Note

Effects of Intravenous Amino Acids on Anesthesia-Induced Hypothermia in Ovariectomized Rats

Satoko ANDO¹, Masahiro KANAZAWA^{1,*}, Michio TSUDA² and Toshiyasu SUZUKI¹

¹Department of Anesthesiology, and ²Department of Molecular Life Science, Division of Basic Medical Science and Molecular Medicine, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259–1193, Japan

(Received September 29, 2011)

Summary A decrease in core temperature during general anesthesia is attenuated by infusion of an intravenous amino acid mixture. The purpose of the present study was to investigate the influence of physical and endocrine changes caused by ovariectomy on the inhibitory effect of amino acid infusion on anesthesia-induced hypothermia. Sprague-Dawley female rats were divided into a sham-operated (Sham) group and an ovariectomized (OVX) group. Saline solution or an amino acid mixture solution was infused for 180 min during sevoflurane anesthesia, and the rectal temperature was measured (4 groups). Intraperitoneal white adipose tissue mass, bilateral gastrocnemius weight and plasma insulin levels were measured. In the Sham rats, no inhibitory effect of the amino acid mixture on anesthesia-induced hypothermia was found, while in the OVX rats, hypothermia was significantly decreased. The intraperitoneal fat weight/body weight ratio was significantly higher in the OVX rats than in the Sham rats, but the gastrocnemius weight/body weight ratio was not significantly different. After amino acid infusion, the plasma insulin level was significantly higher in the OVX rats than in the Sham rats. In conclusion, our findings suggest that, in rats, ovarian function or female hormone affects protein turnover mediated by increase in insulin secretion and, thus, decreases the inhibitory effect of an infusion of amino acid mixture on anesthesia-induced hypothermia.

Key Words hypothermia, amino acids, general anesthesia, ovariectomy

Redistribution hypothermia occurs in the body after induction of general anesthesia. Core body temperature gradually declines as heat is lost from the body surface, and heat production slows because of decreased metabolism. Sellden et al. reported that an amino acid infusion during anesthesia caused nutrient-induced thermogenesis (NIT), preventing the temperature reduction of redistribution hypothermia (1). Fujiwara et al. noted a similar effect after amino acid infusion but not after an infusion of glucose and fat emulsion (2). It is known that the metabolism of amino acids requires a large expenditure of energy (3), and protein synthesis induced by amino acids has been shown to correlate positively with energy expenditure (4). When rats were given the protein synthesis inhibitor puromycin, NIT did not occur after amino acid infusion (5). It has also been suggested that 75% of the increased oxygen uptake by amino acid infusion is produced in tissues other than the internal organs (6). Amino acid infusion during general anesthesia was found to stimulate protein synthesis in skeletal muscle via enhanced insulin secretion and to prevent hypothermia by heat accumulation through NIT (7). We have reported that skeletal

muscle and plasma insulin levels play a major role in changes in body temperature during general anesthesia and the effect of amino acids on body temperature in a rat model of muscle atrophy (8).

On the other hand, sex hormone is known to be involved in thermoregulation. In addition, estrogen is reported to contribute to protein anabolism, affecting thermoregulation via the thermoregulatory center (9). Thus, estrogen may influence the inhibitory effect of an infusion of amino acid mixture during general anesthesia on hypothermia. However, the relationship between the inhibitory effect of an infusion of amino acid mixture during general anesthesia on hypothermia and ovarian function is not yet understood. Menopause causes physical and endocrine changes such as deficiency in female hormone and an increase in white adipose tissue mass. The present study was therefore conducted to investigate the influence of physical and endocrine changes caused by ovariectomy on the inhibitory effect of amino acid infusion on anesthesia-induced hypothermia in the ovariectomized rat model.

Materials and Methods

Animals. The present study was performed in accordance with the research guidelines of Tokai University and approved by the Animal Investigation Committee of

*To whom correspondence should be addressed.
E-mail: kanazawa@is.icc.u-tokai.ac.jp

Table 1. Composition of amino acid mixture solution (Amiparen®).

Amino acid	Concentration (g/L)
L-Leucine	14
L-Isoleucine	8
L-Valine	8
L-Lysine acetate	14.8
L-Threonine	5.7
L-Tryptophan	2
L-Methionine	3.9
L-Cysteine	1
L-Phenylalanine	7
L-Tyrosine	0.5
L-Arginine	10.5
L-Histidine	5
L-Alanine	8
L-Proline	5
L-Serine	3
Glycine	5.9
L-Aspartic acid	1
L-Glutamic acid	1
Total free amino acid	100
EAA concentration	59.1
BCAA concentration	30.0 w/w%

EAA, essential amino acid; BCAA, branched-chain amino acid.

the University. Seven-week-old female Sprague-Dawley rats, weighing 150 to 180 g, were obtained from Charles River Japan (Yokohama, Japan). The rats were housed (one per cage) at constant humidity and temperature ($22 \pm 2^\circ\text{C}$) under a standard light/dark cycle (light from 8:00 AM to 8:00 PM). A standard laboratory diet (CE-2, Charles River Japan) and water were given ad libitum.

Ovariectomy. Ovariectomized ($n=24$) and sham-operated ($n=22$) female rats were prepared as described in a previous report (10). Seven days after the rats were received at the laboratory (at 8 wk old), they were anesthetized by isoflurane and bilaterally ovariectomized or sham operated. After the surgery, penicillin G (1,000 U; Meiji Pharmaceutical, Tokyo, Japan) was injected subcutaneously to prevent infection. After the operation, animals were permitted ad libitum access to food and water for 5 wk.

Experimental protocol. Rats were fasted for 2.5 h before the experiment to exclude the influence of starvation. Room temperature was maintained at 22°C during the experiment. Sham-operated (Sham) rats and ovariectomized (OVX) rats were randomly assigned to 1 of the 2 treatment groups: intravenous infusion of saline (Sal) or amino acid (AA) mixture (4 groups, Sham-Sal and Sham-AA, $n=11$; OVX-Sal and OVX-AA, $n=12$). A 24-gauge over-the-needle catheter was placed in the tail vein and secured with bandage tape. The catheter was connected to a syringe on an infusion pump (TE-332, Terumo Inc., Tokyo, Japan) via the vinyl tube for administration of the test solution. Saline solu-

Fig. 1. Effects of treatment with amino acid administration on rectal temperature. Immediately after the sham-operated rats (Sham) and ovariectomized rats (OVX) received saline (Sal) or amino acid (AA) mixture solution, animals were anesthetized with sevoflurane, and the rectal temperature was measured for 180 min (4 groups: $n=12$ for each group). In the OVX rats, hypothermia was significantly attenuated by AA mixture infusion. In contrast, an inhibitory effect of the amino acid mixture on hypothermia was not observed significantly in the Sham rats. Values are means $\pm$ SE (Sham-Sal and Sham-AA: $n=11$, OVX-Sal and OVX-AA: $n=12$). A two-way repeated measures analysis of variance, followed by Bonferroni post hoc test when appropriate, was used to compare means among the groups. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. OVX-Sal or Sham-AA group. $^\dagger p<0.05$, $^\ddagger p<0.01$, $^\ddagger\ddagger p<0.001$ vs. Sham-Sal group.

tion or the AA mixture solution, which is enriched with branched-chain amino acids (Amiparen®, Otsuka Pharmaceutical Factory Inc., Tokushima, Japan) and stored at room temperature (22°C), was infused continuously at a rate of 14 mL/kg/h. The composition of the amino acid mixture solution is shown in Table 1. All rats were given the test solution at 10:30 AM. Immediately after infusion of the test solution was started, the rats were anesthetized with 3% sevoflurane for 180 min (0 to 180 min). Rectal temperature was measured by a digital thermometer (CTM-303, Terumo) and a thermistor temperature sensor (ME-PDK061, Terumo) throughout the 180-min experiment. No warming devices were used during the experiment.

Blood collection and tissue preparation. At the end of the 180-min period, the rats underwent a median laparotomy, and blood samples were collected from the abdominal artery. The samples were immediately centrifuged at $1,800 \times g$ for 20 min at 4°C to obtain the

Table 2. Effects of ovariectomy on the body weight, relative skeletal muscle weight, and relative intraperitoneal white adipose tissue weight.

	Sham		OVX		Two way ANOVA		
	Sal	AA	Sal	AA	AA	OVX	AA×OVX
Body weight at experiment (g)	299.8±5.4 ^a	285.9±4.9 ^a	354.4±6.8 ^b	366.9±10.6 ^b	$F(1,40)=0.078$ $p=0.78$	$F(1,40)=98.93$ $p<0.001$	$F(1,40)=3.74$ $p=0.07$
Relative fat weight	36.4±2.2 ^a	37.3±2.0 ^a	48.1±2.6 ^b	48.0±3.0 ^b	$F(1,31)=0.025$ $p=0.88$	$F(1,31)=19.44$ $p<0.001$	$F(1,31)=0.034$ $p=0.85$
Relative muscle weight	12.8±0.2	13.0±0.2*	12.4±0.2	12.1±0.1	$F(1,31)=0.073$ $p=0.79$	$F(1,31)=12.23$ $p<0.01$	$F(1,31)=1.83$ $p=0.19$

Rats were fed with chow diet for 5 wk after surgery to remove ovaries. Values are means±SE. Sham-Sal and Sham-AA: $n=11$, OVX-Sal and OVX-AA: $n=12$. Sham, sham-operated rats; OVX, ovariectomized rats. Sal, saline; AA, amino acids. Relative fat weight=retroperitoneal and mesenteric fat weight/body weight ratio ($\text{mg/g} \times 10^3$). Relative muscle weight=wet weight of bilateral gastrocnemius muscle/body weight ratio ($\text{mg/g} \times 10^3$). Means not sharing the same superscript letter are different ($p<0.05$), * $p<0.05$ vs. OVX-AA group, by Tukey-Kramer multiple comparison test.

plasma, which was stored at -80°C until analysis. Retroperitoneal and mesenteric fat were removed from each rat and weighed. Likewise, bilateral gastrocnemius muscle was removed from each rat and weighed.

Measurement of plasma concentrations of insulin and estradiol. Plasma insulin concentrations were analyzed by radioimmunoassay (RIA) with commercial kits (Linco Research, Inc., St Charles, MO, USA). Plasma estradiol concentrations were measured by a liquid scintillation counter (LSC-5100, Aloka, Tokyo, Japan) for radioimmunoassay.

Statistical analysis. Data for each group were represented as means±SE. Statistical evaluation of the data was performed by two-way analysis of variance (ANOVA) followed by a Bonferroni or Tukey-Kramer post hoc test to determine the treatment effect (Prism 4 for Machintosh, GraphPad Software, CA, USA). Differences among the groups were considered significant at the level of $p<0.05$.

Results

Changes in rectal temperature

The changes in rectal temperature are shown in Fig. 1. The results of a two-way repeated-measures ANOVA showed a significant interaction between the study groups (rat model-test solution) and the measurement time points for the rectal temperature (rat model-test solution×time, $F(36, 444)=7.97$, $p<0.0001$) and also showed a significant difference in the rectal temperature by the study group (rat model-test solution) or by the measurement time point (rat model-test solution, $F(3, 444)=9.46$, $p<0.0001$; time, $F(12, 444)=1230$, $p<0.0001$, respectively). With regard to comparison between groups at the same measurement time point, the results of Bonferroni's post tests showed a higher body temperature in the OVX-AA group than in the OVX-Sal group ($p<0.05$ at 105 min, $p<0.01$ at 120 min, and $p<0.001$ at 135–180 min). However, no significant difference was noted in the body temperature between the Sham-Sal group and the Sham-AA group at each measurement time.

Fig. 2. Plasma insulin concentrations. Blood samples were collected from each rat immediately after a 180-min administration of amino acid mixture, and plasma insulin concentrations were measured. Values are means±SE (Sham-Sal and Sham-AA: $n=11$, OVX-Sal and OVX-AA: $n=12$). Values not sharing the same superscript letter are significantly different ($p<0.01$) by the Tukey-Kramer multiple comparison test. Sham, sham-operated rats; OVX, ovariectomized rats. Sal, saline; AA, amino acids.

Effect of ovariectomy on the weights of body, skeletal muscle, and intraperitoneal white adipose tissue

As expected, ovariectomized rats had significantly increased body weight as compared to sham-operated rats (Table 2). Relative fat weight (the retroperitoneal and mesenteric fat weight/body weight ratio) was calculated to exclude the influence of any difference in body weight among the rats. As shown in Table 2, the ratio was significantly higher in the ovariectomized rats than in the sham-operated rats. In contrast, the relative muscle weight (bilateral gastrocnemius weight/body weight ratio) was significantly lower in the OVX-AA group than in the Sham-AA groups (Table 2). These results suggest that ovariectomy increased body weight by adding fat mass, not muscle mass.

Biochemical examination

The results of plasma insulin levels are shown in Fig. 2. The results of a two-way factorial ANOVA showed a

significant interaction between the rat model and the test solution ($F(1, 34)=14.25$, $p<0.0001$). Significant differences were noted in the insulin level by the rat model and by the test solution (OVX, $F(1, 34)=32.05$, $p<0.0001$; AA, $F(1, 34)=20.87$, $p<0.0001$, respectively). The Tukey-Kramer post test showed that the insulin level in the OVX-AA group was significantly higher than that in the other 3 groups, with no significant difference between the Sham-Sal group and the OVX-Sal group. These results suggest an infusion of amino acid mixture had more significant insulin secretion response in the OVX rats than in the Sham rats.

Ovariectomy decreases blood estradiol in rats (11). To confirm the effects of ovariectomy, we measured the plasma estradiol levels in 5 rats each of the treatment groups in the present study, and the levels in all the rats of the OVX group were below the detection limit of 10 pg/mL. On the other hand, the levels in the Sham-Sal and the Sham-AA group were 64.4 ± 18.5 pg/mL and 69.0 ± 26.6 pg/mL respectively (values are mean $\pm$ SE, $n=5$ each group, not shown in Table 2).

Discussion

In the OVX rats, infusion of an amino acid mixture significantly prevented anesthesia-induced hypothermia. In the sham-operated rats, however, the inhibitory effect of amino acid infusion on hypothermia was not seen. In other studies done in male rats, amino acid infusion had a significant inhibitory effect on hypothermia, as measured by central body temperature, during general anesthesia (7, 8, 12, 13). The results of our study suggest that in rats, ovarian function or female hormone decreases the inhibitory effect of amino acid infusion on anesthesia-induced hypothermia.

An amino acid mixture solution rich in BCAA was continuously administered in the present study. BCAA is reported to be required for the inhibitory effect of amino acid infusion during general anesthesia on hypothermia (14). Female hormone, estrogen, exerts its action on protein anabolism. In other words, 17β -estradiol increases activity of branched chain α -keto acid dehydrogenase (BCKDH) kinase, resulting in decreasing activity of BCKDH complex and inhibiting degradation of BCAA (11, 15). However, if the mechanism of the inhibitory effect of amino acid mixture on hypothermia is caused by thermogenesis enhanced by protein synthesis in skeletal muscle (12), the inhibition of degradation of BCAA by female hormone is thought to be more or less meaningful in that BCAA, which is the substance for protein synthesis, may be retained. On the contrary, in the present study, a significant effect of amino acid mixture was found in the ovariectomized rats that are deficient in female hormone, not in the Sham rats that possess estrogen as protein anabolic hormone. In order to find the reason, the metabolism of administered BCAA during general anesthesia, namely, activity of BCKDH complex, degree of protein synthesis in skeletal muscle, and the relationship with the inhibitory effect on hypothermia need to be investigated at molecular level.

The results of the present study confirmed that the blood insulin level plays an important role in the inhibitory effect of an infusion of amino acid mixture on anesthesia-induced hypothermia. Previous studies using male rats have shown that plasma insulin levels increase during general anesthesia after amino acid infusion (7, 8, 12, 16). In the present study, the insulin level after administration of the test solution was significantly and consistently high in the OVX-AA group, in which a significant inhibitory effect on hypothermia was observed. Yamaoka et al. found that the inhibitory effect of amino acid infusion on anesthesia-induced hypothermia disappears in rats with suppressed insulin secretion after an infusion of somatostatin (12). Administration of leucine after an infusion of somatostatin has also been shown to inhibit increases in serum insulin, with no increase in protein synthesis in skeletal muscle (17). Further studies are required to investigate the relationship between insulin levels and degree of protein synthesis in skeletal muscle at the molecular level using the same experimental procedures. In the Sham-AA group, a statistically significantly high insulin level was not observed. The female hormone, estrogen, suppresses insulin secretion stimulated by glucose without inhibiting basal insulin secretion (18, 19). Progesterone is also reported to suppress insulin secretion (20). Therefore, we believe that the ovarian hormone influences insulin secretion in our study, causing a smaller increase in insulin levels in the Sham group than in the OVX group. On the other hand, plasma insulin levels in our study are the values at the end of administration of the test solution. Because previous clinical studies have reported that an increase in insulin levels with the administration of amino acid mixture is transient (16), further studies are required to measure insulin levels over time during continuous administration of amino acids.

We also found that amino acid infusion did not have much inhibitory effect on hypothermia in the Sham-AA group, although gastrocnemius weight/body weight ratio was significantly higher in the Sham-AA group than in the OVX-AA group (Table 2). This result seems to contradict that of the previous study reporting that metabolic heat production by protein synthesis in skeletal muscle plays an important role in the inhibitory effect of amino acid infusion on anesthesia-induced hypothermia (7). However, a previous study shows that there is no statistically significant correlation between the inhibitory effect of amino acid infusion during general anesthesia on hypothermia and muscle weight (21). It has also been shown in rats that the myofibrillar protein degradation product, plasma 3-methylhistidine, increases with amino acid infusion and thus the values of plasma 3-methylhistidine correlate positively with central body temperature (22). These reports suggest that enhanced protein turnover in muscle by amino acid infusion is more important than the weight of skeletal muscle. In addition, another report shows that the fractional protein synthesis rate is higher in gastrocnemius muscle in estrogen-deficient OVX rats than in nor-

mal female rats (23), which supports this result, and also suggests involvement of the female hormone.

Deficiency in female hormone increases mesenteric fat and subcutaneous fat (24, 25). In the present study, as expected, the OVX rats had a significantly large amount of intraperitoneal fat. Because fat has low thermal conductivity (26), heat loss from the body surface may decrease in the OVX rats. It is reported that in humans, there is a correlation between reduced body temperature at the second phase that occurs after redistribution hypothermia and total body fat including subcutaneous fat (27). However, there was no statistically significant difference in the body temperature between the Sham-Sal group and the OVX-Sal group. This suggests that the effect of increased fat weight of the OVX rats on anesthesia-induced hypothermia, namely balance of heat production and heat loss, is small. On the other hand, protein synthesis in fat cells is stimulated by amino acid infusion. Moreover, energy expenditure increases when protein synthesis is stimulated by amino acid infusion (4). Therefore, we cannot deny the possibility that in the OVX rat group, which had increased fat tissues, the added energy expenditure that resulted from amino acid infusion caused heat accumulation, which may have prevented hypothermia. A molecular study is needed to clarify the mechanisms involved.

Thermoregulation is known to be closely related to autonomic nervous activity. In humans, a decrease in female hormone, namely menopause, leads to the development of various autonomic imbalance symptoms. Kobayashi et al. report that tail skin temperature increases more significantly in OVX rats than in Sham rats (28). The changes in the skin temperature are thought to occur as a result of deficiency in estrogen that affects the autonomic nervous system. In addition, the sympathetic nerve stimulates brown adipose tissue to cause non-shivering thermogenesis. It is reported that estrogen acts on the hypothalamus and brown adipose tissue and affects thermoregulation (9, 29). In the present study, changes in central body temperature during general anesthesia were observed. General anesthetics inhibit the functions of the thermoregulatory center in the hypothalamus and the autonomic nervous system. Therefore, the changes in the rectal temperature observed in the OVX-AA group are assumed to have no direct relationship with the changes in the autonomic nervous activity due to estrogen deficiency. Kobayashi et al. also report that there is no difference in rectal temperature (core temperature) between the OVX rats and the Sham rats.

In summary, we investigated the inhibitory effect of an infusion of amino acid mixture on anesthesia-induced hypothermia in ovariectomized rats. In the OVX rats, anesthesia-induced hypothermia was significantly prevented by an infusion of amino acid mixture. A significant inhibitory effect of an amino acid infusion on hypothermia was not observed in the Sham rats. In addition, the insulin level was significantly high after an infusion of amino acid mixture in the OVX rats as

compared with the Sham rats. These results suggest the possibility that in rats, female hormone, through some mechanism, affects protein turnover mediated by increase in insulin secretion and, thus, decreases the inhibitory effect of an infusion of amino acid mixture on anesthesia-induced hypothermia.

REFERENCES

- 1) Selldén E, Brundin T, Wahren J. 1994. Augmented thermic effect of amino acids under general anaesthesia: a mechanism useful for prevention of anaesthesia-induced hypothermia. *Clin Sci* **86**: 611–618.
- 2) Fujiwara H, Sei H, Morita Y. 2002. Effect of intravenous infusion of nutrients on hypothermia induced by propofol anesthesia in rats. *J Surg Metab Nutr* **36**: 215–220 (in Japanese).
- 3) Flatt JP. 1978. The biochemistry of energy expenditure. In: *Recent Advances in Obesity Research* (Bray GA, ed), Vol 2, p 211–228. Newman, London.
- 4) Giordano M, Castellino P. 1997. Correlation between amino acid induced changes in energy expenditure and protein metabolism in humans. *Nutrition* **13**: 309–312.
- 5) Tsujinaka T, Sakaue M, Iijima S, Ebisui C, Kan K, Kishibuchi M, Morimoto T, Kido Y. 1996. Modulation of thermogenic response to parenteral amino acid infusion in surgical stress. *Nutrition* **12**: 36–39.
- 6) Selldén E, Bränström R, Brundin T. 1996. Augmented thermic effect of amino acids under general anaesthesia occurs predominantly in extra-splanchnic tissues. *Clin Sci (Lond)*. **91**: 431–439.
- 7) Yamaoka I, Doi M, Nakayama M, Ozeki A, Mochizuki S, Sugahara K, Yoshizawa F. 2006. Intravenous administration of amino acids during anesthesia stimulates muscle protein synthesis and heat accumulation in the body. *Am J Physiol Endocrinol Metab* **290**: E882–E888.
- 8) Kanazawa M, Ando S, Tsuda M, Suzuki T. 2010. The effect of amino acid infusion on anesthesia-induced hypothermia in muscle atrophy model rats. *J Nutr Sci Vitaminol* **56**: 117–122.
- 9) Uchida Y, Tokizawa K, Nakamura M, Mori H, Nagashima K. 2010. Estrogen in the medial preoptic nucleus of the hypothalamus modulates cold responses in female rats. *Brain Res* **1339**: 49–59.
- 10) Lemieux C, Picard F, Labrie F, Richard D, Deshaies Y. 2003. The estrogen antagonist EM-652 and dehydroepiandrosterone prevent diet- and ovariectomy-induced obesity. *Obes Res* **11**: 477–490.
- 11) Obayashi M, Shimomura Y, Nakai N, Jeoung NH, Nagasaki M, Murakami T, Sato Y, Harris RA. 2004. Estrogen controls branched-chain amino acid catabolism in female rats. *J Nutr* **134**: 2628–2633.
- 12) Yamaoka I, Doi M, Kawano Y, Nakayama M, Watanabe Y, Oba K, Sugahara K, Yoshizawa F. 2009. Insulin mediates the linkage acceleration of muscle protein synthesis, thermogenesis, and heat storage by amino acids. *Biochem Biophys Res Commun* **386**: 252–256.
- 13) Imoto A, Yokoyama T, Suwa K, Yamasaki F, Yatabe T, Yokoyama R, Yamashita K, Selldén E. 2010. Bolus oral or continuous intestinal amino acids reduce hypothermia during anesthesia in rats. *J Nutr Sci Vitaminol* **56**: 104–108.
- 14) Yamaoka I. 2008. Modification of core body temperature by amino acid administration. *Asia Pac J Clin Nutr* **17**: 309–311.

- 15) Kobayashi R, Shimomura Y, Murakami T, Nakai N, Fujitsuka N, Otsuka M, Arakawa N, Popov KM, Harris RA. 1997. Gender difference in regulation of branched-chain amino acid catabolism. *Biochem J* **327**: 449–453.
- 16) Moriyama T, Tsuneyoshi I, Omae T, Takeyama M, Kanmura Y. 2008. The effect of amino-acid infusion during off-pump coronary arterial bypass surgery on thermogenic and hormonal regulation. *J Anesth* **22**: 354–360.
- 17) Anthony JC, Lang CH, Crozier SJ, Anthony TG, MacLean DA, Kimball SR, Jefferson LS. 2002. Contribution of insulin to the translational control of protein synthesis in skeletal muscle by leucine. *Am J Physiol* **282**: E1092–E1101.
- 18) Haouari M, Faure A, Sutter BC. 1986. Oestradiol inhibits the insulin secretion on the first day of treatment in the ovariectomized rat. *Horm Metab Res* **18**: 143–144.
- 19) Faure A, Haouari M, Sutter BC. 1987. Oestradiol and insulin secretion in the rat: when does oestradiol start stimulating the insulin release? *Horm Res* **27**: 225–230.
- 20) Straub SG, Sharp GW, Meglasson MD, De Souza CJ. 2001. Progesterone inhibits insulin secretion by a membrane delimited, non-genomic action. *Biosci Rep* **21**: 653–666.
- 21) Kanazawa M, Ando S, Suzuki T. 2008. A relationship between the effect of amino acids administered for treatment of anesthesia-induced hypothermia and the patient's muscle volume. *Anesth Resus* **44**: 36–39.
- 22) Yamaoka I, Mikura M, Nishimura M, Doi M, Kawano Y, Nakayama M. 2008. Enhancement of myofibrillar proteolysis following infusion of amino acid mixture correlates positively with elevation of core body temperature in rats. *J Nutr Sci Vitaminol* **54**: 467–474.
- 23) Toth MJ, Poehlman ET, Matthews DE, Tchernof A, MacCoss MJ. 2001. Effects of estradiol and progesterone on body composition, protein synthesis, and lipoprotein lipase in rats. *Am J Physiol Endocrinol Metab* **280**: E496–E501.
- 24) Carr MC. 2003. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab* **88**: 2404–2411.
- 25) Gloy V, Langhans W, Hillebrand JJ, Geary N, Asarian L. 2011. Ovariectomy and overeating palatable, energy-dense food increase subcutaneous adipose tissue more than intra-abdominal adipose tissue in rats. *Biol Sex Differ* **2**: 6.
- 26) Orr CS, Eberhart RC. 1995. Overview of bioheat transfer. In: Optical-Thermal Response of Laser-Irradiated Tissue (Welsh AJ, van Gemert MJ, eds). Plenum Pub, New York.
- 27) Yamakage M, Kamada Y, Honma Y, Tsujiguchi N, Namiki A. 2000. Predictive variables of hypothermia in the early phase of general anesthesia. *Anesth Analg* **90**: 456–459.
- 28) Kobayashi T, Tamura M, Hayashi M, Katsuura Y, Tanabe H, Ohta T, Komoriya K. 2000. Elevation of tail skin temperature in ovariectomized rats in relation to menopausal hot flushes. *Am J Physiol Regul Integr Comp Physiol* **278**: R863–R869.
- 29) Uchida Y, Kano M, Yasuhara S, Kobayashi A, Tokizawa K, Nagashima K. 2010. Estrogen modulates central and peripheral responses to cold in female rats. *J Physiol Sci* **60**: 151–160.