

Description

AGENT FOR ENHANCING LIPID METABOLISM

Technical Field

[0001]

The present invention relates to an agent for enhancing lipid metabolism containing arginine, alanine, and/or phenylalanine as active ingredients at high concentrations.

Background Art

[0002]

With westernization of diet and lifestyle, the obese population is steadily increasing. According to "Summary of Results of the National Health and Nutrition Examination Survey (2010)" by the Ministry of Health, Labour and Welfare in Japan, the obesity rate in the Japanese population is 30.4% in men, and is 21.1% in women. Further, 11 million people, which is about half of the obese population estimated to reach 23 million people, concomitantly have metabolic syndrome (lifestyle-related diseases) such as hyperglycemia, hyperlipidemia, and hypertension, resulting in a significant reduction in the quality of life (QOL). For preventing and ameliorating such diseases, a great deal of attention has been recently given, not only to the control of caloric intake through the regulation of food intake, but also to the energy consumption by exercise and the bioregulatory function by nutrients in foods (Non Patent Literature 1).

[0003]

In recent years, the functionality of amino acids has been gaining attention. For example, it is known that exercise after ingestion of "Vespa Amino Acid Mixture (V.A.A.M.)" that is an amino acid composition being a reproduction of the amino acid composition in saliva secreted by the larvae of *Vespa mandarinia* to serve as an energy source for the adults thereof increases serum lipase activity and increase free fatty acids in blood (Patent Literatures 1 to 3 and Non Patent Literature 2). Further, it is known, for example, that an amino acid composition containing alanine, arginine, aspartic acid, glutamic acid, glycine, histidine,

isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, and glutamine at a specific molar ratio enhances the process of using body fats for kinetic energy production during anaerobic exercise (Patent Literature 4). Further, it is also known that a composition containing an amino acid having an insulin secretory function is useful for stamina recovery and nutrition support in the case of physical exhaustion due to exercise or the like (Patent Literature 5). In addition, an amino acid composition containing arginine, glutamine, and a branched-chain amino acid in a certain amount or more (Patent Literature 6), and a physical strength enhancing agent and an athletic performance enhancing agent for infants containing arginine and glutamine in a certain amount or more (Patent Literature 7) are also known.

Citation List

Patent Literature

[0004]

Patent Literature 1: JP Patent Publication (Kokai) No. 4-112825 A

Patent Literature 2: JP Patent Publication (Kokai) No. 6-24977 A

Patent Literature 3: JP Patent Publication (Kokai) No. 5-127258 A

Patent Literature 4: JP Patent Publication (Kokai) No. 2004-352696 A

Patent Literature 5: JP Patent Publication (Kokai) No. 61-215323 A

Patent Literature 6: JP Patent Publication (Kokai) No. 2005-27524 A

Patent Literature 7: JP Patent No. 4526047

Non Patent Literature

[0005]

Non Patent Literature 1: "Obesity and fat energy metabolism - strategies against metabolic syndrome -", KAWADA, Teruo et al., eds., Japan Society of Nutrition and Food Science (supervisor), KENPAKUSHA, pp. 217 to 238 (2008)

Non Patent Literature 2: Tsuchita H et al, "Effects of a Vespa amino acid mixture identical to hornet larval saliva on the blood biochemical indices of running rats.," Nutr Res, 17(6), pp. 999 to 1012 (1997)

Summary of Invention

Technical Problem

[0006]

It is an object of the present invention to provide an agent for enhancing lipid metabolism which is capable of efficiently reducing body fats by enhancing lipid metabolism more effectively.

Solution to Problem

[0007]

As a result of intensive studies for materials that enhance fat metabolism and efficiently reduce body fats, the present inventors have found that an amino acid mixture containing arginine, phenylalanine, and alanine, which are major amino acids that have been eaten, at high concentrations has a high lipid metabolism-enhancing effect and a high anti-obesity effect, thereby accomplishing the present invention.

[0008]

Specifically, it has been found that, as a result of monitoring the amount of change in blood glycerol in animals to which adrenaline is administered, a composition containing a combination of two or more of arginine, phenylalanine, and alanine at high concentrations significantly enhances lipolysis as compared to conventional amino acid compositions. Further, the lipid metabolism-enhancing effect could be observed in animals subjected to high-fat diet feeding and exercise and also in humans subjected to exercise.

[0009]

That is, the present invention includes the following aspects:

[0010]

[1] An agent for enhancing lipid metabolism comprising an amino acid mixture comprising at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine, wherein the agent comprises the at least two amino acids in a molar ratio of 60 mol or more, in total, per 100 mol of an amount of all amino acids;

[0011]

[2] The agent for enhancing lipid metabolism according to the aforementioned aspect [1], wherein the amino acid mixture comprises arginine, alanine, and phenylalanine;

[0012]

[3] The agent for enhancing lipid metabolism according to the aforementioned aspect [1] or [2], wherein the agent comprises arginine, alanine, and phenylalanine in a molar ratio of arginine:alanine:phenylalanine of 8-30 mol:18-30 mol:10-20 mol, per 100 mol of the amount of all amino acids;

[0013]

[4] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [1] to [3], wherein the amounts of arginine, alanine, and phenylalanine contained satisfy at least one weight ratio of (i) arginine:alanine of 1.5:1 to 1:1.5, (ii) alanine:phenylalanine of 4:1 to 1:4, and (iii) arginine:phenylalanine of 1:1 to 1:4;

[0014]

[5] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [1] to [4], wherein the agent comprises arginine, alanine, and phenylalanine in a molar ratio of 100 mol, in total, per 100 mol of the amount of all amino acids;

[0015]

[6] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [1] to [4], wherein the amino acid mixture further comprises glycine, and the agent comprises arginine, alanine, phenylalanine, and glycine in a molar ratio of 85 mol or more, in total, per 100 mol of the amount of all amino acids;

[0016]

[7] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [1] to [4] and [6], wherein the amino acid mixture further comprises exclusively proline, lysine, tyrosine, threonine, leucine, valine, isoleucine, glutamic acid, tryptophan, histidine, serine, methionine, and aspartic acid as other amino acids;

[0017]

[8] The agent for enhancing lipid metabolism according to the aforementioned aspect [7], wherein the amino acid mixture comprises the other amino acids in the following molar ratios per 100 mol of the amount of all amino acids:

0.01 to 4 mol of proline,
0.01 to 2 mol of lysine,
0.01 to 2 mol of tyrosine,
0.01 to 2 mol of threonine,
0.01 to 2 mol of leucine,
0.01 to 2 mol of valine,
0.01 to 2 mol of isoleucine,
0.01 to 1 mol of glutamic acid,
0.01 to 1 mol of tryptophan,
0.01 to 1 mol of histidine,
0.01 to 1 mol of serine,
0.01 to 0.2 mol of methionine, and
0.01 to 0.1 mol of aspartic acid;

[0018]

[9] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [1] to [8], wherein the agent is for use in acting on a condition where adrenaline secretion is increased;

[0019]

[10] The agent for enhancing lipid metabolism according to the aforementioned aspect [9], wherein the condition where adrenaline secretion is increased is a condition of being stimulated by exercise, stress, cold exposure, bathing, or overeating; and

[0020]

[11] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [1] to [10], wherein the agent is for the enhancement of lipid metabolism that is accompanied by at least one selected from the group consisting of increased blood glycerol concentration, suppressed weight gain, increased blood free fatty acid concentration, increased

blood glucagon concentration, reduced blood cortisol concentration, increased blood total ketone body concentration, increased blood 3-hydroxybutyric acid concentration, and increased UCP-1 expression level in brown adipose tissue.

[0021]

The present invention further includes the following aspects:

[0022]

[a] A agent for enhancing lipid metabolism comprising arginine, alanine, and phenylalanine as active ingredients, wherein the total number of moles of arginine, alanine, and phenylalanine is 60 mol or more, per 100 mol of the total number of moles of all amino acids;

[b] The agent for enhancing lipid metabolism according to the aforementioned aspect [a], comprising arginine in a molar ratio of 8 to 30 mol, alanine in a molar ratio of 18 to 30 mol, and phenylalanine in a molar ratio of 10 to 20 mol;

[c] The agent for enhancing lipid metabolism according to the aforementioned aspect [a] or [b], wherein the total number of moles of arginine, alanine, and phenylalanine is 100 mol, per 100 mol of the total number of moles of all amino acids;

[d] An agent for enhancing lipid metabolism comprising arginine, alanine, phenylalanine, and glycine as active ingredients, wherein the total number of moles of arginine, alanine, phenylalanine, and glycine is 85 mol or more, per 100 mol of the total number of moles of all amino acids;

[e] The agent for enhancing lipid metabolism according to the aforementioned aspect [d], comprising arginine in a molar ratio of 8 to 30 mol, alanine in a molar ratio of 18 to 30 mol, phenylalanine in a molar ratio of 10 to 20 mol, and the glycine in a molar ratio of 20 to 27 mol;

[f] The agent for enhancing lipid metabolism according to the aforementioned aspect [d] or [e], wherein the total number of moles of arginine, alanine, phenylalanine, and glycine is 100 mol, per 100 mol of the total number of moles of all amino acids;

[g] The agent for enhancing lipid metabolism according to the aforementioned aspect [a] or [d], further comprising exclusively proline, lysine, tyrosine, threonine, leucine, valine, isoleucine, glutamic acid, tryptophan, histidine, serine, methionine, and aspartic acid as other amino acids;

[h] The agent for enhancing lipid metabolism according to the aforementioned aspect [b] or [e], further comprising exclusively the following amino acids as the other amino acids in the following molar ratios:

0.01 to 4 mol of proline,
0.01 to 2 mol of lysine,
0.01 to 2 mol of tyrosine,
0.01 to 2 mol of threonine,
0.01 to 2 mol of leucine,
0.01 to 2 mol of valine,
0.01 to 2 mol of isoleucine,
0.01 to 1 mol of glutamic acid,
0.01 to 1 mol of tryptophan,
0.01 to 1 mol of histidine,
0.01 to 1 mol of serine,
0.01 to 0.2 mol of methionine, and
0.01 to 0.1 mol of aspartic acid;

[i] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [a] to [h], wherein the agent for enhancing lipid metabolism is ingested in a condition where adrenaline secretion is increased;

[j] The agent for enhancing lipid metabolism according to the aforementioned aspect [i], wherein the condition where adrenaline secretion is increased is a condition of being stimulated by exercise, stress, cold exposure, bathing, or overeating;

[k] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [a] to [j], wherein the agent for enhancing lipid metabolism increases UCP-1 expression level in brown adipose tissue;

[l] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [a] to [j], wherein the agent for enhancing lipid metabolism enhances breakdown of fats into fatty acids;

[m] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [a] to [j], wherein the agent for enhancing lipid metabolism enhances conversion of fatty acids into heat; and

[n] The agent for enhancing lipid metabolism according to any one of the aforementioned aspects [a] to [j], wherein the agent for enhancing lipid metabolism enhances conversion of fatty acids into energy.

Effect of Invention

[0023]

The agent for enhancing lipid metabolism of the present invention has an effect of enhancing lipid metabolism and can efficiently exhibit the lipid metabolism-enhancing effect particularly in a condition where adrenaline secretion is increased.

[0024]

This description includes the disclosure in JP Patent Application No. 2013-168690 to which the present application claims priority.

Brief Description of Drawings

[0025]

[Figure 1] Figure 1 shows the rate of change in blood glycerol concentration determined in Example 1 (the rate of change in glycerol AUC; Glycerol AUC change (%) of Vehicle group).

[Figure 2] Figure 2 shows transitions of the amount of change (Δ glycerol) in blood glycerol concentration determined in Example 2. #: $p < 0.05$ (vs. Vehicle group, Fisher's PLSD test).

[Figure 3] Figure 3 shows transitions of the amount of change (Δ glycerol) in blood glycerol concentration determined in Example 3. Means $\pm$ standard error are indicated. *: $p < 0.05$ (vs. Vehicle group, Fisher's PLSD test).

[Figure 4] Figure 4 shows transitions of body weight measured in Example 4 (Figure 4A), and shows values of feed intake/body weight during the test period (Figure 4B). Means $\pm$ standard error are indicated. Normal group: There is a significant difference in body weight from the 3rd day (Day 3) to the 42nd day (Day 42). Preparation Example 3 group: There is a significant difference in body weight from the 3rd day (Day 3) to the 39th day (Day 39). (vs. High-fat group, $p < 0.05$, Fisher's PLSD test).

[Figure 5] Figure 5 shows perirenal fat weight measured in Example 4. Means $\pm$ standard error are indicated. *: $p < 0.05$ (vs. High-fat group, Fisher's PLSD test).

[Figure 6] Figure 6 shows UCP-1 expression level in brown adipose tissue measured in Example 4. Means $\pm$ standard error are indicated. *: $p < 0.05$ (vs. High-fat group, Fisher's PLSD test).

[Figure 7] Figure 7 shows the measurement results of respiratory exchange ratio over time obtained in Example 5 (Figure 7A), and shows the results of the amount of change in respiratory exchange ratio (Δ respiratory exchange ratio) (Figure 7B). Means $\pm$ standard error are indicated. #: $p < 0.05$ (vs. placebo group, paired-sample t-test).

[Figure 8] Figure 8 shows the measurement results over time obtained in Example 5, of blood glycerol concentration (Figure 8A), and blood free fatty acid concentration (Figure 8B). Means $\pm$ standard error are indicated.

[Figure 9] Figure 9 shows the measurement results over time obtained in Example 5, of blood cortisol concentration (Figure 9A), blood glucagon concentration (Figure 9B), blood insulin concentration (Figure 9C), and blood glucose (Figure 9D). Means $\pm$ standard error are indicated. #: $p < 0.05$, ##: $p < 0.01$ (vs. placebo group, paired-sample t-test).

[Figure 10] Figure 10 shows the measurement results over time obtained in Example 5, of blood total ketone body concentration (Figure 10A), blood 3-hydroxybutyric acid concentration (Figure 10B), and blood acetoacetic acid concentration (Figure 10C). Means $\pm$ standard error are indicated. #: $p < 0.05$ (vs. placebo group, paired-sample t-test).

[Figure 11] Figure 11 shows the measurement results over time obtained in Example 5, of blood growth hormone concentration (Figure 11A), blood adrenaline concentration (Figure 11B), and blood noradrenaline concentration (Figure 11C). Means $\pm$ standard error are indicated.

[Figure 12] Figure 12 shows the measurement results of blood glucagon concentration obtained in Example 9. *: $p < 0.05$ (vs. High-fat group, Fisher's PLSD test).

[Figure 13] Figure 13 shows the measurement results of carnitine palmitoyltransferase activity in the liver obtained in Example 9. *: $p < 0.05$ (vs. High-fat group, Fisher's PLSD test).

[Figure 14] Figure 14 shows the measurement results of acyl-CoA oxidase activity in the liver obtained in Example 9. *: $p < 0.05$ (vs. High-fat group, Fisher's PLSD test).

[Figure 15] Figure 15 shows the amount of change (Δ glycerol) in blood glycerol concentration determined in Example 12.

[Figure 16] Figure 16 shows the measurement results of blood glycerol AUC (min·mg/L) obtained in Example 13. *: $p < 0.05$ (vs. control group, Fisher's PLSD test).

[Figure 17] Figure 17 shows the measurement results of blood glycerol AUC (min·mg/L) obtained in Example 14.

Embodiments for Carrying out the Invention

[0026]

Hereinafter, the present invention will be described in detail. However, the present invention is not limited to the following preferred embodiments, and can be freely modified as long as the effects of the present invention are exerted.

[0027]

The present invention relates to an agent for enhancing lipid metabolism, which comprises an amino acid mixture comprising at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine, wherein the agent comprises the at least two amino acids in a molar ratio of 60 mol or more, in total, per 100 mol of the amount of all amino acids. The present invention further relates to an agent for enhancing lipid metabolism, which further comprises glycine as a component of the amino acid mixture, in addition to the at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine. The agent for enhancing lipid metabolism of the present invention contains arginine, alanine, and phenylalanine; or arginine, alanine, phenylalanine, and glycine as active ingredients.

[0028]

Arginine serving as an active ingredient in the present invention is one of the non-essential amino acids, and has D-form and L-form. The CAS number of L-arginine is 74-79-3, and synonymous names therefor are (S)-2-amino-5-guanidinopentanoic acid, (S)-2-amino-5-(amidino amino) valeric acid, L-(+)-arginine, and Argi-U. As abbreviations for arginine,

Arg and R may be used. Arginine is easily soluble in water and formic acid, soluble in dilute hydrochloric acid, and practically insoluble in ethanol (see, the 16th edition of the Japanese Pharmacopoeia). Arginine is known to be contained in various materials such as various proteins, e.g., meats. Although arginine is biosynthesized as an intermediate in the urea cycle, it is rapidly degraded, and therefore arginine is an essential amino acid for children. Arginine is known to be involved in the growth hormone secretion enhancement and improvement in immune function.

[0029]

Alanine serving as an active ingredient in the present invention is one of the non-essential amino acids, and has D-form and L-form. The CAS number of L-alanine is 56-41-7, and synonymous names therefor are (S)-2-aminopropionic acid, (2S)-2-amino propane acid, α -alanine, and L-(+)-alanine. As abbreviations for alanine, Ala and A may be used. Alanine is easily soluble in water and formic acid, and practically insoluble in ethanol (see, the 16th edition of the Japanese Pharmacopoeia). Alanine is known to be contained in various materials and foods such as various proteins. Alanine is known to be one of the constituent amino acids of proteins, and to be a material for making connective tissue.

[0030]

Phenylalanine serving as an active ingredient in the present invention is one of the essential amino acids, and has D-form and L-form. The CAS number of L-phenylalanine is 63-91-2, and synonymous names therefor are (S)- α -aminobenzenepropanoic acid, (2S)-2-amino-3-phenylpropanoic acid, (S)-3-phenyl-2-aminopropionic acid, L- β -phenylalanine, and L-(-)-phenylalanine. As abbreviations for phenylalanine, Phe and F may be used. Phenylalanine is easily soluble in formic acid, sparingly soluble in water, soluble in dilute hydrochloric acid, and practically insoluble in ethanol (see, the 16th edition of the Japanese Pharmacopoeia). Phenylalanine is known to be contained in various materials and foods such as various proteins.

[0031]

Glycine is one of the non-essential amino acids. The CAS number of glycine is 56-40-6, and synonymous names therefor are 2-aminoacetic acid, α -aminoacetic acid, glycolixir,

and glycocoll. As abbreviations for glycine, Gly and G may be used. Glycine is easily soluble in water and formic acid, and practically insoluble in ethanol (see, the 16th edition of the Japanese Pharmacopoeia). Glycine is known to be contained in various materials and foods such as collagen, gelatin, fish, and shellfish. Glycine is known to be glucogenic, to be components, for example, of hemoglobin and enzymes in the liver, and to be neurotransmitters in the central nerve.

[0032]

In the present invention, arginine, phenylalanine, alanine, glycine or other amino acids may be free bases or hydrates, and may form salts with organic acids (such as acetic acids, tartaric acids, and fatty acids), organic bases, inorganic acids (such as hydrochloric acids, hydrobromic acids, nitric acids, sulfuric acids, and perchloric acids), or inorganic bases (such as potassium, sodium, and zinc). Further, either the D-form or the L-form of arginine, phenylalanine, alanine, and glycine may be used.

[0033]

In the present invention, arginine, phenylalanine, alanine, glycine or other amino acids can be obtained, for example, by juicing, concentration, purification, crystallization, or extraction with various solvents of materials, foods, or the like, which have a high content of arginine, phenylalanine, alanine, glycine, or other amino acids. As the various solvents, water or commonly used solvents such as alcohols, hydrocarbons, organic acids, organic bases, inorganic acids, inorganic bases, and supercritical fluids can be used alone, or a plurality of them can be used in combination. Further, amino acids produced using microorganisms also can be used. Furthermore, amino acids chemically synthesized also can be used. In the present invention, arginine, phenylalanine, alanine, glycine, or other amino acids may be derived from multiple sources for use in combination.

[0034]

The agent for enhancing lipid metabolism of the present invention comprises a combination of at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine, e.g., a combination of arginine, alanine, and phenylalanine, as active ingredients. The agent for enhancing lipid metabolism of the present invention may contain

at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine in amounts such that the total number of moles of arginine, alanine, and phenylalanine is in a molar ratio of 60 mol or more, 70 mol or more, 80 mol or more, 90 mol or more, or 100 mol, per 100 mol of the total number of moles of all the amino acids contained (i.e., amount of all amino acids). In one embodiment, the total amount of arginine, alanine, and phenylalanine in the agent for enhancing lipid metabolism of the present invention may be in a molar ratio of 95 mol or less, 90 mol or less, 80 mol or less, or 75 mol or less, per 100 mol of the total number of moles of all amino acids contained (i.e., amount of all amino acids). The phrase that "the total number of moles of arginine, alanine, and phenylalanine is in a molar ratio of 100 mol, per 100 mol of the total number of moles of all amino acids contained (i.e., amount of all amino acids)" means that all the amino acids contained in the agent for enhancing lipid metabolism are arginine, alanine, and phenylalanine, that is, the amino acid mixture contained in the agent for enhancing lipid metabolism consists of arginine, alanine, and phenylalanine. Other similar phrases in this description are understood in the same way. Composition examples of the agent for enhancing lipid metabolism as described above include agents for enhancing lipid metabolism having compositions shown in Table 2 and Table 3 (Preparation Example 1, Preparation Example 2, and Preparation Example 3), but there is no limitation to these examples.

[0035]

Further, the molar ratios corresponding to the respective amounts of arginine, alanine, and phenylalanine contained in the agent for enhancing lipid metabolism of the present invention may be any values. To mention an example, the agent for enhancing lipid metabolism of the present invention may comprise arginine in a molar ratio of 8 to 30 mol, alanine in a molar ratio of 18 to 30 mol, and phenylalanine in a molar ratio of 10 to 20 mol, per 100 mol of the amount of all amino acids contained therein. In a more preferred embodiment, the agent for enhancing lipid metabolism of the present invention may comprise arginine in a molar ratio of 25 to 30 mol, alanine in a molar ratio of 25 to 30 mol, and phenylalanine in a molar ratio of 10 to 15 mol, per 100 mol of the amount of all amino acids contained therein. Alternatively, in another preferred embodiment, the agent for enhancing

lipid metabolism of the present invention may comprise arginine in a molar ratio of 8 to 50 mol, alanine in a molar ratio of 18 to 50 mol, and phenylalanine in a molar ratio of 10 to 70 mol, per 100 mol of the amount of all amino acids contained therein. For example, in the case where the agent for enhancing lipid metabolism of the present invention comprises arginine, alanine, and phenylalanine, and the total amount of them is equivalent to a molar ratio of 100 mol, per 100 mol of the amount of all amino acids contained in the agent for enhancing lipid metabolism of the present invention (that is, in the case where the amino acids contained therein consist of arginine, alanine, and phenylalanine), the agent for enhancing lipid metabolism of the present invention may comprise arginine in a molar ratio of 8 to 50 mol, alanine in a molar ratio of 18 to 50 mol, and phenylalanine in a molar ratio of 10 to 70 mol, per 100 mol of the amount of all amino acids contained therein. The agent for enhancing lipid metabolism of the present invention also may comprise arginine in a molar ratio of 8 to 30 mol, alanine in a molar ratio of 20 to 35 mol, and phenylalanine in a molar ratio of 10 to 55 mol, per 100 mol of the amount of all amino acids contained therein.

[0036]

The amounts of arginine, alanine, and phenylalanine contained in the agent for enhancing lipid metabolism of the present invention preferably satisfy, but not limited to, at least one weight ratio of: (i) arginine:alanine = 3:1 to 1:3, more preferably 1.5:1 to 1:1.5, for example, 1:1, (ii) alanine:phenylalanine = 7:1 to 1:7, more preferably 4:1 to 1:4, for example, 1:3, and (iii) arginine:phenylalanine = 7:1 to 1:7, more preferably 1:1 to 1:4, for example, 1:3. The agent for enhancing lipid metabolism of the present invention may contain a relatively large amount of phenylalanine. In one embodiment, the amounts of arginine, alanine, and phenylalanine contained in the agent for enhancing lipid metabolism of the present invention may satisfy, but not limited to, the weight ratio of arginine:alanine:phenylalanine = 1:1:1 to 1:1:10, more preferably 1:1:1 to 1:1:6, for example, 1:1:1 to 1:1:3. In another embodiment, the amounts of arginine, alanine, and phenylalanine contained in the agent for enhancing lipid metabolism of the present invention may satisfy, but not limited to, the weight ratio of arginine:alanine:phenylalanine = 2 to 4:1 to 2:1 to 2, for example, 2 to 3:1 to 1.5:1.

[0037]

The agent for enhancing lipid metabolism of the present invention may further comprise glycine as an active ingredient, in addition to the at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine, and may contain, for example, arginine, alanine, phenylalanine, and glycine as active ingredients. The agent for enhancing lipid metabolism of the present invention may contain the at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine, and glycine in amounts such that the total number of moles of arginine, alanine, phenylalanine, and glycine is in a molar ratio of 85 mol or more, 90 mol or more, 95 mol or more, or 100 mol, per 100 mol of the total number of moles of all amino acids contained (i.e., amount of all amino acids). In one embodiment, the total amount of arginine, alanine, phenylalanine, and glycine in the agent for enhancing lipid metabolism of the present invention may be in a molar ratio of 99 mol or less, 95 mol or less, 92 mol or less, or 90 mol or less, per 100 mol of the total number of moles of all amino acids contained (i.e., amount of all amino acids).

[0038]

Further, the molar ratios corresponding to the respective amounts of arginine, alanine, phenylalanine, and glycine contained in the agent for enhancing lipid metabolism of the present invention may be any values. To mention an example, the agent for enhancing lipid metabolism of the present invention may comprise arginine in a molar ratio of 8 to 30 mol, alanine in a molar ratio of 18 to 30 mol, phenylalanine in a molar ratio of 10 to 20 mol, and glycine in a molar ratio of 20 to 27 mol, per 100 mol of the amount of all amino acids contained therein. The agent for enhancing lipid metabolism of the present invention, for example, may comprise arginine in a molar ratio of 25 to 30 mol, alanine in a molar ratio of 25 to 30 mol, phenylalanine in a molar ratio of 10 to 15 mol, and glycine in a molar ratio of 22 to 27 mol, per 100 mol of the amount of all amino acids contained therein.

[0039]

Composition examples of the agent for enhancing lipid metabolism of the present invention include an agent for enhancing lipid metabolism having the composition shown in Table 3 (Preparation Example 3), but there is no limitation to this example. Since arginine, alanine, phenylalanine, and glycine have taste and odor suitable for eating, drinking, and oral

ingestion, the agent for enhancing lipid metabolism of the present invention containing these amino acids at high concentrations is superior in terms of flavor. Therefore, the agent for enhancing lipid metabolism of the present invention has an advantage of not impairing the flavor of foods, drinks, and pharmaceutical products to which this agent is to be added.

[0040]

Further, the agent for enhancing lipid metabolism of the present invention may further comprise other amino acids. The other amino acids may be any of 20 types of amino acids generally constituting proteins in living organisms. The other amino acids may be naturally occurring amino acids or non-naturally occurring amino acids. In a preferred embodiment, the agent for enhancing lipid metabolism of the present invention may further comprise exclusively proline, lysine, tyrosine, threonine, leucine, valine, isoleucine, glutamic acid, tryptophan, histidine, serine, methionine, and aspartic acid, as the other amino acids. As the other amino acids, amino acids that do not inhibit the lipid metabolism-enhancing effect of the agent for enhancing lipid metabolism of the present invention can be preferentially used.

[0041]

Further, the molar ratios corresponding to the respective amounts of the other amino acids contained in the agent for enhancing lipid metabolism of the present invention may be any values. To mention an example, the agent for enhancing lipid metabolism of the present invention may comprise proline in a molar ratio of 0.01 to 4 mol, lysine in a molar ratio of 0.01 to 2 mol, tyrosine in a molar ratio of 0.01 to 2 mol, threonine in a molar ratio of 0.01 to 2 mol, leucine in a molar ratio of 0.01 to 2 mol, valine in a molar ratio of 0.01 to 2 mol, isoleucine in a molar ratio of 0.01 to 2 mol, glutamic acid in a molar ratio of 0.01 to 1 mol, tryptophan in a molar ratio of 0.01 to 1 mol, histidine in a molar ratio of 0.01 to 1 mol, serine in a molar ratio of 0.01 to 1 mol, methionine in a molar ratio of 0.01 to 0.2 mol, and aspartic acid in a molar ratio of 0.01 to 0.1 mol, per 100 mol of the amount of all amino acids contained therein.

[0042]

In a more preferred example, the agent for enhancing lipid metabolism of the present invention may comprise arginine in a molar ratio of 25 to 30 mol, alanine in a molar ratio of

25 to 30 mol, phenylalanine in a molar ratio of 10 to 15 mol, glycine in a molar ratio of 22 to 27 mol, proline in a molar ratio of 0.1 to 4 mol, lysine in a molar ratio of 0.1 to 2 mol, tyrosine in a molar ratio of 0.1 to 2 mol, threonine in a molar ratio of 0.1 to 2 mol, leucine in a molar ratio of 0.1 to 2 mol, valine in a molar ratio of 0.1 to 2 mol, isoleucine in a molar ratio of 0.1 to 2 mol, glutamic acid in a molar ratio of 0.1 to 1 mol, tryptophan in a molar ratio of 0.1 to 1 mol, histidine in a molar ratio of 0.1 to 1 mol, serine in a molar ratio of 0.1 to 1 mol, methionine in a molar ratio of 0.01 to 0.2 mol, and aspartic acid in a molar ratio of 0.01 to 0.1 mol. In a further preferred example, the agent for enhancing lipid metabolism of the present invention may comprise arginine in a molar ratio of 27 to 30 mol, alanine in a molar ratio of 25 to 28 mol, phenylalanine in a molar ratio of 10 to 13 mol, glycine in a molar ratio of 23 to 26 mol, proline in a molar ratio of 2 to 3 mol, lysine in a molar ratio of 0.6 to 1.6 mol, tyrosine in a molar ratio of 0.1 to 1.1 mol, threonine in a molar ratio of 0.5 to 1.5 mol, leucine in a molar ratio of 0.3 to 1.3 mol, valine in a molar ratio of 0.3 to 1.3 mol, isoleucine in a molar ratio of 0.1 to 2.1 mol, glutamic acid in a molar ratio of 0.1 to 1 mol, tryptophan in a molar ratio of 0.1 to 0.5 mol, histidine in a molar ratio of 0.1 to 0.6 mol, serine in a molar ratio of 0.1 to 0.6 mol, methionine in a molar ratio of 0.01 to 0.15 mol, and aspartic acid in a molar ratio of 0.01 to 0.05 mol.

[0043]

The "fats" in the present invention mean neutral fats. Catabolism of neutral fats is classified into breakdown of neutral fats into glycerol and fatty acids, and burning of fatty acids (β -oxidation). The mechanism of fat breakdown (lipolysis) is generally summarized as follows.

[0044]

(1) Adrenaline secretion is increased by exercise, bathing, cold stimulation, or the like.

[0045]

(2) When adrenaline binds to β receptors on the adipocyte membrane, adenylate cyclase present on the inner surface of the cell membrane is activated, and cyclic AMP (cAMP) is synthesized from ATP.

[0046]

(3) As a result of an increase in cellular cAMP concentration, cAMP-dependent protein kinase A is activated, and the protein kinase A phosphorylates and activates hormone-sensitive lipase in adipocytes.

[0047]

(4) The hormone-sensitive lipase converts triacylglycerol into free fatty acids and monoacylglycerol.

[0048]

(5) Monoacylglycerol lipase converts monoacylglycerol into glycerol and free fatty acids.

[0049]

The burning (β -oxidation) of the produced fatty acids is generally summarized as follows.

[0050]

(1) The fatty acids are delivered to the liver, and are catalytically activated by enzyme acyl-CoA synthetase present on the cytoplasmic side of the mitochondrial outer membrane, thereby transforming into fatty acyl-CoA.

[0051]

(2) The produced fatty acyl-CoA is delivered to a mitochondrial inner membrane.

[0052]

(3) Since the mitochondrial inner membrane does not allow the acyl-CoA to directly pass therethrough, fatty acyl-CoA is temporarily bound to carnitine to produce fatty acyl carnitine. Fatty acyl carnitine passes through the mitochondrial inner membrane due to facilitated diffusion via acyl carnitine/carnitine transporters, and translocates into the matrix. Then, fatty acyl group is catalytically transferred from carnitine to coenzyme A present in the mitochondria by enzyme carnitine acyl transferase II, thereby reproducing fatty acyl-CoA.

[0053]

(4) The fatty acyl-CoA after entering the mitochondria is oxidized by enzymes in the matrix. The β -oxidation reaction is composed of repeated cycles of four-step reaction, and two carbons are separated as acetyl-CoA from the carboxyl terminus of the fatty acyl chain in every cycle. Due to desaturation at the α -position and the β -position by dehydrogenase, and

OH addition at the β -position by hydratase, the β -position is bound to a keto group by dehydrogenase, and the CoA is bound to the β -position by thiolase, resulting in cleavage at the α -position and the β -position to produce short-chain fatty acyl-CoA and acetyl-CoA.

[0054]

(5) Some acetyl-CoA is converted into acetoacetyl-CoA, and acetoacetic acid is produced from it through 3-hydroxy 3-methylglutaryl CoA (HMG-CoA). The acetoacetic acid is converted into acetone by decarboxylation, or is converted into β -hydroxybutyric acid by reduction.

[0055]

(6) The acetoacetic acid or β -hydroxybutyric acid produced in the liver is delivered to cells of tissues other than the liver (such as central nervous system, heart muscle, skeletal muscle, kidney, and adrenal gland) and used for ATP production in the TCA cycle and the electron transport system in mitochondria.

[0056]

Further, fatty acids are degraded in brown adipocytes, and are converted into heat by the action of UCP-1 (Uncoupling protein 1; mitochondrial uncoupling proteins). More specifically, when noradrenaline binds to β_3 receptors on the brown fats, the UCP-1, which is also called samogenin, is opened and the membrane potential (mitochondrial proton gradient) is dissipated, and then oxidation of substrates such as fatty acids is significantly increased without ATP being synthesized, resulting in heat production. That is, β -oxidation of fatty acids is enhanced, and thermal energy is released. Further, in the case of poor metabolism of energy derived from glucose in the body, free fatty acids are converted into ketone bodies (such as acetoacetic acid, β -hydroxybutyric acid, and acetone) mainly in mitochondria of the liver, which are to be metabolized as energy sources.

[0057]

In the later-described experimental examples (Example 2, Example 3, and Example 10), it has been found, by monitoring the amount of change in blood glycerol using animals in which adrenaline secretion is artificially increased by administering adrenaline, that the agent for enhancing lipid metabolism of the present invention containing arginine, phenylalanine,

alanine, and glycine at high concentrations, and the agent for enhancing lipid metabolism of the present invention containing a combination of two or more of arginine, phenylalanine, and alanine, enhance lipid metabolism as compared with a conventional amino acid composition.

[0058]

Further, the lipid metabolism-enhancing effect of the agent for enhancing lipid metabolism of the present invention was successfully observed in animals that were fed with high-fat diet, and humans, in a condition where adrenaline secretion is increased by actually applying exercise (Example 4 and Example 5).

[0059]

That is, the agent for enhancing lipid metabolism of the present invention is capable of metabolizing fats more efficiently, particularly in the condition where the adrenaline secretion is continuously increased. The agent for enhancing lipid metabolism of the present invention can be used suitably in acting on a subject in a condition where adrenaline secretion is increased. The condition where adrenaline secretion is increased is not particularly limited, but is preferably a condition of being stimulated, for example, by exercise, stress, cold exposure, bathing, or overeating. In order to allow the agent for enhancing lipid metabolism of the present invention to act on a subject in the condition where adrenaline secretion is increased, the agent for enhancing lipid metabolism of the present invention may be administered to a subject in the condition where adrenaline secretion is increased, or may be administered to a subject expected to become in the condition where adrenaline secretion is increased (for example, a subject before exercise).

[0060]

As shown in Example 4, in animals to which the agent for enhancing lipid metabolism of the present invention was administered, the UCP-1 expression level in brown adipose tissue increased. This demonstrated that the agent for enhancing lipid metabolism of the present invention enhanced the conversion efficiency of fatty acids into heat as well as enhancing lipolysis.

[0061]

Further, as shown in Example 5, in humans to which the agent for enhancing lipid metabolism of the present invention was administered, the production of ketone bodies and the secretion of glucagon during exercise increased. Glucagon acts to enhance the production of ketone bodies as well as enhancing the use of glycogen. It was also found from this that the agent for enhancing lipid metabolism of the present invention enhances the conversion efficiency of fatty acids into energy, in the case of poor metabolism of energy derived from glucose. When attention is given to the transition over time in ketone body concentration, the production of ketone bodies was significantly enhanced after the application of exercise (Time 30 to Time 90). It is shown from this that the increased conversion of fatty acids into energy during exercise was continuously maintained at a high level also after the completion of the exercise.

[0062]

Further, in humans to which the agent for enhancing lipid metabolism of the present invention was administered, the amount of change in respiratory exchange ratio during aerobic exercise decreased. The respiratory exchange ratio is one of the indicators of energy metabolism, and the decrease of this value means that the contribution of lipids to energy metabolism increases. This also revealed that the agent for enhancing lipid metabolism of the present invention enhanced the conversion efficiency of fatty acids into energy (Example 5).

[0063]

Cortisol is one of glucocorticoids, and is a hormone that manifests increased secretion from the adrenal cortex when stress is applied. Since increase of cortisol acts to enhance breakdown of glycogen or proteins into glucose, the increase leads to an enhancement of lipid metabolism. However, when the cortisol secretion is maintained at a high level, muscle glycogen and hepatic glycogen are depleted early, thus exercise performance decreases. Further, it is accompanied with adverse effects to the body such as a reduction in immune function and atrophy of muscles and organs. Therefore, it is preferred that the cortisol level return to a steady-condition value at an early stage of the exercise. In humans who have ingested the agent for enhancing lipid metabolism of the present invention (active groups),

blood cortisol increased immediately after the ingestion (Time 0), but rapidly decreased from 15 minutes after the ingestion (Time 15) and the decrease rate is more rapid than in the placebo group. This revealed that the ingestion of the agent for enhancing lipid metabolism of the present invention makes it possible to perform exercise with less burden on the body, even if the same exercise is applied (Example 5).

[0064]

Meanwhile, in humans to which the agent for enhancing lipid metabolism of the present invention was administered, there was no change in blood insulin concentration and blood glucose. Accordingly, it was found that the agent for enhancing lipid metabolism of the present invention enhances lipid metabolism without affecting insulin secretion or blood glucose (Example 5). Low blood glucose may occur during exercise when the exercise is performed in a condition where insulin secretion is increased. The agent for enhancing lipid metabolism of the present invention does not affect insulin secretion, and therefore can efficiently produce energy derived from lipids without causing low blood glucose during exercise.

[0065]

Further, it was also found that the agent for enhancing lipid metabolism of the present invention exerted the lipid metabolism-enhancing effect without affecting the secretions of growth hormone, adrenaline, and noradrenaline (Example 5).

[0066]

From these results, the agent for enhancing lipid metabolism of the present invention was shown to enhance all the breakdown of fats into fatty acids, conversion of fatty acids into heat, and conversion of fatty acids into energy.

[0067]

Accordingly, the agent for enhancing lipid metabolism of the present invention can be used for enhancing both the breakdown of fats into fatty acids and the conversion of fatty acids into heat. The agent for enhancing lipid metabolism of the present invention can be used for the enhancement of lipid metabolism that is accompanied by at least one, preferably two or more, or more preferably all, selected from the group consisting of an increase in blood

glycerol concentration, a suppression of weight gain, an increase in blood free fatty acid concentration, an increase in blood glucagon concentration, a reduction in blood cortisol concentration, an increase in blood total ketone body concentration, an increase in blood 3-hydroxybutyric acid concentration, and an increase in UCP-1 expression level in brown adipose tissue, which are indicators of the lipid metabolism described above. Further, the agent for enhancing lipid metabolism of the present invention can be used for the enhancement of lipid metabolism that is accompanied by an increase in liver carnitine palmitoyltransferase activity and/or an increase in acyl-CoA oxidase activity in the liver as further indicators. These indicators can be measured by conventional methods, for example, as later described in the Examples. The increase, reduction and the like in these indicators of lipid metabolism may be determined by comparison with the control group (for example, the placebo group) in the same conditions except that the agent for enhancing lipid metabolism of the present invention is not administered or ingested, where the presence of the effects shown by the indicators can be determined when a certain tendency is observed, but it is more preferred that a statistically significant difference be observed.

[0068]

The agent for enhancing lipid metabolism of the present invention can be used in any form, e.g., directly, or as an additive to foods, drinks, or pharmaceutical products for enhancing lipid metabolism.

[0069]

In the present invention, the amounts of arginine, alanine, and phenylalanine to be contained in the agent for enhancing lipid metabolism are not specifically limited, and the amounts may be different, for example, depending on the dosage form, symptom, body weight, and intended use. However, to mention an example, the total amount of arginine, alanine, and phenylalanine contained can be set to 0.2 to 100 w/w% (weight/weight %), preferably 0.3 to 100 w/w%, more preferably 0.4 to 100 w/w%.

[0070]

The present invention further provides a method for enhancing lipid metabolism of a subject, the method comprising administering or ingesting the agent for enhancing lipid

metabolism of the present invention to the subject. The subject is preferably an animal in which enhancement of lipid metabolism is desired, and preferred examples thereof include, but not limited to, mammals including humans, livestock (such as pigs and horses), pet animals (such as dogs and cats), laboratory (test) animals (rodents such as mice and rats, and rabbits). Preferred examples of the subject include subjects with abnormal lipid metabolism or adiposity, subjects with genetic predispositions to obesity, or lifestyle risks or environmental risks of obesity, subjects desired to lose weight or to maintain weight, and subjects who are or will be in a condition where adrenaline secretion is increased due to exercise, stress, or the like. The agent for enhancing lipid metabolism of the present invention has an effect of efficiently enhancing lipid metabolism, and therefore can be used for efficiently burning fats, even in people who are overweight and thus have a tendency of slow and less lipolysis as compared to normal people.

[0071]

In the present invention, the daily intake (dose) of the agent for enhancing lipid metabolism is not specifically limited and it may be different depending, for example, on the age, symptom, body weight, and intended use. However, to mention an example, the intake can be set to an amount such that the total solid content of amino acids in the agent for enhancing lipid metabolism is 0.3 to 30 g, 0.8 to 8 g, 1 to 5 g, 1.3 to 3.5 g, 1.5 g or 3 g.

[0072]

In the present invention, the daily intake of arginine can be 0.06 to 12 g (or 0.4 to 70 mmol), 0.2 to 4 g (or 1 to 21 mmol), 0.4 to 2 g (or 2 to 11 mmol), 1.2 g (7 mmol), 1 g (6 mmol), or 0.6 g (4 mmol), for example.

[0073]

In the present invention, the daily intake of alanine can be 0.03 to 10 g (or 0.3 to 112 mmol), 0.09 to 3 g (or 1 to 34 mmol), 0.2 to 2 g (or 2 to 17 mmol), 1 g (11 mmol), 0.6 g (6 mmol), or 0.3 g (3 mmol), for example.

[0074]

In the present invention, the daily intake of phenylalanine can be 0.02 to 10 g (or 0.1 to 61 mmol), 0.08 to 3 g (or 0.5 to 18 mmol), 0.2 to 2 g (or 0.9 to 9 mmol), 1 g (6 mmol), 0.5 g (3 mmol), or 0.2 g (1 mmol), for example.

[0075]

In the present invention, the daily intake of glycine can be 4 g or less (or 59 mmol or less), 1.3 g or less (or 18 mmol or less), 0.7 g or less (or 9 mmol or less), 0.02 to 4 g (or 0.3 to 59 mmol), 0.07 to 1 g (or 1 to 18 mmol), 0.1 to 0.7 g (or 2 to 9 mmol), 0.4 g (6 mmol) or 0.2 g (3 mmol), for example.

[0076]

For example, in the case where 2.7 g of the agent for enhancing lipid metabolism shown in Table 2 below (Preparation Example 1) is ingested, amounts of arginine, alanine, and phenylalanine to be ingested in the total amount of amino acids of 2.7 g (22 mmol) are 1.2 g (7.0 mmol), 0.57 g (6.4 mmol), and 0.47 g (2.9 mmol), respectively.

[0077]

For example, in the case where 3.0 g of the agent for enhancing lipid metabolism shown in Table 2 (Preparation Example 2) is ingested, amounts of arginine, alanine, and phenylalanine to be ingested in the total amount of amino acids of 3.0 g (23 mmol) are 1.0 g (5.7 mmol), 1.0 g (11 mmol), and 1.0 g (6.1 mmol), respectively.

[0078]

For example, in the case where 3 g of the agent for enhancing lipid metabolism shown in Table 3 (Preparation Example 3) is ingested, amounts of arginine, alanine, phenylalanine, and glycine to be ingested in the total amount of amino acids of 3 g (24 mmol) are 1.2 g (7.0 mmol), 0.57 g (6.4 mmol), 0.47 g (2.9 mmol), and 0.45 g (5.9 mmol), respectively.

[0079]

Further, in the case where 1.5 g of the agent for enhancing lipid metabolism shown in Table 3 (Preparation Example 3) is ingested, amounts of arginine, alanine, phenylalanine, and glycine to be ingested in the total amount of amino acids of 1.5 g (24 mmol) are 0.61 g (3.5 mmol), 0.28 g (3.2 mmol), 0.24 g (1.4 mmol), and 0.22 g (3.0 mmol), respectively.

[0080]

The agent for enhancing lipid metabolism of the present invention may be used simultaneously with the ingestion of conventional foods, drinks, or pharmaceutical products known to have a lipid metabolism-enhancing effect, or may be used in combination before or after the ingestion.

[0081]

Examples of practical forms of the agent for enhancing lipid metabolism of the present invention are forms to be included into tablets, capsules, granules, powders, syrups, liquids, suspensions, drinks, and foods and drinks. They may be ingested or administered orally, via a tube, or intravenously. The agent for enhancing lipid metabolism of the present invention is expected to enhance lipid metabolism, for example, by ingestion of foods for special use such as foods for specified health use, foods with nutrient function claims, dietary supplements, or supplements in which the agent for enhancing lipid metabolism of the present invention is included, or administration of pharmaceutical products in which the agent for enhancing lipid metabolism of the present invention is included. When the agent for enhancing lipid metabolism of the present invention is included in foods for special use such as foods for specified health use, foods with nutrient function claims, dietary supplements, or supplements, consumers can expect the enhancement of lipid metabolism while distinctly distinguishing them from general foods. Herein, the foods and drinks may be also intended to be taken by non-human animals such as livestock and pet animals. The present invention also provides foods and pharmaceutical products containing the agent for enhancing lipid metabolism of the present invention, such as foods, drinks, and pharmaceutical products for enhancing lipid metabolism.

[0082]

The agent for enhancing lipid metabolism of the present invention can be also used to prepare an agent consisting of only the agent for enhancing lipid metabolism of the present invention and an auxiliary agent for formulation. Examples of the auxiliary agent for formulation include excipients, binders, disintegrants, lubricants, flavoring agents, solubilizers, suspending agents, and coating agents. These are commonly used in the field of manufacture technology for foods, drinks, or pharmaceutical products. Further, a suitable amount of

vitamins, minerals, organic acids, saccharides, peptides, amino acids that are not mentioned above, or the like may be added thereto. As other components, components that do not interfere with the lipid metabolism-enhancing effect of the agent for enhancing lipid metabolism of the present invention can be preferentially used.

[0083]

The agent for enhancing lipid metabolism of the present invention may be added to various foods (such as milk, soft drink, fermented milk, yogurt, cheese, bread, biscuit, cracker, pizza crust, formula milk, liquid diet, food for patients, nutritious food, frozen food, processed food, and other commercial food) for its ingestion. These foods may be in any form of liquid form, paste form, gel form, solid form, powder form and the like.

[0084]

The foods to which the agent for enhancing lipid metabolism of the present invention is added can be produced using water, proteins, carbohydrates, lipids, vitamins, minerals, organic acids, organic bases, fruit juices, and flavors, for example. Examples of the proteins include animal and vegetable proteins such as whole milk powder, skimmed milk powder, partially skimmed milk powder, casein, whey powder, whey protein, whey protein concentrate, whey protein isolate, whey protein hydrolysate, α -casein, β -casein, κ -casein, β -lactoglobulin, α -lactalbumin, lactoferrin, soy protein, chicken egg protein, and meat protein, degradation products of these, and various milk-derived ingredients such as butter, whey mineral, cream, whey, non-protein nitrogen, sialic acid, phospholipid, and lactose. Peptides such as casein phosphopeptides and amino acids may be contained. Examples of the carbohydrates include saccharides, modified starches (such as soluble starches, British starches, oxidized starches, starch esters, and starch ethers, as well as dextrin), and dietary fibers. Examples of the lipids include animal oils and fats such as lard, fish oil, and their fractionated oils, hydrogenated oils, and transesterified oils, and vegetable oils and fats such as palm oil, safflower oil, corn oil, rapeseed oil, coconut oil, and their fractionated oils, hydrogenated oils, and transesterified oils. Examples of the vitamins include vitamin A, carotenes, vitamin B group, vitamin C, vitamin D group, vitamin E, vitamin K group, vitamin P, vitamin Q, niacin, nicotinic acid, pantothenic acid, biotin, inositol, choline, and folic acid. Examples of the minerals include calcium,

potassium, magnesium, sodium, copper, iron, manganese, zinc, and selenium. Examples of the organic acids include malic acid, citric acid, lactic acid, tartaric acid, and erythorbic acid. These ingredients can be used individually, or a plurality of them can be used in combination. Synthetic products and/or foods with high content of these may be used. These foods may be in any form of liquid form, paste form, gel form, solid form, powder form and the like.

[0085]

The agent for enhancing lipid metabolism of the present invention is capable of efficiently enhancing lipid metabolism by its ingestion, not only in daily life or during medical care, but also before or in the condition where adrenaline secretion is continuously increased. For example, it is known that adrenaline secretion is continuously increased when exercise, cold exposure, or stress load is applied, or during bathing, or in overeaters. Accordingly, the agent for enhancing lipid metabolism of the present invention may be ingested before the application of exercise, cold exposure, stress load, or the like, or concurrently with the application of exercise, cold exposure, stress load, or the like. Alternatively, the agent for enhancing lipid metabolism of the present invention may be ingested before or during bathing. The agent for enhancing lipid metabolism of the present invention may be ingested by people that tend to overeat.

[0086]

The present invention provides also use (or use method) of a combination of at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine, for example, a combination of arginine, alanine, and phenylalanine, for manufacture of the agent for enhancing lipid metabolism, wherein the total number of moles of arginine, alanine, and phenylalanine is 60 mol or more, per 100 mol of the total number of moles of all amino acids.

[0087]

Further, the present invention provides also a method for enhancing lipid metabolism (except medical practices) comprising administering a composition comprising a combination of at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine, for example, a combination of arginine, alanine, and phenylalanine, in amounts such that the total number of moles of arginine, alanine, and phenylalanine is in a molar ratio

of 60 mol or more, per 100 mol of the total number of moles of all the amino acids contained (i.e., amount of all amino acids).

[0088]

Hereinafter, the present invention is described by way of examples. However, the present invention is not limited by these examples.

Examples

[0089]

(Example 1)

Comparison of various amino acids with a conventional amino acid composition (V.A.A.M.) for lipid metabolism enhancing effect

(1) Material and method

[Test samples]

- V.A.A.M.: An amino acid mixture (V.A.A.M.) having a composition shown in Table 1 was suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) at a concentration of 1 g/10 ml for preparation.

[0090]

- Various amino acids: L-alanine (Ala), L-methionine (Met), L-phenylalanine (Phe), L-arginine (Arg), and glycine (Gly) were each suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) at a concentration of 1 g/10 ml for preparation.

[Table 1]

	V.A.A.M.	
	(w/w%)	(mol/100mol)
L-arginine	4.96	3.56
L-alanine	4.41	6.19
L-phenylalanine	5.12	3.88
Glycine	11.6	19.33
L-proline	16.79	18.24
L-lysine hydrochloride	12.75	8.73
L-tyrosine	6.93	4.78
L-threonine	6.92	7.27
L-leucine	6.53	6.23
L-valine	5.56	5.94
L-isoleucine	4.79	4.57
L-glutamic acid	3.81	3.24
L-tryptophan	3.63	2.22
L-histidine	3.23	2.60
L-serine	2.11	2.51
L-methionine	0.64	0.54
Sodium L-aspartate	0.22	0.18

[0091]

[Animal experiment]

6 week-old Wistar male rats (Japan SLC, Inc.) were preliminarily bred for one week for using in the subsequent test. The rats were divided into groups (7 groups of Vehicle group, V.A.A.M. group, Alanine group, Methionine group, Phenylalanine group, Arginine group, and Glycine group, each with n = 6) by body weight, and were fasted for 18 hours while water was available *ad libitum*.

[0092]

Blood was collected from the tail vein (at Time -30), and immediately thereafter 10 ml/kg of the test sample (a dose of the test sample: 1 g/kg) (for V.A.A.M. group, Alanine group, Methionine group, Phenylalanine group, Arginine group, or Glycine group) or an injection water (for Vehicle group) was orally administered. After 30 minutes, blood was collected again (at Time 0), and immediately thereafter adrenaline ((R)-(-)-Epinephrine, Wako Pure Chemical Industries, Ltd.) was intraperitoneally administered (at 0.2 mg/kg, 4 ml/kg).

Then, blood was collected over time every 15 minutes (at Times 15, 30, 45, 60, and 75) until 75 minutes after the adrenaline administration. Plasma was obtained from the collected blood, and blood glycerol was measured.

[0093]

[Measurement]

Blood glycerol: Plasma glycerol was measured using a glycerol assay kit (Glycerol Assay Kit; Cayman Chemical Company) according to the attached procedure manual. A value was calculated by subtracting the measured value at Time -30 from the measured value at the time of each measurement, and this value was taken as an amount of change in blood glycerol concentration. Subsequently, the area under the curve (AUC) from Time 0 to Time 75 was calculated from the amount of change in blood glycerol concentration. Further, the rate of change (%) of the AUC of each amino acid group relative to the AUC of Vehicle group was calculated.

[0094]

(2) Results

Figure 1 shows the rate of change in blood glycerol concentration (rate of change in AUC of glycerol or Glycerol AUC change). All of Alanine group, Methionine group, Phenylalanine group, Arginine group, and Glycine group exhibited a higher value than V.A.A.M. group.

[0095]

It was found from this that alanine, methionine, phenylalanine, arginine, and glycine exhibited a higher lipid metabolism-enhancing effect than the conventional amino acid composition.

[0096]

However, as a result of checking the flavors of the different amino acids, the taste and odor of methionine are found to be unsuitable for oral ingestion.

[0097]

(Example 2)

Comparison of agent for enhancing lipid metabolism of the present invention (Preparation Example 1) with single amino acid for lipid metabolism-enhancing effect

(1) Material and method

[Test samples]

- "Preparation Example 1": An amino acid mixture (Preparation Example 1) having a composition shown in Table 2 was suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) at a concentration of 2.7 g/10 ml for preparation. 10 ml of the suspension contains 1.2 g of L-arginine, 0.57 g of L-alanine, and 0.47 g of L-phenylalanine.

[0098]

- Arginine: L-arginine was suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) at a concentration of 1.2 g/10 ml for preparation.

[0099]

- Alanine: L-alanine was suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) at a concentration of 0.57 g/10 ml for preparation.

[0100]

- Phenylalanine: L-phenylalanine was suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) at a concentration of 0.47 g/10 ml for preparation.

[Table 2]

	Preparation Example 1		Preparation Example 2	
	(w/w%)	(mol/100mol)	(w/w%)	(mol/100mol)
L-arginine	54.0	43.2	33.3	24.9
L-alanine	25.1	39.3	33.3	48.8
L-phenylalanine	20.8	17.6	33.3	26.3

[0101]

[Animal experiment]

6 week-old Wistar male rats (Japan SLC, Inc.) were preliminarily bred for one week for using in the subsequent test. The rats were divided into groups (5 groups of Vehicle group, "Preparation Example 1" group, Arginine group, Alanine group, and Phenylalanine group, each with n = 6) by body weight, and were fasted for 18 hours while water was available *ad libitum*.

[0102]

Blood was collected from the tail vein (at Time -30), and immediately thereafter 5 ml/kg of the test sample (for V.A.A.M. group, "Preparation Example 1" group, Arginine group, Alanine group, or Phenylalanine group) or an injection water (for Vehicle group) was orally administered. After 30 minutes, blood was collected again (at Time 0), and immediately thereafter adrenaline ((R)-(-)-epinephrine, Wako Pure Chemical Industries, Ltd.) was intraperitoneally administered (at 0.2 mg/kg, 8 ml/kg). Then, blood was collected over time every 15 minutes (at Times 15, 30, 45, 60, 75, and 90) until 90 minutes after the adrenaline administration. Plasma was obtained from the collected blood, and blood glycerol was measured.

[0103]

[Measurement]

Blood glycerol: Plasma glycerol was measured using a glycerol fluorescent assay kit (Glycerol Fluorometric Assay Kit; Cayman Chemical Company) according to the attached procedure manual. A value was calculated by subtracting the measured value at Time -30 from the measured value at the time of each measurement, and this value was taken as an amount of change in blood glycerol concentration.

[0104]

[Statistical processing method]

The measured value was expressed as a mean $\pm$ standard error. Data was tested for the equality of variances by F-test using a statistical software Stat View 5.0-J, and then tested by Fisher's PLSD. The significance level was set at 5%.

[0105]

(2) Results

Figure 2 indicates the amount of change in blood glycerol concentration (Δ glycerol). The amount of change in blood glycerol concentration remained at higher levels in "Preparation Example 1" group than in Vehicle group. At 15 minutes, 30 minutes, 45 minutes, 60 minutes, and 75 minutes (at Times 15, 30, 45, 60, and 75) after the adrenaline administration, "Preparation Example 1" group exhibited significantly higher amounts of

change in blood glycerol concentration as compared with Vehicle group ($p < 0.05$). Phenylalanine group had a significantly higher amount of change in blood glycerol concentration as compared with Vehicle group only at 30 minutes (at Time 30) after the adrenaline administration. No significant difference from Vehicle group was shown in the other groups.

[0106]

It was found from this that alanine, phenylalanine, and arginine exhibit a higher lipid metabolism-enhancing effect when these three amino acids are ingested in combination, than when they are individually ingested.

[0107]

(Example 3)

Comparison of agent for enhancing lipid metabolism of the present invention (Preparation Example 3) with conventional amino acid composition (V.A.A.M.) for lipid metabolism-enhancing effect

(1) Material and method

[Test samples]

- V.A.A.M.: An amino acid mixture (V.A.A.M.) having a composition shown in Table 1 was suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) at a concentration of 3 g/10 ml for preparation.

[0108]

- "Preparation Example 3": An amino acid mixture (Preparation Example 3) having a composition shown in Table 3 was suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) at a concentration of 3 g/10 ml for preparation.

[Table 3]

	Preparation Example 3	
	(w/w%)	(mol/100mol)
L-arginine	40.7	28.81
L-alanine	18.9	26.20
L-phenylalanine	15.7	11.72
Glycine	14.9	24.46
L-proline	2.24	2.40
L-lysine hydrochloride	1.70	1.15
L-tyrosine	0.92	0.63
L-threonine	0.92	0.96
L-leucine	0.87	0.82
L-valine	0.74	0.78
L-isoleucine	0.64	0.60
L-glutamic acid	0.51	0.43
L-tryptophan	0.48	0.29
L-histidine	0.43	0.34
L-serine	0.28	0.33
L-methionine	0.09	0.07
Sodium L-aspartate	0.03	0.02

[0109]

[Animal experiment]

6 week-old Wistar male rats (Japan SLC, Inc.) were preliminarily bred for one week for using the subsequent test. The rats were divided into groups (3 groups of Vehicle group, V.A.A.M. group, and "Preparation Example 3" group, each with n = 7) by body weight, and were fasted for 18 hours while water was available *ad libitum*.

[0110]

Blood was collected from the tail vein (at Time -30), and immediately thereafter 5 ml/kg of the test sample (for V.A.A.M. group or "Preparation Example 3" group) or an injection water (for Vehicle group) was orally administered (Table 4). After 30 minutes, blood was collected again (at Time 0), and immediately thereafter adrenaline ((R)-(-)-epinephrine, Wako Pure Chemical Industries, Ltd.) was intraperitoneally administered (at 0.2 mg/kg, 8 ml/kg). Then, blood was collected over time every 15 minutes (at Times 15, 30, 45,

60, 75, and 90) until 90 minutes after the adrenaline administration. Plasma was obtained from the collected blood, and blood glycerol was measured.

[Table 4]

Group name	Test sample	Dose	n
Vehicle			7
V.A.A.M.	V.A.A.M.	3 g/kg	7
"Preparation Example 3"	Preparation Example 3	3 g/kg	7

[0111]

[Measurement]

Blood glycerol: Plasma glycerol was measured using a Glycerol Assay Kit (Cayman Chemical Company) according to the attached procedure manual. A value was calculated by subtracting the measured value at Time -30 from the measured value at the time of each measurement, and this value was taken as an amount of change in blood glycerol concentration.

[0112]

[Statistical processing method]

The measured value was expressed as a mean $\pm$ standard error. Data was tested for the equality of variances by F-test using a statistical software Stat View 5.0-J, and then tested by Fisher's PLSD. The significance level was set at 5%.

[0113]

(2) Results

Figure 3 indicates the amount of change in blood glycerol concentration. The amount of change in blood glycerol concentration remained at higher levels in "Preparation Example 3" group than in V.A.A.M. group. At 30 minutes (at Time 30) and 45 minutes (at Time 45) after the adrenaline administration, "Preparation Example 3" group exhibited significantly high ($p < 0.05$) amounts of change in blood glycerol concentration as compared with Vehicle group.

[0114]

From the above results, it was found that the agent for enhancing lipid metabolism of the present invention containing alanine, phenylalanine, and arginine at high levels exhibits a higher lipid metabolism-enhancing effect than the conventional amino acid composition.

[0115]

(Example 4)

Lipid metabolism-enhancing effect of agent for enhancing lipid metabolism of the present invention (Preparation Example 3) in high-fat diet-fed animals with exercise applied

(1) Material and method

[Test samples and feeds]

- 0.1% CMC solution: Carboxymethylcellulose (Wako Pure Chemical Industries, Ltd.) was dissolved in distilled water for injection at a concentration of 0.1 w/v% for preparation.

[0116]

- V.A.A.M.: An amino acid mixture (V.A.A.M.) having a composition shown in Table 1 was suspended in 0.1% CMC solution at a concentration of 1 g/10 ml for preparation.

[0117]

- V.A.A.M.+CoQ10+L-Carnitine: An amino acid mixture (V.A.A.M.) having a composition shown in Table 1, coenzyme Q10 (CoQ10, Taiyo Kagaku Co., Ltd.), and L-carnitine (San-Ei Gen F.F.I., Inc.) were suspended in 0.1% CMC solution at concentrations of 1 g/10 ml, 10 mg of CoQ10/10 ml, and 68 mg/10 ml, respectively, for preparation.

[0118]

- "Preparation Example 3": An amino acid mixture (Preparation Example 3) having a composition shown in Table 3 was suspended in 0.1% CMC solution at a concentration of 1 g/10 ml for preparation.

[0119]

- AIN-93M (Oriental Yeast Co., Ltd.): This composition is shown in Table 5. It is a standard purified diet for nutrition research using mice and rats released by the US National Institute of Nutrition (AIN) in 1993 (AIN-93).

[0120]

- HFD-60 (Oriental Yeast Co., Ltd.): This composition is shown in Table 5. It is a high fat diet with 60% of calories from fat.

[Table 5]

	AIN-93M (g/kg)	HFD-60 (g/kg)
Casein	140	260
Beef tallow	0	330
Soybean oil	40	20
β -cornstarch	465.692	0
α -cornstarch	155	160
Sucrose	100	55
Dietary fiber	50	66.1
Mineral mixture	35	35
Vitamin mixture	10	10
L-cystine	1.8	3.6
Choline bitartrate	2.5	2.5
Tertiary butyl hydroquinone	0.008	0
Maltodextrin	0	60
Calcium carbonate	0	1.8

[0121]

[Animal experiment]

3 week-old C57BL/6J male mice (CLEA Japan, Inc.) were preliminarily bred for one week, and acclimatized to the treadmill for one week, and then used for the subsequent test. They were divided into groups (5 groups of Normal group (n =9), High-fat group (n = 10), H+V.A.A.M. group (n =9), H+V.A.A.M.+CoQ10+L-Carnitine group (n = 10), and H+"Preparation Example 3" group (n = 10)) by body weight (at Day 0), and were subsequently fed with AIN-93M (for Normal group) or HFD-60 (for High-fat group, H+V.A.A.M. group, H+V.A.A.M.+CoQ10+L-Carnitine group, and H+"Preparation Example 3" group) *ad libitum* until the last day of the test (Day 42) after 42 days from the division into groups, while water was available *ad libitum*.

[0122]

[Administration of test samples]

The test sample (for H+V.A.A.M. group, H+V.A.A.M.+CoQ10+L-Carnitine group, or H+"Preparation Example 3" group) or a 0.1% CMC solution (for Normal group and High-fat group) was orally administered using a feeding needle every day for 42 days except the last day of the test (from Day 0 to Day 41). The test sample or the 0.1% CMC solution was prepared once a week for use, and was administered at a dose shown in Table 6 (administration volume of 10 mL/kg).

[Table 6]

Group name	Feed	Test sample	Dose	n
Normal	AIN-93M			9
High-fat	HFD-60			10
H+V.A.A.M.	HFD-60	V.A.A.M.	1 g/kg	9
H+V.A.A.M. +CoQ10 +L-Carnitine	HFD-60	V.A.A.M. +CoQ10 +L-Carnitine	1 g/kg 10mg/kg 68mg/kg	10
H+"Preparation Example 3"	HFD-60	Preparation Example 3	1 g/kg	10

[0123]

[Exercise applied using treadmill]

During the period from the division into groups to the last day of the test (from Day 0 to Day 42), exercise was applied using a treadmill (at 15 m/min to 20 m/min, for 40 minutes) three times a week.

[0124]

[Body weight measurement]

During the period from the division into groups to the last day of the test (from Day 0 to Day 42), the body weight was measured once every three days.

[0125]

[Tissue collection]

Dissection was performed on the last day of the test (Day 42), and the weight of perirenal adipose tissue and brown adipose tissue were measured and the UCP-1 expression level in the brown adipose tissue was determined.

[0126]

[Measurement]

UCP-1 expression level in brown adipose tissue: The UCP-1 expression level in brown adipose tissue was determined using a Mouse mitochondrial brown fat uncoupling protein 1 (UCP1) ELISA Kit (CUSABIO) according to the attached procedure manual.

[0127]

[Statistical processing method]

The measured value was expressed as a mean $\pm$ standard error. Data was tested for the equality of variances by F-test using a statistical software Stat View 5.0-J, and then tested by Fisher's PLSD. The significance level was set at 5%.

[0128]

(2) Results

Figure 4 shows the results of the body weight measurement and feed intake/body weight during the test period. In High-fat group, the body weight significantly increased from the 3rd day (Day 3) of administration as compared with Normal group. In "Preparation Example 3" group, a significantly reducing effect on body weight was seen from the 3rd day to the 42nd day (Day 3 to Day 42) of administration as compared with High-fat group. On the other hand, V.A.A.M. group and V.A.A.M.+CoQ10+L-Carnitine group showed no difference in body weight as compared with High-fat group. Each group fed with HFD-60 had a lower value of feed intake/body weight during the test period than the group fed with AIN-93M, but no difference was found among the groups fed with HFD-60.

[0129]

Figure 5 shows perirenal fat weight. In High-fat group, the weight significantly increased as compared with Normal group. In Preparation Example 3 group, the increase in adipose tissue weight was significantly reduced as compared with High-fat group. On the other hand, V.A.A.M. group and V.A.A.M.+CoQ10+L-Carnitine group showed no difference in adipose tissue weight as compared with High-fat group.

[0130]

Figure 6 shows UCP-1 expression level in brown adipose tissue. In High-fat group, there was no change in UCP-1 expression level compared with Normal group. In Preparation Example 3 group, the UCP-1 expression level was significantly increased as compared with High-fat group. On the other hand, V.A.A.M. group and V.A.A.M.+CoQ10+L-Carnitine group showed no difference in UCP-1 expression level as compared with High-fat group.

[0131]

From the above results, the lipid metabolism-enhancing effect of the agent for enhancing lipid metabolism of the present invention during application of exercise was observed.

[0132]

(Example 5)

Confirmation of effect of the agent for enhancing lipid metabolism of the present invention for lipid metabolism-enhancing effect

(1) Material and method

[Test foods]

Using a capsule for which the presence or absence of the content cannot be identified in appearance (white cellulose capsule (of vegetable origin) No. 00 prescribed in the Japanese Pharmacopoeia), the following two types of test foods were prepared.

[0133]

- Placebo: A capsule with no contents

[0134]

- Active: A capsule enclosing 3 g of Preparation Example 3 shown in Table 3

[0135]

[Test subjects]

For selecting the test subjects, men aged 20 years or older were subjected to serum chemistry, hematological test, and bicycle exercise in advance (VS0). In the bicycle exercise, the time required from the start of exercise to total exhaustion and the maximum oxygen intake (VO_{2max}) were determined. Further, lifestyle habits were checked by a preliminary questionnaire survey. Based on these results, test subjects were selected for being health and

having no deviations of the time required from the start of exercise to total exhaustion and VO_2max .

[0136]

In this test, the test subjects were determined to be totally exhausted when two or more of the following (1) to (4) criteria were satisfied. Further, the maximum oxygen intake measured immediately before the determination of total exhaustion was taken as VO_2max .

[0137]

(1) A plateau phenomenon has occurred due to an increase in oxygen intake (VO_2).

[0138]

(2) Respiratory exchange ratio (VCO_2/VO_2) has reached 1.1 or more.

[0139]

(3) Heart rate (HR) has reached 200, which is expected to be the highest heart rate (HRmax) for a 20-year-old man.

[0140]

(4) Rating of Perceived Exertion (RPE) using the Japanese version of Borg scale (Onodera, et al., the correspondence between subjective intensity and objective intensity in whole body endurance exercise, in view of Rating of perceived exertion, Taiikugaku kenkyu (International Journal of Health and Sport Sciences), 21(4), p. 191 to 203 (1976)) has reached 19 (extremely hard (very very hard)) or higher.

[0141]

The bicycle exercise was performed until total exhaustion, using a bicycle ergometer, at a rotation speed of 60 revolutions/minute with an exercise intensity of 100 W from 0 to 1 minute after the start of exercise, which was subsequently increased by 25 W every minute (multistage incremental exercise tolerance test).

[0142]

The thus selected test subjects, 12 men aged 20.5 ± 0.2 years (mean $\pm$ standard error), were subjected to double-blind crossover comparison test.

[0143]

[Test schedule]

The first test (VS1) was performed at an interval of 14 days from VS0. In VS1, one of the two types of test foods was ingested, and bicycle exercise, blood collection, the collection and analysis of respiratory gas, and heart rate measurement were conducted. Further, the second test (VS2) was performed at an interval of 7 days from VS1. In VS2, the test food different from the test food ingested in VS1 was ingested, and bicycle exercise, blood collection, the collection and analysis of respiratory gas, and heart rate measurement were conducted.

[0144]

An instruction was given to the test subjects to restrain themselves from conducting excessive exercise, ingestion, or overeating that significantly deviates from the daily range from the start of the test (VS0) to the completion of the test (VS2). Further, use of supplements, health foods, and pharmaceutical products (including non-prescription drugs) that can affect this test, and smoking were not allowed. Further, an instruction to refrain from drinking alcohol, to finish eating before 22 o'clock, and to sleep by midnight (0 o'clock) on the previous days of the tests (VS0, VS1, and VS2) was given, and the meal was finished 2 hours before the start of bicycle exercise, and thereafter no eating or drinking was imposed (drinking water was allowed) until the completion of the test on the test day. Further, instructions to wake up at the same time on the test day and to perform the tests in the same time zone as far as possible were given.

[0145]

[Implementation of test VS1 and test VS2]

- Ingestion of test foods: The aforementioned test foods were ingested by swallowing with cold or hot water without chewing.

[0146]

- Bicycle exercise: Bicycle exercise was conducted using a bicycle ergometer at a rotation speed of 60 revolutions/minute, continuously for 60 minutes from 30 minutes after the ingestion of the test foods to 90 minutes after the ingestion of the test foods (at Time 30 to 90), with an exercise intensity of 50% of VO_{2max} as measured in VS0.

[0147]

- Blood collection: Blood was collected before the ingestion of the test foods and at 15, 30, 45, 60, 75, 90, 120, 150 minutes (at Times 0, 15, 30, 45, 60, 75, 90, 120, and 150) after the ingestion of the test foods.

[0148]

- Collection and analysis of respiratory gas: The collection and analysis were conducted using the breath-by-breath method, continuously for 150 minutes from immediately after the ingestion of test foods to 150 minutes after the ingestion of test foods (at Time 0 to Time 150).

[0149]

- Heart rate measurement: Heart rate was measured continuously for 150 minutes from immediately after the ingestion of test foods to 150 minutes after the ingestion of test foods (at Time 0 to Time 150).

[0150]

[Measurement]

- Respiratory exchange ratio: Exhaled breath collected was subjected to measurement every breath using a gas analyzer, to determine O₂ and CO₂ concentrations in the exhaled breath and the expiratory flow rate. Based on the measured values, the oxygen intake VO₂ (ml/kg of body weight/min), carbon dioxide production VCO₂ (ml/kg of body weight/min), and respiratory exchange ratio VCO₂/VO₂ were calculated. Further, the amount of change in respiratory exchange ratio (Δ VCO₂/VO₂) was determined by subtracting a mean respiratory exchange ratio of each group at Time 0 from the respiratory exchange ratio at each time.

[0151]

- Blood glycerol: Using an enzymatic method, serum glycerol was measured according to the attached procedure manual.

[0152]

- Blood free fatty acids: Using an enzyme-UV method, serum free fatty acid concentration was measured.

[0153]

- Blood cortisol: Using a CLEIA (Chemiluminescent Enzyme Immunoassay), plasma cortisol concentration was measured.

[0154]

- Blood glucagon: Using RIA2 antibody method (Double-antibody Radioimmunoassay), plasma glucagon concentration was measured.

[0155]

- Blood insulin: Using a CLIA (Chemiluminescent Immunoassay), serum insulin concentration was measured.

[0156]

- Blood glucose: Using an enzymatic method, blood glucose content was measured.

[0157]

- Blood total ketone body: Using an enzymatic method, serum total ketone body concentration (total of 3-hydroxybutyric acid and acetoacetic acid) was measured.

[0158]

- Blood 3-hydroxybutyric acid: Using an enzymatic method, serum 3-hydroxybutyric acid concentration was measured.

[0159]

- Blood acetoacetic acid: Using an enzymatic method, serum acetoacetic acid concentration was measured.

[0160]

- Blood growth hormone: Using a CLEIA, serum growth hormone concentration was measured.

[0161]

- Blood adrenaline: Using an HPLC (High Performance Liquid Chromatography), plasma adrenaline concentration was measured.

[0162]

- Blood noradrenaline: Using an HPLC, plasma noradrenaline concentration was measured.

[0163]

[Statistical processing method]

Each of the measured values, calculated values, or corresponding values obtained by the tests was evaluated by paired-sample t-test.

[0164]

(2) Results

Figure 7 shows the results of the amount of change over time in respiratory exchange ratio ($\Delta VCO_2/VO_2$). In the active group, the values of $\Delta VCO_2/VO_2$ remained low from the start of exercise to the completion of the exercise, and significantly decreased during exercise at Times 85 and 90 (minutes) as compared with the placebo group. After the completion of exercise, in both groups, the values temporarily increased at Times 95 and 100 (minutes), but thereafter drastically decreased until Time 135 (minutes). Particularly between Times 125 and 135 (minutes), the active group showed a lower average, though no significant difference was observed.

[0165]

Figure 8 shows the measurement results of blood glycerol concentration and free fatty acid concentration over time. The active group showed a higher average level of blood glycerol concentration at Times 45 and 90 (minutes) in exercise, though no significant difference was observed.

[0166]

The active group showed a higher average level of blood free fatty acids from the start of exercise (Time 30 (minutes)) to the completion of test (Time 150 (minutes)), and showed the highest average at Time 90 (minutes) in exercise.

[0167]

Figure 9 shows the measurement results of blood cortisol, glucagon, and insulin concentrations, and blood glucose over time.

[0168]

Cortisol level increased from Time 0 (before exercise) to Time 15 (minutes), but thereafter decreased. The active group had lower values from Times 15 to 75 (minutes), and showed a significant difference at Time 45 (minutes).

[0169]

Glucagon level increased from Time 30 (minutes) after the start of exercise, and the active group showed a significantly high value of glucagon at Time 75 (minutes) in exercise.

Further, the active group showed a significantly high value of glucagon also at Time 120 (minutes) after exercise.

[0170]

There was no difference in insulin and blood glucose level between the active group and the placebo group.

[0171]

Figure 10 shows the measurement results of blood concentrations of total ketone bodies, 3-hydroxybutyric acid, and acetoacetic acid over time. Total ketone bodies and 3-hydroxybutyric acid levels temporarily decreased until Time 45 (minutes) (from the start of exercise to 15 minutes after) in exercise in both the active group and the placebo group, but thereafter started to increase, and the active group had a greater increment from Times 45 to 150 (minutes) and the maximum average at Time 120 (minutes). There was a significant difference in the measured value at Time 60 (minutes). Focusing attention on the transition over time, the production of ketone bodies in the active group tended to significantly increase after the application of exercise (Time 120 and Time 150).

[0172]

Acetoacetic acid showed a transition similar to that of total ketone bodies, although there was no significant difference between both groups.

[0173]

Figure 11 shows the measurement results of blood concentrations of growth hormone, adrenaline, and noradrenaline. Growth hormone level increased from the start of the test (Time 0) in both the active group and the placebo group, and showed almost the similar transitions. There was no significant difference between the active group and the placebo group. The values of adrenaline and noradrenaline increased with the start of exercise (Time 30), and the values decreased with the completion of exercise (Time 90). There was no significant difference in both values between the active group and the placebo group.

[0174]

From the above results, the lipid metabolism-enhancing effect of the agent for enhancing lipid metabolism of the present invention during application of exercise could be

confirmed in humans. Further, it was also found that the agent for enhancing lipid metabolism of the present invention exerts the lipid metabolism-enhancing effect without affecting the secretion of insulin, growth hormone, adrenaline, and noradrenaline.

[0175]

(Example 6)

Production of beverage containing agent for enhancing lipid metabolism of the present invention

A beverage was produced using a conventional method by mixing Preparation Example 3 shown in Table 3 at a concentration of 1.5 w/v%, a sweetener at a concentration of 1.0 w/v%, an acidulant at a concentration of 0.9 w/v%, and a thickener at a concentration of 0.2 w/v%. 3 g of Preparation Example 3 can be ingested by ingestion of 200 ml of the beverage.

[0176]

(Example 7)

Production of tablet containing agent for enhancing lipid metabolism of the present invention

A tablet was produced according to a conventional method by mixing Preparation Example 3 shown in Table 3 at 50 w/w%, and an excipient, a binder, a disintegrant, and a lubricant at 50 w/w% in total.

[0177]

(Example 8)

Production of supplement containing agent for enhancing lipid metabolism of the present invention

1 g (5.7 mmol) of L-arginine, 1 g (11 mmol) of L-alanine, 1 g (6.1 mmol) of L-phenylalanine were mixed at this ratio, followed by addition of an excipient thereto, and the mixture was filled into a capsule.

[0178]

(Example 9)

Measurement of indicators of lipid metabolism

(1) Material and method

[Test samples]

As test samples, the amino acid mixture (Preparation Example 3) having a composition shown in Table 3 and the amino acid mixture (V.A.A.M.) having a composition shown in Table 1 were separately suspended in distilled water for preparation.

[0179]

[Animal experiment]

3 week-old male mice C57BL/6J (CLEA Japan, Inc.) were preliminarily bred for 5 days, and acclimatized to the treadmill for 2 days and then used for the experiment. After the completion of the acclimatization period, they were subjected to body weight measurement and divided into groups (Normal group, High-fat group, H+V.A.A.M. group, and H+Preparation Example 3 group) so that the groups had similar average body weights. Water was available *ad libitum*.

[0180]

Table 7 below shows the overview and group structure of the test system. Considering that lipid metabolism is reduced during the ingestion of high-fat diets, the 3 groups other than Normal group were allowed to ingest high-fat diet HFD-60.

[Table 7]

Group name	Feed	Test sample	Dose	n
Normal	AIN-93M	Distilled water	-	16
High-fat	HFD-60	Distilled water	-	18
H+V.A.A.M.	HFD-60	V.A.A.M.	1.0 g/kg	18
H+Preparation Example 3	HFD-60	"Preparation Example 3"	1.0 g/kg	18

*H denotes High-fat diet.

[0181]

The test sample was orally administered to the mice using a feeding needle every day for 4 days from the completion of the acclimatization period except the last day. During the administration period for 4 days, the mice were forced to exercise using the treadmill (forced running) once every 2 days. The exercise was applied using the treadmill for 40 minutes at a speed of 15 m/min. The test sample was administered at 30 minutes before the application of

exercise using the treadmill. The mice were fasted for 18 hours from the 4th day to the 5th day after the start of the administration.

[0182]

Dissection was performed on the 5th day after the start of the administration. On the dissection day, for the purpose of examining the change over time from the administration, each group was divided into three groups. Specifically, the mice in each group were divided into the groups composed of a group to be dissected before the administration (before administration (0)), a group to be dissected at 30 minutes after the administration (before exercise (30)), a group to be dissected after the treadmill exercise for 60 minutes from 30 minutes after the administration (after exercise (90)), and administration of the test sample and application of exercise were performed on the dissection day and then blood collection and dissection were performed. The liver removed was subjected to weight measurement, and immediately thereafter was frozen using liquid nitrogen for freeze preservation at -80°C until measurement of β -oxidation-associated enzymes.

[0183]

[Measurement]

The obtained blood was subjected to measurement of serum glucagon concentration (blood glucagon concentration) using a glucagon colorimetric ELISA kit, Mercodia Glucagon ELISA Kit (Mercodia), according to the attached procedure manual.

[0184]

Further, the activities of β -oxidation-associated enzymes (carnitine palmitoyltransferase activity, and acyl-CoA oxidase activity) in the liver were measured by the following procedure. First, the liver was homogenized and centrifuged to separate a supernatant, thereby preparing a liver total homogenate fraction. This fraction was subjected to measurement of carnitine palmitoyltransferase activity by a partially modified method of Marlwell et al. Specifically, the obtained liver total homogenate fraction was added to 58 mM Tris-hydrochloric acid buffer (pH 8.0) containing 0.04 mM palmitoyl CoA, 0.25 mM DTNB (Ellman's reagent), 1.25 mM EDTA, and 0.1% Triton-X100 at final concentrations, and the absorbance was measured at 30°C and 412 nm to monitor the reaction for 5 minutes.

Thereafter, L-carnitine (at a final concentration of 1.25 mM) was added thereto, and CoA production was monitored for 5 minutes (total amount of 200 μ L), thereby measuring the carnitine palmitoyltransferase activity.

[0185]

Further, the liver total homogenate fraction was subjected to measurement of acyl-CoA oxidase activity by a partially modified method of Hashimoto et al. Specifically, the obtained liver total homogenate fraction was added to 50 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM palmitoyl CoA, 10.6 mM phenol, 0.82 mM 4-aminoantipyrine, 10 μ M FAD, 4U peroxidase (horseradish), and 0.2 mg bovine albumin at final concentrations, and the absorbance was monitored at 30°C and 500 nm for 11 minutes (total amount of 200 μ L).

[0186]

Further, the amount of the proteins in the obtained liver total homogenate fraction was measured using the BCA method. The measurement was performed using a TaKaRa BCA Protein Assay Kit T9300A according to the attached procedure manual.

[0187]

[Statistical processing method]

The various measured values were expressed as means $\pm$ standard error. Data was tested for the equality of variances by F-test using a statistical software Stat View 5.0-J, and then were tested for multiple group comparison by Fisher's PLSD. The significance level was set at 5% on both sides.

[0188]

(2) Results

Figure 12 shows the measurement results of blood glucagon concentration. H+Preparation Example 3 group showed a significant increase in blood glucagon concentration before exercise (at 30 minutes after the administration) as compared with High-fat group. That is, it was demonstrated that the agent for enhancing lipid metabolism of the present invention could efficiently enhance lipid metabolism during exercise by increasing secretion of glucagon having a lipolysis enhancing effect, before the exercise.

[0189]

Figure 13 shows the measurement results of carnitine palmitoyltransferase activity. H+Preparation Example 3 group showed a significant increase in carnitine palmitoyltransferase activity before the administration and before the exercise (at 30 minutes after the administration) as compared with High-fat group.

[0190]

Figure 14 shows the measurement results of acyl-CoA oxidase activity. H+Preparation Example 3 group showed a significant increase in acyl-CoA oxidase activity before the administration as compared with High-fat group.

[0191]

Based on the fact that carnitine palmitoyltransferase and acyl-CoA oxidase, liver β -oxidation-associated enzymes, are known as rate-determining enzymes of lipid metabolism, it was found that the agent for enhancing lipid metabolism of the present invention could enhance lipid metabolism also by enhancing the activities of β -oxidation -associated enzymes.

[0192]

From the above results, it was demonstrated that the agent for enhancing lipid metabolism of the present invention containing alanine, phenylalanine, and arginine at high levels had a high lipid metabolism-enhancing effect.

[0193]

(Example 10)

Measurement of indicators of lipid metabolism

(1) Material and method

[Test samples]

As test samples, (i) alanine (alanine:arginine = 10:0), (ii) a mixture of alanine and arginine at 7.5:2.5, (iii) a mixture of alanine and arginine at 5:5, (iv) a mixture of alanine and arginine at 2.5:7.5, and (v) arginine (alanine:arginine = 0:10) separately suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) for preparation were used.

[0194]

[Animal experiment]

6 week-old Wistar male rats (Japan SLC, Inc.) were preliminarily bred, and thereafter were used for the subsequent test. On the morning of the day before the test, the rats were subjected to measurement for body weight, and were divided into groups having similar average body weights. Table 8 below shows the overview and group structure of the test system.

[Table 8]

Group name	Test sample	Dose	n
Control	Injection water	-	6
Ala:Arg=10:0	Alanine	1 g/kg	6
Ala:Arg=7.5:2.5	Mixture of alanine and arginine in a weight ratio of 3:1	1 g/kg	6
Ala:Arg=5:5	Mixture of alanine and arginine in a weight ratio of 1:1	1 g/kg	6
Ala:Arg=2.5:7.5	Mixture of alanine and arginine in a weight ratio of 1:3	1 g/kg	6
Ala:Arg=0:10	Arginine	1 g/kg	6

[0195]

The rats were fasted for 18 hours from the evening on the day before the test. However, water was available *ad libitum*.

[0196]

On the test day, blood was collected from the tail vein (30 minutes before the administration; at Time-30), and immediately thereafter the test sample was orally administered to the rats at a dose of 1 g/kg of rat body weight (5 ml/kg). Blood was collected again at 30 minutes after the aforementioned blood collection (Time 0), and immediately thereafter adrenaline ((R)-(-)-epinephrine, Wako Pure Chemical Industries, Ltd.) was intraperitoneally administered at a dose of 0.2 mg/kg (8 ml/kg). Thereafter, blood was collected over time every 15 minutes (at Times 15, 30, 45, 60, 75, and 90) until 90 minutes after the adrenaline administration. Plasma was obtained from the collected blood, and blood glycerol was measured.

[0197]

[Measurement]

Plasma glycerol was measured using a glycerol assay kit (Cayman Chemical Company) according to the attached procedure manual. A value was calculated by subtracting the measured value at Time 0 from the measured value in each measurement, and this value was taken as the amount of change in blood glycerol concentration.

[0198]

(2) Results

The amount of change in blood glycerol concentration remained at higher levels in alanine (Ala):arginine (Arg) = 2.5:7.5 group, 5:5 group, 7.5:2.5 group, and 10:0 group than in the control group. Further, it was found that alanine and arginine exhibit the highest lipid metabolism-enhancing effect when they are mixed at 1:1.

[0199]

(Example 11)

Measurement of indicators of lipid metabolism

(1) Material and method

[Test samples]

As test samples, (i) alanine (alanine:phenylalanine = 10:0), (ii) a mixture of alanine and phenylalanine at 7.5:2.5, (iii) a mixture of alanine and phenylalanine at 5:5, (iv) a mixture of alanine and phenylalanine at 2.5:7.5, and (v) phenylalanine (alanine:phenylalanine = 0:10) separately suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) for preparation were used.

[0200]

[Animal experiment]

6 week-old Wistar male rats (Japan SLC, Inc.) were preliminarily bred, and thereafter were used for the subsequent test. On the morning of the day before the test, the rats were subjected to measurement for body weight, and were divided into groups having similar average body weights. Table 9 below shows the overview and group structure of the test system.

[Table 9]

Group name	Test sample	Dose	n
Control	Injection water	-	6
Ala:Phe=10:0	Alanine	1 g/kg	6
Ala:Phe=7.5:2.5	Mixture of alanine and phenylalanine in a weight ratio of 3:1	1 g/kg	6
Ala:Phe=5:5	Mixture of alanine and phenylalanine in a weight ratio of 1:1	1 g/kg	6
Ala:Phe=2.5:7.5	Mixture of alanine and phenylalanine in a weight ratio of 1:3	1 g/kg	6
Ala:Phe=0:10	Phenylalanine	1 g/kg	6

[0201]

The rats were fasted for 18 hours from the evening on the day before the test. However, water was available *ad libitum*.

[0202]

On the test day, blood was collected from the tail vein (at Time -30), and immediately thereafter the test sample was orally administered to the rats at a dose of 1 g/kg of rat body weight (5 ml/kg). Blood was collected again at 30 minutes after the aforementioned blood collection (Time 0), and immediately thereafter adrenaline ((R)-(-)-epinephrine, Wako Pure Chemical Industries, Ltd.) was intraperitoneally administered at a dose of 0.2 mg/kg (8 ml/kg). Thereafter, blood was collected over time every 15 minutes (at Times 15, 30, 45, 60, 75, and 90) until 90 minutes after the adrenaline administration. Plasma was obtained from the collected blood, and blood glycerol concentration was measured.

[0203]

[Measurement]

Plasma glycerol was measured using a glycerol assay kit (Cayman Chemical Company) according to the attached procedure manual. A value was calculated by subtracting the measured value at Time 0 from the measured value in each measurement, and this value was taken as the amount of change in blood glycerol concentration.

[0204]

(2) Results

The amount of change in blood glycerol concentration remained at higher levels in alanine (Ala):phenylalanine (Phe) = 2.5:7.5 group, 5:5 group, 7.5:2.5 group, and 10:0 group than in the control group. Further, it was found that alanine and phenylalanine exhibit the highest lipid metabolism-enhancing effect when they are mixed at 1:3.

[0205]

(Example 12)

Measurement of indicators of lipid metabolism

(1) Material and method

[Test samples]

As test samples, (i) arginine (arginine:phenylalanine = 10:0), (ii) a mixture of arginine and phenylalanine at 7.5:2.5, (iii) a mixture of arginine and phenylalanine at 5:5, (iv) a mixture of arginine and phenylalanine at 2.5:7.5, and (v) phenylalanine (arginine:phenylalanine = 0:10) separately suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) for preparation were used.

[0206]

[Animal experiment]

6 week-old Wistar male rats (Japan SLC, Inc.) were preliminarily bred, and thereafter were used for the subsequent test. On the morning of the day before the test, the rats were subjected to measurement for body weight, and were divided into groups having similar average body weights. Table 10 below shows the overview and group structure of the test system.

[Table 10]

Group name	Test sample	Dose	n
Control	Injection water	-	6
Arg:Phe=10:0	Arginine	1 g/kg	6
Arg:Phe=7.5:2.5	Mixture of arginine and phenylalanine in a weight ratio of 3:1	1 g/kg	6
Arg:Phe=5:5	Mixture of arginine and phenylalanine in a weight ratio of 1:1	1 g/kg	6
Arg:Phe=2.5:7.5	Mixture of arginine and phenylalanine in a weight ratio of 1:3	1 g/kg	6
Arg:Phe=0:10	Phenylalanine	1 g/kg	6

[0207]

The rats were fasted for 18 hours from the evening on the day before the test. However, water was available *ad libitum*.

[0208]

On the test day, blood was collected from the tail vein (at Time -30), and immediately thereafter the test sample was orally administered to the rats at a dose of 1 g/kg of rat body weight (5 ml/kg). Blood was collected again at 30 minutes after the aforementioned blood collection (Time 0), and immediately thereafter adrenaline ((R)-(-)-epinephrine, Wako Pure Chemical Industries, Ltd.) was intraperitoneally administered at a dose of 0.2 mg/kg (8 ml/kg). Thereafter, blood was collected over time every 15 minutes (at Times 15, 30, 45, 60, 75, and 90) until 90 minutes after the adrenaline administration. Plasma was obtained from the collected blood, and blood glycerol concentration was measured.

[0209]

[Measurement]

Plasma glycerol was measured using a glycerol assay kit (Cayman Chemical Company) according to the attached procedure manual. A value was calculated by subtracting the measured value at Time 0 from the measured value in each measurement, and this value was taken as the amount of change in blood glycerol concentration.

[0210]

(2) Results

Figure 15 shows the amount of change (Δ glycerol) in blood glycerol concentration. The amount of change in blood glycerol concentration remained at higher levels in arginine (Arg):phenylalanine (Phe) = 2.5:7.5 group and 5:5 group than in the control group. Further, it was found that arginine and phenylalanine exhibit the highest lipid metabolism-enhancing effect when they are mixed at 1:3.

[0211]

(Example 13)

Measurement of indicators of lipid metabolism

(1) Material and method

[Test samples]

As test samples, (i) a mixture of alanine and arginine in a weight ratio of 1:1, (ii) a mixture of alanine and phenylalanine in a weight ratio of 1:3, and (iii) a mixture of arginine and phenylalanine in a weight ratio of 1:3, which showed high lipolytic activity in Examples 10 to 12 respectively, and (iv) the amino acid mixture (Preparation Example 3) having a composition shown in Table 3 separately suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) for preparation were used.

[0212]

[Animal experiment]

6 week-old Wistar male rats (Japan SLC, Inc.) were preliminarily bred, and thereafter were used for the subsequent test. On the morning of the day before the test, the rats were subjected to measurement for body weight, and were divided into groups having similar average body weights. Table 11 below shows the overview and group structure of the test system.

[Table 11]

Group name	Test sample	Dose	n
Control	Injection water	-	6
Ala:Arg=1:1	Mixture of alanine and arginine in a weight ratio of 1:1	1 g/kg	6
Ala:Phe=1:3	Mixture of alanine and phenylalanine in a weight ratio of 1:3	1 g/kg	6
Arg:Phe=1:3	Mixture of arginine and phenylalanine in a weight ratio of 1:3	1 g/kg	6
Preparation Example 3	Preparation Example 3	1 g/kg	6

[0213]

The rats were fasted for 18 hours from the evening on the day before the test. However, water was available *ad libitum*.

[0214]

On the test day, blood was collected from the tail vein (at Time -30), and immediately thereafter the test sample was orally administered to the rats at a dose of 1 g/kg of their body weight (5 ml/kg). Blood was collected again at 30 minutes after the aforementioned blood collection (Time 0), and immediately thereafter adrenaline ((R)-(-)-epinephrine, Wako Pure Chemical Industries, Ltd.) was intraperitoneally administered at a dose of 0.2 mg/kg (8 ml/kg). Thereafter, blood was collected over time every 15 minutes (at Times 15, 30, 45, 60, 75, and 90) until 90 minutes after the adrenaline administration. Plasma was obtained from the collected blood, and blood glycerol was measured.

[0215]

[Measurement]

Plasma glycerol was measured using a glycerol assay kit (Cayman Chemical Company) according to the attached procedure manual. Based on the value calculated by subtracting the measured value at Time 0 from the measured value in each measurement, blood glycerol AUC (area under the blood concentration-time curve) from Time 0 to Time 90 was calculated.

[0216]

[Statistical processing method]

The measured value was expressed as a mean $\pm$ standard error. Data was tested for the equality of variances by F-test using a statistical software Stat View 5.0-J, and then tested by Fisher's PLSD. The significance level was set at 5%.

[0217]

(2) Results

Figure 16 shows the calculated blood glycerol AUC (min·mg/L). The blood glycerol AUC was higher in all of Ala:Arg = 1:1 group, Ala:Phe = 1:3 group, Arg:Phe = 1:3 group, and Preparation Example 3 group, as compared with the control group.

[0218]

(Example 14)

Measurement of indicators of lipid metabolism

(1) Material and method

[Test samples]

As test samples, (i) a mixture of alanine and arginine in a weight ratio of 1:1, which exhibited the highest lipolytic activity in Example 13, and (ii) a mixture of alanine, arginine, and phenylalanine in a weight ratio of 1:1:3 and (iii) a mixture of alanine, arginine, and phenylalanine in a weight ratio of 1:1:6, of which ratios were set based on the results of Examples 10 to 12, separately suspended in an injection water (Otsuka Pharmaceutical Co., Ltd.) for preparation were used.

[0219]

[Animal experiment]

6 week-old Wistar male rats (Japan SLC, Inc.) were preliminarily bred, and thereafter were used for the subsequent test. On the morning of the day before the test, the rats were subjected to measurement for body weight, and were divided into groups having similar average body weights. Table 12 below shows the overview and group structure of the test system.

[Table 12]

Group name	Test sample	Dose	n
Control	Injection water	-	6
Ala:Arg=1:1	Mixture of alanine and arginine in a weight ratio of 1:1	1 g/kg	6
Ala:Arg:Phe=1:1:3	Mixture of alanine, arginine, and phenylalanine in a weight ratio of 1:1:3	1 g/kg	6
Ala:Arg:Phe=1:1:6	Mixture of alanine, arginine, and phenylalanine in a weight ratio of 1:1:6	1 g/kg	6

[0220]

The rats were fasted for 18 hours from the evening on the day before the test. However, water was available *ad libitum*.

[0221]

On the test day, blood was collected from the tail vein (at Time -30), and immediately thereafter the test sample was orally administered to the rats at a dose of 1 g/kg of rat body weight (5 ml/kg). Blood was collected again at 30 minutes after the aforementioned blood collection (Time 0), and immediately thereafter adrenaline ((R)-(-)-epinephrine, Wako Pure Chemical Industries, Ltd.) was intraperitoneally administered at a dose of 0.2 mg/kg (8 ml/kg). Thereafter, blood was collected over time every 15 minutes (at Times 15, 30, 45, 60, 75, and 90) until 90 minutes after the adrenaline administration. Plasma was obtained from the collected blood, and blood glycerol was measured.

[0222]

[Measurement]

Plasma glycerol was measured using a glycerol assay kit (Cayman Chemical Company) according to the attached procedure manual. A value was calculated by subtracting the measured value at Time 0 from the measured value in each measurement was calculated, and this value was taken as the amount of change in blood glycerol concentration. Based on the value calculated by subtracting the measured value at Time 0 from the measured value in each measurement, blood glycerol AUC (area under the blood concentration-time curve) from Time 0 to Time 90 was calculated.

[0223]

(2) Results

Figure 17 shows the calculated blood glycerol AUC (min·mg/L). The blood glycerol AUC was higher in alanine (Ala):arginine (Arg) = 1:1 group, and alanine (Ala):arginine (Arg):phenylalanine (Phe) = 1:1:3 group and 1:1:6 group, as compared with the control group.

[0224]

From the above results, it was further demonstrated that the agent for enhancing lipid metabolism of the present invention containing a combination of two or three of alanine, phenylalanine, and arginine exhibited a high lipid metabolism-enhancing effect.

Industrial Applicability

[0225]

The agent for enhancing lipid metabolism according to the present invention enables an effective enhancement of fat consumption and an obtainment of a higher body fat reducing effect by the same amount of exercise. The present invention enables a development of an agent for enhancing lipid metabolism expected to have such effects and products using the agent.

[0226]

All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

Claims

1. An agent for enhancing lipid metabolism comprising:
an amino acid mixture comprising at least two amino acids selected from the group consisting of arginine, alanine, and phenylalanine,
wherein the agent comprises the at least two amino acids in a molar ratio of 60 mol or more, in total, per 100 mol of an amount of all amino acids.
2. The agent for enhancing lipid metabolism according to claim 1, wherein the amino acid mixture comprises arginine, alanine, and phenylalanine.
3. The agent for enhancing lipid metabolism according to claim 1 or 2, wherein the agent comprises arginine, alanine, and phenylalanine in a molar ratio of arginine:alanine:phenylalanine of 8-30 mol:18-30 mol:10-20 mol, per 100 mol of the amount of all amino acids.
4. The agent for enhancing lipid metabolism according to any one of claims 1 to 3, wherein the amounts of arginine, alanine, and phenylalanine contained satisfy at least one weight ratio of (i) arginine:alanine of 1.5:1 to 1:1.5, (ii) alanine:phenylalanine of 4:1 to 1:4, and (iii) arginine:phenylalanine of 1:1 to 1:4.
5. The agent for enhancing lipid metabolism according to any one of claims 1 to 4, wherein the agent comprises arginine, alanine, and phenylalanine in a molar ratio of 100 mol, in total, per 100 mol of the amount of all amino acids.
6. The agent for enhancing lipid metabolism according to any one of claims 1 to 4, wherein the amino acid mixture further comprises glycine, and the agent comprises arginine, alanine, phenylalanine, and glycine in a molar ratio of 85 mol or more, in total, per 100 mol of the amount of all amino acids.

7. The agent for enhancing lipid metabolism according to any one of claims 1 to 4 and 6, wherein the amino acid mixture further comprises exclusively proline, lysine, tyrosine, threonine, leucine, valine, isoleucine, glutamic acid, tryptophan, histidine, serine, methionine, and aspartic acid as other amino acids.

8. The agent for enhancing lipid metabolism according to claim 7, wherein the amino acid mixture comprises the other amino acids in the following molar ratios per 100 mol of the amount of all amino acids:

0.01 to 4 mol of proline,
0.01 to 2 mol of lysine,
0.01 to 2 mol of tyrosine,
0.01 to 2 mol of threonine,
0.01 to 2 mol of leucine,
0.01 to 2 mol of valine,
0.01 to 2 mol of isoleucine,
0.01 to 1 mol of glutamic acid,
0.01 to 1 mol of tryptophan,
0.01 to 1 mol of histidine,
0.01 to 1 mol of serine,
0.01 to 0.2 mol of methionine, and
0.01 to 0.1 mol of aspartic acid.

9. The agent for enhancing lipid metabolism according to any one of claims 1 to 8, wherein the agent is for use in acting on a condition where adrenaline secretion is increased.

10. The agent for enhancing lipid metabolism according to claim 9, wherein the condition where adrenaline secretion is increased is a condition of being stimulated by exercise, stress, cold exposure, bathing, or overeating.

11. The agent for enhancing lipid metabolism according to any one of claims 1 to 10, wherein the agent is for the enhancement of lipid metabolism that is accompanied by at least one selected from the group consisting of increased blood glycerol concentration, suppressed weight gain, increased blood free fatty acid concentration, increased blood glucagon concentration, reduced blood cortisol concentration, increased blood total ketone body concentration, increased blood 3-hydroxybutyric acid concentration, and increased UCP-1 expression level in brown adipose tissue.