Diet-induced thermogenesis (DIT) is the increase in whole body energy expenditure following food ingestion, which lasts for several hours and consists of at least two phases. The first ‘cephalic’ phase takes place within the first 40 min of feeding in rats, dogs and humans (Diamond et al. 1985; Allard & LeBlanc, 1988; LeBlanc & Soucy, 1996). The sensory stimulation induced by the sight, smell and taste of food probably activates the brain to produce the cephalic response, because oral ingestion of a meal elicits a larger thermogenic response than intragastric injection of the same liquified meal and because sham-feeding (chewing of food without swallowing) elicits a substantial amount of energy expenditure (Diamond et al. 1985; De Jonge et al. 1991; LeBlanc & Soucy, 1996). The cephalic phase is gradually replaced by the second part of thermogenesis called the ‘gastrointestinal’ or ‘digestive’ phase (Diamond et al. 1985; Acheson, 1993), which is considered to be related to the nutritional content of the meal but not to the palatability of the food (Soucy & LeBlanc, 1999).

Thermogenesis during the gastrointestinal phase contains the obligatory energy costs for digestion, absorption, processing and storage of the nutrients ingested. Accordingly, thermogenesis is considered to take place at tissues that require such obligatory energy. The site of the thermogenic response varies for the different nutrients. The splanchnic tissues account for approximately one-half of the thermogenic response induced by protein meals or intravenous amino acid infusions, suggesting the metabolic costs for hepatic processing of amino acids (Brundin & Wahren, 1994a,b). However, exclusive augmentation of blood flow and the remaining half of the thermogenic response take place in the extrasplanchnic tissues after administration of protein meals or intravenous amino acid infusions, suggesting the metabolic costs for hepatic processing of amino acids (Brundin & Wahren, 1993; Brundin et al. 1996, 1997). Fructose, however, is primarily taken up and phosphorylated by the liver instead of in the

Thermogenesis induced by osmotic stimulation of the intestines in the rat

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1. Infusion of 5–20% glucose, 1.8–3.6% NaCl, 20% methylglucose, 20% fructose, or 5–10% solutions of various amino acids (10 ml kg\(^{-1}\)) into the duodenum induced dose-dependent thermogenesis in urethane-anaesthetized rats. In contrast, infusion of 0.9% NaCl, distilled water, or safflower oil had no effect on the metabolic rate. Infusion of 7.2% urea induced a small and transient increase in the metabolic rate. These results suggested that the thermogenesis was caused mainly by changes in osmolality rather than by a specific action of the different solute molecules.

2. The respiratory exchange ratio increased after the infusion of glucose, fructose, glycine, or serine, did not change after the infusion of NaCl, methylglucose, safflower oil, or distilled water, and decreased after infusion of arginine. Therefore, there was no relationship between substrate utilization and the occurrence of thermogenesis.

3. Intestinal infusion of 3.6% NaCl elevated the plasma osmolality, with a plateau increase of ~20 mosmol kg\(^{-1}\). However, intravenous infusion of the same amount of NaCl induced a significantly smaller thermogenic response, although it elevated the plasma osmolality with a time course and magnitude similar to those obtained after the intestinal infusion. Infusion of NaCl into the hepatic portal vein or the peritoneal cavity also produced a significantly small thermogenic response. These results suggested an intestinal or mesenteric location for osmoreceptors.

4. To test for possible stimulation of intestinal osmoreceptors after intake of a normal meal, we measured the osmolality of the intestinal contents. The osmolality of the duodeno-jejunal contents was 600–800 mosmol kg\(^{-1}\), whereas the plasma osmolality was 306 ± 1 mosmol kg\(^{-1}\), which suggests that the intestinal osmoreceptors are stimulated after meals and are involved in diet-induced thermogenesis.
extraspinal tissues, and a substantial proportion of nutrient glucose is also stored as glycogen in the liver. These studies suggest that the gastrointestinal phase of thermogenesis contains components reflecting energy expended in other than intestinal or hepatic processing of nutrients.

However, the signals by which ingested nutrients stimulate energy expenditure during the gastrointestinal phase are largely unknown, although they are generally assumed to be specific to individual nutrients. At present there is no appropriate experimental model for the study of the signals and mechanisms of thermogenesis during the gastrointestinal phase, although the intravenous infusion of nutrients has been usefully employed as a model to study DIT. In the present study, we employed intestinal infusion of various nutrients and observed metabolic and body temperature responses. For this purpose, we used anaesthetized rats because their baseline metabolic rate is stable and various surgical or pharmacological manipulations are possible. Thus, we infused glucose, fructose, amino acids and safflower oil through an intraduodenal cannula in urethane-anaesthetized rats. The infusion of glucose solutions elicited dose-dependent thermogenic responses, which were similar to those after oral or intragastric administration of meals in awake animals and humans. However, we found that the infusion of hypertonic NaCl solutions also elicited similar thermogenic responses. Accordingly, we infused other non-nutrient solutions such as methylglucose and urea solutions to test the possible involvement of osmoreceptors in the initiation of thermogenesis. In order to investigate the localization of osmoreceptors, we infused a hypertonic NaCl solution into the hepatic portal vein, femoral vein, or peritoneal cavity and compared the resulting thermogenic effects with the effect of the intestinal infusion. Finally, we measured the osmolality of the gastrointestinal contents of rats fed ad libitum to examine the magnitude of osmotic stimulation after normal food intake.

METHODS

Animals and surgery
Male Wistar rats, weighing 250–340 g, were maintained at an ambient temperature of 24 ± 1 °C with lighting between 07.00 and 19.00 h for at least 1 week before the experiments. They had free access to water and laboratory food (MR stock, Nosan, Japan), but were fasted overnight for ~14 h before the experiments unless otherwise mentioned. 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 placed in the supine position on an operating table heated to 30–31 °C. Animals given this dose of urethane remain anaesthetized for at least 10 h and our experiments always lasted less than 8 h. After the abdomen had been clipped and scrubbed with a disinfectant solution (Isodine, Meiji, Japan), a ventral midline incision (~20 mm) was made from the xiphoid caudally. The stomach and a part of the duodenum were exposed. A Teflon cannula (Feeding tube 7207, Fuchigami, Japan) was inserted through a small incision in the wall of the forestomach and then passed 10 mm beyond the pylorus into the duodenum. The cannula and the pylorus were ligated together to prevent a backward flow of the infused solution. The incision in the forestomach was closed with ligature to prevent leakage of gastric contents into the peritoneal cavity. For intraperitoneal infusion of solutions, the tip of cannula was placed on the serosa of the duodenum and the stomach was left intact. The cannula was exteriorized through the abdominal wall and skin and the incision was closed with surgical silk. The abdominal surface was then covered with a quilt to reduce heat dissipation. In some experiments, the femoral vein or hepatic portal vein was cannulated for administration of solutions, and the right jugular vein, for blood sampling. Animals were killed by an overdose of anaesthetic at the end of the experiment.

Experimental procedures
The head of each rat was covered with a cylindrical hood, which was continuously ventilated at a constant rate of 1.01 min⁻¹. The difference in concentrations of O₂ and CO₂ between inflow and outflow air was measured with a differential O₂ analyser (LC700E, Toray, Japan) and two CO₂ sensors (GMW22D, Vaisala, Finland), respectively. Colonic temperature (T) was measured with a thermistor inserted ~60 mm into the anus. Tail skin temperature was measured with a small thermistor, which was taped to the lateral surface of the rat’s tail. All the signals were fed into a computer and recorded at 15 s intervals through a PowerLab system (ADInstruments, Australia) for on-line data display and storage. After experiments, data were averaged over 5 min intervals. The metabolic rate (M; in kJ) was calculated from measurements of O₂ consumption and CO₂ production according to the following equation: $M = 15.8[O_2] + 5.2[CO_2]$ (Kurpad et al. 1994), where $[O_2]$ and $[CO_2]$ are in litres at standard temperature and pressure. Values were corrected for metabolic body size (kg⁰.⁷). The amount of energy expenditure induced by infusion of a solution was calculated as the total area of increase in metabolic rate over resting values. The following solutions were infused into the intestine at a volume of 10 ml kg⁻¹ for 10 min with a syringe pump (KDS100, KD Scientific, USA): 5–20% glucose, 0.9–3.6% NaCl, 20% 3-O-methyl-D-glucose, 20% fructose, 7.2% urea, 10–20% glycine, 10–20% serine, 10–20% threonine, 10–20% arginine, distilled water and safflower oil (~80% oleic acid). The concentrations of the solutions were chosen because 0.9% NaCl and 5% glucose are approximately equiosmotic to normal body fluids, the osmolality of 1.8% NaCl and 10% glucose is twice as high as that of the normal fluids, while that of 3.6% NaCl, 20% glucose, 20% methylglucose, 20% fructose and 7.2% urea is approximately four times higher. For intraperitoneal infusion of NaCl, the concentration (3.6%) and volume (10 ml kg⁻¹) of the solution were identical to those used for the intestinal infusion. However, for intravenous infusion of NaCl, we decreased the rate of infusion and the volume of NaCl solution to avoid rapid expansion of the blood volume. Thus, we infused 10.8% NaCl at a volume of 3.33 ml kg⁻¹ for 30 min, keeping the total amount of NaCl constant. Solutions were warmed to 38–39 °C before administration, but the temperature of the solutions decreased slightly during the infusion. The effects of each solution were tested in separate groups consisting of five or six rats.

Intestinal infusion of hypertonic solutions probably draws body fluids into the gut lumen. Although this load could induce diarrhoea, we did not observe such changes during the experiments. Moreover, we opened the abdominal wall and examined intestinal conditions soon after the infusions in some preliminary experiments and after the 3 h observation period in several experiments. We found that the lower jejunum and ileum were almost empty, suggesting effective absorption of fluids, although the duodenum and upper jejunum always contained a moderate amount of yellow and viscous fluid after experiments.
To examine the plasma osmolality, we obtained blood samples (0.7 ml) from the right atrium through the jugular vein cannula at 0, 20, 40, 60 and 120 min after the intestinal infusion of 3.6% NaCl or 20% glucose or after the intravenous infusion of 10.8% NaCl. After the 0 min sampling, an equal volume of 0.9% NaCl was infused back into the rat. After centrifugation 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 samplings at 20, 40 and 60 min. The osmolality of blood plasma samples was measured with a freezing-point osmometer (model 3CII, Advanced Inc., USA).

Osmolality of gastrointestinal contents

Rats which had been given ad libitum access to food and drink were anaesthetized with Nembutal (60 mg kg⁻¹, i.p.) at 07.00–09.40 h. Blood samples taken from the right atrium, and the contents of the stomach, duodenum and the upper 200 mm of the jejunum and lower 300 mm of the ileum were centrifuged at 2000 g for 8–10 min. The osmolality of the supernatant was measured.

Statistics

Data are presented as the means ± S.E.M. A one-way ANOVA or one-way repeated measures ANOVA was used to determine significant differences. Tukey’s test was used for multiple comparisons. A P value of < 0.05 was considered to indicate a significant difference.

RESULTS

Dose-dependent increases in metabolic rate after intestinal infusion of glucose solutions

Intestinal infusion of glucose solutions increased the metabolic rate, respiratory exchange ratio (RER) and $T_c$ dose dependently (Fig. 1A–C). The metabolic rate rose gradually during the infusion of 20% glucose from a baseline level of $186 ± 7$ J kg⁻⁰.⁷⁵ min⁻¹ to a peak of $217 ± 6$ J kg⁻⁰.⁷⁵ min⁻¹ at 65 min and slowly returned to the baseline level within 3 h (Fig. 1A). The energy expenditure induced by 20% glucose was $2.79 ± 0.45$ kJ kg⁻⁰.⁷⁵ for 3 h (Fig. 4). The RER increased from $0.82 ± 0.01$ to $0.92 ± 0.01$ at 115 min (Fig. 1B), suggesting the oxidation of carbohydrate during the thermogenic response to the glucose infusion. The increase in RER lasted more than 3 h. As a consequence of the thermogenesis, $T_c$ increased from $36.73 ± 0.12$°C to a peak of $37.16 ± 0.07$°C at 95 min (Fig. 1C). Tail skin temperature increased less than 0.5°C after the glucose infusion.

Infusion of 10% glucose also increased the metabolic rate to a peak of $206 ± 7$ J kg⁻⁰.⁷⁵ min⁻¹ at 60 min, and the effect lasted more than 2 h. In spite of the long-lasting increase in metabolic rate, the increase in RER terminated within 80 min. $T_c$ reached a peak of $36.87 ± 0.11$°C at 90 min. Infusion of 5% glucose induced small but significant increases in metabolic rate and RER, but it did not increase $T_c$ significantly. The energy expended was $1.86 ± 0.39$ kJ kg⁻⁰.⁷⁵ after 10% glucose and $0.52 ± 0.24$ kJ kg⁻⁰.⁷⁵ after 5% glucose (Fig. 4). However, energy expenditure as a percentage of energy intake was not statistically different among the rats administered different concentrations of glucose solution (20% glucose, 11.2 ± 1.8%; 10% glucose, 14.9 ± 3.1%; 5% glucose, 5.2 ± 4.0%).

Dose-dependent increases in metabolic rate after intestinal infusion of sodium chloride solutions

Intestinal infusion of hypertonic NaCl solutions also increased the metabolic rate dose dependently (Fig. 2A). The metabolic rate rose during the 10 min infusion period of 3.6% NaCl, stayed at a plateau level of $~205$ J kg⁻⁰.⁷⁵ min⁻¹ between 35 and 120 min and then
slowly declined but was still significantly higher than the baseline level at 3 h. The energy expenditure induced by 3.6% NaCl was 3.49 ± 0.33 kJ kg⁻⁰.₇₅, which was not significantly different from that induced by the infusion of 20% glucose. Administration of 1.8% NaCl also increased the metabolic rate, to a plateau level of ~190 J kg⁻⁰.₇₅ min⁻¹ between 45 and 120 min. Energy expenditure induced by 1.8% NaCl was 2.91 ± 0.59 kJ kg⁻⁰.₇₅, which was not significantly different from that induced by the infusion of 10% glucose. Administration of 0.9% NaCl did not increase the metabolic rate. The RER did not change after infusion of any of the NaCl solutions (Fig. 2B). Tc increased from 36.74 ± 0.06 °C to a peak of 37.20 ± 0.11 °C at 85 min after the infusion of 3.6% NaCl and to a peak of 36.89 ± 0.11 °C at 125 min after the infusion of 1.8% NaCl (Fig. 2C). Tc did not increase after the infusion of 0.9% NaCl.

Effects of intestinal infusion of fructose, methylglucose and urea

The effects of intestinal infusion of 20% fructose and 20% methylglucose on the metabolic rate were similar to the effect of 3.6% NaCl (Fig. 3A). Infusion of these solutions increased the metabolic rate to a plateau level of 205–215 J kg⁻⁰.₇₅ min⁻¹. During the 3 h observation period, infusion of fructose and methylglucose induced energy expenditure levels of 4.38 ± 1.00 kJ kg⁻⁰.₇₅ and 5.69 ± 0.46 kJ kg⁻⁰.₇₅, respectively. No significant difference was found between the thermogenic responses induced by 20% fructose, 20% methylglucose, 20% glucose and 3.6% NaCl. On the other hand, although infusion of 7.2% urea increased the metabolic rate for 10–50 min, the calculated expended energy of 0.30 ± 0.32 kJ kg⁻⁰.₇₅ was significantly smaller than that induced by any of the other solutions. Tc increased after the fructose and methylglucose solutions (Fig. 3C), reflecting the increase in metabolic rate. The change in Tc of rats administered urea was small, although it increased slightly from the baseline level between 35 and 85 min and decreased to a level lower than the baseline after 115 min. The RER of rats with fructose infusion increased with a time course similar to that of the glucose-infused rats. However, infusion of methylglucose or urea solutions had no effect on the RERs (Fig. 3B).

Distilled water and safflower oil

Administration of water and safflower oil had no effect on the metabolic rate, RER and Tc (data not shown), which were similar to those after infusion of 0.9% NaCl. The calculated energy expenditure was 0.04 ± 0.41 kJ kg⁻⁰.₇₅ after infusion of water and 0.69 ± 0.60 kJ kg⁻⁰.₇₅ after infusion of safflower oil.

Amino acids

Glycine, serine, threonine and arginine solutions were infused at concentrations of 5 or 10% into different rats. The molar concentration, which is roughly proportional to the osmolality, of these solutions ranged between 287 and 1330 mM because the molecular weight of these amino acids varies considerably. Infusion of the amino acids gradually increased the metabolic rate to a peak at 30–100 min, which then slowly returned to the baseline level within 3 h. The time course of the increase in metabolic rate was

![Figure 2. Dose-dependent effects of intestinal infusion of NaCl solutions on the metabolic rate (A), RER (B) and Tc (C)](image)

Concentrations of NaCl were 0.9% (△, n = 5), 1.8% (○, n = 5) and 3.6% (●, n = 5). Horizontal bar shows the period of NaCl infusion.
Figure 3. Effects of intestinal infusion of 20% fructose, 20% methylglucose and 7.2% urea on the metabolic rate (A), RER (B) and $T_c$ (C)

- ●, responses to fructose ($n=5$);
- ○, responses to methylglucose ($n=5$);
- ▲, responses to urea ($n=5$).
Horizontal bar shows the period of infusion.

Figure 4. Energy expenditure induced by intestinal infusion of various amino acid solutions
Glycine (Gly, ●), serine (Ser, ○), threonine (Thr, ▲) and arginine (Arg, ▲, △) were infused into the intestine at a concentration of 5% (open symbols) or 10% (filled symbols). The amount of energy expenditure was calculated as the total area of increase in metabolic rate for 3 h after the infusion. Each rat was tested with a single amino acid solution and each solution was tested in two different rats. The amount of energy expenditure correlated significantly with the molar concentration of the infused solution ($r = 0.83$) but not with the weight of the solute. The diagonal line shows the regression line computed from the amino acid-induced energy expenditure. Energy expenditure (means ± S.E.M.) induced by intestinal infusion of 5, 10 and 20% glucose (Gluc) and 20% fructose (Fruc) is also plotted for comparison.
similar to that after the infusion of glucose solutions. The amount of energy expenditure was significantly correlated with the molar concentration of solutions (Fig. 4) but not with the weight of amino acids contained in the solutions. Moreover, the amounts of 5–20% glucose- and 20% fructose-induced energy expenditure were distributed close to the regression line calculated from the amino acid-induced energy expenditure.

The RER increased by 0.05–0.07 between 40 and 60 min after infusion of 10% glycine or 10% serine and then slowly returned to the resting value of 0.76–0.84. Infusion of 10% threonine, 5% glycine, or 5% serine also increased the RER slightly. On the other hand, infusion of 5–10% arginine temporally decreased the RER by 0.07–0.12 at 15–20 min. Infusion of 5% threonine had no obvious effect on the RER.

Comparison of the effects of various routes of NaCl administration on the plasma osmolality and thermogenesis

After an overnight fast, the basal plasma osmolality was 321 ± 2 mosmol kg⁻¹ in the urethane-anaesthetized rats. Intestinal infusion of 3.6% NaCl for 10 min increased the plasma osmolality to a plateau level of ~340 mosmol kg⁻¹ within 40 min (Fig. 5, ○). Infusion of the same amount of NaCl into the femoral vein for 30 min increased the plasma osmolality with a time course and magnitude similar to those after the intestinal infusion (Fig. 5, ●). On the other hand, after intestinal infusion of 20% glucose, the plasma osmolality increased to a peak of 334 ± 5 mosmol kg⁻¹ at 20 min and then gradually returned to the baseline level (Fig. 5, ▲).

Infusion of NaCl into the femoral vein, hepatic portal vein, or peritoneal cavity induced a small but sustained thermogenic response, increasing the $T_c$ by 0.1–0.3 °C, but did not change the RER. However, the amount of energy expenditure and the increase in $T_c$ were significantly smaller than those after the intestinal infusion of the same amount of NaCl (Fig. 6).

Osmolality of gastrointestinal contents

The volumes of gastrointestinal contents were 4–5 ml in the stomach, 1.0–1.5 ml in the duodeno-jejunum and 2.0–2.5 ml in the lower ileum of rats fed ad libitum. A similar volume (300–400 µl) of supernatant was collected after the centrifugation of each of these three contents. The osmolality values of these supernatants were 742 ± 59 mosmol kg⁻¹ in the stomach, 678 ± 27 mosmol kg⁻¹ in the duodeno-jejnum and 556 ± 18 mosmol kg⁻¹ in the lower ileum (Fig. 7), all of which were significantly higher than the plasma osmolality (306 ± 1 mosmol kg⁻¹).

DISCUSSION

Intestinal infusion of 10–20% glucose solutions induced thermogenesis that lasted for ~2 h and was accompanied by an increase in RER in urethane-anaesthetized rats. The thermogenic response corresponded to an expenditure of 5–15% of the infused glucose energy. Because the time course and amount of energy expenditure were similar to those after oral saccharide intake in unanaesthetized animals and humans (Brundin...
However, in the present study, intestinal infusion of hypertonic NaCl, methylglucose, fructose, or amino acid solutions also induced thermogenesis. On the other hand, infusion of 0.9% NaCl, distilled water, or safflower oil had no effect on the metabolic rate. Infusion of 7.2% urea, to which the cell membrane is relatively permeable and which does not afford effective osmotic stimulation in the body, induced a small and transient thermogenesis. These results suggest that the thermogenesis induced by intestinal infusions was caused mainly by the changes in osmolality rather than by a specific action of the different solute molecules.

We used 0.9% NaCl and 5% glucose as isotonic solutions. While 0.9% NaCl had no effect on the metabolic rate, 5% glucose induced a small but significant thermogenic response, indicating that glucose has a specific action on the metabolic rate. However, intestinal infusion of 1.8% NaCl or 10% glucose, which are approximately twice as hypertonic as normal body fluids, induced a comparable amount of energy expenditure. Similarly, 3.6% NaCl, 20% glucose, 20% fructose and 20% methylglucose, which are approximately four times more hypertonic than the isotonic solutions, induced similar amounts of energy expenditure, although ingestion of fructose has been reported to induce a larger thermogenic response. However, the osmolalities of amino acid solutions are generally higher than that of the same weight of glucose in humans (Tappy et al. 1986). Amino acid-induced thermogenesis was correlated with the molar concentration, which is roughly proportional to the osmolality, but not with the weight of amino acids. Therefore, the increase in osmolality is the major factor in the thermogenesis induced by intestinal infusion of hypertonic solutions, although we cannot exclude some contribution via specific thermogenic actions of glucose and other nutrients.

Intestinal infusion of water or safflower oil had no effect on the metabolic rate or the RER. The lack of response to water suggests that signals from the intestinal osmoreceptors do not tonically activate thermogenesis. A very low thermic effect of fat accords well with earlier findings in humans (Jéquier, 1986; Brundin, 1998), although a decrease in RER was reported after oral or intravenous administration of fat, suggesting oxidation of fat.

Intravenous infusion of amino acids reportedly elicits a larger thermogenic response than that of glucose or lipid in terms of the energy content of the infused nutrient (Jéquier, 1986). Intragastric administration of protein also elicits a larger thermogenic effect than that of carbohydrates (Kim et al. 1994). The cause of this difference has been considered to reflect the cost of nutrient storage, in particular, the high energy cost of protein synthesis (Kim et al. 1994; Giordano & Castellino, 1997). In the present study, the intestinal infusion of 5–10% solutions of amino acids produced similar or larger thermogenic responses than that of the same amount of glucose. However, the osmolalities of amino acid solutions are generally higher than that of the same weight of glucose solution, because the average molecular weight of amino acids (~140) is smaller than that of glucose (~180). Accordingly, we consider that the apparent larger thermogenic effect of amino acid solutions can be explained, at least in part, by their higher osmolality. Individual amino acids may have specific effects on the metabolic rate, but we cannot comment on this possibility because no systematic study has been conducted. The present study has demonstrated a common property of amino acid solutions with respect to energy expenditure but did not focus on specific properties of individual amino acids.

Oral or intravenous administration of glucose or fructose reportedly increases the RER (Brundin & Wahren, 1993; Brundin et al. 1996). On the other hand, intravenous infusion of a balanced amino acid mixture increases energy expenditure without changes in the RER (Giordano & Castellino, 1997). In the present study, the RER increased after intestinal infusion of glucose, fructose, glycine, or serine solutions. An increase in RER accompanied by thermogenesis indicates the oxidation of substrates that have a high respiratory quotient (RQ). The RQ of glucose, fructose, glycine and serine is 1.00. On the other hand, the RQ of arginine is 0.71 and the intestinal infusion of arginine decreased the RER. These results suggest that the infused nutrient molecules were utilized as substrates during the nutrient-induced thermogenesis. However, the validity of this interpretation is not immediately apparent. First, the time courses of thermogenesis and RER were different.

Figure 7. Osmolality of plasma and gastrointestinal contents

Samples were collected from Nembutal-anaesthetized rats that had consumed a normal stock diet and water ad libitum. Each symbol and connecting line show data from the same rat. The osmolalities of the gastrointestinal contents were significantly higher than that of plasma.
after infusions of the nutrient solutions. For example, 20% glucose induced a more prolonged increase in the RER than in the metabolic rate and 10% glucose induced a more prolonged increase in the metabolic rate than in the RER. Second, infusions of non-nutrient NaCl and methylglucose elicited thermogenesis that was not accompanied by changes in the RER. Thus, there was no definite relationship between thermogenesis and substrate utilization. Third, intestinal infusion of safflower oil had no effect on the metabolic rate and RER in the present study, although oral or intravenous administration of fat has been reported to result in a small increase in energy expenditure with a significant decrease in RER (Brundin, 1998). Additional research is required to elucidate the mechanism of substrate utilization after intestinal infusion of various nutrients.

Intestinal infusion of 3.6% NaCl elevated the plasma osmolality by 20 mosmol kg\(^{-1}\) to a plateau level after 40 min. Infusion of the same amount of NaCl into the femoral vein increased the plasma osmolality to a similar extent and with a similar time course to values recorded after the intestinal infusion. Because only a small amount of intestinal contents remained after the experiments, intestinally infused NaCl solutions were effectively absorbed from the intestine. However, the energy expenditure induced by infusion of NaCl into the femoral vein or into the portal vein was about half that induced by the intestinal infusion. The results suggest that intestinal or mesenteric osmoreceptors are critically involved in the thermogenesis. Alternatively, an energy cost for absorption could account for the thermogenesis. However, it has been shown that intravenous or intragastric administration of nutrient mixtures induced comparable thermogenic responses, suggesting that the energy cost of absorbing nutrients is only minor (Vernet et al. 1986).

In the present study, the osmolality of the duodenojejunal contents ranged between 600 and 800 mosmol kg\(^{-1}\) in rats that had spontaneously ingested normal solid food with their drinking water. This range of osmolality is slightly higher than that of 1.8% NaCl or 10% glucose and thus should stimulate thermogenesis. Rising in duodenal osmolality (peaking at 430 mosmol kg\(^{-1}\)) associated with meals, and persisting for hours, have been reported to occur in pigs (Houpt, 1991). In normal humans, jejunal osmolality reportedly increased to 380 mosmol kg\(^{-1}\) after a meal of milk and doughnuts and the jejunal hypertonic state lasted for 2 h (Ladas et al. 1983). These studies suggest that a rather long period is required to reach osmotic equilibrium between the intestinal contents and the body fluid after a meal, although water is generally considered to move relatively freely in the body. Therefore, it is possible that the intestinal contents after meals can stimulate osmoreceptors, which in turn elicit a thermogenic response (i.e. DIT). This hypothesis accords well with the finding of Bryant et al. (1984), who showed there is an enhancement of the thermogenic response to a meal in rats drinking saline. However, duodenal osmoreceptors have also been reported to be involved in the slowing of gastric emptying (Barker et al. 1978) and in the signalling of satiety (Houpt et al. 1979).

It has been suggested that osmoreceptors in the hepatoporal or mesenteric area (Arsenijevic & Baertschi, 1985; Choi-Kwon & Baertschi, 1991) or in the brain (Mason, 1980; Osaka et al. 1990) are involved in the regulation of vasopressin release from the neurohypophysis. Vasopressin reportedly has vasoconstriction-associated stimulatory effects on O\(_2\) consumption in isolated rat hindlimb preparations (Ye et al. 1995). Accordingly, it is possible that vasopressin partly mediates the osmotically induced thermogenesis. However, the relatively small thermogenic response to intravenous infusion of NaCl suggests that vasopressin did not play a major role in the osmotic thermogenesis observed in the present study, because the increase in plasma osmolality was sufficient to almost maximally stimulate vasopressin release. Further studies will be necessary to elucidate the mechanisms of thermogenesis induced by intestinal infusion of hypertonic solutions. The present study suggests, however, that the intestinal osmotic pressure, but not specific properties of nutrients, is critically involved in the initiation of DIT.


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Role of diabetes, hypertension, and cigarette smoking on atherosclerosis

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ABSTRACT

Hyperosmolar food causes atherosclerosis. Hyperosmolal food hypothesis encompasses all the factors involved under one heading and, that is, the generation of heat in the body. The involvement of cigarette smoking is obvious. High glycemic index food and diabetes result in high levels of blood glucose, which raises the core body temperature. The ingestion of hyperosmolal salt, glucose, and amino acids singularly or synergistically raise the core body temperature, forcing abdominal aorta to form an insulation wall of fatty material causing atherosclerotic plaques. The osmolarity of food, that is glucose, salt, and amino acids is reduced when water is ingested with food. The incidence of atherosclerosis goes down with increasing intake of water.

Key words: Atherosclerosis, cigarette smoking, diabetes, hypertension

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INTRODUCTION

People all over the world suffer from atherosclerosis. Therefore, etiology of the disease should be the same irrespective of geographical location. Mathur[1-3] has described the effect of hyperosmolality of food consumed in the development of atherosclerosis. These articles state that the ingestion of caloric-rich food containing NaCl, amino acids, and glucose raises the core body temperature. As a result, arteries build insulatory layers of fatty material to protect themselves from temperature fluctuations caused by thermogenesis. Similarly, cigarette smoking raises lung temperature and leads to fatty deposits in arteries. Consumption of fat does not cause atherosclerosis because it does not raise core body temperature. Fatty deposits in arteries are not the cause but the manifestation of the disease. Furthermore, being overweight is also not the cause of disease; it just exacerbates the condition. In addition, diabetes mellitus causes atherosclerosis because it causes hyperglycemia, which leads to excessive thermogenesis. Now let us peruse the literature in the light of hyperosmolal food hypothesis.

THERMOGENESIS

Food intake stimulates the metabolic rate of the whole body and increases core body temperature. This phenomenon has been called diet-induced thermogenesis (DIT), postprandial hyperthermia, and thermic effect of food. According to Jequier,[4] the thermic response to amino acids, glucose, and lipids are 30–40%, 6–8%, and 2–3% of energy infused, respectively. In another study, Westerterp[5] showed that alcohol and protein play the biggest role in thermogenesis. Scott and Devore[6] confirmed Westerterp’s findings by demonstrating that 100% as well as 60% protein shakes produce higher DIT as compared with 60% fat, 30% protein, 10% carbohydrate and 60% carbohydrate, 30% protein, 10% fat, shakes. To determine the mechanism of thermogenesis, Osaka et al.[7-9] infused hypertonic solution of glucose, NaCl, fructose, and amino acids in the intestine of urethane-anesthetized rats. A higher core body temperature was observed with increasing amounts of the above-mentioned nutrients. Furthermore, an intravenous injection (IV) of these nutrients also caused thermogenesis accompanied by an increase in plasma osmolality. However, thermogenesis caused by IV was lesser than that caused by the intestinal infusion of NaCl and the solutions of the other above-mentioned nutrients, suggesting an involvement of intestinal osmoreceptors. This further suggests that it is unlikely that IV and intestinal osmotic stimulation induces identical mechanisms of
thermogenesis. However, it does show that an increase in the plasma osmolality, within the physiological range, elicits thermogenesis. The mechanism of thermogenesis is not clear. However, it may involve intestinal osmoreceptors. The authors also found that food intake stimulated the metabolic rate of the whole body and increased the core body temperature. The core body temperature is measured by inserting a thermister in the anus. The skin or cutaneous body temperature is measured by a thermister taped to the lateral surface of a rat’s tail. The mechanism of core and skin temperatures are regulated differently. It is this thermogenesis that is responsible for the generation of atherosclerotic plaque. Furthermore, it is only the core body temperature that plays the main role and not the whole body temperature or the atmospheric temperature. In addition, there is no evidence of this disease being more prevalent in people running high fever for a prolonged period of time or in those living in warm to hot climates.

**HYPEROSMOLAL FOOD**

The ingestion or intravenous infusion of food nutrients raises body temperature. Westerterp stated that the hierarchy in DIT descends from proteins to carbohydrates to fats, which is similar to macronutrient oxidation in postprandial state. Therefore, it seems that fat produces the least thermic response. This was further elucidated by Nagai et al. They found that high fat meals had lower thermic effects than low fat meals.

Osaka et al. confirmed low thermic response of fat by infusing 100% safflower oil into the duodenum of urethane-anesthetized rats. Safflower oil did not produce any thermic response as compared with 5–20% glucose, 2–4% NaCl, 20% fructose, and 5–10% solutions of various amino acids. Furthermore, infusion of 0.9% NaCl or distilled water also did not produce any heat. However, the thermic response increased with an increase in the osmolality of glucose, fructose, amino acids, and NaCl solutions. Similar effects were observed in conscious animals and humans by Osaka and coworkers.

**CONSUMPTION OF WATER**

Amount of consumption of water is the single most important factor in the development of atherosclerosis. It can increase or decrease the osmolarity of food. The United States Department of Agriculture (USDA) recommends a water intake between 1 and 1.5 mL/kcal of energy expenditure, which translates to 2–3 L of water per day. In the United States and other western countries, water is consumed more in the form of beverages rather than pure water. According to the USDA Nationwide Food Consumption Survey of 1977–1978, the median intake of water as such by a person was only 662 mL/day. In similar USDA surveys of 1994–1996 and 1998, drinking water consumption by adult males and females was 600 and 549 mL/day, respectively. These drinking water consumption figures were far below the USDA recommendations of 2–3 L/day.

Chan et al. in a remarkable cohort study discovered a strong negative multivariate association between the intake of water and the risk for fatal coronary artery disease and, in contrast, a positive association between the intake of fluids other than water and the risk for heart diseases. High intake of water (5 or more glasses/day) compared with low intake (2 or fewer glasses/day) were associated with a very high relative risk of 0.46 in men and 0.59 in women. At the same time, a high versus low intake of fluids other than water was associated with a relative risk of 1.46 in men and 2.47 in women. These statistics remained virtually unchanged in multivariate analysis adjusted for age, education, smoking, hypertension, body mass index, and hormone replacement therapy (in women only). Therefore, it is pertinent to look at the per capita consumption of fluids other than water, that is, sweetened beverages that increase the osmolality of food and also cause hyperglycemia. According to a survey between 1994 and 1996 conducted by Wright et al., the mean consumption of soft drinks by adult males and females in the United States were 752 and 595 mL/day, respectively. This survey also shows that children aged between 2 and 5 years drank on average 266 mL of soft drink per day, which surpassed their water intake of 259 mL/day. Similarly, the consumption of soft drinks by people of other age groups was higher than their water intake. This may be one of the reasons for atherosclerotic plaques appearing in children aged 2–5 years. According to a study by Berenson et al., all persons in the age group between 2 and 39 years had fatty streaks in the aorta. This study states that the prevalence of fatty streaks in coronary arteries increases with age from approximately 50% at 2–15 years of age to 85% at 21–39 years (P = 0.01). The statistics are alarming because 50% of all the children in the United States are already afflicted with the disease by the age of 15 years.

**THE ROLE OF HYPERGLYCEMIA, HIGH GLYCEMIC INDEX FOOD, AND DIABETES MELLITUS**

Ingestion of high glycemic index food creates postprandial hyperglycemia and creates physiologically a diabetic-like
condition in experimental animals.[19] Coutinho et al.[24] stated that postprandial hyperglycemia is an important risk factor for cardiovascular diseases not only among patients with diabetes, but also among the general population. Dickinson and Brand-Miller[21] stated that several lines of evidence indicate that exaggerated postprandial glycemia puts individuals even without diabetes at greater risk of developing cardiovascular disease. Furthermore, Ceriello[20] and de Vegt et al.[23] stated that a high 2-hour postprandial glucose was independently associated with all-cause and cardiovascular mortality in a population even without diabetes.

Renard et al.[24] stated that diabetes causes atherosclerotic lesions regardless of diet. They also discovered that diabetic mice had significantly higher cholesterol on a cholesterol-free diet as compared with nondiabetic mice, which could be attributed only to hyperglycemia in diabetic mice. Therefore, this study suggests that the synthesis of cholesterol is endogenous because it is not coming from the diet. In another study using an animal model, Kunjathoor et al.[25] showed that hyperglycemia and not hyperinsulinemia is responsible for the development of atherosclerosis. Therefore, both diabetes mellitus and high glycemic index food can increase blood sugar and cause excessive thermogenesis.

Sartippour and coworkers[26,27] stated that lipoprotein lipase (LPL) produced by macrophages in vascular walls may favor the development of atherosclerosis by promoting lipid accumulation within the atherosclerotic lesion. They demonstrated that high glucose concentration stimulated in vitro murine and human microphage LPL production. They measured macrophage LPL mRNA expression, immunoreactive mass, and activity in normotriglyceridemic subjects and patients with type 2 diabetes. Monocytes isolated from healthy control subjects and patients with type 2 diabetes were differentiated into macrophages in RPMI media containing 20% autologous serum. After culturing for 5 days in diabetic sera, macrophage LPL mRNA expression increased significantly as compared with its expression in control subjects. Differentiation of macrophages of diabetic patients in sera obtained from control subjects significantly reduced these anomalies. Conversely, culturing macrophages of control subjects in sera of diabetic patients significantly increased the LPL mass, and its activity in these cells. The authors concluded that diabetes may contribute to the development of atherosclerosis.

**CONSUMPTION OF SODIUM CHLORIDE**

A positive correlation between salt intake and cardiovascular diseases has been known to exist for a long time. Menton et al.[28] stated that epidemiologic, migration, intervention, and genetic studies in humans and animals provide very strong evidence of a causal link between high salt intake and high blood pressure. Furthermore, Miura and Nakagawa[29] stated that a reduction in salt intake remarkably decreased blood pressure in the elderly, the middle-aged, and the younger generation in Japan. It is also known that obesity and diabetes mellitus increase a patient’s risk for stroke. The risk for atherosclerosis is even higher when a diabetic patient has high blood pressure. The most plausible explanation again is that both NaCl and glucose in blood synergistically raise both osmolality of blood and core body temperature, resulting in atherosclerotic plaque formation. Because hypertension is a major risk factor for atherosclerosis, Ketonen et al.[30] tested whether high salt intake would aggravate endothelial dysfunction and promote atherosclerosis in apolipoprotein E-deficient mice (ApoE(-/-) mice) and their littermate controls. Their findings suggest a detrimental role of high salt (7%) intake in the development of atherosclerosis and underscore the importance of increased oxidative stress in the pathogenesis of salt-induced vascular damage. Similarly, Weiss and Taylor[31] tested whether atherosclerosis was increased in the setting of a low renin model of hypertension. They observed a dramatic increase in the atherosclerotic lesion areas in the setting of either a low- or high-fat diet. In the hypertensive animals, they observed an increase in angiotensin II staining that was localized to adventitial macrophages. The increase in atherosclerosis was inhibited by the administration of an angiotensin receptor antagonist, an angiotensin-converting enzyme inhibitor, or a renin inhibitor. These data suggest that even in the setting of hypertension, which is not associated with the activation of the systemic renin–angiotensin system, local generation of angiotensin II within the arterial walls may be of pathophysiological relevance to the development of atherosclerosis.

**CONSUMPTION OF PROTEINS, FATS, AND CARBOHYDRATES**

Contrary to current theories, high fat diet does not cause atherosclerosis. It is further substantiated by French paradox.[32] In France, there is a high intake of saturated fat but low mortality from coronary heart disease. It is high carbohydrate and high protein intake that leads to atherosclerosis. In addition, Karst et al.[33] have shown that in DIT, protein was at least 3 times as large a thermic contributor as isocaloric carbohydrate supply. These investigators also discovered that dietary fats produced no
Mathur: Role of diabetes, hypertension, and cigarette smoking on atherosclerosis

evident thermic response. The doubling of energy from either casein or hydrolyzed starch led to an approximate doubling of thermic effect. Kurowska and Carroll[34] have shown that in rabbits, the elevation of cholesterol is produced by feeding a cholesterol-free, semi-purified diet containing 30% casein amino acid mixture or 14.7% casein amino acid mixture, which corresponds to a normal level of dietary protein. Therefore, a diet high in salt, protein, and carbohydrate will maintain an elevated core body temperature and lead to atherosclerosis plaque formation.

CIGARETTE SMOKING

The association between long-term cigarette smoking and coronary artery disease is well established. Furthermore, diabetics who smoke develop more severe cardiovascular diseases early in life. In cigarette smoking, lungs inhale hot smoke because at the time of puff, the temperature at the tip of the cigarette is around 950°C.[35] The smoke also carries numerous chemicals that adversely affect the elasticity of lungs and the hot smoke raises the lung temperature and in turn raises the core body temperature. The lungs become incapable of performing one of their vital physiological functions, that is, cooling or removing the heat from the body. Karim et al.[36] have illustrated that smoking is associated with subclinical atherosclerosis in diabetics and interacts with the duration of diabetes to accentuate atherosclerosis. The association between carotid intima-media thickness and the duration of diabetes increases with both the frequency and duration of smoking. The examination of the Framingham Heart Study by Wolf et al.[31] revealed that regardless of smoking status and sex, hypertensive subjects had twice the incidence of stroke. The report further states that after cessation of cigarette smoking, the risk significantly dropped in 2 years and was at the level of nonsmokers in 5 years. This study suggests that the harmful effect of smoking is reversible as far as the stroke is concerned.

TEST OF HYPEROSMOLAL FOOD HYPOTHESIS

Okinawa centenarians[38] come closest to getting a perfect score on hyperosmolal food hypothesis test. Okinawa centenarians consume food that has very high water content, such as cereals, roots, beans, fish, vegetables, and others. Their beverage is mainly tea, which is close to 99% water. However, they consume about 4 g of NaCl per day, which is too high. In spite of this, they are least affected by cardiovascular diseases as compared with the rest of the world.

CONCLUSION

It is well known that diabetes, excessive salt intake, obesity, and a host of other factors lead to atherosclerosis. However, till now no general theory existed that could explain the involvement of all the above factors in the development of atherosclerosis, but now hyperosmolal food hypothesis explains the etiology of the disease remarkably well. Thus, only this hypothesis can explain the development of atherosclerosis all over the world. The details of hyperosmolal food hypothesis have been described elsewhere.[31]

FUTURE PERSPECTIVE

In perspective, research on atherosclerosis currently is in disarray. The scientists require a paradigm shift in their thinking. We already know that fat intake does not cause atherosclerosis. Lowering blood cholesterol level does not protect people from becoming a prey to the disease. People on low fat diet are also not spared from the disease because the human body is capable of synthesizing cholesterol if needed by the body for insulation from heat. Thus, they too get heart attacks anyway. Furthermore, patients after coronary artery bypass graft (CABG) surgery relapse 50% of the time because the atherosclerotic plaque continues to build up in the grafted arteries and elsewhere. Patients are advised to stay away from fatty foods, which obviously does not help because fatty meal is not the cause for atherosclerosis. Therefore, the researchers should first examine the cause of the disease before trying to cure it; otherwise, we will be treating symptoms rather than curing the disease itself. Likewise, research foundations should increase funding on causal analyses and reduce financial grants on finding cures at this time.

However, there is some good news. The rate of cardiovascular diseases is slowing down, but it is not because of statins or CABG surgery. It is because of the advent of bottled mineral water. Luckily, it has become fashionable to carry a bottle of mineral water anywhere you go. It is this water consumption, which is diluting the hyperosmolar foods we eat.

Finally, this field requires some broad theories and hypotheses explaining the involvement of foods, diabetes, hypertension, cigarette smoking, and others in the
formation of atherosclerotic plaque. We have a mission but are lacking the vision. That is why we have not made any progress even though we have worked on it for more than 50 years.

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