The effect of amino-acid infusion during off-pump coronary arterial bypass surgery on thermogenic and hormonal regulation

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Abstract

Purpose. Amino-acid (AA) infusions promote thermogenesis and prevent perioperative hypothermia, but the mechanism of action is unknown. We sought to verify the hypothesis that AA infusions stimulate the release of metabolic hormones during surgery and increase energy expenditure, resulting in thermogenesis.

Methods. Twenty-four patients were randomly assigned to receive AA ($4 \text{ kJ·kg}^{-1}\text{·h}^{-1}$) or saline, which was infused for 2 h during off-pump coronary artery bypass surgery (OPCABS). Arterial adrenaline, thyroid hormone, insulin, and leptin levels were determined at five defined times during surgery. Oxygen consumption was measured 3 h after the start of infusion.

Results. AA infusion maintained the body core temperature during OPCABS. This effect was accompanied by an increase in oxygen consumption, which depended on increased heart rate. AA infusion prominently stimulated the secretion of insulin and leptin; the insulin level increased rapidly within 2 h after the start of infusion, whereas leptin levels increased gradually over a 6-h period after the start of infusion.

Conclusion. AA infusion significantly increased body core temperature and oxygen consumption during surgery. Given the release of insulin and leptin in response to AA infusion, it is likely that these hormonal signaling pathways may, in part, have contributed to the thermogenic response that occurred during the surgery.

Key words Amino acid · Thermogenesis · OPCAB

Introduction

It is well known that the intravenous infusion of amino acids (AAs) increases heat production and prevents hypothermia during general anesthesia [1–3]. Recent reports have shown that the intravenous infusion of

AAs is likely to enhance the quality of patient care and improve cost-effectiveness [4–6]. AA infusion appears to be a useful new thermoregulatory strategy for preventing hypothermia during surgery.

Despite increasing evidence supporting the clinical usefulness of AA infusion during surgery, the mechanism of action is unknown. According to recent reports, AAs induce an increase in energy expenditure that may be explained by several mechanisms, including an increase in the energy cost of glucose storage [7], increased anabolism [8,9], an influence on central thermoregulatory mechanisms [5], and hormonal regulation of mitochondrial energy production [10]. It has been reported that AA infusion stimulates the release of metabolic hormones such as insulin, cortisol, and glucagon [9], suggesting that hormonal regulation may play a part in energy expenditure. No previous study has examined whether serum levels of other metabolic hormones, such as catecholamines, thyroid hormone (T_3), and leptin change during AA infusion; the secretion patterns of these hormones also have not been studied.

The aim of the present study was to clarify the changes in these metabolic hormones in response to AA infusion during general anesthesia. Off-pump coronary artery bypass surgery (OPCABS) was chosen because the hypothermia that occurs during this type of surgery is difficult to prevent using current strategies [11,12].

Patients and methods

This study was approved by the Human Ethics Committee of Kagoshima University School of Medicine. Twenty-four patients were divided randomly into two groups of 12: an AA infusion group and a saline infusion group (Table 1). Patients were informed of the study protocol and the potential risks before written informed consent was obtained. Patients with renal or hepatic

Table 1. Patient characteristics

Group	Control ($n = 12$)	Amino acid ($n = 12$)	P
Age (years)	62 ± 8	61 ± 7	NS
Male/Female	8/4	8/4	NS
Height (cm)	159 ± 11	157 ± 11	NS
Weight (kg)	58 ± 11	59 ± 12	NS
Operation time (min)	385 ± 25	394 ± 19	NS
Intraoperative blood loss (ml)	2141 ± 1045	1959 ± 1164	NS
Amount of fluid (ml)	4323 ± 1273	4511 ± 1427	NS
Amount of transfusion (ml)	1420 ± 745	1368 ± 814	NS

Values are means ± SD

disorders, acute myocardial infarction, severe diabetes mellitus, thyroid disease, or active infection were excluded from the study. The numbers of patients for whom medications were administered perioperatively in the control and AA infusion groups were: 3 and 3 (β-adrenoceptor blockers), 2 and 3 (angiotensin-converting enzyme [ACE] inhibitors), 6 and 4 (calcium antagonists), and 2 and 0 (diuretics), respectively.

Patients were prepared according to standard preoperative procedures after an overnight fast. Morphine hydrochloride (0.2 mg·kg^{-1}; i.m.) was given 30 min before the induction of anesthesia; a radial artery catheter was inserted to measure blood pressure and to obtain blood samples. Anesthesia was induced by injecting 0.08 mg·kg^{-1} midazolam, 5 μg·kg^{-1} fentanyl, and 0.1 mg·kg^{-1} vecuronium intravenously, and was maintained with a continuous infusion of propofol at a rate of 5 mg·kg^{-1}·h^{-1}. The lungs were ventilated to normocapnia (35–40 mmHg) with 40%–60% oxygen enriched with air. Intravenous fentanyl (total, 20 μg·kg^{-1}) and vecuronium 0.04 mg·kg^{-1} were given as required. After the induction of anesthesia, 0.5 μg·kg^{-1}·min^{-1} glyceryl trinitrate was given to dilate the coronary arteries. To maintain stable experimental conditions, the temperature in the operating room was kept at 24°C, and one surgeon carried out all OPCABS procedures, using a standardized technique. Intraoperative standard heparin therapy was reversed with protamine at the end of the procedure, according to the institutional protocol. The perioperative transfusion policy at our institution is based on international guidelines [13]; the trigger for perioperative allogenic red blood cell transfusion was a hematocrit of 25% or less.

In general, the amount of AA infusion recommended was 400–800 ml·day^{-1}; rapid infusion was avoided because of deterioration of hepatic and renal function. A balanced mixture of 19 AAs (400 ml, Amiparen; Otsuka Pharmaceutical, Tokyo, Japan) was used. The rate of infusion was 200 ml·h^{-1} and infusion was accomplished via a central venous catheter inserted into the internal jugular vein. AA infusion started about 30 min before the start of the procedure and continued for 2 h. The total amount of AA mixture infused was 400 ml. Control subjects received an equal volume of a nutrient-free saline solution. This study was not blinded because the safety of AA infusion has not been clarified.

The temperature of pulmonary arterial blood, measured with a thermistor equipped with a pulmonary artery catheter (OptiQ; Hospira, Donegal, Ireland), was considered to be the core body temperature [14]. Cardiac output was measured using a thermodilution technique, and oxygen uptake was calculated as the product of the measured arterio-venous oxygen difference. Blood samples used to determine the serum concentrations of adrenaline, T_3, insulin, leptin, glucose, and lactate were obtained at five times: preoperatively (baseline) and, 2 h, 4 h, and 6 h after the start of infusion, and postoperatively. Circulating concentrations of insulin, leptin, and T_3 were measured using commercial specific double-antibody immunoassays. Plasma adrenaline level was determined using a fluorescence high-performance liquid chromatography monitor with alumina extraction and electrochemical detection (RF-535; Shimazu, Tokyo, Japan). Plasma glucose and lactate levels were measured using an automated gas analyzer (ABL800 FLEX; Radiometer, Copenhagen, Denmark).

Strategies to prevent intraoperative hypothermia were identical in both groups. For example, in both groups, fluids infused through peripheral veins were warmed by using HOT LINE (Smith Medical, Tokyo, Japan), which warmed the fluids at 40°C. Fluids, including AAs and control saline, were infused through a central venous catheter without warming. Intraoperatively, other warming tools (e.g., forced air warming system) were not used, and OPCABS was done at room temperature (24°C) in both groups.

Postoperatively, patients were continuously sedated with propofol, given at a rate of 5 mg·kg^{-1}·h^{-1}, and transferred to the intensive care unit (ICU) while intubated. In the ICU, to avoid shivering, patients were rewarmed to 37.5°C using a forced-air warming system (Warm-Touch; Mallinckrodt, St Louis, MO, USA) set at 42°C. After rewarming was complete, sedation was stopped, and patients were extubated when fully awake and able to maintain an acceptable respiratory rate and depth.

Statistical analyses

Values for results are expressed as means ± SD (n = number of patients). Statistical analysis was done using one- or two-factor analysis of variance for repeated measures, followed by Scheffé's test (for multiple com-

parisons, including time-dependent changes in temperature and hormone levels in each group) or a two-tailed, unpaired Student's t-test (to compare the control and AA-infused groups). Probability values of less than 0.05 were considered significant.

Results

Patient characteristics for the two groups are shown in Table 1. There were no significant differences in clinical characteristics, operative times, or the number of graft anastomoses between the two groups. There were no significant differences in the durations from the induction of anesthesia to the start of surgery (64 ± 9 min in the AA group, and 68 ± 9 min in the control group) or in the times required from opening the chest to closing the chest (315 ± 22 min in the AA group, and 321 ± 17 min in the control group) between the groups.

The present study demonstrated the clinical efficacy of AA infusion for preventing hypothermia during OPCABS. The baseline core body temperatures did not differ between the groups. After the induction of anesthesia, core body temperature fell from baseline in both groups but, in the AA infusion group, the initial temperature drop was significantly attenuated compared with that in the control group ($P < 0.05$). The reduction in core body temperature from baseline to the end of infusion (2 h after the initiation of the protocol) was 0.7 ± 0.1°C in the control group and 0.4 ± 0.1°C in the AA group. After the initial drop in core body temperature, the temperature in the control group remained low throughout the observation period. Core temperature in the AA group was continuously elevated until the end of the surgery. The temperature in the AA group was significantly ($P < 0.05$) higher than that in the control group at all time points after AA infusion (Fig. 1). However, there were no cases of a temperature exceeding 38°C at the end of surgery in either group. AA infusion did not cause significant febrile responses.

Compared with the control group, plasma insulin concentration in the AA group increased markedly ($P < 0.01$) during the early stage of surgery (2 h after the start of infusion). The increase in insulin was transient; insulin level decreased 4 h after the infusion was started (Fig. 2). Compared with the control group, plasma leptin levels increased slowly in the AA group; plasma leptin levels were significantly increased in the AA group during the late phase of surgery (6 h after the start of the infusion; Fig. 2). T_3 levels did not change in either group (Fig. 3). Adrenaline and blood glucose were significantly increased, compared with baseline levels, in the AA-treated group at 6 h after the start of infusion and at the end of surgery. The same tendencies
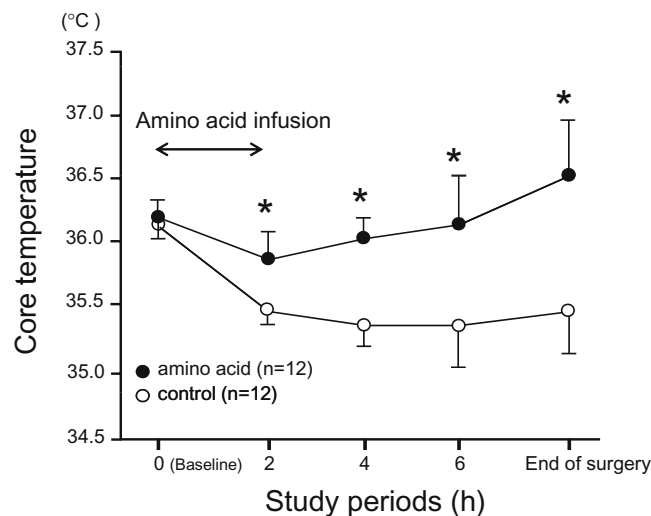

Fig. 1. Changes in core body temperature, measured in the pulmonary artery using a thermistor-equipped pulmonary artery catheter, in 12 patients receiving amino acid infusion (amino acid; *closed circles*) and 12 patients receiving equal volumes of a nutrient-free saline solution (control; *open circles*). As indicated in the Fig., the amino-acid mixture was given from approximately 30 min before the start of the surgery and continued for 2 h. *Vertical bars*, SD; *$P < 0.05$ indicates difference between the groups

occurred in the control group: there were no significant differences between the control and AA-infused groups (Figs. 3 and 4).

Postoperative levels of blood urea nitrogen (BUN) and creatinine were within normal ranges in both groups, and did not change during the procedure in either group. Postoperative levels of creatine kinase-myocardial bound (CK-MB) in the two groups were not significantly different (AA group, 57 ± 102 IU·l^{-1} vs control group, 22 ± 12 IU·l^{-1}), but 3 of the 12 AA-infused patients developed slight increases in CK-MB (100–300 IU·l^{-1}). No control group patients had increased CK-MB levels.

Table 2 shows the hemodynamic changes in the two groups; these parameters were measured before and 3 h after the start of infusion. Compared with the control group, AA infusion significantly ($P < 0.05$) increased oxygen consumption, which was dependent on the increase in heart rate. Three hours from the start of surgery, hemodynamics could not be precisely measured, due to the surgical procedures.

During the surgery, patients required catecholamine therapy with norepinephrine (NE) and dopamine to maintain hemodynamic stability. Neither drug was administered before the measurement of hemodynamics 3 h from the start of AA infusion, so as to avoid the effects of these drugs (Table 2). The total amounts of administered NE and dopamine were 9.1 ± 3.9 µg·kg^{-1} (0.08 ± 0.02 µg·kg^{-1}·min^{-1}, for 137.9 ± 98.7 min) and
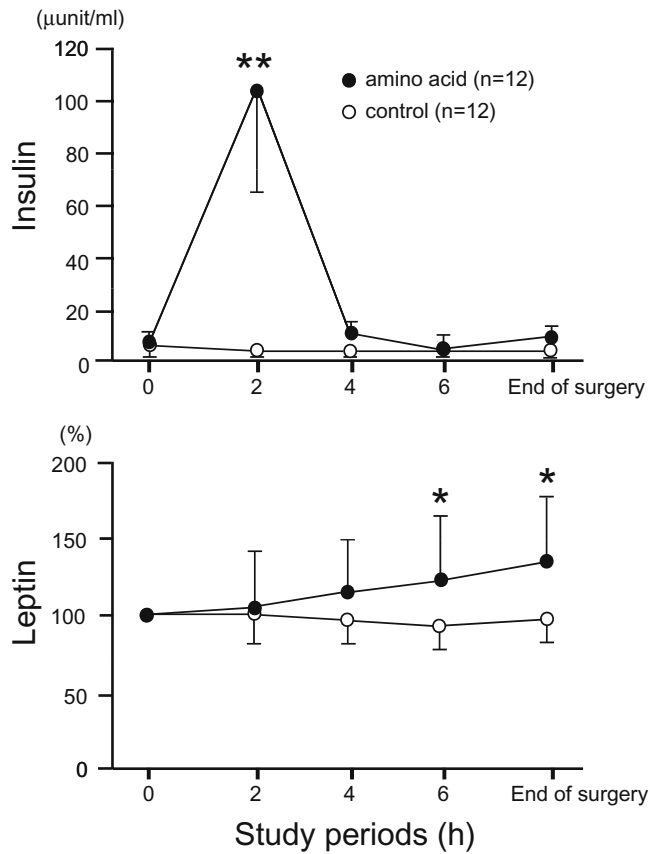
Fig. 2. Arterial plasma insulin (*upper panel*) and leptin (*lower panel*) levels in 12 control subjects (*open circles*) and in 12 patients receiving IV amino acid infusion (*closed circles*) during anesthesia. The leptin level is expressed relative to the initial plasma concentration (i.e., that measured before the start of the infusion, which was given the value of 100%) in the same patient. *Vertical bars*, SD; *$P < 0.05$; **$P < 0.01$ indicate differences between the groups. Details of the experimental protocol are indicated in Fig. 1

Fig. 3. Arterial plasma free thyroid hormone (T$_3$; *upper panel*) and adrenaline (*lower panel*) levels in 12 control subjects (*open circles*) and in 12 patients receiving IV amino acid infusion (*closed circles*) during anesthesia. *Vertical bars*, SD; #$P < 0.05$ indicates difference from the baseline. Details of the experimental protocol are indicated in Fig. 1

$287.1 \pm 128.9\,\mu g \cdot kg^{-1}$ ($2.8 \pm 1.1\,\mu g \cdot kg^{-1} \cdot min^{-1}$, for $99.2 \pm 51.6\,min$), respectively, in the control group; and $8.3 \pm 5.1\,\mu g \cdot kg^{-1}$ ($0.07 \pm 0.04\,\mu g \cdot kg^{-1} \cdot min^{-1}$, for $93.8 \pm 78.5\,min$) and $277.0 \pm 85.5\,\mu g \cdot kg^{-1}$ ($2.9 \pm 1.1\,\mu g \cdot kg^{-1} \cdot min^{-1}$, for $92.9 \pm 60.0\,min$), respectively, in the AA group. The differences between the groups were not significant.

In the ICU, the time required to reach a temperature of 37.5°C in the AA group was significantly shorter than that in the control group. Subsequently, it was found that AA infusion significantly reduced the time required to extubation (intubation duration after surgery was $365 \pm 113\,min$ in the AA group, and $545 \pm 145\,min$ in the control group). There were no significant differences in ongoing or postoperative blood loss, length of stay in the ICU, or duration of hospitalization between the two groups.
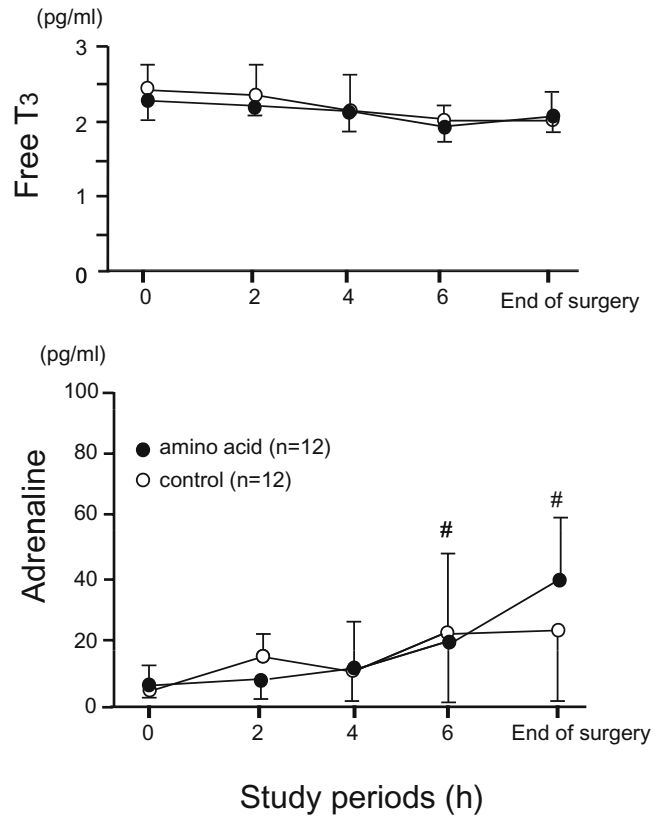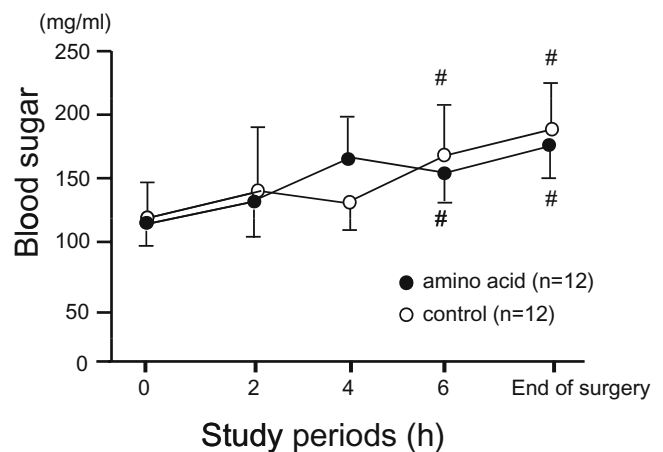

Fig. 4. Blood sugar levels in 12 control subjects (*open circles*) and in 12 patients receiving IV amino acid infusion (*closed circles*) during anesthesia. *Vertical bars*, SD; #$P < 0.05$ indicates difference from the baseline. Details of the experimental protocol are indicated in Fig. 1

Table 2. Hemodynamic parameters before and 3 h after the start of amino-acid infusion

	mAP	HR	CI	SVR	CVP	SV	Hb	VO$_2$
Before								
AA group	78 ± 6	63 ± 19	2.87 ± 0.5	2041 ± 350	8.8 ± 2.9	78 ± 12	12.5 ± 1.9	110 ± 23
Control group	78 ± 10	61 ± 6	2.73 ± 0.3	1931 ± 405	7.8 ± 2.2	86 ± 19	12.3 ± 2.0	109 ± 26
3 h after the infusion								
AA group	77 ± 7	73 ± 9*	3.57 ± 0.7*	1662 ± 407	6.9 ± 2.2	90 ± 15	9.7 ± 1.1	132 ± 25*
Control group	74 ± 6	66 ± 6	3.03 ± 0.4	1764 ± 307	8.1 ± 3.5	83 ± 12	9.4 ± 1.3	105 ± 29

*$P < .05$ compared with control group
AA, amino acid; mAP, mean arterial pressure (mmHg); HR, heart rate (beats·min^{-1}); CI, cardiac index (l·min^{-1}/m^2); SVR, systemic vascular resistance (dyn·s^{-1}·m^2/cm^5); CVP, central venous pressure (mmHg); SV, stroke volume (ml); Hb, hemoglobin (mg·dl^{-1}); VO$_2$, oxygen consumption (ml·min^{-1})

Discussion

In the present study, compared with the control group, the AA infusion group had a significantly increased core body temperature during OPCABS. AA infusion markedly stimulated the secretion of insulin and leptin, whereas there were no significant differences in serum levels of adrenaline and T$_3$ between the two groups. Given the present results and recent reports [15–19], it is possible that two signaling pathways, activated by insulin and leptin, may have contributed, in part, to the AA-mediated thermogenesis during surgery.

Despite accumulating evidence showing the clinical efficacy of AA infusion in preventing intraoperative hypothermia [1–6], the mechanism of action is unknown. We hypothesized that AA infusion induces a thermogenic effect, at least in part through the effects of serum hormones, which, in turn, increase energy expenditure. Few reports have focused on the effects of AA infusion on metabolic hormone levels in humans. The relationship between metabolic hormone levels and thermogenic effects during anesthesia have not been investigated.

The most important finding presented herein was that AA infusion stimulated the release of insulin and leptin during surgery. Recent studies have suggested that these hormones may be important metabolic signals for energy regulation in humans [20]. In the present study, the insulin level increased rapidly within 2 h after the start of AA infusion, whereas the leptin level increased gradually; a significant increase in the leptin level in the AA infusion group compared with the control group was recognized 6 h after the start of infusion. Insulin may induce a thermogenic effect during the early stage of surgery, whereas leptin may have an important role in thermogenesis during the late stage of surgery.

The mechanisms by which insulin affects thermogenesis are complex and incompletely understood. Based on previous reports involving insulin-mediated thermogenesis, it appears that the thermogenic effect of insulin may be the net result of several major components: (i) increased activity of the sympathetic nervous system (SNS) [21]; (ii) stimulation of energy-requiring processes, including protein turnover (anabolism) [9,18]; (iii) production of mitochondrial uncoupling protein (UCP), particularly UCP2 and UCP3 [17,22]; and (iv) the increased energy expenditure associated with glucose storage [7].

In the present study, AA infusion caused a significant increase (13%) in oxygen consumption by increasing the heart rate. In general, the heart rate is primarily controlled by the action of the SNS on the heart and, to a lesser extent, by catecholamines (adrenaline) or thyroid hormone. Neither the levels of epinephrine nor those of thyroid hormone (T$_3$) changed in the present study, suggesting that AA infusion may directly stimulate the SNS. Several studies have suggested that insulin increases energy expenditure in part through direct sympathoadrenal stimulation, or via a direct action of the hormone in the hypothalamus [20,21]. The increased insulin following AA infusion may in our study have increased heart rate through SNS activation, although a relationship between the increased insulin and the increased heart rate could not be directly shown due to various limitations.

It is unlikely that increased glucose metabolism contributed to the thermogenic response we found in the present study; in this study, the serum insulin level was greatly increased after AA infusion, whereas the serum glucose level was unchanged, suggesting that there was little (if any) glycogen cycling when AAs were infused. Consistent with this finding, Donatelli et al. [9] reported little change in plasma glucose level when the insulin level was increased by AA infusion in patients under anesthesia. These results suggest that energy expenditure related to insulin-dependent glucose uptake does not play a major part in the thermogenesis that occurs during AA infusion. Several studies in humans and rats have reported that AA infusion decreases insulin-stimulated peripheral glucose disposal by interfering with skeletal muscle glucose transport/phosphorylation [23,24].

The present study showed, for the first time, that the level of the adipocyte-secreted hormone leptin was increased by AA infusion in humans. Leptin, like insulin, regulates energy homeostasis by upregulating energy consumption; this suggests that it may act as a thermogenic hormone. Leptin and insulin can both lead to the increased expression of UCP3 in skeletal muscle, and the two hormones can lead to the increased expression of UCP2 in pancreatic islets and adipose tissue, respectively [15,16]. UCP2 and UCP3 are likely to have been involved in the thermogenesis induced by AA infusion that occurred in the present study. A recent report suggested that leptin has a primary role in the inflammatory response by activating classical proinflammatory cytokines [19]. Leptin may activate energy expenditure by operating the cytokine network, just as other cytokines do under inflammatory conditions; these actions merit further investigation.

Adverse effects of AA infusion were not observed in the present study, but the risks of adverse effects caused by AA infusion should be considered. AA infusion may be hazardous or harmful for patients with hepatic, renal, or metabolic diseases, as well as those with cardiopulmonary insufficiency [1]. In the present study, no increase in lactate level was noted during AA infusion; this probably indicates that tissue hypoxemia did not worsen and that hepatic clearance did not decrease in these patients. Postoperative levels of BUN and creatinine, compared with the preoperative levels, had not changed in either group; this suggests that AA infusion had little effect on renal function. Our results support the recent finding that AA infusion is a clinically useful method for preventing hypothermia during OPCABS [6]. However, the increased metabolic rate, including increased heart rate, caused by AA infusion appears to overload myocardial function in patients undergoing OPCABS. No episodes of myocardial ischemia or infarction were detected on continuous electrocardiogram monitoring and transesophageal echocardiography during and after the surgery in our study, but some AA-infused patients had increased levels of CK-MB postoperatively. The increase in heart rate may have adverse myocardial effects in patients undergoing cardiac surgery. On the basis of the present study, care must be taken when using AA infusions in patients with cardiac insufficiency, even if they are undergoing general surgery.

In summary, intravenous AA infusion prevents hypothermia during OPCABS. AA infusion stimulated the release of the metabolic hormones insulin and leptin. This finding suggests that hormonal signaling may, in part, contribute to the AA-induced thermogenesis, in addition to the AA-induced stimulation of SNS activity and protein metabolic pathways (including anabolic or uncoupling protein synthesis pathways). Further pharmacological and biochemical studies are required to clarify the roles of insulin and leptin in the regulation of energy expenditure that occurs in response to AA infusion during surgery.

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