Thermogenesis induced by intravenous infusion of hypertonic solutions in the rat

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1. Intravenous administration of 20–60% glucose, 3.2–9.7% NaCl or 20% mannitol solutions (1.66 ml kg^{-1}) for 5 min increased oxygen consumption in urethane-anaesthetized rats, whereas administration of physiological saline had no effect. Administration of 7.7–18.3% urea slightly increased the oxygen consumption, but the increase was significantly smaller than that measured after the administration of other hypertonic solutions. The magnitude of the thermogenic effect correlated with the osmolality of the applied solutions. These results suggest that the thermogenesis was caused mainly by changes in osmolality rather than by a specific action of the different solute molecules.

2. Neither pretreatment with the ganglion blocker hexamethonium (20 mg kg^{-1}, I.P.) or the β-adrenergic antagonist propranolol (10 mg kg^{-1}, I.P.), nor bilateral cervical vagotomy or bilateral adrenalectomy had any effect on the osmotically induced thermogenesis. Therefore, the autonomic nervous system and the adrenal gland were not involved in this metabolic response.

3. In response to osmotic stimulation, the temperature of the skeletal muscle increased significantly, whereas that of brown adipose tissue did not change and that of the colon and liver decreased. Accordingly, the site of osmotic thermogenesis is probably in the skeletal muscle, although osmotic stimulation was not accompanied by electromyographic activity and was not blocked by pretreatment with muscle relaxants such as dantrolene sodium or pancuronium bromide, or with the Na^+–Cl^- co-transport inhibitor bumetanide.

4. The increases in plasma osmolality observed after the administration of 20% (1.3 osmol kg^{-1}) glucose and 4.1% (1.3 osmol kg^{-1}) NaCl were 4.50 ± 0.88 and 5.57 ± 0.71 mosmol kg^{-1}, respectively. Since the slight increase in osmolality is well within the physiological range of changes that occur after food ingestion, diet-induced thermogenesis may have a component that is mediated by an increase in plasma osmolality, which results from the prandial increase in circulating nutrients.

Elevated levels of circulating nutrients occur with early prandial nutrient absorption from the gut. This contributes to the satiety signal for the regulation of nutrient intake, and causes an increase in resting energy expenditure that lasts for several hours after the ingestion of a meal. The increase in energy expenditure also occurs after I.V. administration of glucose, amino acids or lipids (Green & Macdonald, 1981; Acheson *et al.* 1983, 1984; DeFronzo *et al.* 1984; Jéquier, 1986; Brundin *et al.* 1996). The thermogenic response to glucose has been investigated, and 2–7% of the energy content of the infused glucose would account for the increase in energy expenditure above the baseline level. Although most of these studies were performed on humans and not on laboratory animals, Martins *et al.* (1985) reported that I.V. administration of a mixture of glucose and fat increased energy expenditure in conscious rats.

We recently found that intestinal infusion of hypertonic glucose, NaCl, fructose, methylglucose or amino acids elicited thermogenesis lasting for ~3 h in urethane-anaesthetized rats (Osaka *et al.* 2001). The thermogenic response was small after intestinal infusion of urea; the infusion of physiological saline, water or lipids had no effect on the metabolic rate. Accordingly, the thermogenesis elicited by the intestinal infusion was caused by changes in osmolality. Intravenous infusion of NaCl also induced thermogenesis, although to a level approximately half that induced by the intestinal infusion. Therefore, thermogenesis induced by the I.V. infusion of nutrients may have a component that is mediated by the accompanying increases in plasma osmolality. In the present study, we I.V.-infused glucose, NaCl, mannitol and urea solutions into rats to examine the effects of osmotic stimulation on whole-body oxygen consumption ($\dot{V}_{O_2}$).

We then investigated the mechanisms of thermogenesis induced by I.V. infusion of glucose or NaCl. First we examined the possible involvement of the autonomic nervous system and adrenal catecholamines by pretreatment with the ganglion blocker hexamethonium, the β-blocker propranolol, vagotomy or adrenalectomy, because several studies have demonstrated that the thermogenic response to glucose depends, at least in part, upon the sympathetic nervous system (Acheson *et al.* 1983, 1984; DeFronzo *et al.* 1984). We then tested the effects of anti-insulin serum on the thermogenic response to glucose infusion, because administration of glucose stimulates pancreatic B cells to secrete insulin, which is known to facilitate heat production. Next we examined the effects of vasopressin on $\dot{V}_{O_2}$, because a rise in plasma osmolality stimulates the release of vasopressin, which promotes the reabsorption of water in the kidney to lower plasma osmolality, and because it has been shown that the administration of vasopressin increases $\dot{V}_{O_2}$ in isolated hindlimb preparations (Ye *et al.* 1995). Finally, to determine the site of heat production, we measured the temperature of the skeletal muscle, interscapular brown adipose tissue (IBAT) and liver after the infusion of hypertonic solutions. Since changes in temperature were found to be specific to the skeletal muscle, muscle relaxants were also administered to examine the participation of muscle contraction in the observed thermogenesis.

METHODS

Animals and surgery

Male Wistar rats weighing 250–300 g were maintained at an ambient temperature of 24 ± 1 °C on a 12 h:12 h light–dark schedule. All experiments were carried out within the normal range of housing temperatures. The animals had free access to water and laboratory food but were fasted overnight (for ~14 h) before the experiments. The care of animals and all surgical procedures followed institutional and Japanese Physiological Society guidelines.

The rats were anaesthetized with urethane (1.2 g kg^{-1}, I.P.) and kept on a heating pad to maintain their baseline colonic temperature at 36–37 °C during the experiments. Animals given this dose of urethane remain anaesthetized for at least 10 h, and our experiments always lasted less than 8 h. A femoral vein catheter was inserted for injection of solutions. When the animals were administered muscle relaxants, their respiration was maintained through a tracheal cannula connected to an artificial respirator. The electrocardiogram was monitored with the aid of needle electrodes inserted into the limbs, and the heart rate was recorded continuously and was kept in the normal range (300–350 beats min^{-1}) during the artificial ventilation. Some of the rats were subjected to either adrenalectomy or cervical vagotomy. In these animals the effects of I.V. infusion of glucose were tested prior to the surgery. Bilateral adrenalectomy or cervical vagotomy was then performed, and the effects of a second infusion of glucose were examined within 1 h of the surgery. Animals were killed at the end of the experiment by an overdose of anaesthetic.

Measurements of $\dot{V}_{O_2}$, carbon dioxide production and temperature

$\dot{V}_{O_2}$ and the temperature of the colon (T_{co}), tail skin (T_{sk}) and organs (muscle, liver and IBAT) were recorded continuously, according to methods similar to those described elsewhere (Kobayashi *et al.* 1998). Briefly, the head of each rat was covered with a hood that was ventilated continuously at a constant rate of 1.0 l min^{-1}. The difference in concentrations of oxygen and carbon dioxide between inflow and outflow air was measured with a differential oxygen analyser (LC700E, Toray, Japan) and two carbon dioxide sensors (GMW22D, Vaisala, Finland), respectively. T_{co} was measured with a thermistor probe inserted about 60 mm beyond the anus. T_{sk} was measured with a small thermistor taped to the dorsal base of the tail. Temperatures of the IBAT, liver and muscle were measured with other small thermistor probes placed below the IBAT pad, between the liver lobes, and into the femoral muscle, respectively. All of the signals were fed into a computer and recorded every 15 s using a PowerLab system (ADInstruments, Australia) for online data display and storage. After experiments, data were averaged over 1 min intervals. The results of $\dot{V}_{O_2}$ determination are expressed in terms of metabolic mass (kg$^{0.75}$). The time-integrated increase in $\dot{V}_{O_2}$ reflects the total area of increase in $\dot{V}_{O_2}$ over the resting value that was determined as an average during the 5 min before the infusion of a solution. The respiratory exchange ratio was calculated as the ratio of carbon dioxide production to $\dot{V}_{O_2}$. An EMG was recorded with a pair of stainless steel electrodes that were inserted into the musculus biceps femoris, and monitored on an oscilloscope. Direct electrical stimulation of the muscle evoked compound action potentials in the EMG and visible muscle contractions. When vasopressin was used, the electrocardiogram was monitored continuously.

Experimental protocol

The following solutions were infused I.V. at 1.66 ml kg^{-1} for 5 min with a syringe pump (CMA100, Carnegie Medicin, Sweden): 20–60% glucose, 0.9–9.7% NaCl, 20% mannitol or 7.7–18.3% urea. Among these solutions, 20% glucose, 4.1% NaCl, 20% mannitol and 7.7% urea were approximately equiosmotic (1.3 osmol kg^{-1}). Solutions of 44% glucose, 9.7% NaCl and 18% urea were hyperosmotic (3.2 osmol kg^{-1}) and were chosen for comparison. We could not use 3.2 osmol kg^{-1} mannitol because it was insoluble at this concentration. Physiological saline (0.9% NaCl) was infused as an isotonic control. Solutions were warmed to 38–39 °C before administration, but the temperature of the solutions decreased slightly during the infusion. The infusion of solutions was usually repeated 3–4 times at approximately 90 min intervals. The magnitude and time course of the thermogenic response to the same solution were similar among repeated trials in a given rat (see Results). Hexamethonium chloride (20 mg kg^{-1}, I.P.), *dl*-propranolol hydrochloride (10 or 20 mg kg^{-1}, I.P.) or anti-human insulin serum (0.3 ml of 1:3 dilution, I.V.; Oriental Yeast, Suita, Japan) was administered 30 min before infusion of the test solution. Arginine vasopressin (Peptide Institute, Minoh, Japan) was infused I.V. at a dose of 7.5 ng min^{-1} at a volume of 16 μl min^{-1} for 10 min. Dantrolene sodium salt (RBI, MA, USA), which inhibits intracellular Ca^{2+} release from the sarcoplasmic reticulum, was dissolved in 95% physiological saline and 5% propylene glycol and infused I.V. at a dose of 2 mg kg^{-1} in a volume of 1 ml kg^{-1} for 5 min. The neuromuscular blocking agent pancuronium bromide (Sankyo, Tokyo, Japan) was infused I.V. at a dose of 0.4 mg kg^{-1} for 5 min. A specific inhibitor of Na^+–Cl^- co-transport, bumetanide (Biomol, PA, USA) was dissolved in 50% ethanol and 50% distilled water and infused I.V. at a dose of 1.0 mg kg^{-1} in a volume of 1 ml kg^{-1} for 5 min. For the determination of plasma osmolality and levels of catecholamines and glucose, venous blood (1.5 ml) was collected from a catheter inserted into the jugular vein, before and 5 and 20 min after the injection of the test solutions. An equal volume of 0.9% saline was infused back into the rat after the 0 min sampling. After centrifugation (2000 *g*, 6–8 min) and removal of the plasma sample, an equal volume of 0.9% NaCl was added to the erythrocytes. The blood was returned to the rat after the 5 min sampling.

Analytical methods

Plasma osmolality was determined with a freezing-point depression osmometer (Model 3CII, Advanced Instruments, MA, USA). Plasma catecholamines were measured by high-performance liquid chromatography and fluorescence detection (HLC-725CA, TOSOH, Japan). The plasma glucose level was determined with a glucose oxidase method (Glucose B-test, Wako Pure Chemical Industries, Osaka, Japan).

Statistics

Values are expressed as the means ± S.E.M. at 1 min intervals. One-way ANOVA or Friedman's repeated-measures ANOVA was used to determine whether the differences were statistically significant. Newman-Keuls test was used for multiple comparisons. For analysis of correlations, the Pearson product moment correlation and simple linear regression were calculated. Differences between the slopes of regression lines were determined by Student's t test. Statistical significance was defined as $P < 0.05$.

RESULTS

Osmotically induced thermogenesis

Figure 1*A* shows a representative example of changes in $\dot{V}_{O_2}$ after the infusion of glucose solutions in a rat. Simultaneously recorded changes in the respiratory exchange ratio are shown in Fig. 1*B*. Infusion of glucose solutions increased both $\dot{V}_{O_2}$ (Fig. 2*A*) and the respiratory exchange ratio dose dependently. In response to 60 % (4.4 osmol kg^{-1}) glucose, $\dot{V}_{O_2}$ rose significantly from the baseline level at 2 min and reached a peak increase of 1.50 ± 0.05 ml min^{-1} kg$^{-0.75}$ ($n = 5$) at 6 min. Within 50 min of the infusion, the $\dot{V}_{O_2}$ returned to the baseline level. The integrated increase in $\dot{V}_{O_2}$ was 24.5 ± 2.4 ml kg$^{-0.75}$, which corresponds to 4.25 ± 0.45 % of the energy content of the infused glucose. The respiratory exchange ratio ranged between 0.82 and 0.87 during the basal state, increased gradually after the 60 % glucose infusion to a peak of 0.92–0.99 at 19–30 min, and returned to the baseline level within 120 min. Infusion of 44 % (3.2 osmol kg^{-1}) glucose significantly increased $\dot{V}_{O_2}$ at 1 min, giving a peak increase of 0.90 ± 0.03 ml min^{-1} kg$^{-0.75}$ ($n = 5$) at 5 min; the increase lasted for > 20 min. The integrated increase in $\dot{V}_{O_2}$ was 19.9 ± 1.7 ml kg$^{-0.75}$. Infusion of 20 % (1.3 osmol kg^{-1}) glucose significantly increased $\dot{V}_{O_2}$ during the period 3–11 min and induced an integrated increase in $\dot{V}_{O_2}$ of 5.4 ± 0.8 ml kg$^{-0.75}$ ($n = 5$). The respiratory exchange ratio reached a peak of 0.92–0.99 at 22–28 min after infusion of these glucose solutions, and returned to the baseline level within 70 min. In spite of the thermogenic response, T_{co} decreased slightly (~0.07 °C) after the glucose infusion (see below). A small increase in T_{sk} (~0.3 °C) was observed, but it was not statistically significant. The integrated $\dot{V}_{O_2}$ response was significantly correlated with the osmolality of the glucose solution ($r = 0.94$, $P < 0.001$, $n = 21$; Fig. 1*C*).

Infusion of 9.7 % (3.2 osmol kg^{-1}) NaCl also significantly increased $\dot{V}_{O_2}$ at 3 min, giving a peak rise to 0.90 ± 0.13 ml min^{-1} kg$^{-0.75}$ ($n = 5$) at 7 min; this increase lasted for > 20 min (Fig. 2*B*). The response was comparable to that induced by the equiosmotic glucose. Infusion of 4.1 % (1.3 osmol kg^{-1}) NaCl increased $\dot{V}_{O_2}$ with a time course and magnitude similar to those observed after the infusion of 1.3 osmol kg^{-1} glucose. Infusion of physiological saline had no effect on $\dot{V}_{O_2}$. The respiratory exchange ratio did not change after infusion of either NaCl solution. T_{co} decreased with a time course and magnitude similar to those observed after glucose infusion (see below). T_{sk} did not change significantly. The increase in $\dot{V}_{O_2}$ was significantly correlated with the osmolality of the NaCl solution ($r = 0.93$, $P < 0.001$, $n = 16$; Fig. 1*C*). The regression lines of $\dot{V}_{O_2}$ *versus* the osmolality of glucose (continuous line, $y = 6.2x - 1.9$) and *versus* that of NaCl (dashed line, $y = 7.8x - 3.5$) were similar, and the slopes of the regression lines were not statistically different ($P > 0.05$).

To compare the effects of various solutions, we used the 1.3 osmol kg^{-1} mannitol and urea solutions. Infusion of

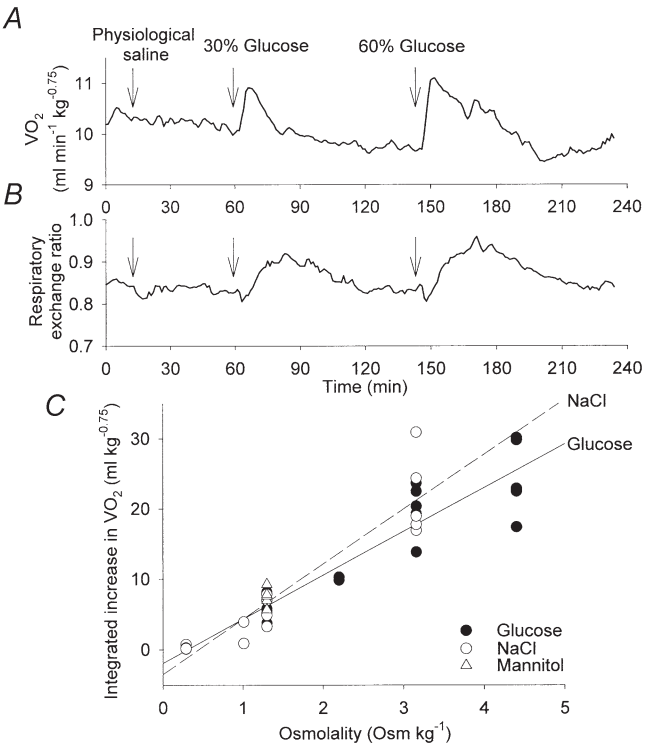

Figure 1. Dose-dependent effects of intravenous infusion of glucose and NaCl solutions

A representative example of changes in oxygen consumption ($\dot{V}_{O_2}$; *A*) and the respiratory exchange ratio (*B*) induced by infusion of physiological saline, and 30 % (2.2 osmol kg^{-1}) and 60 % (4.4 osmol kg^{-1}) glucose solutions in a rat. The arrows show the time of infusion. *C*, the integrated increase in $\dot{V}_{O_2}$ in response to the osmolality of infused glucose (●, $n = 21$), NaCl (○, $n = 16$) and mannitol (△, $n = 5$) solutions. Continuous and dashed lines show linear regressions of $\dot{V}_{O_2}$ *versus* the osmolality of glucose ($r = 0.94$) and *versus* that of NaCl ($r = 0.93$), respectively.

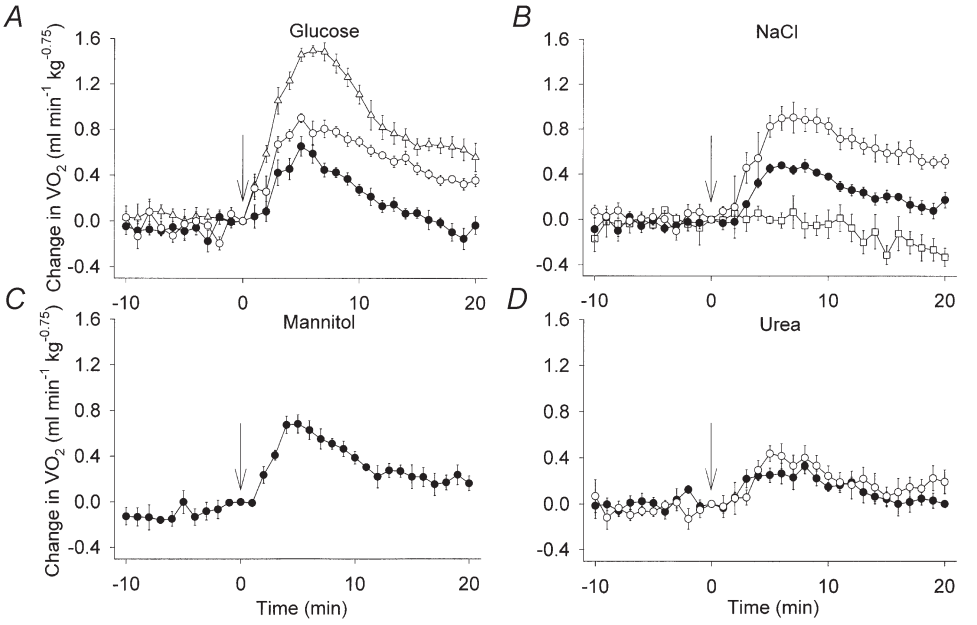

Figure 2. Changes in $\dot{V}_{O_2}$ after infusion of glucose (*A*), NaCl (*B*), mannitol (*C*) and urea (*D*)

A, dose-dependent increases in $\dot{V}_{O_2}$ in response to 4.4 osmol kg^{-1} (△, $n = 5$), 3.2 osmol kg^{-1} (○, $n = 5$) and 1.3 osmol kg^{-1} (●, $n = 5$) glucose solutions. *B*, dose-dependent increases in $\dot{V}_{O_2}$ in response to 3.2 osmol kg^{-1} (○, $n = 5$) and to 1.3 osmol kg^{-1} NaCl (●, $n = 5$). Infusion of physiological saline (□, $n = 4$) had no effect on $\dot{V}_{O_2}$. *C*, the increase in $\dot{V}_{O_2}$ in response to 1.3 osmol kg^{-1} mannitol was similar to that to 1.3 osmol kg^{-1} glucose or NaCl. *D*, increases in $\dot{V}_{O_2}$ after infusion of 1.3 osmol kg^{-1} (●, $n = 5$) and 3.2 osmol kg^{-1} urea (○, $n = 5$). Values are means ± S.E.M. The arrows show the time of infusion.

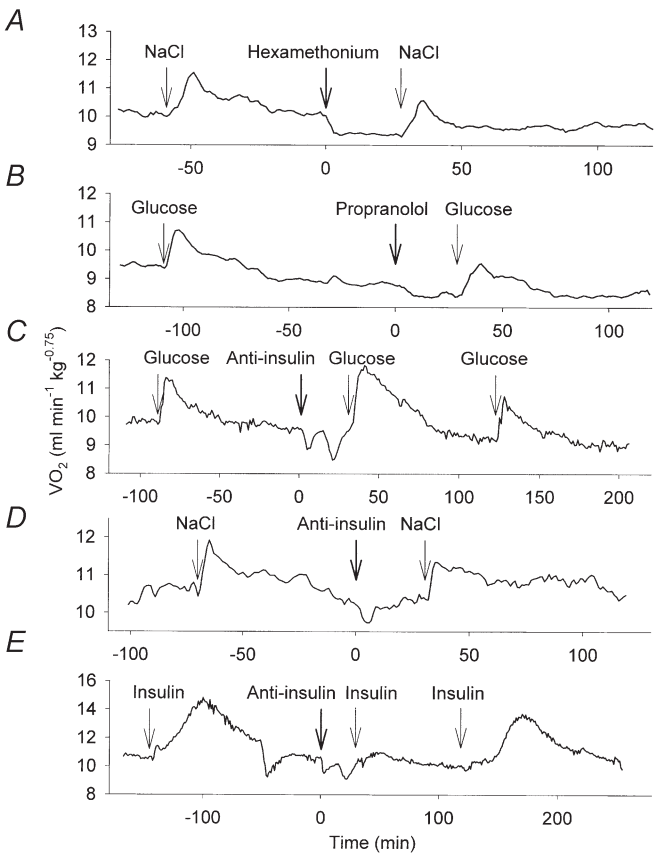

Figure 3. Representative examples of the effects of hexamethonium (*A*), propranolol (*B*), and anti-insulin serum (*C*, *D*, and *E*) on $\dot{V}_{O_2}$ responses to the infusions of glucose or NaCl

A, administration of hexamethonium (20 mg kg^{-1}, I.P., thick arrow) decreased the basal level of $\dot{V}_{O_2}$ but did not affect the increase in $\dot{V}_{O_2}$ induced by an I.V. infusion (thin arrow) of NaCl. *B*, administration of propranolol (10 mg kg^{-1}, I.P.) also decreased the $\dot{V}_{O_2}$ level and had no effect on the glucose-induced $\dot{V}_{O_2}$ response. *C*, administration of anti-insulin serum (0.3 ml of 1:3 dilution, I.V.) enhanced the glucose-induced increase in $\dot{V}_{O_2}$; this effect disappeared at 2 h. *D*, the NaCl-induced increase in $\dot{V}_{O_2}$ was not affected by anti-insulin serum. *E*, administration of insulin (500 mU kg^{-1}, I.V.) increased $\dot{V}_{O_2}$, which was largely attenuated by pretreatment with anti-insulin serum; the insulin effect recovered by 130 min.

1.3 osmol kg^{-1} mannitol (Fig. 2*C*) induced a peak increase of $\dot{V}_{O_2}$ of 0.68 ± 0.08 ml min^{-1} kg$^{-0.75}$ ($n = 5$) with a time course and magnitude similar to those observed after infusion of an equiosmotic solution of glucose or NaCl. The infusion of 1.3 osmol kg^{-1} urea, however, induced only a very small increase in $\dot{V}_{O_2}$ (Fig. 2*D*), which gave a value that was statistically different from the baseline value at 8 min, but not at other time points. The integrated increase in $\dot{V}_{O_2}$ observed after the infusion of 1.3 osmol kg^{-1} urea was 2.4 ± 0.4 ml kg$^{-0.75}$ ($n = 5$), which was significantly smaller than that observed after infusion of the equiosmotic solution of glucose (5.4 ± 0.8 ml kg$^{-0.75}$), NaCl (5.7 ± 0.8 ml kg$^{-0.75}$), or mannitol (7.5 ± 0.6 ml kg$^{-0.75}$). When the effect of 3.2 osmol kg^{-1} urea was compared with that of equiosmotic glucose and NaCl, urea-induced thermogenesis (5.1 ± 1.6 ml kg$^{-0.75}$) was significantly smaller than that induced by either NaCl or glucose. The respiratory exchange ratio remained within baseline levels after the infusion of NaCl, mannitol and urea solutions.

Lack of participation of the autonomic nervous system, insulin and vasopressin in the osmotically induced thermogenesis

At this stage of the experiments, we employed pharmacological or surgical manipulations to examine the mechanisms underlying the thermogenesis induced by 60% glucose or 9.7% NaCl. Figure 3*A* shows a typical example of the effects of hexamethonium on the basal $\dot{V}_{O_2}$ level and NaCl-induced increase in $\dot{V}_{O_2}$. Figure 4*A* summarizes the results ($n = 6$), showing the lack of effect of hexamethonium on the increase in $\dot{V}_{O_2}$ induced by glucose or NaCl. Hexamethonium did not affect the increase in the respiratory exchange ratio elicited by glucose. However, the administration of hexamethonium alone decreased $\dot{V}_{O_2}$ by 1.15 ± 0.13 ml min^{-1} kg$^{-0.75}$ ($n = 6$) and increased T_{sk} by 1.85 ± 0.37 °C, suggesting an effective blockade of autonomic transmission.

Administration of propranolol (10 mg kg^{-1}, I.P.) alone significantly decreased $\dot{V}_{O_2}$ by 0.58 ± 0.11 ml min^{-1} kg$^{-0.75}$ ($n = 4$). A representative record is shown in Fig. 3*B*. However, the infusion of 60% glucose after propranolol treatment resulted in an increase in $\dot{V}_{O_2}$ that did not differ from the value obtained from the same animals before propranolol administration (Fig. 4*B*). Propranolol also had no effect on the glucose-induced increase in the respiratory exchange ratio. A higher dose (20 mg kg^{-1}) of propranolol also did not affect the glucose-induced increase in $\dot{V}_{O_2}$, although it did decrease the basal level of $\dot{V}_{O_2}$ by 0.79 ± 0.19 ml min^{-1} kg$^{-0.75}$ ($n = 3$).

Bilateral adrenalectomy ($n = 5$) significantly decreased the baseline level of $\dot{V}_{O_2}$ by 0.81 ± 0.27 ml min^{-1} kg$^{-0.75}$ and that of T_{co} by 0.43 ± 0.06 °C. The subsequent infusion of 60% glucose resulted in an increase in $\dot{V}_{O_2}$ that was not significantly different from that observed before the

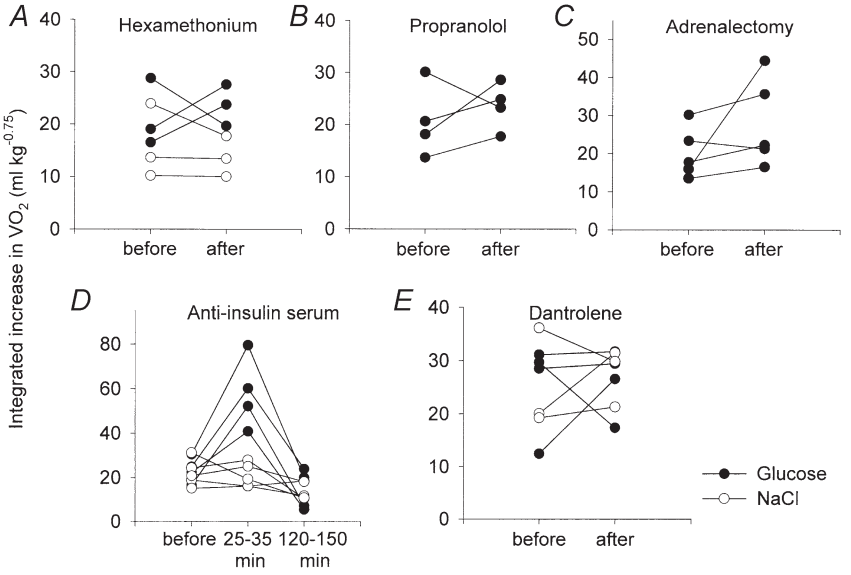

Figure 4. Effects of hexamethonium (*A*), propranolol (*B*), bilateral adrenalectomy (*C*), anti-insulin serum (*D*) and dantrolene (*E*) on integrated increases in $\dot{V}_{O_2}$ induced by infusion of glucose or NaCl

Responses to 4.4 osmol kg^{-1} glucose and 3.2 osmol kg^{-1} NaCl are shown by ● and ○, respectively. Each line indicates an individual rat. The $\dot{V}_{O_2}$ response was not affected by treatment with hexamethonium (*A*), propranolol (*B*), adrenalectomy (*C*), or dantrolene (*E*). However, responses to glucose were enhanced at 25–35 min after administration of anti-insulin serum and returned to the pretreatment level by 150 min (*D*). Amounts of drugs used were the same as those in Fig. 3.

surgery (Fig. 4*C*). Bilateral cervical vagotomy also had no effect on the increase in $\dot{V}_{O_2}$ induced by the infusion of 60 % glucose ($n = 3$).

Administration of insulin (500 mU kg^{-1}, I.V.) gradually increased $\dot{V}_{O_2}$ to give a peak rise of 2.68 ± 0.79 ml min^{-1} kg$^{-0.75}$ ($n = 3$) within 50 min. An example of the effect of insulin on $\dot{V}_{O_2}$ is shown in Fig. 3*E*, which also shows that pretreatment with anti-insulin serum largely attenuated the thermogenic effect of insulin. Administration of anti-insulin serum decreased $\dot{V}_{O_2}$ temporarily by 0.78 ± 0.13 ml min^{-1} kg$^{-0.75}$ ($n = 12$), but the changes in $\dot{V}_{O_2}$ returned to the baseline level within 30 min. However, the insulin-induced increase in $\dot{V}_{O_2}$ was reduced to a peak increase of 0.54 ± 0.07 ml min^{-1} kg$^{-0.75}$ at 32–35 min after administration of the anti-insulin serum, and it recovered to 2.85 ± 0.64 ml min^{-1} kg$^{-0.75}$ at 118–124 min. On the other hand, the glucose-induced increase in $\dot{V}_{O_2}$ was enhanced from 23.5 ± 2.8 ml kg$^{-0.75}$ to 58.3 ± 8.1 ml kg$^{-0.75}$ by pretreatment with the anti-insulin serum (Figs 3*C* and 4*D*). This enhancing effect disappeared within 150 min. The NaCl-induced increase in $\dot{V}_{O_2}$ was not affected by pretreatment with the anti-insulin serum (Figs 3*D* and 4*D*).

Administration of arginine vasopressin (7.5 ng min^{-1}, I.V.) did not affect the resting $\dot{V}_{O_2}$ levels (11.3 ± 0.6 ml min^{-1} kg$^{-0.75}$, $n = 4$), at least for the 20 min observation period ($P > 0.05$). However, it decreased the heart rate by 38.1 ± 10.8 beats min^{-1} at 11 min. This bradycardia suggests a baroreceptor-mediated reflex caused by the hypertensive action of vasopressin, although we did not record arterial blood pressure.

Organ temperature after the administration of hypertonic solutions

Changes in the temperature of the organs were examined in response to infusions of equiosmotic (3.2 osmol kg^{-1}) glucose, NaCl, or urea, and to physiological saline. The muscle temperature (Fig. 5*A*) gradually and significantly increased above the baseline level by 4 min after the infusion of glucose, and the increase reached a peak of 0.17 ± 0.02 °C ($n = 5$) at 16 min. This significant increase continued for > 30 min. The infusion of NaCl also elicited a significant increase in the muscle temperature, with a time course and magnitude similar to those observed after the infusion of glucose. After the infusion of 3.2 osmol kg^{-1} urea, the muscle temperature rose to a small peak of 0.08 ± 0.01 °C ($n = 5$) at 9 min, and rapidly returned to the baseline level. The change was significantly smaller than that observed after the infusion of NaCl or glucose over the 13–30 min period. Infusion of physiological saline did not elicit significant changes in the muscle temperature ($n = 4$). The temperature of IBAT did not change after the infusion of glucose, NaCl or urea

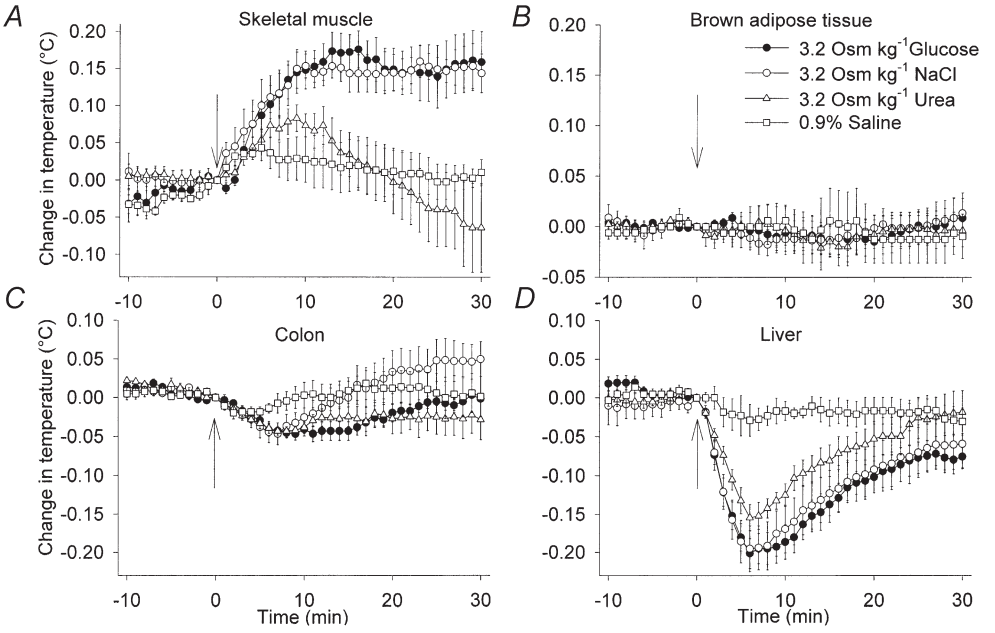

Figure 5. Changes in the temperature of muscle (*A*), brown adipose tissue (*B*), colon (*C*) and liver (*D*) after infusion of equiosmotic (3.2 osmol kg^{-1}) glucose, NaCl or urea solution

A, the increases in muscle temperature after glucose (●, $n = 5$) and NaCl (○, $n = 5$) were higher than that observed after the infusion of urea (△, $n = 5$). *B*, the temperature of brown adipose tissue did not change after infusion of these solutions. *C*, the temperature of the colon decreased slightly (< 0.07 °C) after infusion of these solutions. *D*, the liver temperature decreased immediately and significantly after infusion of these solutions. Infusion of physiological saline (□, $n = 4$) had no effect on the temperature of these organs. Values are means ± S.E.M. The arrows show the time of infusion.

(Fig. 5*B*). The liver temperature decreased to a similar extent (by 0.10–0.31 °C) after the infusion of these solutions, although it was not affected by the infusion of physiological saline (Fig. 5*D*). T_{co} also decreased by 0.02–0.07 °C within 10 min of the infusion of these solutions, although the magnitude of the change was significantly smaller than that observed for the liver (Fig. 5*C*).

Lack of participation of muscle contraction in the osmotically induced thermogenesis

Neither EMG activity nor visible muscle contractions were observed after the infusion of glucose or NaCl solutions. However, muscle compound action potentials could be evoked by direct electrical stimulation of the muscle. Repetitive electrical simulation at 200 Hz elicited visible muscle contraction.

Administration of the muscle relaxant dantrolene did not affect the increase in $\dot{V}_{O_2}$ observed after the infusion of either 4.4 osmol kg^{-1} glucose or 3.2 osmol kg^{-1} NaCl (Fig. 4*E*). Pretreatment with pancuronium also had no effect on the increase in $\dot{V}_{O_2}$ induced by 4.4 osmol kg^{-1} glucose ($n = 4$). Pretreatment with bumetanide had no effect on the increase in $\dot{V}_{O_2}$ induced by 3.2 osmol kg^{-1} NaCl ($n = 3$).

Plasma osmolality, glucose and catecholamine levels after administration of hypertonic solution

Administration of 1.3 osmol kg^{-1} glucose increased the plasma osmolality by 4.50 ± 0.88 mosmol kg^{-1} ($n = 6$) at 5 min (Fig. 6*A*). An increase in plasma osmolality (by 5.57 ± 0.71 mosmol kg^{-1}; $n = 7$) was also observed after the infusion of 1.3 osmol kg^{-1} NaCl, and this increase was not statistically different from that observed after the infusion of glucose. The plasma glucose concentration increased by 10.2 ± 1.0 mM ($n = 4$) at 5 min after the glucose infusion (Fig. 6*B*). The infusion of NaCl did not affect the plasma glucose concentration at 5 min, but slightly increased it by 2.6 ± 1.1 mM ($n = 5$) at 20 min. The infusion of neither glucose nor NaCl had any effect on the plasma level of noradrenaline (Fig. 6*C*). The adrenaline concentration of the plasma had not changed by 5 min after the infusion of glucose or NaCl, although it had increased significantly at 20 min (Fig. 6*D*).

DISCUSSION

The I.V. administration of hypertonic glucose solutions significantly increased $\dot{V}_{O_2}$ in urethane-anaesthetized rats. This result agrees well with previous studies on conscious humans (Green & Macdonald, 1981; Acheson *et al.* 1983,

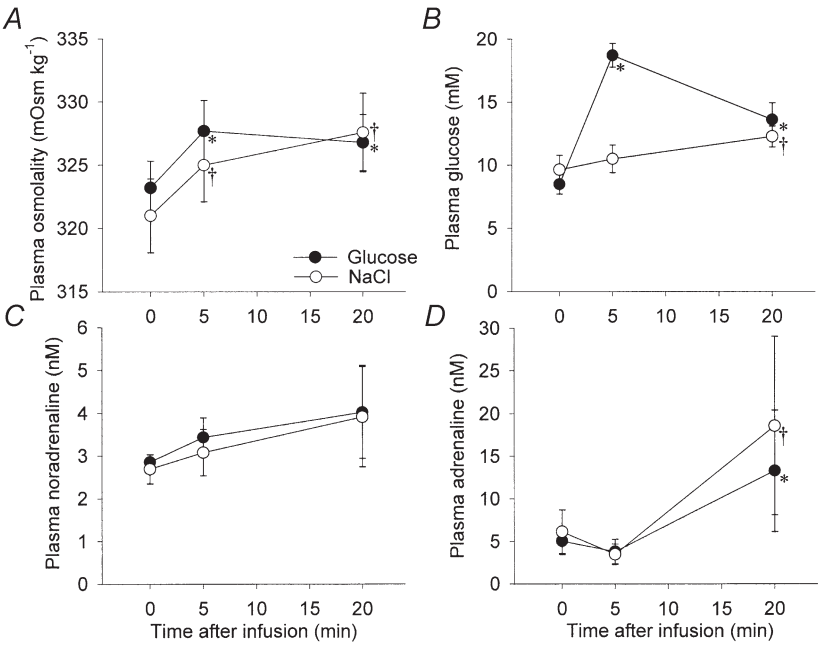

Figure 6. Changes in plasma osmolality (*A*) and levels of glucose (*B*), noradrenaline (*C*) and adrenaline (*D*) after infusion of equiosmotic (1.3 osmol kg^{-1}) glucose or NaCl

A, the plasma osmolality increased similarly after the infusion of glucose (●, $n = 6$) or NaCl (○, $n = 7$). *B*, the plasma glucose concentration increased markedly after the infusion of glucose, and it remained higher than the pretreatment level at 20 min. NaCl infusion did not change the plasma glucose level at 5 min, but slightly increased it at 20 min. *C*, the infusion of neither glucose nor NaCl had any effect on the plasma level of noradrenaline. *D*, the plasma adrenaline level was not changed by the infusion of either glucose or NaCl at 5 min, but was increased at 20 min by both of these solutions. Values are means ± S.E.M. * and † indicate a significant difference from the corresponding pretreatment value ($P < 0.05$).

1984; DeFronzo *et al.* 1984; Brundin *et al.* 1996). However, the most interesting observation in the present study is the comparable increase in $\dot{V}_{O_2}$ that was observed after the infusion of hypertonic NaCl or mannitol solutions. The infusion of isotonic NaCl had no effect on $\dot{V}_{O_2}$, and the $\dot{V}_{O_2}$ responses to hypertonic solutions were closely correlated with the osmolality of the applied solutions. It is noteworthy that the infusion of urea, to which the cell membrane is permeable and which does not afford effective osmotic stimulation in the body, induced only a very small increase in $\dot{V}_{O_2}$. These results show that the heat production induced by I.V. infusion of the hypertonic solutions was caused mainly by changes in osmolality rather than by a specific action of the different solute molecules. Although plasma glucose is gradually taken up by cells and cannot be an effective long-term osmotic stimulant, the plasma glucose concentration remained high for 20 min. The transport of glucose is probably slow enough to permit the development of a transcellular gradient. Thus, the administration of glucose increased the plasma osmolality to a level comparable to that achieved by NaCl. However, $\dot{V}_{O_2}$ returned to the baseline level at 20 min after the I.V. infusion of the hypertonic solutions, even though the plasma osmolality remained at a high level. This result may suggest adaptation of osmoreceptors or an alteration in cell volume induced by the progressive cellular dehydration, although we have no evidence to support these possibilities.

In the present study the maximal change in plasma osmolality observed after the infusion of 1.3 osmol kg^{-1} glucose or NaCl solution was ~5 mosmol kg^{-1}. The slight increase in osmolality is well within the physiological range of changes in plasma osmolality that occur after food ingestion (Houpt, 1991). Intravenous administration of hypertonic saline inhibits food intake and gastric motility (Flanagan *et al.* 1992), suggesting a close relationship between changes in plasma osmolality and feeding control. Moreover, the thermogenic response to food is higher in rats drinking 0.9% NaCl than in those drinking pure water (Bryant *et al.* 1984). These findings suggest that the diet-induced thermogenesis may have a component that is mediated by an increase in plasma osmolality, which results from the circulating nutrients after the ingestion of a meal.

Our previous study (Osaka *et al.* 2001) showed that intestinal infusion of hypertonic NaCl and other solutions elicited a thermogenesis that was larger than that induced by I.V. infusion of the same amount of NaCl, suggesting the involvement of intestinal osmoreceptors. Therefore, it is possible that the thermogenesis induced by the I.V. infusion of hypertonic solutions might be partly mediated by the intestinal osmoreceptors. However, administration of the β-blocker propranolol largely attenuated the thermogenesis induced by intestinal osmotic stimulation (our unpublished observation) but did not affect that induced by I.V. infusion in the present study. Accordingly, it is unlikely that I.V. and intestinal osmotic stimulation activated identical mechanisms of thermogenesis.

The increase in $\dot{V}_{O_2}$ observed after the I.V. infusion of glucose or NaCl solutions was not affected by pretreatment with either a β-blocker or a ganglion blocker. Nor did the plasma concentration of noradrenaline change after the infusion. Therefore, the present results show that activation of the sympathetic nervous system is not essential for the osmotically induced increase in heat production. This conclusion is in accordance with reports showing the lack of involvement of sympathetic activity in glucose-induced thermogenesis in humans (Seaton *et al.* 1984; Vernet *et al.* 1987; Aksnes *et al.* 1994). On the other hand, continuous infusion of hypertonic NaCl for 30–150 min increased plasma noradrenaline concentrations (Peskind *et al.* 1993) and induced a pressor response with regionally non-uniform sympathetic nervous responses (Weiss *et al.* 1996). The duration of infusion and the amount of NaCl used may account for the discrepancy between studies. The present study also showed that adrenalectomy had no effect on the glucose-induced thermogenesis and that plasma adrenaline levels did not change during the peak period of the osmotic thermogenesis, although it increased at 20 min. Therefore, adrenal catecholamines and adrenocortical hormones are also not essential for osmotic thermogenesis. In addition, the lack of effect of cervical vagotomy on glucose-induced thermogenesis shows that the thermogenesis was not mediated by the vagus nerve.

The respiratory exchange ratio increased after the infusion of glucose solution, suggesting oxidation of carbohydrates during the thermogenic response. However, thermogenesis induced by the infusion of NaCl or mannitol solutions was not accompanied by changes in the respiratory exchange ratio, although the magnitude of thermogenesis in these cases was similar to that observed after the infusion of glucose. Accordingly, the thermogenesis occurred without a definite relationship to the substrate utilization.

Pretreatment with the anti-insulin serum, which prevented insulin-induced thermogenesis, did not attenuate the NaCl-induced thermogenesis. Therefore, it seems unlikely that insulin mediated the NaCl-induced thermogenesis. On the contrary, pretreatment with the anti-insulin serum enhanced the glucose-induced thermogenesis. The administration of anti-insulin serum is considered to lower the plasma insulin concentration and thus probably attenuates the uptake of plasma glucose by cells. Consequently, the infusion of hypertonic glucose would become a more effective osmotic stimulus than that seen in animals with normal insulin concentrations. Thus,

pretreatment with anti-insulin serum probably enhanced the osmotic effect of the infused glucose and therefore induced a larger thermogenic response.

Osmotic stimulation is well known to increase the plasma level of vasopressin, which has a thermogenic effect on isolated hindlimb preparations (Ye *et al.* 1995). However, I.V. administration of vasopressin did not affect $\dot{V}_{O_2}$, although the amount of vasopressin that we administered caused baroreceptor-mediated bradycardia (Veelken *et al.* 1989). This result suggests that vasopressin did not contribute to the osmotically induced thermogenesis.

Infusion of physiological saline had no effect on $\dot{V}_{O_2}$ or on the temperature of muscle, IBAT, liver or colon. However, the muscle temperature increased after the infusion of both the glucose and the NaCl solution, the temperatures of the colon and liver decreased, and the IBAT temperature did not change. These results suggest that skeletal muscle is the major site of osmotic thermogenesis. Accordingly, we measured EMG activity to investigate the participation of muscle contraction. However, neither EMG activity nor visible muscle contraction was observed after the infusion of glucose or NaCl solution. Moreover, pretreatment with a muscle relaxant, either dantrolene or pancuronium, did not affect the osmotic thermogenesis, suggesting that a mechanism other than muscle contraction is responsible for the osmotically induced thermogenesis. In isolated superfused muscles, hypertonic conditions induced a substantial increase in heat production; the increase was mediated largely by a bumetanide-sensitive Na^+–Cl^- co-transport mechanism (Chinet 1993), which was also shown to be involved in osmotically induced adjustments of volume regulation of skeletal muscle cells (Dørup & Clausen, 1996). However, Cox & Gibbs (1997) reported that bumetanide had no effect on the osmotically induced thermogenic response in isolated muscles, a finding in line with the present results. Thus, the mechanism of the muscle thermogenesis remains to be clarified.

An acute increase in plasma osmolality (~15 mosmol kg^{-1}) affects whole-body protein, lipid metabolites and glucose kinetics, and enhances the rate of appearance of endogenous glucose (Berneis *et al.* 1999). Moreover, osmotically induced cell shrinkage affects the intracellular metabolic function and stimulates intracellular catabolism, such as activation of glucose and glycogen metabolism in the skeletal muscle (Low *et al.* 1996). Accordingly, hyperosmolality might affect directly the cellular metabolism of muscle and cause heat production, although we have no evidence for this from the present study.

The temperature of IBAT did not change after administration of the hypertonic solutions. Thermogenesis in the IBAT is generally regulated by the sympathetic nervous system, and we have shown that sympathetic activation was not involved in the process of osmotic thermogenesis. Therefore, IBAT is unlikely to be responsible for osmotic thermogenesis. The temperature of both the colon and liver was decreased by the infusion of hypertonic solutions. This hypothermic effect could not be due to an enhancement of heat-loss responses because the T_{sk} did not change. Moreover, increases in $\dot{V}_{O_2}$ and muscle temperature and decreases in the temperatures of the colon and liver occurred almost simultaneously after infusion of the hypertonic solutions. These results suggest that the thermogenesis was not a compensatory response to hypothermia.

Increased plasma osmolality has a vasodilatory action on the vascular smooth muscle in the skin, skeletal muscle, intestine and kidney; this action results in an increase in blood flow in these organs (Gazitùa *et al.* 1971; Järhult *et al.* 1975). A slight decrease in core temperature and increase in T_{sk}, as well as an increase in peripheral blood flow, have been observed in humans after the infusion of glucose or mannitol solutions (Green & Macdonald, 1981). Thus, the changes in organ temperatures observed in the present study may have been caused by the increased regional blood flow in these organs and by the circulatory redistribution of accumulated body heat. The specific increase in the temperature of muscle, therefore, may not suggest the site of thermogenesis. Further studies will be necessary to elucidate the site and mechanisms of osmotically-induced thermogenesis. The present study shows, however, that an increase in plasma osmolality within the physiological range elicits thermogenesis.

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