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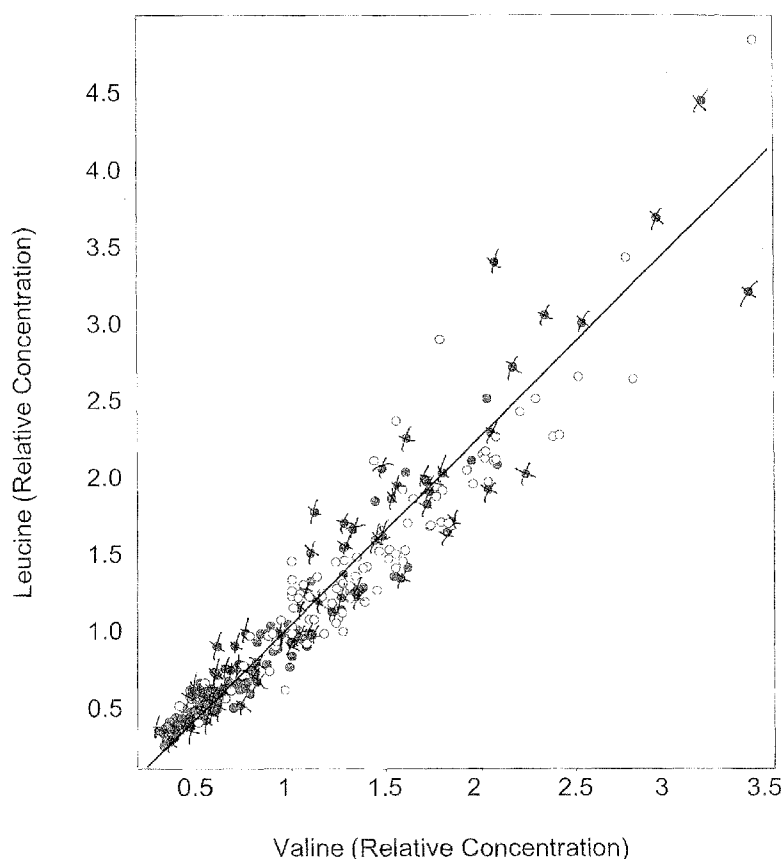
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(54) Title: BIOMARKERS RELATED TO METABOLIC AGE AND METHODS USING THE SAME



(57) Abstract: Biomarkers relating to metabolic age are provided, as well as methods for using such biomarkers as biomarkers for determining metabolic age. In addition, methods for modulating the metabolic age of a subject are also provided. Also provided are suites of small molecule entities as biomarkers for metabolic age.

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## BIOMARKERS RELATED TO METABOLIC AGE AND METHODS USING THE SAME

### CROSS-REFERENCE TO RELATED APPLICATIONS

- 5    **[0001]**       This application claims the benefit of U.S. Provisional Application No. 60/851,468, filed October 13, 2006, the entirety of which is hereby incorporated by reference.

### FIELD OF THE INVENTION

- 10   **[0002]**       This invention relates to biomarkers and methods to determine metabolic age of a subject and methods of using such biomarkers. The invention also relates to biomarkers and methods of using biomarkers relating to gender and race.

### BACKGROUND

- 15   **[0003]**       Interest in metabolomics is growing at a considerable rate. Since 2000, there has been a steady increase in the number of PubMed™ citations per publication year that contain the term “metabolomics” in either the title or abstract fields. Although metabolomics is still an emerging technology, it has already been applied to a diverse set of problems in disparate areas such as pharmaceutical discovery and  
20   development, natural products research, and disease diagnosis, just to name a few. (Griffin, J.L., 2006, Philos Trans R Soc Lond B Biol Sci, 361(1465):147-61; Keun, H.C., 2006, Pharmacol Ther, 109(1-2):92-106; Rochfort, S., J. Nat Prod, 2005, 68(12):1813-2; Kristal, B.S. and Y.I. Shurubor, Sci Aging Knowledge Environ, 2005, 2005(26):pe19; Morris, M. and S.M. Watkins, 2005, Curr Opin Chem Biol 9(4):407-  
25   12; Witkamp, R.F., 2005, J Vet Pharmacol Ther, 28(3):235-45; Watkins, S.M. and J.B. German, 2002, Curr Opin Mol Ther, 4(3):224-8; Fiehn, O., 2002, Plant Mol Biol, 48(1-2):155-71).

- [0004]**       The metabolism of an individual changes with age. Until recently the ability to monitor metabolite changes has been limited to targeted assays. With the  
30   development of metabolomics analysis, changes in metabolites can now be monitored globally in a non-targeted manner. This metabolomic approach allows a metabolic profile to be determined for a group or an individual.

[0005] As more information regarding the impact of nutrition on health-related issues becomes available and as the population ages, interest in health and nutrition has increased. The ability to determine the metabolite levels of an individual and classify the resulting metabolic profile as positive or negative and then to provide guidance as to how to improve a negative profile will have beneficial effects on the health of an individual.

#### SUMMARY

[0006] Methods for determining the metabolic age of a subject are provided. In one aspect, the methods comprise the steps of: (a) analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for metabolic age in the sample, wherein the one or more biomarkers are selected from Tables 2, 7, and/or 8 and combinations thereof; and (b) comparing the level(s) of the one or more biomarkers in the sample to metabolic age reference levels of the one or more biomarkers to determine the subject's metabolic age.

[0007] In another aspect, methods for producing a Biochemical Age Index (BAI) are provided. Such methods comprise the steps of: (a) analyzing biological samples from a plurality of subjects to determine the level(s) of one or more first biomarkers that change with age; (b) identifying one or more second biomarkers that correlate with each of the one or more first biomarkers that change with age to generate one or more groups of biomarkers that change with age; and (c) generating a biochemical age index using the levels of each of the one or more groups of biomarkers.

[0008] Also provided are methods for modifying the metabolic age of a subject comprising the steps of: (a) analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for metabolic age in the sample, wherein the one or more biomarkers are selected from Tables 2, 7, and/or 8 and combinations thereof; (b) comparing the level(s) of the one or more biomarkers in the sample to metabolic age reference levels of the one or more biomarkers to determine the subject's metabolic age; and (c) providing recommendations to modify the metabolic age of a subject through changes in diet, nutrition, lifestyle, and/or administration of metabolites.

[0009] In another aspect, methods of assessing the efficacy of a composition for modulating metabolic age, comprising: (a) analyzing a first biological sample from a

subject having a first metabolic age and currently or previously being treated with a compound or composition to determine the level(s) of one or more biomarkers selected from Tables 2, 7, and/or 8, and (b) comparing the level(s) of the one or more biomarkers in the sample to biomarkers levels selected from the group consisting of

5 (i) level(s) of the one or more biomarkers in a second biological sample from the subject obtained from the subject before being treated with the compound or composition, (ii) metabolic age-positive reference levels of the one or more biomarkers, (iii) metabolic age-negative reference levels of the one or more biomarkers, and (iv) reference levels of the one or more biomarkers for a target

10 metabolic age.

[0010] In addition, methods for screening a test compound for activity in modulating the level of one or more biomarkers of metabolic age are provided. Such methods comprise: (a) contacting one or more cells with a test compound; (b) analyzing at least a portion of the one or more cells or a biological sample associated

15 with the cells to determine the level(s) of one or more biomarkers of metabolic age selected from Tables 2, 7, and/or 8; and (c) comparing the level(s) of the one or more biomarkers with predetermined levels for the biomarkers to determine whether the composition modulated the level(s) of the one or more biomarkers.

## 20 DESCRIPTION OF FIGURES

[0011] Figure 1 is a graph showing the correlation between the relative concentrations of leucine vs. valine as a function of age in humans in three difference age groups. Dark circle, ages 21-35; circle with X, ages 36-50; light circle, ages 51-65.

25 [0012] Figure 2 is a chart illustrating the concept of a metabolic age and the association between metabolic age and chronological age. Changes in metabolic age (MetaboAge) are associated with aging or influence of dietary or lifestyle changes.

[0013] Figure 3 is a stepwise regression plot illustrating the use of age biomarkers to predict the chronological age of a female individual (Figure 3A) or a male

30 individual (Figure 3B) based upon the levels of a set of age biomarkers in the sample.

[0014] Figure 4 is a matrix of scatterplots showing an example of compounds that are increased with age and that are also correlated with one another.

[0015] Figures 5A-F are scatterplots of the individual compounds and the index

comprised of these compounds compared to age. Figure 5A is alanine; Figure 5B is glutamine; Figure 5C is normetanephrine; Figure 5D is ornithine; Figure 5E is valine; and Figure 5F is the Index which is derived from combining the individual compounds versus age. The increase in the level of each of the compounds in this example becomes more pronounced at around age 45, and the variability appears to increase with age.

[0016] Figure 6 is a density contour chart of Biochemical Age Index (BAI) versus age. The density of the data points in each region are illustrated by the contour lines. The BAI is indicated by the solid line.

10 [0017] Figure 7 is a density contour chart of BAI vs. chronological age and shows how determination of a positive MetaboScore is obtained. In the illustration the individual(s) in the black circle have a metabolic age (MetaboAge) of 58 and chronological age of 22 resulting in a MetaboScore of +36, indicating that the individual is biochemically older than the chronological age.

15 [0018] Figure 8 is a density contour chart of BAI vs. chronological age and showing how determination of a negative MetaboScore is obtained. In this illustration the individual in the dark black circle has a metabolic age (MetaboAge) of 26 and chronological age of 54 resulting in a MetaboScore of -28, indicating that the individual is biochemically younger than the chronological age.

20 [0019] Figure 9 is a density contour diagram of tartaric acid concentration versus chronological age. While the majority of individuals have values of tartaric acid  $<2$ , numerous outlying values ( $>3$ ) can be seen at both younger and older ages.

[0020] Figure 10 is a scatterplot diagram of benzoic acid versus chronological age. While the majority of individuals have values of benzoic acid  $<6$ , two individuals with benzoic acid values  $>10$  are observed.

[0021] Figure 11 is a density contour diagram of benzoic acid versus chronological age. A decrease in benzoic acid is illustrated and individuals with low levels ( $<0.5$ ) of benzoic acid are observed.

[0022] Figure 12 is density contour diagram of ornithine concentrations versus chronological age. An increase in ornithine with age is illustrated and individuals with high levels ( $>5$ ) of ornithine are observed.

## DETAILED DESCRIPTION

[0023] The present invention relates to biomarkers of metabolic age, gender and race; methods for determination of metabolic age, methods of monitoring progression/regression of metabolic aging, methods of assessing efficacy of compositions for increasing or decreasing metabolic age of an individual, methods of screening compositions for activity in modulating biomarkers of age, gender or race, methods of modulating metabolic age, as well as other methods based on biomarkers of age, gender or race.

10 [0024] Prior to describing this invention in further detail, however, the following terms will first be defined. Definitions:

[0025] "Biochemical Age Index" (BAI) means the average levels of a group of compounds that change with age and are correlated with one another that can be combined to provide a biochemical age index, BAI. The BAI provides a measure of metabolic age for a population and/or group and/or a plurality of samples that is more robust (i.e. less variable, explains more of the individual variation) than can be obtained with individual compounds alone.

[0026] "Biomarker" means a compound, preferably a metabolite, that is differentially present (i.e., increased or decreased) in a biological sample from a subject or a group of subjects having a first phenotype (e.g., having a particular metabolic age, being within a particular metabolic age range, or having a disease) as compared to a biological sample from a subject or group of subjects having a second phenotype (e.g., having a different metabolic age, being within a different metabolic age range, or not having the disease). A biomarker may be differentially present at any level, but is generally present at a level that is increased by at least 5%, by at least 10%, by at least 15%, by at least 20%, by at least 25%, by at least 30%, by at least 35%, by at least 40%, by at least 45%, by at least 50%, by at least 55%, by at least 60%, by at least 65%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 100%, by at least 110%, by at least 120%, by at least 130%, by at least 140%, by at least 150%, or more; or is generally present at a level that is decreased by at least 5%, by at least 10%, by at least 15%, by at least 20%, by at least 25%, by at least 30%, by at least 35%, by at least 40%, by at least 45%, by at least 50%, by at least 55%, by at least 60%, by at least 65%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at

least 95%, or by 100% (i.e., absent). A biomarker is preferably differentially present at a level that is statistically significant (i.e., a p-value less than 0.05 and/or a q-value of less than 0.10 as determined using either Welch's T-test or Wilcoxon's rank-sum Test).

- 5    **[0027]**     The "level" of one or more biomarkers means the absolute or relative amount or concentration of the biomarker in the sample.

**[0028]**     "Sample" or "biological sample" means biological material isolated from a subject. The biological sample may contain any biological material suitable for detecting the desired biomarkers, and may comprise cellular and/or non-cellular  
10   material from the subject. The sample can be isolated from any suitable biological tissue or fluid such as, for example, blood, blood plasma, urine, cerebral spinal fluid (CSF), crevicular fluid, saliva or breath condensate.

**[0029]**     "Subject" means any animal, but is preferably a mammal, such as, for example, a human, monkey, non-human primate, rat, mouse, dog, cat, horse or rabbit.

- 15   **[0030]**     A "reference level" of a biomarker means a level of the biomarker that is indicative of a particular age, particular age range, disease state, phenotype, or lack thereof, as well as combinations of disease states, phenotypes, or lack thereof. A "reference level" of a biomarker may be an absolute or relative amount or  
20   concentration of the biomarker, a presence or absence of the biomarker, a range of amount or concentration of the biomarker, a minimum and/or maximum amount or concentration of the biomarker, a mean amount or concentration of the biomarker, and/or a median amount or concentration of the biomarker; and, in addition, "reference levels" of combinations of biomarkers may also be ratios of absolute or  
25   relative amounts or concentrations of two or more biomarkers with respect to each other. Appropriate positive and negative reference levels of biomarkers for a particular metabolic ages, age ranges, disease or illness state, phenotype, or lack thereof may be determined by measuring levels of desired biomarkers in one or more appropriate subjects, and such reference levels may be tailored to specific populations of subjects (e.g., a reference level may be age-matched so that comparisons may be  
30   made between biomarker levels in samples from subjects of a certain age and reference levels for a particular disease state, phenotype, or lack thereof in a certain age group). Reference levels may also tailored to specific populations of subjects, including gender populations, race populations, or combinations thereof (e.g. black



males, black females, white males, white females, Hispanic males, or Hispanic females). Such reference levels may also be tailored to specific techniques that are used to measure levels of biomarkers in biological samples (e.g., LC-MS, GC-MS, etc.), where the levels of biomarkers may differ based on the specific technique that is used.

**[0031]** “Metabolite”, or “small molecule”, means organic and inorganic molecules which are present in a cell. The term does not include large macromolecules, such as large proteins (e.g., proteins with molecular weights over 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, or 10,000), large nucleic acids (e.g., nucleic acids with molecular weights of over 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, or 10,000), or large polysaccharides (e.g., polysaccharides with a molecular weights of over 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, or 10,000). The small molecules of the cell are generally found free in solution in the cytoplasm or in other organelles, such as the mitochondria, where they form a pool of intermediates which can be metabolized further or used to generate large molecules, called macromolecules. The term “small molecules” includes signaling molecules and intermediates in the chemical reactions that transform energy derived from food into usable forms. Examples of small molecules include sugars, fatty acids, amino acids, nucleotides, intermediates formed during cellular processes, and other small molecules found within the cell.

**[0032]** “Metabolic profile”, or “small molecule profile”, or “metabolite profile”, means a complete or partial inventory of small molecules within a targeted cell, tissue, organ, organism, or fraction thereof (e.g., cellular compartment). The inventory may include the quantity and/or type of small molecules present. The “small molecule profile” may be determined using a single technique or multiple different techniques. The inventory may include any number of small molecules, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or more small molecules within a targeted cell, tissue, organ, organism, or fraction thereof.

**[0033]** “Non-biomarker compound” means a compound that is not differentially present in a biological sample from a subject or a group of subjects having a first phenotype (e.g., having a particular age, or within a particular age range, or gender or race) as compared to a biological sample from a subject or group of subjects having a second phenotype (e.g., having a different age, or within a different age range, or

gender or race). Such non-biomarker compounds may, however, be biomarkers in a biological sample from a subject or a group of subjects having a third phenotype (e.g., having yet a different age or being within a different age range, or or race) as compared to the first phenotype (e.g., having the first age, age range, gender or race) or the second phenotype (e.g., having a different age, age range, gender or race). Further, these compounds may be useful in determining a BAI.

[0034] “Metabolome” means all of the small molecules present in a given organism. The metabolome includes both metabolites and products of catabolism.

[0035] “Metabolic age” (“MetaboAge” or “MetabAge”) means the age of a subject as determined by the composition of metabolites in cells, tissue and/or fluids. The metabolic age of a subject is determined by comparing the metabolic profile of the subject with metabolic profiles characteristic of various age groups (e.g. <25, 25-35, 36-50, 51-65, >65) (see Figure 2). The metabolic age may also be determined by comparison of a subject’s metabolic profile to a biochemical age index. If the subject profile resembles the profile characteristic of individuals of an age or age group younger than the subject’s chronological age, the subject’s metabolic age is “positive” while if the profile resembles the profile characteristic of an age group older than the subject’s chronological, the subject’s metabolic age is “negative”.

[0036] “MetaboScore” means the calculated value using the Biochemical Age Index (BAI) that represents the difference between the metabolic age (MetaboAge) and the chronological age of a subject. A negative MetaboScore indicates the subject is younger biochemically than chronologically, while a positive MetaboScore indicates the subject is biochemically older than the chronological age.

[0037] “Xenobiotic” means “a chemical which is found in an organism but which is not normally produced or expected to be present in it. A xenobiotic is a compound that is foreign to a living organism. Principle xenobiotics include drugs, carcinogens, and various compounds that have been introduced into the environment by artificial means.” IUPAC Compendium on Chemical Terminology 2003. (available on the worldwide web at iupac.org). Metabolites that are produced by the organism from the xenobiotic may also be considered to be xenobiotics.

[0038] “Xenobiotic Score” means the calculated value that represents the difference between the level(s) of one or more xenobiotics and the reference level(s) of one or more xenobiotics as determined by a Xenobiotic Age Index (XAI). A

negative Xenobiotic Score indicates the subject has lower levels of a xenobiotic than the reference level for their chronological age, while a positive Xenobiotic Score indicates the subject has a higher level of a xenobiotic than the reference level for their chronological age.

5 [0039] Metabolomic studies allow identification of metabolic profiles associated with aging. Metabolomic analysis of a large cohort of individuals has demonstrated that one or more biomarkers may be present in an organism, cell, tissue, or portion thereof at different levels that correlate with the age of the organism, cell, tissue, or portion thereof. The levels change over time according to the age of the organism,  
10 cell, tissue, or portion thereof. Metabolic profiles may be determined that are associated with specific ages or age groups. The metabolic profile for an individual subject can be obtained using metabolomics and by comparison with the characteristic age-related metabolic profiles the metabolic age of the individual can be determined. Based upon the metabolic profile nutritional and/or lifestyle  
15 recommendations can be made to improve the metabolic profile of the individual. In addition to determining the MetaboAge and/or MetaboScore of the individual, the metabolic profile may be evaluated further to identify changes in specific metabolites and the associated biochemical pathways. The disclosed methods may targeted to selected populations of subjects depending on biomarker differences within the  
20 selected population, for example, between race populations, gender populations or combinations of race and gender populations.

[0040] Metabolic or biochemical changes correlated with aging may be identified by the biomarkers and methods disclosed herein and may be used to distinguish groups of individuals according to age. Thus, the metabolic age or status of a subject  
25 can be determined by comparing the metabolic profile of a subject with the metabolic profile of specific age or age groups. Such profiles may be based on gender or race specific profiles. With this information recommendations can be made (e.g. by a physician, a physician's assistant, a nutritionist, etc.) to the subject that will enable the subject to alter his/her metabolic age through therapeutic agents, nutritional  
30 supplements and/or diet. The present invention also describes methods to develop a Biochemical Age Index for determining the metabolic age of a subject and to calculate a MetaboScore value that is the difference between the subject's chronological age and metabolic age.

[0041] When such a method is used to aid in determining the subject's metabolic age, the results of the method may be used along with other methods (or the results thereof) useful in the clinical determination, for example, for: 1) determining fitness for surgery, chemotherapy, physical therapy, or other medical treatments; 2) 5 determining dosage for anesthesia; 3) determining requirement for additional diagnostic assays; and/or 4) determining potential drug treatments, regimens and/or dosages. The metabolic age analysis is also useful for determining risk factors for illness and/or disease.

[0042] The present disclosure provides an understanding of the number and 10 identity of small molecule compounds that are present in biological fluids (e.g. human plasma), along with the distribution of their relative concentrations.

[0043] Additionally, the present invention provides an understanding of the influence of age, gender, and race on the relative levels of observed compounds. Besides understanding the effects of these factors on the small-molecule complement 15 of human plasma, and/or other biological samples (e.g. tissue, saliva, urine, plasma, etc.) such knowledge would also permit us to compare and contrast an efficient, metabolomics-based measurement process with more traditional clinical measurement determinations.

#### **I. Biomarkers**

20 [0044] Metabolic age biomarkers described herein were discovered using metabolic profiling techniques. Such metabolomic profiling techniques are described in more detail in the Examples set forth below as well as in U.S. Patent No. 7,005,255 and U.S. Patent Application Nos. 11/357,732, 10/695,265 (Publication No. 2005/0014132), 11/301,077 (Publication No. 2006/0134676), 11/301,078 25 (Publication No. 2006/0134677), 11/301,079 (Publication No. 2006/0134678), and 11/405,033, the entire contents of which are hereby incorporated herein by reference.

[0045] Generally, metabolic profiles were determined for biological samples from human subjects from various age groups (e.g. <25, 25-35, 36-50, 51-65, >65). The metabolic age of an individual is determined by comparing the metabolic profile 30 with metabolic profiles characteristic of various age groups (e.g. <25, 25-35, 36-50, 51-65, >65) (see Figure 2). If the individual profile resembles the profile characteristic of individuals in an age group younger than the individual's chronological age, the individual's metabolic age is "positive" while if the profile

resembles the profile characteristic of an age group older than the individual's chronological, the individual's metabolic age is "negative". MetaboAge is inversely correlated with the MetaboScore, *i.e.*, a younger MetaboAge will result from a negative MetaboScore and an older MetaboAge will result from a positive

5 MetaboScore.

[0046] Those molecules differentially present, including those molecules differentially present at a level that is statistically significant, in the metabolic profile of samples from subjects from various age, race, and/or gender groups as compared to another group (e.g., subjects from a different age, race, and/or gender group) were  
10 identified as biomarkers to distinguish those groups.

[0047] Biomarkers for use in methods relating to distinguishing metabolic age include those listed in Tables 2, 4, 5, 7, and/or 8, and combinations thereof. Biomarkers for use in methods relating to distinguishing gender include those listed in Table 4; biomarkers for use in methods relating to distinguishing race include those  
15 listed in Table 5; biomarkers that increase with age include those listed in Tables 2, 7, and/or 8 and combinations thereof; and biomarkers that decrease with age include those listed in Table 2, 7, and/or 8, and combinations thereof; and biomarkers that change with age include those listed in Tables 2, 7, and/or 8, and combinations thereof.

20 [0048] Non-biomarker compounds associated with the compared groups may also be identified.

[0049] Although the identities of some of the biomarkers and non-biomarker compounds are not known at this time, such identities are not necessary for the identification of the biomarkers or non-biomarker compounds in biological samples  
25 from subjects, as the "unnamed" compounds have been sufficiently characterized by analytical techniques to allow such identification. The analytical characterization of all such "unnamed" compounds is listed in the Examples. Such "unnamed" biomarkers and non-biomarker compounds are designated herein using the nomenclature "Metabolite" followed by a specific metabolite number.

30 [0050] Xenobiotics, such as, for example, tartaric acid and benzoic acid may also be measured. Xenobiotic compounds from individuals are measured and used to determine a correlation between xenobiotic compounds and age. Measurement of such xenobiotic compounds is used to calculate a xenobiotic score to determine

dietary differences between individuals and a reference level according to age. Xenobiotic levels can also be used to make recommendations to changes in diet, lifestyle or medication.

## II. Analyzing Metabolic Age

- 5 [0051] Methods for determining a subject's metabolic age may be performed using one or more of the biomarkers identified in the respective Tables provided herein. For example, a method for determining the metabolic age of a subject comprises the steps of: (1) analyzing a biological sample from a subject to determine the level(s) of one or more metabolic age biomarkers in the sample, and (2)
- 10 comparing the level(s) of the one or more metabolic age biomarkers in the sample to metabolic age reference levels of the one or more biomarkers in order to determine the subject's metabolic age. The level(s) of the one or more biomarkers may be compared to a biochemical age index (BAI) to determine the subject's metabolic age. The one or more biomarkers that are used are selected from Tables 2, 4, 5, 7, and/or 8
- 15 and combinations thereof.
- [0052] Any suitable method may be used to analyze the biological sample in order to determine the level(s) of the one or more biomarkers in the sample. Suitable methods include chromatography (e.g., HPLC, gas chromatography, liquid chromatography), mass spectrometry (e.g., MS, MS-MS), enzyme-linked
- 20 immunosorbent assay (ELISA), antibody linkage, other immunochemical techniques, and combinations thereof. Further, the level(s) of the one or more biomarkers may be measured indirectly, for example, by using an assay that measures the level of a compound (or compounds) that correlates with the level of the biomarker(s) that are desired to be measured.
- 25 [0053] The levels of one or more of the biomarkers of Tables 2, 4, 5, 7, and/or 8, may be determined in the methods for determining the metabolic age of a subject and methods of aiding in analyzing a metabolic profile. For example, the level(s) of one biomarker, two or more biomarkers, three or more biomarkers, four or more biomarkers, five or more biomarkers, six or more biomarkers, seven or more
- 30 biomarkers, eight or more biomarkers, nine or more biomarkers, ten or more biomarkers, fifteen or more biomarkers, etc., including a combination of all of the biomarkers in Tables 2, 4, 5, 7, and/or 8 or any fraction thereof, may be determined and used in such methods. Determining levels of combinations of the biomarkers

allow greater sensitivity and specificity in analyzing a metabolic profile and aiding in the determining metabolic age, and may allow better differentiation of a metabolic age from other metabolic variations or disorders that may have similar or overlapping biomarkers to metabolic age.

- 5     **[0054]**     Furthermore, ratios of the levels of certain biomarkers (M1/M2) (and non-biomarker compounds) in biological samples may allow greater sensitivity and specificity in determining metabolic age or diagnosis of metabolic disorder, and may allow better differentiation of metabolic age from other metabolic variations or disorders that may have similar or overlapping biomarkers. Also, ratios of
- 10    xenobiotics (X1/X2) in biological samples are also used to measure differences in metabolic profiles of subjects. These ratios may allow better specificity and differentiation in measuring metabolic differences. Further, ratios of certain biomarkers and xenobiotics (M1/X1) also can be used to provide greater sensitivity and specificity in analyzing metabolic profiles of subjects.
- 15    **[0055]**     After the level(s) of the one or more biomarkers in the sample are determined, the level(s) are compared to metabolic age reference levels to aid in analyzing the metabolic profile of an individual to determine the subject's metabolic age. Levels of the one or more biomarkers in a sample matching particular metabolic age reference levels (e.g., levels that are the same as the reference levels, substantially
- 20    the same as the reference levels, above and/or below the minimum and/or maximum of the reference levels, and/or within the range of the reference levels) are indicative of the metabolic age of the subject. Levels of the one or more biomarkers in a sample not matching the metabolic age reference levels (e.g., levels that are the same as the reference levels, substantially the same as the reference levels, above and/or below
- 25    the minimum and/or maximum of the reference levels, and/or within the range of the reference levels) are indicative of that an individual does not fit within a particular metabolic age. In some instances, the reference levels used for such comparisons may be based on gender and/or race differences in biomarker reference levels within a selected population (for example reference levels of biomarkers for black male or
- 30    hispanic female populations). Such biomarkers that may be used to distinguish gender and/or race differences include those identified Tables 4 and/or 5 and combinations thereof.

**[0056]**     The level(s) of the one or more biomarkers may be compared to the

metabolic age reference levels using various techniques, including a simple comparison (*e.g.*, a manual comparison) of the level(s) of the one or more biomarkers in the biological sample to the level in a different metabolic age reference level. The level(s) of the one or more biomarkers in the biological sample may also be compared to the metabolic age reference levels using one or more statistical analyses (*e.g.*, t-test, Welch's T-test, Wilcoxon's rank sum test, random forest).

[0057] In addition, the biological samples may be analyzed to determine the level(s) of one or more non-biomarker compounds. The level(s) of such non-biomarker compounds may also allow differentiation of metabolic age or a metabolic disorder from other metabolic ages or metabolic variations or disorders that may have similar or overlapping biomarkers to a particular metabolic age. For example, a known non-biomarker compound present in biological samples of subjects of a particular metabolic age and subjects of a different metabolic age could be monitored to verify the estimate of a particular metabolic age as compared to another metabolic age when biological samples from subjects having the other metabolic ages do not have the non-biomarker compound.

### III. Methods for monitoring the increasing or decreasing of metabolic age

[0058] The identification of biomarkers for metabolic age also allows for monitoring the increasing or decreasing of metabolic age in a subject. A method of monitoring the increasing or decreasing of metabolic age of a subject comprises the steps of: (1) analyzing a first biological sample from a subject to determine the level(s) of one or more biomarkers for metabolic age selected from Tables 2, 7, and/or 8, the first sample obtained from the subject at a first time point, (2) analyzing a second biological sample from a subject to determine the level(s) of the one or more biomarkers, the second sample obtained from the subject at a second time point, and (3) comparing the level(s) of one or more biomarkers in the first sample to the level(s) of the one or more biomarkers in the second sample in order to monitor the increase or decrease of metabolic age of the subject. The level(s) of the one or more biomarkers may also be compared to a biochemical age index (BAI) to monitor the increase or decrease of metabolic age of the subject. The results of the method are indicative of the metabolic age difference or MetaboScore (*i.e.*, increase or decrease, if any change) in the subject. The change (if any) in the level(s) of the one or more biomarkers over time may be indicative of increase or decrease in the metabolic age



in the subject.

[0059] In order to characterize the course of metabolic age in the subject, the level(s) of the one or more biomarkers in the first sample, the level(s) of the one or more biomarkers in the second sample, and/or the results of the comparison of the levels of the biomarkers in the first and second samples may be compared to metabolic age reference levels of the one or more biomarkers. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time (e.g., in the second sample as compared to the first sample) to become more similar to an older metabolic age reference level (or less similar to a relatively younger metabolic age reference level), then the results are indicative of an increase in metabolic age. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time to become more similar to a younger metabolic age reference level (or less similar to an older metabolic age reference level), then the results are indicative of decreasing metabolic age.

[0060] As with the other methods described herein, the comparisons made in the methods of monitoring increasing/decreasing of metabolic age in a subject may be carried out using various techniques, including simple comparisons, one or more statistical analyses, and combinations thereof.

[0061] The results of the method may be used along with other methods (or the results thereof) useful in the clinical monitoring of increasing/decreasing of metabolic age in a subject. For example, the monitoring of metabolic age can be combined with a lifestyle assessment questionnaire to make recommendations for diet improvements and/or modifications; vitamin, mineral and/or other dietary supplements; and/or lifestyle modifications (e.g. quit smoking, drinking less alcohol, increasing exercise). Further, the measurement of metabolic age and/or monitoring increase/decrease in metabolic age can be combined with a lifestyle assessment questionnaire to determine risk factors for poor health and reduced longevity.

#### **IV. Methods of assessing efficacy of compositions for modulating metabolic age**

[0062] The identification of biomarkers for metabolic age also allows for assessment of the efficacy of a composition for modulating (e.g. increasing or decreasing) the metabolic age of a subject, as well as the assessment of the relative efficacy of two or more compounds or compositions for modulating the metabolic age of a subject. Such assessments may be used, for example, in efficacy studies as well

as in lead selection of compounds or compositions for modulating metabolic age.

[0063] A method of assessing the efficacy of a composition for modulating metabolic age, comprises the steps of: (1) analyzing, from a subject having a first metabolic age, and currently or previously being treated with a compound or composition, a biological sample to determine the level(s) of one or more biomarkers selected from Tables 2, 7, and/or 8, and (2) comparing the level(s) of the one or more biomarkers in the sample to (a) level(s) of the one or more biomarkers in a previously-taken biological sample from the subject, wherein the previously-taken biological sample was obtained from the subject before being treated with the compound or composition, (b) metabolic age-positive reference levels of the one or more biomarkers, (c) metabolic age-negative reference levels of the one or more biomarkers, and/or (d) a target metabolic age reference levels. The results of the comparison are indicative of the efficacy of the composition for modulating metabolic age.

[0064] Thus, in order to characterize the efficacy of the composition for modulating metabolic age, the level(s) of the one or more biomarkers in the biological sample are compared to (1) metabolic age-positive reference levels, (2) metabolic age-negative reference levels, (3) previous levels of the one or more biomarkers in the subject before treatment with the composition, and/or (d) a target metabolic age reference levels.

[0065] When comparing the level(s) of the one or more biomarkers in the biological sample (from a subject having a particular metabolic age, and currently or previously being treated with a composition) to metabolic age-positive reference levels and/or metabolic age-negative reference levels and/or target metabolic age reference levels, level(s) in the sample matching the metabolic age-negative reference levels and/or target metabolic age reference levels (e.g., levels that are the same as the reference levels, substantially the same as the reference levels, above and/or below the minimum and/or maximum of the reference levels, and/or within the range of the reference levels) are indicative of the composition having efficacy for modulating metabolic age. Levels of the one or more biomarkers in the sample matching the metabolic age-positive reference levels (e.g., levels that are the same as the reference levels, substantially the same as the reference levels, above and/or below the minimum and/or maximum of the reference levels, and/or within the range of the

reference levels) are indicative of the composition not having efficacy for modulating metabolic age. The comparisons may also indicate degrees of efficacy for modulating metabolic age, based on the level(s) of the one or more biomarkers.

[0066] When the level(s) of the one or more biomarkers in the biological sample (from a subject having a particular metabolic age and currently or previously being treated with a composition) are compared to level(s) of the one or more biomarkers in a previously-taken biological sample from the subject before treatment with the composition, any changes in the level(s) of the one or more biomarkers are indicative of the efficacy of the composition for modulating metabolic age. That is, if the comparisons indicate that the level(s) of the one or more biomarkers have increased or decreased after treatment with the composition to become more similar to the metabolic age, -negative reference levels and/or target metabolic age reference levels (or less similar to the age-positive reference levels), then the results are indicative of the composition having efficacy for modulating metabolic age. If the comparisons indicate that the level(s) of the one or more biomarkers have not increased or decreased after treatment with the composition to become more similar to the metabolic age -negative reference levels and/or target metabolic age reference levels (or less similar to the metabolic age -positive reference levels), then the results are indicative of the composition not having efficacy for modulating metabolic age. The comparisons may also indicate degrees of efficacy for modulating metabolic age, based on the amount of changes observed in the level(s) of the one or more biomarkers after treatment. In order to help characterize such a comparison, the changes in the level(s) of the one or more biomarkers, the level(s) of the one or more biomarkers before treatment, and/or the level(s) of the one or more biomarkers in the subject currently or previously being treated with the composition may be compared to the metabolic age -positive and/or the metabolic age -negative reference levels and/or target metabolic age reference levels of the one or more biomarkers.

[0067] Another method for assessing the efficacy of a composition in modulating metabolic age, comprises the steps of: (1) analyzing a first biological sample from a subject to determine the level(s) of one or more biomarkers selected from Tables 2, 7, and/or 8, the first sample obtained from the subject at a first time point, (2) administering the composition to the subject, (3) analyzing a second biological sample from a subject to determine the level(s) of the one or more biomarkers, the

second sample obtained from the subject at a second time point after administration of the composition, and (4) comparing the level(s) of one or more biomarkers in the first sample to the level(s) of the one or more biomarkers in the second sample in order to assess the efficacy of the composition for modulating metabolic age. As indicated above, if the comparison of the samples indicates that the level(s) of the one or more biomarkers have increased or decreased after administration of the composition to become more similar to the age -negative reference levels and/or target metabolic age reference levels (or less similar to the metabolic age -positive reference levels), then the results are indicative of the composition having efficacy for modulating metabolic age. If the comparison indicates that the level(s) of the one or more biomarkers have not increased or decreased after administration of the composition to become more similar to the metabolic age -negative reference levels and/or target metabolic age reference levels (or less similar to the metabolic age -positive reference levels), then the results are indicative of the composition not having efficacy for modulating metabolic age. The comparison may also indicate a degree of efficacy for modulating metabolic age, based on the amount of changes observed in the level(s) of the one or more biomarkers after administration of the composition. In order to help characterize such a comparison, the changes in the level(s) of the one or more biomarkers, the level(s) of the one or more biomarkers before administration of the composition, and/or the level(s) of the one or more biomarkers after administration of the composition may be compared to the metabolic age-positive and/or metabolic age -negative and/or target metabolic age reference levels of the one or more biomarkers of the two compositions.

**[0068]** A method of assessing the relative efficacy of two or more compositions for modulating metabolic age comprises the steps of: (1) analyzing, from a first subject having a particular metabolic score, and currently or previously being treated with a first composition, a first biological sample to determine the level(s) of one or more biomarkers selected from Tables 2, 7, and/or 8, (2) analyzing, from a second subject having the same or similar metabolic score, and currently or previously being treated with a second composition, a second biological sample to determine the level(s) of the one or more biomarkers, and (3) comparing the level(s) of one or more biomarkers in the first sample to the level(s) of the one or more biomarkers in the second sample in order to assess the relative efficacy of the first and second

compositions for modulating metabolic age. The results are indicative of the relative efficacy of the two compositions, and the results (or the levels of the one or more biomarkers in the first sample and/or the level(s) of the one or more biomarkers in the second sample) may be compared to metabolic age -positive or metabolic age-

5 negative or target metabolic age reference levels to aid in characterizing the relative efficacy.

[0069] Each of the methods of assessing efficacy may be conducted on one or more subjects or one or more groups of subjects (e.g., a first group being treated with a first composition and a second group being treated with a second composition).

10 [0070] As with the other methods described herein, the comparisons made in the methods of assessing efficacy (or relative efficacy) of compounds or compositions for modulating metabolic age, are carried out using various techniques, including simple comparisons, one or more statistical analyses, and combinations thereof. Any suitable method is used to analyze the biological samples in order to determine the  
15 level(s) of the one or more biomarkers in the samples. In addition, the level(s) of one or more biomarkers, including a combination of all of the biomarkers in Tables 2, 7, and/or 8 or any fraction thereof or using an index derived from all or some of the biomarkers in Tables 2, 7, and/or 8 may be determined and used in methods of assessing efficacy (or relative efficacy) of compositions for modulating metabolic  
20 age.

[0071] Finally, the methods of assessing efficacy (or relative efficacy) of one or more compounds or compositions for modulating metabolic age, may further comprise analyzing the biological sample to determine the level(s) of one or more non-biomarker compounds. The non-biomarker compounds may then be compared  
25 to reference levels of non-biomarker compounds for subjects having (or not having) the target metabolic age.

#### **V. Methods of screening a composition for activity in modulating biomarkers associated with metabolic age**

[0072] The identification of biomarkers for metabolic age also allows for the  
30 screening of compositions for activity in modulating biomarkers associated with age which may be useful in modulating (increasing or decreasing) metabolic age of a subject. Methods of screening compositions useful for increasing or decreasing a subject's metabolic age comprises assaying test compositions for activity in

modulating the levels of one or more metabolic age biomarkers in Tables 2, 4, 5, 7, and/or 8. Such screening assays may be conducted *in vitro* and/or *in vivo*, and may be in any form known in the art useful for assaying modulation of such metabolic age biomarkers in the presence of a test composition such as, for example, cell culture assays, organ culture assays, and *in vivo* assays (*e.g.*, assays involving animal models).

[0073] In one embodiment, a method for screening a composition for activity in modulating one or more biomarkers of metabolic age comprises the steps of: (1) contacting one or more cells with a composition, (2) analyzing at least a portion of the one or more cells or a biological sample associated with the cells to determine the level(s) of one or more biomarkers of metabolic age selected from Tables 2, 4, 5, 7, and/or 8; and (3) comparing the level(s) of the one or more biomarkers with predetermined standard levels for the one or more biomarkers to determine whether the composition modulated the level(s) of the one or more biomarkers. As discussed above, the cells may be contacted with the composition *in vitro* and/or *in vivo*. The predetermined standard levels for the one or more biomarkers may be the levels of the one or more biomarkers in the one or more cells in the absence of the composition. The predetermined standard levels for the one or more biomarkers may also be the level(s) of the one or more biomarkers in control cells not contacted with the composition.

[0074] In addition, the methods may further comprise analyzing at least a portion of the one or more cells or a biological sample associated with the cells to determine the level(s) of one or more non-biomarker compounds of age. The levels of the non-biomarker compounds may then be compared to predetermined standard levels of the one or more non-biomarker compounds.

[0075] Any suitable method may be used to analyze at least a portion of the one or more cells or a biological sample associated with the cells in order to determine the level(s) of the one or more biomarkers (or levels of non-biomarker compounds). Suitable methods include chromatography (*e.g.*, HPLC, gas chromatograph, liquid chromatography), mass spectrometry (*e.g.*, MS, MS-MS), ELISA, antibody linkage, other immunochemical techniques, and combinations thereof. Further, the level(s) of the one or more biomarkers (or levels of non-biomarker compounds) may be measured indirectly, for example, by using an assay that measures the level of a

compound (or compounds) that correlates with the level of the biomarker(s) (or non-biomarker compounds) that are desired to be measured.

#### **VI. Method of identifying potential drug targets**

[0076] The identification of biomarkers for metabolic age also allows for the  
5 identification of potential drug targets for metabolic aging. A method for identifying a potential drug target for metabolic aging comprises the steps of: (1) identifying one or more biochemical pathways associated with one or more metabolic age biomarkers selected from Tables 2, 4, 5, 7, and/or 8 and (2) identifying a protein (e.g., an enzyme) affecting at least one of the one or more identified biochemical pathways,  
10 the protein being a potential drug target for metabolic aging.

[0077] Another method for identifying a potential drug target for metabolic aging comprises the steps of: (1) identifying one or more biochemical pathways associated with one or more biomarkers for metabolic aging selected from Tables 2, 4, 5, 7, and/or 8 and one or more non-biomarker compounds of metabolic aging selected  
15 from Tables 2, 4, 5, 7, and/or 8; and (2) identifying a protein affecting at least one of the one or more identified biochemical pathways, the protein being a potential drug target for metabolic aging.

[0078] One or more biochemical pathways (e.g., biosynthetic and/or metabolic (catabolic) pathway) are identified that are associated with one or more biomarkers  
20 (or non-biomarker compounds). After the biochemical pathways are identified, one or more proteins affecting at least one of the pathways are identified. Preferably, those proteins affecting more than one of the pathways are identified. Examples of biochemical pathways are, but not limited to, glycolysis, the tricarboxylic acid cycle (TCA Cycle/Krebs Cycle/Citric Acid Cycle), the phosphogluconate pathway,  
25 oxidation-reduction and electron transport, oxidative phosphorylation and respiratory metabolism (respiration), fatty acid biosynthesis and oxidation (B-oxidation), amino acid biosynthesis and oxidative degradation, carbohydrate biosynthesis, gluconeogenesis, lipid biosynthesis, the HMG-CoA reductase pathway, the pentose phosphate pathway, the porphyrin synthesis pathway (heme synthesis), nitrogen  
30 metabolism (urea cycle), nucleotide biosynthesis, and DNA replication, transcription, and translation.

[0079] A build-up of one metabolite (e.g., a pathway intermediate) may indicate

the presence of a 'block' downstream of the metabolite and the block may result in a low/absent level of a downstream metabolite (e.g. product of a biosynthetic pathway). In a similar manner, the absence of a metabolite could indicate the presence of a 'block' in the pathway upstream of the metabolite resulting from inactive or non-functional enzyme(s) or from unavailability of biochemical intermediates that are required substrates to produce the product. Alternatively, an increase in the level of a metabolite could indicate a genetic mutation that produces an aberrant protein which results in the over-production and/or accumulation of a metabolite which then leads to an alteration of other related biochemical pathways and result in dysregulation of the normal flux through the pathway; further, the build-up of the biochemical intermediate metabolite may be toxic or may compromise the production of a necessary intermediate for a related pathway. It is possible that the relationship between pathways is currently unknown and this data could reveal such a relationship.

15 [0080] The proteins identified as potential drug targets may then be used to identify compositions that may be potential candidates for treating metabolic aging including compositions for gene therapy.

## **VII. Methods of treating a subject having a positive metabolic score**

[0081] The identification of biomarkers for metabolic age also allows for treatment of a subject having a positive metabolic score (or a negative metabolic age). For example, in order to treat a subject having a positive metabolic score, an effective amount of one or more metabolic age biomarkers that are at lower levels in an individual with a neutral metabolic score (a metabolic score that is not positive or negative) may be administered to the subject. The biomarkers that may be administered may comprise one or more of the biomarkers in Tables 2, 4, 5, 7, and/or 8, that are decreased in metabolic aging. Such biomarkers could be isolated based on the analytical characterizations for the biomarkers listed in Tables 2, 4, 5, 7, and/or 8. In some embodiments, the biomarkers that are administered are one or more biomarkers listed in Tables 2, 4, 5, 7, and/or 8, in particular Tables 2, 4, 5, 7, and/or 8, that are decreased in aging, and that have a p-value less than 0.05 and/or a q-value of less than 0.10.

[0082] In other embodiments, the biomarkers that are administered are one or biomarkers listed in Tables 2, 4, 5, 7, and/or 8, in particular Table 2 that are at



decreased levels in aging, by at least 5%, by at least 10%, by at least 15%, by at least 20%, by at least 25%, by at least 30%, by at least 35%, by at least 40%, by at least 45%, by at least 50%, by at least 55%, by at least 60%, by at least 65%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, or by 100% (i.e., absent).

### **VIII. Methods for producing a Biochemical Age Index**

[0083] The study of metabolomics and metabolic age can be used to determine a subject's fitness for a medical procedure or treatment. For example, one method for producing a Biochemical Age Index (BAI) comprises the steps of: (a) analyzing biological samples from a plurality of subjects to determine the level(s) of one or more biomarkers that change with age; (b) identifying one or more other biomarkers that correlate with each of the one or more initial biomarkers that change with age to generate one or more groups of biomarkers that change with age; and (c) generating a biochemical age index using the levels of each of the one or more groups of biomarkers. One example of a method of producing a BAI may be based on the determining the level(s) of one or more biomarkers listed in Tables 2, 4, 5, 7, and/or 8.

[0084] The samples used for producing a BAI may be based on any number of subjects, including 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, or 100 or more subjects.

[0085] The index may be produced by any method available for generating an index. In one aspect, the index is generated by plotting the level(s) of the one or more biomarkers, for example in a scatter plot. Such a scatter plot may then be fitted with a quadratic curve line.

[0086] In addition, the index may be tailored for a selected population, for example, populations based on gender or race or combinations of gender and race (e.g. black males, black females, white males, white females, Hispanic males, or Hispanic females).

### **IX. Methods of using the metabolic age biomarkers for other age related diseases**

[0087] It is believed that some of the biomarkers for metabolic aging described herein may also be biomarkers for other age related diseases in general. Therefore, it is believed that at least some of the aging biomarkers may be used in the methods

described herein for aging related diseases in general. That is, the methods described herein with respect to aging may also be used for diagnosing (or aiding in the diagnosis of) an aging related disease, methods of monitoring progression/regression of an aging related disease, methods of assessing efficacy of compositions for treating an age related disease, methods of screening a composition for activity in modulating biomarkers associated with an aging related disease, methods of identifying potential drug targets for aging related diseases, and methods of treating an aging related disease. Such methods could be conducted as described herein with respect to metabolic aging.

10 **X. Methods of reporting and making recommendations**

[0088] Following the metabolomic analysis, the resulting metabolic profile of the individual may be compared with metabolic profiles characteristic of different age groups to determine the metabolic age (MetabAge) or MetaboScore of the individual. Such comparisons may allow for the modification of the metabolic age of the subject.

15 In one aspect, methods for the modification of the metabolic age of a subject comprise: (a) analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers associated with metabolic age in the sample; (b) comparing the level(s) of the one or more biomarkers in the sample to metabolic age reference levels to determine the subject's metabolic age; and (c) providing

20 recommendations to modify the metabolic age of the subject. In one aspect, the one or more biomarkers that are used are selected from Tables 2, 4, 5, 7, and/or 8 and combinations thereof.

[0089] A report may be provided that summarizes the results and provides recommendations directed to improving the metabolic age status of the individual.

25 The final report includes, but is not limited to, the metabolic age (MetaboAge); the difference between an individual's chronological age and metabolic age (MetaboScore); a list of affected biochemical pathways (changed positively or negatively), nutritional recommendations (improve if not favorable or maintain if favorable), and therapeutic agents that may be useful in modifying the subject's

30 metabolic age.

[0090] The metabolic age analysis methods can be combined with lifestyle assessment questionnaires to make recommendations for diet improvements and/or modifications; vitamin, mineral and/or other dietary supplements; and lifestyle

modifications (e.g. quit smoking, drinking less alcohol, increasing exercise). Further, the methods and questionnaires can be combined to determine risk factors for poor health and reduced longevity.

5 **XI. Method for determining a subject's fitness for medical procedures or treatments**

[0091] The study of metabolomics and metabolic age can be used to determine a subject's fitness for a medical procedure or treatment. For example, analyzing metabolic age is useful for, but not limited to, determining fitness for surgery, chemotherapy, physical therapy, or other medical treatments; determining dosage for  
10 anesthesia; determining requirement for additional diagnostic test; and determining drug treatments, regimens and/or dosages. The present invention relates to a method for determining a subject's fitness for medical procedures, surgery or treatments comprising the steps of:

- 15 a) determining the metabolic age or profile or level of one or more age related biomarkers in a sample from the subject using the methods described in the present application;
- b) comparing the subject's metabolic age or profile or level to reference level(s) of one or more age related biomarkers or Biochemical Age Index (BAI); and
- 20 c) determining the difference between the subject's metabolic age and chronological age to determine fitness for a medical procedure, surgery or treatment.

[0092] The present invention also relates to a method for determining a subject's fitness for anesthesia comprising the steps of:

- 25 a) determining the metabolic age or profile or level of one or more age related biomarkers in a sample from the subject using the methods described in the present application;
- b) comparing the subject's metabolic age or profile or level to reference level(s) of one or more age related biomarkers or Biochemical Age Index (BAI); and
- 30 c) determining the difference between the subject's metabolic age and chronological age to determine fitness for anesthesia.

**XII. Method for determining risk factors for illness and/or disease**

[0093] Analyzing a subject's metabolic age is also useful for determining a subject's risk factors for illness and/or disease. The present invention relates to a method for determining a subject's risk factors for illness and/or disease comprising the steps of:

- a) determining the metabolic age or profile or level of one or more age related biomarkers in a sample from the subject using the methods described in the present application;
- b) comparing the subject's metabolic age or profile or level to reference level(s) of one or more age related biomarkers or Biochemical Age Index (BAI); and
- c) determining the difference between the subject's metabolic age and chronological age to determine risk factors for illness and/or disease.

**XIII. Methods for analyzing xenobiotics and Xenobiotic Score**

[0094] Xenobiotics are also present in samples from subjects and can be analyzed with relation to age of subjects just as metabolites. A method to determine a Xenobiotic Score for a subject comprises the steps of: a) analyzing a biological sample from a subject to determine the level(s) of one or more xenobiotics in the sample; b) comparing the level(s) of the one or more xenobiotics in the sample to a xenobiotic reference levels of the one or more xenobiotics in order to determine the subject's xenobiotic level; and c) calculating the difference between the subject's xenobiotic level and the xenobiotic reference level to determine the xenobiotic score.

[0095] The Xenobiotic Score can be used to provide recommendations on dietary improvements or modifications, lifestyle changes (e.g. quit smoking, drink less alcohol, increase exercise) and/or vitamins, minerals and/or dietary supplements. Further, lifestyle assessment questionnaires can also be combined with the xenobiotic analysis and score to make recommendations to improve health and xenobiotic levels.

**EXAMPLES**

[0096] The invention will be further explained by the following illustrative examples that are intended to be non-limiting.

**EXAMPLE 1: Identification of Metabolic Age Biomarkers**

[0097] This example describes determination of metabolites that vary in level with age, gender and race of humans.

*Sample Preparation*

- 5 [0098] Human plasma samples shown in Table 1 were obtained from Bioreclamation, Inc. (East Meadow, NY). The sample preparation process was carried out using the automated MicroLab STAR® liquid-handling system from Hamilton Company. Recovery standards were added prior to the first step in the extraction process for Quality Control (QC) purposes. Sample preparation was
- 10 conducted using a proprietary series of organic and aqueous extractions to remove sample proteins, while maximizing the recovery of small molecules. The resulting extract was divided into two fractions, one for analysis by polar liquid chromatography (LC), and the other for analysis by gas chromatography (GC). A TurboVap® (Zymark) was used to remove the organic solvent. Each sample was
- 15 then frozen and dried under vacuum. Samples were then prepared for the appropriate analysis system, either LC/MS or GC/MS.

**Table 1: Composition of subjects by age group, gender, and self-reported race.**

	<b>Age 25-35</b>	<b>Age 36-50</b>	<b>Age 51-65</b>	<b>Total</b>
<b>Gender</b>				
Male	47	34	49	130
Female	43	56	41	140
<b>Race</b>	<i>Male/Female</i>	<i>Male/Female</i>	<i>Male/Female</i>	
Asian	0/0	1/0	0/0	1
Black	28/25 (53)	15/27 (42)	22/15 (37)	132
Hispanic	9/8 (17)	13/13 (26)	21/11 (32)	75
White	10/10 (20)	5/16 (21)	6/15 (21)	62
<b>Total</b>	47/43 (90)	34/56 (90)	49/41 (90)	270

20 *Liquid chromatography/mass spectrometry (LC/MS)*

- [0099] The LC/MS system consisted of a Surveyor HPLC (Thermo-Electron, Waltham, MA) and a LTQ linear ion-trap mass spectrometer (Thermo-Electron, Waltham, MA). Compounds were eluted via an aqueous/organic solvent gradient and ionized via electrospray ionization (ESI). Continuous, alternating polarity switching
- 25 was employed so as to be able to generate both positive and negative ions

consecutively.

[00100] The vacuum-dried sample was dissolved in 100 µl of an injection solvent that contained five or more injection standards at fixed concentrations. The chromatographic system used a binary solvent system, which was delivered as a gradient, where solvent A was water and solvent B was methanol. Both were high purity grade and contained 0.1% formic acid as a pH stabilizer. The HPLC column was a Thermo AquaSil C-18 (100 mm L x 2.1 mm ID). Metabolites that were identified using LC-MS are denoted by the number 35 in the "Library" column in Tables 2-12.

10 *Gas chromatography/Mass Spectrometry (GC/MS)*

[00101] The GC samples were dried under vacuum for a minimum of 24 hours prior to being derivatized under dried nitrogen using bistrimethyl-silyl-trifluoroacetamide (BSTFA). The GC column (Restek, Bellefonte, PA) was 5% phenyl. Elution was effected by a temperature ramp from 40°C to 300°C in a 16 minute period. The mass spectrometer consisted of a TraceDSQ (Thermo-Electron, Waltham, MA) single-quadrupole mass spectrometer. Ionization was accomplished via electron impact (EI). Metabolites that were identified using GC-MS are denoted by the number 50 in the "Library" column in Tables 2-11.

20 *Data processing*

[00102] Data processing was performed using Metabolon's custom developed informatics platform, which consisted of four major components, the Metabolon Laboratory Information Management System (MLIMS), the data extraction and peak-identification software, data processing tools for QC and compound identification, and a collection of information interpretation and visualization tools for use by data analysts.

*Compound identification*

[00103] Compounds were identified by comparison to library entries of purified standards or recurrent, unnamed entities. Identification of known chemical entities was based on comparison to metabolomic library entries of purified standards purchased from a variety of vendors.

*Statistical Calculations*

[00104] Statistical analysis of the data was performed using JMP (SAS, available on the worldwide web at jmp.com), a commercial software package, and “R” (available on the worldwide web at r-project.org), which is a freely available open-source, software package.

[00105] Table 1 contains the data describing the cohort by age, race and gender for the 270 samples used in this study.

[00106] For statistical analysis, ANOVA was performed on the full factorial. A log transform was applied to the observed relative concentrations for each compound because, in general, the variance increased as a function of a compound’s average response. Some compounds, especially xenobiotics, have “sparse” responses, where many samples will not have a reported value. In order to be included in the statistical analysis, a compound had to have a response for at least 80% of the samples in one of age, race and gender combinations shown in Table 1. In cases where a response was missing, we assumed that the value was missing because the compound was below the limit of detection. For these cases, a value was imputed with the minimum response for that compound, which is conservative for estimating the mean. Finally, in order to limit false discoveries, we computed q-values (Benjamini, Y. and Y. Hochberg, 1995, Journal of the Royal Statistical Society, Series B, 57:289-300). In addition to looking for differences by age, race and gender, we also looked for interactions between these factors. For the most part, no strong interactions were observed. However, we did observe that urca levels might be influenced both by age and gender.

[00107] Listed in Table 2 are the named and unnamed metabolites that were detected in the plasma collected from the individuals described in Table 1 and analyzed based upon age groupings. The “Library” column indicates whether the compound was detected using GC-MS or LC-MS. GC-MS metabolites are indicated by “50” while “35” indicates LC-MS metabolites. The mean level for each compound for each age group is indicated in the columns by age group (25-35, 36-50, 51-65). Statistical significance is indicated by the p-value and the the false discovery rate is indicated by the q-value. The “Comp ID” column refers to the internal database tracking number for that compound in our chemical library.

Table 2: Compounds that change with age.

COMP ID	COMPOUND	Library	p-value	q-value	20-35 years	36-50 years	51-65 years	Change with age (youngest to oldest)
16511	Metabolite - 4274	50	2.06E-22	2.09E-20	0.65	1.03	1.62	Increase
9313	Metabolite - 2172	35	1.35E-20	5.18E-19	0.23	0.50	1.37	Increase
12769	Metabolite - 3089	50	1.77E-20	5.18E-19	0.27	0.55	1.20	Increase
1493	ornithine	50	2.04E-20	5.18E-19	0.56	1.10	1.73	Increase
12767	Metabolite - 3087	50	9.85E-20	2.00E-18	0.65	1.00	1.46	Increase
12593	Metabolite - 2973	50	9.56E-19	1.39E-17	0.70	0.97	1.31	Increase
16332	Metabolite - 4164	35	3.26E-18	4.14E-17	0.79	0.96	1.21	Increase
12790	Metabolite - 3108	50	5.17E-16	5.26E-15	0.82	1.00	1.23	Increase
57	glutamic acid	50	6.21E-16	5.74E-15	0.53	1.08	1.47	Increase
11777	glycine	50	2.56E-15	2.17E-14	0.67	1.02	1.47	Increase
21025	iminodiacetic acid	50	3.10E-15	2.42E-14	0.22	0.64	4.39	Increase
1126	alanine	50	3.40E-15	2.47E-14	0.72	1.03	1.42	Increase
12770	Metabolite - 3090	50	1.95E-14	1.32E-13	0.22	0.66	2.47	Increase
19934	inositol	50	3.06E-14	1.94E-13	0.83	1.09	1.18	Increase
60	leucine	50	4.66E-14	2.79E-13	0.71	1.07	1.40	Increase
1649	valine	50	5.20E-14	2.94E-13	0.70	1.01	1.33	Increase
1301	lysine	50	7.10E-13	3.80E-12	0.64	1.07	1.53	Increase
12669	Metabolite - 3036	50	8.86E-13	4.50E-12	0.58	0.80	1.24	Increase
10461	Metabolite - 2313	35	3.20E-12	1.55E-11	0.78	1.04	1.56	Increase
13257	Metabolite - 3218	35	3.49E-12	1.61E-11	0.83	1.00	1.14	Increase
12784	Metabolite - 3102	50	1.01E-11	4.46E-11	0.89	0.99	1.20	Increase
15063	Metabolite - 3772	35	2.58E-11	1.09E-10	0.64	1.16	1.46	Increase
1125	isoleucine	50	3.74E-11	1.52E-10	0.72	1.03	1.35	Increase
594	niacinamide	35	4.32E-11	1.69E-10	0.52	0.81	1.05	Increase



11323	Metabolite - 2711	35	9.42E-11	3.42E-10	0.68	1.10	1.45	Increase
1648	serine	50	1.10E-10	3.86E-10	0.75	1.04	1.30	Increase
20699	meso-erythritol	50	2.56E-10	8.67E-10	0.93	0.98	1.24	Increase
3147	xanthine	35	4.91E-10	1.61E-09	0.58	0.76	1.27	Increase
1647	glutamine	50	5.73E-10	1.82E-09	0.75	0.92	1.49	Increase
527	lactate	50	1.05E-09	3.23E-09	0.73	1.05	1.23	Increase
1303	malic acid	50	3.15E-09	9.15E-09	0.79	1.11	1.31	Increase
12644	Metabolite - 3016	50	4.41E-08	1.18E-07	0.87	0.99	1.10	Increase
1284	threonine	50	1.03E-07	2.68E-07	0.82	1.04	1.28	Increase
59	histidine	50	1.09E-07	2.77E-07	0.77	1.09	1.18	Increase
12774	Metabolite - 3094	50	1.20E-07	2.98E-07	0.85	0.99	1.13	Increase
3127	hypoxanthine	35	3.63E-07	8.58E-07	0.41	0.84	1.36	Increase
569	caffeine	35	4.23E-07	9.77E-07	0.28	0.70	1.16	Increase
16135	Metabolite - 4077	50	5.39E-07	1.22E-06	0.67	0.89	1.19	Increase
1437	succinate	50	5.51E-07	1.22E-06	0.76	1.08	1.09	Increase
1113	isocitrate	35	1.67E-06	3.46E-06	0.76	1.02	1.26	Increase
12795	Metabolite - 3113	50	1.87E-06	3.80E-06	0.61	0.89	1.17	Increase
1584	Metabolite - 1003	35	2.16E-06	4.31E-06	0.78	0.93	1.05	Increase
16070	Metabolite - 4019	50	2.46E-06	4.81E-06	0.85	0.94	1.12	Increase
1107	allantoin	50	3.79E-06	7.13E-06	0.64	0.93	1.15	Increase
1508	pantothenic acid	35	3.91E-06	7.23E-06	0.84	1.05	1.25	Increase
12783	Metabolite - 3101	50	3.99E-06	7.24E-06	0.84	0.97	1.12	Increase
606	uridine	35	4.90E-06	8.74E-06	0.89	0.98	1.18	Increase
5803	Metabolite - 1190	35	5.09E-06	8.92E-06	0.38	0.85	0.88	Increase
1494	5-oxoproline	50	8.40E-06	1.43E-05	0.87	1.05	1.13	Increase
11438	phosphate	50	8.46E-06	1.43E-05	0.90	0.97	1.06	Increase
10347	Metabolite - 2285	35	1.13E-05	1.88E-05	0.67	0.87	1.16	Increase
15529	Metabolite - 3951	35	1.15E-05	1.89E-05	0.92	0.99	1.13	Increase
15140	L-kynurenine	35	1.48E-05	2.39E-05	0.98	0.95	1.16	Increase
6413	Metabolite - 1342-possible-	35	2.20E-05	3.44E-05	0.65	1.05	1.16	Increase

	phenylacetylglutamine								
18254	paraxanthine	35	4.01E-05	1.00E-04	0.32	0.69	0.81	Increase	
13296	Metabolite - 3230	35	1.00E-04	1.00E-04	0.96	1.02	1.12	Increase	
14837	Metabolite - 3707	35	1.00E-04	1.00E-04	0.66	0.88	1.28	Increase	
15234	Metabolite - 3821	35	1.00E-04	1.00E-04	0.75	0.81	0.96	Increase	
7359	Metabolite - 1713	35	1.00E-04	2.00E-04	0.67	1.03	1.17	Increase	
5800	Metabolite - 1188	35	2.00E-04	3.00E-04	0.31	0.75	0.71	Increase	
9130	Metabolite - 2139	35	2.00E-04	3.00E-04	0.90	1.02	1.16	Increase	
1519	sucrose	50	3.00E-04	5.00E-04	0.36	0.61	0.91	Increase	
16067	Metabolite - 4017	50	4.00E-04	5.00E-04	0.90	0.95	1.10	Increase	
1670	urea	50	4.00E-04	5.00E-04	0.88	1.04	1.19	Increase	
1358	octadecanoic acid	50	4.00E-04	6.00E-04	0.93	1.03	1.08	Increase	
7029	Metabolite - 1597	35	5.00E-04	6.00E-04	0.92	1.03	1.03	Increase	
10154	Metabolite - 2258-	35	5.00E-04	7.00E-04	0.78	0.98	1.11	Increase	
1574	histamine	35	6.00E-04	7.00E-04	0.95	0.99	1.14	Increase	
5689	Metabolite - 1111	35	7.00E-04	8.00E-04	0.96	0.93	1.13	Increase	
528	alpha-keto-glutarate	35	7.00E-04	9.00E-04	0.64	1.07	1.00	Increase	
11222	Metabolite - 2688	35	7.00E-04	9.00E-04	0.57	0.89	0.85	Increase	
6424	Metabolite - 1346	35	8.00E-04	0.001	0.93	0.99	1.08	Increase	
6130	Metabolite - 1208	35	9.00E-04	0.001	0.43	0.74	0.86	Increase	
5580	Metabolite - 1067	35	0.0011	0.0012	0.80	1.31	1.45	Increase	
12864	Metabolite - 3124	35	0.0011	0.0013	0.80	0.94	1.02	Increase	
6136	Metabolite - 1211-IHWESALLR	35	0.0013	0.0014	0.38	0.91	0.83	Increase	
12720	Metabolite - 3056	35	0.0014	0.0015	0.86	0.96	1.07	Increase	
16496	Metabolite - 4251	50	0.0015	0.0016	0.72	0.95	1.11	Increase	
1444	Isobar-56: DL-pipecolic acid/1-amino-cyclopentanecarboxylic acid	35	0.0021	0.0021	0.98	1.32	1.19	Increase	
6122	Metabolite - 1206	35	0.0021	0.0021	0.40	0.76	0.59	Increase	

5664	Metabolite - 1215	35	0.0021	0.0021	0.0021	0.48	1.11	0.98	Increase
12785	Metabolite - 3103	50	0.0021	0.0021	0.0021	0.25	0.49	0.75	Increase
15753	hippuric acid	35	0.0022	0.0022	0.0021	0.81	1.14	1.23	Increase
10921	Metabolite - 2558	35	0.0023	0.0022	0.0022	0.30	0.21	0.58	Increase
10672	Metabolite - 2390	35	0.0032	0.0031	0.0031	0.87	0.98	1.12	Increase
6362	Metabolite - 1323-possible-p-cresol-sulfate	35	0.0043	0.004	0.004	0.58	0.81	1.05	Increase
1123	inosine	35	0.0047	0.0044	0.0044	0.62	0.92	1.05	Increase
6236	Metabolite - 1983	35	0.0052	0.0048	0.0048	0.36	0.68	0.63	Increase
12352	Metabolite - 2871	35	0.0053	0.0048	0.0048	0.70	0.92	0.98	Increase
6215	Metabolite - 1261	35	0.0059	0.0052	0.0052	0.48	0.91	0.83	Increase
12754	Metabolite - 3075	50	0.0072	0.0063	0.0063	0.75	0.94	0.93	Increase
15663	Metabolite - 1000	35	0.0078	0.0068	0.0068	0.64	1.00	0.86	Increase
6138	Metabolite - 1213	35	0.008	0.0069	0.0069	0.56	0.90	1.02	Increase
7933	Metabolite - 1911	35	0.0088	0.0074	0.0074	0.51	0.84	0.90	Increase
6112	Metabolite - 1203-HXGXA	35	0.0089	0.0074	0.0074	0.30	0.75	0.64	Increase
15681	4-Guanidinobutanoic acid	35	0.0092	0.0075	0.0075	0.95	1.00	1.06	Increase
13744	Metabolite - 3364	35	0.01	0.008	0.008	0.63	0.83	1.03	Increase
8336	Metabolite - 2005	35	0.0104	0.0083	0.0083	0.90	0.91	1.12	Increase
5687	Metabolite - 1110	35	0.0118	0.0093	0.0093	0.71	0.91	1.11	Increase
10245	Metabolite - 2269	35	0.0135	0.0105	0.0105	0.72	0.89	1.10	Increase
6204	Metabolite - 1252	35	0.0136	0.0105	0.0105	0.35	0.71	0.47	Increase
12626	Metabolite - 3003	50	0.0136	0.0105	0.0105	0.96	1.02	1.07	Increase
10785	Metabolite - 2506	35	0.0137	0.0105	0.0105	0.71	1.01	1.15	Increase
12777	Metabolite - 3097	50	0.0177	0.0134	0.0134	0.86	1.09	1.13	Increase
27718	creatine	35	0.0178	0.0134	0.0134	0.87	1.02	1.11	Increase
6239	Metabolite - 1264	35	0.0187	0.0139	0.0139	0.43	0.90	0.67	Increase
10309	Metabolite - 2277	35	0.0189	0.0139	0.0139	0.66	1.01	0.79	Increase
15991	L-alpha-glycerophosphorylcholine	35	0.0191	0.014	0.014	0.70	0.93	0.97	Increase
7424	Metabolite - 1718	35	0.0192	0.014	0.014	0.26	0.63	0.37	Increase

6231	Metabolite - 1262	35	0.0209	0.0151	0.40	0.83	0.56	Increase
5798	Metabolite - 1187	35	0.0217	0.0155	0.32	0.62	0.47	Increase
7644	Metabolite - 1831	35	0.0238	0.0168	0.84	0.95	1.02	Increase
27741	cis/trans-aconitic acid	35	0.0281	0.0195	0.99	1.06	1.24	Increase
1561	alpha-tocopherol	50	0.0289	0.02	0.91	0.95	1.11	Increase
11111	Metabolite - 2592	35	0.0294	0.0202	0.89	1.40	1.00	Increase
6296	Metabolite - 1303-SHAXQXNR	35	0.031	0.0212	0.48	0.80	0.59	Increase
12646	Metabolite - 3018	50	0.0323	0.0218	0.92	1.01	0.99	Increase
64	phenylalanine	35	0.0323	0.0218	0.96	1.02	1.05	Increase
1431	p-hydroxyphenyllactic acid	35	0.0327	0.0219	0.77	0.93	0.92	Increase
22130	DL-phenyllactic acid	35	0.0331	0.022	0.87	1.05	1.11	Increase
6208	Metabolite - 1254	35	0.0342	0.0226	0.62	1.09	1.14	Increase
12751	Metabolite - 3073	50	0.0364	0.0237	0.89	1.09	0.98	Increase
5724	Metabolite - 1125	35	0.0369	0.0239	0.55	0.77	0.56	Increase
15506	choline	35	0.0381	0.0245	0.99	1.02	1.11	Increase
16138	Metabolite - 4080	50	0.041	0.0261	0.89	1.08	0.92	Increase
1105	linoleic acid	50	0.0415	0.0262	0.93	1.06	1.07	Increase
15677	3-methyl-L-histidine/1-methyl-L-histidine	35	0.0457	0.0284	0.53	0.72	0.82	Increase
10092	Metabolite - 2250	35	0.0493	0.0302	0.41	0.55	0.68	Increase
54	tryptophan	35	0.0506	0.0308	1.00	0.95	1.03	Increase
10083	Metabolite - 2248	35	0.0528	0.032	0.49	0.71	0.79	Increase
5733	Metabolite - 1127	35	0.0599	0.0358	0.90	1.06	0.93	Increase
5577	Metabolite - 1065	35	0.0617	0.0362	0.69	1.31	0.99	Increase
15769	carnitine	35	0.062	0.0362	0.92	0.96	1.03	Increase
18392	theobromine	35	0.0665	0.0381	0.69	0.73	1.00	Increase
5669	Metabolite - 1104	35	0.0669	0.0381	0.92	1.04	1.04	Increase
63	cholesterol	50	0.069	0.039	0.92	0.95	1.12	Increase
6787	Metabolite - 1465	35	0.074	0.0411	0.90	1.04	0.99	Increase

21044	2-hydroxybutyric acid	50	0.0752	0.0415	0.90	1.07	1.08	Increase
10304	Metabolite - 2276	35	0.0841	0.0459	0.67	1.00	0.77	Increase
14933	Metabolite - 3739	35	0.0879	0.0478	0.65	0.59	0.85	Increase
10478	Metabolite - 2317	35	0.0915	0.0493	0.51	0.77	0.63	Increase
16091	Metabolite - 4031	35	0.0916	0.0493	0.89	0.98	1.01	Increase
12710	Metabolite - 3052	35	0.0921	0.0493	0.90	1.03	0.93	Increase
16518	Metabolite - 4276	50	0.095	0.0506	0.78	0.97	0.90	Increase
15596	Metabolite - 3962	35	0.0973	0.0514	1.03	1.15	1.16	Increase
15683	4-methyl-2-oxopentanoate	50	0.0976	0.0514	0.93	1.05	1.02	Increase
12759	Metabolite - 3080	50	0.0982	0.0515	0.98	1.09	1.09	Increase
10317	Metabolite - 2279	35	0.1032	0.0535	0.55	0.67	0.71	Increase
10296	Metabolite - 2273	35	0.1041	0.0535	0.48	0.65	0.71	Increase
1604	uric acid	35	0.1062	0.0542	0.97	1.00	1.02	Increase
1299	tyrosine	35	0.1122	0.0567	0.97	1.00	1.06	Increase
12856	Metabolite - 3123	35	0.1285	0.064	0.75	1.05	0.79	Increase
12791	Metabolite - 3109	50	0.1318	0.0653	0.92	0.82	1.00	Increase
1591	N-acetyl-L-valine	35	0.1459	0.0716	0.83	0.87	0.92	Increase
1336	n-hexadecanoic acid	50	0.1482	0.0721	0.98	1.09	1.07	Increase
1359	oleic acid	50	0.1517	0.0734	0.89	1.05	1.03	Increase
6398	Metabolite - 1335	35	0.1602	0.0768	0.91	0.96	1.09	Increase
5809	3-indoxyl-sulfate	35	0.1642	0.0784	0.93	0.92	1.10	Increase
14785	Isobar-glycochenodeoxycholic acid-glycodeoxycholic acid	35	0.1738	0.0826	0.70	0.98	0.81	Increase
542	3-hydroxybutanoic acid	50	0.1793	0.0844	1.02	1.38	1.29	Increase
14239	Metabolite - 3474	35	0.1853	0.0868	0.83	0.95	0.83	Increase
10424	Metabolite - 2292	35	0.1877	0.0875	0.64	0.55	0.75	Increase
1110	arachidonic acid	50	0.19	0.0882	0.92	0.97	1.02	Increase
5609	Metabolite - 1083	35	0.1989	0.0918	0.12	0.26	0.17	Increase

18349	DL-indole-3-lactic acid	35	0.2003	0.0918	0.95	0.91	1.04	Increase
12781	Metabolite - 3099	50	0.2005	0.0918	0.91	1.05	1.06	Increase
10087	Metabolite - 2249	35	0.2088	0.0939	1.01	0.94	1.05	Increase
15128	Metabolite - 1002	35	0.2126	0.0952	0.99	1.17	1.05	Increase
6402	Metabolite - 3832	35	0.2216	0.0984	1.09	1.10	1.32	Increase
12924	Metabolite - 3131	35	0.2245	0.0992	0.89	1.06	0.99	Increase
9561	Metabolite - 2193	35	0.2255	0.0992	0.50	0.74	0.64	Increase
10782	Metabolite - 2486	35	0.2511	0.1091	0.88	1.05	1.01	Increase
1121	heptadecanoic acid	50	0.2651	0.1147	0.95	0.98	1.04	Increase
16055	Metabolite - 4012	50	0.2678	0.1153	0.89	0.93	1.00	Increase
11499	Metabolite - 2753	35	0.2829	0.1214	0.95	1.03	0.99	Increase
10715	Metabolite - 2395	35	0.3021	0.1274	0.81	1.04	0.83	Increase
8091	glycocholic acid	35	0.3105	0.1304	0.71	0.93	0.87	Increase
10570	Metabolite - 2366	35	0.3118	0.1304	0.86	0.96	1.02	Increase
16071	Metabolite - 4020	50	0.3139	0.1308	0.88	0.87	0.98	Increase
13605	Metabolite - 4868	35	0.3197	0.1326	0.53	0.77	0.60	Increase
10774	Metabolite - 2466	35	0.3641	0.1491	0.50	0.72	0.66	Increase
14755	Metabolite - 3664	35	0.3653	0.1491	0.69	0.83	0.80	Increase
9491	Metabolite - 2185	35	0.3925	0.1589	0.94	0.97	1.04	Increase
15122	glycerol	50	0.3955	0.1596	1.00	1.04	1.09	Increase
10781	Metabolite - 2469	35	0.4135	0.1662	0.85	1.00	0.93	Increase
10414	Metabolite - 2291	35	0.4238	0.1685	0.64	0.67	0.80	Increase
15328	azelaic acid	35	0.4242	0.1685	0.97	1.04	1.01	Increase
15278	Metabolite - 3843	35	0.426	0.1685	0.97	0.92	0.99	Increase
10667	Metabolite - 2389	35	0.4367	0.172	0.94	0.94	1.01	Increase
6254	Metabolite - 1284	35	0.4709	0.1841	1.04	1.14	1.05	Increase
22133	DL-hexanoyl-carnitine	35	0.4843	0.1879	0.97	0.98	1.05	Increase
15220	Metabolite - 3813	35	0.4935	0.1907	0.87	0.94	0.94	Increase
27728	glycerol-2-phosphate	50	0.5162	0.198	0.79	0.86	0.87	Increase
10945	Metabolite - 2560	35	0.5351	0.2037	0.98	0.96	1.00	Increase

10629	Metabolite - 2386	35	0.5582	0.2109	0.81	0.93	0.84	Increase
10492	Metabolite - 2320	35	0.5731	0.2157	0.85	0.84	0.94	Increase
14715	Metabolite - 3653	35	0.5829	0.2186	0.99	0.94	1.18	Increase
15129	Metabolite - 1001	35	0.5888	0.22	0.71	0.78	0.82	Increase
16186	gamma-glu-gly-leu-	35	0.6239	0.2296	0.71	0.80	0.79	Increase
1898	proline	35	0.627	0.2296	1.01	1.05	1.05	Increase
1365	tetradecanoic acid	50	0.6299	0.2296	1.00	1.07	1.06	Increase
1507	palmitoleic acid	50	0.6301	0.2296	0.88	1.01	0.95	Increase
7002	Metabolite - 1576	35	0.6402	0.2308	0.93	0.94	0.97	Increase
6439	Metabolite - 1350	35	0.6454	0.2318	0.54	0.53	0.65	Increase
13142	Metabolite - 3165	35	0.6695	0.2388	0.94	0.99	0.97	Increase
8300	Metabolite - 1988	35	0.7145	0.2507	0.99	1.07	1.00	Increase
12109	Metabolite - 2853	35	0.7191	0.2509	0.63	0.66	0.70	Increase
11292	Metabolite - 2703	35	0.7719	0.2678	0.93	0.98	0.95	Increase
14639	Metabolite - 3603	35	0.7933	0.2739	0.69	0.76	0.70	Increase
10501	Metabolite - 2321	35	0.7968	0.2739	0.94	1.00	1.03	Increase
13038	Metabolite - 3143	35	0.8101	0.2767	0.91	0.97	0.91	Increase
513	creatinine	35	0.8586	0.289	0.97	0.97	0.98	Increase
5727	Metabolite - 1126	35	0.881	0.2946	0.74	0.79	0.77	Increase
12625	Metabolite - 3002	50	0.9626	0.3151	0.99	0.98	1.00	Increase
10551	Metabolite - 2347	35	0.964	0.3151	0.93	0.92	0.96	Increase
5652	Metabolite - 1090	35	0.9896	0.3205	0.28	0.29	0.30	Increase
13200	Metabolite - 3180	35	0.9939	0.3207	0.82	0.82	0.82	Increase
7177	Metabolite - 1656	35	2.58E-19	4.37E-18	1.27	0.80	0.42	Decrease
15113	Metabolite - 3783	35	1.81E-16	2.04E-15	1.20	0.99	0.75	Decrease
16002	Metabolite - 3992	35	7.75E-11	2.92E-10	1.14	0.99	0.80	Decrease
5587	dehydroepiandrosterone- sulfate-	35	1.40E-09	4.19E-09	1.38	0.97	0.59	Decrease
10252	Metabolite - 2271	35	3.96E-09	1.12E-08	1.31	1.01	0.74	Decrease
12763	Metabolite - 3083	50	9.48E-09	2.60E-08	1.09	0.83	0.64	Decrease

10156	Metabolite - 2259	35	1.73E-07	4.19E-07	1.30	1.15	0.68	Decrease
13545	Metabolite - 3322	35	8.43E-07	1.82E-06	1.14	1.02	0.54	Decrease
12768	Metabolite - 3088	50	1.29E-06	2.73E-06	1.05	0.86	0.71	Decrease
11053	Metabolite - 2567	35	3.74E-06	7.13E-06	1.10	1.00	0.81	Decrease
5647	Metabolite - 1088	35	1.93E-05	3.06E-05	1.40	0.94	0.81	Decrease
13589	Metabolite - 3327	35	1.00E-04	1.00E-04	0.96	1.10	0.60	Decrease
13214	Metabolite - 3183- possible-gamma-L- glutamyl-L-phenylalanine- or-aspartame	35	4.00E-04	5.00E-04	1.03	0.86	0.75	Decrease
15686	beta-hydroxypruvic acid	35	5.00E-04	6.00E-04	1.08	0.99	0.91	Decrease
12666	Metabolite - 3033	50	5.00E-04	6.00E-04	1.07	0.93	0.87	Decrease
16506	Metabolite - 4271	50	5.00E-04	6.00E-04	0.84	1.07	0.62	Decrease
6421	Metabolite - 1345	35	0.001	0.0012	1.43	0.92	0.78	Decrease
16509	Metabolite - 4273	50	0.0011	0.0012	0.78	0.49	0.49	Decrease
12726	Metabolite - 3058	50	0.0014	0.0015	1.04	0.92	0.84	Decrease
10727	Metabolite - 2398	35	0.0016	0.0017	1.07	0.98	0.90	Decrease
2761	thyroxine	35	0.0022	0.0022	1.07	0.88	0.96	Decrease
16512	Metabolite - 4275	50	0.0029	0.0028	1.08	0.78	0.79	Decrease
12663	Metabolite - 3030	50	0.0049	0.0045	1.05	0.95	0.89	Decrease
16508	Metabolite - 4272	50	0.005	0.0046	1.03	0.96	0.87	Decrease
10438	gamma-glu-leu	35	0.0055	0.0049	1.02	0.96	0.85	Decrease
6499	Metabolite - 1376	35	0.0082	0.007	0.93	0.83	0.54	Decrease
7650	Metabolite - 1834	35	0.0086	0.0073	0.69	0.93	0.52	Decrease
6266	Metabolite - 1286	35	0.009	0.0074	1.03	0.98	0.93	Decrease
12645	Metabolite - 3017	50	0.01	0.008	1.09	0.95	0.87	Decrease
12757	Metabolite - 3078	50	0.011	0.0088	1.03	0.73	0.76	Decrease
12639	Metabolite - 3012	50	0.0187	0.0139	1.05	0.94	0.91	Decrease
13487	Metabolite - 3310- probable-cotinine	35	0.0236	0.0168	0.34	0.58	0.33	Decrease
6374	Metabolite - 1327	35	0.0243	0.017	1.30	0.83	0.81	Decrease



6108	Metabolite - 1202	35	0.0363	0.0237	0.79	0.94	0.53	Decrease
12609	Metabolite - 2986	50	0.0384	0.0245	1.02	0.97	0.92	Decrease
12601	Metabolite - 2978	50	0.0419	0.0263	1.04	0.86	0.79	Decrease
6137	Metabolite - 1212	35	0.0457	0.0284	1.09	0.84	0.68	Decrease
16229	Isobar-2: arabitol/adonitol/xylitol	35	0.0481	0.0296	1.02	1.07	0.80	Decrease
5765	Metabolite - 1142	35	0.0594	0.0357	0.89	1.09	0.84	Decrease
2129	oxitryptan	35	0.0605	0.0359	0.98	0.87	0.81	Decrease
12647	Metabolite - 3019	50	0.062	0.0362	1.02	0.96	0.92	Decrease
15609	Metabolite - 3969	35	0.0646	0.0375	1.01	1.03	0.94	Decrease
15365	sn-Glycerol-3-phosphate	50	0.0664	0.0381	0.96	1.12	0.93	Decrease
2730	gamma-L-glutamyl-L-glutamine	35	0.0671	0.0381	0.84	0.76	0.66	Decrease
12670	Metabolite - 3037	50	0.07	0.0393	0.97	1.14	0.95	Decrease
10700	Metabolite - 2393	35	0.0721	0.0403	1.03	0.99	0.93	Decrease
6579	Metabolite - 1398-	35	0.08	0.044	1.08	1.03	0.96	Decrease
12658	Metabolite - 3026	50	0.1006	0.0524	1.00	0.93	0.90	Decrease
584	mannose	50	0.104	0.0535	1.02	1.06	0.91	Decrease
10148	Metabolite - 2257	35	0.1067	0.0542	1.05	0.74	0.71	Decrease
12074	glucose	50	0.1234	0.0621	0.96	0.82	0.86	Decrease
12912	Metabolite - 3129	35	0.1245	0.0623	0.98	0.92	0.85	Decrease
13104	Metabolite - 3160	35	0.1359	0.0671	0.97	1.00	0.91	Decrease
12650	Metabolite - 3022	50	0.148	0.0721	1.08	1.03	0.97	Decrease
6852	Metabolite - 1498	35	0.1547	0.0745	0.95	0.87	0.78	Decrease
10047	Metabolite - 2237	35	0.1773	0.0839	0.59	0.72	0.41	Decrease
10145	Metabolite - 2256	35	0.2033	0.0927	0.57	0.66	0.44	Decrease
7096	Metabolite - 1612	35	0.2067	0.0938	0.89	0.76	0.74	Decrease
2734	gamma-L-glutamyl-L-tyrosine	35	0.2086	0.0939	0.90	0.83	0.77	Decrease
10544	Metabolite - 2329	35	0.2172	0.0969	0.92	0.70	0.64	Decrease
10655	Metabolite - 2388	35	0.2283	0.1	0.93	0.86	0.85	Decrease

12771	Metabolite - 3091	50	0.2439	0.1064	0.89	0.96	0.80	Decrease
12756	Metabolite - 3077	50	0.2853	0.1219	1.01	0.99	0.94	Decrease
20675	1,5-anhydro-D-glucitol	50	0.2903	0.1235	0.90	0.93	0.81	Decrease
15626	Metabolite - 3977	35	0.302	0.1274	1.03	1.04	0.96	Decrease
12780	Metabolite - 3098	50	0.3295	0.1362	0.89	0.81	0.73	Decrease
12656	Metabolite - 3025	50	0.3608	0.1485	1.00	0.99	0.94	Decrease
9905	Metabolite - 2231	35	0.3841	0.1562	0.83	0.76	0.77	Decrease
10604	Metabolite - 2370	35	0.4185	0.1675	0.98	1.07	0.95	Decrease
1564	citric acid	35	0.4593	0.1803	1.13	1.01	1.09	Decrease
10476	Metabolite - 2316	35	0.4842	0.1879	0.84	0.98	0.83	Decrease
27738	threonine acid	50	0.5085	0.1958	0.97	0.85	0.90	Decrease
10378	Metabolite - 2287	35	0.5284	0.2019	0.66	0.55	0.62	Decrease
10143	Metabolite - 2255	35	0.5426	0.2058	0.69	0.85	0.65	Decrease
8796	Metabolite - 2074	35	0.597	0.2223	0.64	0.77	0.64	Decrease
1366	trans-4-hydroxyproline	35	0.6026	0.2236	0.59	0.63	0.54	Decrease
10825	Metabolite - 2546	35	0.6117	0.2261	1.01	0.91	1.01	Decrease
9748	Metabolite - 2212	35	0.6327	0.2297	0.94	1.01	0.94	Decrease
16037	Metabolite - 4003	35	0.6375	0.2306	0.63	0.55	0.61	Decrease
5618	Metabolite - 1085	35	0.6565	0.235	0.99	1.00	0.95	Decrease
14753	Metabolite - 3663	35	0.6744	0.2397	0.67	0.68	0.62	Decrease
15612	Metabolite - 3972	35	0.709	0.2507	0.81	0.76	0.77	Decrease
5628	Metabolite - 1086	35	0.7103	0.2507	0.86	0.94	0.84	Decrease
14759	Metabolite - 3667	35	0.7151	0.2507	0.58	0.62	0.54	Decrease
6270	Metabolite - 1288	35	0.7207	0.2509	0.80	0.82	0.74	Decrease
11604	Metabolite - 2774	35	0.7976	0.2739	0.92	0.85	0.88	Decrease
1572	glyceric acid	50	0.8112	0.2767	1.04	0.99	1.02	Decrease
13372	Metabolite - 3249	35	0.8153	0.2772	0.99	0.97	0.97	Decrease
6278	Metabolite - 1289	35	0.827	0.2802	0.82	0.76	0.79	Decrease
12099	Metabolite - 2850	35	0.8505	0.2872	0.86	0.83	0.81	Decrease
12533	Metabolite - 2915	50	0.868	0.2912	1.00	1.01	0.99	Decrease

2137	biliverdin	35	0.8905	0.2968	1.07	1.06	1.02	Decrease
1480	Metabolite - 1005	35	0.9189	0.3053	0.49	0.46	0.47	Decrease
12673	Metabolite - 3040	50	0.9401	0.3113	0.99	0.96	0.96	Decrease
14840	Metabolite - 3708	35	0.9492	0.3133	0.96	0.96	0.94	Decrease
5670	Metabolite - 1105	35	0.9547	0.3141	0.81	0.82	0.77	Decrease
1302	methionine	35	0.9834	0.3204	1.01	1.00	1.00	Decrease
577	fructose	50	0.9899	0.3205	0.63	0.62	0.62	Decrease
13775	Metabolite - 3370	35	0.9974	0.3209	0.93	0.93	0.92	Decrease

[00108] Table 3 contains a list of the biochemical pathways that showed differences either by age, gender or race based upon the changes in metabolites that comprise the pathway.

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**Table 3: Numbers of biomarker compounds showing statistically significant differences per biochemical pathway by age, race and gender.**

Pathway	Age	Race	Gender
Arginine and proline metabolism	11	5	2
ABC transporters	12	2	1
Urea cycle and metabolism of amino groups	8	5	1
Aminoacyl-tRNA biosynthesis	8	2	
Purine metabolism	5	3	2
Alanine and aspartate metabolism	6		2
Lysine degradation	5	2	1
Pyrimidine metabolism	7	1	
Citrate cycle (TCA cycle)	4		3
Glycine, serine and threonine metabolism	6	1	
Neuroactive ligand-receptor interaction	5	1	1
beta-Alanine metabolism	6		
Histidine metabolism	4	1	1
Tryptophan metabolism	5	1	
Tyrosine metabolism	5		1
Valine, leucine and isoleucine degradation	4		2
Glutamate metabolism	4		1
Porphyrin and chlorophyll metabolism	3	1	1
Carbon fixation	3	1	

#### *Age Associated Changes*

- 10 [00109] The majority of compounds that were different between the age groups showed an increase with age. Compounds with levels that showed significant differences with age are shown in Table 2. One interesting observation in this table is

the large number of amino acids that increase with age. In a study of pediatric subjects published by Lepage and coworkers, it was shown that amino acid levels increase steadily after the first year of life (Lepage, N., et al., 1997, Clin Chem. 43(12):2397-402). Although this study only monitored subjects until age 18, its trend is consistent with the data in our study. Also, we frequently see that amino acid levels within subjects are highly correlated. In Figure 1, one can see that leucine and valine levels are highly correlated.

[00110] Several compounds show statistically significant changes in level but no clear upward or downward trend with age (Table 2). Fourteen compounds show highest abundance in subjects aged 36-50. Thirteen compounds with this pattern are unnamed. These are Metabolite – 4271, Metabolite – 1129, Metabolite - 2894, Metabolite – 1834, Metabolite – 3310, Metabolite - 2810, Metabolite - 1142, Metabolite - 3018, Metabolite - 1183, Metabolite - 3487, Metabolite - 1262, Metabolite - 1264, Metabolite - 1187, and Metabolite - 1718. The only named compound in this group is glycerol-3-phosphate. Four compounds are lowest in the middle age group with two unnamed compounds (Metabolite – 2558, Metabolite – 3830) and two named compounds. The named compounds are trans-2-3-4-trimethoxycinnamic acid and tryptophan.

[00111] A search of the literature and the Geigy Tables (Physical Chemistry, Composition of Blood, Hematology, Somatometric Data, 8 ed., 1984, Geigy Scientific Tables, ed. C. Lentner. Vol. 3, Ciba-Geigy Ltd., Basle, Switzerland) was carried out to determine if the changes in levels of the 75 named metabolites that were measured in this study are consistent with previous reports. The Geigy Tables were a useful source of information, with plasma levels of 32 of the 75 compounds listed. The trends for 23 of the 32 reported compounds were in agreement with the Geigy Tables; however, five of 32 showed differing trends. Some of this disparity is due to the fact that not all of the reports were broken out by the same age categories used in our demographic study. If ages were reported, most of the age groups were in the broad categories of newborn, children, adults.

[00112] Literature searches were less informative in terms of age-related changes in the levels of these metabolites. While many of the compounds were studied in the literature, often, the changes with age were not reported, although many compounds were associated with age-related diseases such as Alzheimer's Disease (AD). One

exception is unknown metabolite 1069 which is listed as a possible DHEA-S, a major secretory product of the human adrenal gland. This compound has been reported to gradually decrease with age (Birkenhager-Gillesse, E.G., J. Derksen, and A.M. Lagaay, 1994, Ann. NY Acad Sci, 719(1):543-552). Interestingly, this compound is shown to be increased in AD relative to control subjects. However, the report stated that a significant correlation between DHEAS levels and AD was not observed.

### *Results and Discussion*

[00113] In this example it was demonstrated that metabolic profiles change with age. Thus, characteristic metabolic profiles related to the biochemical or physiological age ("Metabolic Age" or MetaboAge) can be determined using metabolomics.

### *Gender-Associated Changes*

[00114] Table 4 sets forth the metabolites that showed different levels between male and female individuals.

**Table 4: Compounds that vary based on gender.**

<b>Metabolites higher in females than males</b>							
<b>COMP ID</b>	<b>COMPOUND</b>	<b>Library</b>	<b>p-value</b>	<b>q-value</b>	<b>FEMALE</b>	<b>MALE</b>	<b>Female/Male</b>
11438	phosphate	50	7.00E-04	0.0048	1.02	0.93	1.10
1507	palmitoleic acid	50	0.0015	0.0092	1.13	0.79	1.42
15122	glycerol	50	0.0016	0.0092	1.13	0.96	1.18
6108	Metabolite - 1202	35	0.0017	0.0092	0.98	0.55	1.79
10424	Metabolite - 2292	35	0.0021	0.0096	0.79	0.52	1.52
15328	azelaic acid	35	0.0026	0.0115	1.07	0.95	1.12
12609	Metabolite - 2986	50	0.0027	0.0117	1.02	0.92	1.11
12912	Metabolite - 3129	35	0.0035	0.0139	1.00	0.84	1.19
15626	Metabolite - 3977	35	0.0043	0.0156	1.08	0.94	1.14
27718	creatine	35	0.0047	0.0164	1.10	0.90	1.22
16002	Metabolite - 3992	35	0.005	0.017	1.03	0.91	1.13
12774	Metabolite - 3094	50	0.0059	0.0198	1.04	0.93	1.12
12759	Metabolite - 3080	50	0.0072	0.0229	1.12	0.98	1.14
11111	Metabolite - 2592	35	0.0146	0.038	1.28	0.90	1.41
7650	Metabolite - 1834	35	0.0207	0.0494	0.83	0.58	1.42
10667	Metabolite - 2389	35	0.0219	0.0507	1.02	0.91	1.12
6278	Metabolite - 1289	35	0.0247	0.0565	0.88	0.71	1.24
13372	Metabolite - 3249	35	0.031	0.0677	1.01	0.94	1.07
16518	Metabolite - 4276	50	0.0319	0.0687	0.96	0.80	1.20
6852	Metabolite - 1498	35	0.0332	0.0705	0.94	0.79	1.19
1365	tetradecanoic acid	50	0.0537	0.0987	1.10	0.99	1.12

542	3-hydroxybutanoic acid	50	0.0542	0.0987	1.40	1.06	1.31
15612	Metabolite - 3972	35	0.0565	0.1011	0.83	0.73	1.14
10047	Metabolite - 2237	35	0.0573	0.1011	0.71	0.44	1.59
10154	Metabolite - 2258-	35	0.0649	0.1068	1.02	0.88	1.15
13487	Metabolite - 3310- probable-cotinine	35	0.0758	0.1184	0.47	0.34	1.39
11292	Metabolite - 2703	35	0.0781	0.1207	1.00	0.91	1.09
16508	Metabolite - 4272	50	0.0881	0.1334	0.99	0.92	1.08
1561	alpha-tocopherol	50	0.093	0.1369	1.04	0.94	1.11
1359	oleic acid	50	0.0956	0.1394	1.05	0.92	1.14
11604	Metabolite - 2774	35	0.1023	0.1442	0.95	0.82	1.17
12781	Metabolite - 3099	50	0.1435	0.1815	1.06	0.95	1.12
6137	Metabolite - 1212	35	0.1578	0.1916	0.95	0.76	1.25
5618	Metabolite - 1085	35	0.1646	0.1968	1.01	0.95	1.07
7424	Metabolite - 1718	35	0.176	0.204	0.47	0.33	1.41
14639	Metabolite - 3603	35	0.1875	0.2126	0.78	0.67	1.17
1336	n-hexadecanoic acid	50	0.1938	0.2152	1.08	1.01	1.06
2761	thyroxine	35	0.1979	0.2179	1.00	0.94	1.06
1105	linoleic acid	50	0.2016	0.2203	1.05	0.99	1.07
15529	Metabolite - 3951	35	0.2111	0.2266	1.03	0.99	1.05
6215	Metabolite - 1261	35	0.2115	0.2266	0.80	0.64	1.24
6204	Metabolite - 1252	35	0.2117	0.2266	0.55	0.43	1.27
10700	Metabolite - 2393	35	0.2198	0.2336	1.01	0.96	1.05
12785	Metabolite - 3103	50	0.2306	0.2401	0.53	0.38	1.37
12074	glucose	50	0.2376	0.2431	0.91	0.84	1.08
6787	Metabolite - 1465	35	0.2383	0.2431	1.01	0.95	1.06
584	mannose	50	0.2428	0.246	1.03	0.96	1.07
12783	Metabolite - 3101	50	0.2445	0.2462	1.00	0.95	1.06
63	cholesterol	50	0.2745	0.2621	1.04	0.95	1.09
6254	Metabolite - 1284	35	0.2789	0.2621	1.11	1.04	1.07
12663	Metabolite - 3030	50	0.2792	0.2621	0.98	0.94	1.05
15129	Metabolite - 1001	35	0.3014	0.2761	0.82	0.72	1.13
12673	Metabolite - 3040	50	0.3051	0.2779	1.01	0.94	1.08
22133	DL-hexanoyl- carnitine	35	0.3075	0.2783	1.03	0.97	1.06
6266	Metabolite - 1286	35	0.3092	0.2783	0.99	0.97	1.03
5724	Metabolite - 1125	35	0.3226	0.2837	0.66	0.58	1.13
6439	Metabolite - 1350	35	0.3301	0.2876	0.63	0.52	1.21
5800	Metabolite - 1188	35	0.3501	0.2993	0.60	0.50	1.20
7177	Metabolite - 1656	35	0.3681	0.3097	0.78	0.72	1.09
16138	Metabolite - 4080	50	0.3899	0.3218	0.99	0.93	1.06
6296	Metabolite - 1303- SHAXQXNNR	35	0.4569	0.3549	0.65	0.57	1.12
12771	Metabolite - 3091	50	0.462	0.357	0.91	0.85	1.07
12777	Metabolite - 3097	50	0.4828	0.3639	1.05	0.99	1.06
12352	Metabolite - 2871	35	0.4906	0.3644	0.89	0.83	1.06
10782	Metabolite - 2486	35	0.4971	0.3675	1.01	0.95	1.06
569	caffeine	35	0.5074	0.3697	0.65	0.57	1.15
5609	Metabolite - 1083	35	0.5081	0.3697	0.20	0.16	1.25
10304	Metabolite - 2276	35	0.5179	0.3721	0.84	0.76	1.10

1121	heptadecanoic acid	50	0.5243	0.3723	1.00	0.97	1.03
14753	Metabolite - 3663	35	0.5399	0.3789	0.67	0.63	1.06
9561	Metabolite - 2193	35	0.5461	0.3816	0.66	0.59	1.12
13605	Metabolite - 4868	35	0.5922	0.4042	0.66	0.59	1.11
6239	Metabolite - 1264	35	0.5946	0.4042	0.67	0.60	1.12
1584	Metabolite - 1003	35	0.5985	0.4042	0.92	0.90	1.03
10309	Metabolite - 2277	35	0.6028	0.4042	0.83	0.78	1.06
15686	beta-hydroxypyruvic acid	35	0.6034	0.4042	1.00	0.98	1.02
6236	Metabolite - 1983	35	0.6161	0.4066	0.56	0.51	1.09
1110	arachidonic acid	50	0.6196	0.4069	0.98	0.96	1.02
10655	Metabolite - 2388	35	0.6335	0.4092	0.89	0.87	1.02
5803	Metabolite - 1190	35	0.6417	0.4126	0.68	0.64	1.07
18254	paraxanthine	35	0.6485	0.4152	0.59	0.54	1.08
14755	Metabolite - 3664	35	0.6626	0.4162	0.79	0.75	1.05
14759	Metabolite - 3667	35	0.6643	0.4162	0.60	0.57	1.06
9905	Metabolite - 2231	35	0.6705	0.4162	0.79	0.77	1.03
15596	Metabolite - 3962	35	0.6735	0.4162	1.12	1.10	1.02
8796	Metabolite - 2074	35	0.6875	0.4191	0.70	0.65	1.07
1366	trans-4-hydroxyproline	35	0.6967	0.42	0.60	0.57	1.05
16186	gamma-glu-gly-leu-	35	0.7018	0.42	0.78	0.75	1.04
10727	Metabolite - 2398	35	0.7114	0.4216	0.99	0.97	1.02
1572	glyceric acid	50	0.7366	0.4251	1.03	1.01	1.02
10570	Metabolite - 2366	35	0.7453	0.4251	0.96	0.93	1.03
16512	Metabolite - 4275	50	0.7453	0.4251	0.88	0.86	1.03
10945	Metabolite - 2560	35	0.7624	0.4251	0.99	0.98	1.01
13296	Metabolite - 3230	35	0.7664	0.4251	1.04	1.03	1.01
27738	threonic acid	50	0.7737	0.4251	0.92	0.89	1.03
12601	Metabolite - 2978	50	0.7757	0.4251	0.90	0.88	1.03
16509	Metabolite - 4273	50	0.7813	0.4251	0.58	0.56	1.03
12669	Metabolite - 3036	50	0.7834	0.4251	0.84	0.82	1.02
12533	Metabolite - 2915	50	0.8191	0.4339	1.00	0.99	1.01
10145	Metabolite - 2256	35	0.8194	0.4339	0.56	0.54	1.04
10774	Metabolite - 2466	35	0.8418	0.4437	0.64	0.61	1.04
10414	Metabolite - 2291	35	0.8463	0.4437	0.71	0.69	1.03
6499	Metabolite - 1376	35	0.8573	0.4452	0.76	0.74	1.03
15113	Metabolite - 3783	35	0.8583	0.4452	0.97	0.96	1.01
12757	Metabolite - 3078	50	0.87	0.4466	0.84	0.82	1.02
1358	octadecanoic acid	50	0.871	0.4466	1.02	1.01	1.01
21044	2-hydroxybutyric acid	50	0.8821	0.4477	1.02	1.01	1.01
6112	Metabolite - 1203-HXGXA	35	0.8844	0.4477	0.53	0.51	1.04
10715	Metabolite - 2395	35	0.9178	0.4555	0.90	0.88	1.01
10629	Metabolite - 2386	35	0.9198	0.4555	0.86	0.86	1.01
12710	Metabolite - 3052	35	0.9199	0.4555	0.95	0.95	1.01
5798	Metabolite - 1187	35	0.9388	0.4634	0.46	0.45	1.02
6136	Metabolite - 1211-IHWESASLLR	35	0.964	0.4721	0.66	0.66	1.01



5577	Metabolite - 1065	35	0.9931	0.4809	0.96	0.96	1.00
<b>Metabolites higher in males than females</b>							
COMP ID	COMPOUND	LIB_ID	pvalue	qvalue	FEMALE	MALE	Female/Male
10252	Metabolite - 2271	35	2.06E-18	3.15E-16	0.70	1.40	0.50
513	creatinine	35	3.80E-17	2.91E-15	0.86	1.10	0.79
10825	Metabolite - 2546	35	1.68E-15	8.57E-14	0.66	1.44	0.46
15683	4-methyl-2-oxopentanoate	50	1.14E-08	3.70E-07	0.87	1.15	0.76
5647	Metabolite - 1088	35	1.21E-08	3.70E-07	0.76	1.37	0.56
15278	Metabolite - 3843	35	2.31E-08	5.89E-07	0.85	1.09	0.77
1604	uric acid	35	4.27E-07	9.33E-06	0.95	1.04	0.92
15681	4-Guanidinobutanoic acid	35	2.42E-06	4.63E-05	0.93	1.08	0.87
6421	Metabolite - 1345	35	3.29E-06	1.00E-04	0.73	1.40	0.52
7644	Metabolite - 1831	35	3.37E-06	1.00E-04	0.81	1.08	0.75
5587	dehydroepiandrosterone-sulfate-	35	1.46E-05	2.00E-04	0.73	1.17	0.62
12626	Metabolite - 3003	50	3.09E-05	4.00E-04	0.95	1.08	0.88
13038	Metabolite - 3143	35	3.82E-05	4.00E-04	0.77	1.13	0.68
54	tryptophan	35	4.06E-05	4.00E-04	0.94	1.05	0.90
1302	methionine	35	1.00E-04	0.0011	0.94	1.07	0.87
16055	Metabolite - 4012	50	1.00E-04	0.0012	0.84	1.05	0.80
9130	Metabolite - 2139	35	1.00E-04	0.0013	0.92	1.13	0.82
10347	Metabolite - 2285	35	2.00E-04	0.0013	0.73	1.05	0.70
9491	Metabolite - 2185	35	2.00E-04	0.0015	0.86	1.12	0.77
2137	biliverdin	35	5.00E-04	0.0037	0.91	1.21	0.76
10378	Metabolite - 2287	35	8.00E-04	0.0059	0.48	0.76	0.63
12726	Metabolite - 3058	50	0.0011	0.0073	0.86	1.00	0.85
10921	Metabolite - 2558	35	0.0013	0.0083	0.22	0.49	0.46
6398	Metabolite - 1335	35	0.0016	0.0092	0.87	1.12	0.77
16070	Metabolite - 4019	50	0.0017	0.0092	0.90	1.03	0.87
16067	Metabolite - 4017	50	0.0019	0.0096	0.91	1.05	0.87
1303	malic acid	50	0.002	0.0096	0.95	1.16	0.81
12924	Metabolite - 3131	35	0.0021	0.0096	0.86	1.11	0.77
27741	cis/trans-aconitic acid	35	0.0029	0.0123	0.98	1.22	0.81
5733	Metabolite - 1127	35	0.003	0.0126	0.88	1.05	0.84
3147	xanthine	35	0.0035	0.0139	0.71	0.95	0.75
6374	Metabolite - 1327	35	0.004	0.0151	0.76	1.20	0.63
16332	Metabolite - 4164	35	0.0041	0.0154	0.92	1.03	0.90

606	uridine	35	0.0044	0.0156	0.94	1.08	0.87
10148	Metabolite - 2257	35	0.0065	0.0211	0.65	1.03	0.64
6424	Metabolite - 1346	35	0.0094	0.0293	0.96	1.04	0.92
1564	citric acid	35	0.0106	0.0323	0.98	1.18	0.83
18349	DL-indole-3-lactic acid	35	0.0111	0.0329	0.90	1.04	0.86
528	alpha-keto-glutarate	35	0.0113	0.0329	0.76	1.02	0.74
5670	Metabolite - 1105	35	0.0114	0.0329	0.63	1.01	0.63
1444	Isobar-56: DL-pipecolic acid/1-amino-cyclopentanecarboxylic acid	35	0.0121	0.0344	1.06	1.26	0.84
64	phenylalanine	35	0.0128	0.0356	0.97	1.04	0.93
3127	hypoxanthine	35	0.0134	0.0365	0.62	0.97	0.64
1113	isocitrate	35	0.0137	0.0365	0.90	1.10	0.82
5689	Metabolite - 1111	35	0.0138	0.0365	0.95	1.06	0.89
13257	Metabolite - 3218	35	0.0162	0.0414	0.94	1.02	0.92
1431	p-hydroxyphenyllactic acid	35	0.0175	0.0439	0.80	0.94	0.86
8300	Metabolite - 1988	35	0.0178	0.0439	0.93	1.12	0.83
12656	Metabolite - 3025	50	0.0188	0.0456	0.93	1.02	0.92
57	glutamic acid	50	0.0212	0.0498	0.85	1.06	0.80
1494	5-oxoproline	50	0.0271	0.0609	0.96	1.06	0.90
8091	glycocholic acid	35	0.0304	0.0674	0.71	0.98	0.72
15769	carnitine	35	0.034	0.0712	0.93	1.01	0.92
15663	Metabolite - 1000	35	0.0346	0.0716	0.73	0.93	0.78
12791	Metabolite - 3109	50	0.0358	0.073	0.84	0.99	0.84
10672	Metabolite - 2390	35	0.0368	0.0742	0.92	1.05	0.88
13104	Metabolite - 3160	35	0.0386	0.0766	0.92	1.00	0.93
1480	Metabolite - 1005	35	0.0391	0.0767	0.41	0.55	0.75
11222	Metabolite - 2688	35	0.0407	0.0788	0.68	0.85	0.81
16037	Metabolite - 4003	35	0.0455	0.0871	0.53	0.67	0.78
60	leucine	50	0.0465	0.0879	0.95	1.09	0.87
5765	Metabolite - 1142	35	0.0514	0.0959	0.85	1.02	0.83
1437	succinate	50	0.0575	0.1011	0.91	1.02	0.89
13589	Metabolite - 3327	35	0.0595	0.1035	0.76	0.96	0.80
13214	Metabolite - 3183-possible-gamma-L-glutamyl-L-phenylalanine-or-aspartame	35	0.0602	0.1035	0.82	0.93	0.89
14785	Isobar-glycochenodeoxycholic acid-glycodeoxycholic acid	35	0.0625	0.1058	0.72	0.94	0.77
16506	Metabolite - 4271	50	0.0629	0.1058	0.74	0.91	0.81
15140	L-kynurenine	35	0.0641	0.1065	0.99	1.06	0.93
1126	alanine	50	0.0692	0.1127	0.96	1.08	0.89
5809	3-indoxyl-sulfate	35	0.0717	0.1155	0.90	1.06	0.85
15753	hippuric acid	35	0.0734	0.117	0.95	1.14	0.83

10604	Metabolite - 2370	35	0.0747	0.1179	0.94	1.07	0.88
15220	Metabolite - 3813	35	0.0877	0.1334	0.87	0.97	0.90
22130	DL-phenyllactic acid	35	0.0908	0.1362	0.94	1.07	0.87
10781	Metabolite - 2469	35	0.0929	0.1369	0.85	1.01	0.85
12593	Metabolite - 2973	50	0.0973	0.1405	0.92	1.01	0.92
12646	Metabolite - 3018	50	0.1004	0.1436	0.95	1.00	0.95
1125	isoleucine	50	0.1027	0.1442	0.94	1.06	0.89
12751	Metabolite - 3073	50	0.104	0.1447	0.93	1.04	0.90
594	niacinamide	35	0.1068	0.1472	0.71	0.81	0.88
12756	Metabolite - 3077	50	0.1155	0.1578	0.95	1.01	0.94
10438	gamma-glu-leu	35	0.1182	0.1601	0.91	0.98	0.93
6402	Metabolite - 3832	35	0.1228	0.1648	1.08	1.26	0.85
16511	Metabolite - 4274	50	0.124	0.1649	0.97	1.08	0.90
8336	Metabolite - 2005	35	0.1255	0.1655	0.92	1.02	0.90
1107	allantoin	50	0.1287	0.1683	0.82	0.95	0.86
12644	Metabolite - 3016	50	0.1309	0.1697	0.96	1.01	0.95
5628	Metabolite - 1086	35	0.1387	0.1773	0.80	0.96	0.84
12625	Metabolite - 3002	50	0.1391	0.1773	0.96	1.03	0.93
20675	1,5-anhydro-D-glucitol	50	0.15	0.1881	0.83	0.93	0.90
1284	threonine	50	0.152	0.1891	0.98	1.08	0.91
1493	ornithine	50	0.1545	0.1906	0.96	1.09	0.88
15506	choline	35	0.1557	0.1906	1.01	1.07	0.95
1649	valine	50	0.1643	0.1968	0.94	1.03	0.91
15128	Metabolite - 1002	35	0.1674	0.1974	1.01	1.13	0.90
10785	Metabolite - 2506	35	0.1677	0.1974	0.85	1.03	0.82
1898	proline	35	0.1727	0.2017	1.01	1.07	0.95
14239	Metabolite - 3474	35	0.1851	0.2126	0.83	0.91	0.91
1508	pantothenic acid	35	0.1865	0.2126	0.99	1.08	0.92
2730	gamma-L-glutamyl-L-glutamine	35	0.1913	0.2152	0.71	0.80	0.89
12720	Metabolite - 3056	35	0.1941	0.2152	0.93	0.99	0.94
10461	Metabolite - 2313	35	0.2233	0.2355	1.03	1.13	0.91
6138	Metabolite - 1213	35	0.2247	0.2355	0.73	0.89	0.82
1123	inosine	35	0.2361	0.2431	0.77	0.91	0.85
6270	Metabolite - 1288	35	0.2473	0.2463	0.74	0.84	0.88
12767	Metabolite - 3087	50	0.2479	0.2463	0.95	1.02	0.93
5664	Metabolite - 1215	35	0.2567	0.2534	0.72	0.91	0.79
20699	meso-erythritol	50	0.2611	0.2561	1.02	1.06	0.96
12658	Metabolite - 3026	50	0.2638	0.2571	0.92	0.96	0.95
1299	tyrosine	35	0.2691	0.2602	0.99	1.03	0.96
1647	glutamine	50	0.2703	0.2602	0.96	1.06	0.91
11499	Metabolite - 2753	35	0.2775	0.2621	0.97	1.01	0.96
5580	Metabolite - 1067	35	0.2872	0.268	1.07	1.24	0.86
12790	Metabolite - 3108	50	0.2944	0.273	0.98	1.02	0.96
10143	Metabolite - 2255	35	0.3001	0.2761	0.65	0.81	0.81
10492	Metabolite - 2320	35	0.3148	0.2817	0.83	0.92	0.90
12784	Metabolite - 3102	50	0.3181	0.2826	1.00	1.04	0.97
12856	Metabolite - 3123	35	0.3196	0.2826	0.79	0.91	0.87
14933	Metabolite - 3739	35	0.3307	0.2876	0.65	0.74	0.87

1301	lysine	50	0.3373	0.2916	0.97	1.06	0.91
15609	Metabolite - 3969	35	0.3456	0.2971	0.98	1.01	0.97
12754	Metabolite - 3075	50	0.3639	0.3093	0.84	0.89	0.94
7933	Metabolite - 1911	35	0.3703	0.3097	0.67	0.78	0.86
7096	Metabolite - 1612	35	0.3703	0.3097	0.76	0.83	0.92
6208	Metabolite - 1254	35	0.3813	0.3171	0.84	1.01	0.83
13142	Metabolite - 3165	35	0.3932	0.3218	0.94	0.98	0.96
12666	Metabolite - 3033	50	0.3933	0.3218	0.93	0.97	0.96
6122	Metabolite - 1206	35	0.3954	0.3218	0.53	0.60	0.88
1574	histamine	35	0.4008	0.3241	1.00	1.04	0.97
12650	Metabolite - 3022	50	0.4024	0.3241	1.01	1.05	0.96
2129	oxitriptan	35	0.4062	0.3254	0.86	0.91	0.95
9748	Metabolite - 2212	35	0.4252	0.3389	0.93	0.99	0.94
16071	Metabolite - 4020	50	0.4403	0.3491	0.89	0.94	0.95
10083	Metabolite - 2248	35	0.4445	0.3506	0.61	0.69	0.87
12770	Metabolite - 3090	50	0.4499	0.353	0.65	0.78	0.84
14715	Metabolite - 3653	35	0.4563	0.3549	0.96	1.11	0.87
13200	Metabolite - 3180	35	0.4645	0.3572	0.79	0.84	0.94
12795	Metabolite - 3113	50	0.4697	0.3594	0.83	0.89	0.93
527	lactate	50	0.477	0.3618	0.96	1.01	0.95
12780	Metabolite - 3098	50	0.4776	0.3618	0.78	0.84	0.92
21025	iminodiacetic acid	50	0.4853	0.364	0.77	0.94	0.82
577	fructose	50	0.4883	0.3644	0.59	0.65	0.90
7029	Metabolite - 1597	35	0.5036	0.3697	0.98	1.00	0.98
1648	serine	50	0.5098	0.3697	0.98	1.03	0.96
1591	N-acetyl-L-valine	35	0.5166	0.3721	0.86	0.89	0.97
11777	glycine	50	0.5249	0.3723	0.98	1.03	0.95
12647	Metabolite - 3019	50	0.5256	0.3723	0.96	0.98	0.98
19934	inositol	50	0.5299	0.3737	1.01	1.03	0.98
10544	Metabolite - 2329	35	0.5876	0.4042	0.71	0.78	0.91
13744	Metabolite - 3364	35	0.5891	0.4042	0.79	0.85	0.93
12109	Metabolite - 2853	35	0.5904	0.4042	0.64	0.69	0.94
59	histidine	50	0.6	0.4042	0.98	1.02	0.97
12639	Metabolite - 3012	50	0.6049	0.4042	0.95	0.98	0.98
16135	Metabolite - 4077	50	0.6101	0.4059	0.87	0.91	0.96
11053	Metabolite - 2567	35	0.6164	0.4066	0.95	0.98	0.98
10501	Metabolite - 2321	35	0.6288	0.4092	0.96	1.02	0.95
5727	Metabolite - 1126	35	0.6316	0.4092	0.75	0.79	0.94
12099	Metabolite - 2850	35	0.6338	0.4092	0.82	0.85	0.96
15677	3-methyl-L-histidine/1-methyl-L-histidine	35	0.6626	0.4162	0.66	0.70	0.94
5652	Metabolite - 1090	35	0.6639	0.4162	0.28	0.31	0.89
10476	Metabolite - 2316	35	0.6658	0.4162	0.86	0.90	0.95
2734	gamma-L-glutamyl-L-tyrosine	35	0.6691	0.4162	0.82	0.85	0.97
6130	Metabolite - 1208	35	0.6746	0.4162	0.63	0.67	0.94
10156	Metabolite - 2259	35	0.6795	0.4175	0.99	1.03	0.96
9313	Metabolite - 2172	35	0.6821	0.4175	0.52	0.55	0.94
12670	Metabolite - 3037	50	0.6908	0.4195	1.00	1.03	0.97
15365	sn-Glycerol-3-	50	0.7012	0.42	0.99	1.01	0.97

	phosphate						
16091	Metabolite - 4031	35	0.705	0.42	0.95	0.97	0.98
12763	Metabolite - 3083	50	0.7054	0.42	0.82	0.85	0.97
11323	Metabolite - 2711	35	0.714	0.4216	1.01	1.04	0.97
12768	Metabolite - 3088	50	0.7211	0.4216	0.85	0.87	0.98
10551	Metabolite - 2347	35	0.7232	0.4216	0.91	0.96	0.95
1670	urea	50	0.7243	0.4216	1.02	1.04	0.98
15234	Metabolite - 3821	35	0.7247	0.4216	0.83	0.84	0.98
7359	Metabolite - 1713	35	0.738	0.4251	0.91	0.95	0.96
10092	Metabolite - 2250	35	0.7438	0.4251	0.52	0.55	0.94
10245	Metabolite - 2269	35	0.7485	0.4251	0.87	0.91	0.96
15991	L-alpha-glycerophosphoryl choline	35	0.751	0.4251	0.84	0.87	0.97
16229	Isobar-2: arabitol/adonitol/xy litol	35	0.7562	0.4251	0.94	0.97	0.97
14837	Metabolite - 3707	35	0.7674	0.4251	0.89	0.92	0.96
10087	Metabolite - 2249	35	0.7705	0.4251	0.99	1.01	0.98
5687	Metabolite - 1110	35	0.7767	0.4251	0.88	0.91	0.96
6362	Metabolite - 1323-possible-p-cresol-sulfate	35	0.7811	0.4251	0.77	0.81	0.96
10317	Metabolite - 2279	35	0.7826	0.4251	0.63	0.65	0.97
1519	sucrose	50	0.8046	0.4334	0.57	0.60	0.95
6231	Metabolite - 1262	35	0.8069	0.4334	0.56	0.59	0.95
12645	Metabolite - 3017	50	0.8095	0.4334	0.96	0.97	0.99
6579	Metabolite - 1398-	35	0.8101	0.4334	1.02	1.03	0.99
7002	Metabolite - 1576	35	0.8167	0.4339	0.94	0.95	0.99
6413	Metabolite - 1342-possible-phenylacetylglutamine	35	0.8466	0.4437	0.92	0.94	0.98
16496	Metabolite - 4251	50	0.8582	0.4452	0.91	0.92	0.98
27728	glycerol-2-phosphate	50	0.8664	0.4466	0.84	0.85	0.99
12864	Metabolite - 3124	35	0.8727	0.4466	0.91	0.92	0.99
5669	Metabolite - 1104	35	0.8841	0.4477	0.99	1.00	0.99
10478	Metabolite - 2317	35	0.8866	0.4477	0.62	0.64	0.98
12769	Metabolite - 3089	50	0.8967	0.4513	0.56	0.57	0.98
15063	Metabolite - 3772	35	0.9078	0.4555	1.02	1.03	0.99
10296	Metabolite - 2273	35	0.917	0.4555	0.60	0.61	0.98
14840	Metabolite - 3708	35	0.9497	0.4673	0.95	0.96	1.00
13775	Metabolite - 3370	35	0.9666	0.4721	0.93	0.93	1.00
18392	theobromine	35	0.9691	0.4721	0.79	0.80	0.99
13545	Metabolite - 3322	35	0.9718	0.4721	0.85	0.86	1.00

### *Race-Associated Changes*

[00115] There were a total of 24 compounds that are statistically significantly

different based on race in this study. Of the 24 compounds, 7 had differences of greater than 50% in relative concentration between Blacks, Whites, and Hispanics. One of these significant differences is a strictly dietary metabolite, caffeine, while the other 6 are potentially dietary or biologically synthesized. Table 5 provides a listing of metabolites that differed with race.

**Table 5: Compounds that differ by race.**

Compound	p-value	q-value	Black	White	Hispanic
3-phospho-glycerate	4.0E-02	1.2E-01	0.72	0.88	0.86
alanine	2.2E-03	1.7E-02	0.87	1.08	1.07
alpha-keto-glutarate	4.7E-02	1.3E-01	0.83	0.72	1.05
alpha-tocopherol	2.2E-02	8.9E-02	0.90	0.97	1.09
caffeine	3.7E-08	1.0E-05	0.27	1.06	0.75
citric acid	4.4E-02	1.2E-01	1.09	1.34	0.98
creatinine	1.4E-03	1.2E-02	1.04	0.95	0.95
alanyl-alanine	1.8E-05	8.0E-04	0.54	1.00	0.84
homocysteine	2.4E-02	8.9E-02	0.93	1.14	1.14
pipecolic acid	3.7E-04	4.7E-03	0.96	1.26	1.24
guanidineacetic acid	8.0E-03	4.4E-02	0.63	0.38	0.46
guanosine-5-diphosphate	3.3E-03	2.2E-02	0.57	0.70	0.79
hippuric acid	4.2E-02	1.2E-01	0.89	1.07	1.17
histamine	1.3E-02	6.3E-02	0.94	1.07	1.04
isobar: theobromine/theophylline	1.5E-07	2.1E-05	0.44	1.19	0.82
lactate	1.4E-02	6.4E-02	0.87	1.03	1.05
methionine	1.9E-02	7.8E-02	1.07	0.96	0.99
N-6-trimethyl-l-lysine	8.7E-03	4.6E-02	1.03	0.90	0.86
N-N-dimethylarginine	2.7E-02	9.3E-02	0.15	0.26	0.25
ornithine	1.1E-02	5.4E-02	0.85	1.13	1.06
palmitoleic acid	2.8E-03	1.9E-02	0.75	1.11	1.02
pantothenic acid	5.6E-02	1.4E-01	0.97	1.16	0.96
proline	7.4E-04	8.0E-03	0.83	1.09	1.09
tartaric acid	2.4E-02	8.9E-02	0.56	0.67	0.71
myristic acid	1.6E-02	7.0E-02	0.94	1.09	1.10
trans-2-3-4- trimethoxycinnamic acid	2.5E-03	1.8E-02	0.79	0.51	0.68

urca	2.2E-02	8.9E-02	0.95	0.99	1.14
Metabolite - 1911	1.3E-04	2.9E-03	0.98	0.81	0.45
Metabolite - 2005	6.2E-05	2.1E-03	0.83	0.95	1.13
Metabolite - 2150	1.2E-04	2.9E-03	0.84	0.51	0.57
Metabolite - 2250	4.2E-05	1.6E-03	0.80	0.55	0.33
Metabolite - 3088	7.9E-05	2.4E-03	1.04	0.77	0.84
Metabolite - 3230	1.4E-04	2.9E-03	0.97	1.12	1.00

- [00116] One of the more interesting metabolites is palmitoleic acid, which is significantly lower in Blacks as compared to Whites and Hispanics. Palmitoleic acid is one of the major fatty acids in blood and in addition to being synthesized in the liver it is also available from numerous food products including fish, red meat, peanuts, etc. This difference is likely due to diet, although a genetic linkage cannot be ruled out. In the plasma of Black subjects, we observed palmitoleic acid levels that were more than 25% lower relative to the levels observed in Whites and Hispanics. Interestingly, this observation is consistent with two previous studies (Bhattacharyya, A.K., et al., 1987, Am J Clin Nutr. 46(1):41-6; and Kokatnur, M.G., et al., 1979, Am J Clin Nutr. 32(11):2198-205). In Bhattacharyya's study of 714 deceased Black and White men, aged 25-44, they showed significantly elevated levels of palmitoleic acid from fat stores in tissues from Whites compared to Blacks. In Kokatnur's study of 406 men, a similar result was observed.
- [00117] The differences in caffeine levels as a function of race were even greater than for palmitoleic acid. In our study we observed that Blacks generally have significantly reduced levels of caffeine as compared to Whites and Hispanics. Although a dietary difference cannot be ruled out, previous studies have reported elevated levels of potential metabolism of caffeine in Blacks, either due to a less frequent mutation that causes lower levels of CYP2D6 (Evans, W.E., et al., 1993, J Clin Invest. 91(5):2150-4), or increased levels of either CYP1A or n-acetyltransferase (NAT) (Relling, M.V., et al., 1992, Clin Pharmacol Ther. 52(6):643-58; and Butler, M.A., et al., 1992, Pharmacogenetics, 2(3):116-27).

### **Summary**

- [00118] Metabolomic analysis was applied to the plasma from 270 human subjects. The factors considered were age, gender, and race. More than 700 compounds in the plasma had detected differences in levels. Based on our statistical criteria, more than

300 compounds showed statistically significant differences for either age, gender or race. No obviously strong interactions were observed for these three factors, with the possible exception of urea. Finally, many trends were observed within our single study that were consistent with previously published clinical studies.

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**EXAMPLE 2: Analytical characterization of unnamed compounds.**

[00119] Table 6 below includes analytical characteristics of each of the unnamed metabolites listed in Tables 2-5 above. The table includes, for each listed Metabolite, the retention time (RT), retention index (RI), mass, quant. mass, and polarity obtained using the analytical methods described above. “Mass” refers to the mass of the C<sup>12</sup> isotope of the parent ion used in quantification of the compound. The values for “Quant Mass” give an indication of the analytical method used for quantification: “Y” indicates GC-MS and “1” and “2” indicate LC-MS. “Polarity” indicates the polarity of the quantitative ion as being either positive (+) or negative (-).

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**Table 6. Analytical Characteristics of Unnamed Metabolites**

COMPOUND_ NAME	RT	RI	MASS	QUANT MASS	Polarity
Metabolite – 4276	13.92	2262.9	223.1	Y	+
Metabolite – 4275	10.68	1887.0	271.1	Y	+
Metabolite - 4274	10.37	1857.0	158.1	Y	+
Metabolite - 4273	10.34	1845.7	457.2	Y	+
Metabolite - 4272	10.28	1840.2	669.3	Y	+
Metabolite - 4271	9.69	1777.4	419.2	Y	+
Metabolite - 4251	4.09	1130.7	217	Y	+
Metabolite - 4164	1.36	1451.1	484	2	+
Metabolite - 4163	1.35	1444.1	225.3	1	+
Metabolite - 4080	14.02	2270.2	299	Y	+
Metabolite - 4077	14.00	2266.5	227	Y	+
Metabolite - 4046	10.80	1890.5	353.1	Y	+
Metabolite - 4043	10.29	1838.6	317.2	Y	+
Metabolite - 4031- possible- norlevorphenol- sobutylphendienamide- amprolium	14.26	14607	244.2	1	+
Metabolite - 4020	7.91	1561.5	220.1	Y	+
Metabolite - 4019	7.68	1534.5	174	Y	+
Metabolite - 4017	7.62	1527.3	174	Y	+



Metabolite - 4012	7.02	1458.2	357	Y	+
Metabolite - 4003	3.94	4397	205	1	+
Metabolite - 3992- possible-Cl-adduct-of- Formate-dimer	1.4	1400	127.2	1	-
Metabolite - 3977	11.03	11312	187.1	1	-
Metabolite - 3972	6.16	6304	432.6	1	-
Metabolite - 3969	3.81	4302	269.1	1	+
Metabolite - 3968	1.39	1436	327.8	1	+
Metabolite - 3962	10.22	10459	564.1	1	+
Metabolite - 3951	8.41	8705.4	367.1	1	+
Metabolite - 3899	4.41	4818.2	189.1	1	-
Metabolite - 3882	12.6	12949.2	343.1	1	-
Metabolite - 3843	9.54	9721.9	263.1	1	+
Metabolite - 3834	9.2	9410.2	372.2	1	+
Metabolite - 3830	8.42	8725	189	1	-
Metabolite - 3821	7.36	7642	282.1	1	+
Metabolite - 3816	4.16	4350	173.1	1	-
Metabolite - 3813	3.81	4312	212.1	1	+
Metabolite - 3805	2.49	2794	229.1	1	+
Metabolite - 3783	1.37	1464	271.1	1	+
Metabolite - 3772	2.22	2274	109	1	+
Metabolite - 3759	13.81	14203	309.2	1	-
Metabolite - 3752	8.61	8750.4	276.1	1	+
Metabolite - 3739	15.79	16200.3	256.4	1	+
Metabolite - 3708	1.66	1625.3	159.9	1	+
Metabolite - 3707	13.07	13339.5	241	1	+
Metabolite - 3668	9.63	9536	379.1	1	+
Metabolite - 3667	9.17	9410.6	301.1	1	+
Metabolite - 3664	8.72	8784.7	264.8	1	+
Metabolite - 3663	8.4	8649	180.1	1	+
Metabolite - 3653- Possible-stachydrine- or-picolate	4.05	4500	144.1	1	+
Metabolite - 3623	10.9	11553.6	152.1	1	+
Metabolite - 3604	8.99	9551.9	214.2	1	-
Metabolite - 3603	8.41	8971	313.6	1	+
Metabolite - 3498	7.8	8368.7	279.1	1	+
Metabolite - 3487	8.94	9554	648.9	1	+
Metabolite - 3474	15.67	16524.3	228.3	1	+
Metabolite - 3436	8.91	9157.1	157	1	-
Metabolite - 3377	8.86	8963.9	270.2	1	+
Metabolite - 3370	8.11	8529.1	226.2	1	+
Metabolite - 3364	9.06	9172.1	189	1	-
Metabolite - 4868	9.38	9530	531	1	+
Metabolite - 4491	13.34	13588	331.2	1	-

Metabolite - 3327	11.56	11784	385.3	1	-
Metabolite - 3322	11.82	12044	383.2	1	-
Metabolite - 3310	8.58	8787.3	177.1	1	+
Metabolite - 3249	3.28	3298.3	141	1	+
Metabolite - 3230	3.1	3043.2	245	1	+
Metabolite - 3218	2.2	2257	148.1	1	+
Metabolite - 3216	1.68	1743.8	405.7	1	+
Metabolite - 3215	1.67	1733.8	173.8	1	+
Metabolite - 3184	10.28	10364.4	223	1	+
Metabolite - 3183- possible- gamma-L-glutamyl-L- phenylalanine-or- aspartame	9.37	9441	295.2	1	+
Metabolite - 3180	4.14	4356	139	1	+
Metabolite - 3178	3.15	3280	210	1	+
Metabolite - 3176- possible- creatine	1.42	1511.4	132	1	+
Metabolite - 3166	8.69	8746.5	394.2	1	+
Metabolite - 3165	8.38	8472.2	265	1	+
Metabolite - 3160	12.11	12247.3	361	1	+
Metabolite - 3143	9.81	10070	160.1	1	+
Metabolite - 3139	8.82	8934.5	176.1	1	+
Metabolite - 3132	10.14	10392	260.2	1	+
Metabolite - 3131	10.49	10770	192.9	1	+
Metabolite - 3130	9.09	9328	158.2	1	+
Metabolite - 3129	8.8	9012	337.1	1	+
Metabolite - 3125	11.88	12095	187.1	1	+
Metabolite - 3124	4.17	4545.7	307.1	1	+
Metabolite - 3123	8.97	9100	334.2	1	+
Metabolite - 3113	12.73	2113.5	406.2	Y	+
Metabolite - 3110- phthalate-ester	12.68	2107.0	148.9	Y	+
Metabolite - 3109	12.56	2092.6	202.1	Y	+
Metabolite - 3108	12.24	2056.5	246	Y	+
Metabolite - 3103	12.09	2039.8	290.1	Y	+
Metabolite - 3102	11.99	2028.2	217.1	Y	+
Metabolite - 3101	11.93	2022.2	290	Y	+
Metabolite - 3100	11.85	2013.2	204	Y	+
Metabolite - 3099	11.77	2005.2	204	Y	+
Metabolite - 3098	11.75	2003.0	308	Y	+
Metabolite - 3097	11.64	1990.4	204	Y	+
Metabolite - 3094	11.55	1980.6	299	Y	+
Metabolite - 3093	11.50	1975.6	204	Y	+
Metabolite - 3091	11.41	1966.2	232.1	Y	+
Metabolite - 3090	11.31	1955.0	243.1	Y	+

Metabolite - 3089	11.28	1951.5	116.9	Y	+
Metabolite - 3088	11.23	1946.1	372.2	Y	+
Metabolite - 3087	11.19	1942.0	174.1	Y	+
Metabolite - 3086	11.16	1938.5	221	Y	+
Metabolite - 3083	10.94	1916.1	204	Y	+
Metabolite - 3080	10.75	1897.0	116.9	Y	+
Metabolite - 3078	10.65	1887.0	203.1	Y	+
Metabolite - 3077	10.44	1866.2	308.1	Y	+
Metabolite - 3075	10.36	1857.9	204	Y	+
Metabolite - 3074	10.22	1844.5	204.1	Y	+
Metabolite - 3073	10.17	1838.8	362.1	Y	+
Metabolite - 3058	9.70	1786.9	335.1	Y	+
Metabolite - 3056	9.19	9432	185.2	1	+
Metabolite - 3052	8.7	8913.4	426.2	1	+
Metabolite - 3040	9.27	1735.7	274.1	Y	+
Metabolite - 3037	9.16	1722.6	299	Y	+
Metabolite - 3036	9.07	1712.5	119.1	Y	+
Metabolite - 3034	8.92	1694.9	299	Y	+
Metabolite - 3033	8.88	1689.4	116.9	Y	+
Metabolite - 3030	8.62	1659.7	320	Y	+
Metabolite - 3029	8.48	1642.8	117.1	Y	+
Metabolite - 3027	8.21	1610.6	142	Y	+
Metabolite - 3026	8.17	1606.1	274.1	Y	+
Metabolite - 3025	8.11	1600.3	274.1	Y	+
Metabolite - 3022	7.98	1584.9	142	Y	+
Metabolite - 3020	7.81	1564.1	292	Y	+
Metabolite - 3019	7.74	1556.4	260.1	Y	+
Metabolite - 3018	7.69	1550.1	263.2	Y	+
Metabolite - 3017	7.61	1541.4	246.1	Y	+
Metabolite - 3016	7.58	1537.5	186	Y	+
Metabolite - 3012	7.17	1489.8	232	Y	+
Metabolite - 3003	6.79	1446.6	218.1	Y	+
Metabolite - 3002	6.74	1440.8	296.1	Y	+
Metabolite - 2986	5.56	1304.3	201.1	Y	+
Metabolite - 2978	5.01	1244.1	261.8	Y	+
Metabolite - 2973	4.74	1213.4	281	Y	+
Metabolite - 2915	3.77	1099.0	174	Y	+
Metabolite - 2895	10.33	10620	284.1	1	+
Metabolite - 2894	9.94	10320	226.1	1	-
Metabolite - 2871	4.52	5609.5	458.8	1	-
Metabolite - 2853	8.74	8923.5	578.4	1	+
Metabolite - 2850	3.53	3827	522	1	+
Metabolite - 2849- related-to- citric acid	3.17	3045.5	482.6	1	-
Metabolite - 2810	9.18	9363	447.9	1	+

Metabolite - 2809	8.74	8923.5	699.8	1	+
Metabolite - 2774	3.53	3796	230.9	1	+
Metabolite - 2753	3.38	3358	147	1	+
Metabolite - 2711	2.22	2300	123	1	+
Metabolite - 2703	8.86	9054.8	384.1	1	+
Metabolite - 2688	1.42	1614	182	1	-
Metabolite - 2592	10.59	10778.4	697.2	1	-
Metabolite - 2568	8.54	8790.8	342.1	1	+
Metabolite - 2567	7.79	8164.7	247.1	1	+
Metabolite - 2560	14.43	14754	235.2	1	+
Metabolite - 2558	8.14	8674	153.1	1	+
Metabolite - 2548	5.97	6016	202.9	1	-
Metabolite - 2546	1.63	1747.3	129.1	1	+
Metabolite - 2507	14.44	14843	481.4	1	-
Metabolite - 2506	14.05	14437.5	624.4	1	-
Metabolite - 2486	1.52	1667	635.7	1	-
Metabolite - 2469	15.99	16436	502.3	1	+
Metabolite - 2466	9.19	9519.9	624.8	1	+
Metabolite - 2398	13.07	13405.8	404	1	+
Metabolite - 2395	10.13	10447.6	471.9	1	+
Metabolite - 2393	15.02	15461.4	250.4	1	-
Metabolite - 2392	13.08	13460.4	379	1	-
Metabolite - 2390	6.09	6144.9	517.4	1	+
Metabolite - 2389	1.49	1641.5	314.9	1	-
Metabolite - 2388	16.16	16567	259.1	1	-
Metabolite - 2387	8.55	8838.5	182.1	1	-
Metabolite - 2386	11.94	12320.3	539.2	1	-
Metabolite - 2370	16.13	16561.2	476.4	1	-
Metabolite - 2366	8.47	8870.2	271	1	+
Metabolite - 2348	13.91	14293.5	448.3	1	+
Metabolite - 2347	13.65	14091	450.1	1	+
Metabolite - 2329	11.76	12177.6	541.2	1	-
Metabolite - 2326	11.39	11755.8	595.2	1	+
Metabolite - 2321	13.44	13832.6	314.3	1	+
Metabolite - 2320	12.27	12640	288.3	1	+
Metabolite - 2317	9.1	9410	592.9	1	+
Metabolite - 2316	8.82	9163.6	100.1	1	+
Metabolite - 2313	1.56	1685.6	352.9	1	-
Metabolite - 2292	2.4	2513.6	343.9	1	-
Metabolite - 2291	10.55	10921	213.1	1	-
Metabolite - 2287	12.95	13335.6	502.8	1	+
Metabolite - 2285	2	2146	699.6	1	-
Metabolite - 2281	13.93	14325.1	505.2	1	-
Metabolite - 2279	12.38	12781	490.1	1	+
Metabolite - 2277	10.07	10457	201.1	1	-
Metabolite - 2276	9.78	10129.3	199	1	-

Metabolite - 2273	9.28	9643.2	586.5	1	+
Metabolite - 2271	12.95	13348.8	413.2	1	-
Metabolite - 2269	10.36	10727	255.1	1	-
Metabolite - 2267	10	10375.6	663.1	1	+
Metabolite - 2259	11.25	11586	383.2	1	-
Metabolite - 2258	11.09	11425	286.3	1	+
Metabolite - 2257	10.07	10372	245.1	1	-
Metabolite - 2256	9.93	10232	460.8	1	+
Metabolite - 2255	9.08	9394	539.1	1	+
Metabolite - 2250	14.26	14668.4	286.3	1	+
Metabolite - 2249	14.21	14570.9	267.2	1	-
Metabolite - 2248	14.2	14610.4	498.4	1	-
Metabolite - 2247	14	14406	399.3	1	-
Metabolite - 2237	10.14	10453.6	453.1	1	+
Metabolite - 2231	14.31	14629	278.1	1	+
Metabolite - 2212	15.96	16271	478.2	1	+
Metabolite - 2193	8.39	8699	233.1	1	+
Metabolite - 2185	9.22	9499.4	246.2	1	+
Metabolite - 2172	1.53	1639	279.1	1	+
Metabolite - 2150	13.27	13616.5	466.1	1	+
Metabolite - 2139	8.09	8416.7	218.1	1	+
Metabolite - 2100	1.33	1532.9	499	1	+
Metabolite - 2074	2.24	2380.9	280.1	1	+
Metabolite - 2056	1.37	1499	165.1	1	-
Metabolite - 2055	1.37	1502	269.9	1	+
Metabolite - 2026	1.36	1556.2	239.2	1	+
Metabolite - 2005	8.62	9048	232.1	1	+
Metabolite - 1988	11.14	11515	190.1	1	+
Metabolite - 1979-Cl-adduct-of-C6H10O5	1.52	1690.3	199	1	-
Metabolite - 1977	3.56	3815	260.9	1	+
Metabolite - 1951	13.92	14318.6	397.3	1	-
Metabolite - 1911	11.42	11799.6	464.1	1	+
Metabolite - 5233	2.57	2624	138.1	1	+
Metabolite - 1836	2.1	2215.5	205.9	1	-
Metabolite - 1834	1.64	1794.5	104	1	-
Metabolite - 1831-possible-Cl-adduct-of-citrulline	1.46	1638.7	209.9	1	-
Metabolite - 1829	1.43	1600	135	1	-
Metabolite - 1819	1.36	1539.6	244.8	1	-
Metabolite - 1718	8.43	8647	457.9	1	+
Metabolite - 1713	2.73	2770	174	1	-
Metabolite - 1693	14.98	15155	399.2	1	+
Metabolite - 1656	1.46	1509	154.9	1	-
Metabolite - 1638	15.45	15633	627.1	1	-

Metabolite - 1613	8.83	9029.9	454.1	1	+
Metabolite - 1612	8.64	8850.3	230.9	1	+
Metabolite - 1597	3.66	3894	265.9	1	+
Metabolite - 1596	3.66	3902	185	1	-
Metabolite - 1576	2.51	2530	247.1	1	+
Metabolite - 1575	2.25	2243.5	219.1	1	+
Metabolite - 1573	1.63	1669	170.9	1	-
Metabolite - 1498	1.56	1650	143.1	1	-
Metabolite - 1465	3.45	3600	162.1	1	+
Metabolite - 1398- possible- nonanoylmorpholine-	13.21	13698.5	228.2	1	+
Metabolite - 1380	14.43	14937.2	295.1	1	+
Metabolite - 1376	13.93	14421	1487	1	+
Metabolite - 1370	11.46	11912.3	362.2	1	+
Metabolite - 1351	1.77	1936.5	177.9	1	+
Metabolite - 1350	13.75	14248.7	909.8	1	+
Metabolite - 1346	1.27	1449.5	113	1	-
Metabolite - 1345	13.27	13764.5	369.3	1	-
Metabolite - 1342- possible- phenylacetylglutamine- or- formyl-N-acetyl-5- methoxykynurenamine	9.04	9459.4	265.2	1	+
Metabolite - 3832	8.28	8696	173	1	-
Metabolite - 1335	8.74	9162.2	367.2	1	+
Metabolite - 1333	3.05	3194.6	321.9	1	+
Metabolite - 1327- possible- bilirubin	13.22	13705.9	585.4	1	+
Metabolite - 1323- possible-4-sulfobenzyl- alcohol	9.31	9719.8	187	1	-
Metabolite - 1305	14.23	14389.4	264.9	1	+
Metabolite - 1303	9.01	9178	527.8	1	+
Metabolite - 1289	8.96	9139.7	338.4	1	+
Metabolite - 1288	2.11	2120.5	302	1	-
Metabolite - 1287	14.2	14363.9	353.2	1	+
Metabolite - 1286	14.41	14579.8	229	1	+
Metabolite - 1284	9.71	9910	486.9	1	+
Metabolite - 1264	10.69	10878.5	617.8	1	-
Metabolite - 1983	10.25	10418.7	777	1	+
Metabolite - 1262	9.97	10162.7	808.9	1	+
Metabolite - 1261	10.73	10905.1	528.4	1	+
Metabolite - 1254	9.8	9987.5	733.4	1	+
Metabolite - 1252	8.12	8326	229.9	1	+

Metabolite - 1249	9.02	9201.3	630.1	1	+
Metabolite - 1245	9.28	9450.9	553.4	1	+
Metabolite - 1244	15.28	15436.8	343.4	1	-
Metabolite - 1243	8.97	9147.7	751.5	1	+
Metabolite - 1242	8.43	8627.6	355.9	1	+
Metabolite - 1221- possible-phthalate	12.5	12665.8	221.1	1	-
Metabolite - 1220	15.24	15402.5	319.2	1	+
Metabolite - 1213	8.92	9101.8	244.8	1	+
Metabolite - 1212	9.1	9284	584.9	1	+
Metabolite - 1211: IHWESASLLR	9.9	10083.7	606.5	1	+
Metabolite - 1209	8.89	9077.8	426.9	1	+
Metabolite - 1208	15.33	15494	319.4	1	-
Metabolite - 1206- possible- methyltestosterone- and-others	15.32	15475.1	303.2	1	+
Metabolite - 1203- possible- acetylbrownine- tricornine- germine-or-veracevine	9.11	9288	510.2	1	+
Metabolite - 1202	8.96	9142.5	501.7	1	+
Metabolite - 1190	8.83	9130	928.5	1	+
Metabolite - 1188	8.83	9017	619.9	1	+
Metabolite - 1187	8.8	9017	559.9	1	+
Metabolite - 1186	8.83	9000	529.6	1	+
Metabolite - 1183	8.56	8765	365.8	1	+
Metabolite - 1142- possible-5- hydroxypentanoate-or- beta-hydroxyisovaleric acid	8.54	8739	117	1	-
Metabolite - 1129	5.16	5419	260.1	1	+
Metabolite - 1127	12.18	12369	363.1	1	+
Metabolite - 1126	3.04	3188	175.1	1	+
Metabolite - 1125	3.94	4202	221.1	1	+
Metabolite - 1122	4.45	4701	233.1	1	+
Metabolite - 1116	4.2	4480	103.4	1	-
Metabolite - 1114	2.19	2198	104.1	1	+
Metabolite - 1111- possible- methylnitronitrosoguan idine-or-ethyl- thiocarbamoylacetate	2.69	2782	148.1	1	+
Metabolite - 1110	11.66	11841	269.1	1	-

Metabolite - 1105	11.35	11560	229	1	-
Metabolite - 1104	2.43	2410	201	1	-
Metabolite - 1215	9.39	9567	550	1	+
Metabolite - 1090	11.26	11535	719	1	+
Metabolite - 1089	2.01	2017	346.9	1	+
Metabolite - 1088	13.12	13298	369.1	1	-
Metabolite - 1086	4.56	4811	294.1	1	+
Metabolite - 1085- possible-solobinine-or- 4-aminoestra-1-3- 5-10-triene-3-17beta- diol	15.82	15964	288.1	1	+
Metabolite - 1083	10.71	10905	723.5	1	+
Metabolite - 1071- possible-type- of-phthalate	15.23	15445	279.3	1	+
Metabolite - 1069- possible- dehydroepiandrosteron e-sulfate-	12.55	12930	367.2	1	-
Metabolite - 1067	10.03	10216	481.6	1	+
Metabolite - 1065	9.66	9870	769	1	+
Metabolite - 1062- possible-4- hydroxyphenylglyoxyla te-or-phthalate-or- Formylsalicylic acid	9.29	9491	165.1	1	-
Metabolite - 1061- Possible-type- of-phthalate	14.67	14885	279	1	+

### EXAMPLE 3: Application of Metabolomic Analysis to Predict Age and Generate a Biochemical Age Index (BAI)

[00120] This example describes how metabolic analysis is used to predict the  
5 metabolic age of an individual and generate a Biochemical Age Index (BAI) to be  
used to determine the MetaboScore for an individual.

[00121] Regression analysis was performed to evaluate the ability to predict age  
based upon the metabolomic results. The inclusion criterion for addition to the model  
was that the p-value was less than 0.05, which resulted in fewer total variables in the  
10 final model than using either the adjusted R-squared or the Akaike Information  
Criterion (AIC) for variable selection. Due to the number of gender differences,  
regressions were performed separately for each gender. Named compounds and



unnamed compounds, excluding xenobiotics, were considered for the models. The regression results are shown in Table 7 and Figure 3. Table 7 lists the biomarker compounds that comprise the model.

**Table 7. Stepwise regression analysis for predicting age.**

<b>FEMALE</b>	<b>Estimate</b>	<b>StdErr</b>	<b>t-value</b>	<b>p-value</b>
Intercept	13.63	5.46	2.50	0.01416
glutamic acid	2.44	0.73	3.34	0.001178
histidine	-3.44	1.16	-2.96	0.003788
Metabolite - 3078	4.40	1.56	2.83	0.005643
inositol	4.79	1.60	3.00	3.36E-03
Metabolite - 3087	4.97	1.16	4.28	4.14E-05
Metabolite - 3094	4.86	1.28	3.80	0.000244
lysine	-2.50	0.82	-3.07	0.002757
Metabolite - 4077	-2.51	0.98	-2.57	0.011502
oxitryptan	-4.67	0.96	-4.88	3.86E-06
Dehydroepiandrosterone (DHEA-S)	-2.55	0.69	-3.70	0.000342
Metabolite - 1085	3.16	1.14	2.76	0.006751
3-indoxyl-sulfate	2.54	0.64	4.00	0.000118
Metabolite - 1264	-0.36	0.08	-4.38	2.85E-05
Metabolite - 1288	-1.52	0.62	-2.43	0.01683
Metabolite - 1656	-3.72	0.83	-4.46	2.07E-05
Metabolite - 1831	5.04	0.92	5.45	3.38E-07
Metabolite - 2074	2.31	0.35	6.52	2.59E-09
Metabolite - 2231	-7.25	1.12	-6.45	3.63E-09
Metabolite - 2257	-1.17	0.22	-5.28	7.10E-07
Metabolite - 2259	-1.09	0.37	-2.92	0.004242
gamma-glu-leu	6.86	1.72	3.98	1.29E-04
Metabolite - 2393	-5.35	1.48	-3.61	0.000475
Metabolite - 2486	0.73	0.36	1.99	0.048984
Metabolite - 2560	8.40	1.84	4.57	1.37E-05
Metabolite - 2753	-4.39	1.78	-2.46	0.015458
Metabolite - 2871	1.92	0.69	2.81	0.005975
Metabolite - 3056	-4.38	1.18	-3.73	0.000317
Metabolite - 3183	-3.28	0.65	-5.07	1.76E-06
Metabolite - 3218	12.36	1.69	7.32	5.50E-11
Metabolite - 3707	1.24	0.22	5.53	2.38E-07
Metabolite - 3951	7.42	1.39	5.36	5.06E-07
Metabolite - 3962	3.70	0.65	5.68	1.24E-07
Metabolite - 3969	-4.19	2.28	-1.84	0.068653
R <sup>2</sup> =91%. AdjR <sup>2</sup> =89%	AdjR <sup>2</sup> =89			
<b>MALE</b>	<b>Estimate</b>	<b>StdErr</b>	<b>t-value</b>	<b>p-value</b>
Intercept	41.06	5.04	8.15	7.02E-13
Metabolite - 3058	-4.24	1.79	-2.37	0.019657
1,5-anhydro-D-glucitol	-7.99	1.38	-5.80	6.63E-08
inositol	11.62	1.79	6.50	2.55E-09
Metabolite - 4272	-10.96	1.71	-6.41	3.83E-09
succinate	-4.57	1.20	-3.82	0.000227
hypoxanthine	-0.27	0.10	-2.59	0.010974

gamma-L-glutamyl-L-tyrosine	-5.91	1.50	-3.93	0.000148
Metabolite - 1088	-1.28	0.46	-2.81	0.00594
Metabolite - 1206	1.21	0.58	2.08	0.039735
Metabolite - 1718	1.23	0.47	2.61	0.0104
Metabolite - 2237	-0.69	0.25	-2.77	0.006514
Metabolite - 2269	0.56	0.24	2.32	0.022002
Metabolite - 2271	-5.05	0.72	-6.98	2.46E-10
Metabolite - 2273	2.29	0.79	2.88	0.004846
Metabolite - 2285	2.97	1.04	2.87	0.00498
Metabolite - 2486	-1.00	0.49	-2.05	0.042362
Metabolite - 2546	1.93	0.42	4.61	1.1E-05
Metabolite - 3052	11.80	2.46	4.80	5.13E-06
Metabolite - 3123	-0.46	0.14	-3.29	0.001353
creatine	5.51	1.01	5.43	3.57E-07
Metabolite - 4164	7.56	2.10	3.61	0.000472
theobromine	-0.77	0.33	-2.32	0.022258
R2=85%, adj R2=82%				

- [00122] Studentized residuals are used to identify the data points which do not appear to be consistent with the rest of the data (*i.e.* outliers). In this study, outliers represent the individuals that cannot be fitted well to the model; that is, the predicted
- 5 metabolic age is significantly different from the actual chronological age. In this case, the studentized residuals were scaled to have variance equal to one (scaled by the “hat” matrix (Hoaglin, D. C. and R. E. Welsch (1978). "The Hat Matrix in Regression and ANOVA." The American Statistician **32**(1): 17-22.)) and outliers were determined based on studentized residuals greater than absolute value of 2.0.
- 10 Ten individuals, six females and four males, were identified as outliers. Of the six females, four had a predicted age younger than the actual chronological age, while two were predicted as older than the actual chronological age. For the males, two were chronologically older and two were chronologically younger than the predicted age. The data is presented graphically in Figure 3 with the outlying points circled.
- 15 The individuals that are chronologically older than the predicted age are the circled points below the solid diagonal line and the individuals that are chronologically younger than the predicted age are the circled points above the solid diagonal line.
- [00123] To generate a Biochemical Age Index, the first step is to identify named compounds associated with age. Then remove the scores of the obvious outlier
- 20 compounds. Confirm that the compounds are biologically reasonable (not an exogenous compound/xenobiotic, *e.g.* caffeine). Check for obvious gender and racial differences. Find the variables (*i.e.* compounds) that are correlated to the selected

variables (i.e. compounds). That is, identify sets and/or groups of correlated compounds by searching for other compounds/metabolites that are correlated with the compounds/metabolites that are correlated with age. Build a simple index using these compound groups or sets.

- 5 **[00124]** Any of a number of statistical methods can be used to scan the compounds to find those that change with age. For example, polynomial fit analysis of compound concentration versus age, spline fit analysis of compound concentration versus age, simple 2-way scatter plot of compound concentration versus age, etc. In this example, linear regression was used to find compound levels that change with age. The
- 10 compound levels in each individual(s) of each age were analyzed with programs for regression analysis using the Partionater, 4.1.0 (available on the worldwide web at GoldenHelix.com) and SAS JMP 6.0.2 (available on the worldwide web at sas.com). Table 8 lists a few of the named compounds from this regression analysis.

**Table 8 Biomarker compounds that change with age**

15

Compound	Library	P	aP	FDR(aP)	bP	Var. #
normetanephrene-	50	1.15E-15	1.15E-15	1.42E-13	1.42E-13	43
valine	50	4.86E-14	4.86E-14	2.99E-12	5.98E-12	128
ornithine	50	5.29E-14	5.29E-14	2.17E-12	6.51E-12	45
alanine	50	2.11E-13	2.11E-13	6.48E-12	2.59E-11	13
glutamine	50	6.42E-13	6.42E-13	1.58E-11	7.90E-11	28
tyramine	50	5.33E-12	5.33E-12	1.09E-10	6.55E-10	126
leucine	50	8.57E-12	8.57E-12	1.51E-10	1.05E-09	36
L-arabitol	35	1.55E-11	1.55E-11	2.38E-10	1.91E-09	171
glutamic acid	50	3.31E-11	3.31E-11	4.52E-10	4.07E-09	27
glycine	50	7.80E-11	7.80E-11	9.59E-10	9.59E-09	31
lactate	50	1.92E-10	1.92E-10	2.14E-09	2.36E-08	35
serine	50	2.25E-10	2.25E-10	2.31E-09	2.77E-08	120
pyrophosphate	50	2.34E-10	2.34E-10	2.21E-09	2.88E-08	119
isoleucine	50	4.48E-10	4.48E-10	3.94E-09	5.51E-08	34
saccharopine	35	6.14E-10	6.14E-10	5.03E-09	7.55E-08	443
proline	50	8.92E-10	8.92E-10	6.86E-09	1.10E-07	118
dulcitol	50	2.49E-09	2.49E-09	1.80E-08	3.06E-07	21
benzoic acid	50	1.07E-08	1.07E-08	7.33E-08	1.32E-06	17
aspartate	50	4.07E-08	4.07E-08	2.63E-07	5.00E-06	48

threonine	50	7.51E-08	7.51E-08	4.62E-07	9.23E-06	124
aspartate	35	2.44E-07	2.44E-07	1.43E-06	3.00E-05	143
phosphate	50	8.08E-07	8.08E-07	4.52E-06	9.93E-05	47
uridine	35	1.08E-06	1.08E-06	5.80E-06	1.33E-04	451
2-amino-butyrate	50	1.56E-06	1.56E-06	7.99E-06	1.92E-04	6
xanthine	35	3.04E-06	3.04E-06	1.50E-05	3.74E-04	453
histidine	50	3.70E-06	3.70E-06	1.75E-05	4.55E-04	33
urea	50	5.45E-06	5.45E-06	2.48E-05	6.70E-04	127
alpha-L-sorbopyranose	50	5.47E-06	5.47E-06	2.40E-05	6.73E-04	14
malic acid	50	1.04E-05	1.04E-05	4.41E-05	1.28E-03	37

- [00125] Many of these compounds that change in levels and that are correlated with age are also correlated with one another (Figure 1). The first five on the list in Table 8 above were selected to illustrate this point. The correlations for those compounds are shown in Table 9.

**Table 9. Compound correlations**

Correlations	alanine	glutamine	normetanephrine-	ornithine	valine
alanine	1.0000	0.5112	0.6352	0.7723	0.8419
glutamine	0.5112	1.0000	0.4390	0.6075	0.5444
normetanephrine	0.6352	0.4390	1.0000	0.6198	0.6342
ornithine	0.7723	0.6075	0.6198	1.0000	0.8363
valine	0.8419	0.5444	0.6342	0.8363	1.000

- [00126] Scatter plots that illustrate the correlation between the pairs of compounds in Table 9 are shown in Figures 4 and 5. As shown in Table 10, some of these compounds are also correlated significantly with age.

**Table 10. Some compounds with a significant correlation with age.**

Variable (Compound)	R <sup>2</sup>
Alanine	0.183
Glutamine	0.155
Normetanephrine	0.234
Ornithine	0.196
Valine	0.192
Index	0.255

- [00127] A quadratic curve was fit to each of the variables and the R<sup>2</sup> (proportion of

variance explained) was computed (Table 10). For the individual compounds the  $R^2$  ranged from 0.155 to 0.234. As expected, the  $R^2$  for the Index, which is composed of the compounds listed in Table 8, was larger,  $R^2=0.255$ . Thus, the Index reduces variability and provides a larger proportion of variance that can be explained.

5    **[00128]**    As illustrated in the Scatterplots of each of the compounds compared to age shown in Figures 5A-E, the increase in the level of each of the compounds in this example becomes more pronounced at around age 45. Also the variability appears to increase with age. The index derived from alanine, glutamine, normetanephrine, ornithine, and valine has been fit with a Quadratic curve (solid line) and is shown in  
10    Figure 5F

**[00129]**    A number of sources of variability are responsible for variation in individual metabolites. About 19% of the variability in metabolite levels is due to aging, e.g. loss of biochemical control. The remaining variability may result from various sources. Some variability comes from the biochemical analytical process,  
15    although in our platform this is typically <5-8%. Some variability may be due to individual differences in genetics and environment. Some variability comes from life style differences among the people. For example, eating or exercise habits might influence the levels of metabolites or chemicals. People with lower levels of certain metabolites may be considered of younger biochemical age.

20    **[00130]**    As shown in Table 10, one way to reduce the analytical variation in assessing biochemical age is to average a number of compounds that are increasing (or decreasing, although only increasing compounds were used in this example, further it is possible to compute an index using both increasing and decreasing compounds) with age. The average can be considered a biochemical age index (BAI).  
25    Figure 6 shows a density contour chart with BAI versus chronological age. The density of the data points in each region are illustrated by the contour lines. The BAI results from the quadratic curve fit to the variables and is indicated by the thick solid line.

**[00131]**    Compounds can be selected for addition to the index in the following way.  
30    We start with named compounds that increase or decrease with age in a statistically significant manner. Compounds that are obviously associated with food, e.g. caffeine from tea or coffee, tartaric acid from wine, are removed from the list. (Although these xenobiotics can be used to determine a xenobiotic index in a similar manner).

Unnamed compounds that are correlated with named compounds remaining on the list can be added to the list to help reduce the variability of the index.

[00132] Non-parametric density contours are helpful in representing the information in the data (see Figure 6). The Index appears to be low,  $<0.75$ , for individuals, (ages 20-45), and then appears to increase markedly for individuals over 45. Even so, there are a few individuals over 45 that have a low Index. Also, it is clear that there are a large number of individuals under 45 with large values of the Index. These individuals might be considered prematurely old.

[00133] Many named and unnamed compounds have been identified that change with age, and by combining these compounds into an index, we get a more stable relationship of compounds associated with age. This information makes it possible to construct a Biochemical Age Index, BAI.

**EXAMPLE 4: Application of Index to Determine the Metabolic Age Score ("MetaboScore")**

[00134] To determine the metabolic age of an individual, a biological sample (e.g. plasma, urine, saliva, etc.) is obtained from an individual (e.g. human, non-human primate, mammal, dog, horse, cat, etc.) and subjected to metabolomic analysis. The resulting metabolic profile is then used to compute the biochemical age index score for the individual (BAI-Score). The metabolic age (MetaboAge) of the individual then can be determined by locating the BAI-Score of the individual on the chart shown in Figure 7. As shown in Figure 7, each black dot on the chart indicates an individual BAI-score. A line is drawn from the individual to the BAI (diagonal line) and then a line is drawn to the X-axis to determine the metabolic age (or MetaboAge). A line is also drawn from the individual down to the X-axis to determine the chronological age. The difference between the chronological age and the metabolic age (or MetaboAge) is the "MetaboScore". In the illustration in Figure 7 the individual(s) in the black circle have a metabolic age (MetaboAge) of 58 and chronological age of 22 resulting in a MetaboScore of +36, indicating that the individual is metabolically older than their chronological age.

[00135] In a similar manner a negative MetaboScore can be determined. As shown in Figure 8, a line is drawn from the individual to the BAI and then a line is drawn to the X-axis to determine the metabolic age (MetaboAge). A line is also drawn from the individual down to the X-axis to determine the chronological age. The difference

between the chronological age and the metabolic age is the “MetaboScore”. In this illustration in Figure 8, the individual in the dark black circle has a metabolic age (MetaboAge) of 26 and chronological age of 54 resulting in a MetaboScore of -28, indicating that the individual is metabolically younger than the chronological age.

#### 5 **EXAMPLE 5: Refining the Biochemical Age Index**

[00136] This example describes experiments that analyze additional compounds in a variety of individuals under various conditions to enhance and refine the BAI as it relates to different conditions and age. A more stable relationship between age and metabolic profile will be obtained by combining additional named and unnamed  
10 compounds that are shown to change with age into an index such as the BAI. The present invention describes how this information was used to produce an indication of metabolic age and constructed a Biochemical Age Index. This BAI was used in combination with the metabolomic profile of the individual to get an indication of the metabolic age (“MetaboAge”) of the individual and from this the individual’s  
15 MetaboScore was determined.

[00137] With additional data, it is possible to construct a more robust Biochemical Age Index (BAI). To this end, experiments are conducted to identify and remove variability from the measured chemicals. Examples of these experiments are described below and listed in Table 10.

20 [00138] Biological samples will be obtained from groups of subjects as described in Table 11 and subjected to metabolomic analysis. For each age classification a factorial (intervention) study is conducted. Each group consists of, for example, 100 individuals with 25 subjects in each of four age groups (e.g. young adult (18-30), adult (30-45), middle age (46-65), senior (>65)). A BAI is determined based upon the  
25 correlated compounds as described in Example 4.

**Table 11. Example comparisons for further BAI studies**

<b>Group 1</b>	<b>Group 2</b>
Fasting individuals (various times of fast)	Non-fasting individuals
Vegetarian diet	Non-vegetarian diet
Vitamin supplements	Non-supplemented
Caffeine	No caffeine
Coffee	Tea
Alcohol	No alcohol
Exercise regime	No Exercise

[00139] These treatments can be carried out independently and/or in combination (e.g. Alcohol and Vegetarian Diet vs. No Alcohol and Vegetarian Diet; Alcohol and Caffeine vs. No Alcohol and No Caffeine). Alternatively, metadata can be collected from individuals (e.g. enrollment questionnaire) in the simple 2-way experiments designed above to further stratify the cohort and thereby to provide additional discriminating power.

**EXAMPLE 6: Determination of healthful diets and xenobiotic content (e.g. the Xenobiotic Score)**

[00140] This example describes the measurement of xenobiotic compounds from individuals and determines a correlation between xenobiotic compounds and age. Measurement of such xenobiotic compounds is used to calculate a xenobiotic score to determine dietary differences between individuals and according to age.

[00141] Tartaric acid is a white crystalline organic acid. It occurs naturally in many plants, particularly grapes and tamarinds, and is one of the main acids found in wine. It is added to other foods to give a sour taste, and is used as an antioxidant. Salts of tartaric acid are known as tartrates. It is a dihydroxy derivative of dicarboxylic acid. Figure 9 shows a density diagram of the concentration of tartaric acid versus chronological age. People with high levels of tartaric acid, presumably from wine, are older with an average age of 50. Note the outliers with very elevated levels of tartaric acid are presumably serious wine drinkers.

[00142] Benzoic acid (BA) levels were also measured. Benzoic acid is also a common food component. It is used as a preservative in package foods such as pickles and lunch meats, and it occurs naturally in cranberries. "Bacterial deamination of the amino acid phenylalanine produces benzoate, which is conjugated with glycine in the liver to form hippurate. This should be taken into account when interpreting elevated hippurate levels in urine. Whether the source is dietary intake or jejunal bacterial metabolism, benzoate is usually converted to hippurate by conjugation with glycine in the liver. Glycine and pantothenic acid are the limiting factors in this process. Therefore, elevated benzoate is a marker of inadequate levels of these nutrients." (See Bralley and Lord, 2001, Laboratory Evaluations in Molecular Medicine: Nutrients, Toxicants, and Cell Regulators, Chapter 6 Organic Acids, page 206, Institute for Advances in Molecular Medicine (IAMM) Norcross,



Georgia, USA)

[00143] Figure 10 shows a scatterplot of levels of BA versus age. Note the two outlier values (see arrows) for BA; both happen to be females. Since BA is a component of diet soda, and since it is quite common for females to consume diet soft drinks, these results could indicate that the individuals are diet soda drinkers.

[00144] Figure 11 shows a density contour diagram of Benzoic acid compared to age. The levels of BA are somewhat reduced in older individuals, suggesting that older people might metabolize the BA more rapidly than younger people. Alternatively, the difference may be due to differences in the diets. These hypotheses can be verified experimentally; BA could be given to older and younger subjects in order to see if the older subjects get rid of it more efficiently than young people.

#### **EXAMPLE 7: Individual metabolite scores and ratios**

[00145] This example describes the measurement of certain metabolites and calculation of ratios between the metabolite ornithine versus age. Figure 12 shows a density contour diagram of ornithine concentration versus age. The figure shows a shift in ornithine concentration in blood which increases after age 50 and is rather dramatic. The results suggest that there is an age effect that could be modified by diet. Alternatively, this could be an indication of lifestyle, disease, or disease susceptibility. This idea is supported by the observed wide distribution of ornithine from age 35 to 41. The very skewed distribution of ornithine at just about any age could indicate unhealthy effects due to unidentified causes. It has been shown that induced hepatic failure in pigs results in elevation of both ornithine and citrulline.

[00146] While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made without departing from the spirit and scope of the invention.

## CLAIMS

What is claimed is:

1. A method for determining the metabolic age of a subject comprising the steps of:
  - a) analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for metabolic age in the sample, wherein the one or more biomarkers are selected from Tables 2, 4, 5, 7, and/or 8 and combinations thereof; and
  - b) comparing the level(s) of the one or more biomarkers in the sample to metabolic age reference levels of the one or more biomarkers to determine the subject's metabolic age.
2. The method of claim 1, wherein the one or more biomarkers are selected from the biomarkers in Tables 2, 7, and/or 8 and combinations thereof having p-values of less than 0.05 and/or those biomarkers in Tables 2, 4, 5, 7, and/or 8, and combinations thereof having q-values of less than 0.10.
3. The method of claim 1, wherein the level(s) of the one or more biomarkers in the sample are compared to a biochemical age index to determine the subject's metabolic age.
4. The method of claim 1, wherein levels of the one or more biomarkers in the sample are similar to metabolic age reference levels corresponding to an older chronological age are indicative of a negative metabolic age.
5. The method of claim 1, wherein levels of the one or more biomarkers in the sample are similar to metabolic age reference levels corresponding to a younger chronological age are indicative of a positive metabolic age.

6. The method of claim 1, wherein the biological sample is blood plasma and the one or more biomarkers are selected from Tables 2, 4, 5, 7, and/or 8, and combinations thereof.
7. The method of claim 1, wherein the reference level(s) are tailored to a population selected from the group consisting of gender, race and combinations thereof.
8. The method of claim 1, wherein said subject is a male subject and the biomarkers are selected from one or more biomarkers in Tables 4 and 7.
9. The method of claim 1, wherein said subject is a female subject and the biomarkers are selected from one or more biomarkers in Tables 4 and 7.
10. A method for producing a Biochemical Age Index (BAI) comprising the steps of:
  - a) analyzing biological samples from a plurality of subjects to determine the level(s) of one or more first biomarkers that change with age;
  - b) combining the level(s) one or more second biomarkers that correlate with each of the one or more first biomarkers that change with age to generate one or more groups of biomarkers that change with age; and
  - c) generating a biochemical age index using the levels of each of the one or more groups of biomarkers.
11. The method of claim 10, wherein the one or more first biomarkers or the one or more second biomarkers are selected from Tables 2, 4, 5, 7, and/or 8, and combinations thereof.
12. The method of claim 10, wherein the biochemical age index is generated by plotting the levels of the one or more groups of biomarkers versus age of the plurality of subjects.

13. The method of claim 10, wherein the biochemical age index is tailored to a population selected from the group consisting of gender populations and race populations.
14. The method of claim 10, wherein the biological samples are obtained from male or female subjects.
15. A method for modifying the metabolic age of a subject comprising the steps of:
  - a) analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for metabolic age in the sample, wherein the one or more biomarkers are selected from Tables 2, 4, 5, 7, and/or 8 and combinations thereof;
  - b) comparing the level(s) of the one or more biomarkers in the sample to metabolic age reference levels of the one or more biomarkers to determine the subject's metabolic age; and
  - c) providing recommendations to modify the metabolic age of a subject through changes in diet, nutrition, lifestyle, and/or administration of metabolites.
16. The method of claim 15, wherein the one or more biomarkers are selected from the biomarkers in Tables 2, 4, 5, 7, and/or 8 and combinations thereof having p-values of less than 0.05 and/or those biomarkers in Tables 2, 4, 5, 7, and/or 8, and combinations thereof having q-values of less than 0.10.
17. The method of claim 15, wherein the metabolic age reference level(s) are tailored to a population selected from the group consisting of gender, race and combinations thereof.
18. The method of claim 17, wherein the markers are selected from Tables 4 and/or 5.
19. A method of assessing the efficacy of a composition for modulating metabolic age, comprising:

a) analyzing a first biological sample from a subject having a first metabolic age and currently or previously being treated with a compound or composition to determine the level(s) of one or more biomarkers selected from Tables 2, 4, 5, 7, and/or 8, and

b) comparing the level(s) of the one or more biomarkers in the sample to biomarkers levels selected from the group consisting of (a) level(s) of the one or more biomarkers in a second biological sample from the subject obtained from the subject before being treated with the compound or composition, (b) metabolic age-positive reference levels of the one or more biomarkers, (c) metabolic age-negative reference levels of the one or more biomarkers, and (d) reference levels of the one or more biomarkers for a target metabolic age.

20. The method of claim 19, wherein the target metabolic age reference levels correspond to the chronological age of the subject.

21. The method of claim 19, wherein the reference level(s) are tailored to a population selected from the group consisting of gender, race and combinations thereof.

22. The method of claim 20, wherein the method further comprises:

c) identifying compositions capable of modulating metabolic age.

23. A method for screening a test compound for activity in modulating the level of one or more biomarkers of metabolic age, comprising:

a) contacting one or more cells with a test compound;

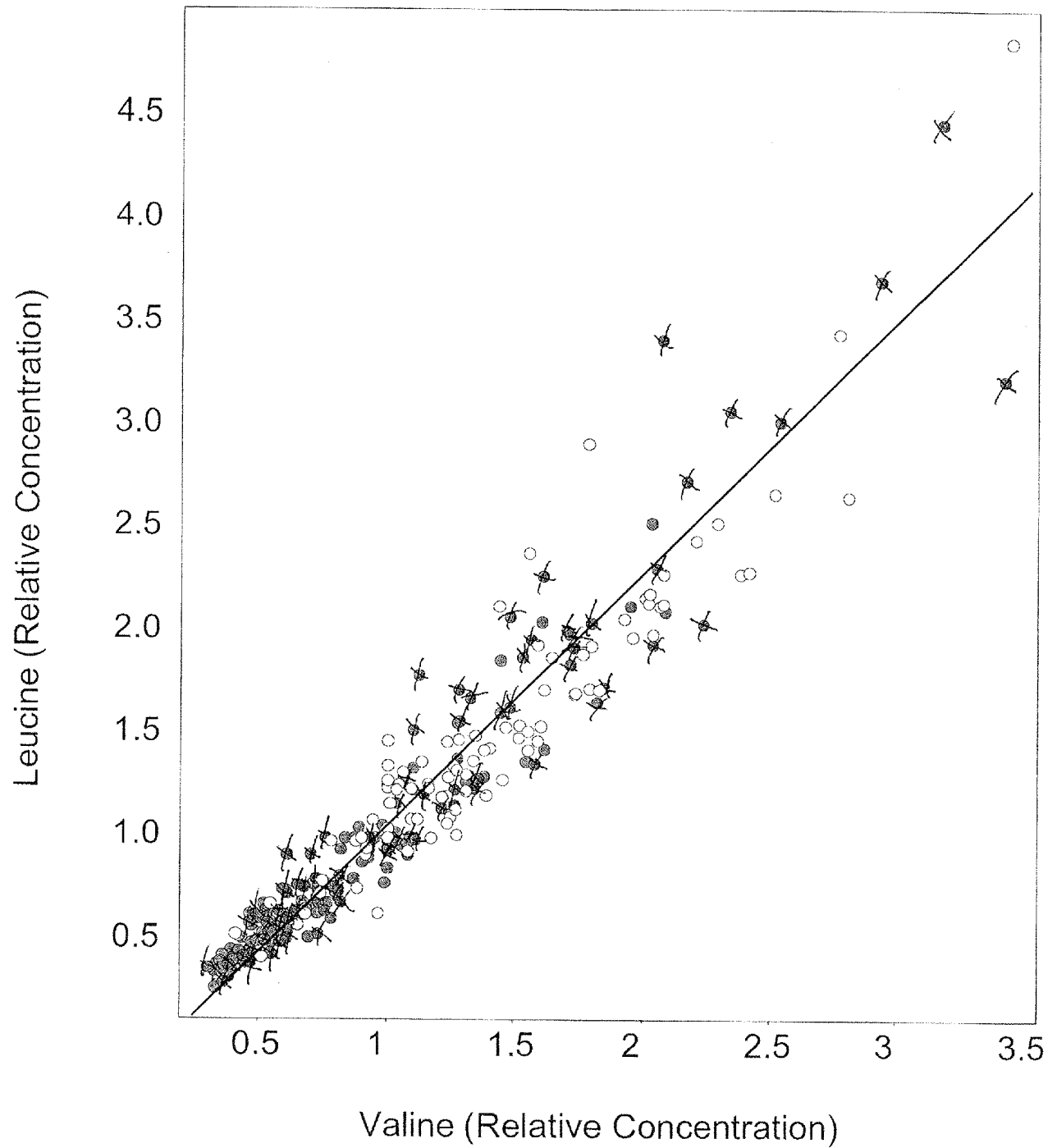
b) analyzing at least a portion of the one or more cells or a biological sample associated with the cells to determine the level(s) of one or more biomarkers of metabolic age selected from Tables 2, 7, and/or 8; and

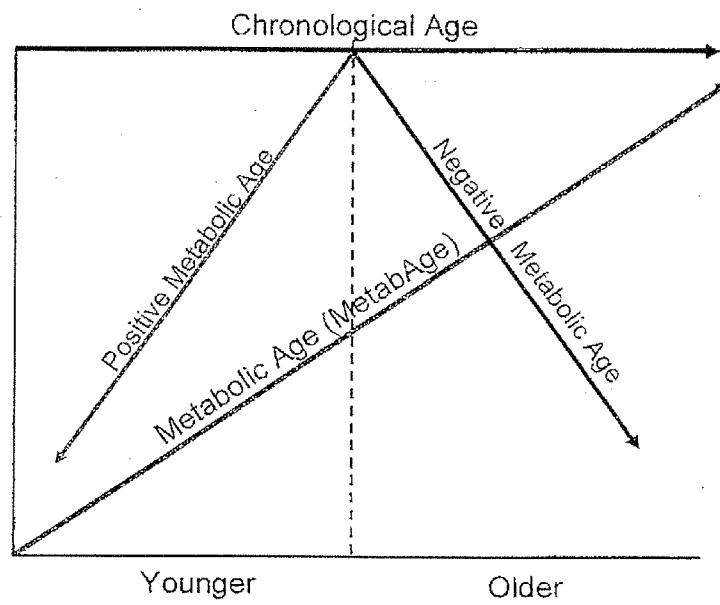
c) comparing the level(s) of the one or more biomarkers with predetermined levels for the biomarkers to determine whether the composition modulated the level(s) of the one or more biomarkers.

24. The method of claim 23, wherein the predetermined standard levels for the biomarkers are selected from the group of level(s) of one or more biomarkers in the one or more cells in the absence of the composition and one or more biomarkers in one or more control cells not contacted with the test compound.

25. The method of claim 23, wherein the method is conducted in vitro or in vivo.

### Figure 1

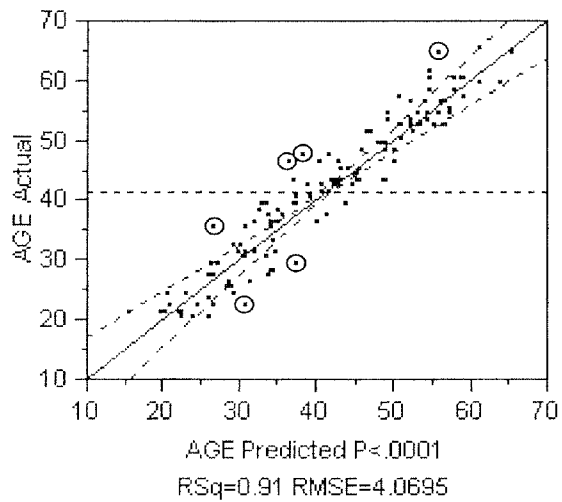


**Figure 2**



**Figure 3 Age prediction by step-wise regression analysis.**

3A. Females



3B. Males

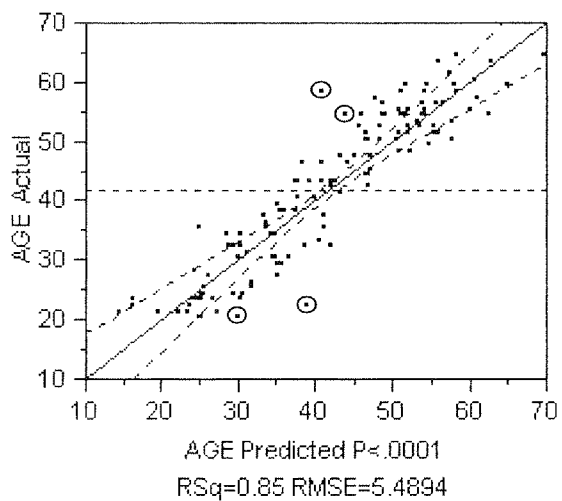
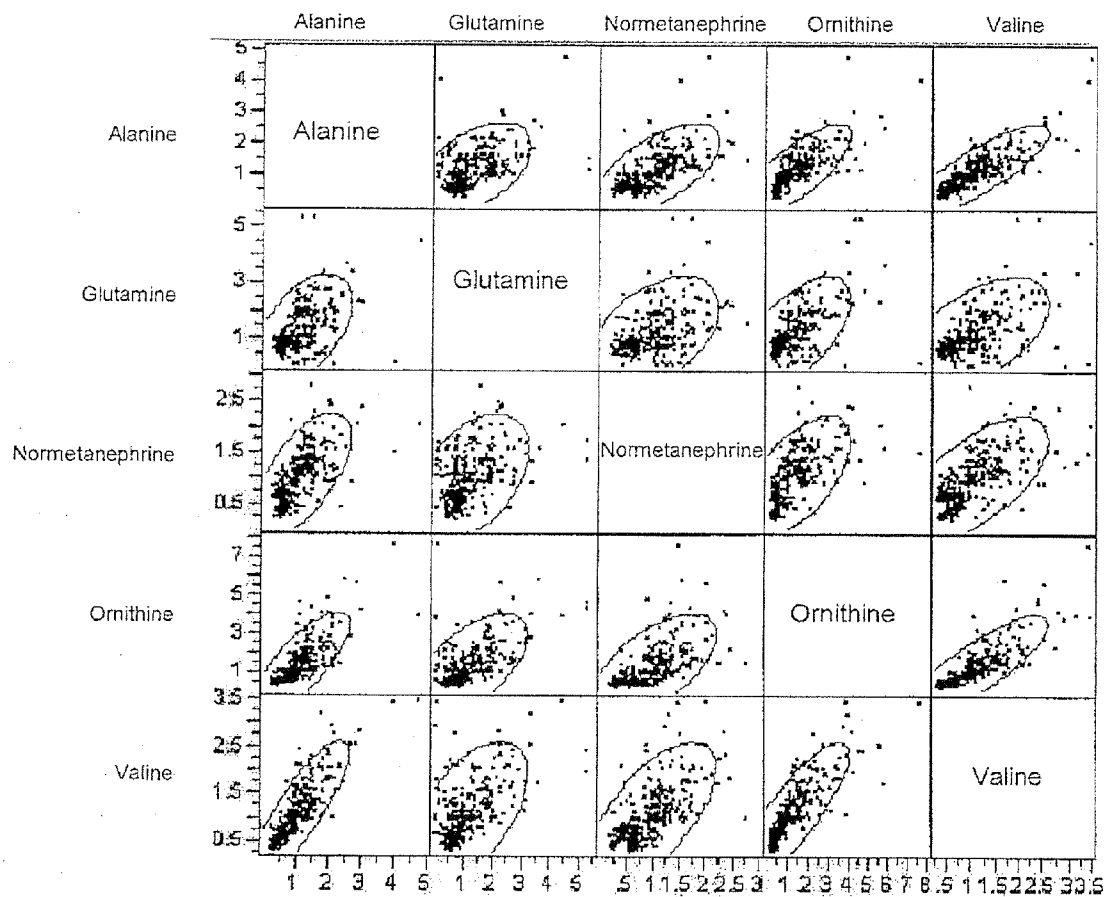
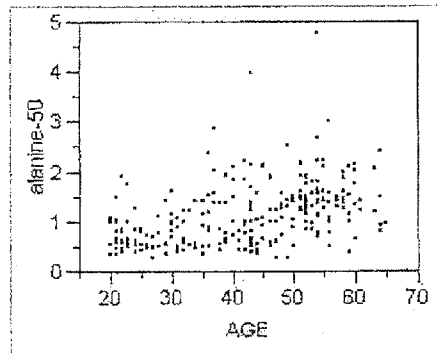


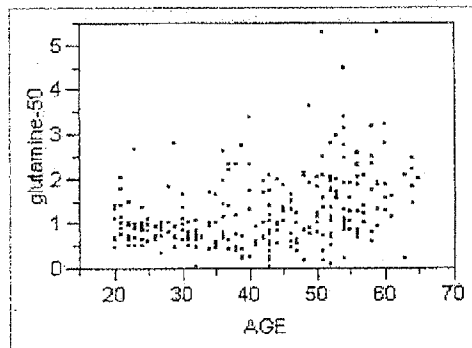
Figure 4



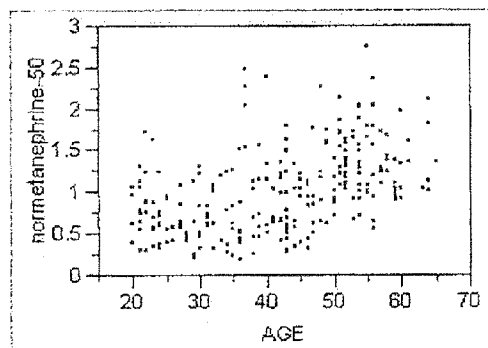
**Figure 5A**



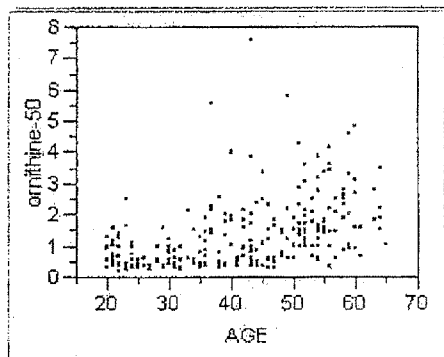
**Figure 5B**



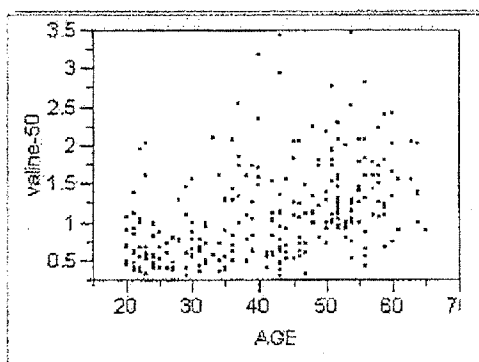
**Figure 5C**



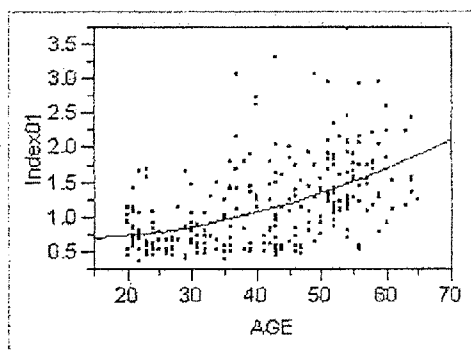
**Figure 5D**



**Figure 5E**



**Figure 5F**



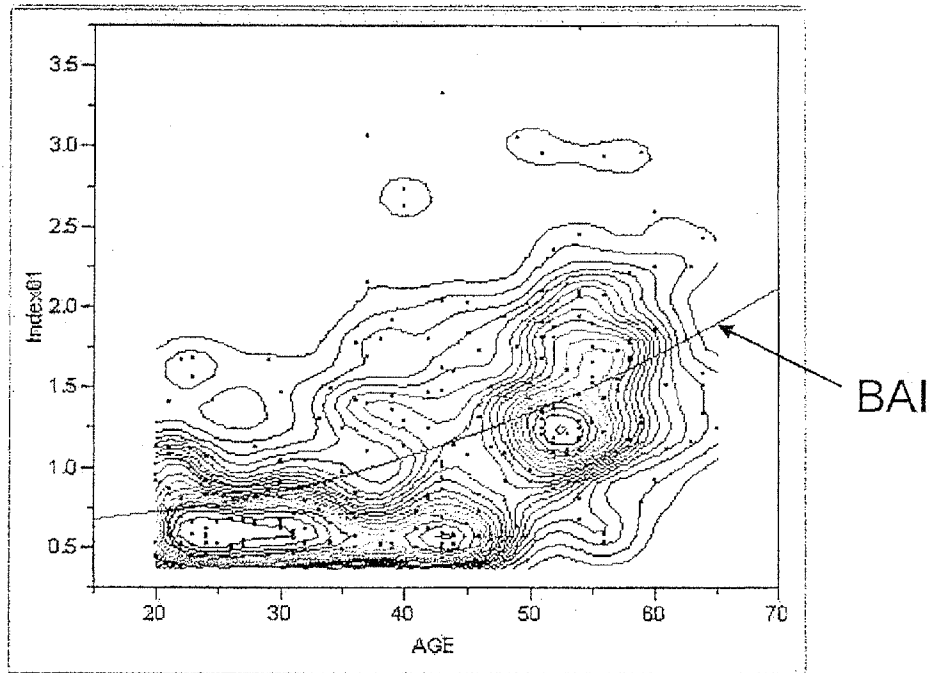
**Figure 6**

Figure 7

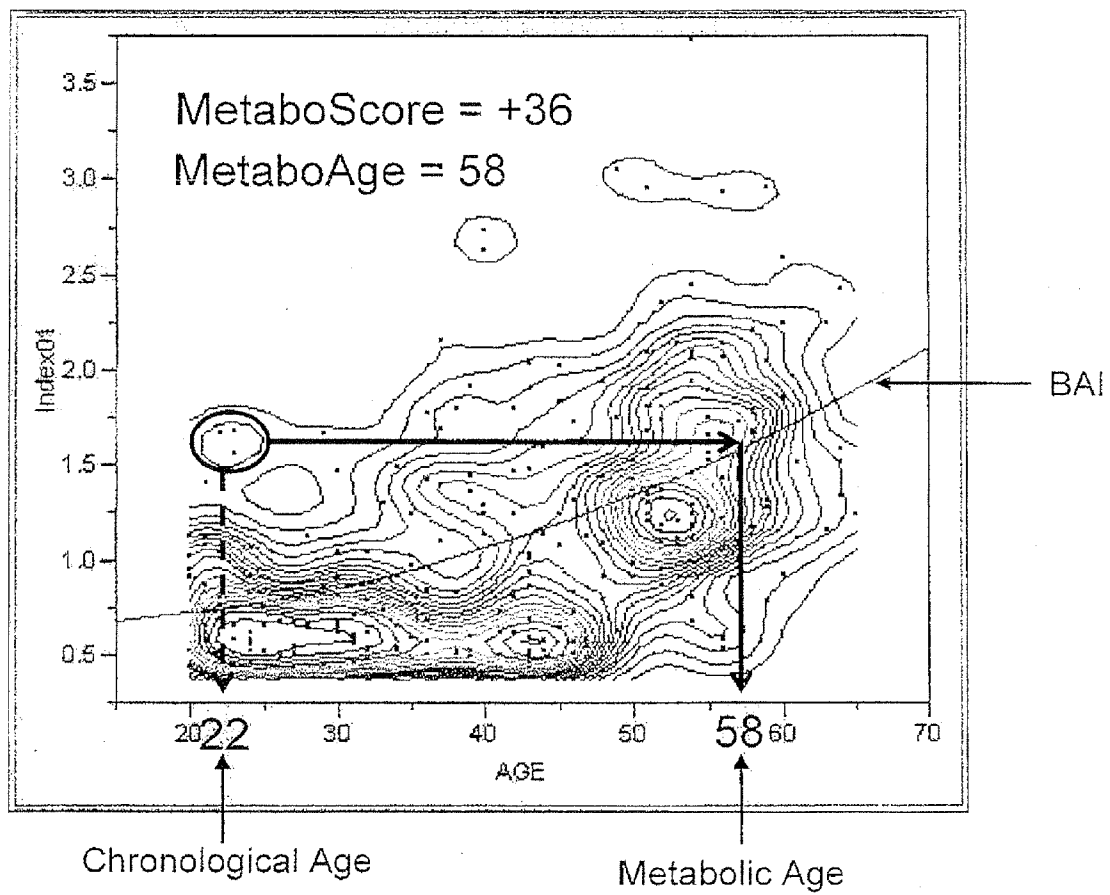
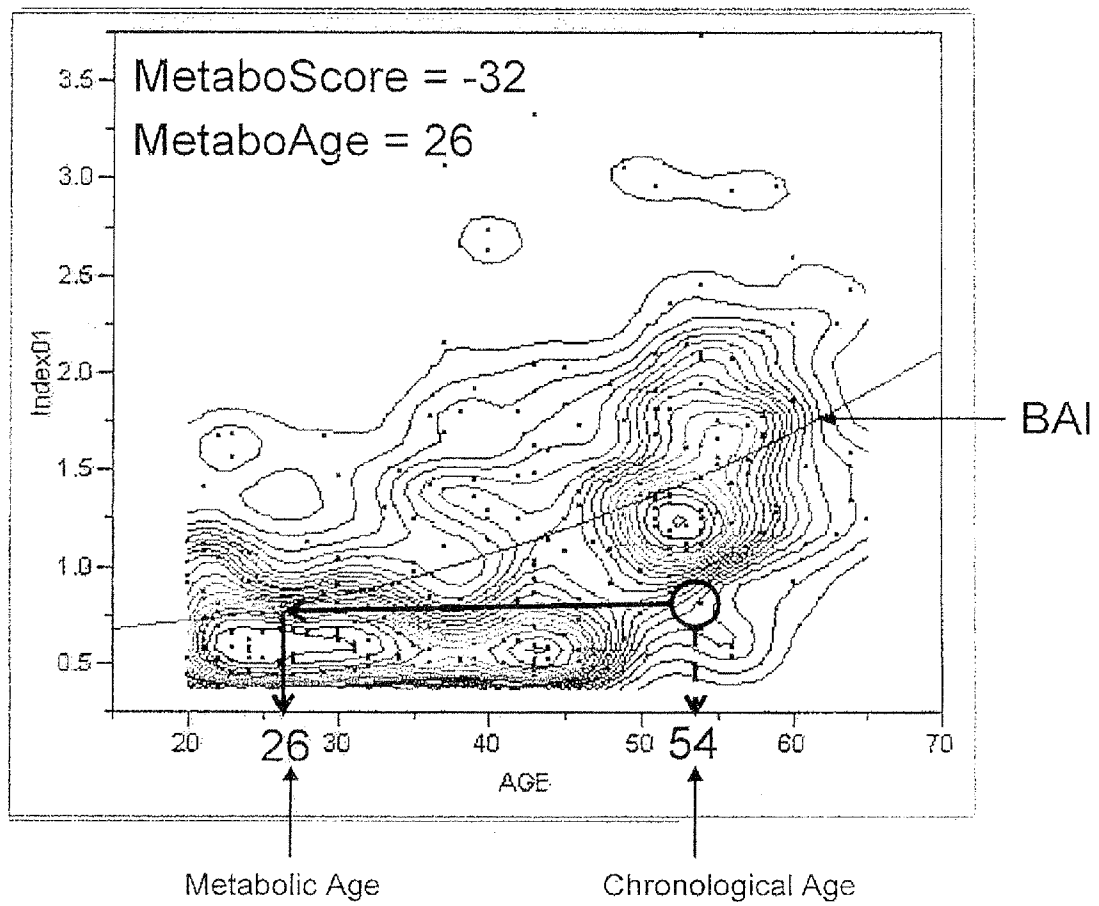


Figure 8



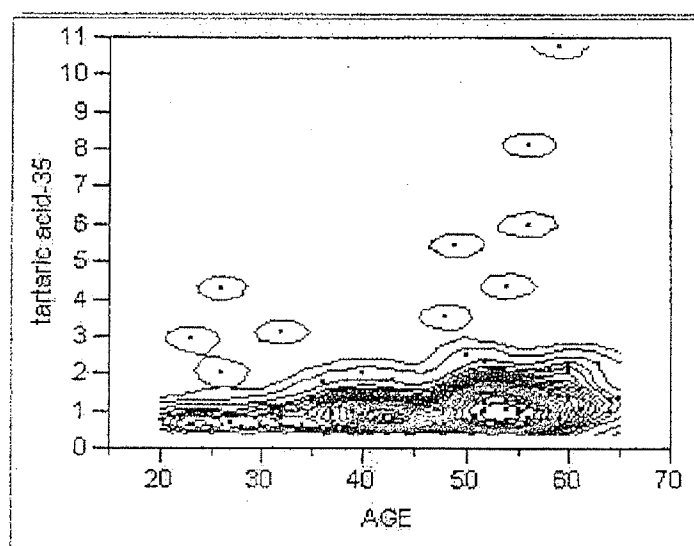
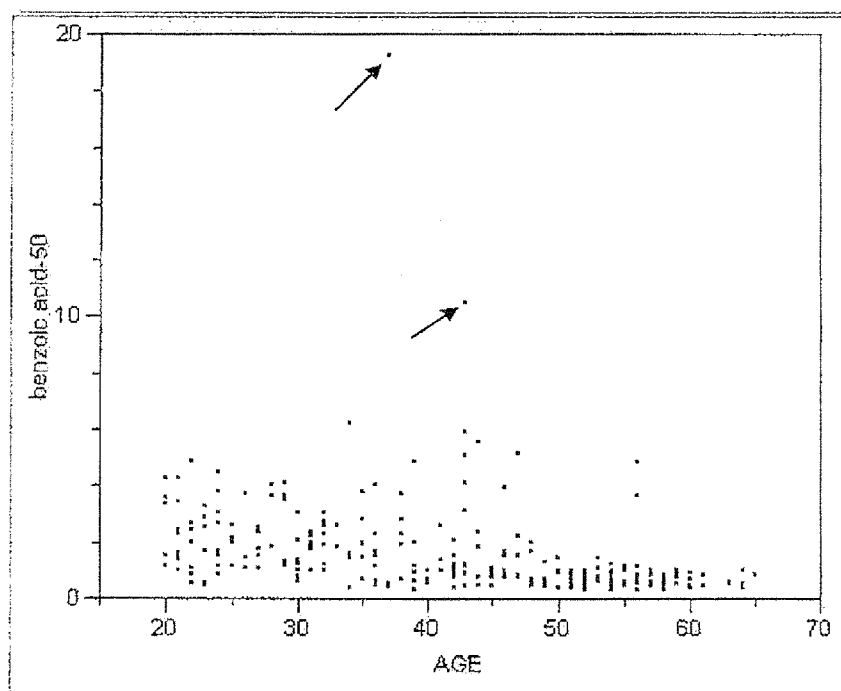
**Figure 9**



Figure 10



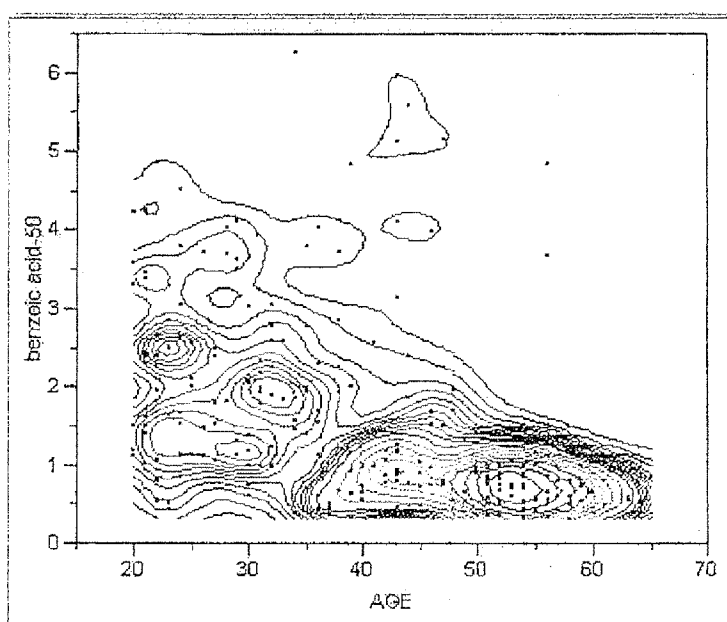
**Figure 11**

Figure 12

