more than 80% of the composition; or

w) a combination of two, three, or four of (s)-(v).

12. The composition of any of claims 9-11, wherein:

x) the ratio of the L-amino acid entity to the I-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5 to 1 or not more than 3:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 2:1;

y) the ratio of L-amino acid entity to V-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5 to 1 or not more than 3:1, e.g., the ratio of L to V is about 2:1;

z) the ratio of the L-amino acid entity to the R-amino acid entity is greater than 1:3, greater than 1.5 to 3, and less than 3:3, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 2:3;

aa) the ratio of the L-amino acid entity to the Q-amino acid entity is greater than 1:4, greater than 1.5 to 4 and less than 4:4, or less than 3:4, e.g., the ratio of the L-amino acid entity to the Q-amino acid entity is about 1:2; or

bb) a combination of two, three, or four of (x)-(aa).

13. The composition of any of claims 9-12, wherein:

cc) the ratio of the I-amino acid entity to the V-amino acid entity is at least .5:1, or at least .75:1, and not more than 1.5 to 1 or not more than 2:1, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 1:1;

dd) the ratio of the I-amino acid entity to the R-amino acid entity is at least .5:3, or at least .75:3, and not more than 2:3, or not more than 1.5:3, e.g., the ratio of the L-amino acid entity to the I-amino acid entity is about 1:3;

ee) the ratio of the I-amino acid entity to the Q-amino acid entity is at least .5:4, or at least .75:4, and not more than 3:4, or not more than 2:4, e.g., the ratio of the L-amino acid entity to the Q-amino acid entity is about 1:4; or

ff) or a combination of two or three of (cc)-(ee).

14. The composition of any of claims 9-13, wherein:

gg) the ratio of the L-amino acid entity to the V-amino acid entity is at least 1.5:1, or at least 1.75:1, and not more than 2.5 to 1 or not more than 3:1, e.g., is the ratio of the L-amino acid entity to the V-amino acid entity is about 2:1;

hh) the ratio of the L-amino acid entity to the R-amino acid entity is greater than 1:3, greater than 1.5 to 3, and less than 3:3, e.g., the ratio of the L-amino acid entity to the R-amino acid entity is about 2:3;

ii) the ratio of the L-amino acid entity to the Q-amino acid entity is greater than 1:4, greater than 1.5 to 4 and less than 4:4, or less than 3:4, e.g., the ratio of the L-amino acid entity to the Q-amino acid entity is about 1:2; or

jj) a combination of two or three of (gg)-(ii).

15. The composition of any of claims 9-14, wherein:

kk) the ratio of the V-amino acid entity to the Q-amino acid entity is at least .5:4, or at least .75:4, and not more than 3:4, or not more than 2:4, e.g., the ratio of the L-amino acid entity to the Q-amino acid entity is about 1:4;

ll) the ratio of the V-amino acid entity to the R-amino acid entity is at least .5:3, or at least .75:3, and not more than 2:3, or not more than 1.5:3, e.g., the ratio of the V-amino acid entity to the R-amino acid entity is about 1:3;

mm) the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, Q-amino acid entity, and NAC or a salt thereof is at least 1:4, or at least 2:3, or not more than 5:7, or not more than 6:7, e.g., the ratio is about 6:11; or

nn) a combination of two or three of (kk)-(mm).

16. The composition of any of the preceding claims, wherein:

oo) a wt. % of the L-amino acid entity in the composition is greater than the wt. % of the NAC or a salt thereof;

pp) a wt. % of the R-amino acid entity in the composition is greater than the wt. % of the NAC or a salt thereof;

qq) a wt. % of the Q-amino acid entity in the composition is greater than the wt. % of the NAC or a salt thereof; or

rr) a combination of two or three of (oo)-(qq).

17. The composition of any of the preceding claims, wherein the L-amino acid entity is chosen from L-leucine, β-hydroxy-β-methybutyrate (HMB), oxo-leucine, isovaleryl-CoA, D-leucine, and n-acetyl-leucine, or a combination thereof.

18. The composition of any of the preceding claims, wherein the R-amino acid entity is chosen from L-arginine, ornithine, argininosuccinate, citrulline, aspartate, glutamate, agmatine, creatine, D-arginine, and N-acetyl-arginine, or a combination thereof.

19. The composition of any of the preceding claims, wherein the Q-amino acid entity is chosen from L-glutamine, glutamate, carbamoyl-P, glutamate, D-glutamine, and n-acetylglutamine, or a combination thereof.

20. The composition of any of the preceding claims, wherein the NAC-amino acid entity is chosen from NAC, serine, acetylserine, cystathionine, glutathione, homocysteine, methionine, D-cysteine, L-cysteine, cystine, and cysteamine, or a combination thereof.

21. The composition of any of the preceding claims, wherein the I-amino acid entity is chosen from L-isoleucine, 2-oxo-3-methyl-valerate, threonine, 2-oxo-3-methyl-valerate, methylbutyrl-CoA, D-isoleucine, and N-acetyl-isoleucine, or a combination thereof.

22. The composition of any of the preceding claims, wherein the V-amino acid entity is chosen from L-valine, 2-oxo-valerate, isobutryl-CoA, 3-HIB-CoA, 3-HIB, D-valine, and N-acetyl-valine, or a combination thereof.

23. The composition of any of the preceding claims, wherein the S-amino acid entity is chosen from L-serine, Phosphoserine, P-hydroxypyruvate, Glycine, Tryptophan, Acetylserine, Cystathionine, Cysteine, Phosphatidylserine, D-Serine, or a combination thereof.

24. A pharmaceutical composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity.

25. The composition of any of the preceding claims, wherein at least one of (a)-(d) is a free amino acid, e.g., two, three, or four of (a)-(d) are a free amino acid, e.g., at least 50 wt. % of the total wt. of the composition is one or more amino acid entities in free form.

26. The composition of any of the preceding claims, wherein at least one of (a)-(d) is in a salt form, e.g., one, two, three, or four of (a)-(d) is in a salt form, e.g., at least 10 wt. % of the total wt. of the composition is one or more amino acid entities in salt form.

27. The composition of any of the preceding claims, wherein at least one of (a)-(d) is a free amino acid, e.g., two, three, or four of (a)-(d) are a free amino acid, e.g., at least 50 wt. % of the total wt. of the composition is one or more amino acid entities in free form.

28. The composition of any of the preceding claims, wherein at least one of (a)-(d) is in a salt form, e.g., one, two, three, or four of (a)-(d) is in a salt form, e.g., at least 10 wt. % of the total wt. of the composition is one or more amino acid entities in salt form.

29. The composition of any of the preceding claims, wherein a wt. ratio of the L-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 0.5 to 3 : 0.5 to 4 : 1 to 4 : 0.1 to 2.5.

30. The composition of claim 23, wherein the wt. ratio of the L-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 1 : 1.5 : 2 : 0.15.

31. The composition of claim 23, wherein the wt. ratio of the L-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 1 : 1.5 : 2 : 0.3.

32. The composition of any of claims 10-31, wherein the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 0.5 to 2 : 0.1 to 1 : 0.1 to 1 : 0.5 to 3 : 0.5 to 4 : 0.1 to 0.5.

33. The composition of claim 32, wherein the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 1 : 0.5 : 0.5 : 1.5 : 2 : 0.15.

34. The composition of claim 32, wherein the wt. ratio of the L-amino acid entity, the I-amino acid entity, the V-amino acid entity, the R-amino acid entity, the Q-amino acid entity, and the NAC-amino acid entity is about 1 : 0.5 : 0.5 : 1.5 : 2 : 0.3.

35. The composition of any of claims 10-31, wherein the composition comprises about 0.5 g to about 10 g of the L-amino acid entity, about 0.25 g to about 5 g of the I-amino acid entity, about 0.25 g to about 5 g of the V-amino acid entity, about 1 g to about 20 g of the R-amino acid entity, about 1 g to about 20 g of the Q-amino acid entity, and about 0.1 g to about 5 g of the NAC-amino acid entity.

36. The composition of claim 35, wherein the composition comprises about 1 g of the L-amino acid entity, about 0.5 g of the I-amino acid entity, about 0.5 g of V-amino acid entity, about 1.5 g of R-amino acid entity, about 2 g of Q-amino acid entity, and about 0.15 g of NAC-amino acid entity.

37. The composition of claim 35, wherein the composition comprises about 1 g of the L-amino acid entity, about 0.5 g of the I-amino acid entity, about 0.5 g of V-amino acid entity, about 1.5 g of R-amino acid entity, about 2 g of Q-amino acid entity, and about 0.3 g of NAC-amino acid entity.

38. The composition of claim 35, wherein the composition comprises about 2 g of the L-amino acid entity, about 1 g of the I-amino acid entity, about 1 g of the V-amino acid entity, about 3 g of the R-amino acid entity, about 4 g of the Q-amino acid entity, and about 0.3 g of the NAC-amino acid entity.

39. The composition of claim 35, wherein the composition comprises about 4 g of the L-amino acid entity, about 2 g of the I-amino acid entity, about 2 g of the V-amino acid entity, about 6 g of the R-amino acid entity, about 8 g of the Q-amino acid entity, and about 0.6 g of the NAC-amino acid entity.

40. The composition of any of the preceding claims, wherein the composition comprises about 10 wt % to about 30 wt % L-amino acid entity, about 5 wt % to about 15 wt % I-amino acid entity, about 5 wt % to about 15 wt % V-amino acid entity, about 15 wt % to about 40 wt % R-amino acid entity, about 20 wt % to about 50 wt % Q-amino acid entity, and about 1 wt % to about 8 wt % NAC-amino acid entity.

41. The composition of any of the preceding claims, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 : 6 : 3 : 9 : 12 : 2.7, optionally wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 : 6 : 3 : 9 : 12 : 2.7 : 18.

42. The composition of any of the preceding claims, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino

acid entity, L-glutamine or a salt thereof, and NAC or a salt thereof is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15%.

43. The composition of any of the preceding claims, wherein the ratio of the L-amino acid-entity, the I-amino acid-entity, and the V-amino acid-entity in combination to the R-amino acid entity, L-glutamine or a salt thereof, NAC or a salt thereof, and the S-amino acid entity is 12 +/- 15% : 6 +/- 15% : 3 +/- 15% : 9 +/- 15% : 12 +/- 15% : 2.7 +/- 15% : 18 +/- 15%.

44. The composition of any of the preceding claims, wherein the composition comprises a combination of 4 to 20 different amino acid entities, e.g., a combination of 5 to 15 different amino acid entities.

45. The composition of any of the preceding claims, wherein at least two, three, four, or more amino acid entities is not a peptide of more than 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues in length.

46. A method for treating one or more symptoms selected from the group consisting of decreased fat metabolism, hepatocyte apoptosis, hepatocyte ballooning, inflammation of adipose tissue, inflammation of hepatic tissue, fibrosis, liver injury, steatosis, glucose tolerance, and oxidative stress, wherein the method comprises administering to a subject in need thereof an effective amount of a composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity.

47. A method for improving liver function, wherein the method comprises administering to a subject in need thereof an effective amount of a composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity.

48. The method of claim 46 or 47, wherein the subject has fatty liver disease.

49. The method of claim 48, wherein the subject has a disease or disorder selected from the group consisting of non-alcoholic fatty liver (NAFL), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic fatty liver disease (AFLD), and alcoholic steatohepatitis (ASH).

50. A method for treating fatty liver disease, wherein the method comprises administering to a subject in need thereof an effective amount of a composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity;

51. The method of claim 50, wherein the subject has a disease or disorder selected from the group consisting of non-alcoholic fatty liver (NAFL), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic fatty liver disease (AFLD), and alcoholic steatohepatitis (ASH).

52. The method of claim 49 or 51, wherein the subject has pediatric NAFLD.

53. The method of any of the preceding claims, wherein the subject has a high BMI or obesity.

54. The method of any of the preceding claims wherein the subject has gut leakiness.

55. The method of any of the preceding claims, wherein the subject has gut dysbiosis or gut microbiome disturbance.

56. The method of any of the preceding claims, wherein the subject has increased levels of inflammatory cytokines relative to a normal subject without a fatty liver disease.

57. The method of claim 56, wherein the subject has increased levels of TNFα relative to a normal subject e.g., without the one or more symptoms or without a fatty liver disease.

58. The method of any of the preceding claims, wherein the patient exhibits muscle atrophy or has a decreased ratio of muscle tissue to adipose tissue relative to a normal subject, e.g., without the one or more symptoms or without a fatty liver disease.

59. The method of claim 58, wherein the patient exhibits muscle atrophy without one or both of fibrosis or cirrhosis.

60. The method of any of the preceding claims, wherein the subject exhibits reverse lipid transport from adipose tissue to liver tissue.

61. The method of any of the preceding claims, wherein the subject has cirrhosis, hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

62. The method of any of the preceding claims, wherein the subject has metabolic syndrome or type 2 diabetes.

63. A dietary composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity

64. The dietary composition of claim 63, wherein the dietary composition is chosen from a medical food, a functional food, or a supplement.

65. The dietary composition of claim 63 or 64, wherein the subject has fatty liver disease.

66. The dietary composition of claim 65, wherein the subject has a disease or disorder selected from the group consisting of non-alcoholic fatty liver (NAFL), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic fatty liver disease (AFLD), and alcoholic steatohepatitis (ASH).

67. The dietary composition of claim 65 or 66, wherein the subject has pediatric NAFLD.

68. The dietary composition of any of the preceding claims, wherein the subject has a high BMI or obesity.

69. The dietary composition of any of the preceding claims wherein the subject has gut leakiness.

70. The dietary composition of any of the preceding claims, wherein the subject has gut dysbiosis or gut microbiome disturbance.

71. The dietary composition of any of the preceding claims, wherein the subject has fibrosis, cirrhosis, hepatocarcinoma, an increased risk of liver failure, or an increased risk of death.

72. The dietary composition of any of the preceding claims, wherein the subject has one, two, three, or four of a high BMI, obesity, metabolic syndrome, or type 2 diabetes.

73. The dietary composition of any of the preceding claims, wherein the composition promotes weight loss in the subject.

74. A method of providing amino acid entities to a subject comprising administering to the subject an effective amount of a composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity.

75. A method of increasing one, two, three, or more (e.g., all) amino acid entities in a subject comprising administering to the subject an effective amount of a composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity.

76. The method of claim 75, wherein the amino acid entities increase in blood, plasma, or serum of the subject.

77. The method of claim 76, wherein the amino acid entities increase in a blood, plasma, or serum sample from the subject.

78. The method or dietary composition for use of any of the preceding claims, wherein administering the composition results in an improvement in one or more metabolic symptoms in the subject.

79. The method or dietary composition for use of claim 78, wherein the improvement in one or more metabolic symptoms is selected from the following: increased free fatty acid and lipid metabolism, improved mitochondrial function, white adipose tissue (WAT) browning, decreased reactive oxygen species (ROS), increased levels of glutathione (GSH), decreased hepatic inflammation, decreased hepatocyte ballooning, improved gut barrier function, increased insulin secretion, or glucose tolerance.

80. The method or dietary composition for use of claim 79, wherein the increased free fatty acid and lipid metabolism occurs in the liver.

81. The method or dietary composition for use of any of the preceding claims, wherein administration of the composition results in an improvement in one or more metabolic symptoms after a treatment period of 24 hours.

82. The method or dietary composition for use of any of the preceding claims, wherein the method further comprises determining the level of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or more (e.g., all) of the following:

a) alanine aminotransferase (ALT);

b) aspartate aminotransferase (AST);

c) adiponectin;

d) N-terminal fragment of type III collagen (proC3);

e) a caspase-cleaved keratin 18 fragments (e.g., M30 or M65);

f) an interleukin (e.g., IL-1β, IL-2, or IL-10);

g) C-reactive protein;

h) PIIINP;

i) a tissue inhibitor of metalloproteinase (TIMP); e.g., TIMP1 or TIMP2;

j) MCP-1;

k) FGF-21;

l) Col1a1;

m) Acta2;

n) a matrix metalloproteinase (MMP), e.g., MMP-13, MMP-2, MMP-9, MT1-MMP, MMP-3, or MMP-10; or

p) NF-kB.

83. The method or dietary composition for use of claim 82, wherein administration of the composition results in an improvement in one or more of a)-n) after a treatment period of 24 hours.

84. The method or dietary composition for use of any of the preceding claims, wherein administration of the composition reduces a level or activity of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or more (e.g., all) of a collagen (e.g., type I and type III collagen), αSMA, a MMP (e.g., MMP-13, MMP-2, MMP-9, MT1-MMP, MMP-3, or MMP-10), a TIMP (e.g., TIMP1 or TIMP2), AST, ALT, proC3, NF-kB, an interferon, an interleukin (e.g., IL-1β, IL-2, or IL-10), MCP-1, MIP-1, a caspase-cleaved keratin 18 fragment (e.g., one or both of M30 or M65), C-reactive protein, ACOX1, and FGF-21.

85. The method or dietary composition for use of any of the preceding claims, wherein administration of the composition reduces a level or activity of adiponectin.

86. The method or dietary composition for use of any of the preceding claims, wherein administration of the composition reduces LPS-induced hepatocyte inflammation.

87. The method or dietary composition for use of any of the preceding claims, wherein the composition enhances fatty acid oxidation.

88. The method or dietary composition for use of any of the preceding claims, wherein the composition increases levels of acylcarnitine.

89. The method or dietary composition for use of any of the preceding claims, wherein the composition reduces levels of unsaturated fatty acids by at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the level of change shown in Table 51.

90. The method or dietary composition for use of claim 88, wherein the increase in levels of acylcarnitine is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the level of change shown in Table 51.

91. The method or dietary composition for use of any of the preceding claims, wherein the composition reduces liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of alanine transaminase (ALT), e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition.

92. The method or dietary composition for use of any of the preceding claims, wherein the composition reduces liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of aspartate transaminase (AST), e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition.

93. The method or dietary composition for use of any of the preceding claims, wherein the composition reduces liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, as detected using an assay of hydroxyproline, e.g., an antibody-based detection assay, e.g., an ELISA, e.g., as described in Example 4, e.g., relative to a reference composition.

94. The method or dietary composition for use of any of the preceding claims, wherein said composition is capable of reducing, or reduces, liver fibrosis or liver injury by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using LX-2 cells, e.g., levels of Col1a1, Acta2, and/or TIMP2 in LX-2 cells, e.g., as assessed using a nucleic acid amplification method, e.g., PCR or qRT-PCR, e.g., as described in Example 7, e.g., relative to a reference composition.

95. The method or dietary composition for use of any of the preceding claims, wherein said composition is capable of reducing, or reduces, hepatocyte inflammation by at least 5%, 10%, 15%, 20%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% as detected using HepG2 cells, e.g., decreased activity, e.g., decreased TNFα-induced activity, of NF-kB in HepG2 cells, as described in Example 8, e.g., relative to a reference composition.

96. The method or dietary composition for use of any of the preceding claims, wherein said composition is capable of increasing, or increases, glucose tolerance by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%, as detected using an assay of glucose levels, e.g., using glucose oxidase, e.g., using a glucometer, e.g., as described in Example 5, e.g., relative to a reference composition.

97. The method or dietary composition for use of any of the preceding claims, wherein the reference composition comprises a single amino acid entity, e.g., a L-amino acid entity, an I-amino acid entity, a V-amino acid entity, a R-amino acid entity, a Q-amino acid entity, or a NAC-amino acid entity, each assayed separately as a free amino acid, or a combination of amino acid entities (e.g., a L-amino acid entity, an I-amino acid entity, and a V-amino acid entity; a R-amino acid entity, a Q-amino acid entity, and a NAC-amino acid entity; a L-amino acid entity, an I-amino acid entity, V-amino acid entity, a R-amino acid entity, and a Q-amino acid entity).

98. The method or dietary composition for use of any of the preceding claims, wherein the reference composition comprises vehicle.

99. The method or dietary composition for use of any of the preceding claims, wherein the composition is administered prior to a meal.

100. The method or dietary composition for use of any of the preceding claims, wherein the composition is administered concurrent with a meal.

101. The method or dietary composition for use of any of the preceding claims, wherein the composition is administered following a meal.

102. The method or dietary composition for use of any of the preceding claims, wherein the composition is administered with a second agent.

103. The method or dietary composition for use of claim 102, wherein the second agent is selected from the group consisting of a farnesoid X receptor (FXR) agonist, a stearoyl CoA desaturase inhibitor, a CCR2 and CCR5 chemokine antagonist, a PPAR alpha and delta agonist, a caspase inhibitor, a galectin-3 inhibitor, an acetyl CoA carboxylase inhibitor, or an ileal sodium bile acid co-transporter inhibitor.

104. The method or dietary composition for use of any of the preceding claims, wherein the composition is administered at a dose of about 15 g/d to about 90 g/d.

105. The method or dietary composition for use of claim 104, wherein the composition is administered at a dose of about 18 g/d, about 24 g/d, about 36/d, about 54 g/d, or about 72 g/d.

106. The method or dietary composition for use of any of the preceding claims, wherein the composition is administered one, two, to three times per day.

107. The method or dietary composition for use of any of the preceding claims, wherein the composition is administered at a dose of about 6 g, about 8 g, about 12 g, about 16 g, about 18 g, or about 24 g three times per day.

108. A method of manufacturing or making a composition comprising forming a composition comprising the following:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity.

109. A composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity, wherein said composition is for use in improving liver function.

110. A composition comprising:

a) a leucine (L)-amino acid entity, a arginine (R)-amino acid entity, and a glutamine (Q)-amino acid entity; and

b) a N-acetylcysteine (NAC) entity, e.g., NAC;

provided that:

c) at least one amino acid entity is not provided as a peptide of more than 20 amino acid residues in length, and

wherein:

(i) an amino acid entity of (a) is selected from Table 2; and

(ii) one or both of the R-amino acid entity and the Q-amino acid entity are present at a higher amount (wt. %) than the L-amino acid entity, or the composition further comprises a serine (S)-amino acid entity, wherein said composition is for use in a method of any of the preceding claims.

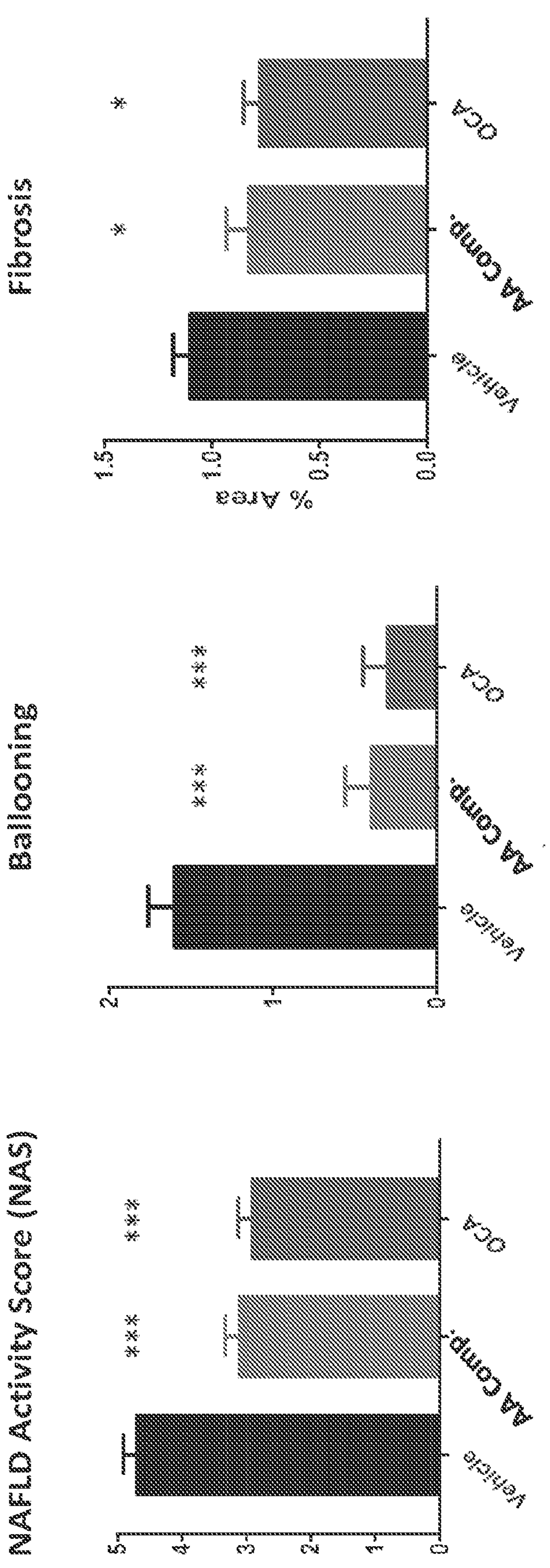

Fibrosis
% Area
1.5
1.0
0.5
0.0
Vehicle
AA Comp.
OCA
*
*
Ballooning
2
1
0
Vehicle
AA Comp.
OCA
***
***
NAFLD Activity Score (NAS)
5
4
3
2
1
0
Vehicle
AA Comp.
OCA
***
***

FIG. 1A

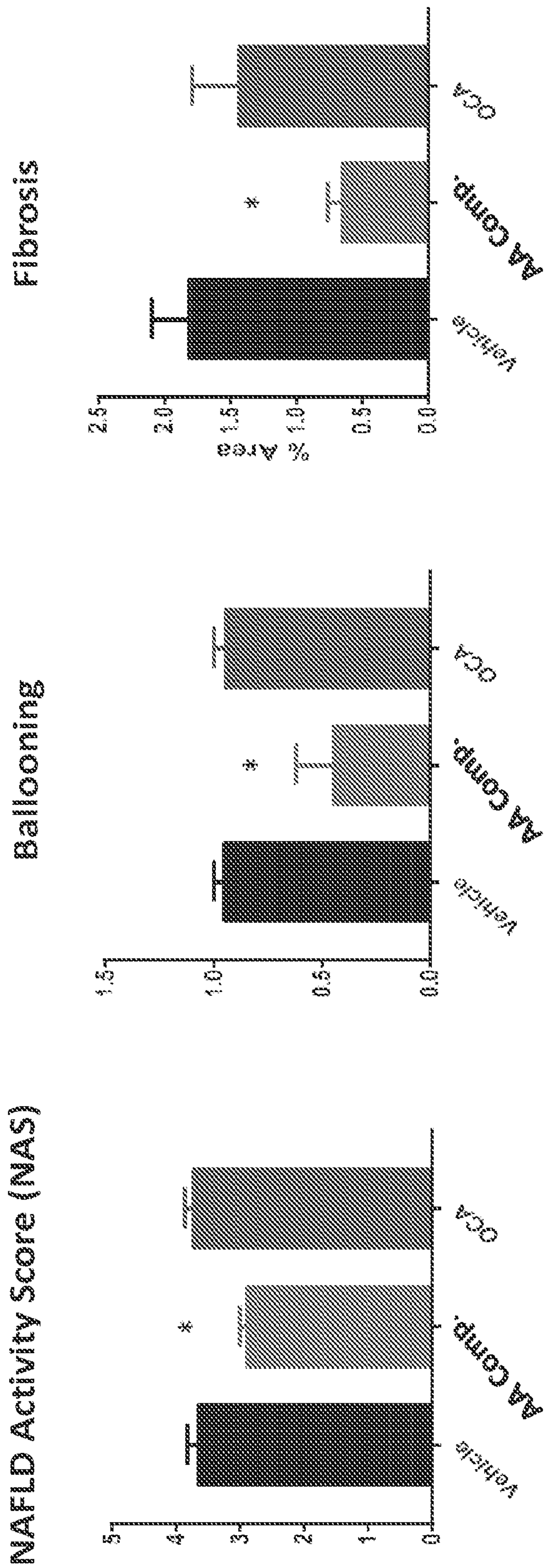

NAFLD Activity Score (NAS)
Ballooning
Fibrosis
% Area
Vehicle
AA Comp.
OCA
*

FIG. 1B

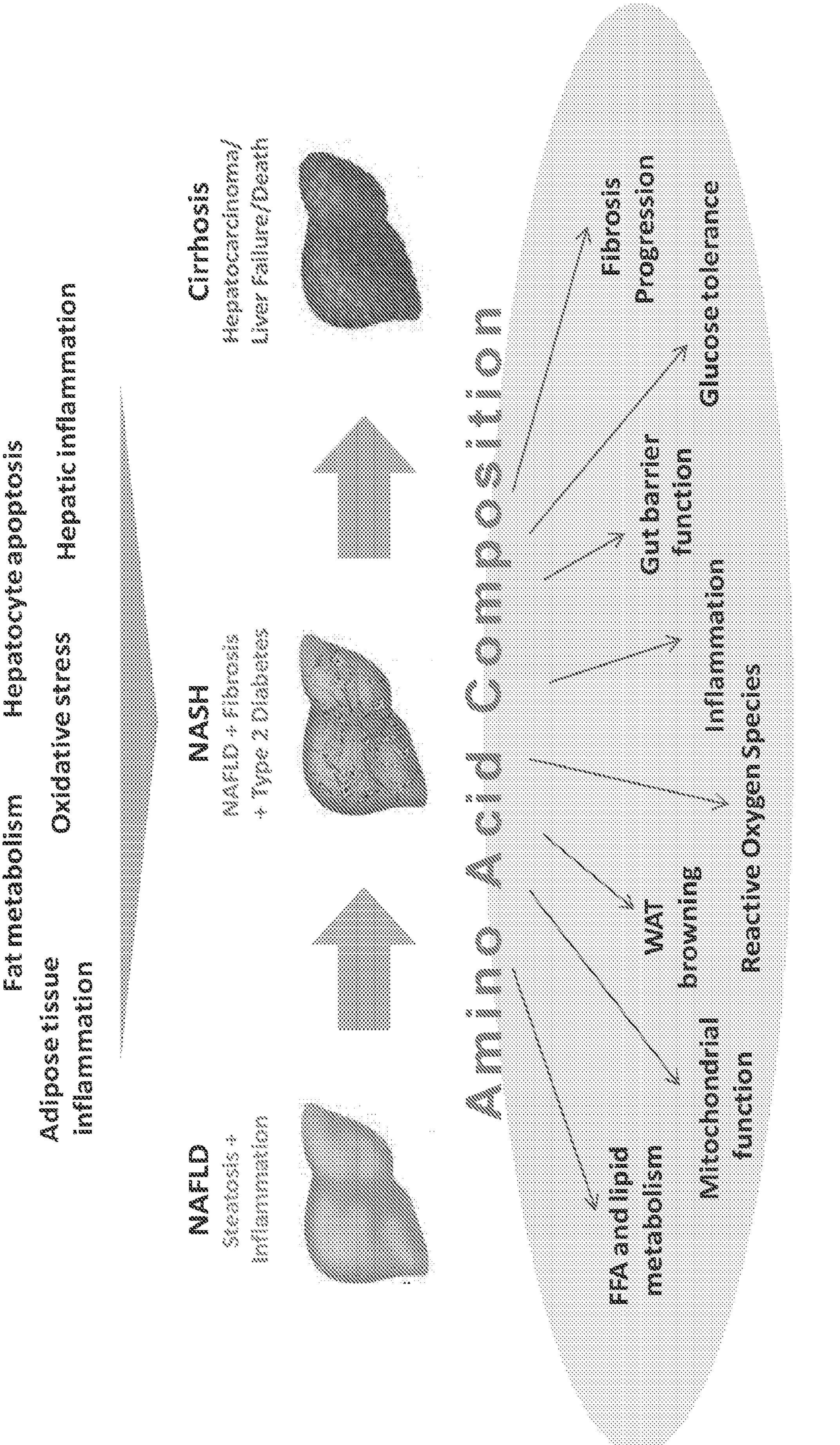

Adipose tissue inflammation
Fat metabolism
Oxidative stress
Hepatocyte apoptosis
Hepatic inflammation
NAFLD
Steatosis + inflammation
NASH
NAFLD + Fibrosis + Type 2 Diabetes
Cirrhosis
Hepatocarcinoma/ Liver Failure/Death
Amino Acid Composition
FFA and lipid metabolism
Mitochondrial function
WAT browning
Reactive Oxygen Species
Inflammation
Gut barrier function
Glucose tolerance
Fibrosis Progression

FIG. 2

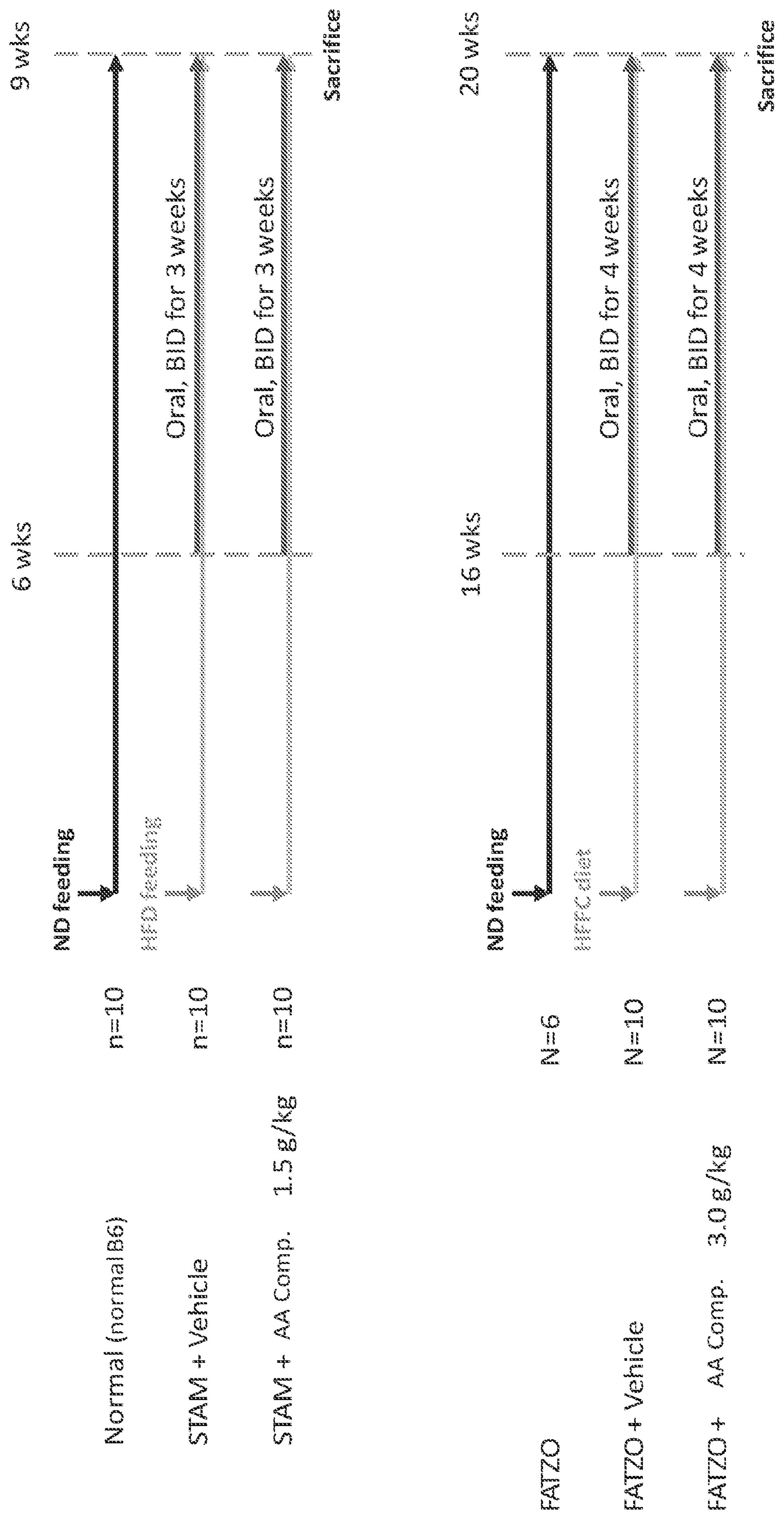

Normal (normal B6)
n=10
STAM + Vehicle
n=10
STAM + AA Comp. 1.5 g/kg
n=10
ND feeding
HFD feeding
6 wks
9 wks
Oral, BID for 3 weeks
Oral, BID for 3 weeks
Sacrifice
FATZO
N=6
FATZO + Vehicle
N=10
FATZO + AA Comp. 3.0 g/kg
N=10
ND feeding
HFFC diet
16 wks
20 wks
Oral, BID for 4 weeks
Oral, BID for 4 weeks
Sacrifice

FIG. 3

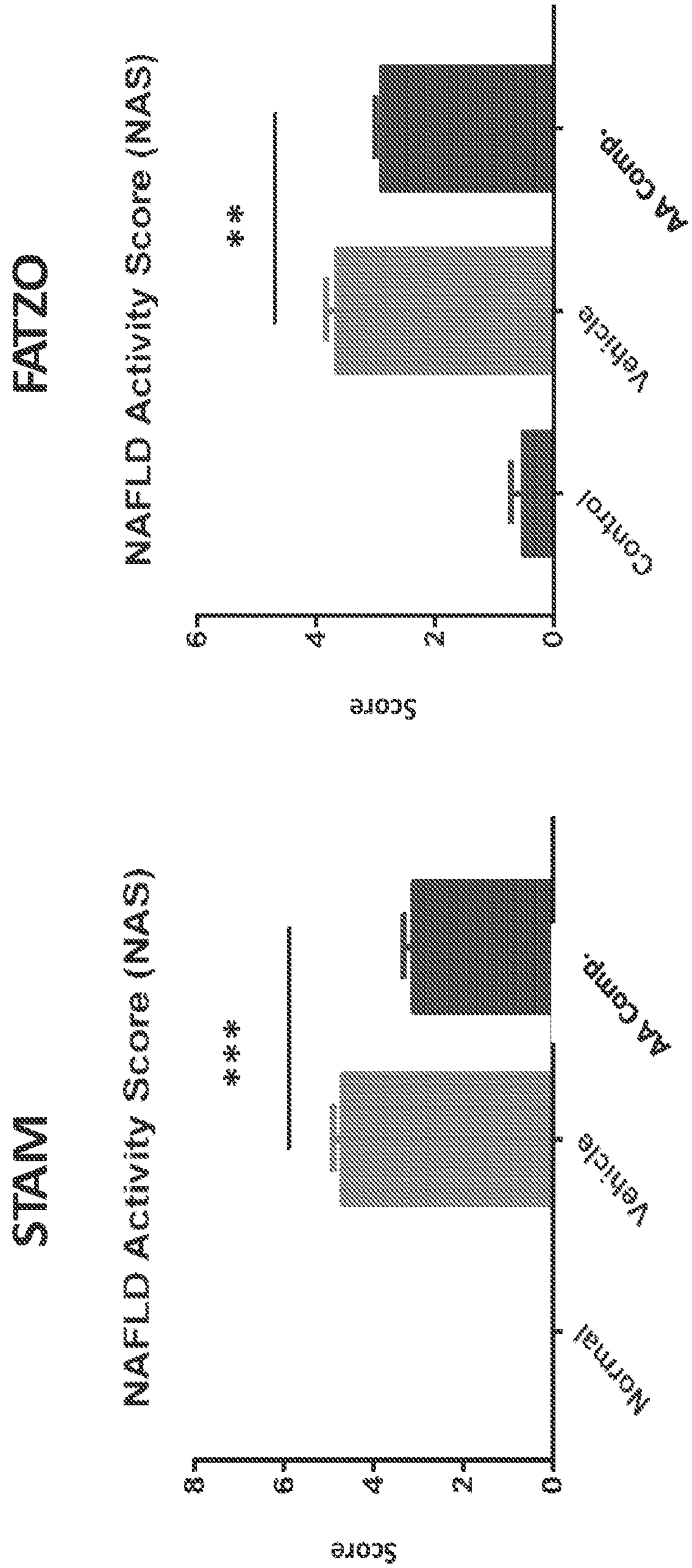

FATZO
NAFLD Activity Score (NAS)
Score
6
4
2
0
Control
Vehicle
AA Comp.
**
STAM
NAFLD Activity Score (NAS)
Score
8
6
4
2
0
Normal
Vehicle
AA Comp.
***

FIG. 4A

STAM

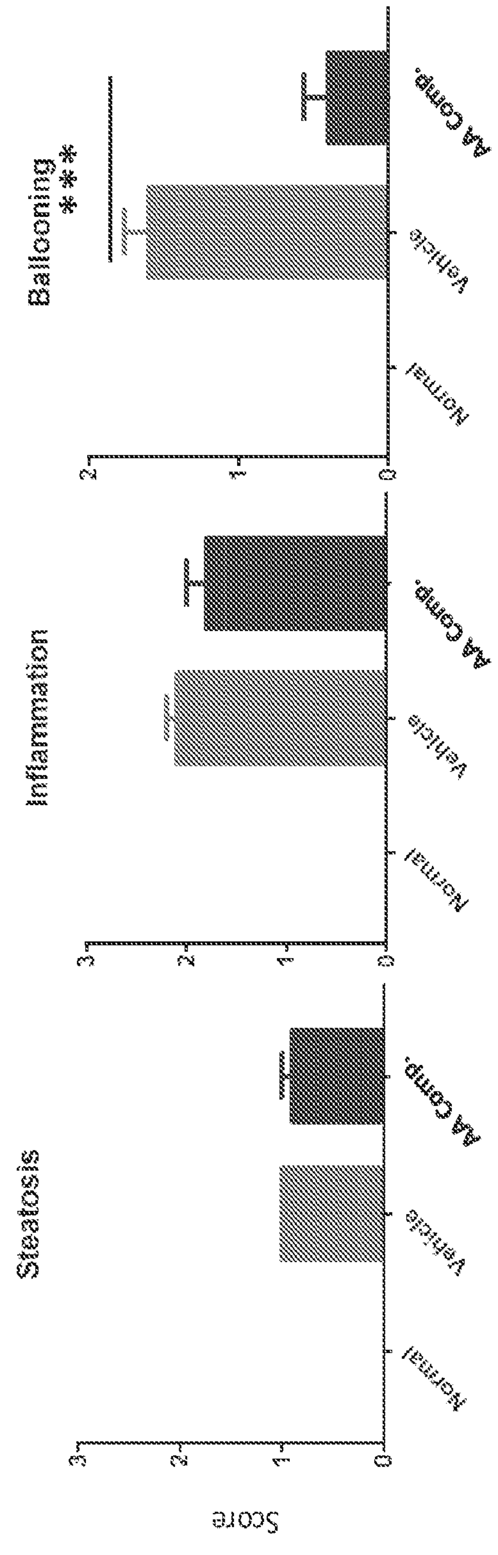

Steatosis
Inflammation
Ballooning
***
Score
0
1
2
3
Normal
Vehicle
AA Comp.

FIG. 4B

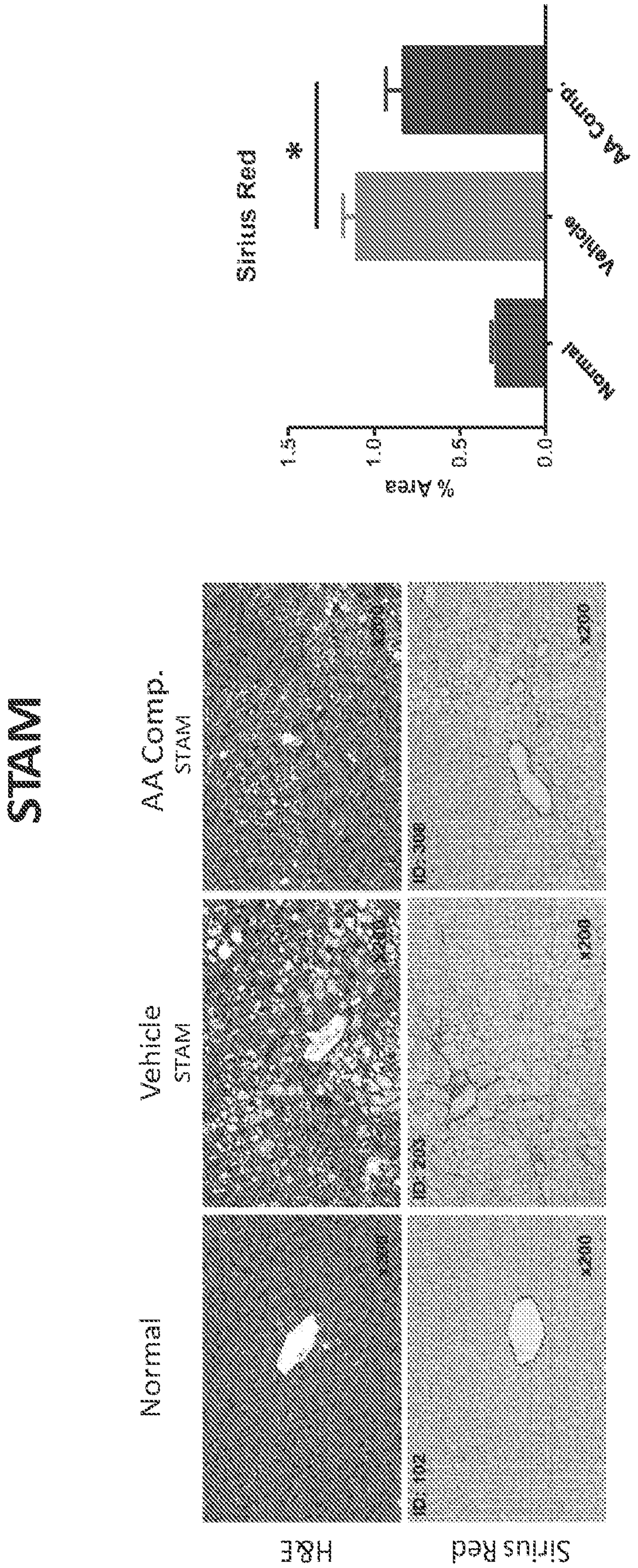

Sirius Red
*
% Area
1.5
1.0
0.5
0.0
Normal
Vehicle
AA Comp.
STAM
Normal
Vehicle
STAM
AA Comp.
STAM
H&E
Sirius Red
ID: 102
ID: 203
ID: 306
x200

FIG. 4C

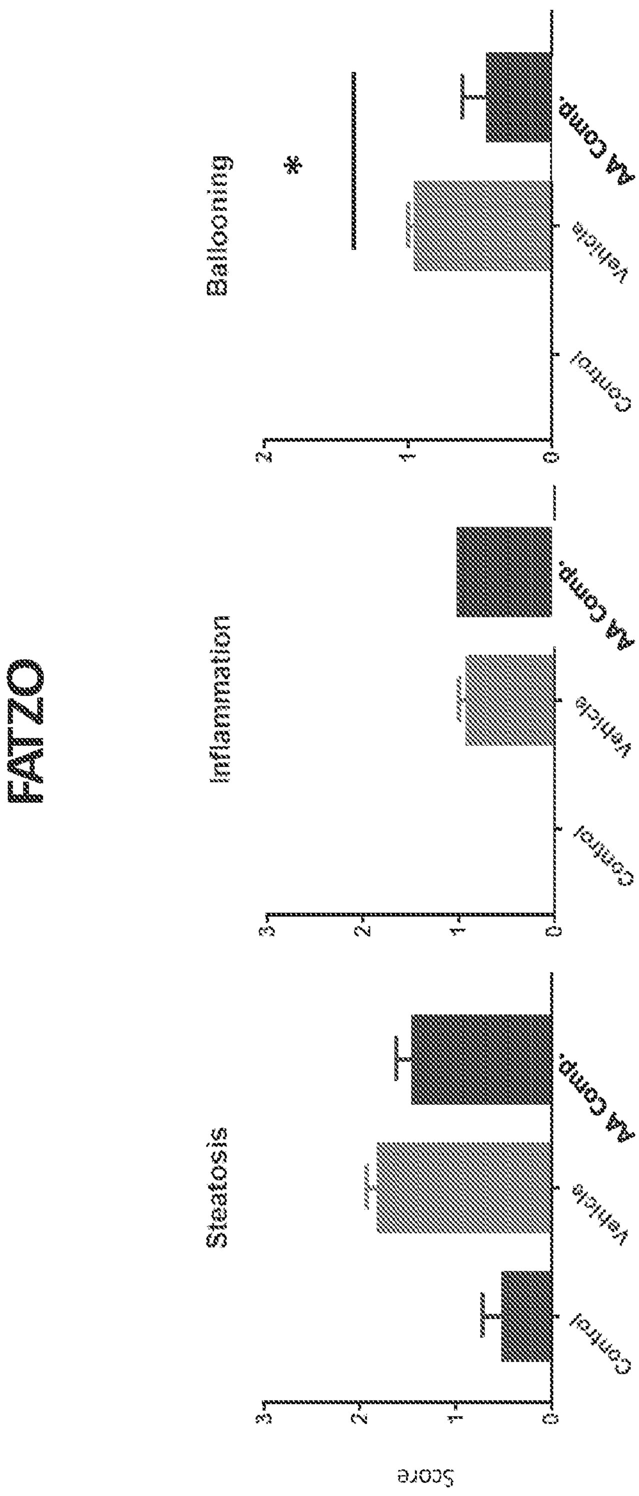

FATZO
Steatosis
Inflammation
Ballooning
Score
0
1
2
3
Control
Vehicle
AA Comp.
*

FIG. 4D

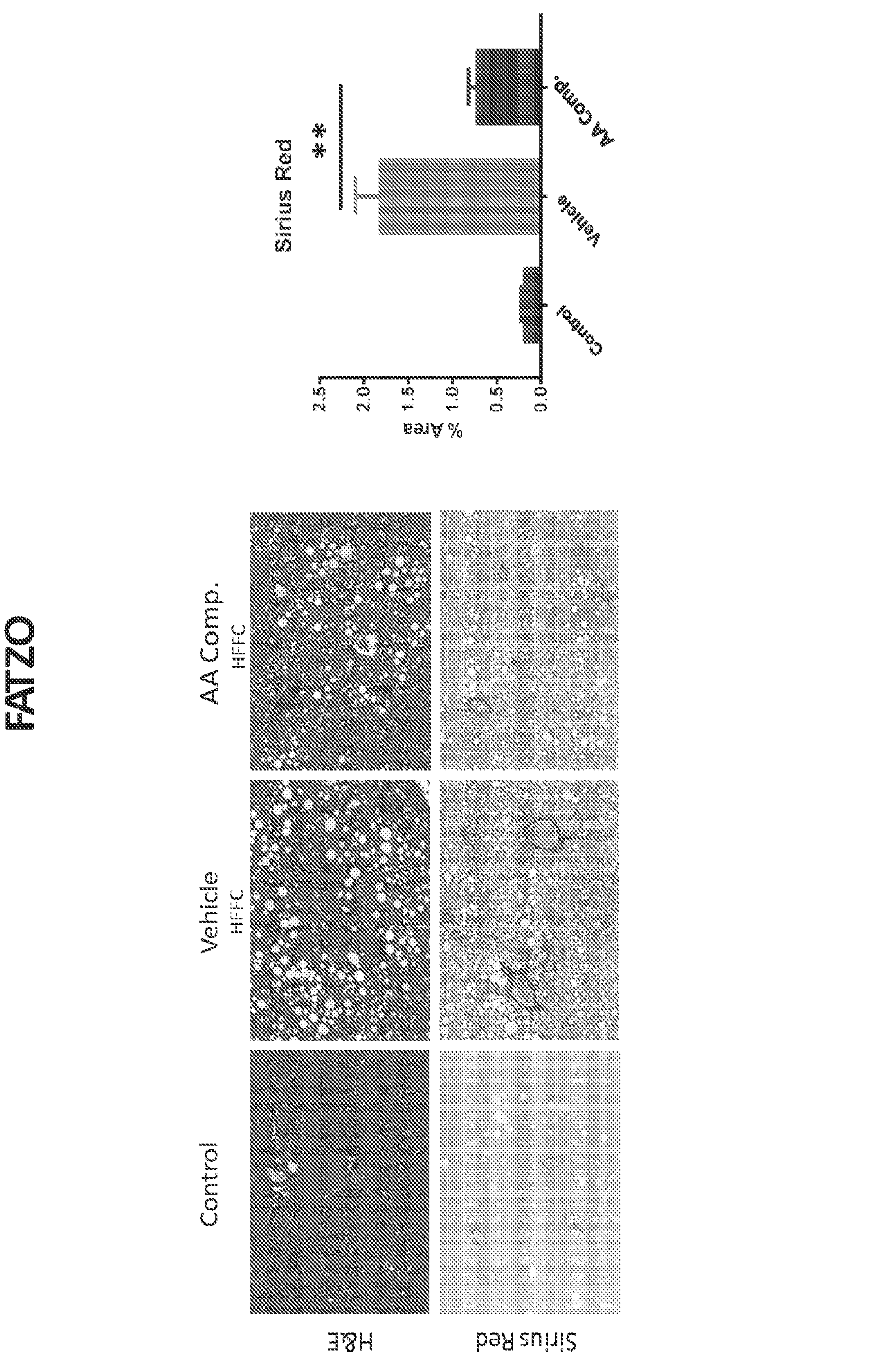

FATZO
Control
Vehicle
HFFC
AA Comp.
HFFC
H&E
Sirius Red
Sirius Red
% Area
0.0
0.5
1.0
1.5
2.0
2.5
**
Control
Vehicle
AA Comp.

FIG. 4E

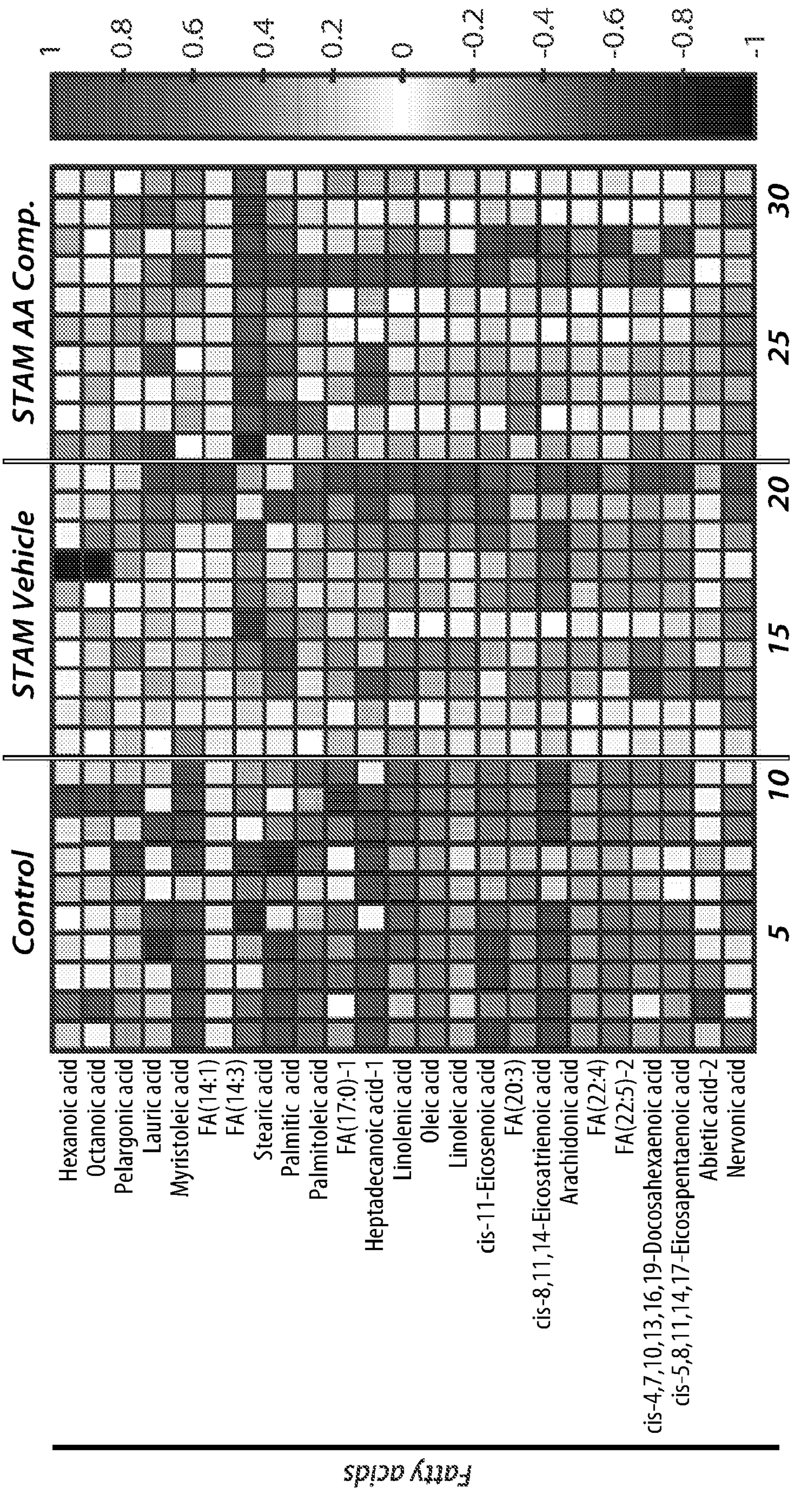

Control
STAM Vehicle
STAM AA Comp.
5
10
15
20
25
30
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
Hexanoic acid
Octanoic acid
Pelargonic acid
Lauric acid
Myristoleic acid
FA(14:1)
FA(14:3)
Stearic acid
Palmitic acid
Palmitoleic acid
FA(17:0)-1
Heptadecanoic acid-1
Linolenic acid
Oleic acid
Linoleic acid
cis-11-Eicosenoic acid
FA(20:3)
cis-8,11,14-Eicosatrienoic acid
Arachidonic acid
FA(22:4)
FA(22:5)-2
cis-4,7,10,13,16,19-Docosahexaenoic acid
cis-5,8,11,14,17-Eicosapentaenoic acid
Abietic acid-2
Nervonic acid
Fatty acids

FIG. 5A

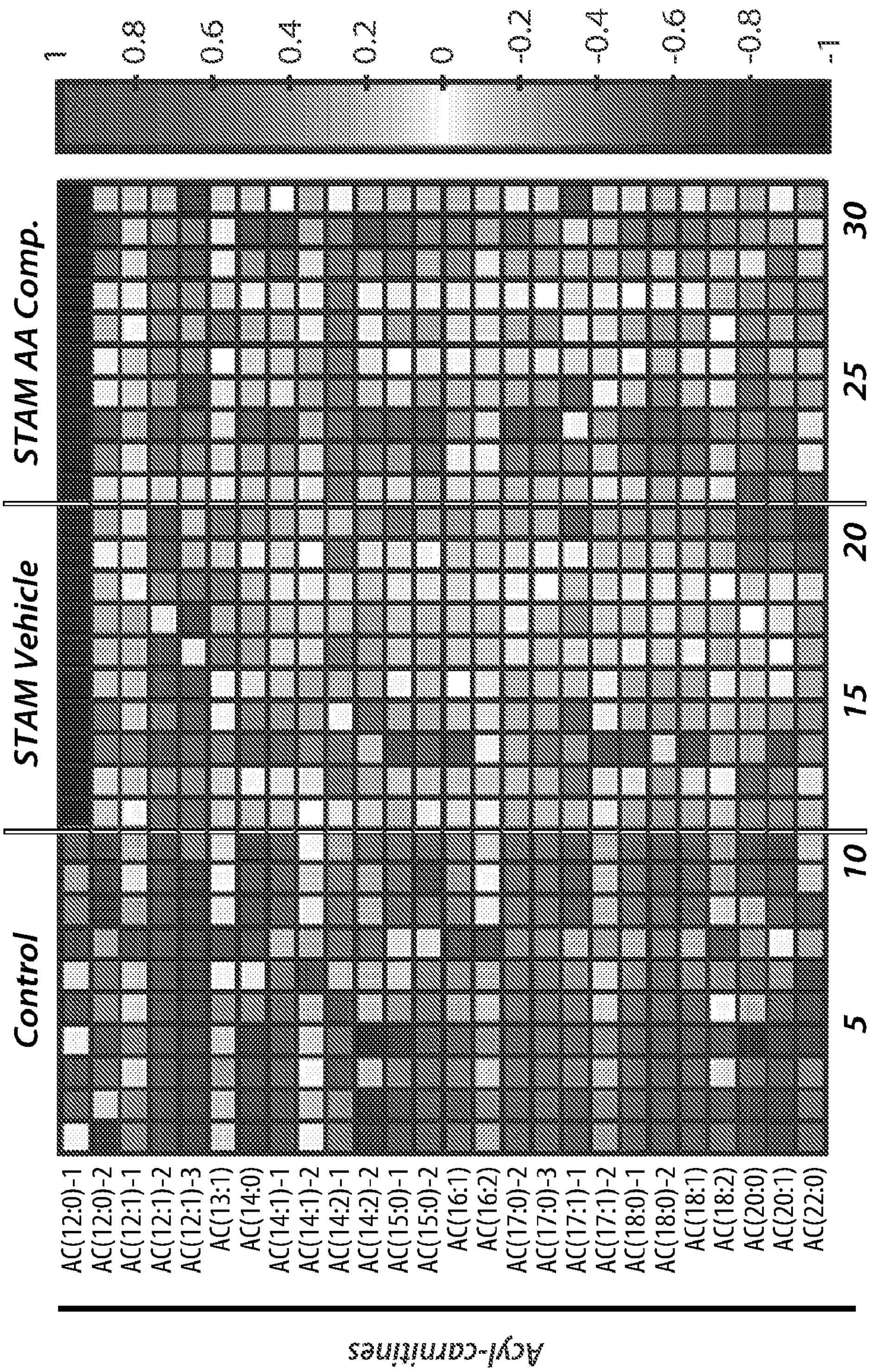

Control
STAM Vehicle
STAM AA Comp.
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
AC(12:0)-1
AC(12:0)-2
AC(12:1)-1
AC(12:1)-2
AC(12:1)-3
AC(13:1)
AC(14:0)
AC(14:1)-1
AC(14:1)-2
AC(14:2)-1
AC(14:2)-2
AC(15:0)-1
AC(15:0)-2
AC(16:1)
AC(16:2)
AC(17:0)-2
AC(17:0)-3
AC(17:1)-1
AC(17:1)-2
AC(18:0)-1
AC(18:0)-2
AC(18:1)
AC(18:2)
AC(20:0)
AC(20:1)
AC(22:0)
5
10
15
20
25
30
Acyl-carnitines

FIG. 5B

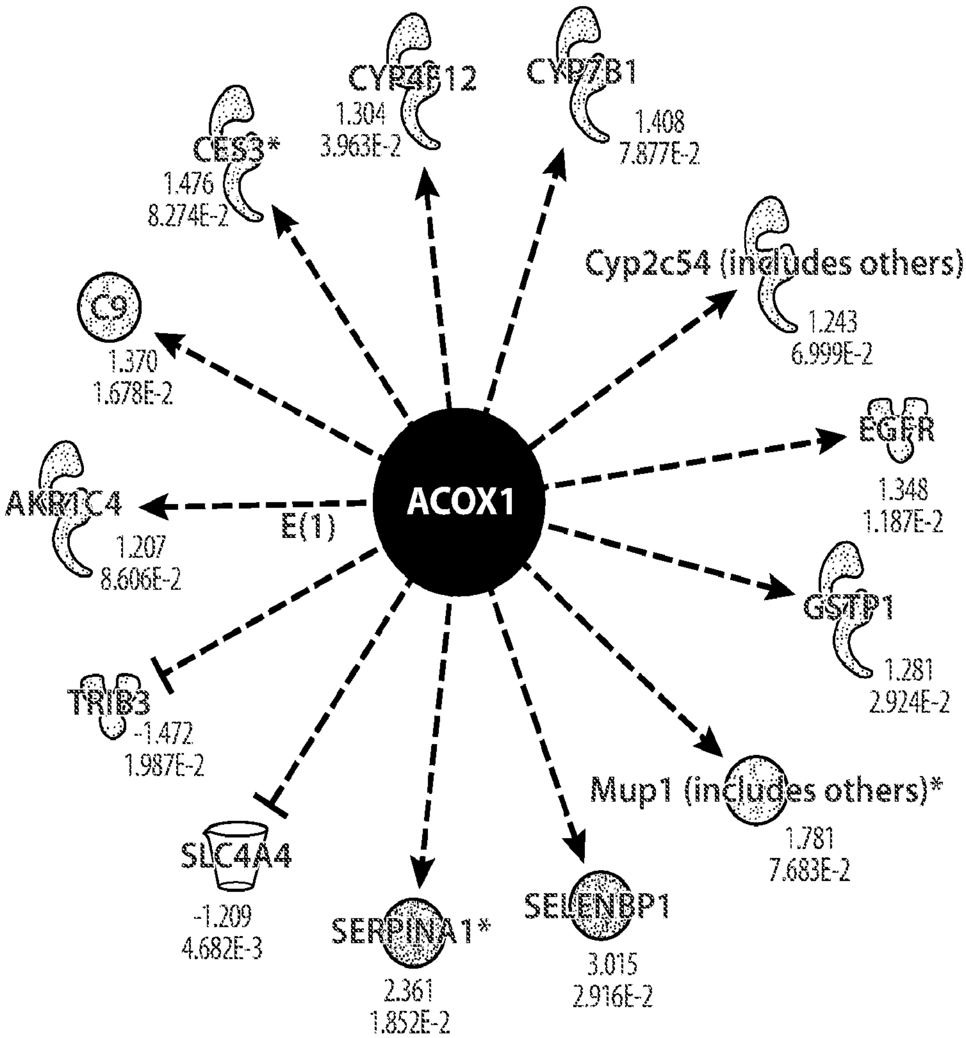

CYP4F12
1.304
3.963E-2
CYP7B1
1.408
7.877E-2
CES3*
1.476
8.274E-2
Cyp2c54 (includes others)
1.243
6.999E-2
C9
1.370
1.678E-2
EGFR
1.348
1.187E-2
AKR1C4
1.207
8.606E-2
E(1)
ACOX1
GSTP1
1.281
2.924E-2
TRIB3
-1.472
1.987E-2
Mup1 (includes others)*
1.781
7.683E-2
SLC4A4
-1.209
4.682E-3
SERPINA1*
2.361
1.852E-2
SELENBP1
3.015
2.916E-2

FIG. 6

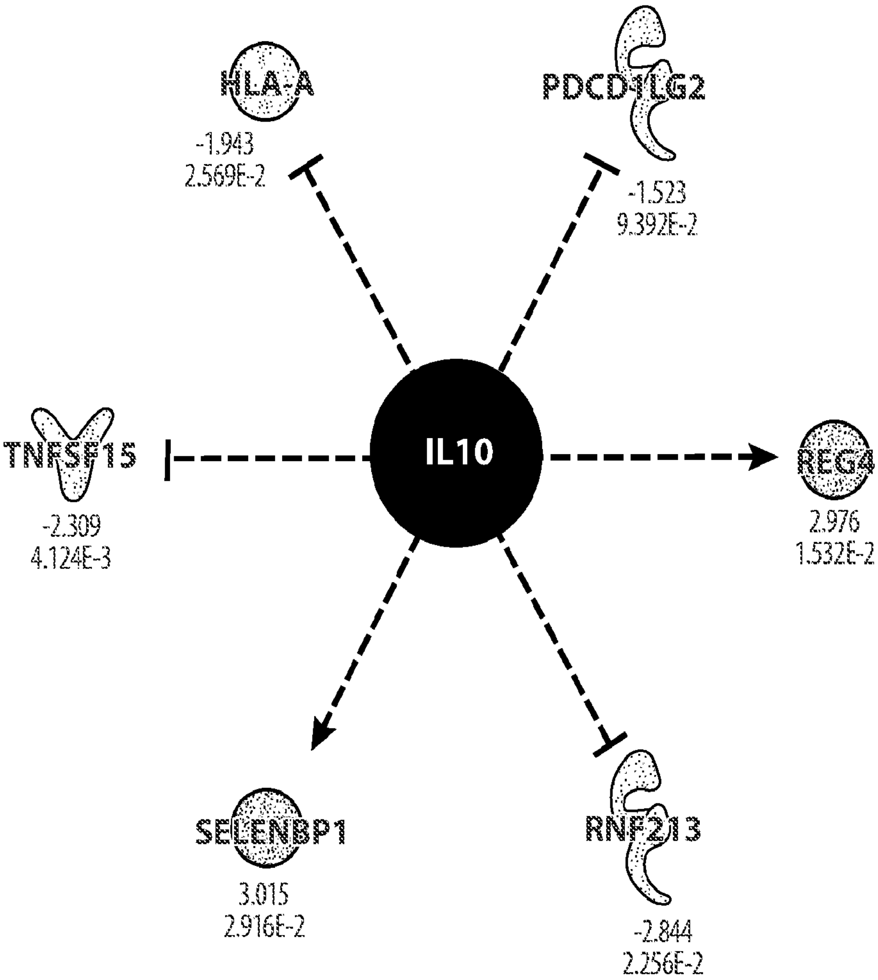

HLA-A
-1.943
2.569E-2
PDCD1LG2
-1.523
9.392E-2
TNFSF15
-2.309
4.124E-3
IL10
REG4
2.976
1.532E-2
SELENBP1
3.015
2.916E-2
RNF213
-2.844
2.256E-2

FIG. 7A

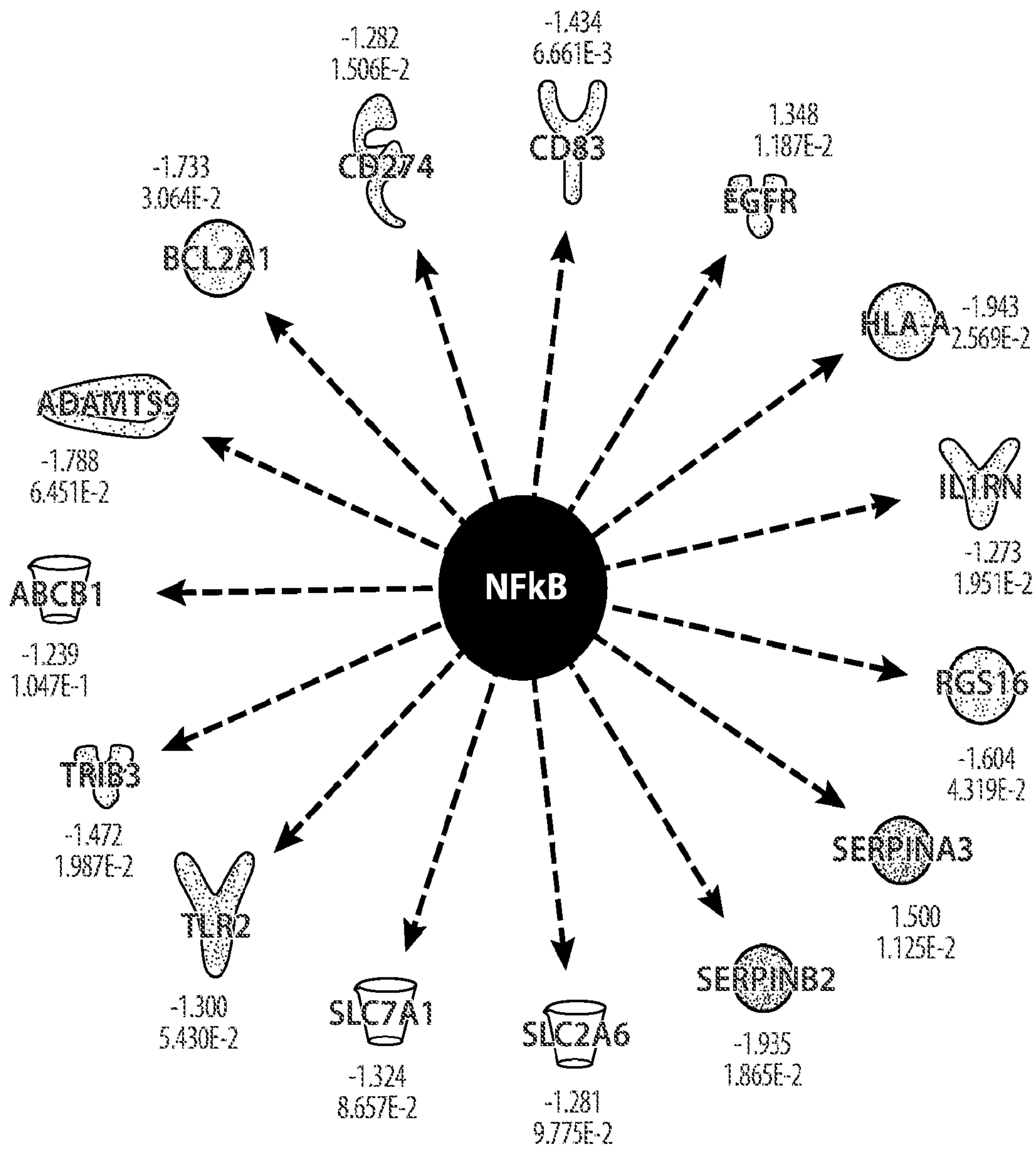

NFkB
CD274
-1.282
1.506E-2
CD83
-1.434
6.661E-3
EGFR
1.348
1.187E-2
BCL2A1
-1.733
3.064E-2
HLA-A
-1.943
2.569E-2
ADAMTS9
-1.788
6.451E-2
IL1RN
-1.273
1.951E-2
ABCB1
-1.239
1.047E-1
RGS16
-1.604
4.319E-2
TRIB3
-1.472
1.987E-2
SERPINA3
1.500
1.125E-2
TLR2
-1.300
5.430E-2
SLC7A1
-1.324
8.657E-2
SLC2A6
-1.281
9.775E-2
SERPINB2
-1.935
1.865E-2

FIG. 7B

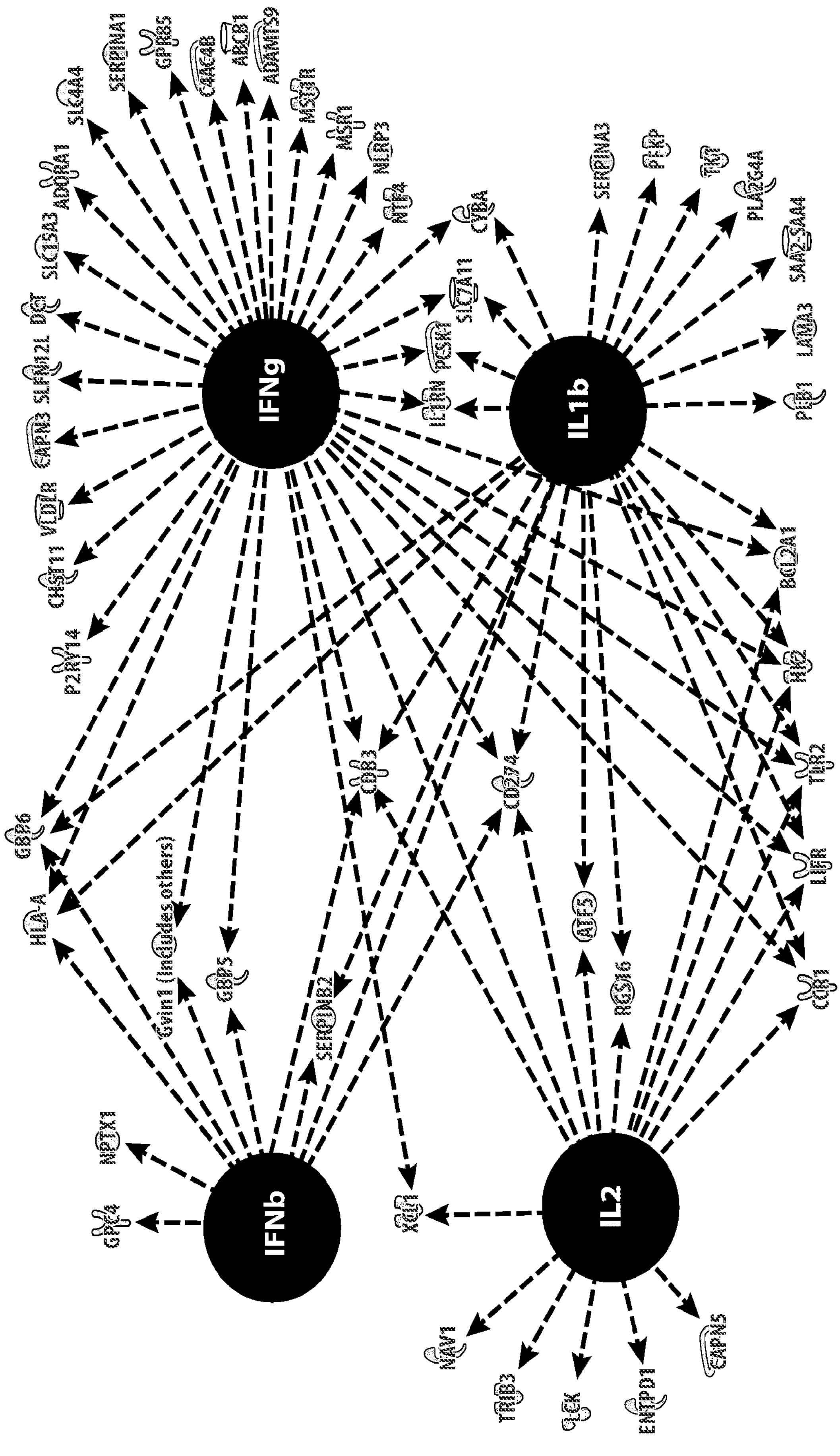

IFNg
IL1b
IFNb
IL2
SLC4A4
SERPINA1
GPR85
ABCB1
ADAMTS9
MST1R
MSR1
NLRP3
CYBA
SLC7A11
PCSK1
IL1RN
SERPINA3
PLA2G4A
SAA2-SAA4
LAMA3
PER1
BCL2A1
HK2
TLR2
LIFR
CCR1
ATF5
RGS16
CD274
CD83
SERPINB2
GBP5
Gvin1 (includes others)
HLA-A
GBP6
P2RY14
CHST11
VLDLR
CAPN3
SLFN12L
DCT
SLC15A3
ADORA1
NPTX1
GPC4
NAV1
TRIB3
LCK
ENTPD1
CAPN5

FIG. 7C

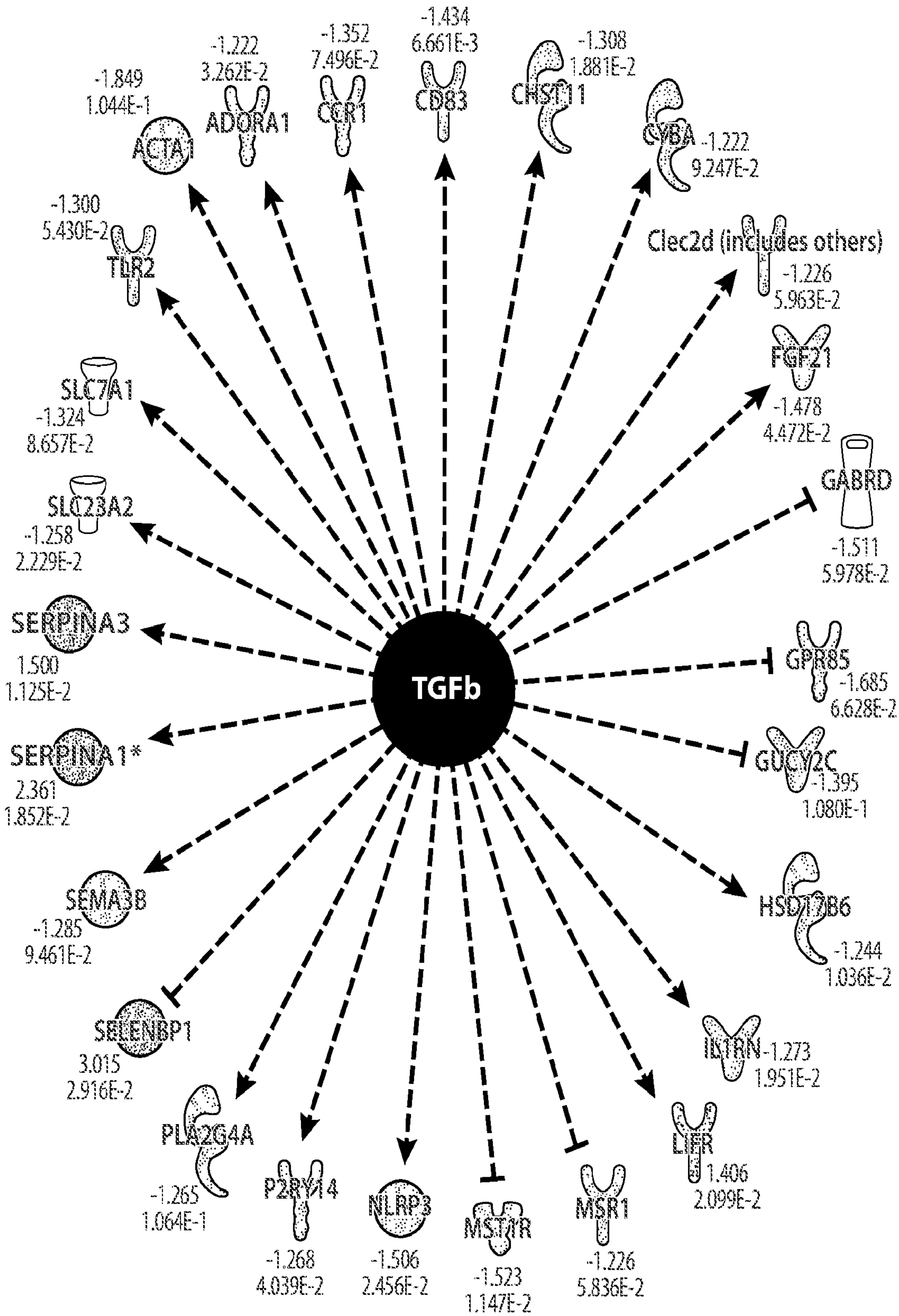

TGFb
ACTA1 -1.849 1.044E-1
ADORA1 -1.222 3.262E-2
CCR1 -1.352 7.496E-2
CD83 -1.434 6.661E-3
CHST11 -1.308 1.881E-2
CYBA -1.222 9.247E-2
Clec2d (includes others) -1.226 5.963E-2
FGF21 -1.478 4.472E-2
GABRD -1.511 5.978E-2
GPR85 -1.685 6.628E-2
GUCY2C -1.395 1.080E-1
HSD17B6 -1.244 1.036E-2
IL1RN -1.273 1.951E-2
LIFR 1.406 2.099E-2
MSR1 -1.226 5.836E-2
MST1R -1.523 1.147E-2
NLRP3 -1.506 2.456E-2
P2RY14 -1.268 4.039E-2
PLA2G4A -1.265 1.064E-1
SELENBP1 3.015 2.916E-2
SEMA3B -1.285 9.461E-2
SERPINA1* 2.361 1.852E-2
SERPINA3 1.500 1.125E-2
SLC23A2 -1.258 2.229E-2
SLC7A1 -1.324 8.657E-2
TLR2 -1.300 5.430E-2

FIG. 7D

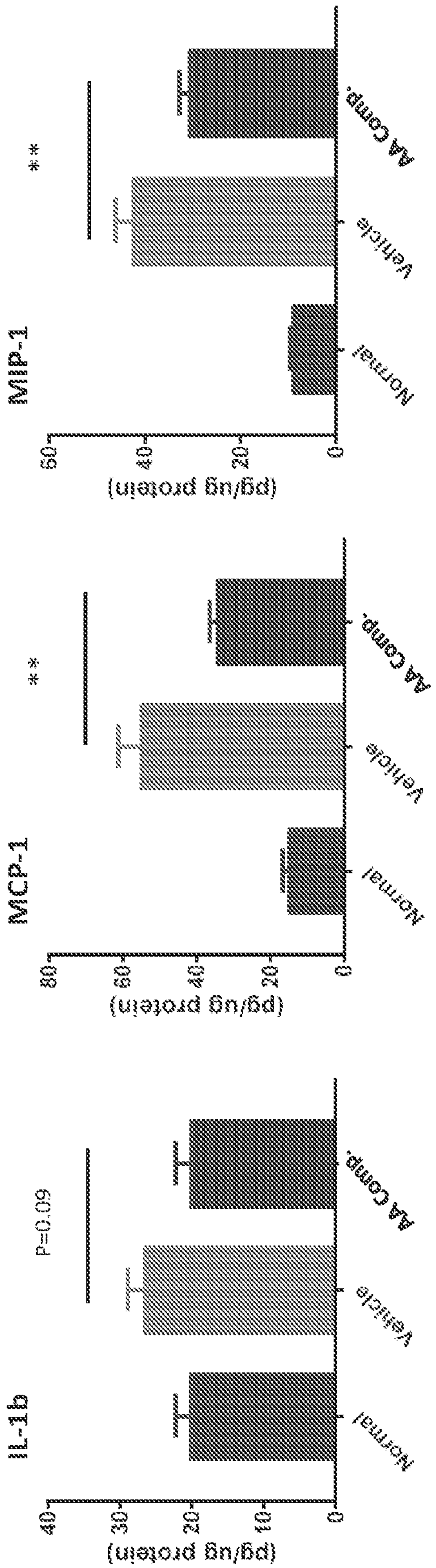

MIP-1
**
(pg/ug protein)
50
40
20
0
Normal
Vehicle
AA Comp.
MCP-1
**
(pg/ug protein)
80
60
40
20
0
Normal
Vehicle
AA Comp.
IL-1b
P=0.09
(pg/ug protein)
40
30
20
10
0
Normal
Vehicle
AA Comp.

FIG. 8

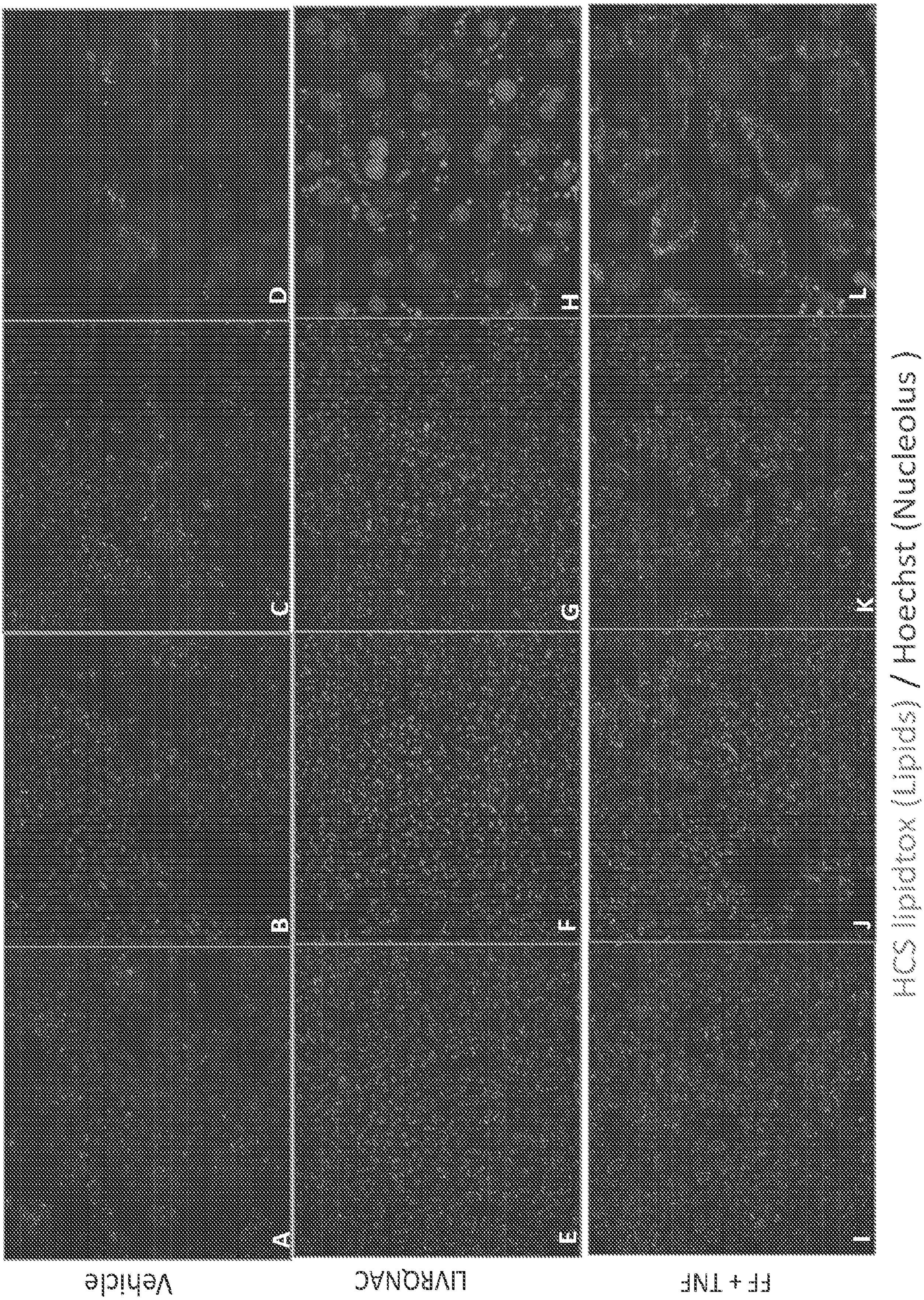

Vehicle
LIVRQNAC
FF + TNF
A
B
C
D
E
F
G
H
I
J
K
L
HCS lipidtox (Lipids) / Hoechst (Nucleolus)

FIG. 9A-9L

PCT/US17/67345 08-03-2018

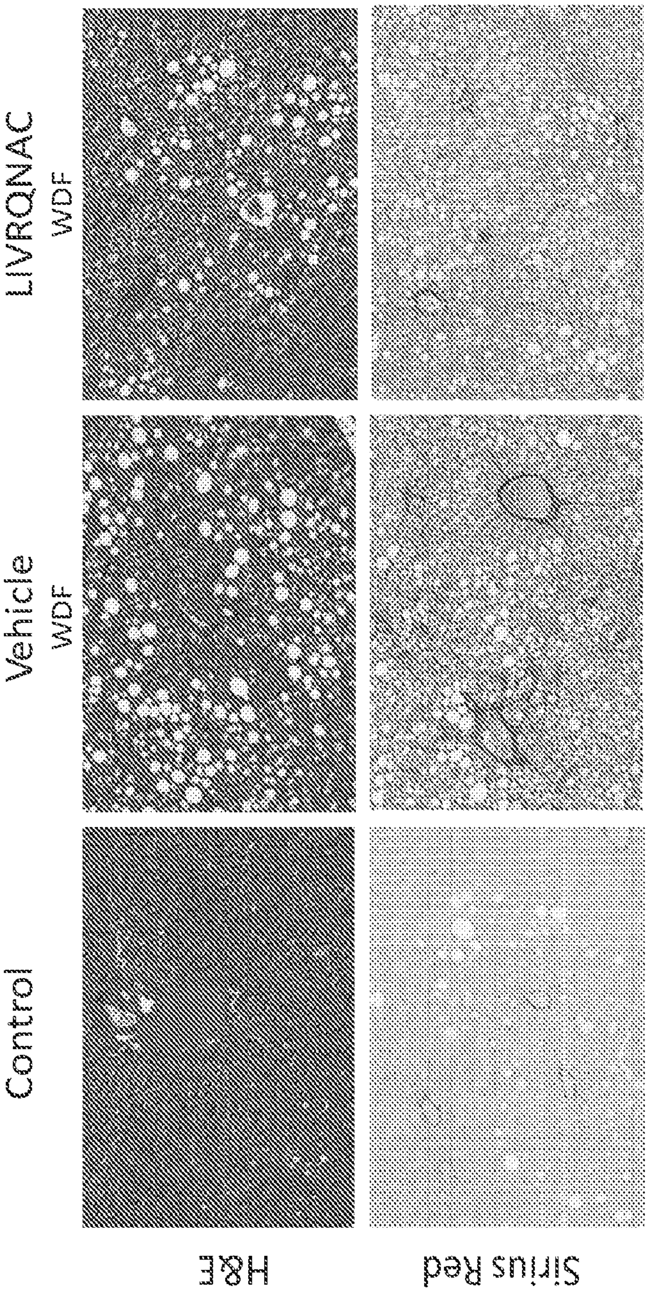


FIG. 10

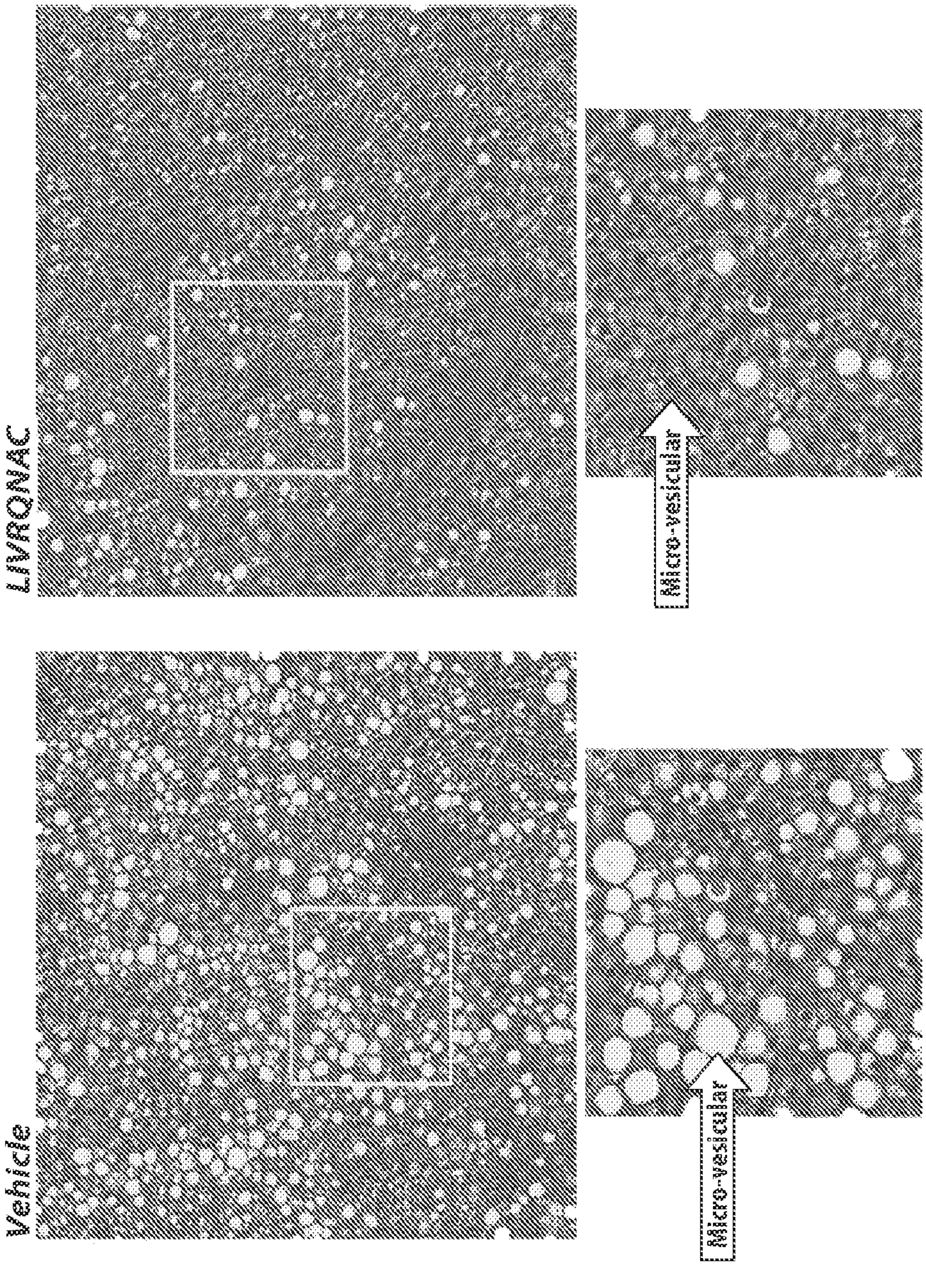
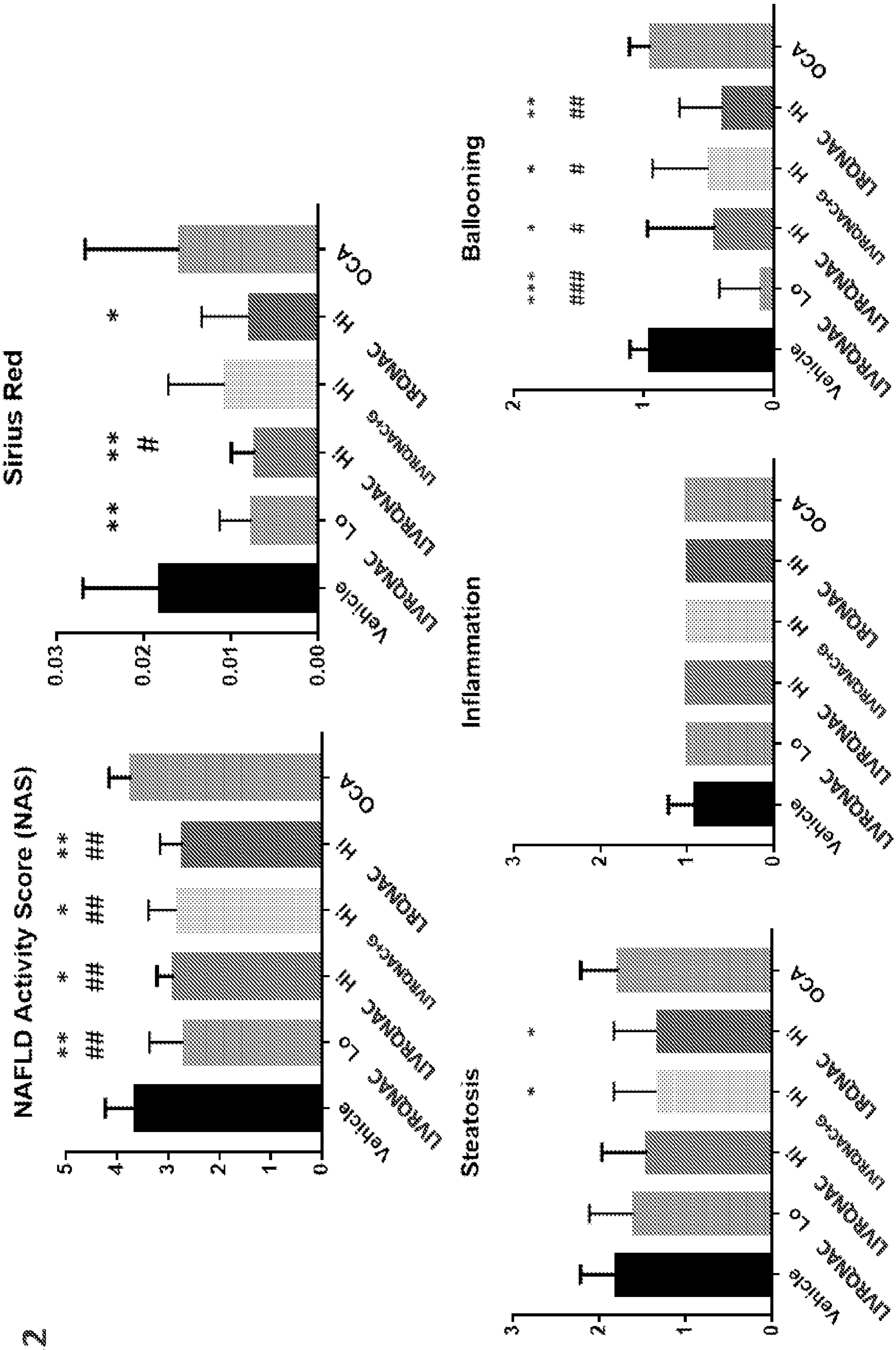


FIG. 11

FIG. 12


NAFLD Activity Score (NAS)
Sirius Red
Steatosis
Inflammation
Ballooning
Vehicle
LIVRQNAC Lo
LIVRQNAC Hi
LIVRQNAC+G Hi
LRQNAC Hi
OCA
**
*
##
#
***
###
0
1
2
3
4
5
0.00
0.01
0.02
0.03

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/067345

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/198 A23L33/175 A23L33/18 A61P1/16
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A23L A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/56402 A2 (MUSCLETECH RES AND DEV INC [CA]) 9 August 2001 (2001-08-09)	1,2,4-6, 8,16-20, 23,24, 29,44, 45, 63-102, 104-108, 110
Y	examples 1-3	1-110

Y	EP 1 541 141 A1 (AJINOMOTO KK [JP]) 15 June 2005 (2005-06-15) example 1; table 1 claims 1, 8	1-110

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2018	09/03/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Collura, Alessandra

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 2 792 354 A2 (OBSHCHESTVO S OGRANICHENNOJ OTVETSTVENNOSTJU BIOTEKHNOLOGII PYSHCHINO) 22 October 2014 (2014-10-22) examples 3, 12 paragraph [0003] -----	1-110
Y	WO 2011/097273 A1 (MARTEK BIOSCIENCES CORP [US]; HOFFMAN JAMES [US]; KURATKO CONNYE [US];) 11 August 2011 (2011-08-11) claims 1, 59 paragraph [0003] -----	1-110
Y	SETSHEDI M ET AL: "S1846 N-Acetylcysteine Improves Hepatic Insulin Resistance Associated With High-Fat Diet and Alcohol-Induced Steatohepatitis", GASTROENTEROLOGY, W.B. SAUNDERS CO, US, vol. 138, no. 5, 1 May 2010 (2010-05-01), pages S-801, XP027026219, ISSN: 0016-5085 [retrieved on 2010-04-27] the whole document -----	1-110
Y	NAGANUMA A ET AL: "SUN-P050: Effect of the Medical Walking and Leucine Enriched Amino Acid Containing Food for Female Non-Alcoholic Fatty Liver Disease : Randomized Controlled Trial", CLINICAL NUTRITION, vol. 35, September 2016 (2016-09), XP029729663, ISSN: 0261-5614, DOI: 10.1016/S0261-5614(16)30393-4 the whole document -----	1-110
Y	SHRESTHA NIRAJAN ET AL: "Glutamine inhibits CCl4induced liver fibrosis in mice and TGF-[beta]1 mediated epithelial-mesenchymal transition in mouse hepatocytes", FOOD AND CHEMICAL TOXICOLOGY, PERGAMON, GB, vol. 93, 29 April 2016 (2016-04-29), pages 129-137, XP029563064, ISSN: 0278-6915, DOI: 10.1016/J.FCT.2016.04.024 abstract -----	1-110
Y	WO 2005/084323 A2 (TECHNICAL SOURCING INTERNATION [US]) 15 September 2005 (2005-09-15) claims 103, 106 -----	1-110

-/--

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/067345

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SELLMANN CATHRIN ET AL: "Oral arginine supplementation protects female mice from the onset of non-alcoholic steatohepatitis", AMINO ACIDS, SPRINGER VERLAG, AU, vol. 49, no. 7, 5 August 2016 (2016-08-05), pages 1215-1225, XP036258517, ISSN: 0939-4451, DOI: 10.1007/S00726-017-2423-4 [retrieved on 2017-04-22] abstract -----	1-110

1

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/067345

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 0156402	A2	09-08-2001	AU	5791701	A	14-08-2001
			CA	2399500	A1	09-08-2001
			CZ	20022927	A3	18-06-2003
			EP	1255454	A2	13-11-2002
			JP	2003521256	A	15-07-2003
			MX	PA02007466	A	26-02-2004
			RU	2002122099	A	10-08-2004
			US	2002006907	A1	17-01-2002
			US	2004023889	A1	05-02-2004
			US	2004208942	A1	21-10-2004
			WO	0156402	A2	09-08-2001
EP 1541141	A1	15-06-2005	AU	2003261825	A1	19-03-2004
			EP	1541141	A1	15-06-2005
			JP	WO2004019928	A1	15-12-2005
			US	2005197398	A1	08-09-2005
			WO	2004019928	A1	11-03-2004
EP 2792354	A2	22-10-2014	EP	2792354	A2	22-10-2014
			RU	2011109884	A	27-09-2012
			WO	2012144938	A2	26-10-2012
WO 2011097273	A1	11-08-2011	NONE			
WO 2005084323	A2	15-09-2005	US	2005233013	A1	20-10-2005
			WO	2005084323	A2	15-09-2005