

Cellular energy expenditure and the importance of uncoupling¹

M.-E. Harper², A. Antoniou, L. Bevilacqua, V. Bezaire, and S. Monemdjou

Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, and Centre for Catalysis Research and Innovation, University of Ottawa, Ottawa, Canada K1H 8M5

ABSTRACT: Just as total body energy expenditure in animals can be broken down into that which supports resting energy metabolism, work, growth, and so on, cellular energy expenditure can similarly be subclassified. The objective in this review is to examine the metabolic origins of cellular energy expenditure, with an emphasis on mitochondrial uncoupling. Mitochondrial uncoupling refers to the dissociation of energy substrate oxidation from the mitochondrial synthesis of ATP. Uncoupling can occur during states of high energy expenditure, as in brown adipose tissue (BAT), or during states of metabolic rest, as in many other tissues. In BAT, uncoupling protein 1 (UCP1) activity can cause very high rates of energy expenditure for the purpose of thermogenesis (heat production). While mitochondrial uncoupling also occurs in other cells of the body, it is of greatest importance to fractional rates of energy

expenditure during periods of relative metabolic rest. The latter form of uncoupling is referred to as *mitochondrial proton leak* and is estimated to account for roughly 20 to 25% of the resting metabolic rate. Leak activity is correlated with thyroid hormone status; scales roughly in proportion with the well-known differences in mass-specific basal metabolic rate in mammals of different body size, and is related to differences in basal metabolic rate between ectothermic and endothermic animals. Proposed functions for mitochondrial uncoupling through proton leak include thermogenesis, energy balance, control of oxidative phosphorylation efficiency, and protection from reactive oxygen species. The mechanisms of mitochondrial proton leak are not well understood; the recently identified uncoupling proteins may play some role, but one or more of these proteins may have other physiological functions, such as fatty acid translocation.

Key Words: Energy Expenditure, Heat Production, Metabolism, Mitochondria, Proton Pump, Proteins

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Introduction

Total body energy expenditure in animals can be categorized into subclasses of expenditure supporting basal metabolic rate (BMR), growth, pregnancy and lactation, diet-induced thermogenesis, cold-induced thermogenesis, and exercise-induced thermogenesis. BMR normally accounts for the largest single fraction of total energy expenditure in animals. The metabolic underpinnings of BMR have been studied largely by examining the major energy demands at the tissue and cellular levels and extrapolating the significance of such processes to the level of the whole body.

The overall aim of this brief review is to examine the metabolic origins of a component of cellular energy

expenditure. We will focus primarily on mitochondrial uncoupling and the role of mitochondrial proton leak and will differentiate between metabolic states in which cells are at relative rest (i.e., when ATP demand is minimal) and those in which cellular energy expenditure is increased. The outcome of mitochondrial uncoupling, regardless of its molecular mechanism, is the release of a significant proportion of the energy as heat. Recent findings suggest that the uncoupling that is mediated by mitochondrial proton leak accounts for 20 to 25% of BMR, making it the single most important predictor of BMR (Rolfe et al., 1999).

Cellular Energy Expenditure in the Context of the Whole Organism

To appreciate the significance and complexities of uncoupled cellular energy expenditure, it would be useful to first review some basic concepts underlying whole animal energy expenditure. At the level of the whole animal, the term *energy balance* refers to a metabolic state in which total body energy expenditure is equal to the energy that is absorbed from the diet and

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²Correspondence: 451 Smyth Rd. (phone: 613 562-5800 x8235; fax: 613 562-5440; E-mail: mharper@uottawa.ca).

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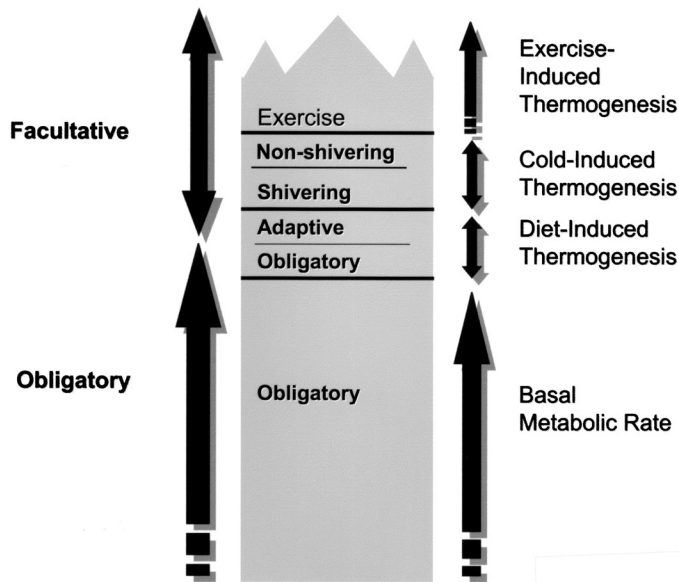


Figure 1. Categories of mammalian thermogenesis. All energy expenditure reactions can be classified as either obligatory or facultative (shown on left). Reactions can be further categorized into more specific types of thermogenesis (shown on right). Diet-induced thermogenesis includes obligatory and adaptive thermogenic reactions. Cold-induced thermogenesis includes shivering and non-shivering reactions. See text for description of tissues that are mainly responsible for groups of thermogenic reactions. Note that the schematic is limited to adult nonpregnant, nonlactating mammals.

that is not lost in the urine or lost as gaseous forms of energy (e.g., methane). Energy balance thus exists when total body energy expenditure is equal to dietary metabolizable energy. Energy expenditure (or “thermogenic”) processes can be partitioned into a number of categories. At the broadest level, reactions can be classified as either obligatory or facultative (Figure 1). Obligatory reactions include basal metabolic rate and those that are essential for the ingestion, digestion, and metabolism of food. The latter reactions account for a significant proportion of diet-induced thermogenesis (**DIT**), the energy costs of assimilating nutrients and retaining net energy (Girardier and Stock, 1983; Himms-Hagen, 1990). Facultative reactions can be switched on and off as needed by the animal. They include reactions that support a further, adaptive form of **DIT**; they also include reactions that are thermoregulatory in function (i.e., cold-induced thermogenesis, **CIT**) and reactions associated with exercise, growth, and reproduction. Obligatory reactions include those that are essential to cells and tissues of the body. Important among these processes are those that are essential for endothermy and those that are essential for postprandial processes, as described above. All cells and tissues of the body contribute to obligatory thermogenesis. Facultative thermogenesis is, how-

ever, predominantly the result of metabolic reactions in two types of tissue, skeletal muscle and brown adipose tissue (**BAT**) (Himms-Hagen, 1990). In muscle, these processes include exercise-induced thermogenesis and cold-induced shivering thermogenesis; both are mechanisms that require coupled oxidative phosphorylation. In **BAT**, facultative thermogenic processes include cold-induced and diet-induced nonshivering thermogenesis; both require uncoupled oxidative phosphorylation. Brown adipose tissue thermogenesis is important in thermoregulation of small mammals. In large mammals, including cattle, sheep, reindeer, and humans, the **BAT** that is present at birth and in infancy atrophies with age and becomes tissue resembling white adipose (Lean et al., 1986; Casteilla et al., 1987; Casteilla et al., 1989; Soppela et al., 1991; Trayhurn et al., 1993; Nicol et al., 1994).

Components of Cellular Energy Expenditure—Coupled vs Uncoupled Energy Metabolism

A large proportion of cellular energy expenditure is used to drive the synthesis of ATP in mitochondria. Oxidative phosphorylation provides most of the ATP, roughly 90% of cellular ATP. Substrate level phosphorylation (through glycolysis and the tricarboxylic acid cycle) provides the remainder. ATP is subsequently used to fuel a large number of cellular reactions (see below).

Cellular and Tissue-Specific Considerations

In reviewing categories of cellular energy expenditure, an important preliminary question is, Does a certain cell or tissue type account for a disproportionately high or low amount of total body energy expenditure? At the individual tissue level, it is safe to state that no one organ or tissue accounts for the majority of basal energy metabolism. However, the contribution of individual tissues varies, to some extent, between species (Schmidt-Nielsen, 1984). On a basis of the percentage contribution to total body weight, some organs (liver, brain, kidney, heart, gastrointestinal tract) contribute a much larger fraction to metabolic rate than would be anticipated based on their fractional mass in the body; others, such as white adipose tissue, bone, skin, and muscle, contribute a much lower fraction of the metabolic rate than would be expected (Rolfe and Brown, 1997). However, the importance of the study of pathways of energy expenditure in the latter types of tissues, such as muscle, should not be underestimated, because some of these tissues, including muscle, by virtue of their fractional mass, contribute substantially to basal metabolic rate. The important contribution of skeletal muscle to energy expenditure under non-resting conditions (e.g., cold-induced, or exercise-induced) is clear.

Cellular oxygen consumption can be subdivided into mitochondrial and nonmitochondrial oxidative reac-

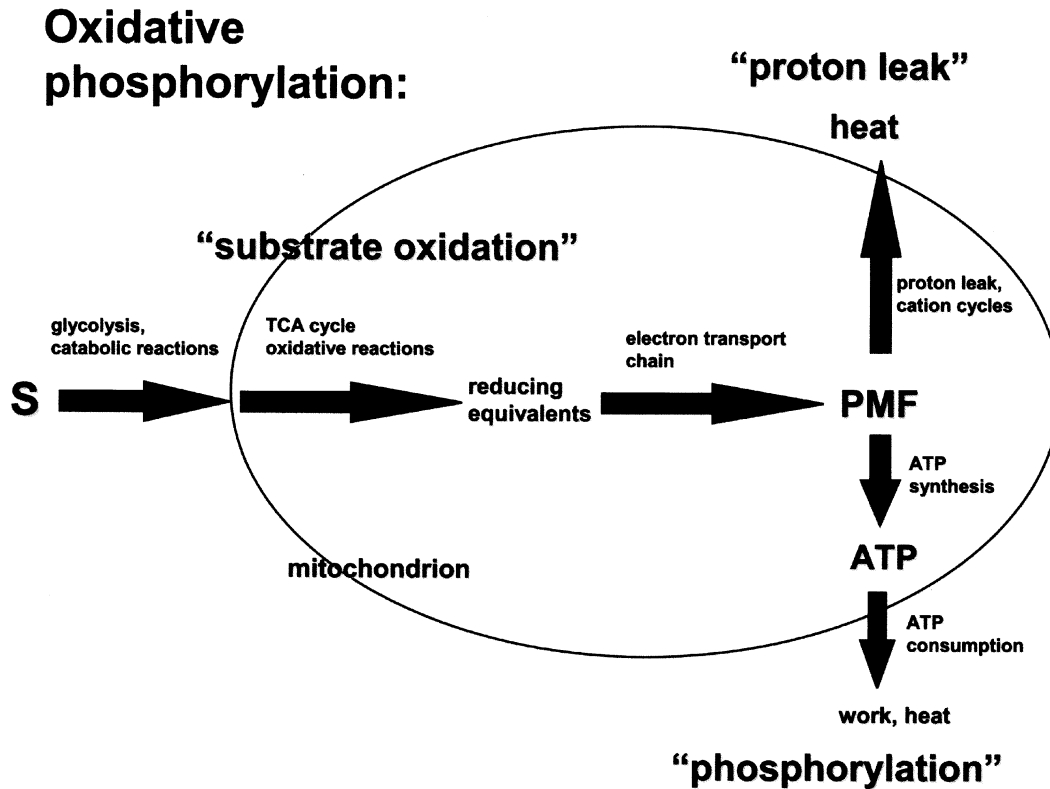


Figure 2. The oxidative phosphorylation system. Substrate oxidation reactions produce reducing equivalents that, in turn, fuel the electron transport chain and the production of protonmotive force (PMF) across the mitochondrial inner membrane. PMF is then used to support ATP synthesis, or proton leak.

tions. While mitochondrial reactions account for the majority of cellular oxygen consumption (roughly 80 to 90%, depending on cell type), the 10 to 20% extramitochondrial oxygen consumption should be considered when estimating effective P:O ratios in cells of the body (Rolfe and Brown, 1997). Proportions of extramitochondrial oxygen consumption amount to approximately 21% in rat liver cells (Brown et al., 1990; Brand et al., 1994), 20% in perfused liver (Scholz and Bucher, 1965), 14% in perfused skeletal muscle in rats (Dora et al., 1992; Rolfe and Brand, 1996), and 20% in rabbit reticulocytes (Siems et al., 1982), but only 2% in isolated thymocytes (Lakin-Thomas and Brand, 1988). Variation between cell types is evident.

Clearly, most of the oxygen consumed by cells is used to support mitochondrial reactions. Mitochondrial oxygen consumption can be further partitioned into that which supports ATP synthesis and that which is uncoupled from ATP synthesis (Brand, 1990). In other words, not all mitochondrial oxygen consumption is coupled to ATP synthesis. Figure 2 depicts oxidative phosphorylation processes as a tripartite system including blocks of reactions representing the substrate oxidation reactions, ATP synthesis and turnover reactions, and proton leak reactions. The latter two groups of reactions are fueled by mitochondrial protonmotive force (PMF), which, in turn, is maintained largely by the proton pumping components of

the electron transport chain. Because the ultimate reaction of the electron transport chain is cytochrome c oxidase, the activity of the block of substrate oxidation reactions can be measured as oxygen consumption. Protonmotive force is used to support ATP synthesis and turnover reactions and mitochondrial proton leak reactions.

ATP Synthesis and Turnover Reactions

The focus in this brief review is on the importance of uncoupling to the efficiency of energy metabolism, so we will acknowledge the importance of, but not elaborate on, the many subgroups of reactions comprising the block of ATP turnover reactions. Moreover, several very useful reviews are available (Kelly and McBride, 1990; Clausen et al., 1991; Buttgureit and Brand, 1995; Rolfe and Brown, 1997; Hulbert and Else, 2000). As described in the excellent review by Rolfe and Brown (1997), the contributions of different ATP-consuming processes within a cell have often been determined by 1) assaying the rate of the process of interest, and then estimating the corresponding oxygen consumption needed to support that rate or 2) specifically inhibiting the process of interest and measuring the degree of inhibition of oxygen consumption. In most instances, the application of either approach has been flawed. With the first approach, a mechanistic

P:O ratio of 3.0 (for NADH substrate-fueled mitochondria), or 2.0 (for succinate-fueled mitochondria) has commonly been used. These values are incorrectly high because they do not take into account either the proportion of cellular oxygen consumption that is extramitochondrial (0 to 20%, depending on cell type, described above), or the proportion of mitochondrial oxygen consumption that is used to balance mitochondrial proton leak (0 to 50%, depending on tissue, described below) (Rolfe and Brown, 1997). With the second approach, inhibitors that are used to specifically inhibit a given process (e.g., ouabain, an inhibitor of the Na, K pump) often have secondary effects on other metabolic processes (Himms-Hagen, 1976).

Nonetheless, the most important subgroups of ATP-consuming reactions in cells are thought to include protein synthesis; the activities of Na/K ATPase, Ca ATPase, and actinomycin ATPase; and the processes of gluconeogenesis (in liver and kidney), of ureagenesis (in liver), of mRNA synthesis, and of substrate cycling. The contributions of these processes toward total cellular ATP consumption are approximately 28% for protein synthesis, 19 to 28% for Na/K ATPase, 4 to 8% for Ca ATPase, 2 to 8% for actinomycin ATPase, 7 to 10% for gluconeogenesis, and 3% for ureagenesis (Rolfe and Brown, 1997). Clearly, the relative importance of groups of ATP-demanding reactions can vary substantially between cell types.

Uncoupled Oxidative Phosphorylation

The most pronounced example of uncoupled oxidative phosphorylation is found in BAT, in which the exclusive presence of uncoupling protein -1 (UCP1) allows the return of protons from the intermembrane space into the matrix, bypassing ATP synthase (Nicholls and Locke, 1984; Himms-Hagen 1985). Mitochondrial protonmotive force decreases as a result, and the activity of the respiratory chain increases to rectify protonmotive force. The consequence is the increased oxidation of fuel substrates, increased oxygen consumption, and thermogenesis. UCP1-mediated thermogenesis is significant, particularly in small rodents, and in neonatal mammals (see above, and, Himms-Hagen 1990). Brown adipose tissue thermogenesis is controlled primarily through the activity of the sympathetic nervous system. Uncoupling is activated by norepinephrine acting upon adrenergic receptors. The capacity for thermogenesis in brown adipocytes is directly related to the concentration of mitochondrial UCP1; both are inversely related to the temperature at which the animal has been living (Himms-Hagen and Ricquier, 1998).

Uncoupling of another sort also occurs in tissues of the body. This uncoupling is referred to as mitochondrial proton leak and has been studied extensively by Brand since the late 1980s (Brand, 1990). Several physiological functions have been proposed for proton leak, including a means for heat production, regula-

tion of the efficiency of oxidative phosphorylation, minimizing the production of reactive oxygen species (ROS), and, finally, a means to maintain the NAD⁺/NADH ratio sufficiently high to support carbon fluxes in biosynthetic processes (Brand et al., 1994; Stuart et al., 1999). The bioenergetic implications of proton leak are that a finite amount of mitochondrial oxygen consumption is not coupled to ATP synthesis, but is instead released simply as heat. Estimates of proton leak-dependent oxygen consumption in isolated rat liver cells and perfused rat skeletal muscle show that approximately 26% of resting energy expenditure is due to leak in liver cells (Brown et al., 1990; Nobes et al., 1990; Harper and Brand, 1993; Brand et al., 1994) and 52% in resting skeletal muscle (Brand et al., 1994; Rolfe and Brand, 1996). In the heart, Rolfe and Brown (1997) estimated that a maximum of 10 to 13% of resting oxygen consumption is due to proton leak, based on studies in arrested heart (Challoner, 1968). At the level of the whole body, mitochondrial proton leak accounts for approximately 15 to 20% of basal energy metabolism (Rolfe and Brown, 1997).

Results thus indicate that mitochondrial proton leak is a very significant contributor to resting cellular energy metabolism. How does the proportion of leak-dependent oxygen consumption change with increases in ATP demand in cells? In studies of isolated mitochondria, it is possible to create metabolic situations in which the demand for ATP synthesis ranges from high to low. By incubating mitochondria in the presence of an ADP-regenerating system, such as hexokinase and glucose, the rate of ATP synthesis is high, and mitochondria are said to be functioning in "state 3." When ATP demand is high, the rate of proton leak reactions is low (Brand, 1990). Conversely, when ATP demand is low, the rate of leak is highest. Brand and colleagues, using top-down metabolic control analyses, have further shown that control of leak, substrate oxidation, and ATP turnover reactions changes significantly when ATP demand changes (i.e., from state 4 to state 3) (Brand et al., 1993). Overall, these results show that the proportional contribution of leak to total cellular oxygen consumption is highest when cells are at rest (i.e., when ATP demand is low). Leak, therefore, exerts a tonic effect on changes in oxygen consumption: when ATP demand is low, oxygen consumption drops, but it does not drop as much as it would if leak did not occur.

Relationship Between Proton Leak Rates and Factors Known to Affect BMR

The absolute rate of mitochondrial proton leak is affected by several factors that are related to BMR. These factors include body size in mammals, thyroid status, and endothermy.

Body Size in Mammals

In the 1930s and 1940s Kleiber and Brody explored the empirical relationships between body weight and

BMR of mammals, ranging from mice to elephants, and showed that BMR was proportional to the three-quarters power of their body weight ($W^{0.75}$) (reviewed in Kleiber, 1977; Schmidt-Nielsen 1984; Blaxter, 1989). Later explored was the relationship between body weight and mass-specific metabolic rate (i.e., metabolic rate per unit body weight). Metabolic rate (per unit of body weight) varies inversely with body weight in mammals. As an example, the expected specific metabolic rate of a 30-g mouse would be $168.2 \text{ kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, whereas the value for a 300-kg cow would be $16.82 \text{ kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ (Schmidt-Nielsen, 1984). In their studies of proton leak in liver mitochondria of mammals having a wide range of body masses, Porter and Brand established that proton leak rate varies inversely with body mass (Porter and Brand, 1993). However, in their later studies it was demonstrated that in intact cells (hepatocytes) the proportion of respiration due to leak is remarkably constant at approximately 20% in mammals of widely differing body weight (Porter and Brand, 1995). Thus, it has been suggested that leak may be a general phenomenon of high energetic cost, having common important physiological roles (Brand, 2000).

Thyroid Status

Thyroid hormones are important endocrine regulators of basal metabolic rate. Thyroid hormones produce increases in proton leak and as well as in the mitochondrial inner membrane surface area (Brand et al., 1992). In hypothyroid rats, the decrease in proton leak in hepatocytes accounts for all of the decrease in mitochondrial oxygen consumption observed (Harper and Brand, 1993), and the effective P:O ratio is increased 10 to 15% (Brand et al., 1993). In hyperthyroid rats, the increase in oxygen consumption of hepatocytes was shown to be due to equal increases in proton leak and ATP turnover, such that there were no significant changes in effective P:O ratios (Harper and Brand, 1993, 1994).

Endothermy

There have been several studies supporting the idea that proton leak significantly contributes to the cost of endothermy (warm-bloodedness). Reptiles (ectotherms) have resting metabolic rates that are four to five times lower than those of mammals of the same body weight. Experiments have shown that in the ectothermic bearded dragon lizard, which has about the same body mass as a rat and a similar preferred body temperature (37°C), proton leak rate in liver mitochondria is one-fifth of that in mitochondria from the rat (Brand et al., 1991). However, intact hepatocytes of the lizard consume about one-fifth of the oxygen that rat hepatocytes do (Brand et al., 1991). It is thus apparent that the contribution of proton leak to cellular thermogenesis (about 20 to 25%) is conserved. The

conserved contribution of leak as a proportion of cellular thermogenesis, and likely of BMR, is similar to the conservation observed for the effects of body mass in mammals (above).

The Question of Mechanism

Is proton leak mediated by a protein, or is it a property of the lipid-protein composition of the mitochondrial inner membrane? Its mechanisms are the subject of intense debate at this time, and much of the debate centers on whether the recently identified uncoupling proteins mediate proton leak to any extent (Brand et al., 1999; Ricquier and Bouillaud, 2000). Since the identification of the novel uncoupling proteins, including UCP2 (Fleury et al., 1997; Gimeno et al., 1997), UCP3 (Boss et al., 1997; Gong et al., 1997), BMCP1 (Sanchis et al., 1998), and UCP4 (Mao et al., 1999), many studies have examined the hypothesis that proton leak was mediated by these novel UCP (see review of Boss et al., 2000). The expectation has been that the novel uncoupling proteins caused uncoupling through a mechanism similar to UCP1, and that these novel uncoupling proteins were therefore very important in controlling resting metabolic rate. Many types of experimental approaches have been used to study the function of the uncoupling proteins, including heterologous expression in yeast systems; reconstitution in liposomes; studies of human genetic linkage, association and variants; physiological induction of expression through means of diet composition, diet-restriction, and exercise; and creation of UCP knockout and UCP overexpression mice. Unfortunately, none of these approaches has proven unequivocally that the physiological function of the novel UCP is either proton leak or uncoupling (see reviews of Harper and Himms-Hagen, 2001; Himms-Hagen and Harper, 2001; Stuart et al., 2001).

The literature describing the physiological induction of the uncoupling proteins includes a number of paradoxical findings. The most pronounced paradox is the significant increases in Ucp2 and Ucp3 gene expression during fasting and severe food restriction, conditions in which the efficiency of energy metabolism is well recognized, not situations in which energy wastage would be desirable. Cadenas et al. (1999) examined the increased expression of Ucp3 mRNA and protein caused by fasting in rats. While there were significant increases in Ucp3 expression in muscle, there were no changes in mitochondrial proton leak. Very recently, Bezaire and colleagues conducted a similar study in wild-type mice and Ucp3 knockout mice (Bezaire et al., 2001). Fasting caused a fourfold increase in Ucp3 and a twofold increase in Ucp2 in muscle of wild-type mice, but similar to the findings of Cadenas and colleagues (1999), there were no changes in mitochondrial proton leak reactions. Mitochondria of Ucp3 knockout mice have higher protonmotive force than those from wild-type mice (Gong et al., 2000;

Bezaire et al., 2001), and fasting results in further small increases in protonmotive force (Bezaire et al., 2001). It seems that fasting exacerbates the metabolic aberrations occurring in the absence of Ucp3. Nedergaard and colleagues have shown that norepinephrine-induced thermogenesis in brown-fat cells is absolutely dependent on UCP1, despite the high levels of UCP2/UCP3 mRNA observed in BAT of UCP1-ablated mice (Nedergaard et al., 2001). Moreover, UCP1-ablated mice are cold-insensitive (Enerback et al., 1997), isolated brown-fat mitochondria are not uncoupled, and the kinetics of mitochondrial proton leak are similar to those of wild-type mitochondria in the presence of GDP, a well known inhibitor of UCP1-mediated uncoupling (Monemdjou et al., 1999). Perhaps Ucp2 and Ucp3 cause uncoupling under some situations, but it is becoming increasingly clear that they have more important physiological functions in the transport of metabolites (e.g., those related to fatty acid oxidation) (Samec et al., 1998).

In fact, there is a tight correlation between the expression of Ucp3 and metabolic states in which fatty acid oxidation is high. Most prominent is the correlation of Ucp3 expression with fasting and food restriction. A mechanism to explain how UCP3 could enhance fatty acid oxidation was recently proposed (Himms-Hagen and Harper, 2001). This hypothesis describes how UCP3 might facilitate rapid rates of fatty acid oxidation by acting as a mitochondrial fatty acid efflux protein. It was proposed that UCP3 functions in concert with mitochondrial thioesterase(s) (**MTE**) to remove free fatty acid (produced by MTE) from the matrix and liberate CoA-SH. The latter is in relative high demand during fatty acid oxidation. That the direct interaction of fatty acid with UCP1 is necessary for its function (Garlid et al., 1996; Klingenberg and Huang, 1999) lends some support to the idea that its homologue, UCP3, interacts with fatty acid. A number of very recent studies are providing further support for the idea that UCP3 may indeed function as a fatty acid efflux protein. In the mouse that overexpresses human UCP3 in muscle, Moore et al. (2001) studied the expression of carnitine palmitoyl transferase, lipoprotein lipase, MTE-1, CD36 (a fatty acid transporter, also referred to as FAT), among other genes, and found a threefold increase in MTE-1 mRNA. Lipoprotein lipase expression also increased significantly, but to a lesser extent (50%). Moreover, Bezaire and colleagues (2001) found impaired fatty acid oxidation, as reflected in high respiratory quotients, in the Ucp3 knockout mouse. Thus, the evidence supporting the idea that Ucp3 functions physiologically in fatty acid metabolism is accruing.

Summary and Conclusions

Basal metabolic rate normally accounts for the largest single fraction of total body energy expenditure in mammals. Its metabolic origins are not well under-

stood, but many of the processes occurring in cells and tissues of the body have been widely studied and are briefly reviewed here. The importance of uncoupled states of cellular energy expenditure due to mitochondrial proton leak reactions is becoming increasingly appreciated. Proton leak is a process thought to occur in all cell types and is described as being the largest single contributor to BMR. The proportional contribution of proton leak-dependent energy expenditure is greatest when cells are in a relative state of rest (i.e., when energy expenditure and ATP demand is low). This is in contrast to the situation in BAT, whose primary physiological function is heat production (thermogenesis). The unique presence of UCP1 in brown adipocytes allows very high absolute rates of uncoupled energy expenditure.

The mechanism(s) responsible for mitochondrial proton leak are poorly understood. The identification within the last few years of novel uncoupling proteins led immediately to the hypothesis that these proteins caused mitochondrial proton leak. While they may cause proton leak under some conditions, it is becoming increasingly clear that they likely have other important physiological functions. In particular, evidence suggests that UCP3 plays an important role in fatty acid metabolism, and may act as a fatty acid anion translocator. Thus, the search for the molecular origins of mitochondrial proton leak continues.

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