Effects of leptin on mitochondrial 'proton leak' and uncoupling proteins: implications for mammalian energy metabolism

Richard K. Porter[1]* and J. Fred Andrews[2]

[1]*Department of Biochemistry and* [2]*Department of Physiology, Trinity College Dublin, Dublin 2, Republic of Ireland*

Since the discovery of the *ob* gene (Zhang *et al.* 1994), much research has focused on its product leptin and its role in energy balance and metabolism (Campfield *et al.* 1996; Caro *et al.* 1996; Trayhurn, 1996; Flier, 1997; Lönnqvist & Schalling, 1997).

The leptin gene is expressed in white adipose tissue (WAT; and brown adipose tissue (BAT)) under conditions when high energy surplus leads to triacylglycerol accumulation (Zhang *et al.* 1994; Trayhurn, 1996). Such conditions include obesity, feeding, increased glucose and insulin concentrations, and also administration of cytokines and endotoxins. Conversely, leptin expression is decreased by weight reduction, fasting, cold exposure and β_3-receptor agonists, thiazolidinediones, and in insulin-dependent diabetes (Lönnqvist & Schalling, 1997). Leptin administration to *ob/ob* mice reduces appetite, increases metabolic rate and decreases fat mass (Pelleymounter *et al.* 1995; Hwa *et al.* 1997).

Leptin has been shown to act centrally both to suppress appetite and increase energy metabolism (Halaas *et al.* 1995; Pelleymounter *et al.* 1995). Leptin receptors in the hypothalamus (Tartaglia *et al.* 1995) have been shown to mediate the satiety effects by depressing neuropeptide Y production (Campfield *et al.* 1995; Stephens *et al.* 1995) and increase metabolism by activation of the efferent sympathetic system (Campfield *et al.* 1996).

More recently, it has been found that leptin has direct peripheral effects on metabolism. Receptors for leptin are found on the cell surface of many tissues (Caro *et al.* 1996) and leptin has been shown to stimulate metabolism in cultured cells (Pallett *et al.* 1997; Shimabukuro *et al.* 1997*b*) and isolated tissues (Liu *et al.* 1997), particularly by intracellular lipolysis and fatty acid oxidation (Koyama *et al.* 1997; Shimabukuro *et al.* 1997*a*).

Clearly, leptin can affect whole-body fat energy storage by influencing both sides of the energy balance equation, either input (feeding) and/or output (metabolism), whichever is appropriate to biological circumstances (Caro *et al.* 1996). Thus, leptin satisfies the signal postulated by Kennedy (1953) in his lipostatic hypothesis.

The present review discusses the role of proton leak in general in whole-body metabolism and in the regulation of energy balance. It examines evidence for the role of leptin in controlling metabolism, and discusses the different leak mechanisms by which leptin may potentially affect the efficiency of oxidative phosphorylation.

It is first necessary to give some background in bioenergetics to enable an understanding of the leak mechanisms and their role.

Oxidative phosphorylation

A convenient and accurate measure of resting whole-body metabolism in mammals can be achieved by using indirect calorimetry to measure O_2 consumption and CO_2 production rates (Blaxter, 1989). The main site of O_2 consumption and CO_2 production is the mitochondrion, where ATP synthesis is coupled to O_2 consumption by a process termed oxidative phosphorylation (Nicholls & Ferguson, 1992). This process is best described by the chemiosmotic theory of Mitchell (1961). Reducing equivalents such as $NADH_2$ and $FADH_2$, derived from the oxidation of reduced C compounds (the carbohydrate or fatty acid molecules derived directly from the diet or released from the fuel stores of the body), feed electrons into the electron transport chain. This chain is situated in the mitochondrial inner membrane. As electrons pass down the chain, protons are translocated across the mitochondrial inner membrane from the matrix space to the inter-membrane space, thus setting up a delocalized trans-membrane proton electrochemical gradient (Δp). Δp can drive useful work such as (1) metabolite transport across the inner membrane (e.g. ADP–ATP exchange and phosphate/proton symport) and (2) ATP synthesis from matrix ADP and phosphate by driving protons through the ATP synthase which is also located in the inner membrane and extends into the matrix. Hence, ATP synthesis is coupled to O_2 consumption via the electrochemical gradient. This is the principal source of ATP at rest. However, it has long been known from H^+/O stoichiometric studies of the electron transport chain and H^+/ATP stoichiometric studies of the ATP synthase, that ATP synthesis is not perfectly coupled to O_2 consumption in intact mitochondria (Brand, 1977). The reason for this discrepancy is that the mitochondrial inner membrane is not absolutely impermeable to protons, i.e. there is an inefficiency in the system, namely a leakage of protons across the mitochondrial inner membrane.

Abbreviations: BAT, brown adipose tissue; Δp, proton electrochemical gradient; UCP, uncoupling protein; WAT, white adipose tissue.

***Corresponding author:** Dr R. K. Porter, fax +353 1 6772400, email rkporter@mail.tcd.ie

https://doi.org/10.1079/PNS19980065 Published online by Cambridge University Press

Leakage of protons across the mitochondrial inner membrane: 'proton leak'

A quantitatively very important mechanism contributing to whole-body metabolism is diffusion-mediated 'proton leak' (Brand, 1990). This 'proton leak' is thought to be a non-enzymic diffusion process that occurs across the inner membrane of all mitochondria and displays a non-linear dependence on its driving force, the Δp, such that the leak is maximal when mitochondria are not making ATP and minimal when they are (Nicholls & Rial, 1974; Brown & Brand, 1986; Rolfe *et al.* 1994; Nicholls, 1997). Diffusion of other ions shows a similar kinetic profile (Brown & Brand, 1986). The mechanism of this leak is not fully understood. Under certain conditions studied, there is a correlation between 'proton leak' and inner membrane surface area (Porter *et al.* 1996), as one would expect for a diffusion process. In addition, 'proton leak' can be measured directly through liposomes (Brookes *et al.* 1997*a*,*b*). However, the role played by inner membrane composition on differences in 'proton leak' rate is still unclear (Brown & Brand, 1991; Porter *et al.* 1996; Brookes *et al.* 1997*a*,*b*).

'Proton leak' can be measured indirectly by measuring the rate of O_2 consumption by mitochondria in the presence of saturating amounts of oligomycin, which prevents any ATP synthesis by proton flux through ATP synthase. 'Proton leak' is found in mitochondria from all tissues studied so far: liver, thymus, lymphocytes, skeletal muscle, heart and even BAT mitochondria (Nicholls & Rial, 1974; Brand *et al.* 1994; Rolfe & Brand, 1997). Furthermore, 'proton leak' is not just observable in isolated mitochondria. A similar non-ohmic dependence of O_2 consumption on Δp is observed for non-phosphorylating mitochondria *in situ* in a variety of cells (hepatocytes, thymocytes and lymphocytes) and tissue (skeletal muscle; Rolfe & Brand, 1996*a*). In fact, 'proton leak' accounts for approximately 20 % of the resting O_2 consumption of cells, and up to approximately 30 % of the resting O_2 consumption of rat hindquarter skeletal muscle. Indeed, 'proton leak' is a significant contributor to resting O_2 consumption in the whole animal and *per se* must be important in the energy budget (Rolfe & Brand, 1996*b*).

Apart from being quantitatively important, 'proton leak' seems to vary in accordance with factors that determine standard metabolic rate, such as body mass, thyroid status and phylogeny (Brand *et al.* 1994; Rolfe & Brown, 1997). It has clearly been demonstrated that smaller mammals, which have a greater mass-specific metabolic rate than larger mammals, have greater (liver) mitochondrial 'proton leak' rates (Porter & Brand, 1993, 1995*a*,*b*). Rats made hyperthyroid have higher liver mitochondrial 'proton leak' rates when compared with their euthyroid controls (Hafner *et al.* 1988; Harper & Brand, 1993, 1994; Harper *et al.* 1993), and *vice versa*; rats made hypothyroid have lower 'proton leak' rates. Also bearded dragon lizards (*Amphibolurus vitticeps*) of equivalent mass to rats have lower whole-body resting metabolic rates and lower 'proton leak' rates (Brand *et al.* 1991). This area has been reviewed recently (Rolfe & Brand, 1997; Rolfe & Brown, 1997).

Obese (*ob/ob*) mice, which lack functional leptin, have been shown to have increased 'proton leak' in experiments on isolated liver mitochondria compared with mitochondria from lean controls (Porter *et al.* 1997), although liver mitochondrial 'proton leak' was not increased in *fa/fa* rats compared with their littermate controls (RK Porter, JA Buckingham and MD Brand, unpublished results). A high dose of leptin once daily for 2–3 d restores the kinetic leak profile of *ob/ob* mice to that of lean controls (Melia *et al.* 1997). The result is consistent with the general 'normalization' effects observed when leptin is administered to *ob/ob* mice (Caro *et al.* 1996).

Leakage of protons across the mitochondrial inner membrane: uncoupling proteins

In addition to diffusion-mediated 'proton leak', it is widely known that the inner mitochondrial membrane of the brown adipocyte contains an uncoupling protein (UCP; originally called thermogenin) enabling dissipation of the protein gradient independently of ATP utilization, thus enabling fuel to be oxidized for direct production of heat in thermoregulation (and, at least in rodents, also for energy regulatory thermogenesis; Nicholls & Locke, 1984). Recent research has shown that there is a family of UCP occurring in BAT and other tissues, which has necessitated the renaming of the classic UCP of BAT to UCP1 (Fleury *et al.* 1997). As a consequence of its relatively early discovery (Nicholls *et al.* 1978; Ricquier & Kader, 1978) there is a lot of information on the mechanism and regulation of UCP1 activity.

UCP1 is a 32 kDa protein incorporated into the mitochondrial inner membrane to form a 'proton conductance channel', as a result of sympathetic nervous system action via novel β_3-adrenergic receptors on the surface of BAT cells (Rohlfs *et al.* 1995). Noradrenaline release as a result of cold exposure increases UCP1 expression in BAT. Active BAT is the principal site of non-shivering thermogenesis, and although it accounts for approximately 1% of the body mass of a rat (Foster, 1986), it accounts for 50 % of the heat production in non-shivering thermogenesis, at least in the cold-acclimated rat (Foster & Frydman, 1979). Diet, in particular a high-carbohydrate cafeteria diet, can also induce UCP1 expression in BAT of rodents (Rothwell & Stock, 1986). BAT UCP1 activity is also modulated by thyroid hormone (Silva & Rabelo, 1997). Recent studies on cultured human pre-adipocytes show a marked increase in UCP1 in response to peroxisomal proliferating activator receptor agonist, troglitazone (Paulik & Lenhard, 1997; Digby *et al.* 1998). Retinoic acid also increases UCP1 expression in mice *in vivo* and *in vitro* (Puigserver *et al.* 1996). UCP1 is predicted to have six trans-membrane helices and has three amino acid motifs common to all mitochondrial inner membrane transporters cloned so far (Klaus *et al.* 1991; Palmieri, 1994). The *in vitro* binding of GDP to a purine-nucleotide binding site of UCP1, located on the cytosolic side in isolated mitochondria, prevents proton conductance through UCP1 (Nicholls & Rial, 1974). UCP1 activity has also been shown to be regulated by free fatty acids which promote its proton conductance activity (Nicholls & Rial, 1974). There is also evidence that UCP1 is a chloride channel.

In fully active BAT, where extensive uncoupling protein resides within the inner membrane of mitochrondria in that tissue, only small Δp values are obtainable, and O_2

consumption is essentially uncoupled from ATP synthesis. The leak rate of protons across the inner membrane of mitochondria isolated from active BAT displays a linear dependence on Δp (Nicholls, 1974; Nicholls & Rial, 1974). Little information exists about the effect of leptin on BAT activity; however, leptin administration has been shown to increase noradrenaline turnover in interscapular BAT, increase BAT mass and increase UCP1 expression in BAT in normal rats (Collins *et al.* 1996; Scarpace *et al.* 1997).

The existence of other UCP only came to light recently (Boss *et al.* 1997*b*; Fleury *et al.* 1997; Vidal-Puig *et al.* 1997). UCP2 was cloned using primers to UCP1 and was found to be widespread in mammalian tissues, with highest levels being found in WAT, BAT, heart, spleen, thymus, macrophages, bone marrow and stomach. In the liver, UCP2 is present only in Kupffer cells, not the hepatocytes themselves (Larrouy *et al.* 1997). UCP2 has an approximate molecular mass of 30 kDa and has approximately 56 % homology to UCP1 in rats (Fleury *et al.* 1997).

A third UCP (UCP3) has also been cloned. It has two isoforms: a long form (UCP3L) and a short form (UCP3S; Boss *et al.* 1997*b*; Vidal-Puig *et al.* 1997). The short form has 275 amino acid residues and the long form has an additional thirty-seven residues. The two human isoforms have amino acid homologies of 57 and 73 % to UCP1 and UCP2 respectively. UCP3S lacks the sixth potential trans-membrane region and the putative nucleotide binding site. Expression of UCP3 is most abundant in skeletal muscle and BAT (Boss *et al.* 1997*b*). Like UCP1, UCP2 and UCP3 expression is increased in BAT on cold exposure of rodents (Boss *et al.* 1997*a*; Larkin *et al.* 1997; Vidal-Puig *et al.* 1997; for a contradictory view on UCP3, see Boss *et al.* 1998). It has also been shown that in UCP1-knockout mice, UCP2 expression is dramatically increased on cold exposure (Enerbäck *et al.* 1997). In addition, like UCP1, β_3-agonists increase expression of UCP3 but have not been found to affect UCP2 expression (Gong *et al.* 1997). Thyroid hormone has been shown to modulate UCP2 expression in WAT, heart and skeletal muscle, and UCP3 expression in skeletal muscle and BAT (Gong *et al.* 1997; Lanni *et al.* 1997; Larkin *et al.* 1997; Masaki *et al.* 1997). High-fat diet, 48 h starvation and dexamethasone increase UCP3 expression in skeletal muscle but decrease UCP3 expression in BAT (Matsuda *et al.* 1997). High-fat diets increase UCP2 expression in WAT and the peroxisomal proliferating activator receptor agonist troglitazone (a thiazolidinedione) has been shown to increase UCP2 expression in pancreatic islet cells, adipocytes and pre-adipocytes (Aubert *et al.* 1997; Matsuda *et al.* 1997; Shimabukuro *et al.* 1997*a*). Fasting has been shown to increase UCP2 and UCP3 expression in human subjects (Millet *et al.* 1997; Boss *et al.* 1998). UCP2 and UCP3 have been mapped to adjacent genes on chromosome 11q13 in human subjects, close to markers of resting metabolic rate (Bouchard *et al.* 1997; Solanes *et al.* 1997). Again, information about the role played by leptin in the activity of these novel UCP is limited. Suffice it to say, in normal rats over-expression of leptin increased UCP2 expression in both islet cells and in WAT (particularly epididymal, retroperitoneal and subcutaneous depots; Zhou *et al.* 1997). Leptin increased UCP3 expression in muscle and BAT in *ob/ob* mice (Gong *et al.* 1997).

There is no doubt that the extent of the evidence for a physiological role for these novel UCP has to date been rather limited. It has been demonstrated that yeast cells (Fleury *et al.* 1997) and myoblasts (Boss *et al.* 1998) transfected with UCP have decreased mitochondrial membrane potential and, as described previously, factors that regulate metabolism have been shown to affect UCP expression (as detected by Northern blot analysis). It is also possible that the diffusion-mediated 'proton leak' measured in different tissues (Brand *et al.* 1994; Rolfe & Brand, 1997) may be due, in part, to the presence of submaximal amounts of UCP in those tissues (with the possible exception of liver parenchyma cells, where no UCP have been shown to be expressed; Larrouy *et al.* 1997). Clearly now, the differential role played by UCP in physiology, medicine and energy metabolism requires a concerted multidisciplinary investigation.

Leptin effects on mitochondrial 'proton leak' and uncoupling proteins: implications for mammalian energy metabolism

Mitochondrial 'proton leak' has been demonstrated to be an important mechanism by which basal metabolism is determined (Rolfe & Brand, 1997). It has been shown that 'proton leak' in liver mitochondria is greater in *ob/ob* mice compared with lean mice, and that a short course of leptin administration (without significant changes in body mass) normalizes the 'proton leak' of *ob/ob* mice to that of controls. Clearly, constitutive leptin production plays a role in determining metabolic homeostasis.

The increased expression of UCP in rodents as a result of leptin administration is consistent with the increased metabolic rates observed for these animals when leptin is administered (Pelleymounter *et al.* 1995). Although the liver is quantitatively important when accounting for basal metabolism in mammals (20 % of the basal metabolism of a rat; Rolfe & Brand, 1997), the increased BMR in the whole animal most probably reflects the increase in UCP expression and activity within mitochondria of muscle, BAT and other highly-metabolic visceral organs.

And finally, leptin levels and UCP activity may have roles in determining insulin resistance and insulin sensitivity of islets cells (Koyama *et al.* 1997; Shimabukuro *et al.* 1997*a*; Zhou *et al.* 1997). The observations that leptin promotes intracellular triacylglycerol oxidation and increases UCP2 expression in islet cells have implications for lipotoxicity, which is postulated to be the basis of islet insulin resistance and insulin insensitivity.

Clearly, knowledge of the role of leptin in determining the efficiency of mitochondrial oxidative phosphorylation via 'proton leak'/UCP expression has implications for our understanding of basic physiology, but also has clinical implications in terms of anti-obesity and anti-diabetic strategies.

References

Aubert J, Champigny O, Saint-Marc P, Negrel R, Collins S, Ricquier D & Ailhaud G (1997) Up-regulation of UCP2 gene expression by PPAR agonists in preadipose and adipose cells.

Biochemical and Biophysical Research Communications **238**, 606–611.

Blaxter K (1989) *Energy Metabolism in Animals and Man*. Cambridge, UK: Cambridge University Press.

Boss O, Samec S, Dulloo A, Seydoux J, Muzzin P & Giacobino JP (1997*a*) Tissue-dependent upregulation of rat uncoupling protein-2 expression in response to fasting or cold. *FEBS Letters* **412**, 111–114.

Boss O, Samec S, Kühne F, Bijlenga P, Assimacopoulos-Jeannet F, Seydoux J, Giacobino J-P & Mizzin P (1998) Uncoupling protein-3 expression in rodent skeletal muscle is modulated by food intake but not by changes in environmental temperature. *Journal of Biological Chemistry* **273**, 5–8.

Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, Muzzin P & Giacobino JP (1997*b*) Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Letters* **408**, 39–42.

Bouchard C, Pérusse L, Chagnon YC, Warden C & Ricquier D (1997) Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. *Human Molecular Genetics* **6**, 1887–1889.

Brand MD (1977) The stoichiometric relationships between electron transport, proton translocation and adenosine triphosphate synthesis and hydrolysis in mitochondria. *Biochemical Society Transactions* **5**, 1615–1620.

Brand MD (1990) The proton leak across the mitochondrial inner membrane. *Biochimica et Biophysica Acta* **1018**, 128–133.

Brand MD, Chien L-F, Ainscow EK, Rolfe DFS & Porter RK (1994) The causes and functions of mitochondrial proton leak. *Biochimica et Biophysica Acta* **1187**, 132–139.

Brand MD, Couture P, Else PL, Withers KW & Hulbert AJ (1991) Evolution of energy metabolism. *Biochemical Journal* **275**, 81–86.

Brookes PS, Hulbert AJ & Brand MD (1997*a*) The proton permeability of liposomes made from mitochondrial inner membrane phospholipids: no effect of fatty acid composition. *Biochimica et Biophysica Acta* **1330**, 157–164.

Brookes PS, Rolfe DFS & Brand MD (1997*b*) The proton permeability of liposomes made from mitochondrial inner membrane phospholipids – comparison with isolated mitochondria. *Journal of Membrane Biology* **155**, 167–174.

Brown GC & Brand MD (1986) Changes in the permeability to protons and other cations at high proton motive force in rat liver mitochondria. *Biochemical Journal* **234**, 75–81.

Brown GC & Brand MD (1991) On the nature of the mitochondrial proton leak. *Biochimica et Biophysica Acta* **1059**, 55–62.

Campfield LA, Smith FJ & Burn P (1996) The OB protein (leptin) pathway – a link between adipose tissue mass and central neural networks. *Hormone and Metabolic Research* **28**, 619–632.

Campfield LA, Smith FJ, Guisez Y, Devos R & Burn P (1995) Recombinant mouse ob protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269**, 546–549.

Caro JF, Sinha MK, Kolaczynski JW, Zhang PL & Considine RV (1996) Leptin: the tale of an obesity gene. *Diabetes* **45**, 1455–1462.

Collins S, Kuhn CM, Petro AE, Swick AG, Chrunyk BA & Surwit RS (1996) Role of leptin in fat regulation. *Nature* **380**, 677.

Digby JE, Montague CT, Sewter CP, Sanders L, Wilkinson WO, O'Rahilly S & Prins JB (1998) Thiazolidinedione exposure increases the expression of uncoupling protein 1 in cultured human adipocytes. *Diabetes* **47**, 138–141.

Enerbäck S, Jacobsson A, Simpson EM, Guerra C, Yamashits H, Harper M-E & Kozak LP (1997) Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* **387**, 90–94.

Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F, Seldin MF, Surwit RS, Ricquier D & Warden CH (1997) Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nature Genetics* **15**, 269–272.

Flier JS (1997) Leptin expression and action: new experimental paradigms. *Proceedings of the National Academy of Sciences USA* **94**, 4242–4245.

Foster DO (1986) Quantitative role of brown adipose tissue in thermogenesis. In *Brown Adipose Tissue*, pp. 31–52 [P Trayhurn and DG Nicholls, editors]. London: Arnold.

Foster DO & Frydman ML (1979) Tissue distribution of cold-induced thermogenesis in conscious warm- and cold-acclimated rats reevaluated from changes in tissue blood flow. *Canadian Journal of Physiology and Pharmacology* **57**, 257–270.

Gong D-W, He Y, Karas M & Reitman M (1997) Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, β_3-adrenergic agonists and leptin. *Journal of Biological Chemistry* **272**, 24129–24132.

Hafner RP, Nobes CD, McGown AD & Brand MD (1988) Altered relationship between proton motive force and respiration rate in non-phosphorylating liver mitochondria isolated from rats of different thyroid hormone status. *European Journal of Biochemistry* **178**, 511–518.

Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK & Friedman JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269**, 543–546.

Harper M-E, Ballantyne JS, Leach M & Brand MD (1993) Effects of thyroid hormones on oxidative phosphorylation. *Biochemical Society Transactions* **21**, 785–792.

Harper M-E & Brand MD (1993) The quantitative contribution of mitochondrial proton leak and ATP turnover reactions to the changed respiration rates of hepatocytes from rats of different thyroid status. *Journal of Biological Chemistry* **268**, 14850–14860.

Harper M-E & Brand MD (1994) Hyperthyroidism stimulates mitochondrial proton leak and ATP turnover in rat hepatocytes but does not change the overall kinetics of substrate oxidation reactions. *Canadian Journal of Physiology and Pharmacology* **72**, 899–908.

Hwa JJ, Fawzi AB, Graziano MP, Ghibaudi L, Williams P, van Heek M, Davis H, Rudinski M, Sybertz E & Strader CD (1997) Leptin increases energy expenditure and selectively promotes fat metabolism in *ob/ob* mice. *American Journal of Physiology* **272**, R1204–R1209.

Kennedy GC (1953) The role of depot fat in the hypothalamic control of food intake in the rat. *Proceedings of the Royal Society* **140**B, 578–592.

Klaus S, Casteilla L, Bouillaud F & Ricquier D (1991) The uncoupling protein UCP: a membraneous mitochondrial ion carrier in brown adipose tissue. *International Journal of Biochemistry* **23**, 791–801.

Koyama K, Chen G, Lee Y & Unger RH (1997) Tissue triglycerides, insulin resistance, and insulin production: implications for hyperinsulinemia of obesity. *American Journal of Physiology* **273**, E708–E713.

Lanni A, De Felice M, Lombardi A, Moreno M, Fleury C, Ricquier D & Goglia F (1997) Induction of UCP2 mRNA by thyroid hormones in rat heart. *FEBS Letters* **418**, 171–174.

Larkin S, Mull E, Miao W, Pittner R, Albrandt K, Moore C, Young A, Denaro M & Beaumont K (1997) Regulation of the third member of the uncoupling protein family, UCP3, by cold and thyroid hormone. *Biochemical and Biophysical Research Communications* **240**, 222–227.

Larrouy D, Laharrague P, Carrera G, Viguerie-Bascands N, Levi-Meyrueis C, Fleury C, Pecqueur C, Nibbelink M, André M, Casteilla L & Ricquier D (1997) Kupffer cells are a dominant site of uncoupling protein 2 expression in rat liver. *Biochemical and Biophysical Research Communications* **235**, 760–764.

Liu YL, Emilsson V & Cawthorne MA (1997) Leptin inhibits glycogen synthesis in the isolated soleus muscle of obese (*ob/ob*) mice. *FEBS Letters* **411**, 351–355.

Lönnqvist F & Schalling M (1997) Role of leptin and its receptor in human obesity. *Current Opinion in Endocrinology and Diabetes* **4**, 164–171.

Masaki T, Yoshimatsu H, Kakuma T, Hidaka S, Kurokawa M & Sakata T (1997) Enhanced expression of uncoupling protein 2 gene in rat white adipose tissue and skeletal muscle following chronic treatment with thyroid hormone. *FEBS Letters* **418**, 323–326.

Matsuda J, Hosoda K, Itoh H, Son C, Doi K, Tanaka T, Fukunaga Y, Inoue G, Nishmura H, Yoshimasa Y, Yamori Y & Nakao K (1997) Cloning of rat uncoupling protein-3 and uncoupling protein-2 cDNAs: their gene expression in rats fed high-fat diet. *FEBS Letters* **418**, 200–204.

Melia HP, McBennett SM, Andrews JF & Porter RK (1997) Leptin reverses the increased proton leak observed in liver mitochondria from obese mice to leak rates observed in lean controls. *International Journal of Obesity* **21**, S33.

Millet L, Vidal H, Andreelli F, Larrouy D, Riou JP, Ricquier D, Laville M & Langin D (1997) Increased uncoupling protein-2 and -3 mRNA expression during fasting in obese and lean humans. *Journal of Clinical Investigation* **100**, 2665–2670.

Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. *Nature* **191**, 144–148.

Nicholls DG (1974) The influence of respiration and ATP hydrolysis on the proton-electrochemical gradient across the inner membrane of rat liver mitochondria as determined by ion distribution. *European Journal of Biochemistry* **50**, 305–315.

Nicholls DG (1997) The non-ohmic proton leak – 25 years on. *Bioscience Reports* **17**, 251–257.

Nicholls DG, Bernson V & Heaton G (1978) The identification of the component in the inner membrane responsible for regulating energy dissipation. In *Effectors of Thermogenesis*, pp. 119–134 [L Girardier and J Seydoux, editors]. Basel: Birkhauser Verlag.

Nicholls DG & Ferguson SJ (1992) *Bioenergetics 2*. London: Academic Press.

Nicholls DG & Locke RM (1984) Thermogenic mechanisms in brown fat. *Physiological Reviews* **64**, 1–64.

Nicholls DG & Rial E (1974) Measurement of proton leakage across mitochondrial inner membranes and its relation to proton motive force. *Methods in Enzymology* **174**, 85–94.

Pallett AL, Morton NM, Cawthorne MA & Emilsson V (1997) Leptin inhibits secretion and reduces insulin mRNA levels in rat isolated pancreatic islets. *Biochemical and Biophysical Research Communications* **238**, 267–270.

Palmieri F (1994) Mitochondrial carrier proteins. *FEBS Letters* **346**, 48–54.

Paulik MA & Lenhard JM (1997) Thiazolidines inhibit akaline phosphatase while increasing expression of uncoupling protein, deiodinase, and increasing mitochondrial mass in C3H10T/2 cells. *Cell and Tissue Research* **290**, 79–87.

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T & Collins F (1995) Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science* **269**, 540–543.

Porter RK & Brand MD (1993) Body mass dependence of H^+ leak in mitochondria and its relevance to metabolic rate. *Nature* **268**, 628–630.

Porter RK & Brand MD (1995*a*) Cellular oxygen consumption depends on body mass. *American Journal of Physiology* **268**, R641–R650.

Porter RK & Brand MD (1995*b*) Causes of differences in respiration rate of hepatocytes from mammals of different body mass. *American Journal of Physiology* **269**, R1213–R1224.

Porter RK, Hulbert AJ & Brand MD (1996) Allometry of mitochondrial proton leak: influence of membrane surface area and fatty acid composition. *American Journal of Physiology* **271**, R1550–R1560.

Porter RK, McBennett SM, MacDonald ZI & Andrews JF (1997) Increased proton leak in liver mitochondria from obese mice compared with lean controls. *Proceedings of the Nutrition Society* **56**, 198A.

Puigserver P, Vazquez F, Bonet ML, Pico C & Palou A (1996) *In vitro* and *in vivo* induction of brown adipocyte uncoupling protein (thermogenin) by retinoic acid. *Biochemical Journal* **317**, 827–833.

Ricquier D & Kader J-C (1978) Mitochondrial protein alteration in active brown fat: a sodium dodecyl sulfate-polyacrylamide gel electrophoretic study. *Biochemical and Biophysical Research Communications* **873**, 577–583.

Rohlfs EM, Daniel KW, Premont RT, Kozak LP & Collins S (1995) Regulation of the uncoupling protein gene (UCP) by beta 1, beta 2, and beta 3-adrenergic receptor subtypes in immortalised brown adipose cell lines. *Journal of Biological Chemistry* **270**, 10723–10732.

Rolfe DFS & Brand MD (1996*a*) Proton leak and control of oxidative phosphorylation in perfused, resting rat muscle. *Biochimica et Biophysica Acta* **1276**, 45–50.

Rolfe DFS & Brand MD (1996*b*) The contribution of mitochondrial proton leak to the rate of respiration in rat skeletal muscle and SMR. *American Journal of Physiology* **271**, C1380–C1389.

Rolfe DFS & Brand MD (1997) The physiological significance of mitochondrial proton leak in animal cells and tissues. *Bioscience Reports* **17**, 9–16.

Rolfe DFS & Brown GC (1997) Cellular energy utilization and molecular origin of standard metabolic rate. *Physiological Reviews* **77**, 731–758.

Rolfe DFS, Hulbert AJ & Brand MD (1994) Characteristics of mitochondrial proton leak and control of oxidative phosphorylation in the major oxygen-consuming tissues of the rat. *Biochimica et Biophysica Acta* **1118**, 405–416.

Rothwell NJ & Stock MJ (1986) Brown adipose tissue and diet-induced thermogenesis. In *Brown Adipose Tissue*, pp. 269–298 [P Trayhurn and DG Nicholls, editors]. London: Arnold.

Scarpace PJ, Matheny M, Pollock BH & Tumer N (1997) Leptin increases uncoupling protein expression and energy metabolism. *American Journal of Physiology* **273**, E226–E230.

Shimabukuro M, Koyama K, Chen G, Wang MY, Trieu F, Lee Y, Newgard CB & Unger RH (1997*a*) Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proceedings of the National Academy of Sciences USA* **94**, 4637–4641.

Shimabukuro M, Zhou YT, Lee Y & Unger RH (1997*b*) Induction of uncoupling protein-2 mRNA by troglitazone in pancreatic islets of Zucker diabetic fatty rats. *Biochemical and Biophysical Research Communications* **237**, 359–361.

Silva JE & Rabelo R (1997) Regulation of the uncoupling protein gene expression. *European Journal of Endocrinology* **136**, 251–264.

Solanes G, Vidal-Puig A, Grucjic D, Flier JS & Lowell BB (1997) The human uncoupling protein-3 gene. *Journal of Biological Chemistry* **272**, 25433–25436.

Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A,

MacKellar W, Rosteck PR Jr, Smith D, Tinsley FC, Zhang X-Y & Heiman M (1995) The role of neuropeptide Y in the anti-obesity action of the obese gene product. *Nature* **337**, 530–532.

Tartaglia LA, Demdksi M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutho JS, Mays GG, Woolf EA, Monroe CA & Tepper RI (1995) Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83**, 1263–1271.

Trayhurn P (1996) New insights into the development of obesity: obese genes and the leptin system. *Proceedings of the Nutrition Society* **55**, 783–791.

Vidal-Puig A, Solanes G, Grujic D, Flier JS & Lowell BB (1997) UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochemical and Biophysical Research Communications* **235**, 79–82.

Zhang Y, Procenca R, Maffei M, Barone M, Leopold L & Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425–432.

Zhou Y-T, Shimabukuro M, Koyama K, Lee Y, Wang M-Y, Trieu F, Newgard CB & Unger RH (1997) Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. *Proceedings of the National Academy of Sciences USA* **94**, 6386–6390.

https://doi.org/10.1079/PNS19980065 Published online by Cambridge University Press