Thermic effect of food in hypothyroid rats

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Abstract

The regulatory and obligatory components of cephalic and gastrointestinal phases of the thermic effect of food (TEF) were measured in control and hypothyroid rats. A significant decrease ($P<0·05$) in regulatory and obligatory components of cephalic and gastrointestinal TEF, after either a control or energy-dense meal, was found in hypothyroid rats compared with control rats. Our findings indicate that

hypothyroidism is associated with a decreased thermogenic response to food which contributes to the reduced energy expenditure of hypothyroid rats. Our results also suggest that tri-iodothyronine is involved in the regulation of postprandial thermogenesis directly as well as through its influence on β-adrenergic response and insulin release.

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Introduction

It is well known that the acute increase in metabolic rate which occurs after a meal, the thermic effect of food (TEF), has two distinct phases. The initial cephalic phase lasts about 30 min and is induced by sensory stimulations (Diamond *et al.* 1985, Allard & LeBlanc 1988, LeBlanc 1991), the subsequent gastrointestinal phase is explained by the composition and the caloric content of the ingested nutrients (LeBlanc & Diamond 1986). We have shown recently that in rats, as well as in dogs (Diamond & LeBlanc 1987), the phases of TEF consist of two components: a regulatory component which is under the control of the sympathetic nervous system (SNS) as it is abolished by propranolol (Liverini *et al.* 1995), and an obligatory component not affected by propranolol (Liverini *et al.* 1995). We have also shown that the regulatory component can play a role in preventing the development of obesity (Liverini *et al.* 1995).

Previous studies have demonstrated that TEF is related to pre-existing levels of thermogenesis. In fact, rats with high levels of thermogenesis, such as cold-adapted, hyperthyroid and hyperphagic rats, exhibit an increase in TEF (Rothwell *et al.* 1982a, 1983, Rothwell & Stock 1983). On the other hand, fasting or obese rats which exhibit low levels of thermogenesis show a reduced TEF (Rothwell *et al.* 1981, 1982a, Rothwell & Stock 1983). Since low levels of thermogenesis are also characteristic of the hypothyroid state (Donner Denkla & Marcum 1973, Guernsey & Edelman 1983, Hayashi & Nagasaka 1983), it was of interest to verify whether changes in TEF could contribute to the reduced energy expenditure of hypothyroid rats. To this end we have measured the regulatory and obligatory components of the

cephalic and gastrointestinal phases of TEF in hypothyroid rats.

Materials and Methods

Animals and experimental design

Forty male Wistar rats (Charles River, Calco, Italy), weighing 60 ± 2 g, were used for the experiments. Half of the rats were made hypothyroid by administration of a 0·1% solution of propylthiouracil (PTU) in drinking water for 27 days; the remainder were used as controls. All rats were given free access to a control diet and water, and were maintained one per cage (in grid-bottomed cages) at 24 °C under an artificial circadian 12 h light:12 h darkness cycle. Food intake (corrected for spillage) and body weight were measured daily. Animal care, housing and killing met the guidelines of the Italian Health Ministry.

Oxygen consumption measurements were performed as previously described (Iossa *et al.* 1992, Liverini *et al.* 1992). Briefly, oxygen consumption was measured with an oxygen consumption monitor (Columbus Instruments, Columbus, Ohio, USA) in a chamber at 24 °C. All rats were allowed to adapt to the conditions for a minimum of 30 min before beginning the measurements. Values were taken only when the animals were resting.

Cephalic and gastrointestinal phases of TEF were measured by using two test meals, one composed of a control diet (29% protein, 10·6% lipid and 60·4% carbohydrate, J/100 J; 12·5 kJ metabolizable energy/g) and the other composed of an energy-dense diet (28% control diet, 39·5% lyophilized meat, 17·8% butter, 12% alphacel, 0·7% AIN vitamin mix, 2% AIN mineral mix, g/100 g; composition=29% protein, 50% lipid and 21% carbohydrate,

J/100 J, 15·8 kJ metabolizable energy/g). The latter diet is characterized by a higher caloric content and by the presence of a meat component which is among the flavours most preferred by rats (Naim *et al.* 1985). It was chosen because of its ability to stimulate SNS activity after both acute (Liverini *et al.* 1995) and chronic (Liverini *et al.* 1994) administration.

Measurement of total TEF

On the 27th day the rats were starved for 16 h from 1700 h; at the end of the fasting period, rats were injected with saline, the fasting resting metabolic rate (RMR) was measured and the values obtained served as baseline values in the calculation of total TEF.

Cephalic TEF was measured in five control and five hypothyroid rats by giving them a small portion (7 kJ; 0·56 g) of a test meal composed of the control diet, while five control and five hypothyroid rats were given a small portion (7 kJ; 0·44 g) of a test meal composed of the energy-dense diet. We used very small meals (7 kJ) to minimize TEF due to digestion, absorption and storage of food. To avoid the possibility that part of the increase in oxygen consumption could be due to the movements of the rats during eating, the measurements started after the rats had completely eaten the test meal. This was possible because the amount of food given was very small (about 0·5 g): it took less than 2 min to be ingested and then the rats rapidly resumed the initial resting daytime position. After the end of the meal, oxygen consumption was continuously monitored for 30 min. The integrated increase in the 30-min period was calculated using the trapezoid method.

Gastrointestinal TEF was measured in five control and five hypothyroid rats by giving them a larger portion of the control meal (35 kJ; 2·8 g), while five control and five hypothyroid rats were given a larger portion of the energy-dense meal (35 kJ; 2·2 g). The rats ate the food in about 10 min. Oxygen consumption was measured every 10 min for 180 min after meal consumption (values over 2 min were taken only when the animals were resting). The integrated increase was calculated using the trapezoid method.

Measurement of obligatory TEF

Three days later, the rats were again starved for 16 h from 1700 h; at the end of the fasting period the rats were injected with propranolol (2 mg/100 g body weight), the fasting RMR was measured and the values obtained served as baseline values in the calculation of the obligatory component of cephalic and gastrointestinal TEF. The measurements were repeated as described above.

Serum thyroid hormone levels

At the end of TEF measurements, rats were anesthetized by i.p. injection of chloral hydrate (40 mg/100 g body

weight), blood was collected via the abdominal aorta and the thyroid state of the animals was monitored by measuring serum-free tri-iodothyronine (T_3) and tetra-iodothyronine (T_4) levels according to the method of Romelli *et al.* (1978).

Statistics

Data are given as means ± s.e.m. of five different rats. Statistical significance between the means was examined by multi-way analysis of variance (only for main effects) followed by two-tailed Student's *t*-test. Probability values less than 0·05 were considered to indicate a significant difference.

Materials

DL-Propranolol was purchased from Sigma Chemical Co., St Louis, MO, USA. Alphacel, AIN mineral and vitamin mix were purchased from ICN Pharmaceuticals Inc., Costa Mesa, CA, USA. Lyophilized meat (Liomellin, Star s.p.a., Milano, Italy) and butter (Lurpak, Denmark) were purchased locally.
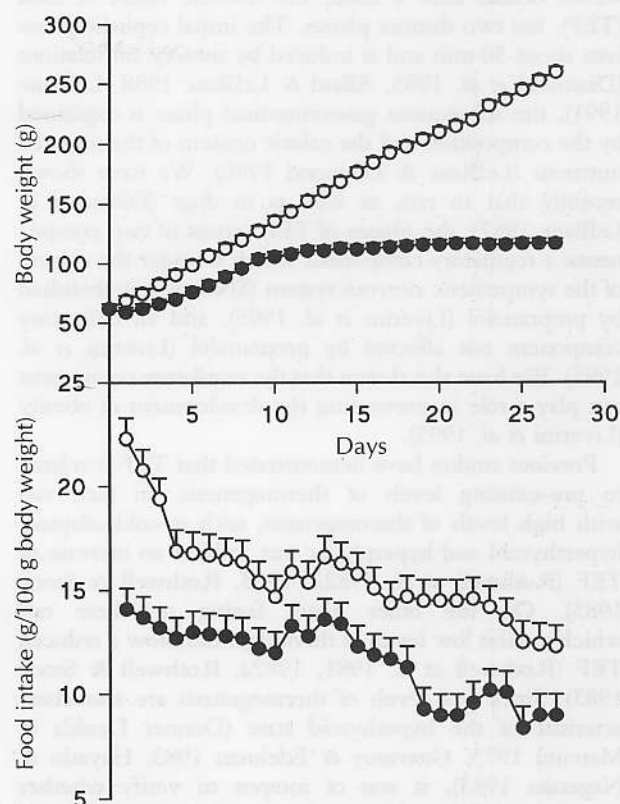

Figure 1 Body weight gain and food intake in control (○) and hypothyroid (●) rats. The values are reported as means+s.e.m. of twenty different rats. Values of hypothyroid rats are significantly ($P<0·05$) different compared with those of control rats.
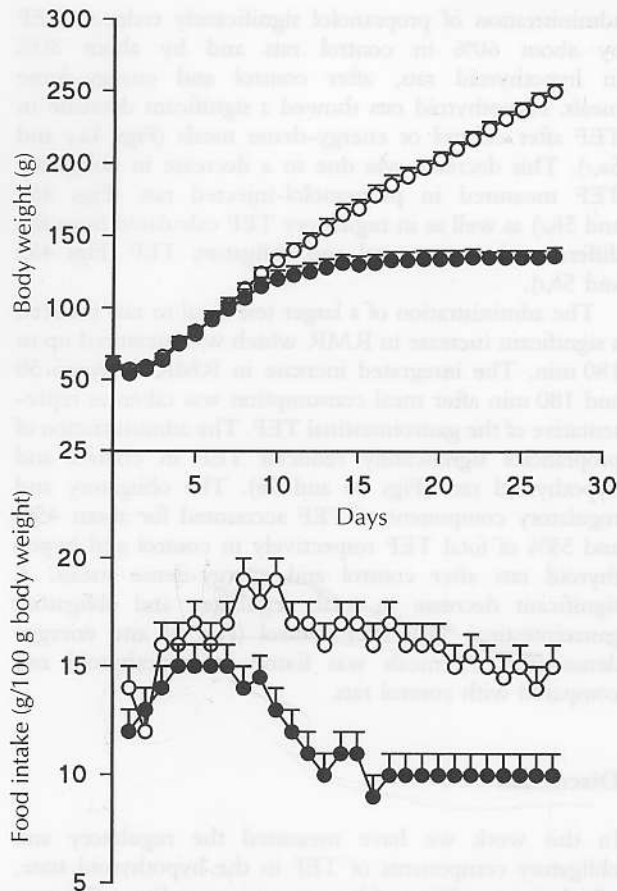
Figure 2 Body weight gain and food intake in PTU+saline-treated (●) and PTU+T$_3$-treated (○) rats. PTU+saline-treated rats were given PTU (0·1% in drinking water) and daily intraperitoneal injections of saline, while PTU+T$_3$-treated rats were given PTU (0·1% in drinking water) and daily intraperitoneal injections of T$_3$ (500 ng/100 g body weight) for 27 days. The values are reported as means+S.E.M. of five different rats. Values of food intake of PTU+T$_3$-treated rats are significantly (P<0·05) different compared with those of PTU+saline-treated rats from the eighth day onwards.

Figure 3 Fasting resting metabolic rate (RMR) in control (open bars) and hypothyroid (hatched bars) rats after saline or propranolol injection. Rats were fasted for 16 h from 1700 h; at the end of the fasting period they were injected with either saline or propranolol and the fasting RMR was measured. The values are reported as means±S.E.M. of twenty different rats. *P<0·05 compared with control rats. **P<0·05 compared with saline-injected hypothyroid rats.

Results

To test the influence of thyroid hormones on TEF, hypothyroidism was induced in rats by treatment with PTU. The hypothyroid state of PTU–treated rats was confirmed by the measurements of serum-free T$_3$ and T$_4$ which were 4·0±0·2 and 11±1 ng/l respectively in control rats and were below the limit of detection in hypothyroid rats.

Body weight gain and food intake in control and hypothyroid rats are shown in Fig. 1. Control rats gained weight at a constant rate during the experimental period, while hypothyroid rats gained weight slowly during the first ten days and then stopped growing. Food intake/ 100 g body weight was also significantly lower in
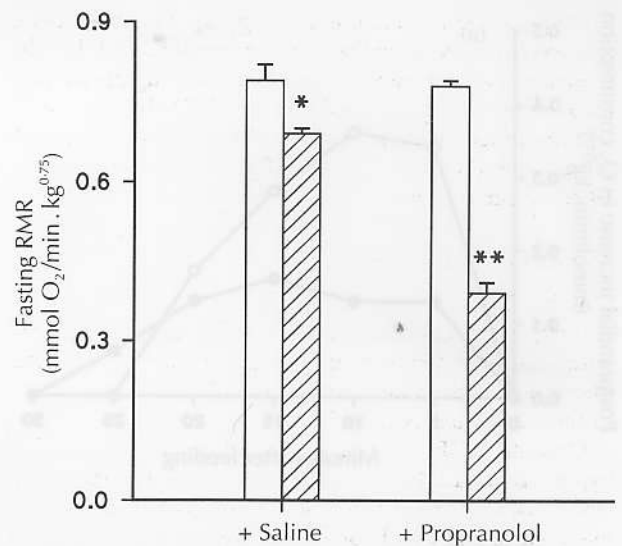
hypothyroid rats compared with control rats. To determine if the decrease in food intake was due to a direct action of PTU or to the decrease in serum thyroid hormone levels, rats were treated with PTU plus a replacement dose of T$_3$ (500 ng/100 g body weight daily) or PTU plus saline for 27 days. Serum-free T$_3$ levels of PTU+T$_3$-treated rats were 3·0±0·1 ng/l. The results show that PTU exerts a direct action on food intake only in the first days of treatment (Fig. 2). In fact, starting from about the eighth day of treatment, PTU+T$_3$-treated rats ate and gained weight to a greater extent than PTU+ saline-treated rats and to a similar extent as control rats (Figs 1 and 2). We think that food intake was lower in the first days of PTU treatment because of the bitter taste of PTU-supplemented water. Thus the rats drink and eat less until they get used to the taste of PTU.

Fasting RMR measurements in control and hypothyroid rats after the injection of either saline or propranolol are shown in Fig. 3. In saline-injected rats, fasting RMR was 12% lower in hypothyroid rats compared with control rats; propranolol injection caused a significant decrease in fasting RMR (−43%) in hypothyroid rats but it was without effect in control rats.

The results of the determination of cephalic TEF in control and hypothyroid rats after the control or energy-dense meals (Figs 4 and 5) show a rapid increase in RMR that reaches a peak value after about 10 min and returns to the prefeeding level after about 30 min. The
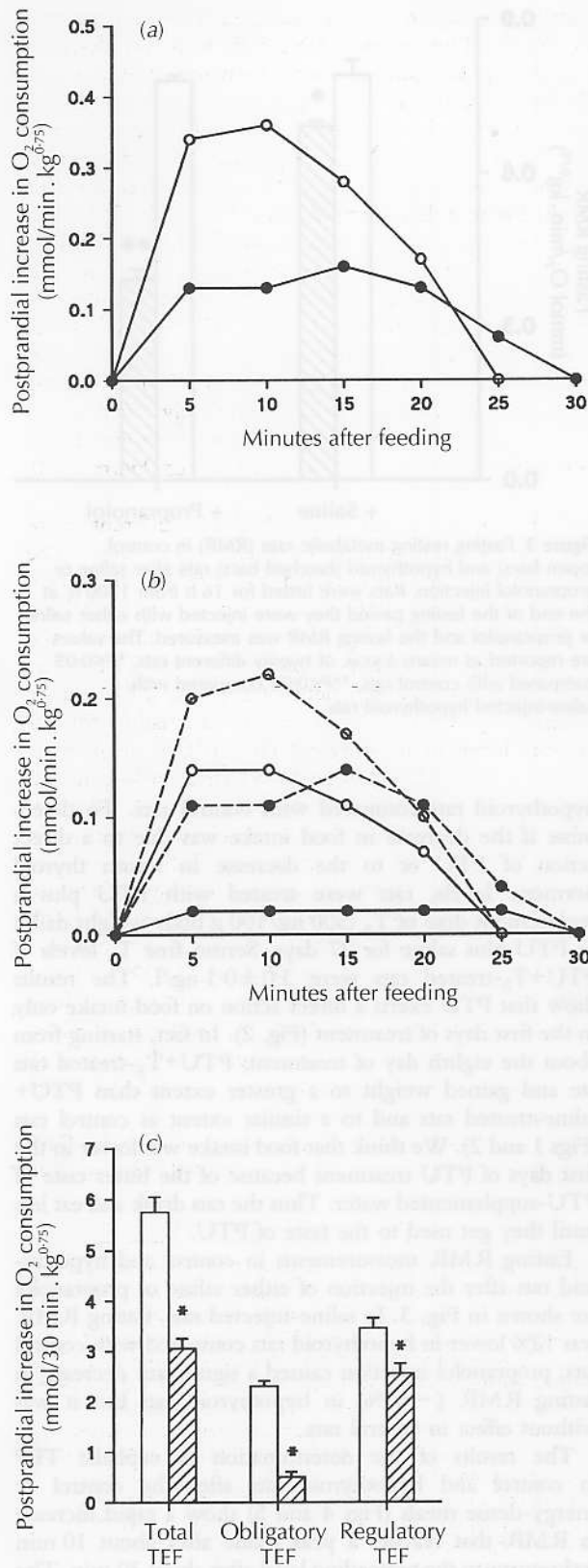
administration of propranolol significantly reduced TEF by about 60% in control rats and by about 80% in hypothyroid rats, after control and energy-dense meals. Hypothyroid rats showed a significant decrease in TEF after control or energy-dense meals (Figs 4a,c and 5a,c). This decrease was due to a decrease in obligatory TEF measured in propranolol-injected rats (Figs 4b,c and 5b,c) as well as in regulatory TEF calculated from the difference between total and obligatory TEF (Figs 4b,c and 5b,c).
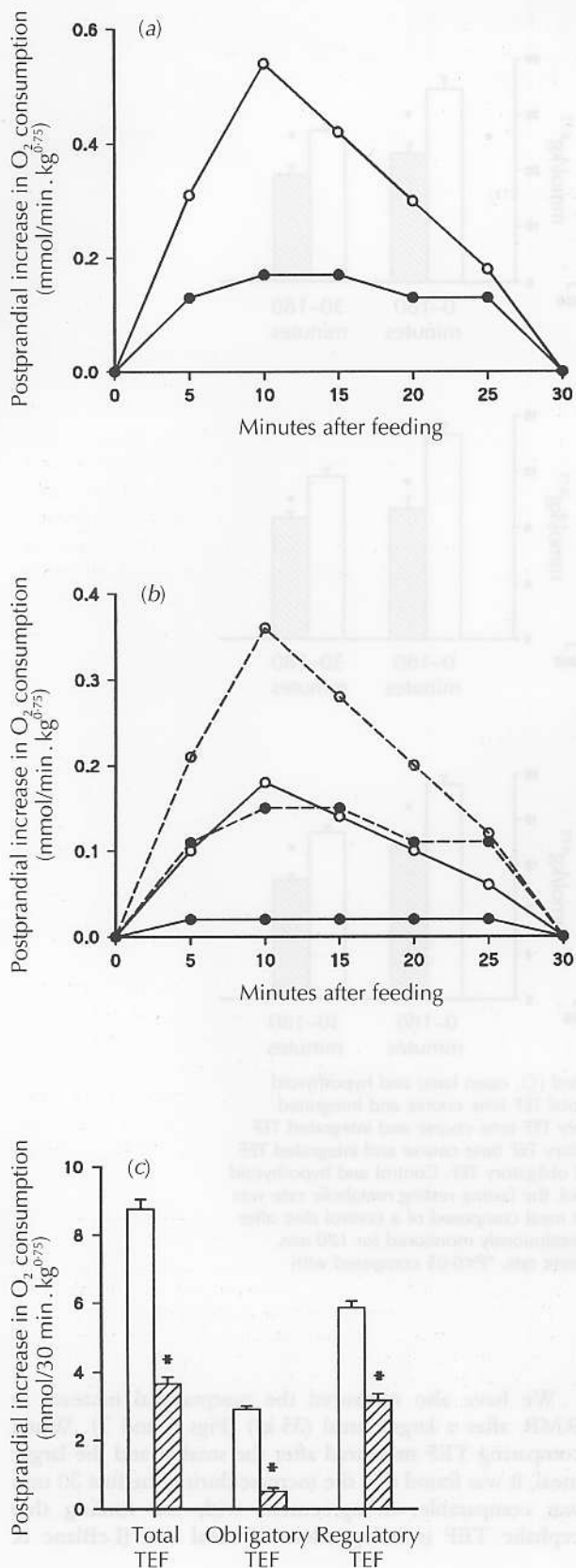
The administration of a larger test meal to rats induced a significant increase in RMR which was measured up to 180 min. The integrated increase in RMR between 30 and 180 min after meal consumption was taken as representative of the gastrointestinal TEF. The administration of propranolol significantly reduced TEF in control and hypothyroid rats (Figs 6b and 7b). The obligatory and regulatory component of TEF accounted for about 45% and 55% of total TEF respectively in control and hypothyroid rats after control and energy-dense meals. A significant decrease in total, regulatory and obligatory gastrointestinal TEF after control (Fig. 6) and energy-dense (Fig. 7) meals was found in hypothyroid rats compared with control rats.

Discussion

In this work we have measured the regulatory and obligatory components of TEF in the hypothyroid state, which is a condition of low energy expenditure (Donner Denkla & Marcum 1973, Guernsey & Edelman 1983, Hayashi & Nagasaka 1983), and we have found that fasting RMR (Fig. 3), as well as food intake and body weight gain (Fig. 1), decreased in hypothyroid rats.

We have also measured fasting RMR after the injection of the β-blocking agent propranolol both in control and in hypothyroid rats (Fig. 3). The results show that there is a significant decrease in fasting RMR only in hypothyroid rats. This finding confirms previous observations that hypothyroid rats show an increased activity of the SNS (Henley *et al.* 1991, Silva & Landsberg 1991), which

Figure 4 Cephalic thermic effect of food (TEF) in control (○, open bars) and hypothyroid (●, hatched bars) rats after a control meal. (a) Total TEF time course measured in saline-injected rats. (b) Obligatory TEF time course measured in propranolol-injected rats (unbroken lines) and regulatory TEF time course calculated from the difference between total and obligatory TEF (broken lines). (c) Integrated TEF calculated using the trapezoid method. Control and hypothyroid rats were injected with either saline or propranolol, the fasting resting metabolic rate was measured and then the rats were fed a 7 kJ test meal composed of the control diet; after the end of the meal, oxygen consumption was continuously monitored for 30 min. Values are reported as means ± S.E.M. of five different rats. *P<0·05 compared with control rats.

contributes by about 50% to fasting RMR in hypothyroid rats (Fig. 3). The increased SNS activity is considered to be an attempt to maintain normal body temperature (Silva 1993), expecially in rats kept at room temperature (24 °C) as in this study. In agreement with this, several authors have found that brown adipose tissue, the main site of facultative thermogenesis, shows some characteristics of its recruited state in hypothyroid rats (Mory *et al.* 1981, Park *et al.* 1989, Dicker *et al.* 1992). However, hypothyroidism induces a reduced tissue responsiveness to SNS stimuli (Dicker *et al.* 1992) due to a reduced number of β-receptors (Bilezikian & Loeb 1983) as well as to post-receptor defects (Dimitriadis *et al.* 1991, Pracyk & Slotkin 1992, Wahrenberg *et al.* 1994). Therefore, the increased SNS activity is not completely effective in maintaining normal RMR (Fig. 3) as well as normal core temperature (Park *et al.* 1989, Abelenda & Puerta 1990, Henley *et al.* 1991).

We measured cephalic TEF after two different meals composed of either control or energy-dense diets. The results show that hypothyroidism elicits a significant decrease in cephalic TEF whatever the meal (Figs 4 and 5). This decrease is due to a reduction in both the obligatory and regulatory components of TEF. The reduced obligatory component of cephalic TEF can be explained by the virtual absence of circulating thyroid hormone levels in hypothyroid rats. In fact, an increase in plasma T_3 levels has been found after feeding in rats (Rothwell *et al.* 1982*b*) and pigs (Dauncey & Morovat 1993), although the extent to which the increase in thyroid hormone could contribute to TEF is at present unclear. It has also been shown that feeding induces an early plasma insulin increase (de Jong *et al.* 1977, Diamond & LeBlanc 1987), which in turn stimulates SNS activity (Diamond & LeBlanc 1988). Since hypothyroidism is characterized by a reduction in both plasma insulin levels and peak plasma insulin response to glucose load (Loeb 1986), the decreased obligatory TEF could also be due to an impairment in insulin response to feeding.

The reduced regulatory component of cephalic TEF could be due to the decreased tissue responsiveness to SNS

Figure 5 Cephalic thermic effect of food (TEF) in control (○, open bars) and hypothyroid (●, hatched bars) rats after an energy-dense meal. (*a*) Total TEF time course measured in saline-injected rats. (*b*) Obligatory TEF time course measured in propranolol-injected rats (unbroken lines) and regulatory TEF time course calculated from the difference between total and obligatory TEF (broken lines). (*c*) Integrated TEF calculated using the trapezoid method. Control and hypothyroid rats were injected with either saline or propranolol, the fasting resting metabolic rate was measured and then the rats were fed a 7 kJ test meal composed of an energy-dense diet; after the end of the meal, oxygen consumption was continuously monitored for 30 min. Values are reported as means ± S.E.M. of five different rats. *$P < 0.05$ compared with control rats.
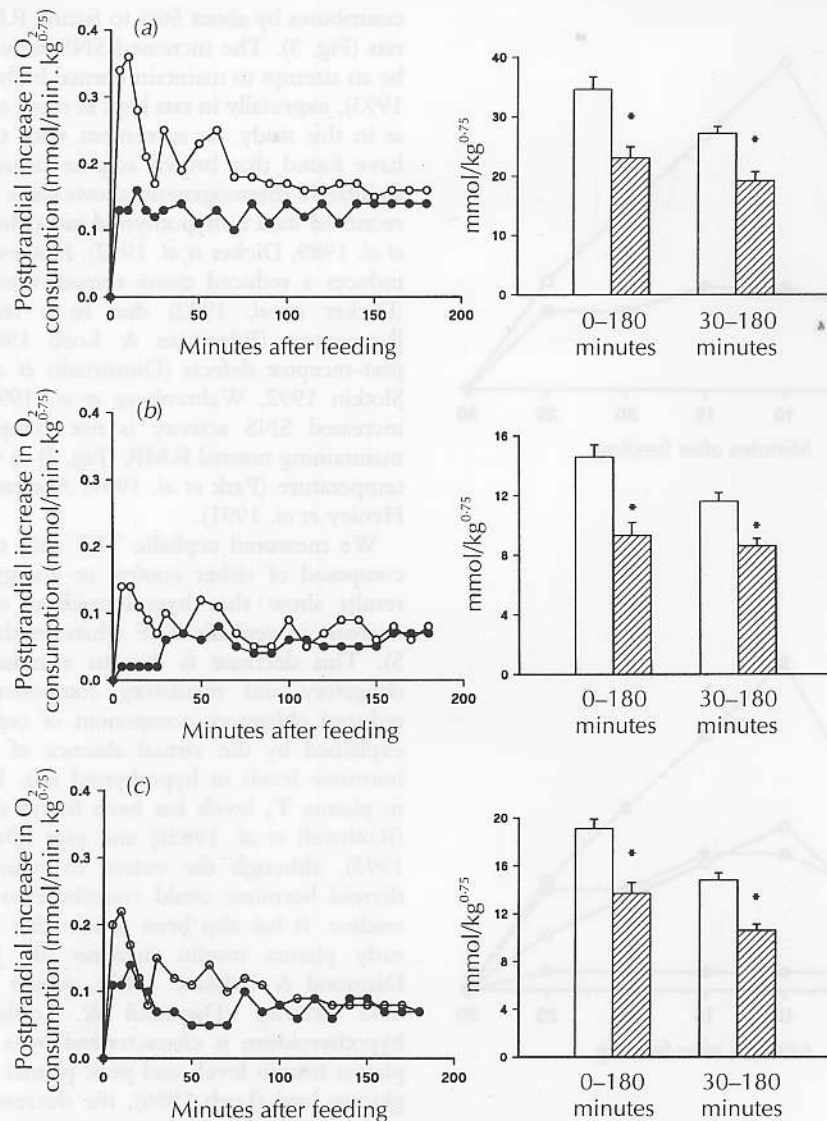
Figure 6 The thermic effect of food (TEF) in control (○, open bars) and hypothyroid (●, hatched bars) rats after a control meal. (a) Total TEF time course and integrated TEF measured in saline-injected rats. (b) Obligatory TEF time course and integrated TEF measured in propranolol-injected rats. (c) Regulatory TEF time course and integrated TEF calculated from the difference between total and obligatory TEF. Control and hypothyroid rats were injected with either saline or propranolol, the fasting resting metabolic rate was measured and then the rats were fed a 35 kJ test meal composed of a control diet; after the end of the meal, oxygen consumption was continuously monitored for 180 min. Values are reported as means ± S.E.M. of five different rats. *$P<0.05$ compared with control rats.

found in hypothyroid rats (Dicker *et al.* 1992), as well as to a lower SNS stimulation caused by the impaired insulin response. It should be noted that hypothyroidism also abolishes the increase in the regulatory component of cephalic TEF induced in control rats by an energy-dense meal (Fig. 5).

We have also measured the postprandial increase in RMR after a larger meal (35 kJ) (Figs 6 and 7). When comparing TEF measured after the smaller and the larger meal, it was found that the increase during the first 30 min was comparable, in agreement with the finding that cephalic TEF is independent of meal size (LeBlanc &
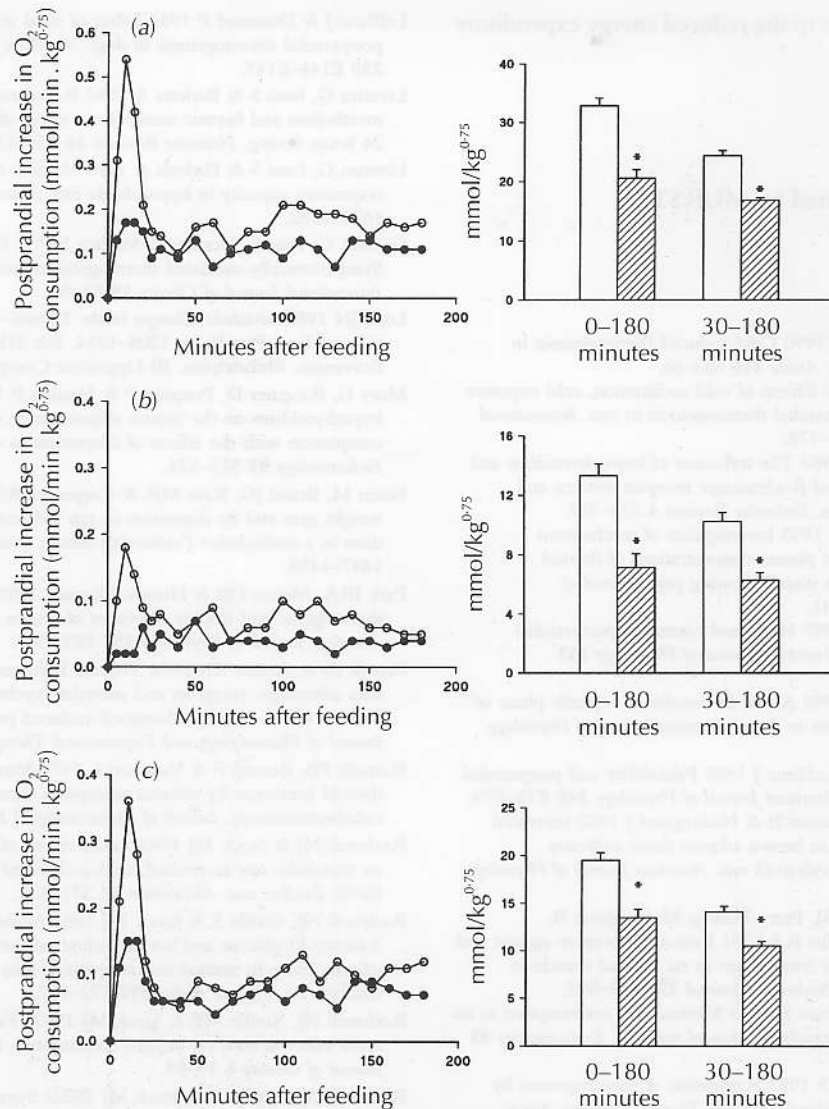
Figure 7 The thermic effect of food (TEF) in control (○, open bars) and hypothyroid (●, hatched bars) rats after an energy-dense meal. (a) Total TEF time course and integrated TEF measured in saline-injected rats. (b) Obligatory TEF time course and integrated TEF measured in propranolol-injected rats. (c) Regulatory TEF time course and integrated TEF calculated from the difference between total and obligatory TEF. Control and hypothyroid rats were injected with either saline or propranolol, the fasting resting metabolic rate was measured and then the rats were fed a 35 kJ test meal composed of an energy-dense diet; after the end of the meal, oxygen consumption was continuously monitored for 30 min. Values are reported as means ± S.E.M. of five different rats. *P<0·05 compared with control rats.

Diamond 1986, Allard & LeBlanc 1988). The integrated postprandial increase in RMR between 30 and 180 min after the larger meal was taken as representative of the gastrointestinal TEF. The results show that hypothyroidism significantly decreases both the regulatory and the obligatory component whatever the meal. The above decrease cannot be attributed to changes in food

absorption since preliminary experiments have shown that food absorption is not reduced in hypothyroid rats. However, we cannot exclude the possibility that the decreased TEF found in hypothyroid rats could be due, at least in part, to delayed food absorption.

In conclusion, our findings indicate that hypothyroidism is associated with a decreased thermogenic response to

food which contributes to the reduced energy expenditure of hypothyroid rats.

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