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Effect of a high-fat diet on energy balance and thermic effect of food in hypothyroid rats

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Abstract

We have carried out measurements of energy balance in hypothyroid rats fed a low-fat or a high-fat diet for eighteen days. We have also measured cephalic and processing thermic effect of food (TEF) after a low-fat or a high-fat meal.

Body lipid gain, carcass lipid content and gross efficiency were significantly ($P < 0{\cdot}05$) higher in hypothyroid rats fed a high-fat diet compared with hypothyroid rats fed a low-fat diet, while metabolizable energy intake and energy expenditure remained unchanged. Cephalic TEF after a low-fat meal was significantly ($P < 0{\cdot}05$) lower in hypothyroid rats fed a high-fat diet compared with hypothyroid rats fed a low-fat diet, while it was significantly ($P < 0{\cdot}05$) higher after a high-fat meal than after a low-fat meal in hypothyroid rats fed a high-fat diet. No significant variation was found in processing TEF after a low-fat or a high-fat meal.

Our results indicate that hypothyroid rats are unable to develop increased energy expenditure and increased TEF in response to a high-fat diet.

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Introduction

It is well known that thyroid hormones regulate energy metabolism: hyperthyroidism results in large increases in metabolic rate (1, 2), whereas hypothyroidism causes a decrease in energy expenditure (3, 4). Thyroid hormones have also been involved in the thermogenic response to hyperphagia and food ingestion. In fact, a significant increase in thyroid hormone serum levels occurs in hyperphagic rats exhibiting diet-induced thermogenesis (DIT) (5, 6), as well as after food ingestion in rats (7) and pigs (8). We have previously shown that rats fed a high-fat diet, which exhibit high circulating tri-iodothyronine (T_3) serum levels (5), overeat but resist becoming obese through a sympathetically-mediated increase in energy expenditure (5, 9). The above increase in energy expenditure is due not only to a chronic increase in fasting resting metabolic rate (RMR), but also to an increase in the acute thermic effect of food (TEF) (10). On the other hand, we have found that hypothyroid rats show a decrease in both RMR and TEF (11).

In view of these observations, it was considered of interest to investigate whether the administration of a high-fat diet to hypothyroid rats was able to induce adaptive responses capable of preventing excess energy deposition as it does in euthyroid rats (5, 9, 10). To this purpose, we have carried out energy balance measurements in hypothyroid rats fed a low-fat or a high-fat diet. In addition, we have measured cephalic and processing TEF after a low-fat or a high-fat meal.

Materials and methods

Animals and experimental design

Forty-five male Wistar rats (Charles River, Calco, Como, Italy), weighing 60 ± 2 g (about 27 days of age), were made hypothyroid by administration of propylthiouracil (PTU, 0·1% in drinking water) for 12 days. On day 13, five rats (B_0 rats) were killed for the determination of the initial carcass energy content and serum free T_3 (12) using a radioimmunoassay kit, while PTU treatment continued in the other forty rats. Twenty rats were used as controls and were fed a low-fat diet for eighteen days (Mucedola 4RF21, Settimo Milanese, Milan, Italy; percentage of total metabolizable energy: 29 protein, 10·6 lipid and 60·4 carbohydrate, J/J; 15·88 kJ gross energy/g). The other twenty rats were fed a high-fat diet for eighteen days (28% stock diet, 39·5% lyophilized meat, 17·8% butter, 12% alphacel, 0·7% AIN vitamin mix, 2% AIN mineral mix, g/g; percentage of total metabolizable energy: 29 protein, 50 lipid, 21 carbohydrate, J/J; 19·85 kJ gross energy/g). The latter diet is characterized by a higher fat content and by the presence of a meat component which is among the flavours most preferred by rats (13); it was chosen because of its ability to stimulate sympathetic nervous system activity after both acute (10) and chronic (5, 9) administration. All rats were given free access to food 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. Animal care, housing and killing met the guidelines of the Italian Health Ministry.

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.

Measurement of TEF

Cephalic and processing phases of TEF were measured by using two test meals, one composed of the low-fat diet and the other composed of the high-fat diet.

Total TEF After 15 days of diet treatment, the rats were starved for 16 h from 1700 h. At the end of the fasting period, rats were injected with saline, fasting RMR was measured and the values obtained served as baseline values in the calculation of total TEF.

Cephalic TEF was measured in five hypothyroid rats fed a low-fat diet and five hypothyroid rats fed a high-fat diet by giving them a small portion (7 kJ metabolizable energy; 0·56 g) of a test meal composed of the low-fat diet, while another five hypothyroid rats fed a low-fat diet and five hypothyroid rats fed a high-fat diet were given a small portion (7 kJ metabolizable energy; 0·44 g) of a test meal composed of the high-fat diet. Oxygen consumption was continuously monitored for 30 min after the rat had completely eaten the test meal and had resumed the initial resting daytime position. The integrated increase in the 30-min period was calculated using the trapezoid method. The above procedure has been previously used to measure cephalic TEF in rats (14); in fact, the use of very small portions of food minimizes TEF due to digestion, absorption and storage of food and allows measurement of the cephalic phase of TEF, which is independent of the meal size (14, 15).

Processing TEF was measured in five hypothyroid rats fed a low-fat diet and five hypothyroid rats fed a high-fat diet by giving them a larger portion of the low-fat meal (35 kJ metabolizable energy; 2·8 g), while another five hypothyroid rats fed a low-fat diet and five hypothyroid rats fed a high-fat diet were given a larger portion of the high-fat meal (35 kJ metabolizable energy; 2·2 g). The rats ate the food in about 10–15 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.

Obligatory TEF Three days later, the rats were again starved for 16 h from 17.00 h. At the end of the fasting period the rats were injected with propranolol (2 mg/100 g body weight), fasting RMR was measured and the values obtained served as baseline values in the calculation of the obligatory component of cephalic and processing TEF. The measurements were repeated as described above. Control experiments showed that fasting RMR in rats treated with propranolol did not change in any of the rats during the experiments.

Energy balance measurements

Body weights and food intakes were monitored daily to allow calculations of body weight gain and gross energy intake. The faeces were also collected daily for energy content measurements.

Digestible energy intake (taking into account the food spillage) was obtained by subtracting the energy measured in the faeces from the total energy intake as measured from daily food consumption. Metabolizable energy intake was expressed as digestible energy intake × 0·96 (16). The gain in energy during the 18-day period of dietary treatment was obtained by subtracting the energy content of the B_0 group from that of each of the two experimental groups. At the end of TEF measurements, the animals were killed by decapitation. After gut content removal, the carcasses were autoclaved, chopped into small pieces, thoroughly mixed, and homogenized in water (volumes equal to twice the carcass weight) with a Polytron. Samples of homogenates as well as samples of faeces were desiccated into a dry powder from which small pellets (about 200 mg) were made. The energy content of the pellets and the gross energy content of the low-fat and high-fat diets were measured with a Parr adiabatic calorimeter calibrated with dry benzoic acid standard. Energy expenditure was calculated from the difference between metabolizable energy intake and energy gain. Gross efficiency was expressed as percentage of metabolizable energy intake stored as body energy. Carcass lipid content was measured according to the method of Folch *et al.* (17).

Statistics

Data are given as means ± S.E.M. of five different rats. Statistical significance between the means was examined by two-tailed Student's *t*-test or by two-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

Table 1 Energy balance measurements in hypothyroid rats fed a low-fat or a high-fat diet. Values are the means± S.E.M. of 20 different rats and refer to the whole period of dietary treatment (18 days).

	Low-fat	High-fat
Initial body weight (g)	93 ± 3	95 ± 4
Final body weight (g)	119 ± 4	122 ± 3
Body weight gain (g)	26 ± 3	27 ± 4
Metabolizable energy intake (kJ)	1874 ± 102	1916 ± 58
Body energy gain (kJ)	353 ± 49	444 ± 32*
Lipid gain (g)	4·3 ± 0·4	8·6 ± 1·3*
(kJ)	162 ± 15	323 ± 49*
Carcass lipid content (%)	11·4 ± 0·5	14·8 ± 0·7*
Energy expenditure (kJ)	1521 ± 111	1472 ± 39
Gross efficiency (%)	19 ± 3	23 ± 2*
Fasting RMR (mmol O_2/min × $kg^{0.75}$)		
Saline-injected rats	0·70 ± 0·02	0·73 ± 0·02
Propranolol-injected rats	0·40 ± 0·02	0·42 ± 0·02

Gross efficiency = body energy gain/metabolizable energy intake.
* $P < 0{\cdot}05$ compared with hypothyroid rats fed a low-fat diet (two-tailed Student's *t*-test).

Inc., Costa Mesa, CA, USA. Lyophilized meat (Liomellin, Star s.p.a., Milano, Italy) and butter (Lurpak, Denmark) were purchased locally.

Results

The hypothyroid state of rats at the beginning of dietary treatment was confirmed by the measurements of undetectable serum levels of free T_3 in B_0 rats.

Table 1 shows the results of energy balance measurements in hypothyroid rats fed a low–fat or a high-fat diet. Mean initial and final body weights were not significantly different between the two groups, yielding a daily body weight gain of about 1·5 g in both groups. There were no significant differences in metabolizable energy intake and energy expenditure between hypothyroid rats fed a low-fat or a high-fat diet, although energy expenditure tends to be lower. On the other hand, body energy and lipid gain as well as % carcass lipid content of hypothyroid rats fed a high-fat diet were significantly higher (+26%, +99% and +30% respectively) than those of hypothyroid rats fed a low-fat diet. Consequently, gross efficiency was significantly higher (+21%) in hypothyroid rats fed a high-fat diet than in those fed a low-fat diet. Finally, fasting RMR was the same in hypothyroid rats fed a low-fat or a high-fat diet, both in saline-injected and in propranolol-injected rats.

The results of the determination of cephalic TEF in hypothyroid rats fed a low-fat or a high-fat diet after low-fat and high-fat meals are shown in Figs 1 and 2. Total integrated TEF after a low-fat meal was significantly lower in hypothyroid rats fed a high-fat diet compared with those fed a low-fat diet (Fig. 2). This decrease was due to a decrease in facultative TEF calculated from the difference between total and obligatory TEF, while no variation was found in obligatory TEF measured in propranolol-injected rats (Fig. 2). On the other hand, no significant variation in cephalic TEF after a high-fat meal was found in hypothyroid rats fed a high-fat diet compared with those fed a low-fat diet (Fig. 2). In addition, cephalic TEF after a high-fat meal was significantly higher than after a low-fat meal in hypothyroid rats fed a high-fat diet but not in those fed a low-fat diet. This increase was completely due to an increase in facultative TEF (Fig. 2).

Postprandial increase in RMR measured for 180 min after the end of a 35 kJ test meal is shown in Fig. 3. The integrated increase in RMR between 30 and 180 min was taken as representative of the processing TEF and is shown in Fig. 4. No significant variation was found in total TEF, although TEF after a high-fat meal tends to be lower than that after a low-fat meal. A significant decrease in obligatory TEF after a high-fat meal compared with TEF after a low-fat meal was found in both groups of rats (Fig. 4). In addition, a significant decrease in the obligatory component (Fig. 4) and an increase in the facultative component (Fig. 4) of processing TEF after low-fat and high-fat meals was found in hypothyroid rats fed a high-fat diet compared with those fed a low-fat diet.

Discussion

In this work we have carried out energy balance measurements (Table 1) in hypothyroid rats fed low-fat or high-fat diets. The two groups of rats were consuming approximately the same amount of energy; however, hypothyroid rats fed a high-fat diet gained significantly more body energy as fat and exhibited higher carcass lipid content than rats fed a low-fat diet. Although energy expenditure was not significantly altered, gross efficiency was higher in hypothyroid rats fed a high-fat diet than in those fed a low-fat diet (Table 1). The increased efficiency of hypothyroid rats fed a high-fat diet presumably results from the reduced energy cost of

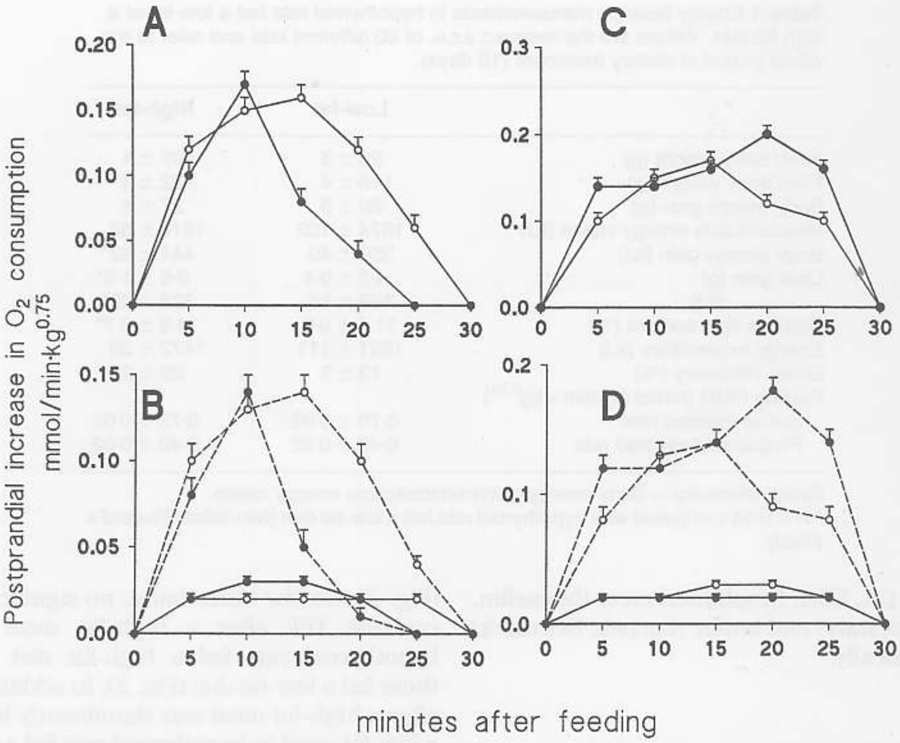

Figure 1 Cephalic TEF after low-fat and high-fat meals measured in hypothyroid rats fed a low-fat (○) or a high-fat (●) diet. (A) Total TEF time course measured in saline-injected rats after a low-fat meal. (B) Obligatory TEF time course measured in propranolol-injected rats (unbroken line) and facultative TEF time course calculated from the difference between total and obligatory TEF (dotted line) after a low-fat meal. (C) Total TEF time course measured in saline–injected rats after a high-fat meal. (D) Obligatory TEF time course measured in propranolol-injected rats (unbroken line) and facultative TEF time course calculated from the difference between total and obligatory TEF (dotted line) after a high-fat meal. Values are reported as means ± S.E.M. of five different rats.

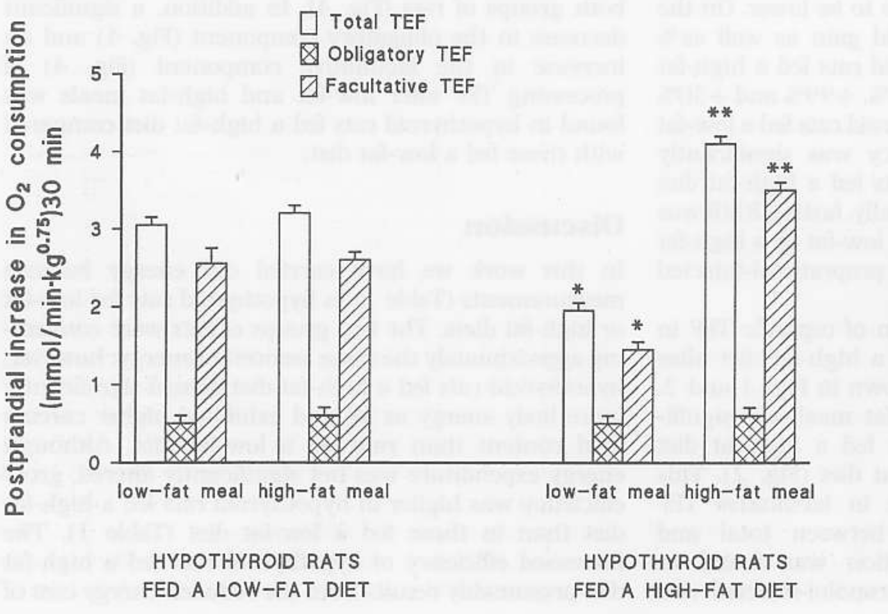

Figure 2 Integrated cephalic TEF after low-fat and high-fat meals in hypothyroid rats fed a low-fat or a high-fat diet. Integrated TEF over the 30-min period was calculated using the trapezoid method. Values are reported as means ± S.E.M. of five different rats. $^*P < 0{\cdot}05$ compared with hypothyroid rats fed a low-fat diet (two-way analysis of variance followed by two-tailed Student's *t*-test). $^{**}P < 0{\cdot}05$ compared with TEF after a low-fat meal in hypothyroid rats fed a high-fat diet (two-way analysis of variance followed by two-tailed Student's *t*-test).

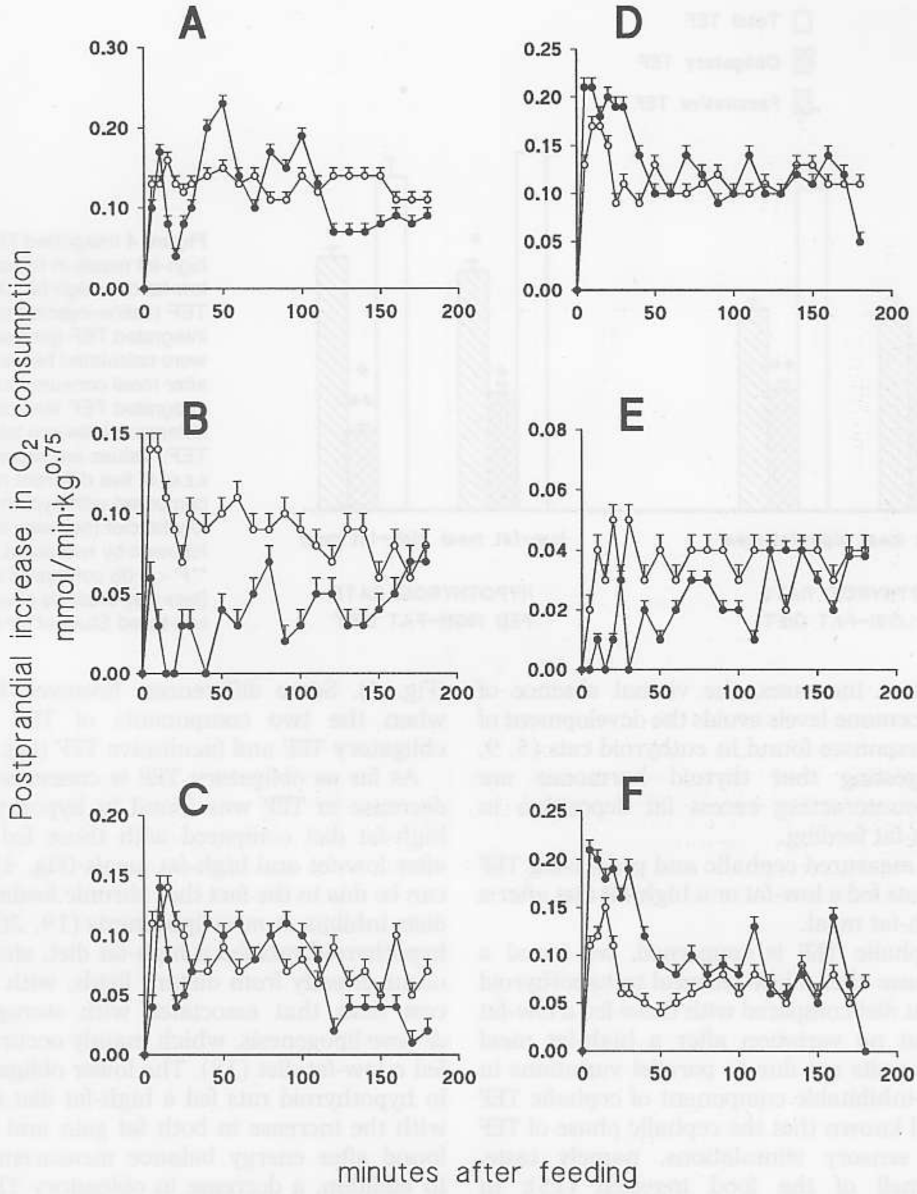

Figure 3 TEF after low-fat and high-fat meals measured in hypothyroid rats fed a low-fat (○) or a high-fat (●) diet. (A) Total TEF time course measured in saline-injected rats after a low-fat meal. (B) Obligatory TEF time course measured in propranolol-injected rats after a low-fat meal. (C) Facultative TEF time course calculated from the difference between total and obligatory TEF after a low-fat meal. (D) Total TEF time course measured in saline-injected rats after a high-fat meal. (E) Obligatory TEF time course measured in propranolol-injected rats after a high-fat meal. (F) Facultative TEF time course calculated from the difference between total and obligatory TEF after a high-fat meal. Values are reported as means ± S.E.M. of five different rats.

fat synthesis. In fact, in rats consuming standard laboratory diets almost all of the body fat will be synthesized *de novo* from glucose, with a relatively high energy cost (0·36 kJ/kJ fat deposited) (18). When the level of fat in the diet is increased body fat is derived directly from dietary lipid, with a much lower energy cost (0·16 kJ/kJ fat deposited) (18). In hypothyroid rats, the energy saved in this way is not dissipated but stored as fat. The above results are different from those previously obtained by us on euthyroid rats (5, 9, 10). In fact, we have found that euthyroid rats fed a high-fat diet showed an increase in energy intake and energy expenditure, a decrease in gross efficiency and no variation in lipid gain compared with rats fed a low-fat diet, so that carcass lipid content was about 11% in both groups of rats. Carcass lipid content was also about 11% in hypothyroid rats fed a low-fat diet (Table 1), indicating that, when dietary fat content is low, rats are able to avoid excess fat deposition even in the absence of thyroid hormones. On the other hand, when

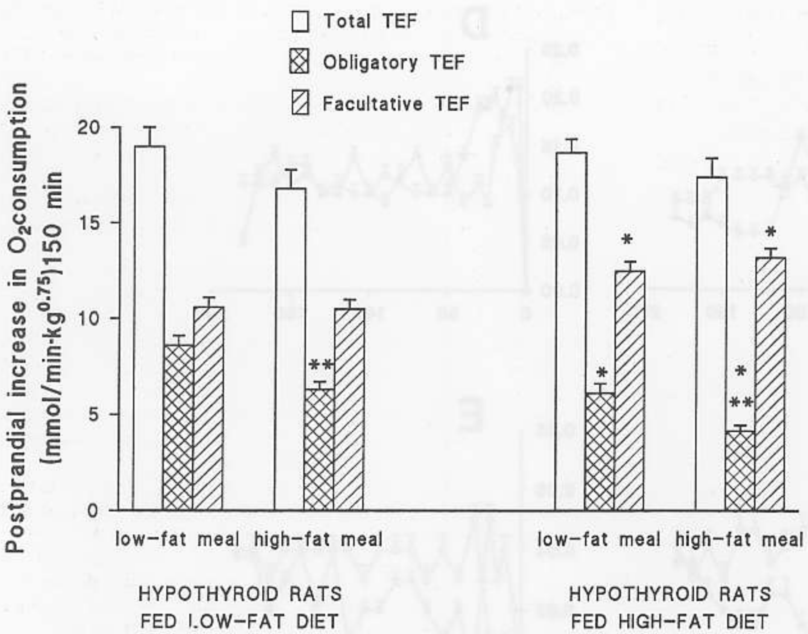

Figure 4 Integrated TEF after low-fat and high-fat meals in hypothyroid rats fed a low-fat or a high-fat diet. Total integrated TEF (saline-injected rats) and obligatory integrated TEF (propranolol-injected rats) were calculated between 30 and 180 min after meal consumption. Facultative integrated TEF was calculated from the difference between total and obligatory TEF. Values are reported as means ± S.E.M. of five different rats. *$P < 0.05$ compared with hypothyroid rats fed a low-fat diet (two-way analysis of variance followed by two-tailed Student's *t*-test). **$P < 0.05$ compared with a low-fat meal (two-way analysis of variance followed by two-tailed Student's *t*-test).

dietary fat content increases, the virtual absence of serum thyroid hormone levels avoids the development of those adaptive responses found in euthyroid rats (5, 9, 10), thus suggesting that thyroid hormones are important in counteracting excess fat deposition in response to high-fat feeding.

We have also measured cephalic and processing TEF in hypothyroid rats fed a low-fat or a high-fat diet after a low-fat or a high-fat meal.

As far as cephalic TEF is concerned, we found a significant decrease after a low-fat meal in hypothyroid rats fed a high-fat diet compared with those fed a low-fat diet (Fig. 2), but no variation after a high-fat meal (Fig. 2). These results are due to parallel variations in the propranolol-inhibitable component of cephalic TEF (Fig. 2). It is well known that the cephalic phase of TEF is induced by sensory stimulations, namely taste, texture and smell of the food ingested (15); in agreement with this, we have found previously that cephalic TEF after a high-fat meal (which is preferred to a low-fat meal in a two-choice preference test) was higher than TEF after a low-fat meal (10). The present results show that in hypothyroid rats which are used to the energy-dense diet a low-fat meal elicits a significantly lower TEF than a high-fat meal (Fig. 2); this decrease is completely due to a decrease in the facultative component of TEF (Fig. 2). This result suggests that previous feeding experience can affect facultative cephalic TEF: rats familiar with a more palatable food are less responsive to a less palatable food.

We have also measured the postprandial increase in RMR after a larger meal (35 kJ) (Fig. 3). Our results show that there is no variation in processing TEF after low-fat and high-fat meals in hypothyroid rats fed a high-fat diet compared with those fed a low-fat diet (Fig. 4). Some differences, however, become evident when the two components of TEF are examined: obligatory TEF and facultative TEF (Fig. 4).

As far as obligatory TEF is concerned, a significant decrease in TEF was found in hypothyroid rats fed a high-fat diet compared with those fed a low-fat diet, after low-fat and high-fat meals (Fig. 4). This decrease can be due to the fact that chronic feeding with high fat diets inhibits *de novo* lipogenesis (19, 20). Therefore, in hypothyroid rats fed a high-fat diet, storage of fat may occur directly from dietary lipids, with a lower energy cost than that associated with storage of lipids via *de novo* lipogenesis, which mainly occurs when rats are fed a low-fat diet (18). The lower obligatory TEF found in hypothyroid rats fed a high-fat diet is in agreement with the increase in both fat gain and gross efficiency found after energy balance measurements (Table 1). In addition, a decrease in obligatory TEF after a high-fat meal compared with TEF after a low-fat meal was found in both groups of rats (Fig. 4). This result can be explained by taking into account the fact that obligatory TEF is influenced by the amount and nature of the nutrient consumed (15); since the fraction of energy dissipated during digestion, absorption and storage of nutrients ranges from 5 to 10% for carbohydrate and from 3 to 5% for fat (21), a high-fat diet, which has a higher fat and a lower carbohydrate content, is associated with a lower cost of nutrient assimilation.

As far as facultative TEF is concerned, a significant increase was found in hypothyroid rats fed a high-fat diet after low-fat and high-fat meals (Fig. 4). This result can be explained by taking into account the fact that brown adipose tissue (BAT) has been involved in the processing TEF (14, 22) and high-fat diets stimulate

BAT activity (18). In addition, it has been shown that BAT shows some characteristics of its recruited state in hypothyroid rats (23–25). Therefore, the increased facultative TEF in hypothyroid rats fed a high-fat diet could be due to an increased BAT thermogenesis.

In conclusion, our results indicate that hypothyroid rats are unable to develop those adaptive mechanisms which are useful to counteract obesity. In fact, the lack of T_3 inhibits both acute and chronic thermogenic responses found in euthyroid rats fed a high-fat diet.

References

1 Iossa S, Liverini G & Barletta A. Relationship between the resting metabolic rate and hepatic metabolism in rats: effect of hyperthyroidism and fasting for 24 hours. *Journal of Endocrinology* 1992 **135** 45–51.

2 Loeb JN. Metabolic changes in thyrotoxicosis. In *The Thyroid. A Fundamental and Clinical Text*, pp 845-853. Eds LE Braveman & RD Utiger. Philadelphia: JB Lippincott Company, 1991.

3 Liverini G, Iossa S & Barletta A. Relationship between resting metabolism and hepatic metabolism: effect of hypothyroidism and 24 hours fasting. *Hormone Research* 1992 **38** 154–159.

4 Loeb JN. Metabolic changes in hypothyroidism. In *The Thyroid. A Fundamental and Clinical Text*, pp 1064-1071. Eds LE Braveman & RD Utiger. Philadelphia: JB Lippincott Company, 1991.

5 Liverini G, Iossa S & Barletta A. Hepatic mitochondrial respiratory capacity in hyperphagic rats. *Nutrition Research* 1994 **14** 1671–1682.

6 Rothwell NJ & Stock MJ. A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 1979 **281** 31–35.

7 Rothwell NJ, Saville E & Stock MJ. Sympathetic and thyroid influences on metabolic rate in fed, fasted and refed rats. *American Journal of Physiology* 1992 **243** R339–R346.

8 Dauncey MJ & Morovat A. Investigation of mechanisms mediating the increase in plasma concentrations of thyroid hormones after a meal in young growing pigs. *Journal of Endocrinology* 1993 **139** 131–141.

9 Iossa S, Mollica MP, Lionetti L, Barletta A & Liverini G. Hepatic mitochondrial respiration and transport of reducing equivalents in rats fed an energy dense diet. *International Journal of Obesity* 1995 **19** 539–543.

10 Liverini G, Iossa S, Lionetti L, Mollica MP & Barletta A. Sympathetically-mediated thermogenic response to food in rats. *International Journal of Obesity* 1995 **19** 87–91.

11 Iossa S, Lionetti L, Mollica MP, Barletta A & Liverini G. Thermic effect of food in hypothyroid rats. *Journal of Endocrinology* 1996 **148** 167–174.

12 Romelli PB, Pennisi F & Vancheri L. Measurement of free thyroid hormones by column absorption chromatography and radioimmunoassay. *Journal of Endocrinological Investigation* 1978 **1** 89–94.

13 Naim M, Brand JG, Kare MR & Carpenter RG. Energy intake, weight gain and fat deposition in rats fed nutritionally controlled diets in a multichoice ('cafeteria') design. *Journal of Nutrition* 1985 **115** 1447–1458.

14 Allard M & LeBlanc J. Effect of cold acclimation, cold exposure and palatability on postprandial thermogenesis in rats. *International Journal of Obesity* 1988 **12** 169–178.

15 LeBlanc J & Diamond P. Effect of meal size and frequency on postprandial thermogenesis in dogs. *American Journal of Physiology* 1986 **250** E144–E147.

16 Barr HG & McCracken KJ. High efficiency of energy utilization in 'cafeteria' and force-fed rats kept at 29 °C. *British Journal of Nutrition* 1984 **51** 379–387.

17 Folch J, Lees N & Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 1957 **226** 497–510.

18 Rothwell NJ, Stock MJ & Warwick BP. Energy balance and brown fat activity in rats fed cafeteria diets or high-fat, semisynthetic diets at several levels of intake. *Metabolism* 1985 **34** 474–480.

19 Pegorier JP, Duee PH, Herbin C, Laulan PY, Bladè C, Peret J & Girard J. Fatty acid metabolism in hepatocytes isolated from rats adapted to high-fat diets containing long- or medium-chain triacylglycerols. *Biochemical Journal* 1988 **249** 801–806.

20 Rothwell NJ, Stock MJ & Trahyurn P. Reduced lipogenesis in cafeteria-fed rats exhibiting diet-induced thermogenesis. *Bioscience Reports* 1983 **3** 217–227.

21 Flatt JP. The biochemistry of energy expenditure. In *Obesity*, pp 100-116. Eds P Bjorntorp & N Brodoff. Philadelphia: JB Lippincott Company, 1992.

22 Glick Z & Raum WJ. Norepinephrine turnover in brown adipose tissue is stimulated by a single meal. *American Journal of Physiology* 1986 **251** R13–R17.

23 Mory G, Ricquier D, Pesquies P & Hemon P. Effects of hypothyroidism on the brown adipose tissue of adult rats: comparison with the effects of adaptation to cold. *Journal of Endocrinology* 1981 **91** 515–524.

24 Park IRA, Mount DB & Himms-Hagen J. Role of T_3 in thermogenic and trophic responses of brown adipose tissue to cold. *American Journal of Physiology* 1989 **257** E81–E87.

25 Dicker A, Raasmaja A, Cannon B & Nedergaard J. Increased α_1-adrenoceptor density in brown adipose tissue indicates recruitment drive in hypothyroid rats. *American Journal of Physiology* 1992 **263** E654–E662.

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