Biochimica et Biophysica Acta, 1107 (1992) 159–164

BBAMEM 75651

Effect of sympathetic de-activation on thermogenic function and membrane lipid composition in mitochondria of brown adipose tissue

Marc Goubern [a], Marie-France Chapey [a], Claude Senault [b], Marie-Claude Laury [a], Jocelyne Yazbeck [a], Bruno Miroux [c], Daniel Ricquier [c] and René Portet [a]

[a] *Laboratoire d'Adaptation Energétique à l'Environnement, EPHE, Paris (France),*
[b] *U 286 INSERM, Faculté de Médecine X. Bichat, Paris (France)*
and [c] *Centre de Recherche sur l'Endocrinologie Moléculaire et le Développement, CNRS, Meudon (France)*

(Received 30 December 1991)

Key words: Brown adipose tissue; Phospholipid composition; Mitochondrion; GDP binding; Uncoupling protein; Energization parameter; Proton conductance

Male Long-Evans rats (9 weeks of age) were exposed to cold (5°C) for 10 days. Then, sympathetic de-activation of brown adipose tissue (BAT) was performed either by BAT surgical denervation (Sy) or by warm re-exposure at 28°C (WE) for 4 days. The incidence of the two treatments on thermogenic activity of BAT mitochondrial membranes and their lipid composition was investigated. Sy and WE induced a large decrease in GDP binding on the uncoupling protein (UCP) (43% and 82%, respectively). Several parameters of mitochondrial energization were investigated. Sy and WE substantially decreased UCP-dependent proton conductance (CmH^+) over the whole range of protonmotive force. CmH^+ showed greater variation than GDP binding. The low basal UCP-independent CmH^+ was the same in all groups. Comparison of GDP binding and CmH^+ with UCP content which is not modified revealed a masking of both the nucleotide binding site and the proton channel. Sy and WE induced the same increase of phosphatidylcholine to phosphatidylethanolamine ratio (16%) but had opposite effects on fatty acid unsaturation. The results were discussed with reference to functional significance of these variations in BAT mitochondrial thermogenic activity and lipid composition.

Introduction

Brown adipose tissue (BAT) is the major site of both non-shivering thermogenesis and diet-induced thermogenesis [1,2]. It has been found to be a common effector for both thermic and weight regulation [3]. In this tissue the respiration rate is controlled by an uncoupling protein located in the inner mitochondrial membrane which acts as a proton translocator [4]. The proton extrusion linked to substrate oxidation is dissipated to produce heat rather than used to generate a protonmotive force driving ATP synthesis.

The possibility of relationships between the composition and the function of biomembranes has attracted attention [5,6]. Stimulation of BAT by exposure to cold is accompanied by substantial modifications in mitochondrial composition. Fatty acid unsaturation increases and the relative proportion of phospholipid classes is modified [7,8]. However, a study of BAT thermogenesis induced by essential fatty acid deficiency has shown no relationship between BAT activity as assessed by GDP binding and fatty acid unsaturation [8]. Consequently further studies are required to resolve the question of relationships between the composition and function of BAT mitochondrial membranes.

The development of thermogenesis in BAT is under the control of hypothalamic thermoregulatory centers and the effector pathway involves the sympathetic system via the release of norepinephrine [9,10].

In the present study the possible involvement of the sympathetic system on the structure/function relationships is investigated by decreasing cold-induced BAT thermogenesis either by surgical denervation of the tissue or by warm re-exposure.

The aim of the work is to study the functional importance of such deactivation which is demonstrated by comparing several parameters of mitochondrial energization including membrane potential, protonmotive force and effective proton conductance with classical GDP binding test and uncoupling protein content. In addition the possibility of modifications of mitochon-

Correspondence: M. Goubern, Laboratoire d'Adaptation Energétique à l'Environnement, EPHE, 11 place Marcellin Berthelot, 75231 Paris Cedex 05, France.

drial membrane lipid composition being related to changes in thermogenic activity is examined.

Material and Methods

Animals and diets

Male Long-Evans rats reared at 23°C were exposed to cold (5°C) at 9 weeks of age. They received ad libitum a standard laboratory diet (UAR A03: proteins 23.5%, lipids 5%, carbohydrates 49.8%). After 10 days of cold exposure a first group of rats was warm re-exposed at 28°C (thermal neutrality) for 4 days. In a second group, interscapular BAT was surgically denervated: animals were anesthetized with chloral hydrate (300 mg/kg i.p.), the five pairs of nerves supplying the two BAT lobes were isolated and cut without damage to the tissue. Then they were kept at 5°C for 4 days. Two control groups kept at 5°C and 28°C were killed at the same time by decapitation after a 14 day experimental period. These control groups were introduced to indicate the lower limit (thermal neutrality) and the upper limit (10-days cold exposure which corresponded to minimal and maximal stimulation of BAT mitochondrial proton conductance in the rat [17]). Interscapular BAT was rapidly dissected out and used for experiments.

Isolation of mitochondria

Mitochondria were isolated by Cannon and Lindberg's method [11]. After the last centrifugation, they were kept in 250 mM sucrose, 5 mM Tes (pH 7.2). Mitochondrial protein yield was determined by spectrophotometric assay of cytochrome-*c* oxidase method described by Yonetany and Ray [12].

GDP binding

Purine nucleotide binding was assessed by the method described by Nicholls [13]. Various concentrations of GDP were used (0.1, 1, 3 μM) to test the linearity of a Scatchard plot and to obtain a satisfactory estimation of the high-affinity binding sites.

Respiratory studies

Oxygen consumption and membrane potential were determined simultaneously. Oxygen uptake was measured polarographically with a Clark-type electrode and membrane potential with a laboratory constructed tetraphenylphosphonium (TPP^+) selective electrode [14]. Measurements were carried out in a medium containing 100 mM sucrose, 20 mM Tes, 4 mM KH_2PO_4, 2 mM $MgCl_2$, 1 mM EGTA, 1% w/v fatty acid free serum albumin, 5 μM rotenone (final volume 1.5 ml). 0.5 mg mitochondrial protein was added per assay. Respiration rate was modulated by varying α-glycerophosphate substrate concentration. Being dependant on the native CmH^+, state 4, which is investigated here, provides a valuable criterion for the assessment of BAT thermogenic activity [15].

Membrane potential was corrected to take into account the activity coefficient of TPP^+ into the matrix according to Rottenberg [16] (about 50 mV in these experimental conditions).

To calculate the proton conductance of the mitochondrial inner membrane CmH^+ (nmol protons per mg proteins flowing through the membrane per min per mV of protonmotive force Δp), proton current J_{H^+} was calculated from the respiratory rate, J_O, on the assumption that six protons were extruded by the respiratory chain per two electrons transferred to oxygen (α-glycerophosphate oxidation). CmH^+ was calculated according to the following equation:

$$CmH^+ = \frac{J_{H^+}}{\Delta p} = \frac{J_O(H^+/O)}{\Delta p}$$

where J_O was expressed in natom O per min per mg protein and $\Delta p = \Delta\psi - 59\Delta pH$ was expressed in mV at 25°C.

Δp was calculated according to the linear relationship previously established experimentally between $\Delta\psi$ and Δp (in these conditions: $59\Delta pH = 0.73\Delta\psi - 76$ (in mV)). Technical conditions applied here to calculate CmH^+ have been discussed in details elsewhere [17,18].

Uncoupling protein determination

Western blots were used. Ewes were injected with purified BAT uncoupling protein of cold-adapted rats in order to raise antibodies. Rat mitochondrial proteins were separated by polyacrylamide gel electrophoresis and electroeluted from the gels to nitrocellulose. Activity staining was effected with anti-ewe IgG conjugated to alkaline phosphatase to develop coloration. Densitometric scanning of blots was performed with a Shimazu CS930 densitometer [19,20].

Mitochondrial proteins were assayed by the method of Lowry et al. [21].

Lipid composition

Total lipids were extracted as described by Folch et al. [22]. Phospholipid classes were separated by two-dimensional thin-layer chromatography [23]. Fatty acids were methylated with sulfuric methanol, after extraction methyl esters were analyzed by gas-liquid chromatography using a 15% CP-SIL 84 on a chromosorb WHP 80-100 mesh column previously described [8]. Organic phosphorus was determined as described by Bartlett [24].

Statistics

Data were expressed as mean $\pm$ S.E. The significance of the differences between different groups was analyzed using Student's *t*-test.

Results

BAT composition

As shown in Table I, comparison of BAT denervated rats and warm re-exposed rats with 5°C controls indicates an accumulation of neutral lipids in BAT (44% and 58% increases, respectively). Mitochondrial protein content was 2.7-times higher in 5°C control rats than in 28°C controls. Sympathectomy and warm re-exposure did not decrease mitochondrial protein content significantly compared to cold exposed control rats.

GDP binding

Compared to the 28°C control group, cold exposure led to a large 4.7-fold increase in specific GDP binding expressed per mg mitochondrial proteins (classical indicator of the thermogenic state of the tissue (Table I). Sympathectomy and warm re-exposure induce a large decrease (43% and 82%, respectively). The same level of specific GDP binding was observed in warm re-exposed and in 28°C control rats. An estimate of total GDP binding can be obtained if mitochondrial yield is combined with specific GDP binding. In this case, 51% and 85% decreases were observed in sympathectomized and warm re-exposed groups, respectively. However, total GDP binding was about 60% higher in warm re-exposed rats than in 28°C controls.

Uncoupling protein

Estimation of the uncoupling protein content by densitometric tracing of immunological blots as shown in Fig. 3 indicated 2.8-times more protein in the mitochondria of 5°C control rats than in 28°C controls. BAT denervation and warm re-exposure led to a slight decrease (12% and 18%, respectively) which did only reach statistical significance ($P < 0.05$) in warm re-exposed group.

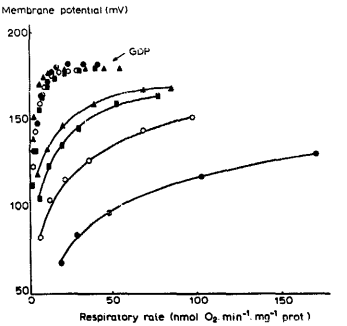

Fig. 1. Representative variations of membrane potential ($\Delta\psi$) with respiration rate (V_{ss_4}) in brown adipose tissue mitochondria of sympathectomized and warm re-exposed rats. Respiration rate was modulated with α-glycerophosphate additions (final concentration 0.1–10 mM). Uncoupled state without GDP, coupled state with 1.2 mM GDP. ▲, 28°C control rats; ○, sympathectomized rats; ■, warm re-exposed rats; ●, 5°C control rats.

Energization parameters

Fig. 1 shows typical experiments in which values of respiration rate were plotted against values of membrane potential. Respiration rate was modulated by varying α-glycerophosphate concentration (0.1–10 mM) in the absence of Ca^{2+}. In the absence of GDP, a much lower membrane potential was observed over the whole range of respiration rate in the cold exposed control rats compared to 28°C control rats, indicating a high level of uncoupling. The effect of cold exposure was decreased by BAT surgical denervation. 4 days warm

TABLE I

Effects of surgical denervation or warm re-exposure on interscapular brown adipose tissue (BAT)

Results are expressed as mean ± S.E. with the number of observations in parentheses. Comparison with cold exposed rats: significant effect of sympathectomy: [a] $P < 0.01$, [b] $P < 0.001$: significant effect of warm re-exposure: [c] $P < 0.001$.

	5°C		28°C	
	control (7)	denervated (6)	W re-exposed (6)	control (6)
BAT wet weight (mg)	394 ±23	430 ±5	440 ±15	414 ±15
Total lipids (mg)	109 ± 5	157 ±4 [b]	172 ± 7 [c]	243 ± 6
Mitochondria:				
Proteins (mg)	19.4 ± 2.0	16.3 ±0.8	15.3 ± 1.2	7.2 ± 1.1
GDP binding (nmol)				
per mg proteins	0.93± 0.05	0.53±0.04 [a]	0.17± 0.01 [c]	0.20±0.02
per BAT	17.6 ± 0.4	8.6 ±0.4 [b]	2.6 ± 0.2 [c]	1.4 ± 0.2

re-exposure was sufficient to obtain respiratory rates and potentials comparable to those observed in 28°C control rats.

With uncoupling protein completely blocked by an optimal concentration of GDP [17], membrane potential was increased. Relationships between membrane potential and oxidation rate were the same in the four groups.

Fig. 2 shows the relationship between the calculated values of proton conductance, CmH^+ and those of protonmotive force Δp (calculated as described in Materials and Methods). CmH^+ was dependent on Δp and establishement of the force/flux relationship make comparison of CmH^+ easier over the whole range of Δp. As expected, surgical denervation and warm re-exposure substantially decreased CmH^+ over the whole range of Δp. CmH^+ levels were similar in de-adapted rats and in 28°C control rats. In the presence of GDP, residual conductance was found to be very low except for higher values of Δp and was superposable for the four groups.

Acute injection of isoproterenol (200 μg/kg intramuscularly 90 min before killing) to sympathectomized or warm re-exposed rats greatly enhanced CmH^+. In sympathectomized rats CmH^+ reached the level observed in the cold control group whereas in warm re-exposed rats only 80% of the control cold group CmH^+ was observed (results not shown). This treatment had no effect in the cold control group. UCP

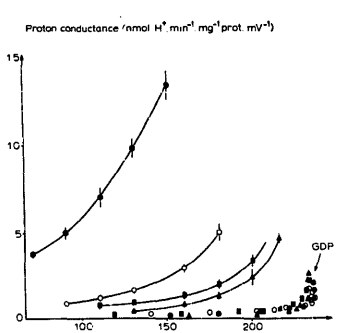

Fig. 2. The variations of proton conductance (C_{mH^+}) with protonmotive force (Δp) in brown adipose tissue mitochondria of sympathectomized and warm re-exposed rats. Uncoupled state without GDP, coupled state with 1.2 mM GDP. Six animals per group, values are mean ± S.E. ▲, 28°C control rats; ○, sympathectomized rats; ■, warm re-exposed rats; ●, 5°C control rats.

TABLE II

Effects of surgical denervation or warm re-exposure on the proportions of the main phospholipid classes and fatty acid unsaturation indexes in interscapular brown adipose tissue mitochondria

PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin; TU, total unsaturation indexes; PU, polyunsaturation indexes (number of double bonds per 100 total fatty acids). Results are expressed as mean ± S.E. with the number of observations in parentheses. Comparison with cold exposed rats: significant effect of sympathectomy: $^a P < 0.05$, $^b P < 0.01$, $^c P < 0.001$; significant effect of warm re-exposure: $^d P < 0.05$, $^e P < 0.01$.

	5°C		28°C	
	control (7)	denervated (6)	W re-exposed (6)	control (6)
PC	35.8 ± 0.8	37.9 ± 0.3 ^{a}	37.6 ± 0.2 ^{d}	40.0 ± 0.9
PE	43.4 ± 1.0	39.5 ± 0.4 ^{b}	39.4 ± 0.3 ^{e}	35.5 ± 0.9
CL	11.6 ± 1.0	12.1 ± 1.0	12.9 ± 0.5 ^{e}	13.3 ± 0.7
PC/PE	0.83 ± 0.03	0.96 ± 0.01 ^{c}	0.96 ± 0.02 ^{e}	1.12 ± 0.03
TU	141 ± 2	149 ± 1 ^{b}	140 ± 2	142 ± 2
PU	131 ± 2	137 ± 1 ^{a}	120 ± 2 ^{e}	120 ± 2

content was not modified by this acute stimulation which indicates a pure unmasking process.

Mitochondrial phospholipids

Based on biochemical and histological studies [25,26] it is admitted that the outer membrane only accounts for less than 5% in BAT mitochondria. Thus our results reflects essentially variations in composition of the inner mitochondrial membrane. No significant variation in the percentage of cardiolipin was observed between the four groups. Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipid (CL) were found to represent 90% of total mitochondrial phospholipids (Table II). Compared to cold exposed control rats, BAT denervation and warm re-exposure significantly increased the percentage of PC (about 6%) and decreased that of PE to a greater extend (about 9%). The ratio of PC to PE was significantly increased by 16% and tended towards the PC and PE percentage and ratio observed in 28°C control rats.

Acute injection of the potent β-agonist isoproterenol (200 μg/kg intramuscularly 90 min before killing) in warm re-exposed rats led to a small decrease of PC to PE ratio in BAT mitochondria (0.91 ± 0.01, $P < 0.05$).

Whereas BAT sympathectomy and de-adaptation exerted the same effect on phospholipid classes, they had the opposite effects on fatty acid unsaturation (Table II). Total unsaturation index (number of double bonds per 100 total fatty acids) was higher in the BAT denervated group (5%) compared to the three other groups. Sympathectomy increased (5%) the polyunsaturation index (reflecting essentially linoleic and arachi-

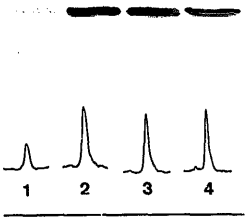

Fig. 3. Immunological characterization of BAT uncoupling protein in sympathectomized and warm re-exposed rats. 1, 28°C control rats; 2, sympathectomized rats; 3, warm re-exposed rats; 4, 5°C control rats.

donic acids) whereas warm re-exposure decreased it (7%) to the same level as in 28°C control rats.

Discussion

In this experiment alterations induced in BAT mitochondrial membrane following BAT sympathetic deactivation either by surgical denervation or by de-adaptation to cold were compared. We observed a decrease in thermogenic activity evidenced by a decrease in GDP binding and proton conductance and an increase in PC to PE ratio. For warm re-exposed rats, the values were close to those of control rats (28°C), whereas a higher activity was observed in sympathectomized rats. This difference could be explained by a residual content of norepinephrine (15% to 20%) [27] which is believed to be the primary mediator of the thermogenic response of BAT, acting directly on adipocytes [26] but such bilateral denervation completely prevents trophic response when rats are exposed to cold [29]. Several studies suggest that other neural or hormonal influences can participate in the control of BAT thermogenesis [30,31]. Factors such as thyroid hormones, insulin and adrenocortical hormones play a role in the adaptive changes that occur in BAT during cold exposure. Another possibility to explain the persistance of thermogenic activity is the well known hypersensitivity of denervated structures to β-agonist stimulation [32].

The variations of proton conductance were more marked than those of GDP binding although they followed the same trend. In fact CmH^+ is the primary functional parameter of the uncoupling protein which function is to channel protons. Recent work of Katyiar et al. [33] had shown that the proton translocation site is structurally different and distinct from the GDP binding site. It is likely that various factors other than the number of GDP binding sites may contribute as modulators of proton transport [34]. For instance, membrane potential may enhance proton leak through the uncoupling protein. Such factors may explain the larger variation of CmH^+.

Uncoupling protein content was not modified during the first days after surgical denervation, results at variance with the findings of Park and Himms-Hagen who report a 50% decrease 3 days after BAT denervation [35]. In our experiment the very large increase in proton conductance after acute injection of a β-agonist confirms the stability of the uncoupling protein.

In the same way, during the first days of warm re-exposure uncoupling protein content is also largely maintained which confirms the marked lag in the decrease in the concentration of the protein previously observed [36]. Under the conditions of the present experiment, it is likely that the decrease in mitochondrial protein mass is mainly related to the decrease in BAT protein mass as a whole. Thus, we conclude that switching off sympathetic stimulation does not lead to selective loss of the uncoupling protein during the first days.

Comparison of direct immunological titration of the uncoupling protein with GDP binding or proton conductance revealed a masking of both the nucleotide binding sites and the proton channels of the protein. Masking of GDP regulatory sites by warm re-exposure is a known phenomenon [36]. However, such large variations of the true functional parameter using a direct chemiosmotic approach is not presented in previous publications. A masking/unmasking capacity of proton conductance over one order of magnitude was clearly demonstrated here. Even in the cold, BAT surgical denervation led to an important masking of both GDP sites and proton conductance. Thus masking was the consequence of the impaired direct sympathetic stimulation of BAT.

Although in many situations such as cold exposure, thermogenesis induced by a high fat diet or chronic administration of norepinephrine, BAT activity seems to be related to the degree of unsaturation in the fatty acids of mitochondrial phospholipids [7,37,38] no such evidence was found here. In fact, the decreased BAT activity was associated with increased unsaturation in sympathectomized rats or with decreased fatty acid unsaturation in warm re-exposed rats. Following a previous study bearing on the effects of essential fatty acid deficiency on BAT mitochondria composition, we have reported a similar lack of correspondance between unsaturation index or fluidity and thermogenic activity [8].

The stimulation of BAT thermogenic activity whether obtained by cold exposure or by essential fatty acid deficiency is accompanied by a decrease of PC to PE ratio [7,3]. Conversely, decreasing cold-induced

BAT thermogenesis by sympathectomy or by warm re-exposure led to an increase in the PC to PE ratio. PC to PE ratio showed more rapid modifications than uncoupling content. Thus, the present work evidenced no link between uncoupling protein content and PC to PE ratio variations. PC to PE ratio is the only modification of inner membrane phospholipids observed here which follows systematically the modulation of functional activity which is modulated here by a masking/unmasking mechanism. Thus, the present results complete and reinforce our previous observations [8].

The mechanisms involved in the masking/unmasking of GDP binding sites or unactivation/activation of the proton channel have not been established. Conformational changes in the protein may be one possibility. Direct relationship between membrane lipid composition and GDP binding or proton conductance is not obvious since, to our knowledge nothing is known about the lipids near the uncoupling protein. Thus, the possibility that modifications of the phospholipid environment can modify uncoupling protein activity remains to be elucidated.

The present work demonstrates sympathetic control and functional importance of UCP masking in BAT mitochondria and further, supports the hypothesis that the modulation of phospholipid composition may have functional significance.

References

1 Smith, R.E. (1961) Physiologist 4, 113–114.
2 Himms-Hagen, J., Triandafillou, J. and Gwilliam, C. (1981) Am. J. Physiol. 241, E116-E120.
3 Himms-Hagen, J. (1985) Annu. Rev. Nutr. 5, 69–94.
4 Shrago, E. and Strieleman, P.J. (1987) World Rev. Nutr. Diet. 53, 171–217.
5 Field, C.J. and Clandinin, M.T. (1984) Nutr. Res. 4, 743–755.
6 Stubbs, C.D. and Smith, A.D. (1984) Biochim. Biophys. Acta 779, 89–137.
7 Ricquier, D., Mory, G., Nechad, M. and Hemon, P. (1978) J. Physiol. 74, 695–702.
8 Senault, C., Yazbeck, J., Goubern, M., Portet, R., Vincent, M. and Galley, J. (1990) Biochim. Biophys. Acta 1023, 283–289.
9 Derry, D.M., Schonbaum, E. and Steiner, G. (1969) Can. Physiol. J. Pharmacol. 47, 57–63.
10 Cottle, M.K.W. and Cottle, W.H. (1970) J. Histochem. Cytochem. 18, 116–119.
11 Cannon, B. and Lindberg, O. (1979) Methods Enzymol. 60, 65–78.
12 Yonetany, J. and Ray, G.S. (1965) J. Biol. Chem. 240, 3392–3398.
13 Nicholls, D.G. (1976) Eur. J. Biochem. 62, 223–228.
14 Kamo, N., Maratsugu, M., Hongo, R. and Kobatake, Y. (1979) J. Membr. Biol. 81, 127–138.
15 Nicholls, D.G. (1982) Bioenergetics, Academic Press, London.
16 Rottenberg, H. (1984) Biochem. J. 222, 685–693.
17 Goubern, M., Chapey, M. and Portet, R. (1991) Comp. Physiol. Biochem. 100B, 727–732.
18 Goubern, M., Yazbeck, J., Chapey, M., Diolez, Ph. and Moreau, F. (1990) Biochim. Biophys. Acta 1015, 334–340.
19 Ricquier, D., Barlet, J.P., Garel, J.M., Combes-George, M. and Dubois, M.P. (1983) Biochem. J. 210, 859–866.
20 Casteilla, L., Forest, C., Robelin, J., Ricquier, D., Lombet, A. and Ailhaud, G. (1987) Am. J. Physiol. 252, E627–E636.
21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
22 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509.
23 Portoukalian, J., Meister, R. and Zwingelstein, G. (1978) J. Chromatogr. 152, 569–574.
24 Bartlett, G.R. (1959) J. Biol. Chem. 234, 462–466.
25 Cannon, B., Polnaszek, C.K., Butler, K.W., Eriksson, L.E.G. and Smith, I.C.P. (1975) Arch. Biochem. Biophys. 167, 505–518.
26 Zaluska, H., Brabcova, J., Wroniszewska, A., Zborowski, J., Drahota, Z. and Wojtczak, L. (1975) Expl. Cell. Res. 91, 63–72.
27 Laury, M.C., Beauvallet, M. and Portet, R. (1982) Horm. Metab. Res. 14, 107.
28 Rafael, J., Fesser, W. and Nicholls, D.J. (1986) Am. J. Physiol. 250, C228–C235.
29 Rothwell, N.J. and Stock, M.J. (1984) J. Physiol. London 355, 457–463.
30 Ricquier, D. and Mory, G. (1984) Clin. Endocrinol. Metab. 13, 501–520.
31 Desautels, M., Dulos, R.A. and Mozaffari, B. (1986) Biochem. Cell. Biol. 64, 1125–1134.
32 Arch, J.R.S. (1989) Proc. Nutr. Soc. 48, 215–223.
33 Kativar, S.S. and Shrago, E. (1989) Proc. Natl. Acad. Sci. USA 86, 2957–2960.
34 Jezek, P., Houstek, J. and Drahota, Z. (1988) J. Bioenerg. Biomembr. 20, 603–622.
35 Park, I.R.A. and Himms-Hagen, J. (1988) Am. J. Physiol. 255, R874–R881.
36 Trayhurn, P., Ashwell, M., Jennings, G., Richard, D. and Stirling, D.M. (1987) Am. J. Physiol. 252, E237–E243.
37 Laury, M.C., Goubern, M., Razanoelina, M. and Portet, R. (1988) Ann. Nutr. Metab. 32, 324–331.
38 Mory, G., Gawer, M. and Kader, J.C. (1988) Biosci. Rep. 8, 465–469.