International Journal of Obesity (1995) 19, 539–543

Hepatic mitochondrial respiration and transport of reducing equivalents in rats fed an energy dense diet

S Iossa, MP Mollica, L Lionetti, A Barletta and G Liverini

Department of General and Environmental Physiology, University of Naples, I-80134 Italy

OBJECTIVE: To measure hepatic mitochondrial respiration as well as the ability of hepatic mitochondria to transport reducing equivalents by shuttle systems in rats fed an energy dense diet.

DESIGN: Rats were fed a control (CD) or energy dense (ED) diet for 15 days.

MEASUREMENTS: State 3 and State 4 oxygen consumption were measured in isolated mitochondria using glutamate+malate or pyruvate+malate as substrate. We also measured malate-aspartate shuttle activity and mitochondrial α-glycerophosphate dehydrogenase activity.

RESULTS: ED rats, in comparison with CD rats, showed a significantly greater energy intake without a corresponding greater body weight gain and carcass lipid content. Compared to CD rats, ED rats also showed a significant increase in resting metabolic rate, which was abolished by propranolol. Hepatic mitochondrial state 3 respiration using glutamate+malate or pyruvate+malate as substrate as well as malate-aspartate shuttle activity significantly decreased, while mitochondrial α-glycerophosphate dehydrogenase significantly increased in ED rats compared to CD rats.

CONCLUSIONS: Mitochondrial NADH oxidation is reduced and a greater fraction of cytoplasmic NADH can be transported to the mitochondria through the α-glycerophosphate shuttle and oxidized through the respiratory chain from complex II onwards. This mechanism could lead to a decrease in hepatic metabolic efficiency which is in line with the increased energy expenditure occurring in rats fed an energy dense diet.

Keywords: energy dense diet; liver; diet-induced thermogenesis

Introduction

Young rats provided with a variety of highly palatable human food items (the so-called 'cafeteria diet') overeat, but may resist becoming obese through a facultative increase in energy expenditure known as diet-induced thermogenesis (DIT).[1,2] Much evidence has suggested brown adipose tissue (BAT) [3–5] and liver [6–9] as the possible sites of the DIT in rats fed a cafeteria diet.

As far as BAT is concerned, its role in DIT is suggested by changes found in its thermogenic capacity, which is due to the presence of the unique proton conductance pathway of BAT mitochondria.[3–5]

The possibility of the liver being a site of DIT was put forward by the observation of Berry *et al.*[6] of an increased oxygen consumption of isolated hepatocytes from rats fed a cafeteria diet. Subsequent studies of partial hepatectomy carried out by Ma *et al.*[8–9] also indicated that the liver is involved in DIT. However, unlike the BAT, the mechanism by which the liver could contribute to DIT remains substantially obscure and little is known about the modifications in hepatic metabolism induced by cafeteria feeding.

Our previous results have shown that rats fed an energy dense diet exhibited a propranolol-inhibitable, chronic increase in fasting metabolic rate.[7,10] In these rats, hepatic mitochondrial respiration using succinate as substrate significantly increased, with no variation in ADP/O ratio, and hence in the degree of coupling.[7] However, the use of succinate only allows for the evaluation of the respiratory chain activity from complex II onwards, but gives no information on the variations of the mitochondrial oxidative systems which feed electrons to the respiratory chain. Taking this consideration into account, we have studied, in rats fed an energy dense diet, the respiratory activities of the liver mitochondrial compartment using substrates which involve different modes of transportation into the mitochondria, different dehydrogenases and different sites of entry of reducing equivalents into the mitochondrial respiratory chain. We have also measured the ability of hepatic mitochondria to transport reducing equivalents by shuttle systems.

The results obtained indicate that in rats fed an energy dense diet a decreased hepatic metabolic efficiency could contribute to preventing obesity development.

Materials and methods

Animals and experimental design

Forty male Wistar rats (MORINI, 42020 S.Polo D'Enza (R.E.), Italy) of about 30 days of age were divided into two groups with the same mean body weight (about 85 g), which were given free access to either a control diet (CD) or an energy dense diet (ED) (see Table 1) for 15 days.

All rats were allowed free access to water and were

Correspondence: Prof. G Liverini, Dipartimento di Fisiologia Generale ed Ambientale, Via Mezzocannone 8, I-80134, Napoli, Italy.
Received 21 November 1994; revised 10 February 1995; accepted 24 March 1995

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
Lyophilized meat[e]	–	169
Energy density, kJ/g diet[f]	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 (Nx6.25), 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, 37.56, and 0 for carbohydrate, protein, fat and fiber, respectively.*

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.

Body weights and food intakes were monitored daily to allow calculations of body weight gains and energy intakes (corrected for spillage).

The thyroid state of the animals was monitored by measuring serum total triiodothyronine (T_3) and tetraiodothyronine (T_4) concentrations according to Murphy and Pattee[11] and Brown *et al.*[12] respectively, as well as free T_3 and T_4 according to Romelli *et al.*,[13] using radioimmunoassay kits (Byk Guldem Italia, Milano, Italy).

Measurement of resting metabolic rate

Resting metabolic rate (RMR) was measured to all the rats between 11:00 and 11:30 in the morning with an oxygen consumption monitor (Columbus Instruments, Columbus, OH, USA) in a chamber at 24°C. Although most of the rats became quiet after about 30 min in the chamber, all were allowed to adapt to the conditions for a minimum of 60 min before beginning the measurements. RMR in each animal was obtained over a period of at least 10 min during which time the rat remained quiet. Five CD and five ED rats were injected with saline, RMR was measured and then the rats were anesthetized by i.p. administration of chloral hydrate (40 mg/100 g body wt) and blood and liver were collected. Other five CD and five ED rats were injected with propranolol (dl-propranolol hydrochloride in 0.15 mol/L NaCl, 1 mg/100 g body wt, s.c.) and RMR was measured after 30–60 min. The remaining ten CD and ten ED rats were food-deprived (at 5.00 p.m.) for 16 h, to measure fasting RMR 30–60 min after the injection of either saline or propranolol.

Preparation of isolated mitochondria

After removal, the livers were quickly weighed. All subsequent operations were done at ice-melting temperature and using sterilized solutions and glassware. The livers were finely minced and washed with a medium containing 220 mM mannitol, 70 mM sucrose, 20 mM Tris, pH 7.4, 1 mM EDTA, and 0.1% fatty acid free bovine serum albumin. Tissue fragments were gently homogenized with the same medium (1:10, w/v), in a Potter Elvehjem homogenizer set at 500 rpm (four strokes/min). The homogenate was filtered through sterile gauze and freed of debris and nuclei by centrifugation at 1000 g_{av} for 10 min; the resulting supernatant was then centrifuged at 3000 g_{av} for 10 min, the mitochondrial pellet was washed twice and finally resuspended in a medium containing 80 mM KC1, 50 mM Hepes, pH 7.0, 5 mM KH_2PO_4. The protein content of the mitochondrial suspension was determined by the method of Hartree[14] using bovine serum albumin as the protein standard. Mitochondrial β-hydroxybutyrate and acetoacetate content were measured enzymatically following the appearance or disappearance of NADH by standard spectrophotometric procedures,[15] as described previously.[16] β-hydroxybutyrate/acetoacetate ratio (B/A ratio) was obtained from the ratio of β-hydroxybutyrate to acetoacetate content.

Mitochondrial respiration

Mitochondrial oxygen consumption was measured polarographically with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH, USA), maintained in a chamber at 30°C, using the above suspension medium supplemented with 0.1% bovine serum albumin as the incubation medium. Measurements were made within 2 h, following the isolation of the mitochondria. The mitochondria were allowed to oxidize their endogenous substrates for a few minutes. Pyruvate (10 mM) + malate (2.5 mM), or glutamate (10 mM) + malate (2.5 mM) were then added to determine state 4 oxygen consumption rate. Six minutes later, ADP (at a final concentration of 0.3 mM) was added and state 3 rate was measured. Both the ratio between state 3 and 4 (RCR) and the ADP/O ratios were calculated according to Estabrook.[17]

Shuttle systems activities

The malate-aspartate shuttle system was reconstituted by incubating mitochondria with extramitochondrial components of the shuttle: 2 mM malate, 2 mM aspartate, 5 mM glutamate, 1 mM ADP, 0.15 mM NADH, 20 units of malic dehydrogenase and 25 units of glutamic-oxalacetic transaminase to a final volume of 2 ml. The shuttle activity was measured following the decrease in absorbance at 340 nm of NADH and was expressed as nanomoles of NADH converted to NAD/min × mg protein. Blanks which omitted either mitochondria or shuttle components were routinely assayed and subtracted from the complete system.

Mitochondrial α-glycerophosphate dehydrogenase activity was measured in isolated mitochondria by the method of Lee and Lardy.[18]

Carcass lipid content

Other five CD and five ED rats, after fifteen days of dietary

treatment, were killed for determination of carcass lipid content according to the method of Folch.[19]

Statistical analysis

Data are given as means ± s.e.m. Statistical significance between the means was examined by two-tailed Student's *t*-test or by three-way analysis of variance followed by two-tailed Student's *t*-test. Probability values less than 0.05 were considered to indicate a significant difference.

Materials

ADP, pyruvate, malate, glutamate, aspartate, malic dehydrogenase, glutamic-oxalacetic transaminase, NADH, NAD, acetoacetate, β-hydroxybutyrate, β-hydroxybutyrate dehydrogenase, hydrazine hydrate, dl-propranolol, phenazine methosulfate and iodonitrotetrazolium violet were purchased from Sigma Chemical Co., St Louis, MO, USA. All other reagents used were of the highest purity commercially available.

Results

Body weights, carcass lipid content and energy intakes of CD and ED rats are shown in Table 2. Mean initial and final body weights were not significantly different between the two groups, with a daily body weight gain of about 6.4 and 6.8 g in CD and ED rats, respectively. The energy intake of ED rats during the whole period of treatment was 50% higher than that of CD rats. At the end of the experimental period, carcass lipid content was about 12% both in

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

	CD rats	*ED rats*
Initial body weight, g	87 ± 3	85 ± 3
Final body weight, g	183 ± 10	187 ± 12
Carcass lipid content, %	11.4 ± 0.5	11.9 ± 0.5
Energy intake, kJ[a]	3703 ± 105	5576 ± 248*
Daily energy intake, kJ/g	1.8 ± 0.1	2.5 ± 0.1*

Data are the means ± s.e.m. of five different rats.
**P < 0.0002 compared to CD rats (Two-tailed Student's t-test).*
[a]*The reported values (not corrected for faecal and urinary N losses) are referred to the whole period of treatment (15 days).*

Table 3 Serum total and free T_3 and T_4 levels in CD and ED rats

	CD rats	*ED rats*
Total T_3 (ng/100 ml)	57 ± 3	76 ± 4*
Free T_3 (pg/100 ml)	360 ± 20	516 ± 25**
Total T_4 (μg/100 ml)	4.2 ± 0.2	3.5 ± 0.2
Free T_4 (ng/100 ml)	1.1 ± 0.1	1.2 ± 0.1

Data are the means ± s.e.m. of five different rats.
**P < 0.01 compared to CD rats (Two-tailed Student's t-test).*
***P < 0.001 compared to CD rats (Two-tailed Student's t-test).*

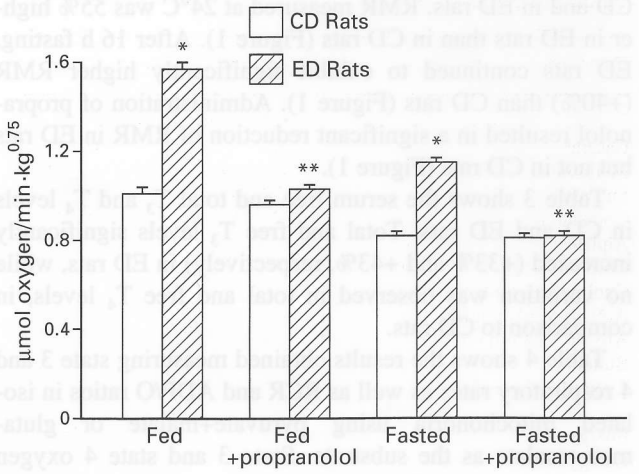

Figure 1 Resting metabolic rate of CD and ED rats: effect of propranolol administration and 16 h food deprivation. Resting metabolic rate was measured at 24°C after the subcutaneous administration of either saline or propranolol, in fed rats and after 16 h of food deprivation, in CD and ED rats. Bars represent means ± s.e.m. of five different rats.
*Significant effect ($P < 0.05$) of diet (Three-way analysis of variance followed by Student's *t*-test).
**Significant effect ($P < 0.05$) of propranolol (Three-way analysis of variance followed by Student's *t*-test).

Table 4 Respiratory parameters in isolated mitochondria from CD and ED rats using pyruvate+malate or glutamate+malate as substrate

	CD rats	*ED rats*
Pyruvate+malate		
State 3	31.7 ± 0.8	25.0 ± 1.1*
State 4	7.9 ± 0.3	6.2 ± 0.6**
RCR	4.0	4.0
ADP/O	2.7 ± 0.1	2.7 ± 0.1
Glutamate+malate		
State 3	96.5 ± 2.3	72.8 ± 2.8*
State 4	12.1 ± 0.7	9.1 ± 0.8**
RCR	8.0	8.0
ADP/O	2.7 ± 0.1	2.7 ± 0.1

Data are the means ± s.e.m. of five different rats.
State 3 and State 4 respiratory rates are expressed as nmol O/min × mg protein
**P < 0.01 compared to CD rats (Two-tailed Student's t-test).*
***P < 0.05 compared to CD rats (Two-tailed Student's t-test).*

Table 5 Reducing equivalents shuttle systems activities in CD and ED rats

	CD rats	*ED rats*
Mitochondrial α-GPDH activity nmol/min × mg protein	33 ± 1	47 ± 1*
Malate-aspartate shuttle activity nmol NADH/min × mg protein	38.6 ± 2.5	21.1 ± 1.3**

Data are the means ± s.e.m. of five different rats.
**P < 0.001 compared to CD rats (Two-tailed Student's t-test).*
***P < 0.01 compared to CD rats (Two-tailed Student's t-test).*

CD and in ED rats. RMR measured at 24°C was 55% higher in ED rats than in CD rats (Figure 1). After 16 h fasting, ED rats continued to exhibit significantly higher RMR (+40%) than CD rats (Figure 1). Administration of propranolol resulted in a significant reduction of RMR in ED rats but not in CD rats (Figure 1).

Table 3 shows the serum free and total T_3 and T_4 levels in CD and ED rats. Total and free T_3 levels significantly increased (+33% and +43%, respectively) in ED rats, while no variation was observed in total and free T_4 levels, in comparison to CD rats.

Table 4 shows the results obtained measuring state 3 and 4 respiratory rates as well as RCR and ADP/O ratios in isolated mitochondria using pyruvate+malate or glutamate+malate as the substrate. State 3 and state 4 oxygen consumption significantly decreased (–21% and –25%, respectively) in ED rats with both the substrates, while no significant variation was found in RCR and ADP/O ratios.

Table 5 shows the results obtained measuring the activity of mitochondrial α-glycerophosphate dehydrogenase, used as an index of the activity of α-glycerophosphate shuttle, as well as the activity of the reconstituted malate-aspartate shuttle. In ED rats we found a significant increase of 42% in α-glycerophosphate dehydrogenase activity and a significant decrease of 45% in malate-aspartate shuttle activity, compared to CD rats.

Discussion

In this work we have studied hepatic mitochondrial oxidative capacity in rats fed an energy dense diet, exhibiting DIT (Table 2 and Figure 1). We have measured mitochondrial respiration with pyruvate+malate or glutamate+malate as the substrate. Glutamate enters the citric acid cycle at α-ketoglutarate and pyruvate can be converted to acetyl-CoA; both the substrates are NAD-linked and electrons enter at site 1 of the electron transport chain. In addition, we have measured NADH transport into the mitochondria by the reconstituted malate-aspartate shuttle, as well as mitochondrial B/A ratio, an indicator of the mitochondrial NADH/NAD^+ ratio.

The results show that state 3 and state 4 oxygen consumption significantly decreased in ED rats (Table 4), while B/A ratio was higher in ED rats compared to CD rats (CD rats = 2.9 ± 0.1; ED rats = 4.2 ± 0.2, $P < 0.05$). The lower respiratory rates could be due to a decrease either in NADH production by the different dehydrogenases or in NADH oxidation by the respiratory chain. However, the higher NADH/NAD^+ ratio found in ED rats allows us to exclude the possibility of a decrease in NADH production and suggests a decrease in NADH oxidation by the respiratory chain. The decreased NADH oxidation is due to a decrease in NADH dehydrogenase activity rather than to an impairment of ATP synthesis system, as the oxygen consumption measured in the presence of an uncoupler was still significantly lower in ED rats compared to CD rats (data not shown). The increased mitochondrial NADH/NAD^+ ratio also causes a significant decrease in the transport of reducing equivalents into the mitochondria by the reconstituted malate-aspartate shuttle in ED rats compared to CD rats (Table 5).

We also assessed the activity of mitochondrial α-glycerophosphate dehydrogenase; this enzyme is rate limiting for α-glycerophosphate shuttle[19] which exchange one cytosolic NADH molecule for one mitochondrial $FADH_2$ molecule. A significant increase in α-glycerophosphate dehydrogenase activity was found in ED rats compared to CD rats (Table 5). This result suggests that in ED rats a greater fraction of cytoplasmic NADH is transported to mitochondria through the α-glycerophosphate shuttle as $FADH_2$. Since $FADH_2$ enters the electron transport chain at a point after the first reaction that translocates hydrogen ions, during $FADH_2$ oxidation less of a gradient is established for driving ATP synthesis and hence there is a decrease in hepatic metabolic efficiency. This mechanism has been proposed to explain DIT found in rats fed low-protein diets.[20,21]

Our present results could have additional metabolic implications. In fact, the increase in mitochondrial NADH/NAD^+ ratio which occurs in ED rats causes a shift of the reaction catalyzed by malate dehydrogenase in favour of malate production at the expense of oxalacetate[22] and this in turn favours the export of malate to the cytosol. Here malate can be converted to oxalacetate and NADH by malic dehydrogenase enzyme, as well as to pyruvate and NADPH by malic enzyme, whose activity greatly increases in overfed rats.[23] It has been recently shown that the cytosolic form of α-glycerophosphate dehydrogenase can use both NADH and NADPH to reduce dihydroxyacetone phosphate to α-glycerophosphate;[24] therefore both NADH and NADPH could be re-transported into the mitochondria through the α-glycerophosphate shuttle. This mechanism would thus lead to a far greater fall in hepatic metabolic efficiency, since it involves not only the reducing equivalents which are generated in the cytosol during glycolysis, but also those which are produced into the mitochondria during the oxidation of pyruvate and fatty acids, whose serum levels increase in rats fed an energy dense diet.[7]

It has been suggested that metabolic signals may participate in the regulation of body weight[25] and it has been proposed the possibility that metabolic products from fat or carbohydrate oxidation may also provide a post-absorptive effect which is involved in the maintenance of energy balance.[25] This mechanism is abolished by hepatic vagotomy, which implies that the liver is essential for the above effect.[26] Hypothalamic injections of β-hydroxybutyrate have been shown to activate sympathetic nervous system (SNS)[27] and to reduce body weight without affecting energy intake.[28] Since β-hydroxybutyrate serum levels increase in ED rats (data not shown), β-hydroxybutyrate can act as a metabolic signal for the activation of the systems responsible for the dietary control of energy metabolism, such as the SNS and thyroid hormones. However, it is also possible that the increased T_3 serum levels are due to enhanced SNS activity, as it is well known that SNS regulates peripheral deiodinase activity.[29]

The observed modifications in the activity of the α-glycerophosphate shuttle can be correlated to the measured increases in serum total and free T_3 levels in ED rats (Table

3). In fact, it has been suggested that thyroid hormones may regulate thermogenesis by increasing the oxidation of cytoplasmic NADH by the α-glycerophosphate shuttle.[30–32] On the other hand, the observed decrease in mitochondrial NADH oxidation can be hardly attributed to enhanced T_3 serum levels. In fact, we have previously shown that hyperthyroidism[33] and cold exposure (that enhances serum T_3 levels)[34] induce an increase in mitochondrial respiratory rates. Thus, it seems that the decreased hepatic mitochondrial NADH oxidation capacity found in the present study should be attributed to other hormonal factors, such as norepinephrine, whose hepatic turnover increases during overfeeding.[35]

This work was supported by C.N.R. and M.U.R.S.T.

References

1 Rothwell NJ, Stock MJ. Energy expenditure of 'cafeteria-fed' rats determined from measurements of energy balance and indirect calorimetry. *J Physiol* 1982; **328**: 371–377.
2 Rothwell NJ, Stock MJ. Diet-Induced Thermogenesis. In: *Mammalian Thermogenesis*, L Girardier, MJ Stock (eds), Chapman and Hall: London, 1983, pp. 208–233.
3 Rothwell NJ, Stock MJ. A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 1979; **281**: 31–35.
4 Rothwell NJ, Stock MJ. Influence of noradrenaline on blood flow to brown adipose tissue in rats exhibiting diet-induced thermogenesis. *Pflug Arch* 1981; **389**: 237–242.
5 Rothwell NJ, Stock MJ. Brown adipose tissue and diet-induced thermogenesis. In: *Brown Adipose Tissue*, P Trayhurn, DG Nicholls (eds), Edward Arnold: London, pp. 269–286.
6 Berry MN, Clark DG, Grivell AR, Wallace PG. The contribution of hepatic metabolism to diet-induced thermogenesis. *Metab* 1985; **34**: 141–147.
7 Liverini G, Iossa S, Barletta A. Hepatic mitochondrial respiratory capacity in hyperphagic rats. *Nutr Res* 1994; **14**: 1671–1682.
8 Ma SWY, Foster DO. Brown adipose tissue, liver, and diet-induced thermogenesis in cafeteria diet-fed rats. *Can J Physiol Pharmacol* 1989; **67**: 376–381.
9 Ma SWY, Nadeau BA, Foster DO. Evidence for liver as the major site of diet-induced thermogenesis of rats fed a 'cafeteria' diet. *Can J Physiol Pharmacol* 1987; **65**: 1802–1804.
10 Liverini G, Iossa S, Lionetti L, Mollica MP, Barletta A. Sympathetically-mediated thermogenic response to food in rats. *Int J Obes* 1995; **19**: 87–91.
11 Murphy BEP, Patte CJ. Determination of thyroxine utilizing the property of protein binding. *J Clin Endocrinol Metab* 1964; **24**: 186.
12 Brown DL, Elkins RP, Ellis SM, Reith WS. Specific antibodies to triiodothyronine hormones. *Nature* 1970; **26**: 359–365.
13 Romelli BP, Pennisi F, Vancheri L. Measurement of free thyroid hormones by column adsorption chromatography and radioimmunoassay. *J Endocrinol Invest* 1978; **1**: 89–94.
14 Hartree EF. Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal Biochem* 1972; **48**: 422–427.
15 Williamson JR, Corkey BE. Assays of intermediates of the citric acid cycle and related compounds by flourometric enzyme methods. *Meth Enzymol* 1969; **13**: 434–513.
16 Iossa S, Barletta A, Liverini G. Different effects of cold exposure and cold acclimation on rat liver mitochondrial fatty acid oxidation and ketone bodies production. *Int J Biochem* 1994; **26**: 425–431.
17 Estabrook RW. Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. *Meth Enzym* 1967; **10**: 41–47.
18 Lee YP, Lardy HA. Influences of thyroid hormones on L-alfa-glicerophosphate and other dehydrogenases in various organs of the rat. *J Biol Chem* 1965; **240**: 1427–1436.
19 Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957; **226**: 497–510.
20 Tyzbir RS, Kunin AS, Sims NM, Danforth E. Influence of diet composition on serum triiodothyronine (T_3) concentration, hepatic mitochondrial metabolism and shuttle system activity in rats. *J Nutr* 1981; **111**: 252–259.
21 Rothwell NJ, Stock MJ, Tyzbir RS. Energy balance and mitochondrial function in liver and brown fat of rats fed 'cafeteria' diets of varying protein content. *J Nutr* 1982; **112**: 1663–1672.
22 Seifter S, England S. Energy metabolism. In: *The Liver: Biology and Pathobiology*, IM Arias, B Jakobi, H Popper, D Schachter, DA Schafritz (eds), Raven Press: New York, 1988, pp. 279–315.
23 Su CY, Lardy H. Induction of hepatic mitochondrial glycerophosphate dehydrogenase in rats by dehydroepiandrosterone. *J Biochem* 1991; **110**: 207–213.
24 Bobyleva V, Kneer N, Bellei M, Battelli D, Lardy HA. Concerning the mechanism of increased thermogenesis in rats treated with dehydroepiandrosterone. *J Bionerg Biomemb* 1993; **25**: 313–321.
25 Bray GA, York DA, Fisler JS. Experimental obesity: a homeostatic failure due to defective nutrient stimulation of the sympathetic nervous system. *Vit Horm* 1989; **45**: 1–125.
26 Langhans W, Egli G, Scharrer E. Selective hepatic vagotomy eliminates the hypophagic effect of different metabolites. *Appetite* 1985; **13**: 255–262.
27 Sakaguchi T, Takahashi M, Bray GA. The lateral hypothalamus and sympathetic firing rate. *Am J Physiol* 1988; **255**: R507–R512.
28 Davis JD, Wirtshafter D, Asin KE, Brief D. Sustained intracerebroventricular infusion of brain fuels reduces body weight and food intake in rats. *Science* 1981; **212**: 81–82.
29 Kaplan MM. Regulatory influences on iodothyronine deiodination in animal tissues. In: *Thyroid hormone metabolism*, G Hennemann (ed). Marcel Dekker: New York, 1986, pp. 231–254.
30 Lardy H, Shrago E. Biochemical aspects of obesity. *Ann Rev Biochem* 1990; **59**: 689–710.
31 Rognstad R. Futile hydrogen cycling in liver cells from triiodothyronine treated rats. *Biochem Biophys Res Comm* 1977; **78**: 881–888.
32 Werner HV, Berry MN. Stimulatory effects of thyroxine administration on reducing-equivalent transfer from substrate to oxygen during hepatic metabolism of sorbitol and glycerol. *Eur J Biochem* 1974; **42**: 315–324.
33 Iossa S, Liverini G, Barletta A. Effect of thyroid state and cold exposure on mitochondrial protein mass and function. *J Endocrinol* 1991; **131**: 67–73.
34 Iossa S, Liverini G, Barletta A. Physiological changes due to cold adaptation in rat liver. *Cell Physiol Biochem* 1991; **1**: 226–236.
35 Young JB, Landsberg L. Effect of diet and cold exposure on norepinephrine turnover in pancreas and liver. *Am J Physiol* 1979; **236**: E524–E533.