An arginine, alanine, and phenylalanine mixture increases synthesis of ketone bodies during low-intensity exercise via stimulating glucagon secretion in men with obesity

Keisuke Ueda^{1,2*}, Chiaki Sanbongi¹ and Shuji Ikegami¹

¹Food Science Research Labs, R&D Division, Meiji Co., Ltd., 540 Naruda, Odawara, Kanagawa 250-0862, Japan

²Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8574, Japan

Received: May 23, 2017 / Accepted: July 3, 2017

Abstract During exercise, levels of several hormones are acutely increased in the blood. We previously reported that pre-exercise ingestion of a specific combination of amino acids (arginine, alanine, and phenylalanine; A-mix) increases fat mobilization and ketone body synthesis by increasing secretion of adrenalin and glucagon in healthy active young men. Herein, we sought to determine whether this acute hormone response could be induced upon administration of A-mix combined with exercise in patients with obesity during periods of low-intensity exercise. We performed a randomized crossover study of eleven middle-aged men with obesity without regular exercise habits, administered either A-mix (3 g/dose) or a placebo (3 g of dextrin/dose). Thirty minutes after ingestion, each subject subsequently performed workload tests on a cycle ergometer at 40% of peak oxygen consumption for 1 h. Following oral intake of A-mix, the concentration of plasma ketone bodies was significantly increased during exercise. This was accompanied by a significant increase in the area under the concentration-time curve for glucagon. Taken together, these results indicate that pre-exercise ingestion of the A-mix supplement significantly accelerated hepatic ketone body synthesis via stimulation of glucagon secretion during exercise in men with obesity.

Keywords : pre-exercise nutrition, amino acid supplementation, metabolism, hormones

Introduction

Obesity is a worldwide public health problem¹⁾, and a major risk factor for insulin resistance, type 2 diabetes, atherosclerosis, stroke, hypertension, and some types of cancer²⁾. Obesity results from a chronic imbalance in energy metabolism. To prevent and/or mitigate these concerns, weight loss through dietary restriction and regular exercise is considered the best available non-surgical, non-pharmacologic treatment strategy³⁾.

Regular exercise is one important element in the approach to prevent obesity⁴⁾. According to the exercise prescription recommended in the American College of Sports Medicine⁵⁾ guidelines, 45-60 min of exercise should be targeted to ensure sufficient energy expenditure in obese people. In particular, the blood levels of several hormones such as catecholamines, glucagon, growth hormone and cortisol are increased by acute exercise⁶⁾. Moreover, several studies reported on acute exercise and lipid utilization, and related hormones in obese subjects⁷⁻⁹⁾. Therefore, we hypothesize that a combination of acute exercise and the factors that affect the secretion of some of these hormones may be effective for increasing fat catabolism.

We recently focused our research efforts on amino acids (AAs) that affect glucagon secretion, as it was previously reported that ingestion or infusion of some AAs increases insulin and/or glucagon concentrations in the blood¹⁰⁾. Glucagon is one of the key hormones implicated in fat catabolism during exercise^{11,12)}. Tan et al. reported that glucagon infusion acutely increased energy expenditure in humans¹³⁾. Moreover, glucagon administration in rodents was shown to increase brown adipose tissue mass and activity¹⁴⁾. We hypothesized that a specific combination of AAs that affects glucagon secretion may acutely promote fat catabolism and energy expenditure when combined with exercise. Several reports suggested that when combining AAs with similar functions, the additive effect is expected for the mixture^{15,16)}. Moreover, arginine and phenylalanine increase GTP cyclohydrolase-I expression and activity that increases tetrahydrobiopterin availability for NO synthesis and aromatic AA hydroxylation¹⁷⁾. Therefore, in earlier reports, we developed a new AA mixture containing arginine, alanine, and phenylalanine (A-mix), and investigated the acute effects of A-mix supplementation combined with 60-min moderate-intensity (50% of maximal oxygen uptake [$\dot{V}O_{2max}$]) exercise using cycle ergometry. Pre-exercise ingestion of A-mix increased levels of ketone bodies and glycerol in the blood both during

*Correspondence: keisuke.ueda@meiji.com

and after a bout of exercise in healthy active young men, by increasing secretion of adrenalin and glucagon¹⁸⁾.

Herein, we sought to determine whether this acute response induced by administration of A-mix combined with exercise also occurs in patients with obesity during periods of low-intensity exercise. Previous cross-sectional studies reported that the exercise intensity that corresponds to a maximal fat oxidation is 42% of $\dot{V}O_2\text{max}$ ¹⁹⁾. Therefore, we conducted a randomized, double-blind, placebo-controlled crossover study to investigate glucagon secretion induced by A-mix and fat catabolism combined with 40% of $\dot{V}O_2\text{peak}$ intensity exercise in middle-aged men with obesity without regular exercise habits.

Materials and Methods

Trial design. This was a randomized, double-blind, placebo-controlled crossover study, conducted at the Chiyoda Paramedical Care Clinic in Chiyoda-ku, Japan, from June 2015 to August 2015. The study protocol was approved by the Institutional Review Board of the Chiyoda Paramedical Care Clinic (No.15061803) and the Meiji Institutional Review Board (No.57). All study participants provided written informed consent prior to participation in the study. The study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. The study protocol was registered in the UMIN Clinical Trials Registry (UMIN000018161) on July 1, 2015.

Participants. Participants were recruited via a contract research organization (CRO). Overweight (BMI ≥ 25 but $< 30 \text{ kg/m}^2$) men aged 30 to 50 years old without regular exercise habits were included in the study. A regular exercise habit was defined as engaging in at least 30 min of exercise twice a week for more than one year, according to the criteria of the National Health and Nutrition Examination Survey²⁰⁾. Exclusion criteria consisted of individuals with a history or current condition of severe disease (such as liver disorder, cardiovascular disorder, respiratory disorder, endocrine disorder, and/or metabolic disorder), severe systemic disease, heavy drinkers ($\geq 40 \text{ g}$ of ethanol per day), smokers, those with extremely irregular eating patterns due to shift work or late-night work, those who regularly consumed functional foods or nutritional supplements, those who donated blood over the past 6 months, patients with phenylketonuria or hyperphenylalaninemia, and those who were judged ineligible by the study physician due to abnormal blood and urine tests or for other reasons. Characteristics of the study participants are included in Table 1.

Experimental procedures. Study participants visited the clinic a total of three times throughout the study. During the first visit, all participants underwent a physical examination, and a baseline blood sample (10 mL) was collected for analysis of the following parameters: platelet,

white blood cell and red blood cell counts, levels of hemoglobin, hematocrit, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, albumin, total protein, blood urea nitrogen, creatinine, uric acid, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, sodium, potassium, and chloride. Body weight and percent fat mass were measured using multi-frequency bioelectrical impedance analysis (Lookin'Body 3.2, Biospace, Seoul, Korea). Height was measured to the nearest 0.1 cm using a portable stadiometer (AD-6351, A&D Co., Ltd., Tokyo, Japan) at baseline to estimate BMI. BMI was computed as weight in kilograms divided by squared height in meters. The physical working capacity (PWC) at 75% of the maximal heart rate (HR) was measured on a ramp test (8 W/min) using a bicycle ergometer (Aero Bike 75XL-III, Konami Sports Life Co., Ltd., Kanagawa, Japan). The age-related maximal HR was calculated as: $209 - 0.69 \times \text{age}$, in beats per min (BPM). Results of the $\text{PWC}_{75\%HR\text{max}}/\text{weight}$ were used to estimate the power output equivalent to 40% of $\dot{V}O_2\text{peak}$ ²¹⁾.

The remaining two study visits were separated by at least six days. On the day of the second and third visits, the subjects consumed meals provided at least 6 h before each test. All meals had the same carbohydrate:fat:protein ratio (71:16:13) and contained 612 kcal. The subjects consumed no food or drink except water from the last meal to the start of each test. Individual tests were performed at a similar time of day for each subject ($\pm 3 \text{ h}$) to avoid any influence of circadian rhythm on the results. Each subject continued his usual diet and was instructed to refrain from binge eating, strenuous exercise throughout the study, and drinking alcohol for 24 hours prior to each test. Dietary intake was self-recorded by the subjects from their first visit until their last.

During the second and third visits, subjects participated in the main study tests. After measurement of blood pressure and HR, blood samples were drawn from the antecubital vein. The subjects were randomized to ingest 50 mL

Table 1. Characteristics of subjects

Age (years)	39.7 $\pm$ 5.2
Height (cm)	170.0 $\pm$ 4.4
Body weight (kg)	76.0 $\pm$ 6.1
BMI (kg/m ²)	26.3 $\pm$ 1.4
Percent fat mass (%)	24.7 $\pm$ 3.2
$\dot{V}O_2 \text{ max}$ (ml/kg/min)	32.7 $\pm$ 6.3

Values are mean $\pm$ SD (n = 11)

of ordinary tap water and a cellulose capsule containing either 3 g of A-mix (Kyowa Hakko Bio Co., Ltd., Tokyo, Japan) as the active sample or 3 g of dextrin (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan) as the placebo (designated 0 min). The treatments were subsequently switched during the crossover phase of the study. After sitting for 30 min (rest period), the subjects mounted a cycle ergometer and commenced cycling for 60 min at a constant power output equivalent to 40% of $\dot{V}O_{2\text{peak}}$ (exercise period). In order to prevent dehydration, 200 ml of tap water was consumed by all subjects at 30 minutes during the exercise. After exercising, subjects rested for 60 min in the supine position (post-exercise period). Blood samples were collected every 30 min throughout the test. HR was tracked from the earlobe during exercise. Blood pressure and HR were recorded again at the end of the post-exercise period. The tests were conducted in a quiet controlled room environment at a temperature of $21 \pm 2^\circ\text{C}$ and humidity of $45 \pm 5\%$. The study design is summarized in Fig. 1.

Blood sampling. The assays used to measure glucagon, insulin, blood glucose, acetoacetic acid, 3-hydroxybutyrate, lactate, FFAs, growth hormone, adrenalin, and blood chemistry panels were performed at the LSI Medience Corporation (Tokyo, Japan), while the glycerol assay was performed at IMUH Co., Ltd. (Tokyo, Japan) as described previously¹⁸⁾. In brief, glucagon concentrations were measured by a double-antibody radioimmunoassay (Glucagon RIA SML, Euro-Diagnostica AB, Malmö, Sweden), while insulin levels were measured by a chemiluminescent immunoassay (Architect Insulin, Abbott Japan, Japan). Enzymatic methods were used to measure the levels of blood glucose (Iatoro LQ GLU, Unitica, Japan), acetoacetic acid, 3-hydroxybutyrate (Total Ketone Bodies,

Kainos, 3-HB Kainos, Kainos Co., Ltd., Japan), glycerol (Glycerol Colorimetric Assay Kit, Cayman Chemical, Ann Arbor, MI, USA), and lactate (Detamina-LA, Kyowa Medex Co., Ltd., Japan). Blood FFAs (NEFA-SS Eiken, Eiken Chemical Co., Ltd., Japan) were measured by the enzyme-ultraviolet (UV) method, and growth hormone (Access hGH, Beckman Coulter, Inc., USA) and cortisol (Access cortisol, Beckman Coulter, Inc., USA) concentrations were measured by a chemiluminescent enzyme immunoassay. Adrenalin and noradrenalin were measured using high-performance liquid chromatography (HLC-725CAII, Tosoh Corporation, Tokyo, Japan).

Statistical analysis. Data were expressed as mean $\pm$ standard deviation (SD), and were analyzed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). The total area under the concentration-time curve (AUC) for each circulating plasma hormone was calculated using the Trapezium Rule. Repeated measures 2-factor analysis of variance (ANOVA, time-treatment) was used to examine differences between the biochemical parameters in the two tests. When the ANOVA showed significant effects or interactions between factors, Tukey's *post-hoc* test was used to detect significant differences between the two treatments. To specifically compare the AUCs for each hormone between treatments, the experiment was divided into three time-phases (*i.e.*, rest, exercise, and post-exercise). All variables were tested for normal distribution by the F-test using StatView-J 5.0 software (Abacus Concepts, Berkeley, CA, USA). Statistical significance of differences between the two experimental tests was analyzed by a paired-sample *t* test using Microsoft Excel for normally distributed data and the Wilcoxon signed-rank test using the StatView-J 5.0 software for data with a skewed distribution. Correlations were tested using a two-

Fig. 1A

Fig. 1B

Fig. 1 Study design: Study schedule (A) and schedule on experimental trial days (Visit 2 and Visit 3; B).

tailed Pearson's test. Statistical significance was set at $P < 0.05$. To investigate the amount of the difference between the groups, effect size (Cohen's d) was calculated.

Results

Cardiorespiratory responses. Two-factor ANOVA revealed significant effects of time post ingestion on the mean HR during exercise, but no significant treatment-time interaction. There were no differences in HR during exercise in subjects receiving either A-mix supplementation or a placebo (112 ± 10 vs. 108 ± 7 BPM, respectively, $P = 0.16$).

Biochemical parameters. Various biochemical parameters measured following ingestion of each of the two treatments are summarized in Fig. 2, and the AUCs during rest, exercise, and recovery are summarized in Table 2. Two-factor ANOVA showed significant effects of time post ingestion on the AUC, but no significant treatment-time interaction. However, the AUC for ketone bodies during exercise was significantly higher following supplementation with A-mix compared with the placebo, while the AUC for blood glycerol, FFAs, glucose, and lactate measurements did not differ between the two treatments.

Circulating hormones. The concentrations of circulating hormones following ingestion of A-mix and a placebo are summarized in Fig. 3, while the AUC for each during rest, exercise, and recovery is summarized in Table 3. Two-factor ANOVA showed a significant treatment-time interaction for plasma glucagon concentration (treatment, $P = 0.60$; time, $P < 0.01$; interaction, $P = 0.018$), with Tukey's *post-hoc* test revealing significant differences between treatments at 30 min post-ingestion ($P < 0.05$). Moreover, the AUC for glucagon during exercise was significantly higher following supplementation with A-mix compared to the placebo ($P = 0.026$). Moreover, the [glucagon]:[insulin] ratio during exercise positively correlates with ketone bodies AUC ($r = 0.44$, $P = 0.038$, Fig. 4.). Two-factor ANOVA revealed significant effects of time post ingestion on blood levels of insulin, adrenalin, nor-adrenalin, growth hormone, and cortisol concentrations; but no significant treatment-time interaction, and no significant differences between the two treatments throughout the experimental period.

Discussion

This study investigated the acute effects of A-mix supplementation combined with low-intensity exercise in middle-aged men with obesity without regular exercise habits. The study showed that ingestion of the A-mix supplement significantly increased the concentration of ketone bodies during exercise, compared to ingestion of a placebo (Table 2). The amount of fat oxidation from

adipose tissue can be estimated from the production of ketone bodies, since acetyl-CoA produced from the oxidation of FFAs gives rise to ketone bodies via a reaction during the HMG-CoA cycle in the liver²²). These results suggest that the ingestion of A-mix stimulates hepatic ketone body synthesis, and that such an effect might lead, at least in part, to an increase in fat oxidation during exercise. Moreover, the ingestion of A-mix significantly increased the concentration of glucagon in the blood (Table 3), and the [glucagon]:[insulin] ratio positively correlated the AUC of ketone bodies during exercise. Therefore, the acceleration of ketone body synthesis by A-mix ingestion can be estimated via the increase in glucagon secretion. These findings lay the groundwork for further investigations on specific amino acid supplementation combined with exercise and related hormones in patients with obesity.

In this study, following A-mix ingestion, ketone levels during exercise were significantly increased compared to pre-exercise ingestion of the placebo, and the [glucagon]:[insulin] ratio during exercise was positively correlated with ketone bodies AUC (Fig. 4). One possible mechanism for the increase in ketone levels is via a glucagon-stimulated cascade. A-mix also significantly increased the concentration of glucagon during exercise. McGarry & Foster²²) previously reported that elevation of the [glucagon]:[insulin] ratio suppresses the synthesis of malonyl-CoA and lipogenesis, with concomitant activation of fatty acid oxidation and ketogenesis. Simultaneous increases in hepatic carnitine and fatty acyl-CoA content further enhance β -oxidation and the production of ketone bodies such as acetoacetic acid and 3-hydroxybutyric acid. Therefore, increasing the [glucagon]:[insulin] ratio is the key to controlling hepatic fatty acid oxidation and production of ketone bodies.

The balance of several hormones, including glucagon, insulin, catecholamine, growth hormone, and cortisol, plays a critical role in fat catabolism, both at rest and during exercise^{23,24}). We speculate that elevated blood amino acid levels by A-mix ingestion directly leads to elevated glucagon secretion. This notion is supported by a previous study¹⁰) showing that specific amino acids stimulate insulin and glucagon secretion in humans. In a previous report, we suggested that ingestion of A-mix caused a significant increase in the concentration of catecholamines, especially adrenalin, both during and after exercise¹⁸). In this study, however, no significant differences in levels of adrenalin and noradrenalin were noted between the different treatments. Blood adrenalin levels were previously found to increase with exercise intensity²⁵). Taken together, these studies clearly indicate that the primary effect of A-mix ingestion is in stimulating glucagon secretion, and that, depending on the exercise intensity, can contribute to glycemic control.

Our study has several noteworthy strengths. First, this trial adopted the research design of a placebo-controlled

Fig. 2 Concentrations of biochemical parameters assessed during the experimental trials: glycerol (A), FFAs (B), total ketone bodies (C), glucose (D), and lactate (E). Values are $M \pm SD$ ($n = 11$). A = A-mix treatment; FFAs = free fatty acids; P = placebo treatment.

Table 2. Effects of amino acid mixture on the AUC for biochemical parameters

	Rest (0-30 min)				Exercise (>30-90 min)				Recovery (>90-150 min)			
	A-mix	Placebo	<i>p</i> *	d	A-mix	Placebo	<i>p</i> *	d	A-mix	Placebo	<i>p</i> *	d
Glycerol (min-mg/L)	97 ± 18	94 ± 33	.77	.14	473 ± 154	411 ± 154	.33	.40	481 ± 164	418 ± 187	.28	.36
FFAs (min-mEq/L)	14.0 ± 4.1	12.2 ± 3.8	.29	.45	39.0 ± 14.8	34.4 ± 10.9	.38	.36	55.7 ± 16.6	49.6 ± 16.3	.28	.37
Total ketone bodies (min-mmol/L)	3.83 ± 3.26	2.26 ± 2.12	.051	.57	10.0 ± 8.08	5.49 ± 5.79	.047	.65	28.2 ± 23.3	17.5 ± 18.3	.094	.51
Glucose (min-g/L)	25.3 ± 1.5	25.8 ± 1.1	.17	.35	47.3 ± 3.1	47.0 ± 3.1	.74	.083	46.4 ± 4.1	46.2 ± 2.7	.85	.055
Lactate (min-g/L)	3.00 ± 1.25	2.74 ± 0.95	.46	.23	10.5 ± 5.2	9.53 ± 3.35	.27	.22	6.78 ± 2.35	6.49 ± 1.66	.59	.14

Values are mean ± SD (n=11). FFA = free fatty acids.

**p* value for the paired-sample *t* test if data were normally distributed and the Wilcoxon signed-rank test if not.

Table 3. Effects of amino acid mixture on the AUC for circulating hormones

	Rest (0-30 min)				Exercise (>30-90 min)				Recovery (>90-150 min)			
	A-mix	Placebo	<i>p</i> *	d	A-mix	Placebo	<i>p</i> *	d	A-mix	Placebo	<i>p</i> *	d
Glucagon (min-μg/L)	4.59 ± 1.39	4.14 ± 1.06	.064	.36	9.97 ± 2.79	9.10 ± 2.42	.026	.33	9.94 ± 3.05	9.57 ± 2.59	.24	.13
Insulin (min-mU/L)	168 ± 81	160 ± 93	.62	.099	221 ± 112	221 ± 108	.96	.0075	215 ± 110	213 ± 101	.93	.023
Adrenalin (min-μg/L)	0.71 ± 0.29	0.72 ± 0.42	.85	.038	2.99 ± 1.10	3.14 ± 1.95	.74	.095	2.39 ± 1.66	2.29 ± 1.55	.85	.060
Noradrenalin (min-μg/L)	8.17 ± 1.19	8.58 ± 2.42	.66	.22	30.6 ± 8.3	32.2 ± 14.0	.48	.14	23.9 ± 6.8	23.4 ± 8.2	.72	.069
Growth hormone (min-μg/L)	13.7 ± 21.7	7.31 ± 6.77	.86	.40	94.5 ± 89.9	86.7 ± 55.7	.73	.10	83.8 ± 78.9	76.9 ± 45.1	.75	.11
Cortisol (min-mg/L)	2.42 ± 0.60	2.10 ± 0.37	.061	.65	4.44 ± 1.35	4.47 ± 0.90	.90	.032	4.31 ± 1.87	4.65 ± 2.46	.60	.16

Values are mean ± SD (n=11).

**p* value for the paired-sample *t* test if data were normally distributed and the Wilcoxon signed-rank test if not.

Fig. 3 Concentrations of circulating hormones during the experimental trials: glucagon (A), insulin (B), adrenalin (C), noradrenalin (D), growth hormone (E), and cortisol (F). Values are $M \pm SD$ ($n = 11$). A = A-mix treatment; P = placebo treatment.

Fig. 4 Total ketone bodies AUC versus ratio of glucagon/insulin during exercise.

double-blind cross-over randomized trial. Therefore, the findings are highly reliable. Second, in this study, we employed low-intensity exercise (*i.e.*, 40% of $\dot{V}O_{2peak}$). Low- to moderate-intensity exercise may be better than high-intensity exercise for promoting loss of fat mass, because the substrate oxidation achieved during high-intensity exercise is primarily reflective of carbohydrate oxidation²⁶). In addition, it is wise to encourage moderate-intensity physical activity in patients with obesity, as it is more feasible and acceptable than high-intensity exercise. Therefore, supplements that promote fat mobilization during periods of low-intensity exercise are considered optimal for exercise motivation in patients with obesity.

In contrast, we must note some limitations. First, we did not investigate the oxygen uptake and whole body energy expenditure. Therefore, the data robustness may not be high. Second, this study only investigated 11 middle-aged men. Future clinical trials are necessary in humans with obesity, especially including women.

In conclusion, pre-exercise ingestion of the A-mix supplement significantly accelerated secretion of glucagon during exercise in men with obesity. Furthermore, fat catabolism - especially hepatic ketogenesis - during exercise increased significantly, indicating a shift towards fat catabolism. Thus, these results suggest that ingestion of A-mix stimulates hepatic ketone body synthesis, and this effect might lead to, at least in part, an increase in fat oxidation during exercise. Further studies are needed to investigate whether this acute response induced by the administration of A-mix is sustained if the supplement is ingested over several weeks.

Conflict of Interests

This work was supported by a grant from Meiji Co., Ltd. K Ueda, C Sanbongi, and S Ikegami are employees of Meiji Co., Ltd.

Acknowledgments

The authors thank all the volunteers for taking part in these studies, and thank Dr Shukuko Ebihara and the staff of CPCC Co., Ltd. for data collection.

References

- 1) Batsis JA, Nieto-Martinez RE and Lopez-Jimenez F. 2007. Metabolic syndrome: from global epidemiology to individualized medicine. *Clin Pharmacol Ther* 82: 509-524. doi: 10.1038/sj.clpt.6100355.
- 2) Zou C and Shao J. 2008. Role of adipocytokines in obesity-associated insulin resistance. *J Nutr Biochem* 19: 277-286. doi: 10.1016/j.jnutbio.2007.06.006.
- 3) Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, Hu FB, Hubbard VS, Jakicic JM, Kushner RF, Loria CM, Millen BE, Nonas CA, Pi-Sunyer FX, Stevens J, Stevens VJ, Wadden TA, Wolfe BM, Yanovski SZ and Jordan HS et al. 2013. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation* 129: S102-S138. doi: 10.1161/01.cir.0000437739.71477.ee.
- 4) Jakicic JM. 2003. Exercise in the treatment of obesity. *Endocrinol Metab Clin North Am* 32: 967-980.
- 5) Mitchell HW, Peter HB, Robert MO and Lawrence EA. 2006. American College of Sports Medicine: clinical exercise testing. In *ACSM's guidelines for exercise testing and prescription (7th edition)* (Whaley MH eds.): 93-114.
- 6) Richter EA, Kiens B, Mizuno M and Strange S. 1989. Insulin action in human thighs after one-legged immobilization. *J Appl Physiol* 67: 19-23.
- 7) Numao S, Hayashi Y, Katayama Y, Matsuo T, Tomita T, Ohkawara K, Nakata Y and Tanaka K. 2006. Effects of obesity phenotype on fat metabolism in obese men during endurance exercise. *Int J Obes* 30: 1189-1196. doi: 10.1038/sj.ijo.0803282.
- 8) Miyashita M. 2008. Effects of continuous versus accumulat-

- ed activity patterns on postprandial triacylglycerol concentrations in obese men. *Int J Obes* 32: 1271-1278. doi: 10.1038/ijo.2008.73.
- 9) Saunders TJ, Palombella A, McGuire KA, Janiszewski PM, Després JP and Ross R. 2012. Acute exercise increases adiponectin levels in abdominally obese men. *J Nutr Metab* 2012: 148729. doi: 10.1155/2012/148729.
 - 10) Gannon MC and Nuttall FQ. 2010. Amino acid ingestion and glucose metabolism - a review. *IUBMB Life* 62: 660-668. doi: 10.1002/iub.375.
 - 11) Wasserman DH, Williams PE, Lacy DB, Green DR and Cherrington AD. 1988. Importance of intrahepatic mechanisms to gluconeogenesis from alanine during exercise and recovery. *Am J Physiol* 254: E518-E525.
 - 12) Wolfe RR, Nadel ER, Shaw JHF, Stephenson LA and Wolfe MH. 1986. Role of changes in insulin and glucagon in glucose homeostasis in exercise. *J Clin Invest* 77: 900-907. doi: 10.1172/JCI112388.
 - 13) Tan TM, Field BC, McCullough KA, Troke RC, Chambers ES, Salem V, Gonzalez Maffe J, Baynes KC, De Silva A, Viardot A, Alsafi A, Frost GS, Ghatei MA and Bloom SR. 2013. Coadministration of glucagon-like peptide-1 during glucagon infusion in humans results in increased energy expenditure and amelioration of hyperglycemia. *Diabetes* 62: 1131-1138. doi: 10.2337/db12-0797.
 - 14) Billington CJ, Bartness TJ, Briggs J, Levine AS and Morely JE. 1987. Glucagon stimulation of brown adipose tissue growth and thermogenesis. *Am J Physiol* 252: R160-R165.
 - 15) Suzuki T, Morita M, Hayashi T and Kamimura A. 2017. The effects on plasma L-arginine levels of combined oral L-citrulline and L-arginine supplementation in healthy males. *Biosci Biotechnol Biochem* 81: 372-375. doi: 10.1080/09168451.2016.1230007.
 - 16) Shinozaki F and Abe T. 2008. Synergistic effect of vespa amino acid mixture on lipolysis in rat adipocytes. *Biosci Biotechnol Biochem* 72: 1860-1868.
 - 17) Shi W, Meininger CJ, Haynes TE, Hatakeyama K and Wu G. 2004. Regulation of tetrahydrobiopterin synthesis and bioavailability in endothelial cells. *Cell Biochem Biophys* 41: 415-434.
 - 18) Ueda K, Sanbongi C, Takai S, Ikegami S and Fujita S. 2017. Combination of aerobic exercise and an arginine, alanine, and phenylalanine mixture increases fat mobilization and ketone body synthesis. *Biosci Biotechnol Biochem* 81: 1417-1424. doi: 10.1080/09168451.2017.1303359.
 - 19) Dériaz O, Dumont M, Bergeron N, Després JP, Brochu M and Prud'homme D. 2001. Skeletal muscle low attenuation area and maximal fat oxidation rate during submaximal exercise in male obese individuals. *Int J Obes Relat Metab Disord* 25: 1579-1584. doi: 10.1038/sj.ijo.0801809.
 - 20) Ministry of Health, Labour and Welfare. Report on National Health and Nutrition Survey 2015. Available from: <http://www.mhlw.go.jp/bunya/kenkou/eiyou/dl/h27-houkoku.pdf>. Accessed March 27, 2017.
 - 21) Gore CJ, Booth ML, Bauman A and Owen N. 1999. Utility of pwc75% as an estimate of aerobic power in epidemiological and population-based studies. *Med Sci Sports Exerc* 31: 348-351.
 - 22) McGarry JD and Foster DW. 1980. Regulation of hepatic fatty acid oxidation and ketone body production. *Annu Rev Biochem* 49: 395-420. doi: 10.1146/annurev.bi.49.070180.002143.
 - 23) Wasserman DH, Spalding JA, Lacy DB, Colburn CA, Goldstein RE and Cherrington AD. 1989. Glucagon is a primary controller of hepatic glycogenolysis and gluconeogenesis during muscular work. *Am J Physiol* 257: E108-E117.
 - 24) Wolfe RR, Nadel ER, Shaw JH, Stephenson LA and Wolfe MH. 1986. Role of changes in insulin and glucagon in glucose homeostasis in exercise. *J Clin Invest* 77: 900-907. doi: 10.1172/JCI112388.
 - 25) McMurray RG, Forsythe WA, Mar MH and Hardy CJ. 1987. Exercise intensity-related responses of beta-endorphin and catecholamines. *Med Sci Sports Exerc* 19: 570-574.
 - 26) Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E and Wolfe RR. 1993. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol* 265 (Pt 1): E380-E391.