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(54) Title: METHODS AND COMPOSITIONS FOR INCREASING THE ANAEROBIC WORKING CAPACITY IN TISSUES

(57) Abrégé/Abstract:
The invention provides compositions comprising beta-alanylhistidine peptides and beta-alanines, and methods for administering these peptides and amino acids. In one aspect, the compositions and methods cause an increase in the blood plasma concentrations of beta-alanine and/or creatine.

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(54) Title: METHODS AND COMPOSITIONS FOR INCREASING THE ANAEROBIC WORKING CAPACITY IN TISSUES

(57) Abstract: The invention provides compositions comprising beta-alanylhistidine peptides and beta-alanines, and methods for administering these peptides and amino acids. In one aspect, the compositions and methods cause an increase in the blood plasma concentrations of beta-alanine and/or creatine.

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METHODS AND COMPOSITIONS FOR INCREASING THE ANAEROBIC WORKING CAPACITY IN TISSUES

Related Applications

This application claims the benefit of priority under 35 U.S.C. Section 119(e) of U.S. Provisional Application No. 60/462,238 filed April 10, 2003. This application is also a continuation-in-part (CIP) of U.S. application serial no. 10/209,169, filed July 30, 2002, which is a continuation of U.S. application serial no. 09/757,782, filed January 9, 2001, now U.S. Patent No. 6,426,361, which is a continuation of U.S. application serial no. 09/318,530, filed May 25, 1999, now U.S. Patent No. 6,172,098, which is a divisional of U.S. application serial no. 08/909,513, filed August 12, 1997, now U.S. Patent No. 5,965,596, which claims the benefit of foreign priority under 35 USC 119 to United Kingdom application nos. 9621914.2, filed October 21, 1996, and 9616910.7, filed August 12, 1996.

TECHNICAL FIELD

This invention relates to the fields of pharmaceuticals and physiology. In one aspect, the invention provides methods for increasing the buffering capacity of muscles and decreasing muscle fatigue. The invention also provides methods and compositions for increasing the anaerobic working capacity of muscle and other tissues.

BACKGROUND

Natural food supplements are typically designed to compensate for reduced levels of nutrients in the modern human and animal diet. In particular, useful supplements increase the function of tissues when consumed. It can be particularly important to supplement the diets of particular classes of animals whose normal diet may be deficient in nutrients available only from meat and animal products (e.g., human vegetarians and other animals who consume an herbivorous diet).

For example, in the sporting and athletic community, natural food supplements which specifically improve athletic ability are increasingly important, such as supplements that promote or enhance physical prowess for leisure or employment purposes. In another example, anaerobic (e.g., lactate-producing) stress can cause the onset of fatigue and discomfort that can be experienced with intense exercise (e.g., continuous or

intermittent sprinting in soccer or ice-hockey), where oxygen availability may be limited (e.g., peripheral vascular disease, free diving or synchronized swimming) and with aging. Anaerobic stress can also result from prolonged submaximal isometric exercise when the local circulation is partially or totally occluded by the increase in intra-muscular pressure (e.g., during rock climbing). Excessive lactate production can result in the acidification of the intracellular environment.

Creatine (i.e., N-(aminoiminomethyl)-N-glycine, N-amidinosarcosine, N-methyl-N-guanylglycine, or methylglycocyamine) is found in large amounts in skeletal muscle and other "excitable" tissues (e.g., smooth muscle, cardiac muscle, or spermatozoa) characterized by a capacity for high and variable energy demand. Creatine is converted into phosphorylcreatine in energy-generating biochemical pathways within cells. In mammalian skeletal muscle, the typical combined content of creatine (i.e., creatine and phosphorylcreatine) may vary from less than 25 to about 50 mmol per kilogram fresh muscle (i.e., 3.2 to 6.5 grams per kilogram fresh muscle).

Creatine is formed in the liver and taken up into tissues, such as muscle, by means of an active transport system. Creatine synthesis in the body may also be augmented by the ingestion of creatine present in meat (e.g., 5-10 milligrams per kilogram body weight per day in the average meat-eating human and approximately zero in a vegetarian diet).

During sustained intense exercise, or exercise sustained under conditions of local hypoxia, the accumulation of hydronium ions formed during glycolysis and the accumulation of lactate (anaerobic metabolism) can severely reduce the intracellular pH. The reduced pH can compromise the function of the creatine-phosphorylcreatine system. The decline in intracellular pH can affect other functions within the cells, such as the function of the contractile proteins in muscle fibers.

Dipeptides (also referred to herein as peptides) of beta-alanine and histidine, and their methylated analogues, which include carnosine (beta-alanyl-L-histidine), anserine (beta-alanyl-L-1-methylhistidine), or balenine (beta-alanyl-L-3-methylhistidine), are present in the muscles of humans and other vertebrates. Carnosine is found in appreciable amounts in muscles of, for example, humans and equines. Anserine and carnosine are found in muscles of, for example, canines, camelids and numerous avian species. Anserine is the predominant beta-alanylhistidine dipeptide in many fish. Balenine is the predominant beta-alanylhistidine dipeptide in some species of aquatic mammals and reptiles. In humans, equines, and camelids, the highest concentrations of the beta-

alanylhistidine dipeptides are found in fast-contracting glycolytic muscle fibers (type IIA and IIB) which are used extensively during intense exercise. Lower concentrations are found in oxidative slow-contracting muscle fibers (type I). See, e.g., Dunnett, M. & Harris, R.C. Equine Vet. J., Suppl. 18, 214-217 (1995). It is known that carnosine contributes to hydronium ion buffering capacity in different muscle fiber types, and up to 50% of the total in equine type II fibers.

SUMMARY

The invention provides methods of increasing anaerobic working capacity in a tissue, comprising the following steps: (a) providing a beta-alanylhistidine dipeptide and a glycine, an insulin, an insulin mimic, or an insulin-action modifier; and (b) administering the beta-alanine and at least one of the glycine, insulin mimic, or insulin-action modifier to the tissue in an amount effective to increase beta-alanylhistidine dipeptide synthesis in the tissue, thereby increasing the anaerobic working capacity in the tissue. The invention provides methods of regulating hydronium ion concentrations in a tissue comprising the following steps: (a) providing a beta-alanylhistidine dipeptide and a glycine, an insulin an insulin mimic, or an insulin-action modifier; and (b) administering the beta-alanine and at least one of the glycine, insulin mimic, or insulin-action modifier to the tissue in an amount effective to increase the hydronium ion concentration in the tissue.

In one aspect of the methods, the step of administering the beta-alanine and at least one of the glycine, insulin mimic, or insulin-action modifier to the tissue comprises oral administration, administration to a blood or blood plasma or a combination thereof. The beta-alanylhistidine dipeptide can comprise a carnosine, an anserine, or a balenine, or analogs or mimetics thereof.

The invention provides compositions comprising a mixture of a glycine, an insulin, an insulin mimic or an insulin-action modifier, and a composition comprising an amino acid or an active derivative thereof selected from the group consisting of a beta-alanine, a chemical derivative of beta-alanine and a peptide comprising a beta-alanine or analogs thereof. In one aspect, the beta-alanine comprises a beta-alanylhistidine dipeptide, such as a carnosine, an anserine or a balenine or analogs thereof. The compositions can further comprise at least a creatine or a carbohydrate.

In one aspect, the insulin mimic comprises a D-pinitol (3-O-methyl-chiroinositol), a 4-hydroxy isoleucine, a demethyl-asterriquinone B-1 compound, an alpha lipoic acid, a R-alpha lipoic acid, a guanidiniopropionic acid, a vanadium compound, a

vanadium complex or a synthetic phosphoinositolglycan peptide. The insulin-action modifier can be a sulphonylurea, a thiazolidinedione or a biguanide.

In alternative aspects, the composition is a pharmaceutical composition, a dietary supplement or a sports drink. The dietary supplement or sports drink can be a supplement for humans. The pharmaceutical composition can be formulated for humans.

The invention provides compositions comprising at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 or more grams of a peptide or an ester comprising a beta-alanine or analogs or mimetics thereof. The invention provides compositions comprising at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5 or more grams of a peptide or an ester comprising a beta-alanine (or analogs or mimetics thereof) in an injectable form. In one aspect, the peptide comprises a beta-alanylhistidine dipeptide, such as a carnosine, an anserine or a balenine, or analogs or mimetics thereof.

The invention provides compositions formulated for humans comprising at least 200, 225, 250, 275, 300, 325, 350, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975 or 1000 or more mg of a beta-alanine or beta-alanine analogs or mimetics. In one aspect, the composition is formulated in an ingestible or an injectable formulation. The ingestible formulation can be a drink, a gel, a food or a tablet. The peptide can comprise a beta-alanylhistidine dipeptide, such as a carnosine, an anserine or a balenine, or analogs or mimetics thereof.

The invention provides methods of increasing the anaerobic working capacity of a tissue in a subject comprising the following steps: (a) providing a composition comprising (i) a mixture of a glycine, an insulin, an insulin mimic or an insulin-action modifier, and a composition comprising an amino acid or an active derivative thereof selected from the group consisting of a beta-alanine, a chemical derivative of beta-alanine and a peptide comprising a beta-alanine, or analogs or mimetics thereof; (ii) at least 0.5 gram of a peptide or an ester comprising a beta-alanine in an injectable form; or, (iii) at least 200 mg of a beta-alanine; and (b) administering the composition to the subject in an amount effective to increase the anaerobic working capacity of the tissue. In one aspect, the total dosage of the beta-alanine for a 24-hour period is at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5 or more grams. The total dosage of the beta-alanine for a 24-hour period can be between about 0.2 gram and about

6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0 or more grams. The composition can be given over a period of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 or more days. The composition can be given over a period of at least about 3 days to about two, three, four or more weeks. The beta-alanine can comprise a beta-alanylhistidine dipeptide, such as a carnosine, an anserine or a balenine, or analogs or mimetics thereof. The total dosage of the beta-alanylhistidine dipeptide over a 24 hour period can be at least about 0.5 gram, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5 or more grams. The total dosage of the beta-alanylhistidine dipeptide over a 24 hour period can be greater than about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more grams. The total dosage of the beta-alanylhistidine dipeptide over a 24 hour period can be more than about 5 gram to about 16 gram. The composition can be administered in multiple doses. The composition can be administered at least two times to eight times in a 24-hour period. In one aspect, about 200 mg, 225, 250, 275, 300, 325, 350, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975 or 1000 mgm of a beta-alanine (or analogs or mimetics thereof) and/or about 500 mg (or, about 200 mg, 225, 250, 275, 300, 325, 350, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975 or 1000 mgm) of carnosine (or analogs or mimetics thereof) is administered about two to eight, or more, times a day (e.g., 2, 3, 4, 5, 6, 7, 8 or more times a day) over a period of several weeks. In one aspect, at least about 2 g of a beta-alanine or at least about 5 g of carnosine is administered about two to eight times a day over a period of about two, three or four days.

In one aspect, the amount of a composition of the invention administered is increased daily. The amount of the composition of the invention administered can be increased weekly. The composition can be administered in treatment periods that last for at least about four weeks.

While the invention is not limited by any particular mechanism of action, the invention provides methods of regulating hydronium ion concentration in tissue in a subject comprising the following steps: (a) providing a composition comprising (i) a mixture of a glycine, an insulin, an insulin mimic or an insulin-action modifier, and a composition comprising an amino acid or an active derivative thereof selected from the group consisting of a beta-alanine, a chemical derivative of beta-alanine and a peptide comprising a beta-alanine or analogs or mimetics thereof; (ii) at least 0.5 gram of a peptide or an ester comprising a beta-alanine in an injectable form; or, (iii) at least 200 mg of a

beta-alanine; and (b) administering the composition to the subject in an amount effective to regulate the hydronium ion concentration in the tissue.

In one aspect, the invention features methods and compositions for increasing the anaerobic working capacity of muscle and other tissues. The methods and compositions of the invention provide for the simultaneous accumulation of creatine and/or beta-alanylhistidine dipeptides, or beta-alanine and L-histidine analogues, within a tissue in the body. The methods include ingesting or infusing compositions into the body. In one aspect, the compositions are mixtures of compounds capable of increasing the availability and uptake of creatine and of precursors for the synthesis and accumulation of beta-alanylhistidine dipeptides in human and animal tissue. The compositions of the invention can induce the synthesis and accumulation of beta-alanylhistidine dipeptides in a human or animal body when introduced into the body.

The compositions can include beta-alanine, chemical derivatives and analogs of beta-alanine such as esters of beta-alanine, peptides of beta-alanine, such as carnosine, anserine, and balenine, as well as analogues thereof. The compositions may also include L-histidine and mixtures thereof. Each of the beta-alanine and/or L-histidine can be formulated or administered as individual amino acids, or, as components of dipeptides (e.g., carnosine, anserine, and/or balenine), oligopeptides, or polypeptides. The beta-alanine, L-histidine, carnosine, anserine, and/or balenine, or peptides of beta-alanine can be active derivatives. An active derivative is a compound derived from, or is a precursor of, a substance and performs in the same or similar way in the body as the substance, or which is processed into the substance when placed into the body. Examples include, for example, esters and amides. Compositions can also include any one or more of a creatine, a carbohydrate, insulin, an insulin mimic, an insulin-action modifier or a glycine. The compositions of the invention can be used for the preparation of a dietary supplement (including, e.g., drinks, gels, foods) or pharmaceutical composition for humans or animals. The compositions of the invention can be used in any of the methods of the invention.

In one aspect, the invention features compositions for and a method of regulating hydronium ion concentrations in a tissue. The method includes the steps of providing an amount of beta-alanine to blood or blood plasma effective to increase beta-alanylhistidine dipeptide synthesis in a tissue and exposing the tissue to the blood or blood plasma, whereby the concentration of beta-alanylhistidine is increased in the tissue. The beta-alanylhistidine may be a carnosine, anserine, or a balenine. The method can include

the step of providing an amount of L-histidine to the blood or blood plasma effective to increase beta-alanylhistidine dipeptide synthesis.

In another aspect, the invention features a method of increasing the anaerobic working capacity of a tissue. The method includes the steps of providing an amount of beta-alanine to blood or blood plasma effective to increase beta-alanylhistidine dipeptide synthesis in a tissue, providing an amount of L-histidine to the blood or blood plasma effective to increase beta-alanylhistidine dipeptide synthesis in a tissue, and exposing the tissue to the blood or blood plasma. The concentration of beta-alanylhistidine is increased in the tissue.

In alternative aspects, the methods can include the step of increasing a concentration of creatine in the tissue. The increasing step can include providing an amount of creatine to the blood or blood plasma effective to increase the concentration of creatine in the tissue (e.g., by providing creatine to the blood or blood plasma).

The providing steps of the methods can include ingestion, infusion (e.g., injection) or a combination of ingestion and infusion, of a composition including an amount of beta-alanine, a peptide of beta-alanine such as carnosine, anserine and balenine which are hydrolyzed to their constituent amino acids on ingestion and are a source of beta-alanine for the body. Methods of the invention also include providing L-histidine, creatine, carbohydrate, insulin, insulin mimics, insulin-action modifiers and/or glycine.

In yet another aspect, the methods can include increasing a concentration of insulin in the blood or blood plasma. The concentration of insulin can be increased, for example, by injection of insulin. Methods of the invention can also include injection ingestion, or other modes of delivery, known to those of skill in the art, to a body (also referred to as a subject) of insulin mimics. Examples of insulin mimics include, but are not limited to, D-pinitol (3-O-methyl-chiroinositol), 4-hydroxy isoleucine, L783,281 (a demethyl-asterriquinone B-1 compound), alpha lipoic acid, R-alpha lipoic acid, guanidiniopropionic acid, vanadium compounds such as vanadyl sulfate or vanadium complexes such as peroxovanadium, and synthetic phosphoinositolglycans (PIG peptides). Additionally or alternatively, methods of the invention can include the use of insulin-action modifiers to enhance or inhibit the action of insulin in the body. Examples of insulin-action modifiers can include, but are not limited to, sulphonylureas, thiazolidinediones, and biguanides.

In still another aspect, the methods include providing glycine to a body. It is thought that glycine may suppress blood glucose release in the blood after ingestion of a

meal. It may be that glycine enhances insulin sensitivity by promoting greater glucose uptake. Accordingly, the methods include providing glycine alone or in conjunction with insulin, insulin mimics or insulin-action modifiers in the compositions and methods of the invention. Glycine may be provided in various forms, for example, alone or in combination with other substances, such as in dietary supplements. Alternatively, glycine can be derived from other sources, such as gelatin.

The tissue referred to in the invention can be a skeletal muscle.

In one aspect, the invention provides compositions for practicing the methods of the invention. Accordingly, one aspect of the invention contemplates a composition having one or more active ingredient, including beta-alanine, beta-alanylhistidine peptides (or analogues or derivatives thereof), creatine, insulin, insulin mimics or insulin-action modifiers, glycine, and carbohydrate, to carry out the methods of the invention. The invention further contemplates the use of multiple compositions formulated to provide one or more active ingredient to the body for carrying out the methods of the invention.

Therefore, in an exemplary aspect, the invention features a composition consisting essentially of beta-alanine or a peptide source of beta-alanine, between about 39 and about 99 percent by weight of a carbohydrate, and up to about 60 percent by weight of water. The composition can include between about 0.1 and about 20 percent by weight of the beta-alanine (in the free or a bound form). The composition can include between about 0.1 and about 20 percent by weight of L-histidine.

The carbohydrate can be a simple carbohydrate (e.g., glucose).

In another aspect, the invention features a composition consisting essentially of beta-alanine or a peptide source of beta-alanine, between about 1 and about 98 percent by weight of a creatine source, and up to about 97 percent by weight of water. The composition includes between about 0.1 and about 98 percent by weight of the beta-alanine. The peptide source can include L-histidine and the composition can include between about 0.1 and about 98 percent by weight of L-histidine from this source.

The peptide source can be a mixture of amino acids, dipeptides, oligopeptides, polypeptides, or active derivatives thereof.

The composition can be a dietary supplement. The creatine source can be creatine monohydrate.

The concentrations of components in blood or blood plasma, including beta-alanine, can be increased by infusion (i.e., injection) or ingestion of an agent operable to

cause an increase in the blood plasma concentration. The composition can be ingested in doses of between about 100 milligrams and about 800 grams or more per day. The doses can be administered in one part or multiple parts each day.

An increase of creatine and beta-alanylhistidine dipeptides in the muscles can increase the tolerance of the cells to an increase in hydronium ion production with anaerobic work and lead to an increase in endurance during exercise before the onset of fatigue. The compositions and methods can contribute to correcting the loss of beta-alanine, L-histidine, or creatine due to degradation or leaching of these constituents during the cooking or processing of food. The compositions and methods can also contribute to correcting the absence of these components from a vegetarian diet.

The methods and compositions can be used to increase beta-alanylhistidine dipeptides in sportsmen, athletes, body-builders, synchronized swimmers, soldiers, elderly people, horses in competition, working and racing dogs, and game birds, to avoid or delay the onset of muscular fatigue.

In another aspect, the invention provides a dietary supplement comprising: glycine, and an amino acid of beta-alanine or ester or amide thereof; or glycine, an amino acid of beta-alanine or ester or amide thereof, and L-histidine, wherein the amino acid of beta-alanine is not part of a dipeptide, an oligopeptide or a polypeptide.

In another aspect, the invention provides use of an effective amount of a dietary supplement composition comprising: and an amino acid of beta-alanine or ester or amide thereof; or glycine, an amino acid of beta-alanine or ester or amide thereof, and L-histidine to increase the anaerobic working capacity in a tissue, and wherein the amino acid of beta-alanine is not part of a dipeptide, an oligopeptide or a polypeptide.

In another aspect, the invention provides use of a dietary supplement comprising: and an amino acid of beta-alanine or ester or amide thereof; or glycine, an amino acid of beta-alanine or ester or amide thereof, and L-histidine in the

manufacture of a dietary supplement to increase the anaerobic working capacity in a tissue, and wherein the amino acid of beta-alanine is not part of a dipeptide, an oligopeptide or a polypeptide.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other advantages and features of the invention will be apparent to the skilled artisan from the detailed description, drawings, and claims.

DESCRIPTION OF DRAWINGS

Fig. 1 is a graph depicting changes in the concentrations of beta-alanine in blood plasma of five horses, before and at 2 hour intervals following the feeding of beta-alanine and L-histidine (100 milligrams per kilogram body weight and 12.5 milligrams per kilogram body weight, respectively, three times per day) over a period of 30 days.

Fig. 2 is a graph depicting changes in the concentrations of L-histidine in blood plasma of five horses, before and at 2 hour intervals following the feeding of beta-alanine and L-histidine (100 milligrams per kilogram body weight and 12.5 milligrams per kilogram body weight, respectively, thee times per day) over a period of 30 days.

Figures 3a, 3b, 3c, 3d, 3e and 3f are graphs depicting the contrast in the changes in the concentrations of beta-alanine in blood plasma of six horses, before and at hourly intervals following the feeding of beta-alanine and L-histidine, as described in detail, below.

Figures 4a, 4b, 4c, 4d, 4e and 4f are graphs depicting the contrast in the changes in the concentrations of L-histidine in blood plasma of six horses, before and at hourly intervals following the feeding of beta-alanine and L-histidine, as described in detail, below.

Fig. 5 is a graph depicting the contrast in the changes in the mean concentrations of beta-alanine in equine blood plasma (n = 6), before and at hourly intervals following the feeding of beta-alanine and L-histidine, as described in detail, below.

Fig. 6 is a graph depicting the contrast in the changes in the mean concentrations of L-histidine in equine blood plasma (n = 6), before and at hourly intervals following the feeding of beta-alanine and L-histidine (100 milligrams per kilogram body weight and 12.5 milligrams per kilogram body weight, respectively, three times per day) on the first and last day of a 30 day period of dietary supplementation.

Fig. 7 is a graph depicting the correlation between the increase in 6 thoroughbred horses in the carnosine concentration in type II skeletal muscle fibers (the average of the sum of type IIA and IIB fibers) and the increase, between the 1st and 30th day of supplementation, in the area under the blood plasma beta-alanine concentration-time curve over the first 12 hours of the day ($AUC_{(0\text{-}12\ hr)}$).

Fig. 8 is graph depicting the mean results of the administration of beta-alanine, broth, or carnosine to test subjects.

Fig. 9 is a graph depicting mean changes in plasma beta-alanine over nine hours of treatment.

Fig. 10 is a graph depicting the mean changes in plasma beta-alanine over 9 hours following the oral ingestion of 10 milligrams per kilogram body weight of beta-alanine.

Fig. 11 is a graph depicting the mean (n = 6) plasma beta-alanine concentration over the 24 hours of Day 1 and Day 30 of the treatment period.

Fig. 12 is a graph depicting changes in muscle carnosine concentration pre and post treatment in different subjects. The red circles indicate the muscle concentrations prior to supplementation.

Fig. 13 is a graph depicting muscle concentration (mean +SD) of carnosine before and post supplementation in three different treatment groups.

Fig. 14 is a graph depicting muscle concentration (mean +SD) of histidine before and post supplementation in three different treatment groups.

Figure 15 is a graph illustrating data showing muscle concentration (mean +SD) of taurine before and post supplementation in four different treatment groups.

Figure 16 is a graph illustrating data showing muscle concentration (mean +SD) of taurine before and post supplementation in different subjects.

Figure 17 illustrates a table of data, described in detail as Table 9, below.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

The invention provides compositions comprising beta-alanine, peptides of beta-alanine, analogues and derivatives thereof, beta-alanylhistidine dipeptides (e.g., carnosine, anserine, and balenine) and methods using these compositions for increasing the anaerobic working capacity of a tissue comprising providing an amount of beta-alanine to blood or blood plasma effective to increase beta-alanylhistidine dipeptide synthesis in a tissue. Beta-alanylhistidine dipeptides can include peptides of beta-alanine, such as carnosine, anserine, and balenine. In one aspect, they can have pKa values between approximately 6.8 and 7.1. In one aspect, they can be involved in the regulation of intra-cellular pH homeostasis during muscle contraction and the development of fatigue. The content of other substances involved in hydronium ion buffering, such as amino acid residues in proteins, inorganic and organic phosphates and bicarbonate, can be constrained by their involvement in other cell functions. In one aspect, the beta-alanylhistidine dipeptides provide an effective way of accumulating pH-sensitive histidine residues into a cell. Variations in the muscle beta-alanylhistidine dipeptide concentrations affect the anaerobic work capacity of individual athletes.

The beta-alanylhistidine dipeptides are synthesized within the body from beta-alanine and L-histidine. These precursors can be generated within the body or are made available via the diet, including from the breakdown of an ingested beta-alanylhistidine dipeptide. Within the body, beta-alanine is transported to tissues such as muscle. In a typical fed state, the concentration of beta-alanine is low in comparison with the concentration of L-histidine in human and equine blood plasma. These concentrations should be viewed in relation to the affinity of the carnosine synthesizing enzyme, carnosine synthetase, for its substrates as determined by the Michaelis-Menton constant (Km). The Km for histidine is about 16.8 μM. The Km for beta-alanine is between about 1000 and 2300 μM. The low affinity of carnosine synthetase for beta-alanine, and the low

concentration of beta-alanine in muscle, demonstrate that the concentration of beta-alanine in muscle is limiting to the synthesis of the beta-alanylhistidine dipeptides.

Increasing the amount of beta-alanylhistidine dipeptides within a muscle favorably affects muscular performance and the amount of work that can be performed by the muscle. Accordingly, it is desirable to increase the synthesis and accumulation of beta-alanylhistidine dipeptides in a tissue in a human or animal body.

The synthesis and accumulation of beta-alanylhistidine dipeptides in a human or animal body can be increased by increasing creatine within the body, increasing the blood or blood plasma concentrations of beta-alanine, increasing the blood or blood plasma concentrations of beta-alanine and creatine, or increasing the blood or blood plasma concentrations of beta-alanine, L-histidine, and creatine. The increase in dipeptides can be simultaneous with the increase in beta-alanine concentrations.

In one aspect, the compositions and methods of the invention can be used to increase blood plasma concentrations of beta-alanine, L-histidine and/or creatine by ingestion or infusion of beta-alanine, peptides of beta-alanine, L-histidine, creatine, carnosine, anserine, and/or balenine and/or active derivatives or analogs thereof alone or in various combinations. The compositions of the invention can be administered orally, enterally, or parenterally. For example, compositions of the invention can be orally ingested or infused through the skin through a topical cream or a patch.

The composition can include carbohydrates (e.g., simple carbohydrates), insulin, or agents that stimulate the production of insulin. Compositions can also include glycine, insulin, insulin mimics, and/or insulin-action modifies.

The compositions can be a dietary supplement that can be ingested, injected, or absorbed through the skin. Preferably, the compositions can be administered in one or more doses per day. The beta-alanine dosage can be between about 1 milligram and about 200 milligrams per kilogram body weight or the dose of a peptide of beta-alanine (e.g. carnosine) from 2.5 milligrams to 500milligrams per kilogram body weight. In one aspect, the total amount of beta-alanine (or other composition of the invention) administered can be at least 200 mg, from 200 mg to 5 g, or from 5 g or more per day for a human. A single dose of active ingredient, e.g., beta-alanine, carnosine, anserine, or balenine, or mixtures thereof, may be formulated to be in the amount about 200, 400, 800 mg or more. The creatine (e.g., creatine monohydrate) dosage, or dosage of other compositions of the invention, can be between about 5 milligrams to 200 milligrams per kilogram body weight. The L-histidine dosage, or dosage of other compositions of the invention, can be between

about 1 milligram to 100 milligrams per kilogram body weight. The simple carbohydrate (e.g., glucose) dosage, or dosage of compositions of the invention, can be between about 0.5 and 2.0 grams per kilogram body weight.

In an 80 kilogram person, suitable dosages per day can be between 0.08 grams to 16.0 grams of beta-alanine or 200 milligrams to 40 grams of a peptide of beta-alanine, 0.4 grams to 16.0 grams of creatine monohydrate, 0.08 grams to 8.0 grams of L-histidine, or 40 grams to 160 grams of glucose or other simple carbohydrate. The composition can be in a solid form or a liquid form or in a suspension which can be ingested or infused into the body. The composition is can be ingested by humans in an amount of between 0.08 grams and 1000 grams or more per day , which may be taken in one or more parts throughout the day. In animals, the daily intake will be adjusted by body weight.

In one aspect, the total amount of a peptide of beta-alanine, for example, carnosine, anserine or balenine that can be administered per day may be at least 500 mg, between about 500 mg to about 5 g, between about 5 g to about 16 g, or greater than 16g. A single dose of a peptide of beta-alanine creatine, anserine or balenine, or mixtures thereof, may be formulated to be in the amount of 0.5, 1, 1.5, or 2 g (for each, or all, or the peptides in a formulation comprising a mixture).

For humans and animals, the compositions can be:

(a) 1% to 99% by weight of beta-alanine or 1% to 99% by weight of a peptide of beta-alanine;
1% to 99% by weight of creatine monohydrate; and
0% to 98% by weight of water;

(b) 1% to 98% by weight of beta-alanine or 1% to 98% by weight of a peptide of beta-alanine;
1% to 98% by weight of L-histidine;
1% to 98% by weight of creatine monohydrate; and
0% to 97% by weight of water;

(c) 1% to 20% by weight of beta-alanine or 1% to 20% by weight of a peptide of beta-alanine;
39% to 99% by weight of glucose or other simple carbohydrate; and
0% to 60% by weight of water; or

(d) 1% to 20% by weight of beta-alanine or 1% to 20% by weight of a peptide of beta-alanine;

1% to 20% by weight of L-histidine

39% to 99% by weight of glucose or other simple carbohydrate; and

0% to 60% by weight of water.

In one aspect, compositions are applied to a body for at least three days, from 3 days to 2 weeks, from 2 weeks to 4 weeks, or longer. In certain regimens, the daily dosages are gradually increased or decreased. This can be done daily, every couple of days, or weekly.

EXAMPLES

The following are specific examples of the methods and compositions for increasing the anaerobic working capacity of muscle and other tissues.

Example 1

The effect of supplementation of a normal diet with multiple daily doses of beta-alanine and L-histidine on the carnosine concentration in type I, IIA, and IIB skeletal muscle fibers of thoroughbred horses was assessed. Six experimental thoroughbred horses of normal health (three fillies and three geldings), aged 4 to 9 years, underwent one month (30 days) of dietary conditioning (pre-supplementation period) prior to the commencement of the supplementation period. During the dietary conditioning period each horse was fed a diet comprising 1 kilogram of pelleted feed (Spillers racehorse cubes) and 1 kilogram of soaked sugar beet pulp as a source of complex and simple carbohydrates, three times per day (at 08:30, 12:30, and 16:30, respectively). Soaked hay (3 kilograms dry weight) was also provided twice daily (at 09:00 and 17:00). Water was provided *ad libitum*.

During the supplementation period, an identical feeding regime was implemented. However, each hard feed meal was supplemented with beta-alanine and L-histidine (free base). Beta-alanine and L-histidine were mixed directly into the normal feed. Individual doses of beta-alanine and L-histidine were calculated according to body weight. Beta-alanine was administered at 100 milligrams per kilogram body weight and L-histidine at 12.5 milligrams per kilogram body weight. Dietary supplementation was begun on day 1 of the protocol and discontinued at the end of day 30. Heparinized blood samples (5 milliliters) were collected on days 1, 6, 18, 24, and 30. On day 1 and day 30, blood samples were collected prior to the first feed and at hourly intervals for a total of 12 hours each day. On the three intervening sampling days, blood was collected prior to the first feed and 2 hours after each subsequent feed. On the day before the start of supplementation (day 0) a muscle biopsy was taken, following application of local

anesthesia of the skin, from the right middle gluteal muscle (m. gluteus medius) of each horse using a Bergstrom-Stille percutaneous biopsy needle. Subsequent muscle biopsies were collected immediately after the end of the supplementation period (day 31) as close as possible to the original sampling site. Clinical monitoring of the horses was performed daily. This comprised a visual examination and measurement of body weight, twice-daily measurement of rectal temperature, and weekly blood sampling for clinical biochemistry and hematology. During the course of the study, the horses received no formal training or exercise, although they were allowed one hour of free exercise each day.

Fragments of individual muscle fibers dissected from freeze-dried muscle biopsies were characterized as either type I, IIA or IIB by histochemical staining for myosin ATPase activity at pH 9.6 following pre-incubation at pH 4.5 by a modification of the method described in, Kaiser and Brook, Arch. Neurol., 23:369-379 (1970).

Heparinized blood plasma samples were extracted and analyzed for beta-alanine and L-histidine concentrations by high-performance liquid chromatography (HPLC). Individual weighed muscle fibers were extracted and analyzed for carnosine by HPLC according to the method described in, Dunnett and Harris, "High-performance liquid chromatographic determination of imidazole dipeptides, histidine, 1-methylhistidine and 3-methylhistidine in muscle and individual muscle fibers," J. Chromatogr. B. Biomed. Appl., 688:47-55 (1997).

Differences in carnosine concentrations within fiber types before and after supplementation were established within horses using one-way analysis of variance (ANOVA). In instances where differences were detected, significance was determined using a multiple comparison test (Fisher's PLSD).

No palatability problems were encountered with the addition of beta-alanine and L-histidine to the feed. No adverse physiological or behavioral effects of the supplemented diet were observed in any of the horses during the thirty days of supplementation. No significant changes in body weight were recorded, and rectal temperatures remained within the normal range. No acute or chronic changes in clinical biochemistry or hematology were observed. Beta-alanine was not detected in the plasma of any of the horses prior to the start of supplementation. The lower limit of quantitation for beta-alanine in plasma by the assay used was 3 micromolar (μM). Plasma L-histidine concentrations in the six horses prior to the start of supplementation were between 36.6 and 54.4 μM.

Individual changes in blood plasma beta-alanine and L-histidine concentrations for five of the six horses over all the sampling days are shown in Figs. 1 and 2,

respectively. There was a trend towards an increase in the pre-feeding concentrations of blood plasma beta-alanine and L-histidine with increasing time of supplementation. Furthermore, over the thirty day supplementation period, the blood plasma concentration response to supplementation was also increased. The response was greater for beta-alanine.

Comparisons of the changes in blood plasma beta-alanine and L-histidine concentrations prior to the first feed of the day, and hourly thereafter between the first and last days of the supplementation period, for the six individual horses, are shown in Figs. 3a and 3b, and Figs. 4a and 4b, respectively. Figures 3a, 3b, 3c, 3d, 3e and 3f are graphs depicting the contrast in the changes in the concentrations of beta-alanine in blood plasma of six horses, before and at hourly intervals following the feeding of beta-alanine and L-histidine (100 milligrams per kilogram body weight and 12.5 milligrams per kilogram body weight, respectively, three times per day) on the first and last day of a 30 day period of dietary supplementation. Figures 4a, 4b, 4c, 4d, 4e and 4f are graphs depicting the contrast in the changes in the concentrations of L-histidine in blood plasma of six horses, before and at hourly intervals following the feeding of beta-alanine and L-histidine (100 milligrams per kilogram body weight and 12.5 milligrams per kilogram body weight, respectively, three times per day) on the first and last day of a 30 day period of dietary supplementation. Fig. 5 is a graph depicting the contrast in the changes in the mean concentrations of beta-alanine in equine blood plasma (n = 6), before and at hourly intervals following the feeding of beta-alanine and L-histidine (100 milligrams per kilogram body weight and 12.5 milligrams per kilogram body weight, respectively, three times per day) on the first and last day of a 30 day period of dietary supplementation. The mean (SD) changes (n = 6) in blood plasma beta-alanine concentration over time during the 24 hours of the first (day 1) and last (day 30) days of the supplementation period are contrasted in Fig. 5. The area under the mean blood plasma beta-alanine concentration versus time curve over 24 hours ($AUC_{(0\text{-}24hr)}$) was much greater on day 30 of the supplementation.

The mean (SD) changes (n = 6) in blood plasma L-histidine concentration over time during the 24 hours of the first (day 1) and last (day 30) days of the supplementation period are contrasted in Fig. 6. The area under the mean blood plasma beta-alanine concentration vs. time curve over 24 hours ($AUC_{(0\text{-}24hr)}$) was greater on day 30 of the supplementation. The greater AUC for blood plasma beta-alanine on the last day of supplementation (day 30) in contrast to the first day of supplementation (day 1) suggests the increased uptake of beta-alanine from the equine gastro-intestinal tract with progressive

supplementation. A similar effect was observed for changes in blood plasma L-histidine concentration during the supplementation period. Peak blood plasma concentrations of beta-alanine and L-histidine occurred approximately one to two hours post-feeding in each case.

A total of 397 individual skeletal muscle fibers (192 pre-supplementation; 205 post-supplementation) from the six horses were dissected and analyzed for carnosine. Mean (SD) carnosine concentration, expressed as millimoles per kilogram dry weight (mmol kg^{-1} dw), in pre- and post-supplementation type I, IIA, and IIB skeletal muscle fibers from the six individual horses are given in Table 1 where n is the number of individual muscle fibers analyzed. Following thirty days of beta-alanine and L-histidine supplementation the mean carnosine concentration was increased in type IIA and IIB fibers in all six horses. These increases were statistically significant in seven instances. The increase in mean carnosine concentration in type IIB skeletal muscle fibers was statistically significant in five out of six horses. The increase in mean carnosine concentration in type IIA skeletal muscle fibers was statistically significant in two out of six horses.

Table 1

Horse	Day	Type 1	n	Type IIA	n	Type IIB	n
6	0 31	32.3 (14.5) -	3	72.1 (47.7) 16.2 (20.9)	11 17	111.8 (22.8) 117.7 (38.7)	14 12
5	0 31	59.5 (3.9) 55.5	2 1	102.6 (12.7) 112.2 (17.1)	12 18	131.2 (26.6) 153.3 (28.0)**	26 22
4	0 31	44.8 (6.6) 37.0 (9.3)	4 2	59.9 (19.5) 88.0 (34.2)*	13 17	108.6 (41.5) 152.4 (65.0)*	19 19
1	0 31	56.7 (5.3) 57.8	2 1	88.5 (20.9) 96.1 (17.3)	15 19	101.3 (15.2) 14.3 (13.3)*	13 11
2	0 31	- 65.9 (13.2)	4	89.6 (16.2) 102.2 (22.1)	13 18	104.2 (22.2) 142.0 (35.4)***	14 12
3	0 31	30.9 (4.0) -	2	85.1 (20.3) 105.0 (17.6)*	6 23	113.5 (20.4) 135.4 (24.9)*	23 9
Mean	0 31	44.8 54.1	13 8	83.0 96.6*	70 112	111.8 135.9**	109 85

* significantly different to pre-supplementation, $p < 0.05$
** significantly different to pre-supplementation, $p < 0.01$
*** significantly different to pre-supplementation, $p < 0.005$

The absolute (e.g. mmol kg^{-1} dw) and percentage increases in the mean carnosine concentrations in type IIA and IIB skeletal muscle fibers from the six horses are listed in Table 2.

Table 2

Horse	Type IIA	Type IIA	Type IIB	Type IIB
	Absolute increase	% increase	Absolute increase	% increase
6	4.1	5.7	5.6	5.3
5	9.6	9.4	22.1	16.8
4	28.1	46.9	43.8	40.3
1	7.6	8.6	13.0	12.8
2	12.6	14.1	37.8	36.3
3	19.9	23.4	21.9	19.3
Mean	13.6	18.0	24.1	21.8

It was observed that the individual horses which showed the greater increase in muscle carnosine concentration following thirty days of supplementation also demonstrated the greater increase in blood plasma beta-alanine AUC between day 1 and day 30 of the supplementation period. Referring to Fig. 7, a significant correlation ($r = 0.986$, $p < 0.005$) for five of the six horses was observed between the increase in mean carnosine concentration, averaged between type IIA and IIB skeletal muscle fibers and the increase, between the 1st and 30th day of supplementation, in blood plasma beta-alanine AUC, over the first 12 hours($AUC_{(0\text{-}12\ hr)}$). Only five horses were used to calculate the regression line. Horse 6 (filled circle) showed no appreciable increase in blood plasma beta-alanine concentration greater than that observed on day 1 until the last day of supplementation. This was unlike the other five horses, which showed a progressive increase with each sampling day. For this reason horse 6 was excluded from the calculation of the regression equation.

Increases in muscle carnosine concentration following thirty days of supplementation with beta-alanine and L-histidine will cause a direct increase in total muscle buffering capacity. This increase can be calculated by using the Henderson-Hasselbach Equation. Calculated values for the increases in muscle buffering capacity in type IIA and IIB skeletal muscle fibers in the six thoroughbred horses are shown in Table 3.

Table 3

Horse	Day	Type IIA	Type IIA	Type IIA	Type IIB	Type IIB	Type IIB
		βmcar	βmtotal	Δβmtotal (%)	βmcar	βmtotal	Δβmtotal (%)
6	0 31	23.9 25.3	93.9 95.3	+1.5	37.1 39.0	107.1 109.0	+1.8
5	0 31	34.0 37.2	104.0 107.2	+3.1	43.5 50.8	113.5 120.8	+6.4
4	0 31	19.9 29.2	89.9 99.2	+10.3	36.0 50.5	106.0 120.5	+13.7
1	0 31	29.3 31.9	99.3 101.9	+2.6	33.6 37.9	103.6 107.9	+4.2
2	0 31	29.7 33.9	99.7 103.9	+4.2	34.5 47.1	104.5 117.1	+12.1
3	0 31	28.2 34.8	98.2 104.8	+6.7	37.6 44.9	107.6 114.9	+6.8
Mean	0 31	27.5 32.1	97.5 102.1	+4.7	37.1 45.0	107.1 115.0	+7.5

Example 2

The effect of supplementation of a normal diet with single and multiple daily doses of beta-alanine in free or peptide bound form on the beta-alanine and beta-alanyl dipeptide concentrations of plasma of humans was assessed. The plasma concentration of beta-alanine in six normal subjects following the consumption of a broth delivering approximately 40 milligrams per kilogram body weight of beta-alanine was monitored. Doses of 10 and 20 milligrams per kilogram body weight of beta-alanine were also given.

The broth was prepared as follows. Fresh chicken breast (skinned and boned) was finely chopped and boiled for fifteen minutes with water (1 liter for every 1.5 kg of chicken). Residual chicken meat was removed by course filtration. The filtrate was flavored by the addition of carrot, onion, celery, salt, pepper, basil, parsley and tomato puree, and reboiled for a further fifteen minutes and then cooled before final filtration

though fine muslin at 4°C. The yield from 1.5 kilograms of chicken and one liter of water was 870 mL of broth. A portion of the stock was assayed for the total beta-alanyl-dipeptide content (e.g., carnosine and anserine) and beta-alanine. Typical analyses were:

total beta-alanyl-dipeptides	74.5 mM
free beta-alanine	5.7 mM

The six male test subjects were of normal health and between 25 - 53 years of age, as shown in Table 4. The study commenced after an overnight fast (e.g., a minimum of 12 hours after the ingestion of the last meat containing meal). Subjects were given the option to consume a small quantity of warm water prior to the start of the study. Catheterization was begun at 08:30 and the study started at 09:00.

As a control, 8 milliliters per kilogram body weight of water was ingested (e.g., 600 mL in a subject weighing 75 kilograms).

In one session, 8 milliliters per kilogram body weight of broth containing approximately 40 milligrams per kilogram body weight of beta-alanine (e.g., in the form of anserine and carnosine) was ingested. For a subject weighing 75 kilograms, this amounted to the ingestion of 600 milliliters of broth containing 3 grams of beta-alanine. In another session, 3 milliliters per kilogram body weight of a liquid containing the test amount of beta-alanine with an additional 5 milliliters per kilogram body weight of water was ingested. In all sessions, subjects additionally consumed a further 8 milliliters per kilogram body weight of water (in 50 mL portions) during the period of 1 to 2 h after ingestion. A vegetarian pizza was provided after 6 hours. An ordinary diet was followed after 8 hours.

2.5 milliliter venous blood samples were drawn through an indwelling catheter at 10 minute intervals for the first 90 minutes and then after 120, 180, 240 and 360 minutes. The blood samples were dispensed into tubes containing lithium-heparin as an anti-coagulant. The catheter was maintained by flushing with saline. Plasma samples were analyzed by HPLC according to the method described in Jones & Gilligan (1983) J. Chromatogr. 266:471-482 (1983).

Table 4 summarizes the allocation of treatments during the beta-alanine absorption study. The estimated equivalent doses of beta-alanine are presented in Table 3.

Table 4

Subject	Age yrs	Weight kg	Broth 40 mg/kg bwt	β-ala 0 mg/kg bwt	β-ala 10 mg/kg bwt	β-ala 20 mg/kg bwt	β-ala 40 mg/kg bwt	Carnosine 20 mg/kg bwt
1	53	76	+			+	+	+
2	33	60	+			+	+	
3	29	105	+	+	+	+		
4	31	81	+	+	+		+	
5	30	94	+	+	+		+	
6	25	65	+	+	+	+		

Plasma concentration curves following each treatment are depicted graphically in Fig. 8. Mean results of the administration of beta-alanine, broth, or carnosine according to the treatments schedule in Table 4. Plasma beta-alanine was below the limit of detection in all subjects on the control treatment. Neither carnosine or anserine were detected in plasma following ingestion of the chicken broth or any of the other treatments. Ingestion of the broth resulted in a peak concentration in plasma of 427.9 (SD 161.8) μM. Administration of carnosine equivalent to 20 milligrams per kilogram body weight of beta-alanine in one test subject resulted in an equivalent increase in the plasma beta-alanine concentration.

Administration of all treatments except control resulted in an increase in the plasma taurine concentration. The changes in taurine concentration mirrored closely those of beta-alanine. Administration of broth, a natural food, caused an equivalent increase in plasma taurine, indicating that the response occurs normally following the ingestion of most meals.

Example 3

The effect of administration of three doses of 10 milligrams per kilogram body weight of beta-alanine per day (i.e., administered in the morning, noon, and at night) for seven days on the plasma concentration profiles of beta-alanine and taurine were investigated. The plasma concentration profiles following administration of 10 milligrams per kilogram body weight of beta-alanine were studied in three subjects at the start and end of a seven-day period during which they were given three doses of the beta-alanine per day.

Three male subjects of normal health, aged between 33 - 53 years were studied. Test subjects received three doses per day of 10 milligrams per kilogram body weight of beta-alanine for eight days. In two subjects, this was followed by a further 7 days (days 9 - 15) when three doses of 20 milligrams per kilogram body weight per day were given. Subjects reported at 8 am to the blood collection laboratory on days 1 (prior to any treatment given), 8 and 15 following an overnight fast. Subjects were asked not to consume any meat containing meal during the 12 hours preceding the study. On each of these three test days subjects were catheterized and an initial blood sample taken when the beta-alanine was administered at or close to 9 am, 12 noon, and 3 pm. Blood samples were drawn after 30, 60, 120 and 180 minutes, and analyzed for changes in the plasma concentration of beta-alanine and taurine. 24-hour urine samples were collected over each day of the study and analyzed by HPLC to determine the excretion of beta-alanine and taurine. The treatments are summarized in Table 5.

Table 5

Treatment Day beta-alanine	Day 1 10 mg/kg bwt	Day 8 10 mg/kg bwt	Day 15 20 mg/kg bwt
1	+	+	+
2	+	+	+
3	+	+	

The plasma beta-alanine concentrations are summarized in Fig. 9. Each dose resulted in a peak beta-alanine concentration at one-half hour or one hour after ingestion followed by a decline to a 0-10 micromolar basal level at three hours, just prior to administration of the next dose. The response on day 8 of the treatment tended to be less than on day 1, as indicated by the area under the plasma concentration curve.

Example 4

The effect of administration of three doses of 40 milligrams per kilogram body weight of beta-alanine per day (i.e., administered in the morning, noon, and at night) for 2 weeks on the carnosine content of muscle and isometric endurance at 66% of maximal voluntary contraction force was investigated.

Six normal male subjects, aged 25 to 32 years, that did not have evidence of metabolic or muscle disease were recruited into the study. The subjects were questioned regarding their recent dietary and supplementary habits. None of subjects was currently taking supplements containing creatine, or had done so in recent testing supplementation procedures. The physical characteristics of the test subjects are summarized in Table 6.

Table 6

Subject	Age (years)	Weight (kg)
1	29	78
2	31	94
3	29	105
4	25	65
5	31	81
6	25	75
7	53	76

Two days before treatment, a preliminary determination of maximal voluntary (isometric) contraction force (MVC) of knee extensors with the subject in the sitting position was carried out. MVC was determined using a Macflex system with subjects motivated by an instantaneous visual display of the force output. For each subject, two trials were carried out to determine endurance at 66% MVC sustained until the target force could no longer be maintained despite vocal encouragement. This first contraction was subsequently followed by a rest period of 60 seconds, with the subject remaining in the isometric chair. After the rest period, a second contraction was sustained to fatigue. Following a second rest of 60 seconds, a third contraction to fatigue was undertaken.

One day before treatment, the subjects reported to the isometric test laboratory between 8 and 10 am. MVC was determined and endurance at 66% MVC over three contractions with 60 second rest intervals, as described above, was determined. Measurements were determined using the subject's dominant leg. A biopsy of the lateral portion of the *vastus lateralis* was taken again from the dominant leg.

On day 1 of the treatment study, subjects reported to the blood sampling laboratory at 8 am following an overnight fast and a minimum of 12 hours since the last meat containing meal. Following catheterization and a basal blood sample, each subject followed the supplementation and blood sampling protocol described in Example 3. A dose of 10 milligrams per kilogram body weight of beta-alanine was administered at time 0 (9 am), 3 hours, and 6 hours.

On days 2-15, subjects continued to take three doses of 10 milligrams per kilogram body weight of beta-alanine.

In the morning of day 14, post-treatment isometric exercise tests were conducted on the dominant leg to determine MVC and endurance at 66% MVC relative to the 66% MVC measured on the day prior to treatment. In the afternoon, a muscle biopsy was taken of the *vastus lateralis* from close to the site of the biopsy taken on the day before treatment.

On day 15, the procedures followed on day 1 were repeated to determine any overall shift in the plasma concentration profile of beta-alanine and taurine over the 15 days of supplementation. Mean changes in plasma beta-alanine over 9 hours following the oral ingestion of 10 milligrams per kilogram body weight of beta-alanine at 0, 3 and 6 hours on days 1 and 15 when dosing at 3 x 10 milligrams per kilogram body weight per day are shown in Fig. 10.

One additional test subject (number 7) followed the study, taking three doses 10 milligrams per kilogram body weight for 7 days followed by three doses of 20 milligrams per kilogram body weight for 7 days. No muscle biopsies were taken from this test subject.

There was no apparent change in the muscle carnosine content in the muscle of the six subjects biopsied. Changes in plasma taurine concentrations in the six subjects mirrored those of beta-alanine, as noted in Example 2.

Values from the MVC and endurance at 66% MVC measurements one day before treatment and after 14 days after treatment with three doses of 10 milligrams per kilogram body weight of beta-alanine are listed in Table 7. The mean endurance time at

66% MVC increased in 5 of the 6 subjects. An increase was also seen in subject 7 who had taken the higher dose.

Table 7

Subject	MVC 1st try N	MVC 2nd try N	time @ 66% MVC 1st seconds	time @ 66% MVC 2nd seconds	time @ 66% MVC 3rd seconds	Total Contraction Time seconds
Pre						
1	784.5	821.9	48.53	29.03	23.78	100.83
2	814.4	886.2	48.40	26.03	16.90	91.33
3	984.9	970.4	38.15	26.03	16.78	80.95
4	714.6	740.4	89.03	56.15	45.65	190.83
5	1204.8	1217.2	37.65	27.64	21.53	86.83
6	722.4	716.8	46.78	29.40	21.90	98.08
Pre mean	870.9	892.1	51.4	32.4	24.3	108.1
Pre SD	190.6	184.6	19.1	11.7	10.8	41.2
Post						
1	895.6	908.0	47.08	30.38	24.03	101.48
2	832.2	908.0	46.65	31.28	18.40	96.33
3	973.7	952.2	42.65	25.03	16.03	83.70
4	814.1	863.9	114.40	64.28	48.53	227.20
5	1246.6	1233.0	42.03	22.78	19.40	84.20
6	760.8	773.3	52.28	31.53	25.95	109.73
Post mean	920.5	939.7	57.5	34.2	25.4	117.1
Post SD	175.7	156.0	28.1	15.2	11.9	54.9
Subject 7						
Pre	858.18	861.54	54.0			
Post	792.54	851.41	62.0			

Example 5

The effect of 4 weeks of beta-alanine supplementation using two dosing regimens and an isomolar dose of L-carnosine (beta-alanylhistidine) administered over 4 weeks on the muscle carnosine content were investigated.

Fifteen male subjects, aged 20 to 29 years with no obvious signs of clinical disease and with heights and weights within the normal range, were recruited into the study (Table 8). All subjects participated in one or more sports and all ate a mixed diet containing variable amounts of meat. A record of each subject's approximate intake of meat during the course of the investigation was made.

Table 8: Summary of subjects' physical characteristics for each of the three treatment groups:

	AGE	HEIGHT	MASS
Treatment	Mean ± SD	Mean ± SD	Mean ± SD
1	24.4 ± 2.7	182.3 ± 7.5	80.0 ± 15.9
2	23.8 ± 1.9	180.9 ± 5.4	80.6 ± 8.6
3	24.0 ± 3.8	180.1 ± 3.8	80.4 ± 12.1

Five subjects were allocated to one of three treatment groups (1, 2, and 3). During the study, their diet was supplemented with either beta-alanine or carnosine as described in Table 9 (Figure 17). The supplements were provided in soft gelatine capsules containing either 400mg beta alanine or 500mg carnosine.

In Group 1, beta-alanine was administered in 4 separate doses throughout the day (qid) at a steady rate for four weeks.

In Group 2, beta-alanine was administered as 8 separate doses throughout the day, rather than as 4 doses, in an attempt to maintain a more even increase in the blood-plasma concentration. In addition, the dose was increased progressively each week by 800mg per day.

In Group 3, carnosine was administered at approximately the same isomolar dose as in Group 2, again divided into 8 doses. This treatment, therefore, contained approximately the same amount of beta-alanine as in Group 2, when hydrolyzed to its constituent amino acids.

The subjects took the supplements at the times indicated in Table 9 (Figure 17). A single muscle biopsy of the *vastus lateralis* was taken before and at the end of the supplementation using the percutaneous needle biopsy procedure of Bergström (1962). In brief, the procedure involves the insertion of a hollow bored needle under local anesthetic and sterile conditions to obtain specimens around 20-40 mg containing approximately 100-700 muscle fibers. The skin and subcutaneous tissue is anesthetized with 1% lignocaine (avoiding contact with the muscle). An incision is made to the skin and deep fascia with a scalpel blade. The needle minus central rod is inserted into the muscle. The muscle bulk is pressed into a needle side-window. A sample is cut by ramming an inner, sharpened cylinder along the needle. The needle is removed and the central rod is used to evacuate the specimen. The wound is then closed.

Muscle samples from the subjects were frozen in liquid nitrogen, freeze-dried and analyzed for muscle carnosine and taurine contents by HPLC. Table 9 (Figure 17) shows a breakdown of the dosing strategies employed in each of the three treatment groups. Results: There was no change in body mass in either beta-alanine or carnosine supplemented subjects.

Changes in muscle carnosine (Table 10 and figures 12 and 13).

A significant increase in muscle carnosine content was recorded for the subjects in Groups 1 and 3. In Group 2, one subject (no. 10) with the highest initial carnosine content (initial carnosine content: 33.3 mmol/kg dry muscle) showed no change in his muscle carnosine content (post content: 33.7 mmol/kg dm). When this subject was deleted from Group 2, this group showed a significant increase of the same order as seen in the other Groups. Subject 10 was a medium to high consumer of meat and otherwise unremarkable.

Supplementation with either beta-alanine or carnosine at the same dose (Groups 2 and 3) appeared to be equally effective in increasing the muscle carnosine content.

The pattern of change is reminiscent of the changes observed with creatine loading and may suggest that there is a threshold which is quickly reached, with further supplementation having no further effect. In the case of subject 10, while not wishing to be bound by this theory, a threshold appears to have been reached even before the start of supplementation. However, there are exceptions to the notion of an upper threshold, notably subjects 6 (post supplementation carnosine: 45.9 mmol/kg dry muscle) and 15

(post supplementation carnosine: 68.9 mmol/kg dm). Subjects 6 and 15 were unremarkable in either their dietary patterns or participation in physical exercise.

Table 10 is a summary of data for carnosine muscle concentrations for treatment Groups 1 to 3. Treatment Group 2, in italics, is without subject 10 who did not exhibit an increase in muscle carnosine concentration. The initial carnosine concentration in subject 10 was the highest of all subjects and may have already been at an "upper threshold" level prior to supplementation.

Table 10

Treatment	1	2	*2*	3
	n = 5	n = 5	*n = 4*	n = 5
Mean pre	19.58	24.23	*21.96*	23.15
SD pre	3.71	5.27	*1.64*	5.07
Mean post	27.38	35.27	*35.67*	39.52
SD post	2.96	6.18	*7.08*	16.95
Mean difference	7.80	11.04	*13.72*	16.37
SD difference	0.81	9.20	*8.08*	12.06
Sign	***	ns	*	*
Min difference	6.99	0.35	*8.62*	7.05
Max difference	9.08	25.77	*25.77*	37.39
Mean% change	42.1	51.6	*64.2*	65.8
SD % change	14.9	46.5	*42.7*	31.8
Min % change	31.5	1.1	*41.0*	38.2
Max % change	68.0	128.2	*128.2*	118.7

Changes in muscle beta-alanine and histidine (Table 11 and figure 14)

The muscle beta-alanine concentration was below the limit of detection (< 0.2 mmol/kg dm) before and at the end of supplementation. In some subjects, a final dose of beta-alanine was taken within 1 to 2 hours of the final muscle biopsy.

There was no change in the muscle histidine concentration with supplementation with either beta-alanine or carnosine, the latter having the potential to release histidine into the general circulation. There was no decrease in the histidine concentration in response to the increased synthesis of carnosine (each mole requiring one mole of histidine).

Changes in muscle Taurine (Table 12 and figure 15 and figure 16)

While beta-alanine at high concentrations may interfere with the uptake of taurine into tissues, previous observations show an increase in the plasma taurine concentration and loss of taurine in urine following both beta-alanine and carnosine administration, no loss of muscle taurine was noted in this study in any of the three Groups. Marked changes in the muscle taurine content occurred in some individuals, but both increases and decreases were observed. Figure 15 is a graph illustrating data showing muscle concentration (mean +SD) of taurine before and post supplementation in four different treatment groups. Figure 16 is a graph illustrating data showing muscle concentration (mean +SD) of taurine before and post supplementation in different subjects.

Conclusions

These studies demonstrate that the supplements of beta-alanine and carnosine of the invention have the potential to increase the muscle carnosine content. Based on the test results, they appear to be equally effective in increasing carnosine in tissue.

The changes in the muscle buffering capacity help maintain the intracellular microenvironment during intense exercise, countering the accumulation of H^+. As such, supplementation with beta-alanine or compounds delivering beta-alanine on ingestion may have a positive effect on exercise capacity in sports and those general daily activities leading to lactate accumulation. In view of the other chemical activities ascribed to carnosine (as an anti-oxidant and anti-glycating agent), an increase in carnosine concentration may have other beneficial effects apart from those arising from an increase in muscle buffering capacity.

Four weeks of supplementation did not result in any apparent loss of taurine in the muscles.

Table 11: summary data for histidine muscle concentrations in treatment Groups 1 to 3:

Treatment	1	2	3
	n = 5	n = 5	n = 5
mean pre	5.76	5.56	7.01
SD pre	0.59	0.63	2.60
mean post	5.12	5.51	5.38
SD post	1.17	0.87	1.39
mean diff	-0.64	-0.05	-1.63
SD diff	1.24	0.70	2.87
Sign	ns	ns	ns
% change	-10.59	-0.78	-17.47
SD % change	21.65	12.65	29.56

Table 12: Summary data for taurine muscle concentrations in treatments Groups 1 to 3:

Treatment	1	2	3
	n = 5	n = 5	n = 5
Mean pre	36.52	28.68	35.40
SD pre	7.77	5.29	8.92
Mean post	33.70	27.54	32.32
SD post	7.98	8.77	15.19
Mean difference	-2.82	-1.15	-3.08
SD difference	7.96	6.04	13.61
Sign	ns	ns	ns
% change	-6.28	-4.46	-7.66
SD % change	21.54	24.06	34.97

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

51978-12

CLAIMS:

1. A dietary supplement comprising:

glycine, and an amino acid of beta-alanine or ester or amide thereof; or

glycine, an amino acid of beta-alanine or ester or amide thereof, and L-histidine,

wherein the amino acid of beta-alanine is not part of a dipeptide, an oligopeptide or a polypeptide.

2. The dietary supplement of claim 1, further comprising at least a creatine or a carbohydrate.

3. The dietary supplement of claim 1, further comprising an insulin mimic, and wherein the insulin mimic comprises a D-pinitol (3-O-methyl-chiroinositol), a 4-hydroxy isoleucine, a demethyl-asterriquinone B-1 compound, an alpha lipoic acid, a R-alpha lipoic acid, a guanidiniopropionic acid, a vanadium compound, a vanadium complex or a synthetic phosphoinositolglycan peptide.

4. The dietary supplement of claim 1, further comprising an insulin-action modifier, and wherein the insulin-action modifier is a sulphonylurea, a thiazolidinedione or a biguanide.

5. The dietary supplement of claim 1, wherein the dietary supplement is a sports drink.

6. The dietary supplement of claim 1, wherein the dietary supplement is a supplement for humans.

7. The dietary supplement of claim 1, comprising at least 0.2, 0.3, 0.4,0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 or 5 grams of the amino acid of beta-alanine or ester or amide thereof.

51978-12

8. The dietary supplement of claim 1, comprising at least 0.2,0.3,0.4,0.5, 1.0, 1.5,2.0, 2.5, 3.0 grams of the amino acid of beta-alanine or ester or amide thereof in an injectable form.

9. The dietary supplement of claim 1, comprising at least 200, 250, 300, 450, 500, 550, 600, 650, 700, 750 or 800 mg of the beta-alanine or ester or amide thereof.

10. The dietary supplement of claim 9, wherein the dietary supplement is formulated in an ingestible or an injectable formulation.

11. The dietary supplement of claim 10, wherein the ingestible formulation is a drink, a gel, a food or a tablet.

12. Use of an effective amount of a dietary supplement comprising:

glycine, and an amino acid of beta-alanine or ester or amide thereof; or

glycine, an amino acid of beta-alanine or ester or amide thereof, and L-histidine to increase the anaerobic working capacity in a tissue, and

wherein the amino acid of beta-alanine is not part of a dipeptide, an oligopeptide or a polypeptide.

13. Use of a dietary supplement comprising:

glycine, and an amino acid of beta-alanine or ester or amide thereof; or

glycine, an amino acid of beta-alanine or ester or amide thereof, and L-histidine in the manufacture of a dietary supplement to increase the anaerobic working capacity in a tissue, and

wherein the amino acid of beta-alanine is not part of a dipeptide, an oligopeptide or a polypeptide.

14. The use according to claim 13, wherein said dietary supplement is formulated for oral administration, administration to a blood or blood plasma or a combination thereof.

15. The use according to claim 13, wherein said dietary supplement is for administration at a total dosage of the beta-alanine of at least 0.2 gram for a 24-hour period.

16. The use according to claim 13, wherein said dietary supplement is for administration at a total dosage of the beta-alanine of between about 0.2 gram and about 6.4 gram for a 24-hour period.

17. The use according to claim 13, wherein said dietary supplement is for administration over a period of at least 3 days.

18. The use according to claim 13, wherein said dietary supplement is for administration over a period of at least about 3 days to about two weeks.

19. The use according to claim 13, wherein the dietary supplement is formulated for administration in multiple doses.

20. The use according to claim 19, wherein the dietary supplement is formulated for administration at least two times to eight times in a 24-hour period.

21. The use according to claim 13, wherein the amount of the dietary supplement for administration is increased daily.

22. The use according to claim 13, wherein the amount of the dietary supplement for administration is increased weekly.

23. The use according to claim 13, wherein the dietary supplement is for administration in treatment periods that last for at least about four weeks.

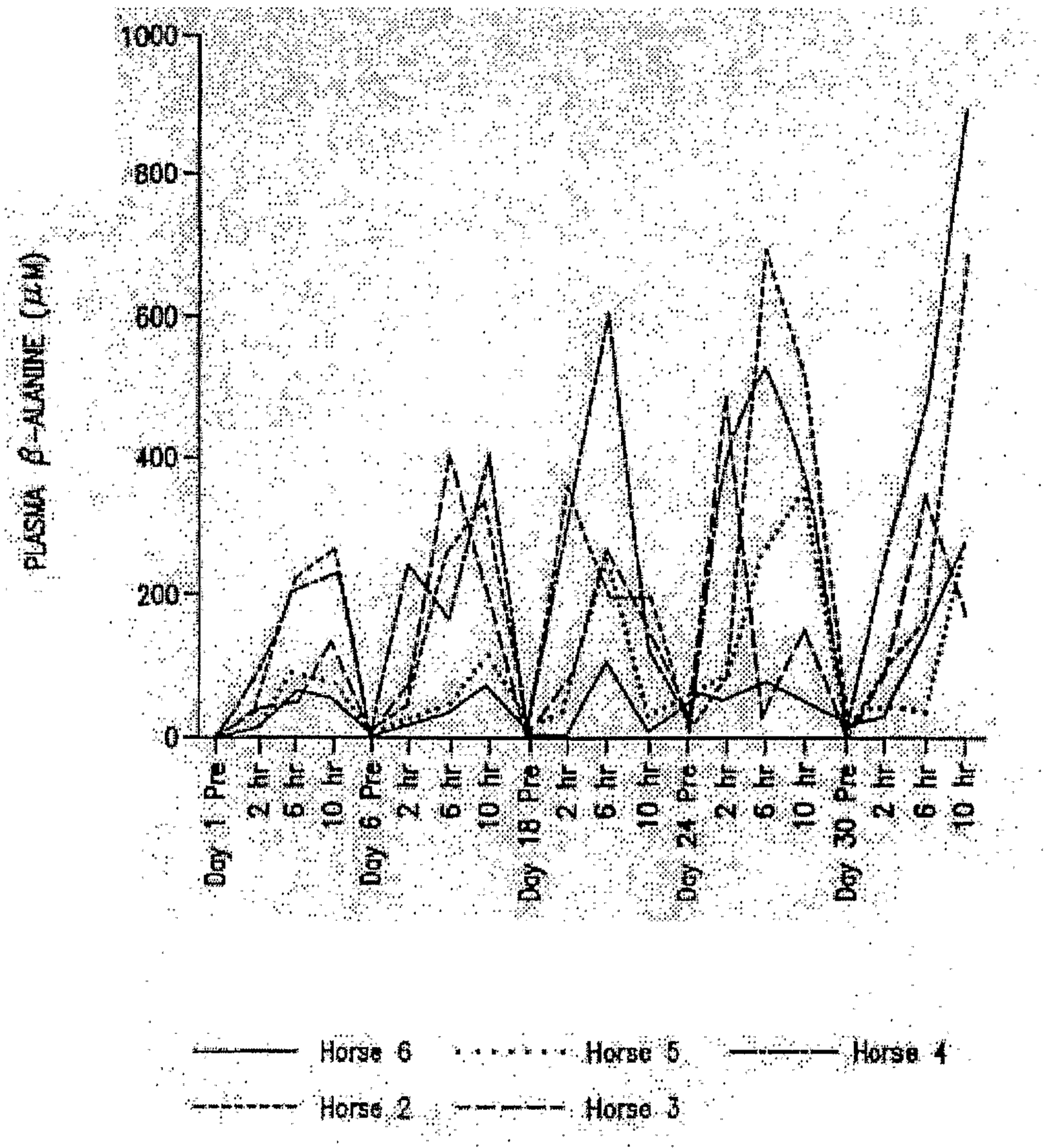
PLASMA β-ALANINE (μM)
1000
800
600
400
200
0
Day 1 Pre
2 hr
6 hr
10 hr
Day 6 Pre
2 hr
6 hr
10 hr
Day 18 Pre
2 hr
6 hr
10 hr
Day 24 Pre
2 hr
6 hr
10 hr
Day 30 Pre
2 hr
6 hr
10 hr
Horse 6
Horse 5
Horse 4
Horse 2
Horse 3

FIG. 1

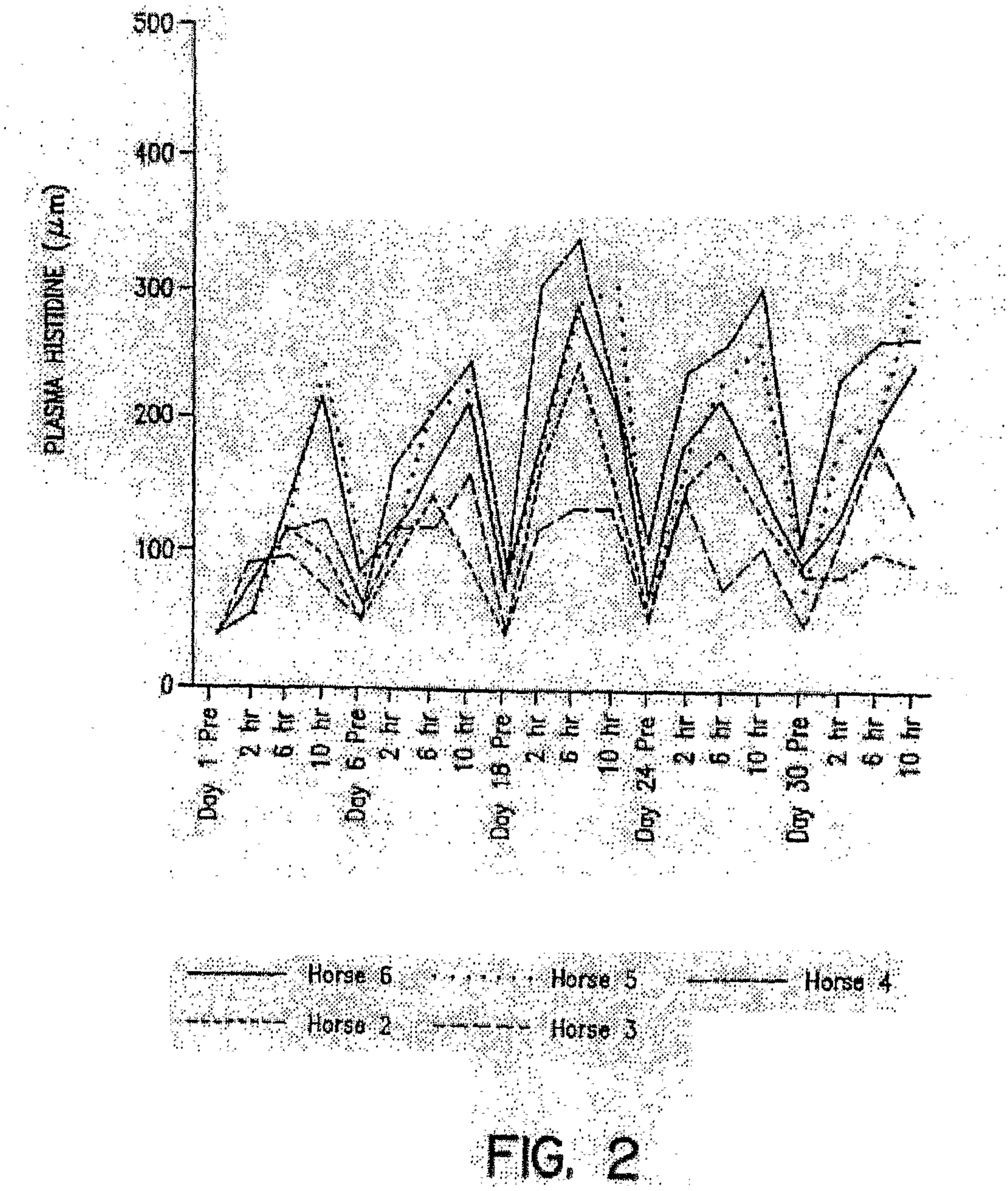
PLASMA HISTIDINE (μm)
500
400
300
200
100
0
Day 1 Pre
2 hr
6 hr
10 hr
Day 6 Pre
2 hr
6 hr
10 hr
Day 18 Pre
2 hr
6 hr
10 hr
Day 24 Pre
2 hr
6 hr
10 hr
Day 30 Pre
2 hr
6 hr
10 hr
Horse 6
Horse 5
Horse 4
Horse 2
Horse 3

FIG. 2

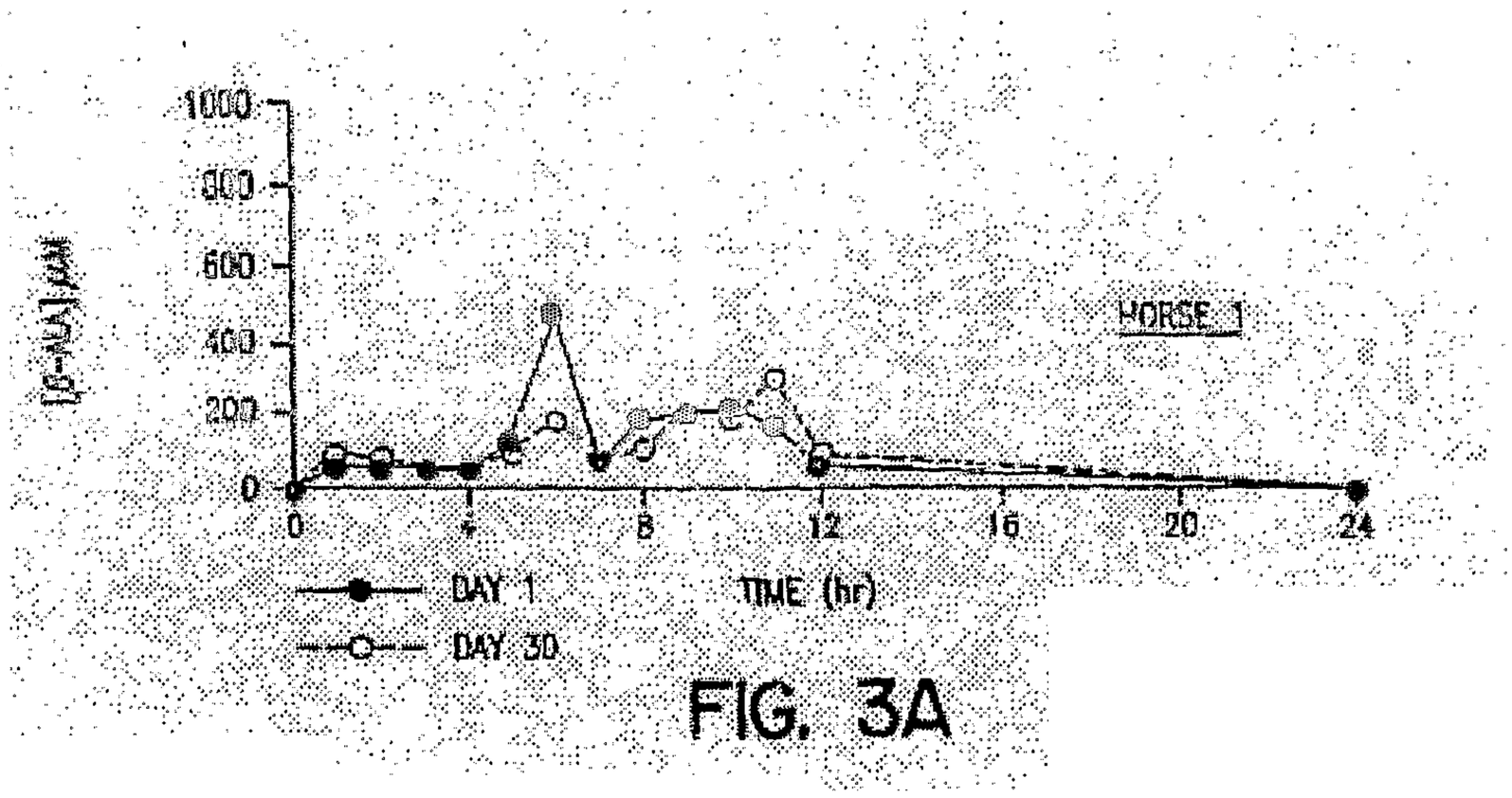

FIG. 3A

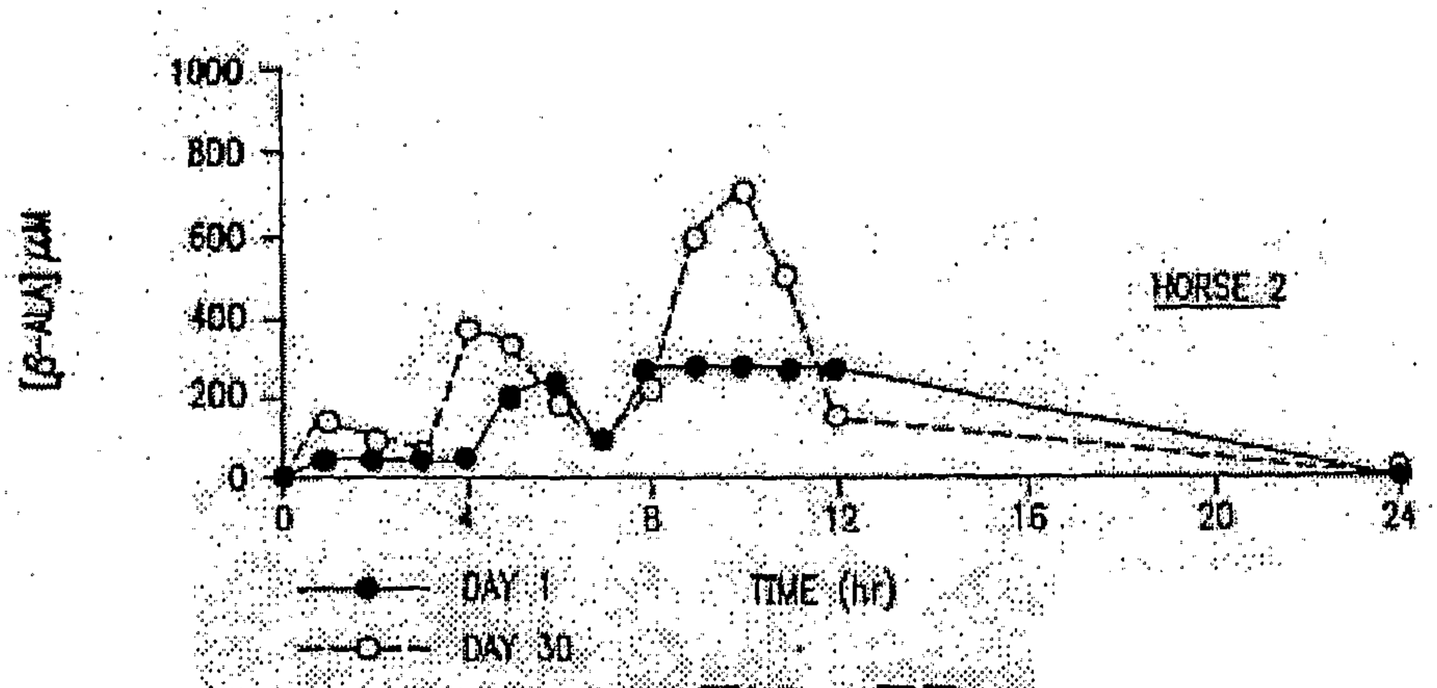

FIG. 3B

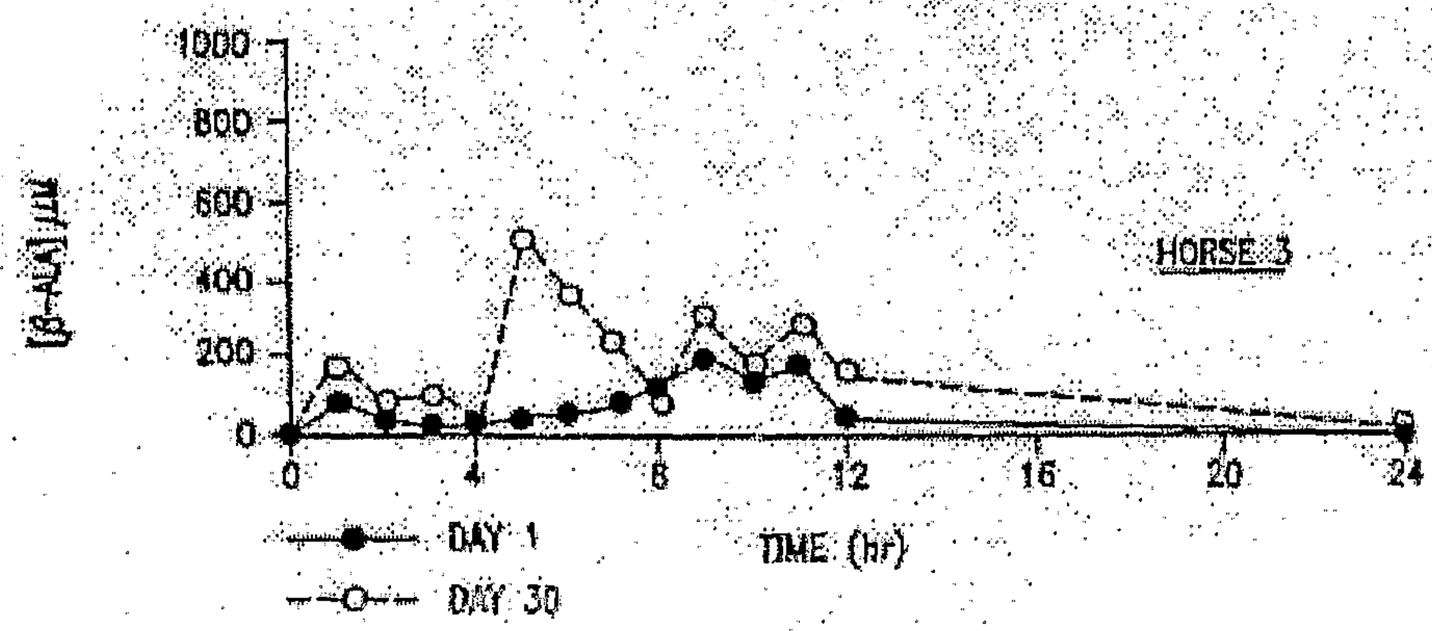

FIG. 3C

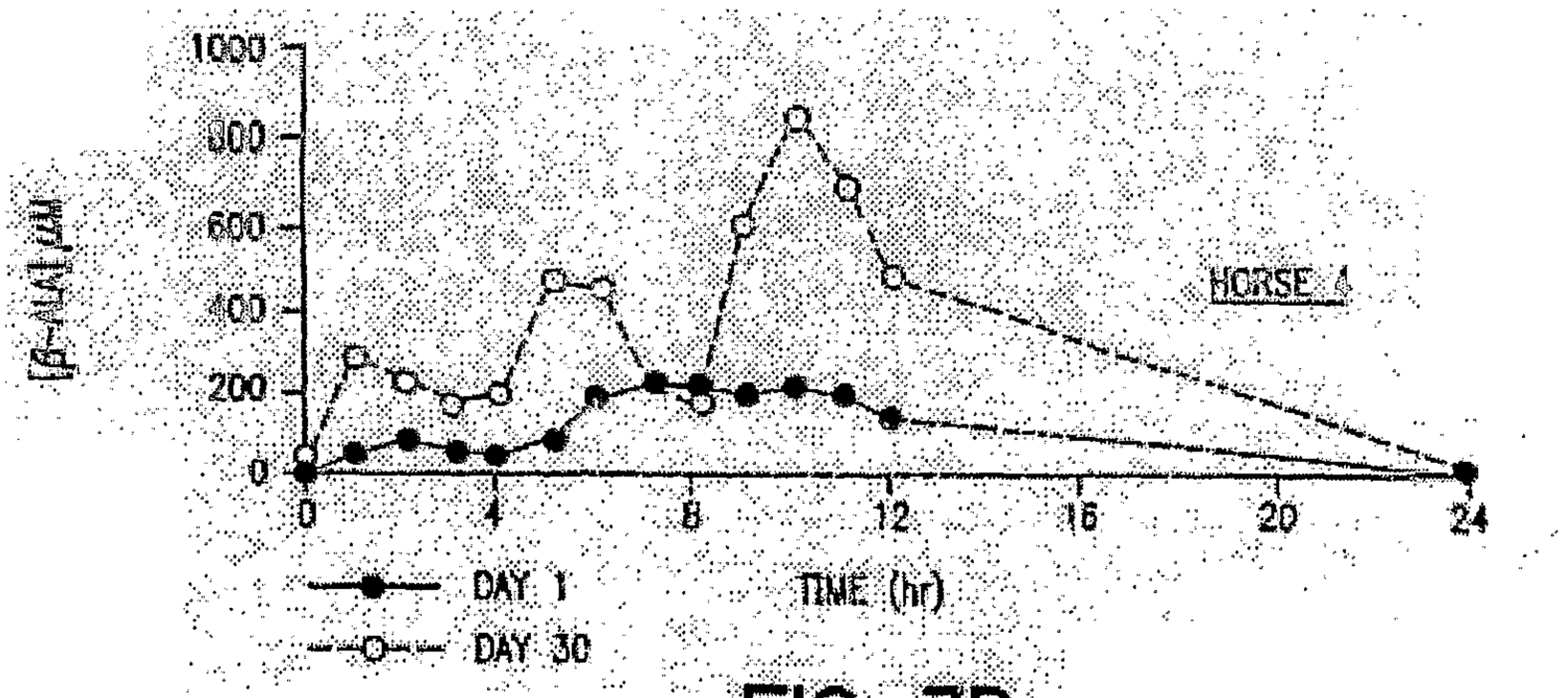

FIG. 3D

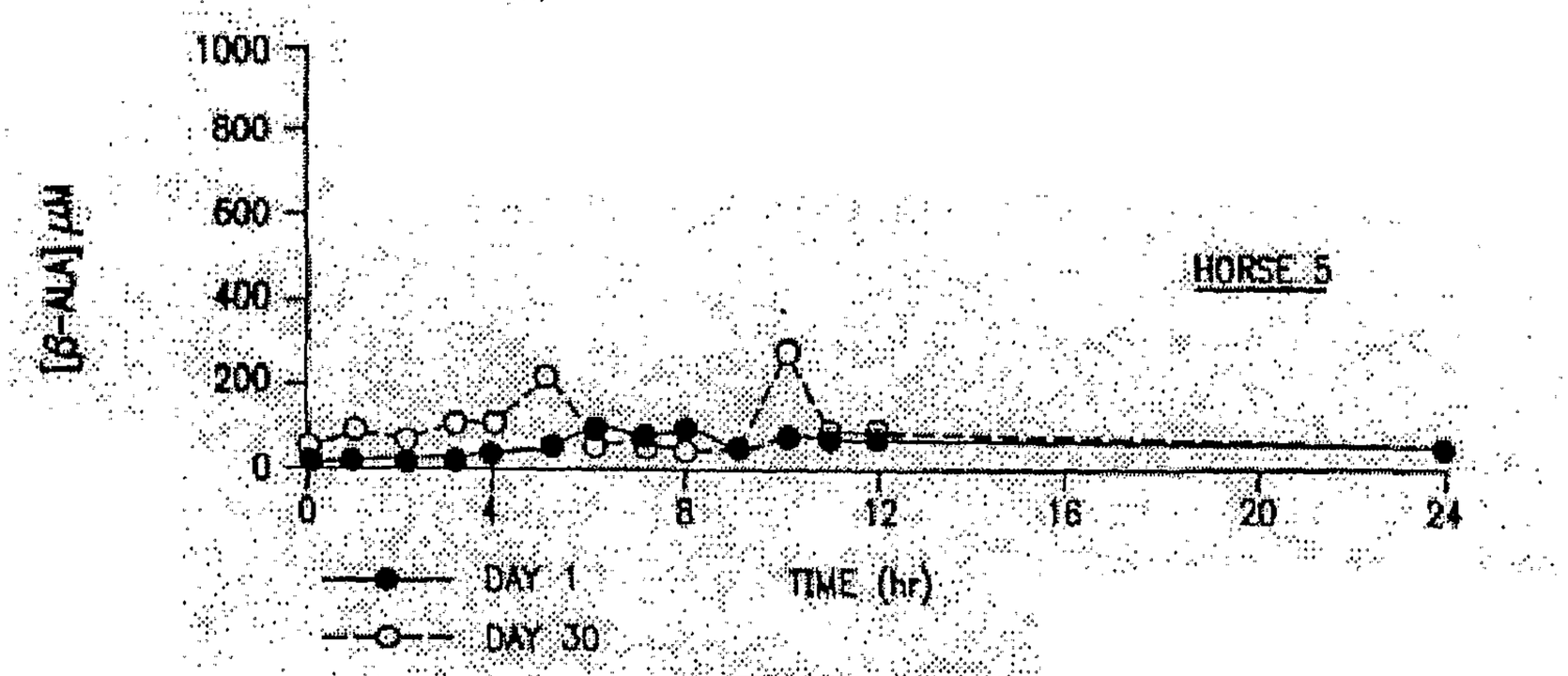

FIG. 3E

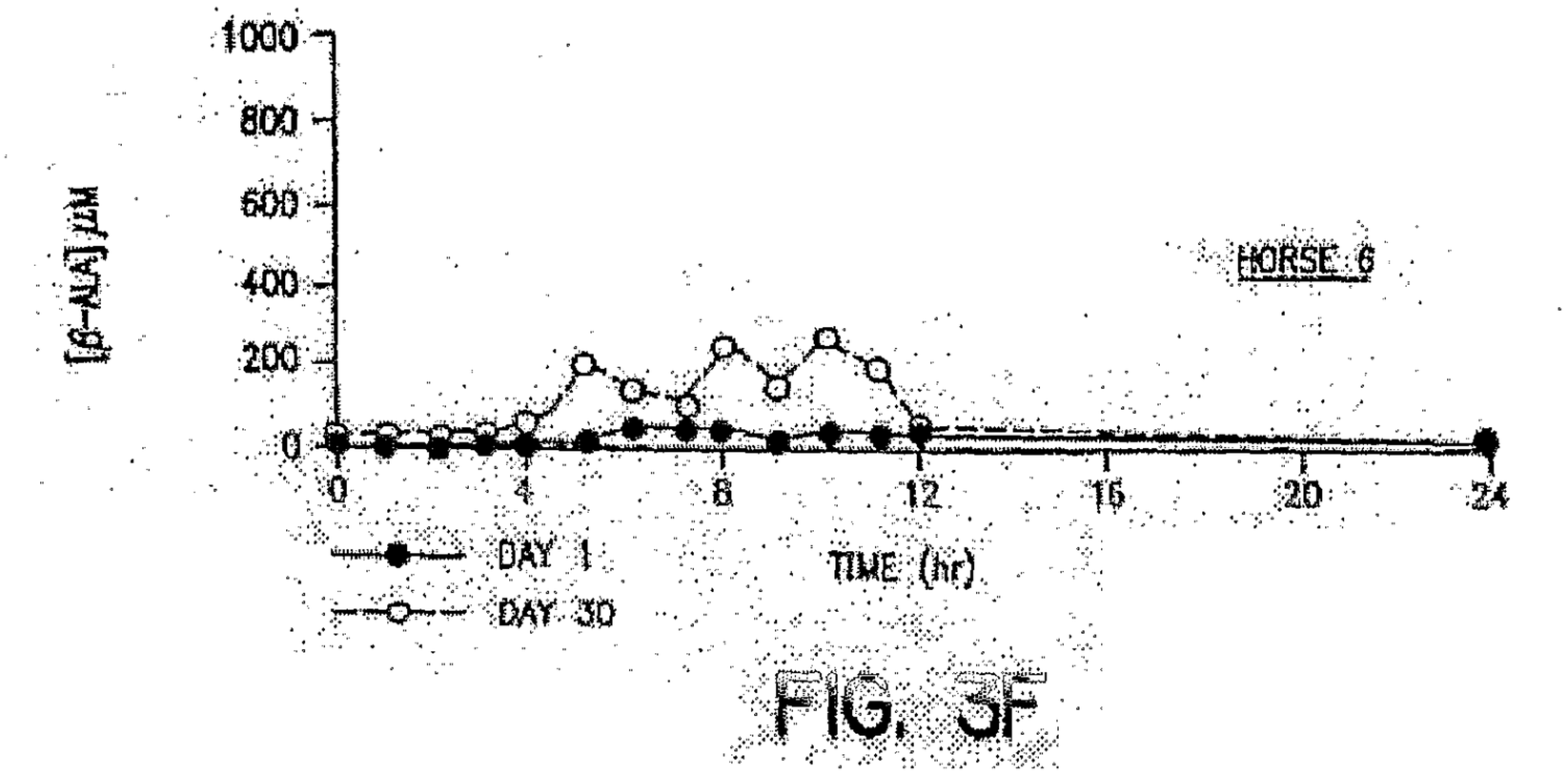

FIG. 3F

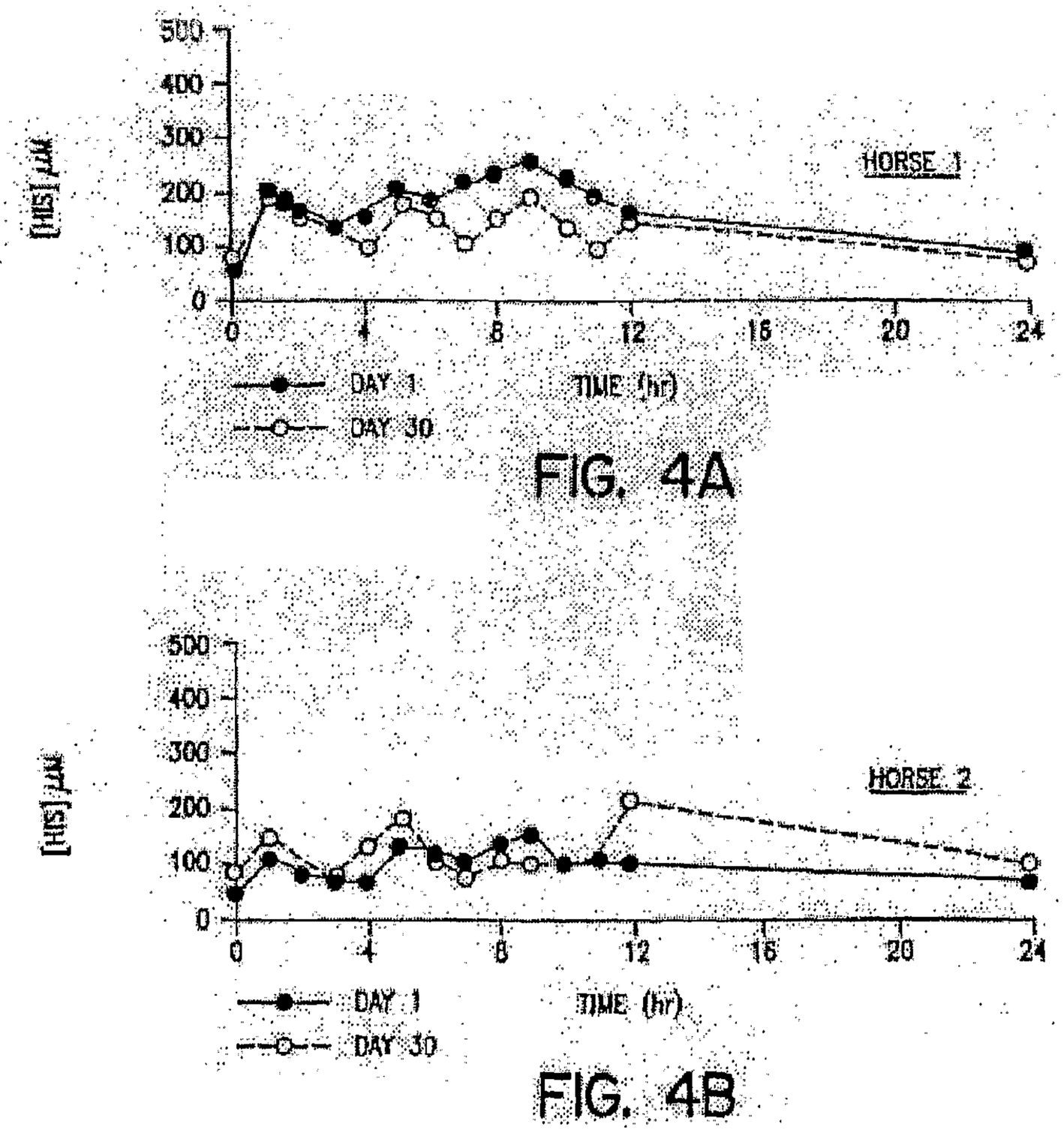

FIG. 4A

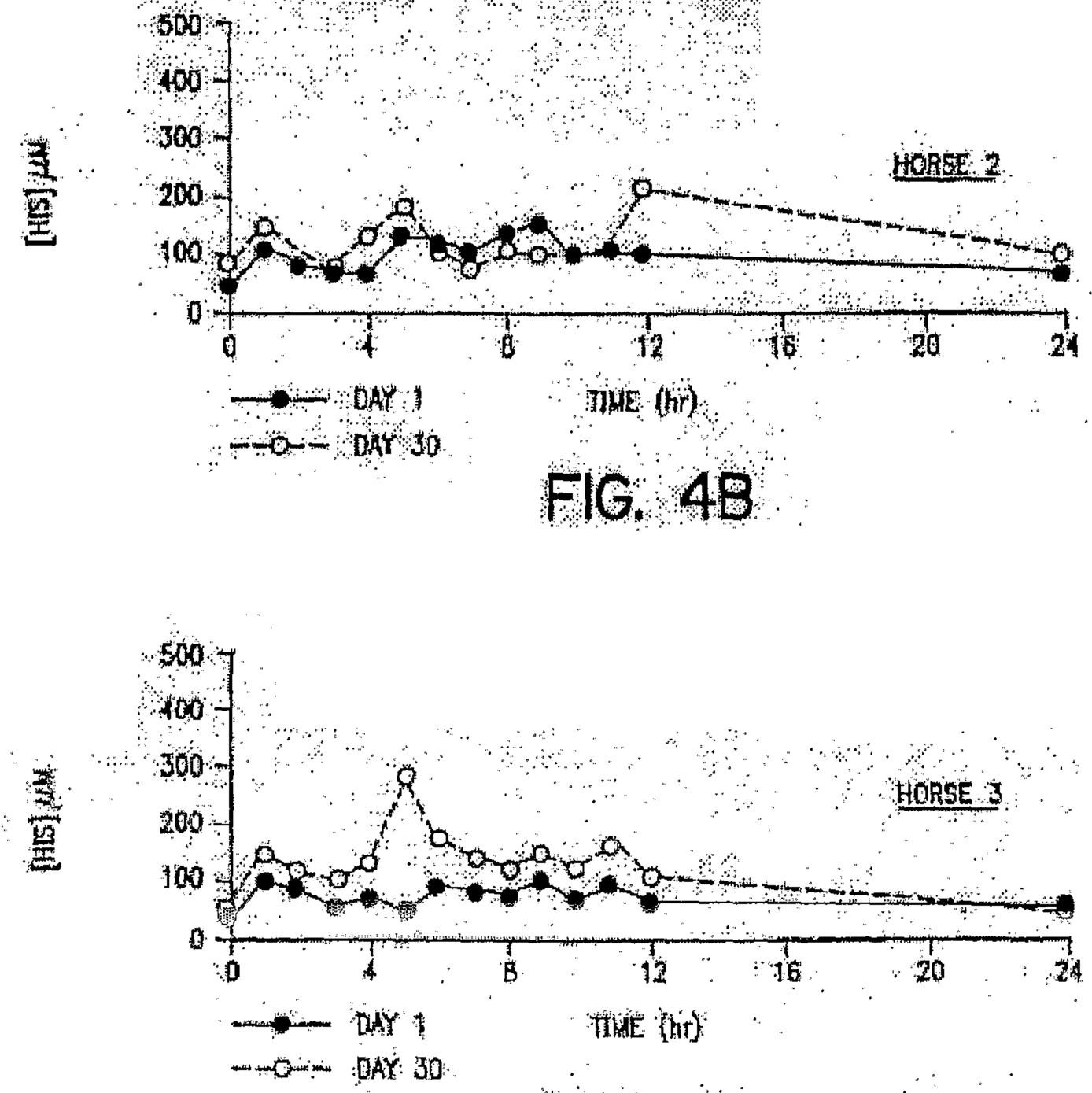

FIG. 4B

FIG. 4C

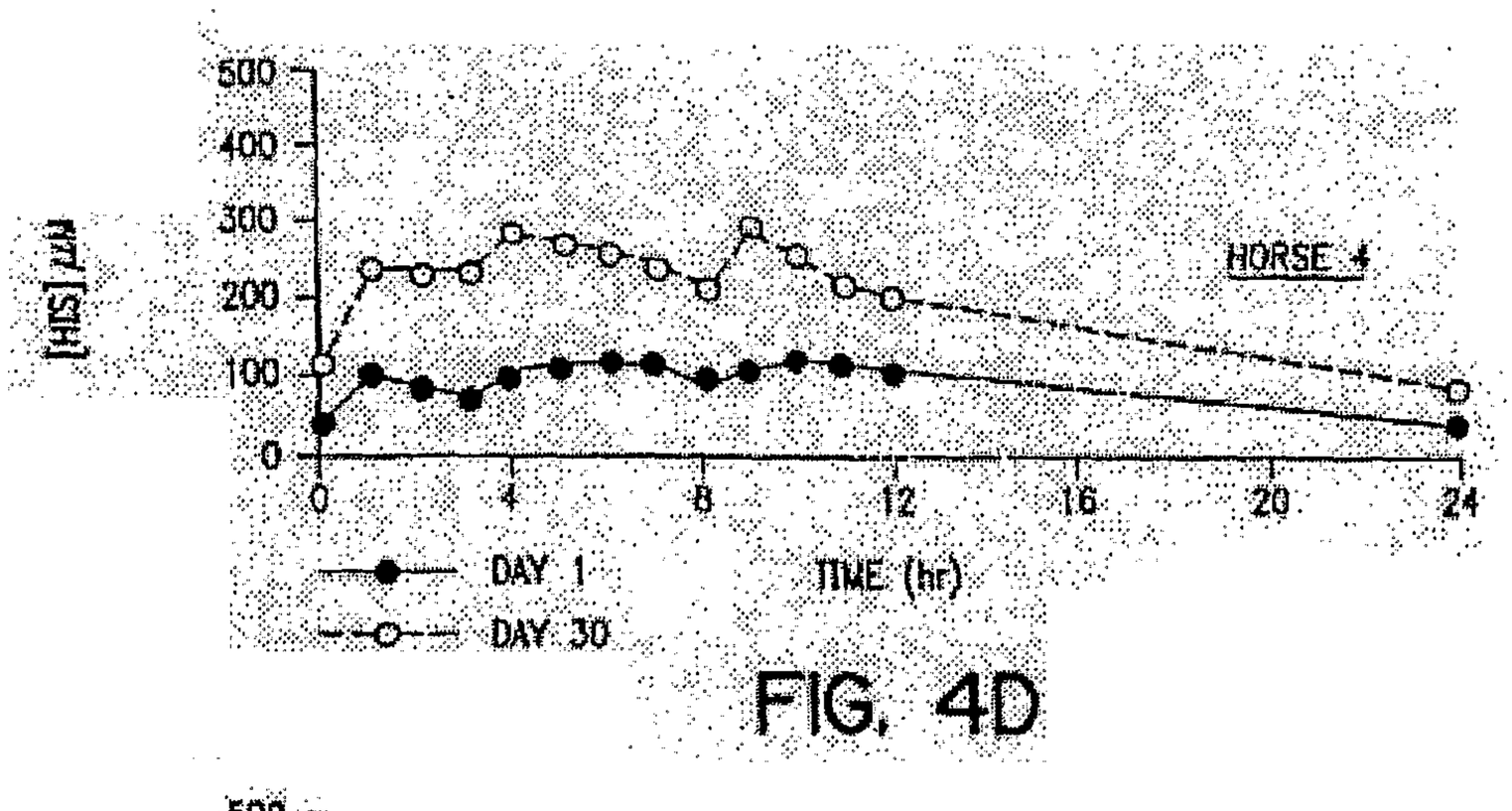

FIG. 4D

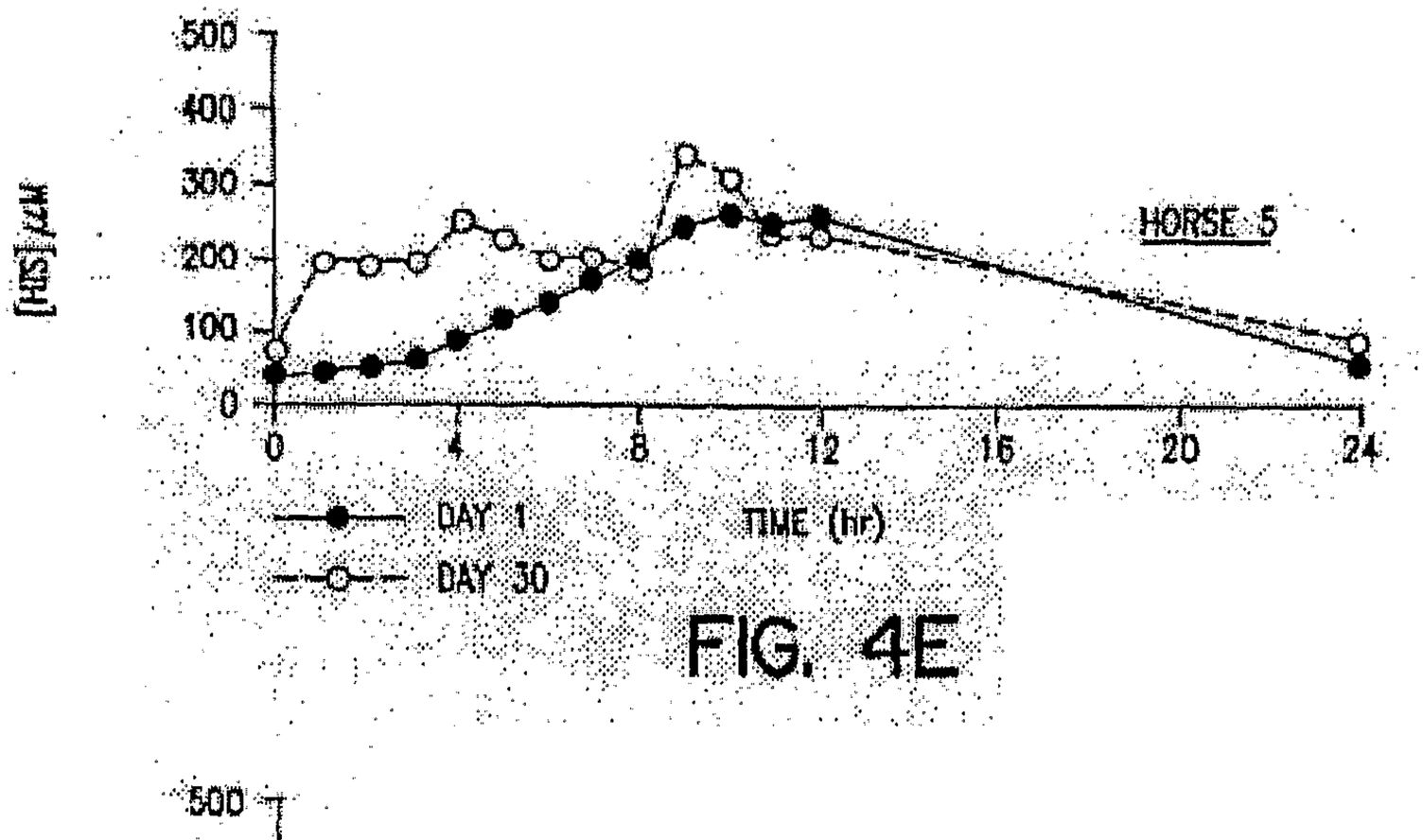

FIG. 4E

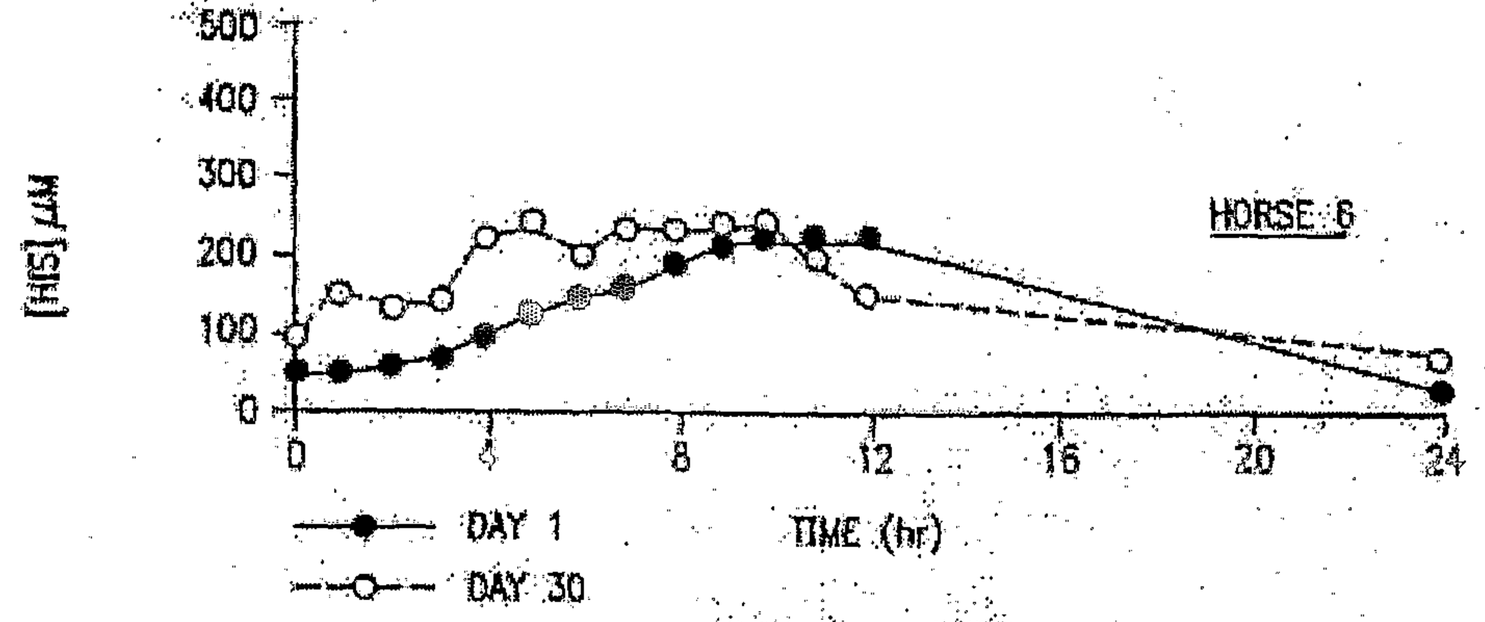

FIG. 4F

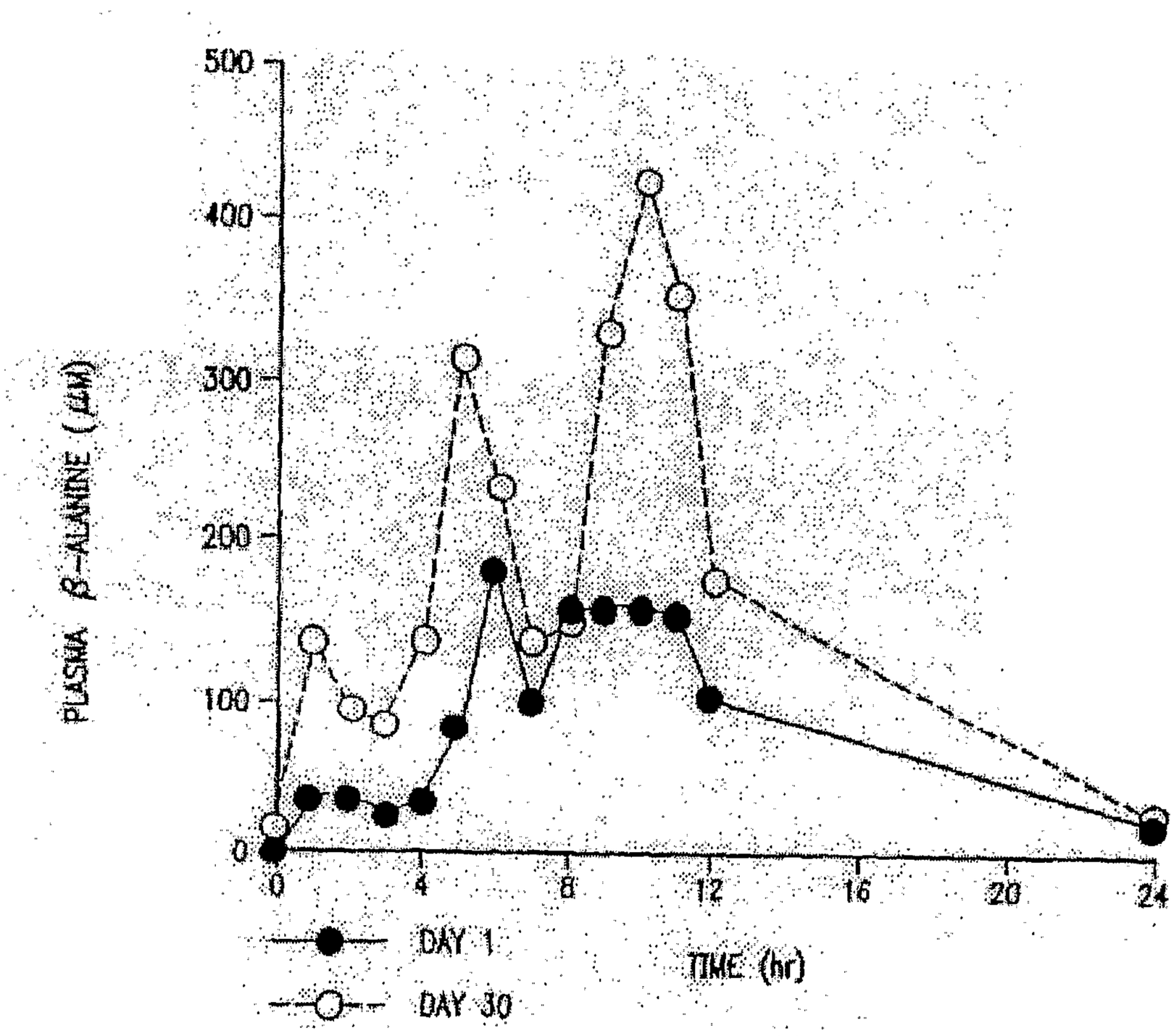
500
400
300
200
100
0
PLASMA β-ALANINE (μM)
0
4
8
12
16
20
24
TIME (hr)
DAY 1
DAY 30

FIG. 5

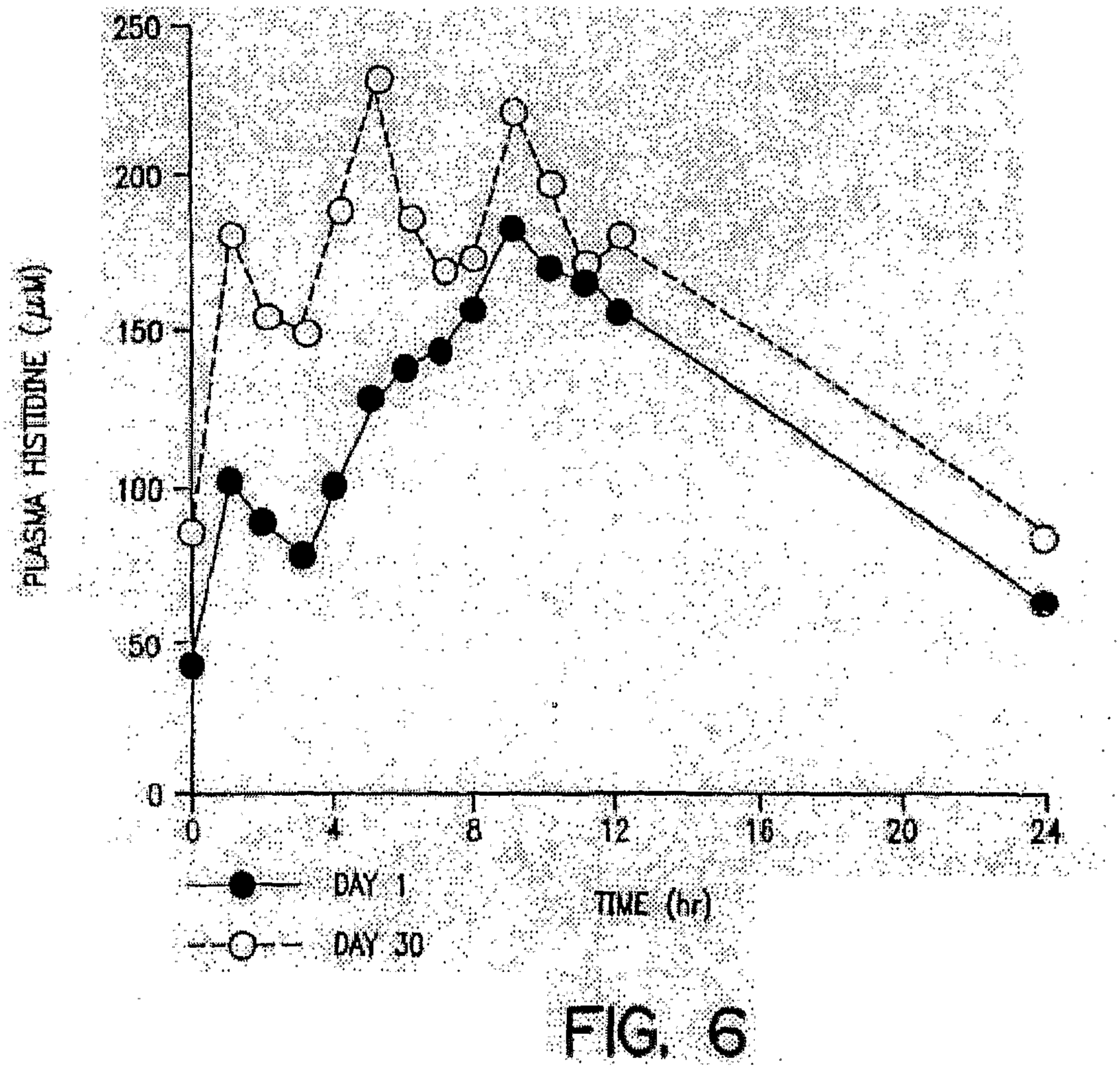
250
200
150
100
50
0
PLASMA HISTIDINE (μM)
0
4
8
12
16
20
24
TIME (hr)
DAY 1
DAY 30

FIG. 6

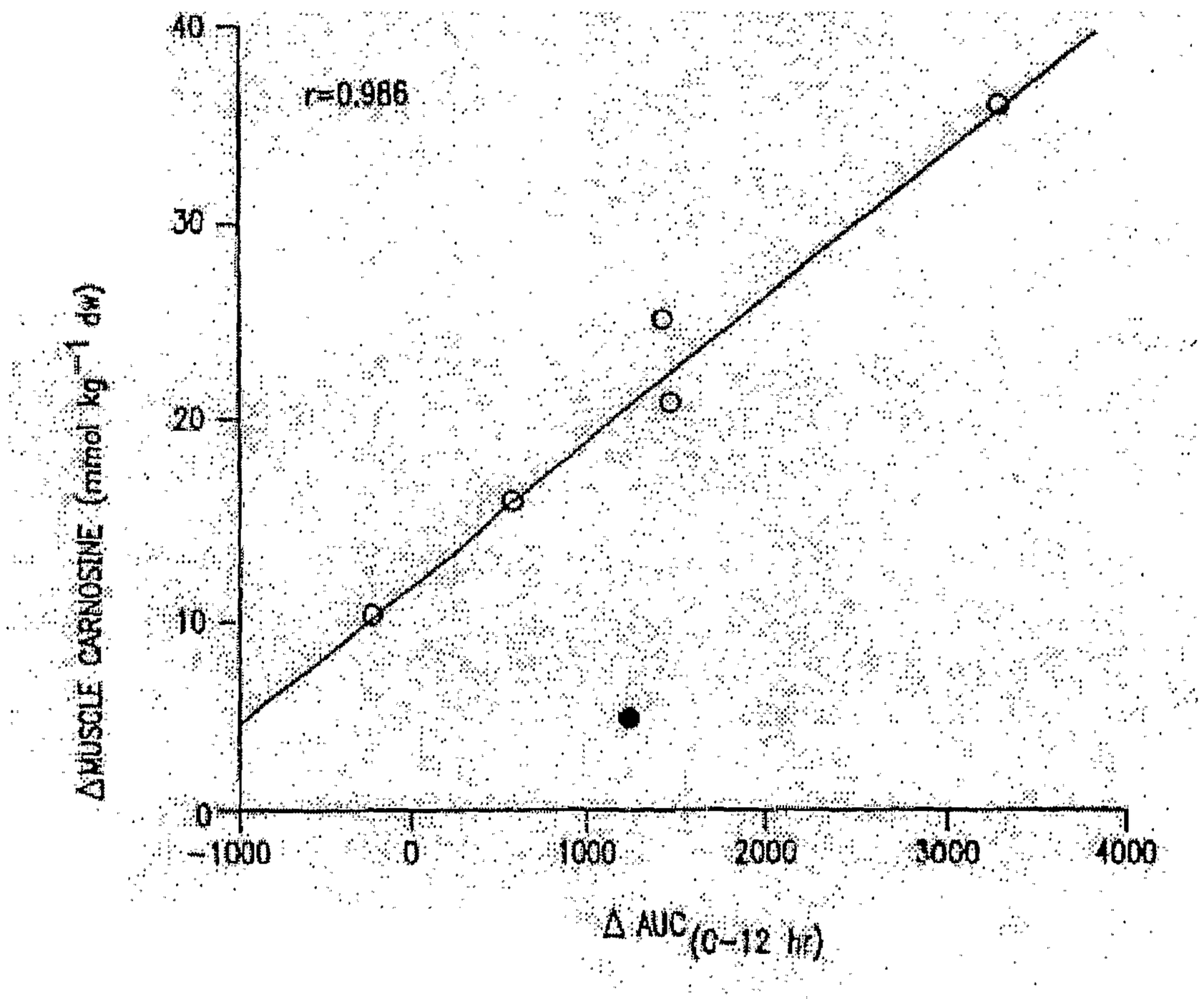
r=0.986
40
30
20
10
0
−1000
0
1000
2000
3000
4000
Δ MUSCLE CARNOSINE (mmol kg⁻¹ dw)
Δ AUC (0–12 hr)

FIG. 7

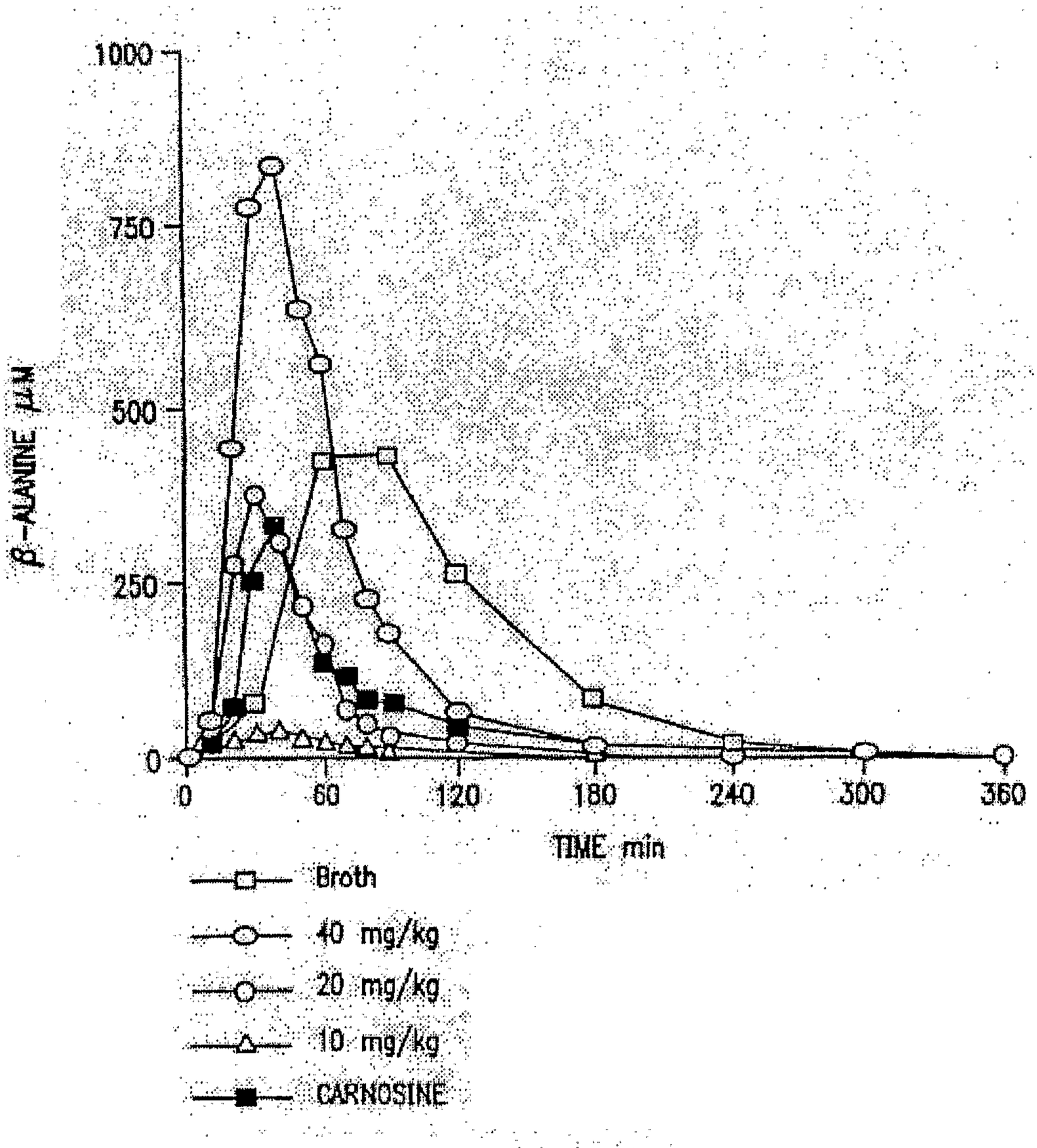
1000
750
500
250
0
β-ALANINE μM
0
60
120
180
240
300
360
TIME min
Broth
40 mg/kg
20 mg/kg
10 mg/kg
CARNOSINE

FIG. 8

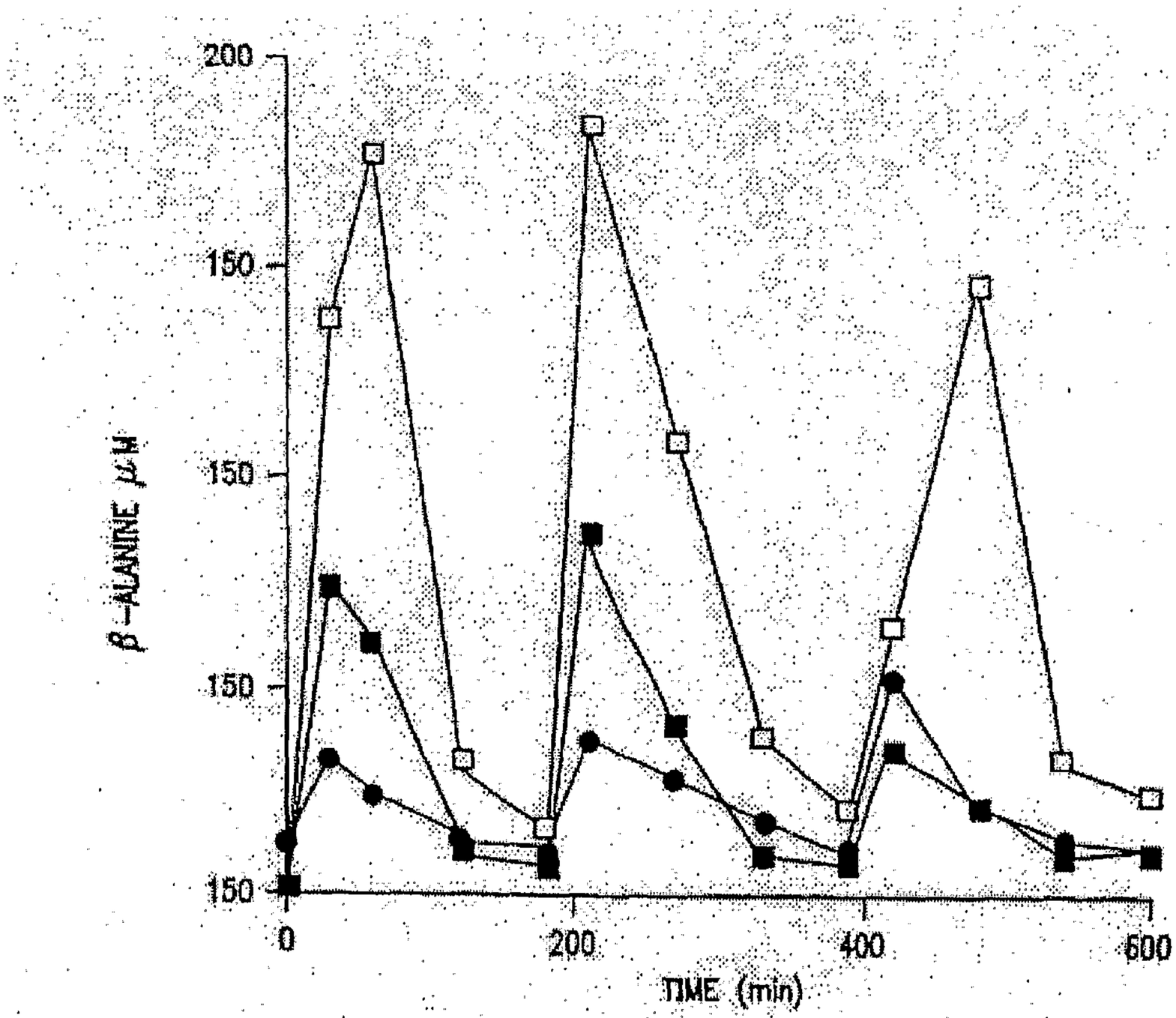
200
150
150
150
β-ALANINE μM
0
200
400
600
TIME (min)
day 1: 3 x 10mg/kg bwt
day 8: 3 x 10mg/kg bwt
day 15: 3 x 20mg/kg bwt

FIG. 9

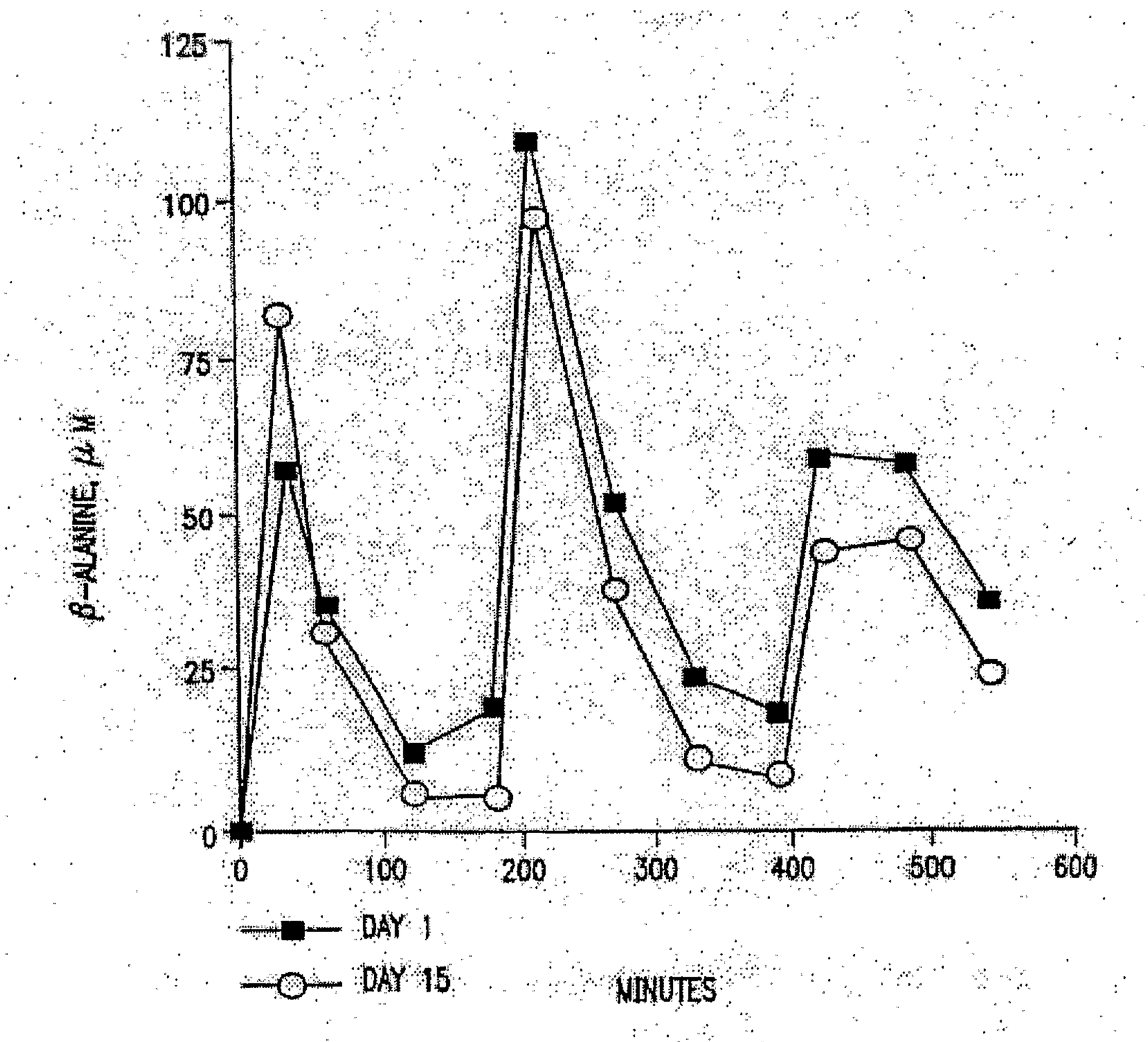
125
100
75
50
25
0
β-ALANINE, μ M
0
100
200
300
400
500
600
DAY 1
DAY 15
MINUTES

FIG. 10

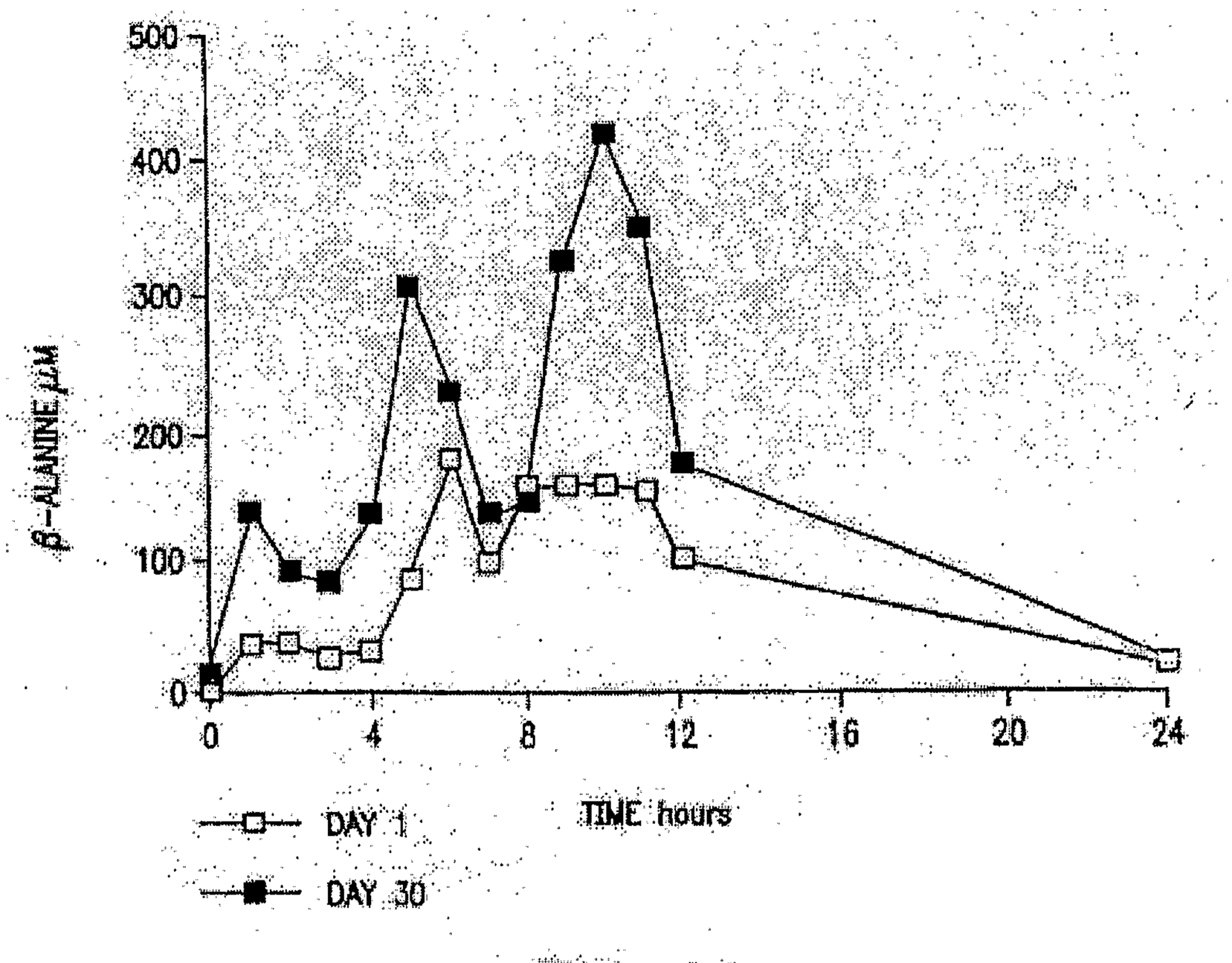
500
400
300
200
100
0
β-ALANINE μM
0
4
8
12
16
20
24
TIME hours
DAY 1
DAY 30

FIG. 11

Figure 12

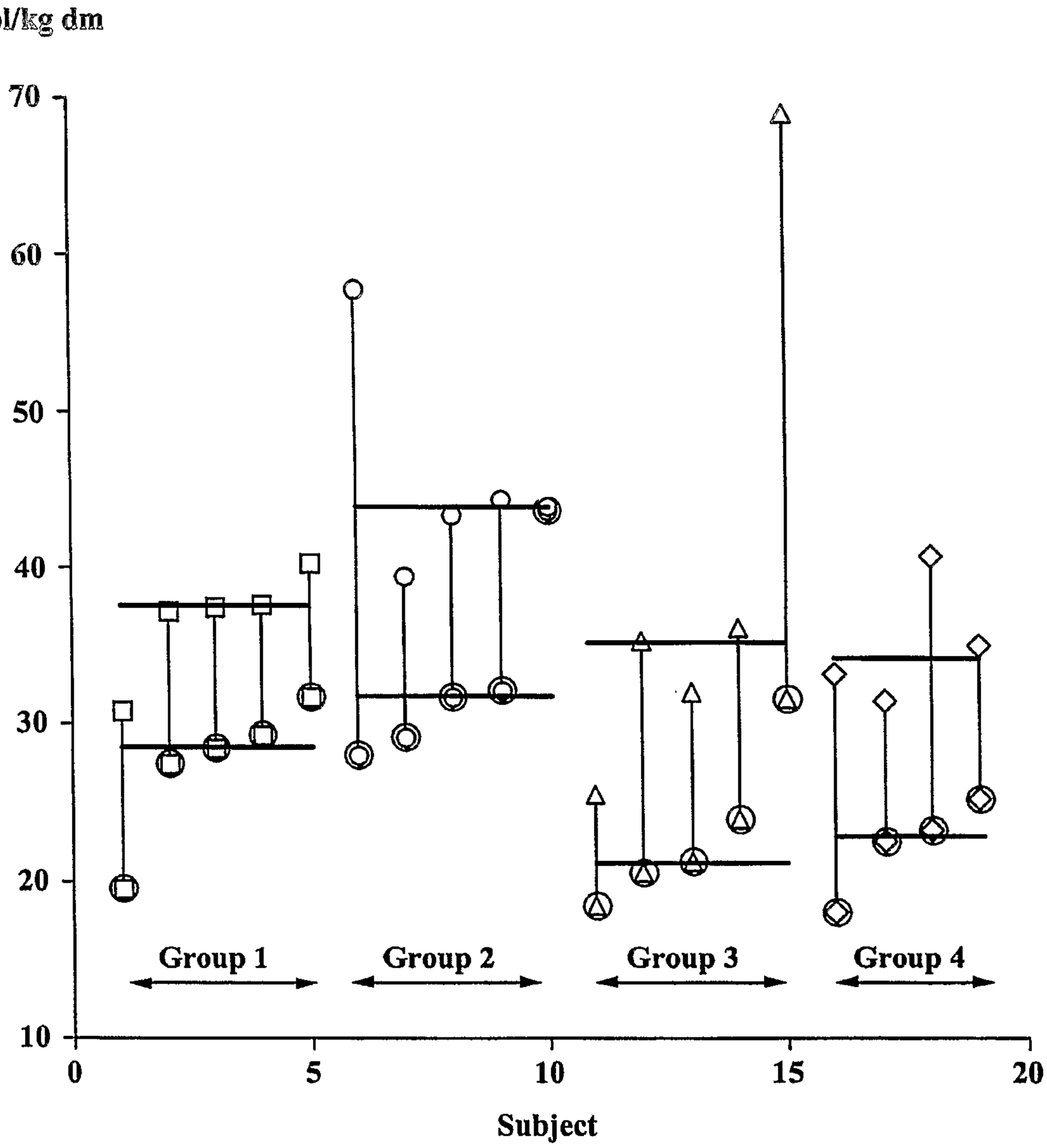
mmol/kg dm
70
60
50
40
30
20
10
0
5
10
15
20
Group 1
Group 2
Group 3
Group 4
Subject

Figure 13

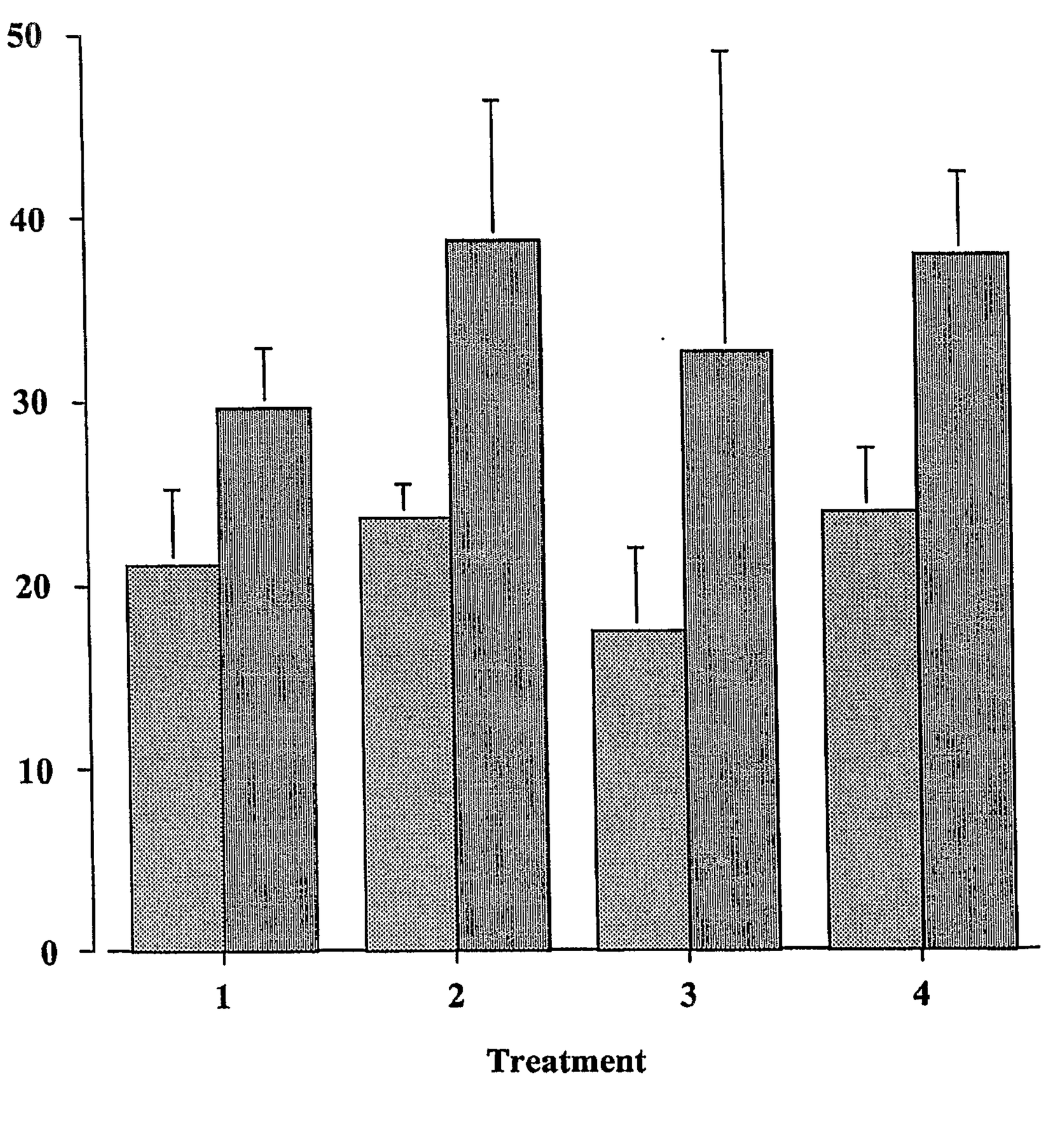

Figure 14

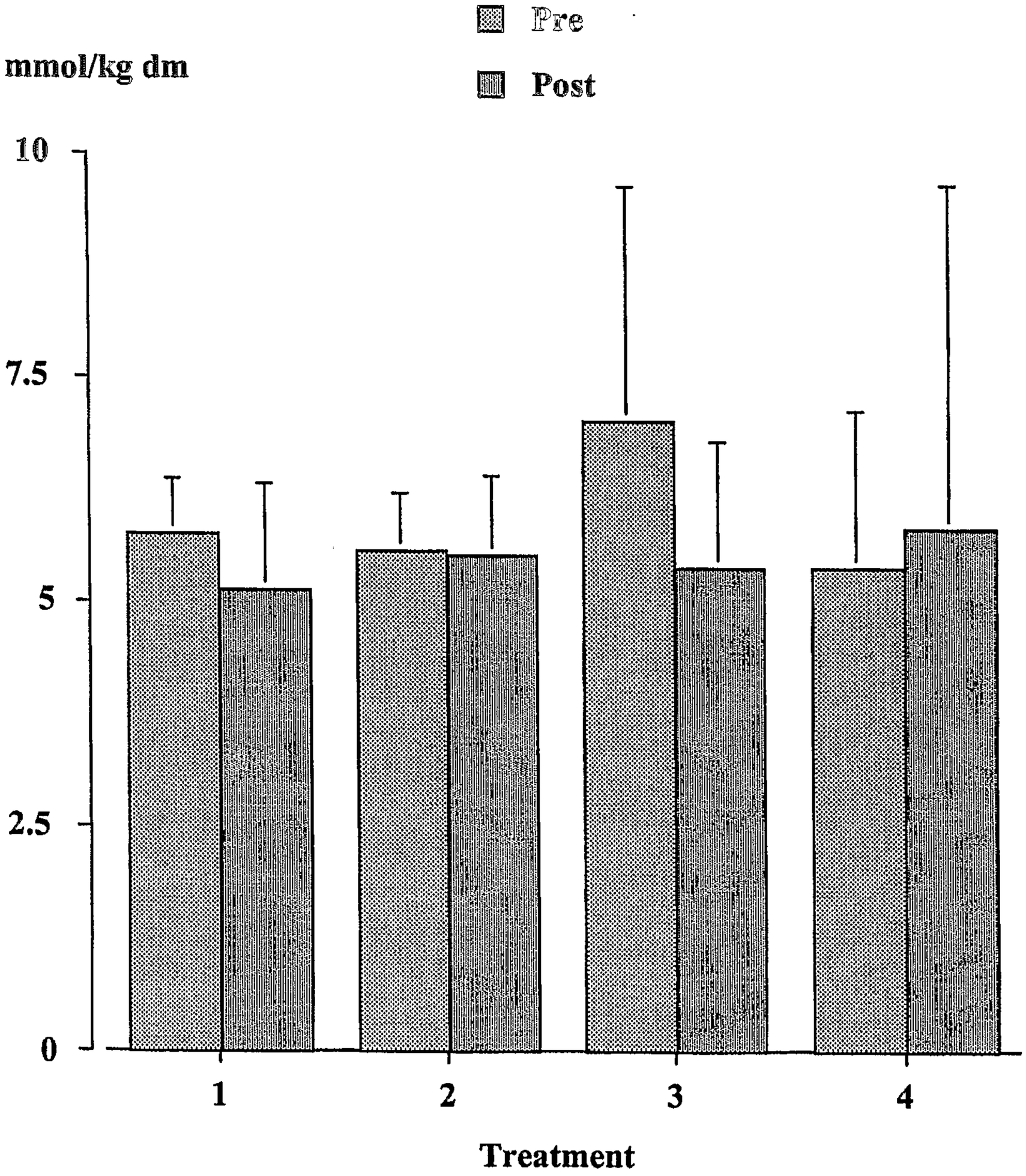

Figure 15

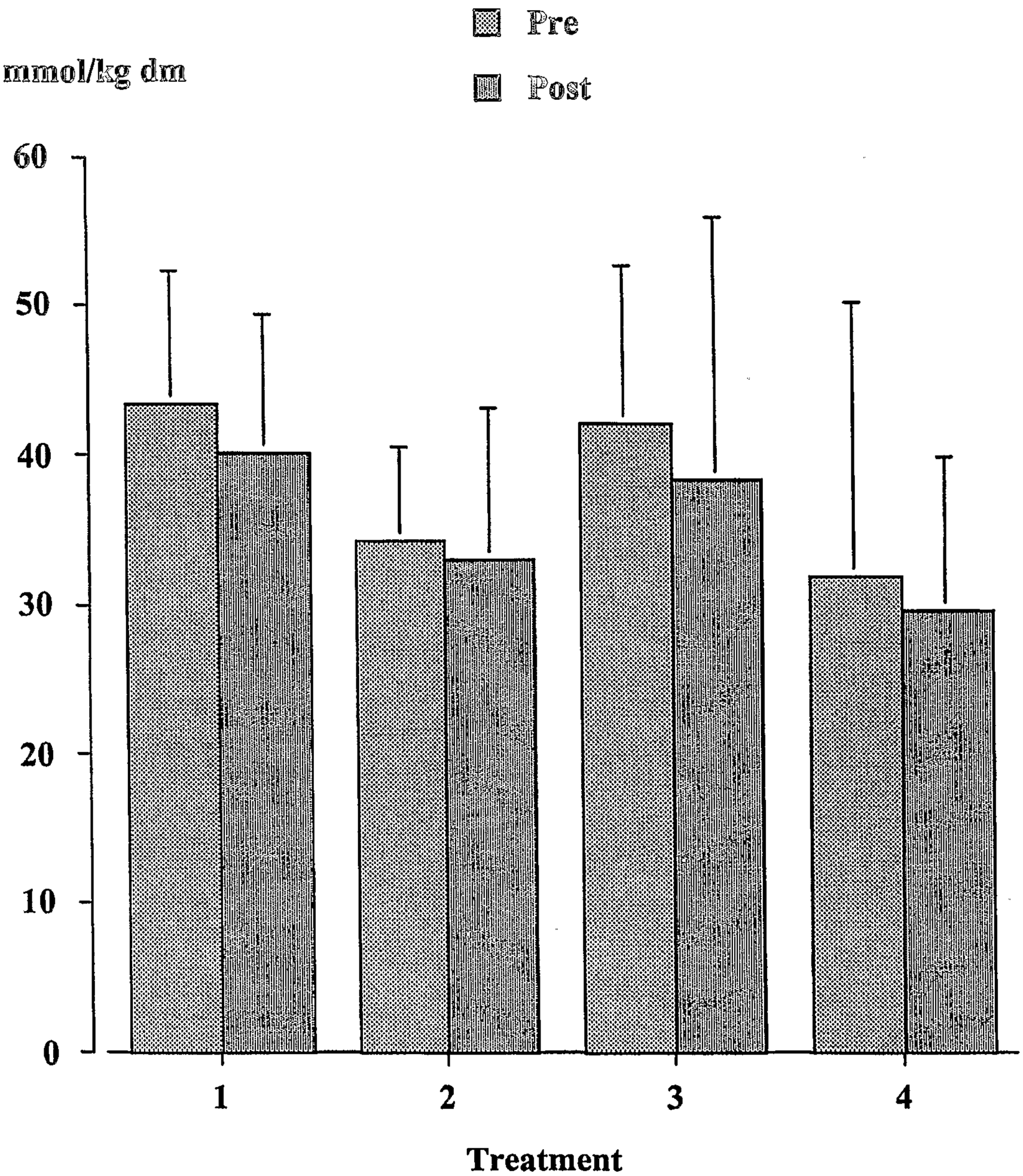

Figure 16

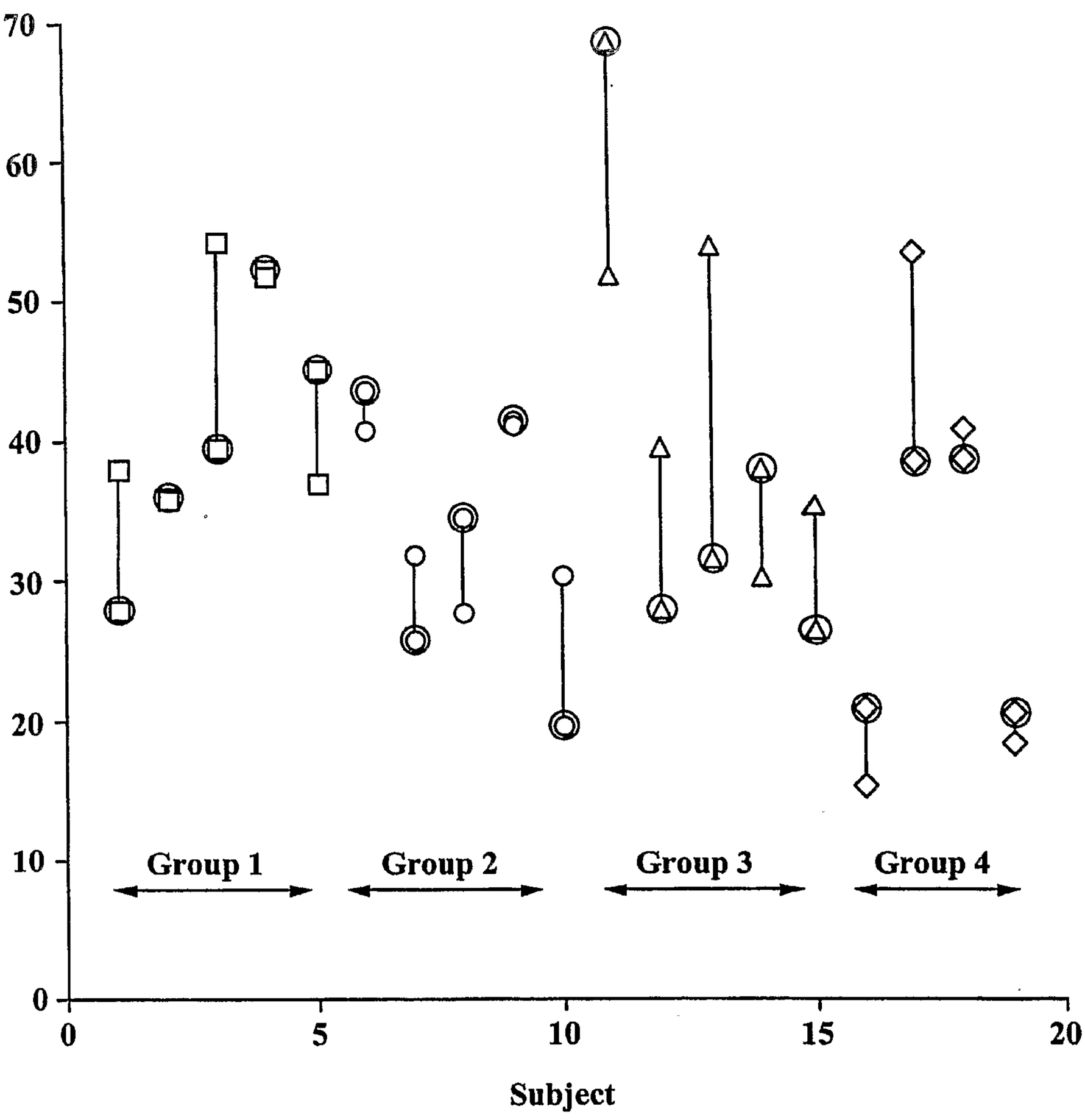
mmol/kg dm
70
60
50
40
30
20
10
0
Group 1
Group 2
Group 3
Group 4
0
5
10
15
20
Subject

Figure 17

Table 9

TREAT-MENT		DOSING TIMES								AVG DOSE (mg x times)	PER DAY	
		9am	10am	11am	12noon	3pm	4pm	5pm	6pm		GIVEN	as ß-Ala
1 Beta alanine (ß-Ala) n = 5	Week 1		800mg		800mg		800mg		800mg	800 x 4	3.2g	3.2g
	2		800mg		800mg		800mg		800mg	800 x 4	3.2g	3.2g
	3		800mg		800mg		800mg		800mg	800 x 4	3.2g	3.2g
	4		800mg		800mg		800mg		800mg	800 x 4	3.2g	3.2g
										Total 90g ß-Ala in 4W		
2 Beta alanine (ß-Ala) n = 5	Week 1	800mg	400mg	400mg	400mg	800mg	400mg	400mg	400mg	500 x 8	4.0g	4.0g
	2	800mg	400mg	400mg	800mg	800mg	400mg	400mg	800mg	600 x 8	4.8g	4.8g
	3	800mg	400mg	800mg	800mg	800mg	400mg	800mg	800mg	700 x 8	5.6g	5.6g
	4	800mg	800mg	800mg	800mg	800mg	800mg	800mg	800mg	800 x 8	6.4g	6.4g
										Total 146g ß-Ala in 4W		
3 Carnosine (C) n = 5	Week 1	1500mg	1500mg	1000mg	1000mg	1500mg	1500mg	1000mg	1000mg	1250 x 8	10g	4.0g
	2	1500mg	1500mg	1500mg	1500mg	1500mg	1500mg	1500mg	1500mg	1500 x 8	12g	4.8g
	3	2000mg	1500mg	1500mg	2000mg	2000mg	1500mg	1500mg	2000mg	1750 x 8	14g	5.6g
	4	2000mg	2000mg	2000mg	2000mg	2000mg	2000mg	2000mg	2000mg	2000 x 8	16g	6.4g
										Total 364g C in 4W (145g ß-Ala)		