International Journal of Obesity (1995) 19, 87–91

Sympathetically-mediated thermogenic response to food in rats

G. Liverini, S. Iossa, L. Lionetti, M.P. Mollica and A. Barletta

Department of General and Environmental Physiology, University of Naples, Italy

The aim of this work was to assess the participation of the sympathetic nervous system in the thermogenic response to food in control and hyperphagic rats. Rats were fed either a control (CD) or energy dense (ED) diet. After 15 days, CD rats received a small (7 kJ) meal composed of either control or energy dense diet, while ED rats received a small meal composed of energy dense diet. The experiment was then repeated, with the exception that rats received a larger portion (35 kJ) of the test meal.

The postprandial increase in oxygen consumption was measured for 30 min after the small meal and 90–180 min after the completion of the large meal. The measurements were made in saline-injected and propranolol-injected rats.

ED rats exhibited hyperphagia as well as an increase of 32% in resting metabolic rate after a 16 h fast. The sympathetically-mediated postprandial increase in oxygen consumption was greater after an energy dense meal than after a control meal in CD rats, and was higher in ED rats than in CD rats fed an energy dense meal.

It was concluded that the sympathetically-mediated increase in the thermogenic response to food, as well as the increase in fasting metabolic rate can help prevent obesity development in hyperphagic rats.

Keywords: thermic effect of food, sympathetic nervous system, hyperphagic rats

Introduction

It has been known for many years that meal feeding is followed by an increased metabolic rate, called thermic effect of food (TEF), and it has been demonstrated that two phases occur in the thermogenic response to a meal in dogs.[1,2] The early phase, lasting 45 min, is elicited by the sensory inputs that are triggered by food and is independent of the meal size.[3] The late phase is related to the mechanical process of digestion, absorption and substrate storage.[3] Diamond and LeBlanc[4,5] have shown that, in dogs, both of these phases consist of two components: an 'obligatory' component and a 'regulatory' one, the latter being under the control of the sympathetic nervous system (SNS). Although the occurrence of both these phases of TEF have also been found in rats and hens,[6–9] the role of SNS has not been established.

One purpose in this study is to assess the participation of SNS in the thermogenic response to food in rats. To this end, TEF was measured both in the presence and in the absence of the β-adrenergic blocker, propranolol.

Chronic administration of energy dense food to rats can induce hyperphagia, together with an increase in SNS activity and energy expenditure.[10–14] In these hyperphagic rats an increase in TEF induced by tube-feeding has been seen.[15] However, to our knowledge, a complete study on the effect of hyperphagia on the regulatory component of TEF has not been carried out. Therefore we have also measured regulatory TEF in rats chronically fed an energy dense diet.

Methods

Animals and experimental design

Sixty male Wistar rats (MORINI, 42020 S.Polo D'Enza (R.E.), Italy) of about 30 days of age (90 ± 2 g) were used for the experiments. The rats were divided into two groups: 40 rats were given free access to a control diet (called CD rats) and 20 rats were given free access to an energy dense diet (called ED rats) for 15 days (see Table 1 for diets). All rats were allowed free access to water and were maintained one per cage (in grid-bottomed cages) at 24°C under an artificial circadian 12.12 light-dark cycle. Animal care, housing and killing met the guidelines of the Italian Health Ministry.

Oxygen consumption measurements were performed as previously described.[16–17] Briefly, oxygen consumption was measured at 10.00 in the morning 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.

CD and ED rats were used for the determination of obligatory and regulatory components of TEF. The rats were starved for 16 h from 5.00 p.m.; at the end of the fasting period, half of the rats were injected with propranolol (2 mg/100 g b.w.), while the other half were injected with saline. Oxygen consumption was measured in all the rats (only when the animals were resting) and the values obtained served as baseline values in the calculation of

Correspondence to: Prof. G. Liverini, Dipartimento di Fisiologia Generale ed Ambientale, Via Mezzocannone 8, I-80134, Napoli, Italy.
Received 8 December 1993; accepted 15 September 1994

Table 1 Composition of diets

Component	*Control diet*	*Energy dense diet*
	g/kg diet	
Casein[a]	200	135
Methionine	3	3
Sucrose	250	160
Cornstarch	400	200
Alphacel	50	50
Corn oil	50	50
Choline bitartrate	2	2
AIN 76 Mineral mix[b]	35	35
AIN 76 Vitamin mix[c]	10	10
Butter[d]	---	186
Lyophilised meat[e]	---	169
Energy density[f], kJ/g diet	15.66	19.35
Energy (J/100 J) from protein	19.5	19.5
lipid	12	46.1
carbohydrate	68.5	34.4

[a] *Purified high nitrogen casein, containing 88% protein.*
[b] *American Institute of Nutrition (1977).*
[c] *American Institute of Nutrition (1980).*
[d] *Lurpak, Denmark, locally purchased, containing 10% water.*
[e] *Liomellin, STAR s.p.a., Milano, Italy, containing (in 10 g): 5.8 g protein (Nx 6.25), 1.2 g lipid, 2.57 g carbohydrate, 0.2 g minerals, 0.2 g water.*
[f] *The energy density was estimated applying the coefficients (kJ/g) 16.51, 17.34 and 37.56 for carbohydrate, protein and fat, respectively.*

TEF. It should be noted that oxygen consumption in rats treated with propranolol did not change over the whole time course of the experiments both in CD and ED rats (data not shown). The rats were then divided into 12 groups of five rats each.

Experiment I

One group of CD rats which had received propranolol and one group of CD rats which had received saline were given a small portion (7 kJ; 0.44 g) of a test meal composed of control diet (see Table 1), while one group of CD and one group of ED rats which had received propranolol and one group of CD and one group of ED rats which had received saline were given a small portion (7 kJ; 0.36 g) of a test meal composed of an energy dense diet (see Table 1). 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. It should be mentioned that the animals ate the food within 2 min, and thereafter resumed the initial resting position typical of rats during the daytime.

Experiment II

Experiment I was repeated with the exception that the rats were given a larger portion of the test meal (35 kJ, corresponding to 2.2 g of control diet and to 1.8 g of energy dense diet). The rats ate the food in about 10–15 min. Oxygen consumption was measured every ten minutes between 90 and 180 min after meal consumption (values over 2 min were taken only when the animals were resting) and was reported as the average increase during the whole period of measurement. The time range selected for determining postprandial increase in oxygen consumption was based on the observation that during this period a steady state was achieved.

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. dl-propranolol was purchased from Sigma Chemical Co., St. Louis, MO.

Results

Body weights and energy intakes of CD and ED rats are shown in Table 2. Mean initial and final body weights are not significantly different between the two groups, showing a daily body weight gain of about 7g in both groups. The energy intake (not corrected for energy loss through faeces and urine) of ED rats during the whole period of treatment is 40% higher than in CD rats. After an overnight fast (16 h), ED rats exhibit a significantly higher RMR (+32%) than CD rats (Table 3). The administration of propranolol results in a significant reduction of RMR in ED rats but not in CD rats (Table 3).

Experiment I

The results show that after a test meal, there is a rapid increase in RMR, that reaches a peak value after about 10 min and that goes back to prefeeding level about 30 min

Table 2 Body weight and energy intake of CD and ED rats

	CD rats	*ED rats*
Initial body weight, g	90 ± 3	90 ± 3
Final body weight, g	198 ± 10	202 ± 12
Energy intake, kJ[a]	3514 ± 102	4920 ± 200*

Data are the means ± s.e.m. of 40 CD rats and 20 ED rats.
[a] *The reported values (not corrected for energy loss through faeces and urine) are referred to the whole period of treatment (15 days).*
* *$P < 0.0002$ compared to CD rats (Student's t-test).*

Table 3 Postprandial oxygen consumption in CD and ED rats

		CD rats Control meal	*CD rats Energy dense meal*	*ED rats Energy dense meal*
Saline injected	Preprandial	1.31 ± 0.02[a]	1.28 ± 0.03[a]	1.69 ± 0.03[b]
	Postprandial	1.56 ± 0.03[b]	1.51 ± 0.03[b]	1.96 ± 0.05[c]
	% Increase	19	18	16
Propranolol injected	Preprandial	1.29 ± 0.02[a]	1.26 ± 0.02[a]	1.28 ± 0.02[a]
	Postprandial	1.45 ± 0.02[d]	1.42 ± 0.02[d]	1.43 ± 0.03[d]
	% Increase	12	13	12

Preprandial oxygen consumption was measured after 16 h fasting. Postprandial oxygen consumption was measured between 90 and 180 min after the rats had eaten 35 kJ of either a control meal or an energy dense meal (values were taken only when the animals were resting) and was reported as the average increase during the whole period of measurement. Values are reported as means ± s.e.m. of five different rats and are expressed as µmol O_2/ min × g body weight. Values with different superscript [(a-d)] are significantly different ($P < 0.05$) (multi-way analysis of variance followed by two-tailed unpaired Students t-test).

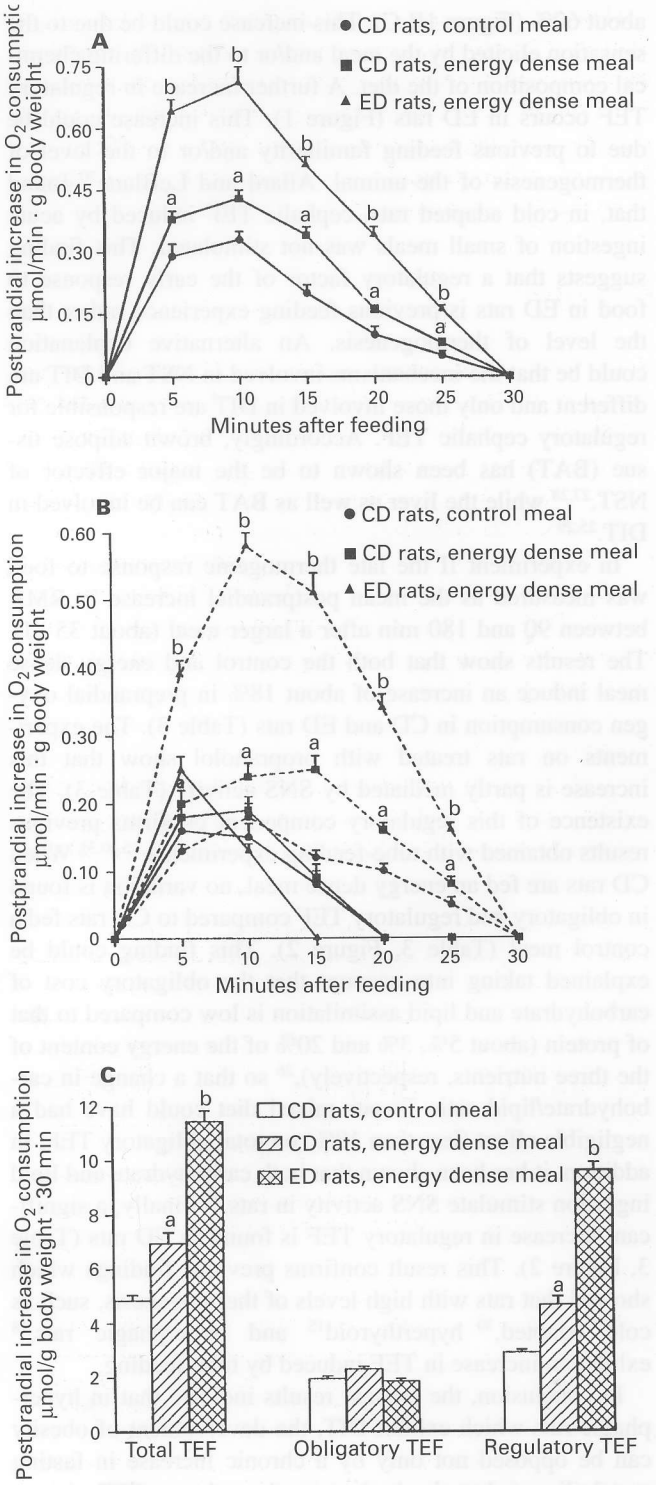

Figure 1 Early postprandial increase in oxygen consumption. **(A)** Total TEF time course measured in saline injected rats. **(B)** Obligatory TEF time course measured in propranolol-injected rats (unbroken line) and regulatory TEF time course calculated from the difference between total and obligatory TEF (dotted line). **(C)** Integrated TEF calculated using the trapezoid method. Preprandial oxygen consumption was measured after 16 h fasting and served as baseline value (1.33 ± 0.02 and 1.30 ± 0.02 µmol O_2/min × g body weight for CD rats fed control meal, with or without propranolol; 1.34 ± 0.02 and 1.30 ± 0.02 µmol O_2/min × g body weight for CD rats fed an energy dense meal, with or without propranolol; 1.29 ± 0.02 and 1.70 ± 0.02 µmol O_2/min × g body weight for ED rats, with or without propranolol) for the calculation of TEF. 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. **a** $P < 0.05$ compared to CD rats fed control meal. **b** $P < 0.05$ compared to CD rats fed energy dense meal.

after both a control meal and an energy dense meal (Figure 1A). TEF is significantly higher after an energy dense meal than after a control meal in CD rats and is significantly higher in ED rats than in CD rats (Figure 1A,C). The administration of propranolol significantly reduces TEF by about 50% in CD rats fed a control meal, by about 70% in CD rats fed an energy dense meal and by about 80% in ED rats (Figure 1B,C), showing that obligatory TEF is the same in the three groups of rats. The regulatory TEF, calculated as the difference between total and obligatory TEF, is significantly higher after an energy dense meal than after a control meal in CD rats and is significantly higher in ED rats than in CD rats (Figure 1B,C).

Experiment II

The administration to rats of a 35 kJ-test meal induces a significant increase in preprandial oxygen consumption in the three groups of rats (Table 3). The administration of propranolol significantly reduces postprandial RHR in CD and ED rats (Table 3). The obligatory and regulatory components of TEF accounted for about 70 and 30% of total TEF, respectively, in CD rats, after both control and energy dense meals (Figure 2). In ED rats, the regulatory TEF was significantly higher than in CD rats fed an energy dense meal, and it accounted for 44% of the total TEF (Figure 2).

Discussion

In the present work we have induced hyperphagia in rats by using a semipurified diet supplemented with lyophilized meat and butter (Table 1): this diet combines two important characteristics of diets which normally induce hyperphagia

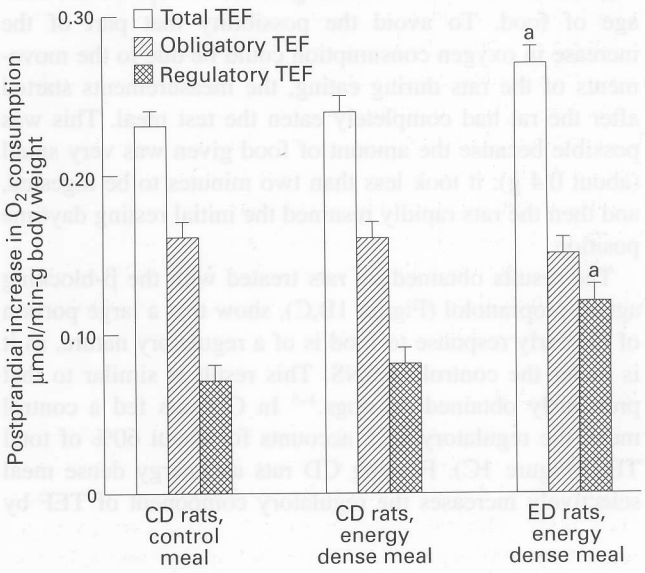

Figure 2 Late postprandial increase in oxygen consumption. TEF values were obtained from the difference between postprandial and preprandial oxygen consumption reported in Table 3: total TEF was obtained from saline-injected rats and obligatory TEF from propranolol-injected rats. Regulatory TEF was obtained from the difference between total and obligatory TEF. Values are the means ± s.e.m. of five different rats. **a** $P < 0.05$ compared to CD rats fed energy dense meal.

in rats (i.e. cafeteria diets): high fat content[18] and the presence of a meat component, which is among the flavours most preferred by rats.[19,20]

After 15 days of ad libitum consumption of the energy dense diet, ED rats showed an increase of about 40% in energy intake compared to CD rats (Table 2); however, there was no difference in body weight gain between CD and ED rats (Table 2). When MR was measured after an overnight fast, ED rats showed 32% higher RMR compared to CD rats (Table 3). This increase was almost completely abolished by propranolol administration (Table 3). Therefore, it can be concluded that ad libitum consumption of the energy dense diet for 15 days induces hyperphagia, together with a propranolol-dependent increase in energy expenditure. These variations are similar to those observed in cafeteria diet-fed rats, exhibiting diet-induced thermogenesis (DIT).[10–12]

Our present results indicate the persistence of DIT even after overnight fasting, which is generally assumed to eliminate the contribution of food processing and storage to the metabolic rate but not to alter normal maintenance energy expenditure significantly.[21,22] Thus, DIT appears to be independent of the immediate energy intake. This finding is in contrast with the suggestion that DIT produced during prolonged overeating only reflects the summation of the TEF of single meals[23,24] and supports the idea that DIT involves whatever increase in the fasting metabolic rate may be induced by overeating.[25,26] In our conditions DIT accounts for about 39% of the increased energy intake.

In this work we have also assessed the participation of SNS in the thermogenic response to food in rats.

The purpose of experiment I was to evaluate the early response to food. As this response is independent of the meal size,[6] we used very small portions of food (about 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 rat had completely eaten the test meal. This was possible because the amount of food given was very small (about 0.4 g): it took less than two minutes to be ingested, and then the rats rapidly resumed the initial resting daytime position.

The results obtained on rats treated with the β-blocking agent, propranolol (Figure 1B,C), show that a large portion of the early response to food is of a regulatory nature, as it is under the control of SNS. This result is similar to that previously obtained on dogs.[4–5] In CD rats fed a control meal, the regulatory TEF accounts for about 60% of total TEF (Figure 1C). Feeding CD rats an energy dense meal selectively increases the regulatory component of TEF by about 60% (Figure 1B,C). This increase could be due to the sensation elicited by the meal and/or to the differing chemical composition of the diet. A further increase in regulatory TEF occurs in ED rats (Figure 1). This increase could be due to previous feeding familiarity and/or to the level of thermogenesis of the animal. Allard and LeBlanc[20] found that, in cold adapted rats, cephalic TEF induced by acute ingestion of small meals was not stimulated. This finding suggests that a regulatory factor of the early response to food in ED rats is previous feeding experience rather than the level of thermogenesis. An alternative explanation could be that the mechanisms involved in NST and DIT are different and only those involved in DIT are responsible for regulatory cephalic TEF. Accordingly, brown adipose tissue (BAT) has been shown to be the major effector of NST,[27,28] while the liver as well as BAT can be involved in DIT.[25,29]

In experiment II the late thermogenic response to food was measured as the mean postprandial increase in RMR between 90 and 180 min after a larger meal (about 35 kJ). The results show that both the control and energy dense meal induce an increase of about 18% in preprandial oxygen consumption in CD and ED rats (Table 3). The experiments on rats treated with propranolol show that this increase is partly mediated by SNS activity (Table 3). The existence of this regulatory component confirms previous results obtained with tube-feeding experiments.[15,30,31] When CD rats are fed an energy dense meal, no variation is found in obligatory and regulatory TEF compared to CD rats fed a control meal (Table 3, Figure 2). This finding could be explained taking into account that the obligatory cost of carbohydrate and lipid assimilation is low compared to that of protein (about 5%, 3% and 20% of the energy content of the three nutrients, respectively),[26] so that a change in carbohydrate/lipid ratio in our mixed diet could have had a negligible effect (less than 10%) on total obligatory TEF. In addition, it has been shown that both carbohydrate and lipid ingestion stimulate SNS activity in rats.[32] Finally, a significant increase in regulatory TEF is found in ED rats (Table 3, Figure 2). This result confirms previous findings which showed that rats with high levels of thermogenesis, such as cold adapted,[30] hyperthyroid[15] and hyperphagic rats,[18] exhibit an increase in TEF induced by tube-feeding.

In conclusion, the present results indicate that in hyperphagic rats which exhibit DIT, the development of obesity can be opposed not only by a chronic increase in fasting metabolic rate but also by increased regulatory TEF.

Acknowledgements

This work was supported by C.N.R.

References

1 Diamond, P., Brondel, L. & LeBlanc, J. (1985): Palatability and postprandial thermogenesis in dogs. *Am J Physiol* **248**, E75–E79.

2 LeBlanc, J. (1991): Hormonal and metabolic responses to meals of various composition. In *Obesity: dietary factors and control,* eds. Romsos, D.R., Himms-Hagen, J. & Suzuki, M., pp. 59–66. Basel: Karger.

3 LeBlanc, J. & Diamond, P. (1986): Effect of meal size and frequency on postprandial thermogenesis in dogs. *Am J Physiol* **250**, E144–E147.

4 Diamond, P. & LeBlanc, J. (1987): Role of autonomic nervous system in postprandial thermogenesis in dogs. *Am J Physiol* **252**, E719—E726.

5 Diamond, P. & LeBlanc, J. (1987): Hormonal control of postprandial thermogenesis in dogs. *Am J Physiol* **253**, E521–E529.
6 Allard, M. & LeBlanc, J. (1988): Effects of cold acclimation, cold exposure and palatability on postprandial thermogenesis in rats. *Int J Obes* **12**, 169–178.
7 Griggio, M.A., Luz, J. & Carvalho, S.M.T. (1992): The effect of fasting and refeeding on oxygen consumption by rats. *Braz J Med Biol Res* **25**, 205–208.
8 Viarouge, C., Caulliez, R. & Nicolaidis, S. (1992): Umami taste of monosodium glutamate enhances the thermic effect of food and affects the respiratory quotient in the rat. *Physiol Behav* **52**, 879–884.
9 LeBlanc, J. & Brondel, L. (1985): Role of palatability on meal-induced thermogenesis in human subjects. *Am J Physiol* **248**, E333–E336.
10 Rothwell, N.J. & Stock, M.J. (1979): A role for brown adipose tissue in diet-induced thermogenesis. *Nature* **281**, 31–35.
11 Rothwell, N.J. & Stock, M.J. (1981): Influence of noradrenaline on blood flow to brown adipose tissue in rats exhibiting diet-induced thermogenesis. *Pflug Arch* **389**, 237–242.
12 Rothwell, N.J. & Stock, M.J. (1982): Energy expenditure of 'cafeteria'-fed rats determined from measurements of energy balance and indirect calorimetry. *J Physiol* **328**, 371–377.
13 Young, J.B. & Landsberg, L. (1979): Effect of diet and cold exposure on norepinephrine turnover in pancreas and liver. *Am J Physiol* **236**, E524–E533.
14 Young, J.B., Saville, M.E., Rothwell, N.J., Stock, M.J., & Landsberg, L. (1982): Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue of the rat. *J Clin Invest* **69**, 1061–1071.
15 Rothwell, N.J., Saville, M.E. & Stock, M.J. (1982): Factors influencing the acute effect of food on oxygen consumption in the rat. *Int J Obes* **6**, 53–59.
16 Iossa, S., Liverini, G. & Barletta, A. (1992): Relationship between the resting metabolic rate and hepatic metabolism in rats: effect of hyperthyroidism and fasting for 24 hours. *J Endocrinol* **135**, 45–51.
17 Liverini, G., Iossa, S. & Barletta, A. (1992): Relationship between resting metabolism and hepatic metabolism: effect of hypothyroidism and 24 hours fasting. *Horm Res* **38**, 154–159.
18 Rothwell, N.J., Stock, M.J. & Warwick, B.P. (1983): The effect of high fat and high carbohydrate cafeteria diets on diet-induced thermogenesis in the rat. *Int J Obes* **7**, 263–270.
19 Naim, M., Brand, J.G., Kare, M.R. & Carpenter, R.G. (1985): Energy intake, weight gain and fat deposition in rats fed nutritionally controlled diets in a multichoice ('cafeteria') design. *J Nutr* **115**, 1447–1458.
20 Allard, M. & LeBlanc, J. (1988): Effect of cold acclimation, cold exposure and palatability on postprandial thermogenesis in rats. *Int J Obes* **12**, 169–178.
21 Ma, S.W.Y. & Foster, D.O. (1986): Starvation-induced changes in metabolic rate, blood flow and regional energy expenditure in rats.*Can J Physiol Pharmacol* **64**, 1252–1258.
22 Griggio, M.A., Luz, J. & Carvalho, S.M.T. (1992): The effect of fasting and refeeding on oxygen consumption by rats. *Braz J Med Biol Res* **25**, 205–208.
23 Glick, Z., Teague, R.J. & Bray, G.A. (1981): Brown adipose tissue: thermic response increased by a single low protein, high carbohydrate meal. *Science* **213**, 1125–1127.
24 Glick, Z. & Raum, W.J. (1986): Norepinephrine turnover in brown adipose tissue is stimulated by a single meal. *Am J Physiol* **251**, R13–R17.
25 Ma, S.W.Y. & Foster, D.O. (1989): Brown adpose tissue, liver, and diet-induced thermogenesis in cafeteria diet-fed rats. *Can J Physiol Pharmacol* **67**, 376–381.
26 Flatt, J.P. (1992): The biochemistry of energy expenditure. In *Obesity*, eds Bjorntorp, P. & Brodoff, B.N., pp. 100–116. Philadelphia: J.B. Lippincott Company.
27 Foster, D.O. & Frydman, M.L. (1979): Tissue distribution of cold-induced thermogenesis in conscious warm-or cold-acclimated rats reevaluated from changes in tissue blood flow: The dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can J Physiol Pharmacol* **57**, 257–280.
28 Himms-Hagen, J. (1987): Brown adipose tissue and cold acclimation. In *Brown Adipose Tissue*, eds Trayhurn P. & Nicholls, D.G., pp. 214– 268. London: Edward Arnold.
29 Rothwell, N.J. & Stock, M.J. (1987): Brown adipose tissue and diet-induced thermogenesis. In *Brown Adipose Tissue*, eds Trayhurn P. & Nicholls, D.G., pp. 269–298. London: Edward Arnold.
30 Rothwell, N.J., Saville, E. & Stock, M.J. (1981): Acute effects of food, 2-deoxy-D-glucose and noradrenaline on metabolic rate and brown adipose tissue in normal and atropinized lean and obese (fa/fa) Zucker rats. *Pflüg Arch* **392**, 172–177.
31 Rothwell, N.J. & Stock, M.J. (1983): Acute effects of fat and carbohydrate on metabolic rate in normal, cold-acclimated and lean and obese (fa/fa) Zucker rats. *Metabolism* **32**, 371–376.
32 Landsberg, L. & Young, Y.B. (1983): Autonomic regulation of thermogenesis. In *Cellular Thermogenesis*, eds Girardier, L. & Stock, M.J., pp. 99–140. London: Chapman and Hall.