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Relationship between membrane potential and respiration rate in isolated liver mitochondria from rats fed an energy dense diet

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Abstract

We studied the relationship between membrane potential and respiration rate in isolated liver mitochondria from rats fed an energy dense diet. We conceptually divided the system into blocks of reactions that produced or consumed mitochondrial membrane potential and then measured the kinetic response of these blocks of reactions to this potential using NAD-linked and FAD-linked substrates. We show that decreased respiration rate with an NAD-linked substrate is accounted for by decreased kinetic response of the substrate oxidation pathway to the potential. No variation in the kinetic response of the above blocks of reactions to the potential was found using an FAD-linked substrate. These results indicate that FAD-linked and NAD-linked pathways are differently affected in rats fed an energy dense diet. (Mol Cell Biochem **158:** 133–138, 1996)

Key words: mitochondria, oxygen consumption, top-down elasticity analysis, energy dense diet

Introduction

We have previously shown that rats fed an energy dense diet become hyperphagic and exhibit a propranolol-inhibitable chronic increase in fasting metabolic rate (diet-induced thermogenesis, DIT) which avoids obesity development [1–3]. Brown adipose tissue (BAT) was initially suggested as the major effector organ of DIT in hyperphagic rats [4, 5]. On the other hand, the results of Berry *et al.* [6] and Ma *et al.* [7, 8] suggested that, in addition to BAT, the liver can play a role in DIT in hyperphagic rats. Moreover, in rats fed an energy dense diet we found that liver mitochondrial respiratory rates significantly increased using succinate as substrate while significantly decreased using NAD-linked substrates [1, 2].

Changes in state 3 and state 4 respiration in isolated mitochondria may reflect changes in either respiratory chain or adenine nucleotide translocase or ATP synthase or substrate permeation or substrate dehydrogenation activity [9–11]. Therefore, we thought it would be suitable to apply top-down elasticity analysis to identify the sites responsible for the changes in the respiration rates found in isolated mitochondria from rats fed an energy dense diet. In fact, the application of top-down elasticity analysis allows the identification of the sites of action of external effectors within metabolic pathways [12, 13]. This approach involves dividing a system conceptually into blocks of reactions that produce and that consume a common intermediate [12], which is the mitochondrial membrane potential ($\Delta\Psi$) in the case of respiration in isolated mitochondria. Analysis involves measuring the changes in the overall kinetic responses to $\Delta\Psi$ of the block of reactions that produce $\Delta\Psi$ (substrate oxidation pathway) and those that consume it (proton leak and phosphorylating system) [12, 13]. This approach has been previously used to investigate the mechanism by which thyroid hormone [13–16] and glucagon [17] alter the respiration rates of isolated liver mitochondria.

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We applied top-down elasticity analysis to isolated liver mitochondria using an FAD-linked substrate (succinate) or an NAD-linked substrate (glutamate+malate) to involve different modes of transportation into the mitochondria, different dehydrogenases and different sites of entry of reducing equivalents into the mitochondrial respiratory chain.

This study shows a decrease in substrate oxidation pathway with the NAD-linked substrate in isolated mitochondria from rats fed an energy dense diet compared to rats fed a control diet, while no variation was found with the FAD-linked substrate.

Materials and methods

Treatment of animals

Male Wistar rats of about 30 days of age (bred in the animal house of the Department of Biochemistry, University of Cambridge, UK) were divided into two groups with the same mean body weight (about 80 g). One group (CD rats) was fed a control diet (percentage of total metabolizable energy: 29 protein, 10.6 lipid and 60.4 carbohydrate, J/J; 15.88 kJ gross energy/g) while the other group (ED rats) was fed 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/g; percentage of total metabolizable energy: 29 protein, 50 lipid, 21 carbohydrate, J/J, 19.85 kJ gross energy/g) [18]. The diet treatment lasted fifteen days. Body weights and food intakes were monitored daily to allow calculations of body weight gain and gross energy intake. At the end of the experimental period these rats were used for preparations of isolated mitochondria.

Other male Wistar rats of 30 days of age (Charles River Italia, Calco, Como, Italy) were kept in the animal house of the Department of General and Environmental Physiology, University of Naples, Italy and were divided into two groups which were fed either the control diet or the energy dense diet for fifteen days. At the end of the experimental period these rats were used for energy balance measurements.

All rats were housed individually in suspended wire-bottom cages at 24°C under an artificial circadian 12 h light/12 h dark cycle.

Energy balance measurements

Body weights and food intakes were monitored daily to allow calculations of body weight gain and gross energy intake.

Digestible energy intake (taking into account the food spillage) was obtained by subtracting the energy measured in the faeces from the gross energy intake as measured from daily food consumption. Metabolizable energy (ME) intake was expressed as digestible energy intake × 0.96 [19]. The gain in energy was obtained by subtracting the energy content of an initial group (five rats killed for energy content at the beginning of the study) from that of each of the two experimental groups. At the end of the experiment the animals were anesthetized by the intraperitoneal injection of chloral hydrate (40 mg/ 100 g b.w.) and were killed by decapitation. Then, 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 dessicated 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 control and energy dense diet, were measured with a Parr adiabatic calorimeter calibrated with dry benzoic acid standard. Energy expenditure was calculated from the difference between ME intake and energy gain.

Preparation of mitochondria

Rats were killed by decapitation between 08:30 and 09:30 h, without any previous food deprivation. The order in which CD and ED rats were killed was random. Mitochondria were prepared simultaneously from CD and ED rats. Livers were quickly removed, weighed and finely minced and washed with a medium containing 220 mM mannitol, 70 mM sucrose, 20 mM Hepes, 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 large dounce homogenizer with medium fit plunger. The homogenate was filtered through sterile gauze and freed of debris and nuclei by centrifugation at 1,000 × g_{av} for 10 min; the resulting supernatant was centrifuged at 3,000 × g_{av} for 10 min, the mitochondrial pellet was washed twice and finally resuspended in a medium containing 80 mM KCl, 50 mM Hepes, pH 7.0, 5 mM KH_2PO_4, 1 mM EGTA. The protein content of the mitochondrial suspension was determined by the method of Hartree [20] using bovine serum albumin as the protein standard.

Measurement of oxygen consumption and mitochondrial membrane potential ($\Delta\Psi$)

Mitochondrial oxygen consumption was measured polarographically with a Clark-type electrode (Rank Brothers, Bottisham, Cambridge). $\Delta\Psi$ was measured with an electrode sensitive to methyltriphenylphosphonium cation ($TPMP^+$) as described by Brown and Brand [21]. This was coupled to a reference electrode, and both electrodes were inserted

through an air-tight port into the oxygen vessel to monitor oxygen consumption and $\Delta\Psi$ simultaneously. The $TPMP^+$ electrode was calibrated for each incubation with 1 μM additions of $TPMP^+$ up to 5 μM. A binding correction factor for $TPMP^+$ of 0.4 [21] was assumed. $\Delta\Psi$ was calculated as 60•log(0.4•accumulation ratio of $TPMP^+$). Incubations were carried out at 30°C in 3.3 ml of the above suspension medium supplemented with 0.1% defatted bovine serum albumin. Stock 9% bovine serum albumin was defatted by the method of Chen [22].

For mitochondria (1 mg of protein/ml) oxidizing succinate (10 mM) the above medium also contained 5 μM rotenone and nigericin (80 ng/ml) which was used to collapse the pH difference across the mitochondrial inner membrane (ΔpH) and allow the whole of the protonmotive force across the mitochondrial inner membrane to be expressed as $\Delta\Psi$.

For mitochondria (0.8 mg of protein/ml) oxidizing glutamate (10 mM) and malate (2.5 mM) the medium was supplemented with $MgCl_2$ (5 mM), glucose (20 mM) and ADP (100 μM) to allow the titrations in presence of hexokinase. Nigericin was not used with glutamate+malate as substrate, as it inhibited respiration, perhaps because of secondary effects of a relatively acid matrix pH on NADH dehydrogenase [23]. It is valid to ignore any effects of ΔpH as long as there is a reasonably constant relationship between $\Delta\Psi$ and ΔpH [24].

Experimental approach to top-down elasticity analysis

Kinetic response of proton leak to $\Delta\Psi$

If the activity of the respiratory chain is titrated with inhibitors in the presence of oligomycin (1 μg/mg of protein) to prevent ATP synthesis, the resulting titration curve of $\Delta\Psi$ against respiration rate represents the kinetic response of the proton leak to changes in $\Delta\Psi$ [12–15]. For mitochondria oxidizing succinate, titrations were made with malonate up to 5 mM. For mitochondria oxidizing glutamate and malate, titrations were made with KCN up to 970 μM.

Kinetic response of phosphorylating system to $\Delta\Psi$

If the titrations with respiratory chain inhibitors are repeated in State 3 in the absence of oligomycin and the values are corrected for the amount of oxygen required to balance the rate of proton leakage at each $\Delta\Psi$ measured, the resulting titration curve reflects the kinetic response of the phosphorylating system to $\Delta\Psi$ [12–17]. For mitochondria oxidizing succinate, state 3 was achieved by adding ADP 0.3 mM and titrations were made with malonate up to 5 mM. For mitochondria oxidizing glutamate and malate, state 3 was achieved by adding hexokinase (1.25 units/ml) and titration were made with KCN up to 970 μM.

Kinetic response of substrate oxidation pathway to $\Delta\Psi$

If we manipulate the ATP synthesis capacity, the relationship between the rate of respiration and $\Delta\Psi$ will reflect the kinetics of substrate oxidation pathway to $\Delta\Psi$ [12–14]. For mitochondria oxidizing succinate, titrations were made with oligomycin up to 1 μg/mg of protein. For mitochondria oxidizing glutamate and malate, titrations were made with hexokinase from 0–1.25 units/ml.

Statistics and materials

All statistical analysis was performed by the two tailed Students *t*-test; P values less than 0.05 were considered significant. Hexokinase, oligomycin, nigericin, malate, glutamate and ADP were obtained from SIGMA Chemical Co. (Poole, Dorset, UK). All other reagents used were of the highest purity commercially available.

Results

Table 1 shows the results of energy balance measurements in CD and ED rats. Mean initial and final body weights were not significantly different between the two groups, yielding a daily body weight gain of about 7 g in both groups. The ME intake and energy expenditure of ED rats during the whole period of treatment were 28 and 51% higher, respectively, than in CD rats. As far as the rats bred in the animal house of the Department of Biochemistry, University of Cambridge, there were no significant differences in daily body weight gain between CD and ED rats, while ED rats showed a significant increase in gross energy intake (47%). State 3 and state 4 oxygen consumption was measured in isolated mitochondria using succinate or glutamate+malate as substrate. No significant difference was found in state 3 and state 4 respiration using succinate as substrate (Fig. 1c). State 3 and state 4 oxygen consumption significantly decreased in ED rats with glutamate+malate as substrate (State 3: 178.5 ± 2.7 and 153.2 ± 1.5 in CD and ED rats, respec-

Table 1. Energy balance of CD and ED rats

	CD Rats	ED Rats
Initial body weight, g	87 ± 3	85 ± 3
Final body weight, g	183 ± 10	187 ± 12
ME intake, W	1.97 ± 0.02	2.53 ± 0.15*
Energy gain, W	0.78 ± 0.04	0.74 ± 0.04
Energy expenditure, W	1.19 ± 0.07	1.79 ± 0.08*
Gross efficiency, %	40 ± 3	29 ± 2*

Data are the means ± S.E.M. of 4 different rats. ME intake, energy gain and energy expenditure are referred to the whole period of treatment (15 days). *$p < 0.05$ compared to CD rats. Gross efficiency = (body energy gain, ME intake) × 100.

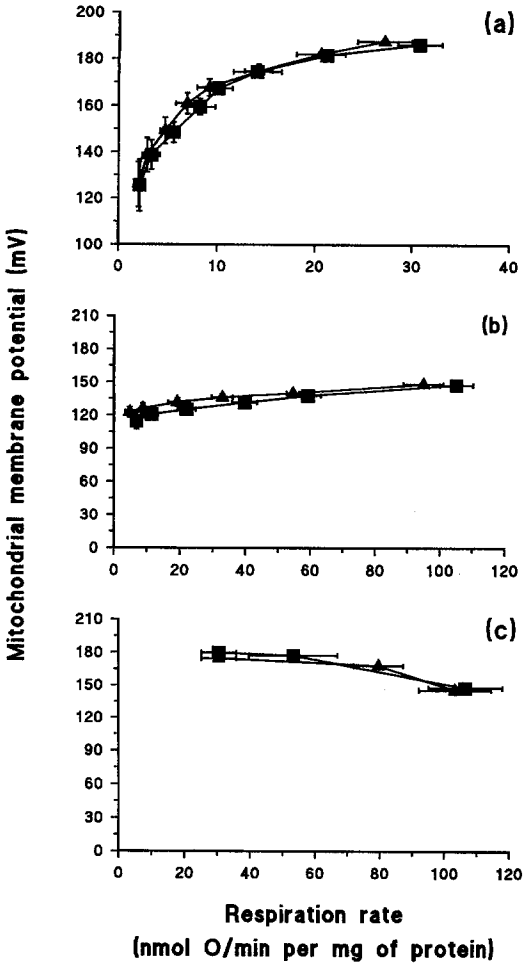

Fig. 1. Comparison of the kinetic responses of the proton leak (a), phosphorylating system (b) and substrate oxidation pathway (c) to $\Delta\Psi$ in liver mitochondria, oxidizing succinate as substrate, isolated from CD (■) and ED (▲) rats. (a) Kinetic response of the mitochondrial proton leak to $\Delta\Psi$: malonate titration (up to 5 mM) of oligomycin-inhibited respiration (1 μg/mg protein); (b) Kinetic response of the phosphorylating system to $\Delta\Psi$: malonate titration (up to 5 mM) of the state 3 (0.3 mM ADP) in the absence of oligomycin, minus the malonate titration in the presence of oligomycin; (c) Kinetic response of the substrate oxidation to $\Delta\Psi$: oligomycin titration (up to 1 μg/mg protein) from state 3 (0.3 mM ADP) to state 4. Measurements of respiration rate and mitochondrial membrane potential to obtain titration curves, were performed in triplicate. These replicates were averaged to give single values for each CD and ED rat. The means of three independent experiments were averaged to give the mean values reported in each point. All errors shown refer to the error of these means of means.

tively, n = 3, $p < 0.05$; State 4: 14.5 ± 1.6 and 11.2 ± 1.1 in CD and ED rats, respectively, n = 3, $p < 0.05$).

Figure 1 shows the comparison of the kinetic responses of proton leak (a), phosphorylating system (b) and substrate oxidation pathway (c) to $\Delta\Psi$ in liver mitochondria from CD and ED rats using succinate as substrate. No difference was found between CD and ED rats.

Top-down elasticity analysis was repeated in mitochondria from CD and ED rats using glutamate+malate as substrate.

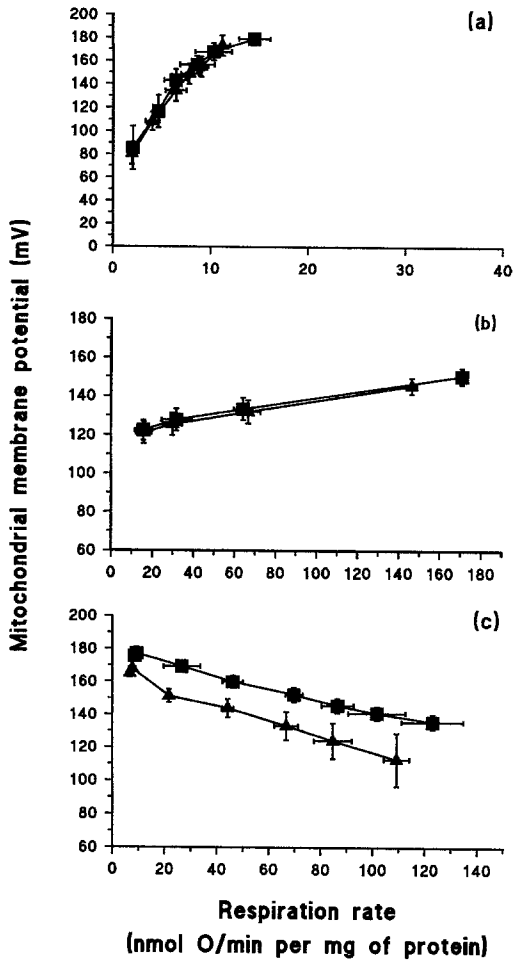

Fig. 2. Comparison of the kinetic responses of the proton leak (a), phosphorylating system (b) and substrate oxidation pathway (c) to $\Delta\Psi$ in liver mitochondria, oxidizing glutamate+malate as substrate, isolated from CD (■) and ED (▲) rats. (a) Kinetic response of the mitochondrial proton leak to $\Delta\Psi$: KCN titration (up to 970 μM) of oligomycin-inhibited respiration (1 μg/mg protein); (b) Kinetic response of the phosphorylating system to $\Delta\Psi$: KCN titration (up to 970 μM) of state 3 (hexokinase 1.25 units/ml) in the absence of oligomycin, minus the KCN titration in the presence of oligomycin; (c) Kinetic response of the substrate oxidation to $\Delta\Psi$: Titration with hexokinase (up to 1.25 units/ml), state 4 was achieved by adding saturating amount of oligomycin (1 μg/mg protein). Measurements of respiration rate and mitochondrial membrane potential to obtain titration curves, were performed in triplicate. These replicates were averaged to give single values for each CD and ED rat. The means of three (a, b) or four (c) independent experiments were averaged to give the mean values reported in each point. All errors shown refer to the error of these means of means.

Figure 2a, b shows no difference in the kinetic responses of proton leak and phosphorylating system to $\Delta\Psi$ between CD and ED rats. On the other hand, Fig. 2c shows that the kinetic response of substrate oxidation pathway to $\Delta\Psi$ is lower in mitochondria from ED rats. In fact, the plot of mitochondrial oxygen rate against $\Delta\Psi$, obtained with mitochondria from ED rats, lies under the plot obtained with mitochondria from CD

rats, such that, at any given $\Delta\Psi$, the oxygen consumption rate is lower in mitochondria from ED rats than in mitochondria from CD rats. This result indicates that the substrate oxidation pathway is inhibited in mitochondria from ED rats compared to CD rats with glutamate+malate as substrate.

Discussion

In the present study, ED rats showed a significant increase in ME intake as well as in energy expenditure with no variation in body weight gain compared to CD rats (Table 1). Therefore, ED rats showed a decreased gross efficiency (Table 1). These variations are similar to those observed in cafeteria-diet fed rats, exhibiting DIT [4, 5]. In fact, our energy dense diet combines two important characteristics of cafeteria diets: an high-fat content and the presence of a meat component, which is among the flavours most preferred by rats [25, 26]. At present we cannot identify the factors responsible for the variations found by us in ED rats; however, it should be noted that a number of works have shown that high-fat-low-carbohydrate diets 'per se' are unable to induce hyperphagia and/or increase in energy expenditure [27–30]. In addition, high-fat diet tend to increase rather than decrease gross efficiency because of the lower cost of lipid storage.

We also investigated the oxidative capacities and the kinetic responses of proton leak, phosphorylating system and substrate oxidation to $\Delta\Psi$ in isolated liver mitochondria from CD and ED rats using top-down elasticity analysis. This analysis was applied using various substrates (succinate and glutamate+malate) to involve different modes of transportation into the mitochondria, different dehydrogenases and different sites of entry of reducing equivalents into the mitochondrial respiratory chain. Succinate is FAD-linked and enters the electron transport chain at site 2, while glutamate is NAD-linked and electrons enter at site 1 of the electron transport chain. The results show that state 4 and state 3 respiratory rates significantly decreased in ED rats using glutamate+malate as substrate, while no variation was found using succinate as substrate. It should be noted that, using an energy dense diet with lower protein content, we have previously found an increase in succinate supported respiration in isolated liver mitochondria [1].

The unchanged respiration rate using succinate as substrate could be due to simultaneous and opposite changes in the activity of substrate oxidation pathway and proton leak+phosphorylating system. The application of top-down elasticity analysis shows that the unchanged respiratory rate is due to unchanged kinetic responses of proton leak, phosphorylating system and substrate oxidation to $\Delta\Psi$ (Fig. 1).

The lower respiratory rates with glutamate+malate could be due to variations in the activity of substrate oxidation pathway and/or proton leak + phosphorylating system. The plot of the kinetic response of the proton leak to $\Delta\Psi$ (Fig. 2a) shows that there is no difference in the rate of the proton leak pathway at any given $\Delta\Psi$ between CD and ED rats. Similarly, there is no difference in the rate of the phosphorylating system at any given value of $\Delta\Psi$ (Fig. 2b). Therefore, changes in state 4 and state 3 respiration in ED rats using glutamate+malate as substrate are not caused by changes in the kinetic response of proton leak and phosphorylating system to $\Delta\Psi$, respectively. On the other hand, Fig. 2c shows a decrease in the rate of the substrate oxidation reactions at a given $\Delta\Psi$ with glutamate+malate as substrate. It follows that changes in respiration can be due to changes in respiratory chain, substrate transport systems and substrate dehydrogenases. The unchanged kinetic response of substrate oxidation pathway to $\Delta\Psi$ obtained using succinate as substrate suggests that there is no difference in the respiratory chain from complex II onwards. Therefore, the decrease in mitochondrial respiration in ED rats with glutamate+malate as substrate involves significant changes in NADH dehydrogenase and/or in NADH production (substrate transport systems and substrate dehydrogenases). Our present results do not allow us to discriminate between changes in NADH production and NADH dehydrogenase. However, in previous experiments we showed an increase in the $NADH/NAD^+$ ratio in mitochondria from ED rats [2]: this result excludes the possibility of a decrease in NADH production and suggests a decrease in NADH oxidation by NADH dehydrogenase.

Since it is well known that cellular respiration is principally NAD-linked [31], the decreased NADH-oxidation should lead to a fall in hepatic respiration. However, preliminary results obtained in isolated hepatocytes have shown that hepatic respiration significantly increased in ED rats. This apparent discrepancy can be explained taking into account our previous hypothesis [2] that the impairment of NADH dehydrogenase and the following increase in mitochondrial NADH would favor the production of malate from oxalacetate [32] and its export to the cytosol. Here malate can be converted to pyruvate or oxalacetate, with the formation of NADPH or NADH. The reducing equivalents can be finally retransported into the mitochondria through the α-glycerophosphate shuttle, whose activity increases in ED rats [2]. This mechanism would thus lead to a fall in hepatic metabolic efficiency, which is in line with the increased energy expenditure found in ED rats.

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References

1. Liverini G, Iossa S, Barletta A: Hepatic mitochondrial respiratory capacity in hyperphagic rats. Nutr Res 14: 1671–1682, 1994
2. 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. Int J Obes 19: 539–543, 1995
3. Liverini G, Iossa S, Lionetti L, Mollica MP, Barletta A: Sympathetically-mediated thermogenic response to food in rats. Int J Obes 19: 87–91, 1995
4. Rothwell NJ, Stock MJ: A role for brown adipose tissue in diet-induced thermogenesis. Nature 281: 31–35, 1979
5. Rothwell NJ, Stock MJ: Brown adipose tissue in diet-induced thermogenesis. In: P. Trayhurn and D.G. Nicholls (eds). Brown Adipose Tissue. Edward Arnold, London,1986, pp 269–286
6. Berry MN, Clark DG, Grivell AR, Wallace PG: The contribution of hepatic metabolism to diet induced thermogenesis. Metabolism 34: 141–147, 1985
7. Ma SWY, Nadeu BA, Foster DO: Evidence for liver as the major site of the diet-induced thermogenesis of rats fed a 'cafeteria' diet. Can J Physiol Pharmacol 65: 1802–1804, 1987
8. Ma SWY, Foster DO: Brown adipose tissue, liver, and diet-induced thermogenesis in cafeteria diet-fed rats. Can J Physiol Pharmacol 67: 367–381, 1989
9. Hansford RG: Bioenergetics in aging. Biochim Biophys Acta 726: 41–80, 1983
10. La Noue KF, Schoolwerth AC: Metabolite transport in mitochondria. Annu Rev Biochem 48: 871–922, 1979
11. Nicholls DG: Bioenergetics, an Introduction to the Chemiosmotic Theory. Academic Press, London, 1982
12. Brand MD: The proton leak across the mitochondrial inner membrane. Biochim Biophys Acta 1018: 128–133, 1990
13. Harper ME, Ballantyne JS, Leach M, Brand MD: Effects of thyroid hormones on oxidative phosphorylation. Bioch Soc Trans 21: 785–792, 1993
14. Hafner RP, Nobes CD, McGown AD, Brand MD: Altered relationship between protonmotive force and respiration rate in non-phosphorylating liver mitochondria isolated from rats of different thyroid hormone status. Eur J Biochem 178: 511–518, 1988
15. Hafner RP, Leake MJ, Brand MD: Hypothyroidism in rats decreases mitochondrial inner membrane cation permeability. FEBS Lett 248: 175–178, 1989
16. Hafner RP, Brown GC, Brand MD: Thyroid-hormone control of state-3 respiration in isolated rat liver mitochondria. Biochem J 265: 731–734, 1990
17. Brand MD, D'Alessandri L, Reis HMGPV and Hafner RP: Stimulation of the electron transport chain in mitochondria isolated from rats treated with mannoheptulose or glucagon. Arch Biochem Biophys 283: 278–284, 1990
18. Iossa S, Lionetti L, Mollica MP, Barletta A and Liverini G: Thermic effect of food in hypothyroid rats. J Endocrinol 148: 167–174, 1996
19. Barr HG, McCracken KJ: High efficiency of energy utilization in 'cafeteria' and force-fed rats kept at 29°C. Br J Nutr 51: 379–387, 1984
20. Hartree EF: Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal Biochem 48: 422–427, 1972
21. Brown GC, Brand MD: Thermodynamic control of electron flux through mitochondrial cytochrome bc1 complex, Biochem J 225: 399–405, 1985
22. Chen RF: Removal of fatty acid from serum albumin by charcoal treatment. J Biol Chem 242: 173–181, 1967
23. Brand MD, Harper ME, Taylor HC: Control of the effective P/O ratio of oxidative phosphorylation in liver mitochondria and hepatocytes. Biochem J 291: 739–748, 1993
24. Brown GC, Hafner RP, Brand MD: A 'top-down' approach to the determination of control coefficients in metabolic control theory. Eur J Biochem 188: 321–323, 1990
25. Naim M, Brand JG, Kare MR, Carpenter RG: Energy intake, weight gain and fat deposition in rats fed flavored nutritionally controlled diets in a multichoice ('cafeteria') design. J Nutr 115: 1447–1458, 1985
26. Allard M, LeBland J: Effect of cold acclimation, cold exposure and palatability on postprandial thermogenesis in rats. Int J Obes 12: 169–178, 1988
27. Brady LJ, Hopper CL: Effect of diet and starvation on hepatic mitochondrial function in the rat. J Nutr 113: 2129–2137, 1983
28. Rothwell NJ, Stock MJ, Warwick BP: Energy balance and brown fat activity in rats fed cafeteria diet or high-fat, semisynthetic diets at several levels of intake. Metab 34: 474–480, 1985
29. Brady PS, Knoeber CM, Brady LJ: Hepatic mitochondrial and peroxisomal oxidative capacity in riboflavin deficiency: effect of age, dietary fat and starvation. J Nutr 116: 1992–1999, 1986
30. Mazier MJP, LeBland J: How dietary fat intake affects cold tolerance and energy balance in the rat. Nutr Res 11: 807–818, 1991
31. Murphy MP: Slip and leak in mitochondrial oxidative phosphorylation. Biochim Biophys Acta 977: 123–141, 1989
32. Seifter S, England S: Energy Metabolism. In: I.M. Arias, B. Jakobi, H. Popper, D. Schachter, D.A. Schafritz (eds). The liver: Biology and Pathobiology. Raven Press: New York, 1988, pp 279–315