The Efficiency of Cellular Energy Transduction and Its Implications for Obesity

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Abstract

We assess the existence, mechanism, and functions of less-than-maximal coupling efficiency of mitochondrial oxidative phosphorylation and its potential as a target for future antiobesity interventions. Coupling efficiency is the proportion of oxygen consumption used to make adenosine triphosphate (ATP) and do useful work. High coupling efficiency may lead to fat deposition; low coupling efficiency to a decrease in fat stores. We review obligatory and facultative energy expenditure and the role of a futile cycle of proton pumping and proton leak across the mitochondrial inner membrane in dissipating energy. Basal proton conductance is catalyzed primarily by the adenine nucleotide translocase but can be mimicked by chemical uncouplers. Inducible proton conductance is catalyzed by specific uncoupling proteins. We discuss the opportunities and pitfalls of targeting these processes as a treatment for obesity by decreasing coupling efficiency and increasing energy expenditure, either directly or through central mechanisms of energy homeostasis.

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INTRODUCTION

If we are prone to obesity, is this because our metabolism is too efficient for a Western lifestyle? The converse, at least, is true: Patients with Luft's syndrome have inefficient mitochondria, leading to high caloric intake, high metabolic rate with profuse sweating, and low body weight (but they also suffer from debilitating muscle weakness) (98). Perhaps humans have a spectrum of efficiencies, with the most efficient individuals prone to obesity on an excess, palatable food supply. Could we increase heat production pharmacologically to decrease fat storage by judiciously lowering metabolic efficiency and raising metabolic rate? How efficient is our metabolism? Thermodynamic efficiency is the proportion of input energy that is output as work on the external environment. In contrast, by metabolic efficiency we usually mean the coupling efficiency, which is the proportion of calories burned and oxygen consumed that is coupled to metabolic work through synthesis of adenosine triphosphate (ATP). At high coupling efficiency, most of our metabolic rate makes ATP to drive essential maintenance reactions or to store dietary calories as fat. At low coupling efficiency, we burn fat and produce heat. **Figure 1** shows the chemiosmotic mechanism of mitochondrial ATP synthesis: As the electrons derived from respiratory substrates like sugars or fatty acids pass down the respiratory chain to reduce oxygen to water, protons are pumped across the mitochondrial inner membrane (reaction 1), generating a membrane potential and a pH gradient (the protonmotive force). This source of potential energy drives protons through the ATP synthase (reaction 2), coupling respiration to ATP synthesis. If only reactions 1 and 2 occur, the coupling efficiency is 100%. The coupling efficiency is decreased if any protons leak back across the membrane through other proton conductance pathways (reaction 3); if they all leak back, then oxidation is completely uncoupled from phosphorylation and the coupling efficiency is zero. The coupling efficiency thus depends on the relative rates of reactions 2 and 3, and is defined as the rate of reaction 2 divided by the rate of reaction 1.

Although it could be argued that physiological regulation of body weight is failing in Western societies, regulation must exist since most adults maintain a stable body weight for years. The set-point theory posits that body weight is regulated through feedback and feedforward mechanisms. Dietary maintenance of a 10% change in body weight results in changed energy expenditure, even after correction for changes in body weight and body composition (88). When body weight is altered, peripheral signals are transmitted to metaboregulatory sensor sites in the brain, and effector signals

Efficiency: useful power output/total power input

Coupling efficiency: percentage of oxygen consumed that is used to drive ATP synthesis

ATP: adenosine triphosphate

are released to modulate energy intake and expenditure, resulting in resistance to deviations from the body weight set point. In discussions of energy expenditure in obesity, there has been much debate about alterations in coupling efficiency. Clearly, weight gain or loss will depend, at a given level of dietary energy, on the amount of work done (e.g., exercise, growth, repair) and on the coupling efficiency of that work. In the following sections, we review energy expenditure and coupling efficiency, and their influence on obesity development and other processes.

ENERGY EXPENDITURE

Obligatory and Facultative Energy Expenditure

The largest contributor to obligatory energy expenditure is the basal metabolic rate (BMR): resting energy expenditure at thermoneutrality in the unfed state. In sedentary adults, it can be 75% of energy expenditure (122). Processes underlying BMR include maintenance of ion gradients across cell membranes (e.g., for transmission of neural impulses and transmembrane transport of metabolites); basal synthetic reactions (e.g., RNA and protein synthesis); cellular turnover and repair, and, as detailed below, mitochondrial proton leak. Other obligatory energy expenditure is associated with the digestion, absorption, and metabolism of dietary constituents and accounts for 10%–15% of dietary energy intake (81).

Facultative energy expenditure is associated with specific physiological functions that are not "obligatory," including pregnancy, lactation, growth, and adaptive diet-induced thermogenesis (43, 133, 141). The weight gained or lost by different individuals is highly variable (see, e.g., 14, 71), so differences in compensatory thermogenesis probably contribute to poor long-term efficacy in treatment of obesity in some people. Facultative reactions also include cold-induced thermogenesis: shivering in skeletal muscle and nonshivering thermogenesis in brown adipose tissue. Brown adipose tissue thermogenesis is one mechanism known to decrease coupling efficiency and mitigate obesity development (133, 141). It can be substantial in newborn humans (and may play a role in thermoregulatory feeding) (74) but diminishes dramatically thereafter. However, small diffuse depots of active brown adipose tissue persist during adulthood (67, 109). Perhaps these could be targeted to alter metabolic efficiency and treat obesity. Beyond these facultative reactions, there is a small but significant energy expenditure to support fidgeting activity (93). Finally, exercise can result in huge increases in total body energy expenditure but is highly variable between individuals.

The best predictor of BMR is lean body mass. Studies of obese, lean, and underweight subjects demonstrate that resting energy expenditure can be calculated accurately from organ and tissue masses (determined by imaging) using organ-specific metabolic rates (12). Nevertheless, 20%–30% of the variability in BMR remains unaccounted for and may be due to differences between individuals in mass-specific energy expenditure of the tissues (138). A classic study of overfeeding in monozygotic twins (14) concluded that genetic factors were involved in determining weight gain. Although genetic variation cannot explain the recent and rapid rise in obesity, it can explain why some people are more susceptible than others. The number of genes associated with obesity has grown exponentially in recent years (see 120 and **http://obesitygene.pbrc.edu**). Could genetically determined differences in coupling efficiency underlie some of this variability in mass-specific energy expenditure of the tissues?

Mechanisms of Inefficiency of Coupling

Most ATP synthesized in the resting state is used to maintain posture, respiration, blood circulation, and other motile processes, to degrade and resynthesize macromolecules (protein, RNA, DNA) for regulation or repair, and to maintain ion gradients (Na^+, K^+, Ca^{2+}) to allow basal nervous and muscular activity (29, 129, 130). It is not known whether the rates

Protonmotive force: the electrochemical proton gradient across the mitochondrial inner membrane that drives ATP synthesis, made up of a membrane potential and a pH gradient

BMR: basal metabolic rate

Obese: body mass index [(body mass in kg)/(height in m)^2] >30

UCP: uncoupling protein

ROS: reactive oxygen species

of these essential processes differ substantially between individuals. However, metabolic efficiency can be lowered if ATP is synthesized but then hydrolyzed without doing useful work. Such thermogenic ATP cycles are well known, for example in the flight muscle of bumblebees to warm the muscle before flight (36) or in the heater organ of endothermic fish such as marlin and tuna to warm the muscle, viscera, brain, and eyes to allow active hunting in cold marine environments (9). Other than shivering, thermogenic futile cycles of ATP synthesis and hydrolysis are not characterized in mammals.

The most prominent metabolic inefficiency occurs when ATP is synthesized, because the coupling efficiency of mitochondrial ATP synthesis is intrinsically variable. Given oxidizable substrates, isolated mitochondria consume oxygen at a low rate even in the absence of added ADP. In this condition (state 4), no oxygen consumption is used for ATP synthesis and the coupling efficiency is zero. In the presence of ADP (state 3), respiration is much faster because of rapid ATP synthesis (reaction 2 in **Figure 1**) and the coupling efficiency approaches 90% (24). The cause of the low coupling efficiency in state 4 is the leak of protons across the mitochondrial inner membrane (reaction 3 in **Figure 1**). An alternative process, a slip reaction in the electron transport chain allowing electron transport without concomitant proton pumping, does not occur significantly at 37°C in either isolated mitochondria (21) or intact cells (3, 117).

There are two general classes of mitochondrial proton leak: basal and inducible. Basal proton leak occurs in mitochondria from all tissues and species investigated (15). It is greater when metabolic rate is high: in small mammals (115) or birds (26) compared to large ones, in warm-blooded homeotherms compared to cold-blooded poikilotherms (76), or when thyroid hormones are elevated (70). It is caused mostly by the presence in the membrane of the adenine nucleotide translocase, the protein that exports ATP synthesized in mitochondria, which is more abundant in mitochondria with high rates of ATP synthesis, potentially explaining the correlation between proton conductance and BMR (25).

Inducible proton leak is catalyzed by the adenine nucleotide translocase and by specific uncoupling proteins, UCP1, UCP2, and UCP3 (23, 52). The classic uncoupling protein is UCP1, which uncouples brown adipose tissue mitochondria in response to β-adrenergic stimulation, causing facultative thermogenesis (32, 111). Importantly, UCP1 is inactive in the absence of β-agonists because it is fully inhibited by physiological concentrations of purine nucleotides, primarily ATP. Norepinephrine activates the proton conductance of UCP1 by releasing fatty acids from triacylglycerol stores; these overcome the inhibition by ATP and also act as the fuel for thermogenic respiration. When fully activated, UCP1 in brown adipose tissue can increase metabolic rate in rodents fourfold (73). UCP2 is more widely distributed, and UCP3 is found mostly in skeletal muscle (125). They are activated by superoxide and its derivatives (44, 45), but because they are present at much lower concentrations than UCP1, their contribution to thermogenesis is much less, and may be negligible. Their main functions may be in regulation of reactive oxygen species (ROS) production and in regulation of hormone secretion (23, 52) (see below).

Coupling efficiency has been measured in several cell types (17). It can be approximated as the proportion of mitochondrial respiration in a cell that is inhibited by oligomycin, a specific inhibitor of ATP synthase. Since such inhibition allows the protonmotive force that drives the leak to build up, proton leak rates are higher in the presence of oligomycin than in its absence, so oligomycin-sensitive respiration underestimates coupling efficiency slightly (generally by about 5%) unless the change in protonmotive force is corrected for. Initial studies in resting or metabolically active rat hepatocytes yielded corrected coupling efficiencies of about 70%–80% (20, 112, 130). Similar values are found in hepatocytes from other mammals (116), birds (48), and ectotherms (8, 22, 76, 135), and in other cell types such as thymocytes

(30), primary cerebellar granule neurons (80), lung carcinoma cells (4, 153), and C2C12 myoblasts (1). An exception is INS-1E clonal pancreatic β-cells, which have much lower coupling efficiency, about 25% (1), perhaps related to the regulation of insulin secretion by UCP2 (see below).

Coupling efficiency has also been estimated in intact tissues and whole organisms. In perfused rat muscle, estimated from oligomycin-sensitive oxygen consumption, it is about 50% at rest (127) or 66% during work (130), although estimates using nuclear magnetic resonance give higher values (102). Combining the values from cells and tissues with data for the contribution of the different tissues to BMR leads to an overall estimate for the coupling efficiency of 75%–80% in rats (17, 130). In other words, 20%–25% of BMR in rats (and, by extension, in humans and other mammals) may be used to drive a futile cycle of proton pumping and proton leak across the mitochondrial inner membrane. It appears that humans may have a coupling efficiency of less than 100% not only under basal conditions, but also even at normal exercise levels. If so, there may be scope for increasing or decreasing the coupling efficiency. However, the difficulties of estimating coupling efficiencies in vivo make it hard to assess whether there is any variation in this efficiency between individuals that might explain different propensities to obesity, although new methods may make this feasible in the future (38, 102, 103).

Why Are We Inefficient? Protective Functions of Cellular Inefficiency

The molecular mechanisms of inefficiency described above are the products of evolutionary selection and must have conferred some selective advantage. The function of certain "futile" substrate cycles is to amplify metabolic signals, but what are the ancestral functions of basal and inducible mitochondrial proton leak? Possibilities include thermogenesis, protection against ROS, endowment of metabolic sensitivity, and maintenance of carbon fluxes (128).

An attractive theory to explain the persistence of mitochondrial proton leak throughout evolution is "uncoupling to survive," which proposes that proton leak decreases excessive mitochondrial superoxide production that causes oxidative damage and, ultimately, aging (16). The discovery that UCPs 1, 2, and 3 are activated by superoxide fits this hypothesis and suggests that UCPs may have an ancestral function as preventative antioxidants, responding to elevated superoxide by decreasing its production (18). The persistence of mitochondrial proton leak may represent an evolutionary trade-off between the coupling efficiency of ATP synthesis and superoxide production.

In view of the preceding discussion of energy balance, it seems clear that increased energy expenditure resulting from cellular inefficiency has a potential antiobesity effect. Although evolutionary studies concerning obesity focus on so-called thrifty mechanisms, these are simply the corollary of inefficiency mechanisms. Any thrifty (i.e., efficient) gene selected for must by definition operate on an inefficient background. Furthermore, advantageous thrifty phenotypes should show plasticity in response to environmental changes: Efficiency may be selected in times of famine, but this is likely to be counterbalanced by the adverse effects of morbid obesity in times of plenty, such that antiobesity effects of inefficiency may have undergone positive selective pressure (118). By increasing glucose catabolism (and thereby decreasing plasma glucose), cellular inefficiency may confer an antidiabetogenic advantage. Consistent with this idea, mice ectopically expressing UCP3 show significantly lower plasma glucose levels than wild-type mice (35) and, conversely, chow-fed *Ucp3*$^{-/-}$ mice show slightly higher fasting plasma glucose levels (146).

IS OBESITY A PROBLEM OF DECREASED ENERGY EXPENDITURE?

As discussed above, there is substantial unexplained variability in resting energy expenditure. Because resting metabolic rate is

the single greatest determinant of total energy expenditure, and since low rates are a risk factor for weight gain, many studies have attempted to identify factors associated with this residual variation.

However, as the genetic and environmental origins of human obesity are numerous and complex, it is a challenge to conduct well-controlled studies. An approach that has been used (by one of us) is to compare carefully matched groups of obese people who have dramatically differing rates of weight loss during a defined clinical weight-loss program. Intrigued by a tenfold variability in rate of weight loss in highly compliant subpopulations, we demonstrated that about half of this variability could be explained by known variables, including initial weight, age, and plasma free triiodothyronine concentrations (71). A fivefold variability remained, so we tested whether differences in mitochondrial coupling efficiency in skeletal muscle explain differences in diet responsiveness. The highest and lowest quintiles for rate of weight loss were defined as diet-responsive and diet-resistant, respectively. After body weight was stable for at least 10 weeks, 12 of 70 subjects in each group donated a muscle biopsy and blood for measurements of mitochondrial proton leak, UCP mRNA expression, and genetic studies. Despite similar baseline weight and age, weight loss was 43% greater, mitochondrial proton leak–dependent (state 4) respiration was 51% higher, and expression of UCP3 mRNA was 25% greater in diet-responsive than in diet-resistant subjects. There were no differences in UCP2 mRNA abundance. None of the published polymorphisms in *Ucp3* or its 5′ flanking sequence was associated with weight loss (or abundance of UCP3 mRNA). These findings are consistent with the idea that differences in metabolic efficiency explain some of the variability in weight-loss success. Ongoing studies corroborate these findings and reveal lower proportions of oxidative (versus glycolytic) fibers and total mitochondrial content in the diet-resistant subpopulation (58).

Antiobesity Therapies

Obesity is treated by achieving an extended period of negative energy balance followed by maintenance of the new body mass at energy balance. Weight-management programs that combine approaches of reduced energy intake through energy-restricted diets, behavior modification, and increased physical activity can be very effective in the short term, but the long-term success rates are usually poor.

Currently, very few pharmacological treatments are available, and more are greatly needed. In the United States, three drugs have approval for long-term use: sibutramine, orlistat, and rimonabant (27). Sibutramine inhibits norepinephrine and serotonin reuptake at nerve endings; to some extent it also inhibits dopamine reuptake. Food intake is reduced 25% in subjects receiving 30 mg/d for 7–14 days (131). The combination of sibutramine and behavior modification strategies increases the efficacy of weight loss (147). Orlistat is a lipase inhibitor and increases the excretion of dietary fat in the feces. Its effects are dose-dependent, and about one-third of the fat in a 30% fat diet is lost in fecal matter. In a review of meta-analyses of the effects of orlistat, Bray & Ryan (27) report a mean weight loss of 3.26 kg (95% CI 4.15–2.37 kg) after two years of use. Rimonabant is a specific antagonist of type 1 cannabinoid receptors and thus inhibits ingestion of highly palatable food. According to phase III clinical trial results, it appears promising for obesity treatment (27). However, because it acts as a central nervous system pleasure response inhibitor, there are psychiatric concerns for its use in susceptible populations.

All three drugs affect either satiety and food intake or the absorption of dietary energy, although there may be a component of increased energy expenditure in the effects of both sibutramine and rimonabant. Currently no antiobesity drugs act primarily to decrease coupling efficiency and increase energy expenditure. Substantial potential exists for the development of thermogenic agents as an additional means to treat obesity. Obviously, exercise-induced

thermogenesis is effective at encouraging negative energy balance. It has a wide range of additional benefits, including increased insulin sensitivity in muscle, increased strength, and improved cognitive function.

Could Uncoupling Be a Useful Target in Antiobesity Pharmacotherapies?

The idea of using synthetic uncoupling agents to treat obesity is not new. Synthetic uncouplers are amphipathic weak acids that dissolve in membranes. In the mitochondrial inner membrane, they create a selective conduit for protons to bypass ATP synthase; the potential energy generated from the oxidation of fuels is simply released as heat. Examples of uncouplers include 2,4 dinitrophenol (DNP); carbonylcyanide *m*-chloro phenylhydrazone (CCCP); and *p*-trifluoromethoxycarbonylcyanide phenylhydrazone (FCCP).

Synthetic uncouplers have been used clinically to treat obesity. DNP was used as a weight-loss drug in the 1930s (68). The association between DNP and weight loss followed observations during World War I of workers in munitions factories where compounds related to DNP were used. Subsequent clinical studies in the 1930s demonstrated that doses of up to 5 mg/kg led to few side effects other than a feeling of warmth and some increased perspiration. Notably, DNP caused a specific loss of body fat, indicating a qualitative difference between uncoupler-mediated and diet-induced weight loss (we can speculate that uncoupling β-cell mitochondria lowered plasma insulin, see below, increasing lipolysis in DNP-treated individuals). The acute effect of doses of 3–5 mg/kg was a 20%–30% increase in energy expenditure, which was induced within 1 hour and maintained for 24 hours before gradually dissipating. Chronic effects of the same dose for more than 10 weeks included a 40% increase in metabolic rate and no indication of acquired tolerance. Doses greater than 10 mg/kg were associated with tachycardia and increases in body temperature (40).

Thereafter, the use of DNP as a treatment for obesity became widespread, and by 1934 roughly 100,000 people had used DNP. Unfortunately, some prescriptions were not carefully tailored to patients. Moreover, DNP was included in nonprescription elixir-type products. Problems arose, including rashes, cataracts, and, in some cases, death. Since 1938, the clinical use of DNP and the sale of DNP-containing elixirs have been illegal in many countries. One problem with DNP and related uncouplers is that they have a narrow therapeutic window: The pharmacologically effective dose is only an order of magnitude less than the dose that can cause death by hyperpyrexia. Other uncouplers activate the proton conductance of the adenine nucleotide carrier at low concentrations and uncouple conventionally at much higher concentrations, giving them a much wider dynamic range (96) and raising the possibility that they could be used as a starting point for developing safer chemical uncouplers that could be used to treat obesity by lowering coupling efficiency.

DNP:
2,4 dinitrophenol

Uncoupling Proteins as a Target

With the identification of the novel uncoupling proteins ten years ago came great interest in new approaches to treat obesity. The original hypothesis for UCPs 2–5 was that they uncoupled like UCP1; thus, they were proposed to be important in controlling resting metabolic rate (11, 49, 60, 61). However, their concentrations are orders of magnitude lower than that of UCP1 in brown adipose tissue (69), so their thermogenic effects will be much less. Research using *Ucp1* knockout mice demonstrated that the absence of UCP1 did not lead to obesity, only to cold intolerance (49). Subsequent studies revealed that *Ucp2* and *Ucp3* knockout and *Ucp2/3* double-knockout mice had no increased susceptibility to obesity (6, 62, 146). However, as UCP3 expression is restricted mainly to skeletal muscle and brown adipose tissue, there has been much interest in specifically targeting this UCP to increase metabolic rate and treat obesity. Because the

expression of UCP2 is widespread and that of UCP4 and UCP5 is highest in the central nervous system, there has been less interest in targeting them. Moreover, based on phylogenetic analyses, UCP4 and UCP5 are not really members of the UCP family (32).

Many experimental approaches have been used to study the function of the UCPs, including (*a*) direct measurement of native function in isolated mitochondria, (*b*) heterologous expression in yeast, (*c*) reconstitution in liposomes, (*d*) studies of human genetic linkage, association, and variants, (*e*) physiological induction of expression by diet composition, diet restriction, and exercise, and (*f*) creation of *Ucp*-knockout and UCP-overexpression mice. Many studies suggest that the physiological functions of UCPs do not increase metabolic rate. In particular, UCP2 and UCP3 expression increase in skeletal muscle during fasting and severe food restriction—conditions in which hypometabolism is well recognized.

The tight correlation between UCP3 expression in skeletal muscle and metabolic states with high fatty acid oxidation has led to hypothesized functions in fatty acid metabolism (134). A mechanism to explain how UCP3 could enhance fatty acid oxidation (75) proposes that UCP3 is a fatty acid anion transporter that removes free fatty acid (produced by mitochondrial thioesterase) from the matrix, liberating CoA. Because CoA is in high demand during fatty acid oxidation, UCP3 would indirectly facilitate fatty acid oxidation and remove potentially damaging fatty acid anions. This hypothesized mechanism still requires empirical testing.

Early observations suggested that UCP2 and UCP3 protect against oxidative stress (6, 110, 145). A second major hypothesis is that they protect cells against excessive mitochondrial superoxide production by mild uncoupling of oxidative phosphorylation (18, 19). In support of this hypothesis, the uncoupling function of UCPs is activated by superoxide and its derivatives (44, 45), and their presence and activity lowers the production of ROS in isolated mitochondria, in cells, and in vivo (23, 52). A third hypothesis, that UCP2 is part of a signaling mechanism through mild uncoupling and regulation of cellular ATP/ADP ratios, is discussed below.

Although the absence of the UCPs does not cause obesity in mice, the ectopic expression and overexpression of UCPs is associated with protection from obesity. Prior to the identification of the novel UCPs, Kopecky et al. (85) ectopically expressed UCP1 in white adipose tissue. The transgenic mice fed a low-fat diet gained body weight similarly to wild-type mice; however, on a high-fat diet, the transgenic mice had twofold lower feed efficiency and significantly lower body weight than did wild-type mice. Overexpression of UCP3 in skeletal muscle in mice caused hyperphagia and weight loss (specifically, loss of fat) (35). The mitochondria from these mice had higher proton conductance, implying that the phenotype was caused by uncoupling. Later work (31) showed that the uncoupling was not regulated, suggesting that it was not an expression of the native function of the protein (a general problem with overexpression studies of UCP2 and UCP3), although this finding does not affect the conclusion that mild uncoupling of skeletal muscle mitochondria can be an effective antiobesity treatment in vivo. Similarly, Li et al. (94) found that low expression of UCP1 in skeletal muscle doubled muscle oxygen consumption. Transgenic mice had the same food intake as wild-type mice, but lower body weights and circulating glucose and triglycerides. Glucose tolerance was also improved in the transgenic mice, and they were protected from high-fat-diet-induced obesity. Whole-body metabolic rate was increased at rest and with exercise. These findings show that increased uncoupling of oxidative phosphorylation (and lowering of coupling efficiency) in muscle is a viable treatment for obesity and insulin resistance (see Sidebar).

Some evidence links UCP3 to a role in energy expenditure in mice in the context of the hyperthermia associated with the recreational drug 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy"). *Ucp3* knockout mice were protected from hyperthermia and the sometimes-lethal toxic effect of MDMA

(105). Subsequent studies linked the effects of MDMA to substantial increases in circulating norepinephrine and free fatty acids and to thyroid hormone status (140). Thus, under these specific pharmacological conditions in which there are dramatic increases in sympathetic activity, it is possible to discern a thermogenic role for UCP3.

However, short of these transgenic approaches to increase the expression of UCP1 or UCP3, none of the physiological approaches (e.g., fasting) to increase UCP2 or UCP3 expression is associated with increased energy expenditure. Targeting UCP3 to treat obesity may require pharmacological compounds or mixtures that both increase UCP3 expression and acutely activate its proton translocation activity. It also remains possible that methods could be devised to increase the amount of brown adipose tissue and the expression and activity of UCP1 in the residual pockets of brown adipose tissue in adult humans. In rodents, this is possible through treatment with β-3 adrenergic agonists. In rats and mice, the β-3 adrenergic receptor is highly selectively expressed in white and brown adipose tissues, and acute doses of a β-3-selective agonist, such as CL 316,243, double resting energy expenditure (see, e.g., 142). Chronic treatment of rodents with β-3 receptor agonists also causes brown adipose tissue growth and the remodeling of white adipose to a phenotype similar to brown adipose tissue (5, 41, 59). These observations in rodents parallel findings in adult humans afflicted with catecholamine-secreting pheochromocytoma of the adrenal glands, where there is an expansion of brown adipose tissue depots (126). However, a major challenge for the coordinated induction of lipolysis in white adipose tissue and uncoupled thermogenesis in brown adipose tissue in humans is that rodent and human β-3-adrenergic receptors differ in relative levels of expression. Humans express β-3-adrenergic receptors abundantly in brown but not white adipocytes, whereas rodents express β-3-adrenergic receptor mRNA abundantly in both cell types. Studies in transgenic mice indicate that expression of the receptor in both brown and white adipose tissues is required for a significant thermogenic response to a β-3-selective agonist (78). This challenge will need to be overcome before β-3-selective adrenergic agonists can be used to stimulate the growth and activity of brown adipose tissue in adult humans. More research is needed to advance understanding of the apparent conversion of white adipose tissue to thermogenic brown adipose tissue. It is possible that future "cocktails" of agonists could simultaneously activate lipolysis in white adipose tissue and induce brown adipose tissue activity and growth to treat obesity by altering coupling efficiency in humans.

RAISING COUPLING EFFICIENCY TO INCREASE GROWTH RATE

The inverse of decreasing coupling efficiency to help increase energy expenditure and treat obesity is to increase coupling efficiency to increase growth rate or fat accumulation. Although the concept of increasing coupling efficiency underpinned the development of methods to isolate well-coupled mitochondria from different sources (by employing media lacking calcium or fatty acids or by adding purine nucleotides to inhibit native UCP1 activity in isolated brown adipose tissue mitochondria), increased coupling efficiency has not been achieved experimentally in whole animals (e.g., by removing activators or by employing inhibitors of inducible proton conductance). However, it may be desirable in a number of situations, such as in cachexia or postoperative recovery, when weight gain is clinically desirable. We may have produced this effect inadvertently in domesticated animals during intensive selection to increase feed efficiency (weight gain divided by food consumed). For example, such selection may have led to high mitochondrial coupling efficiency in chickens (13).

CENTRAL CONTROL OF EFFICIENCY

All three antiobesity drugs mentioned above exert their effects primarily by decreasing energy intake. Although feeding behavior is governed by many factors, including social, psychological, and economic influences (10), regulation by a central homeostatic system undeniably plays an important role. Our understanding of how

ARC: arcuate nucleus
NPY: neuropeptide Y

the brain regulates feeding behavior has improved enormously over the past 15 years, most notably since the cloning of the gene for leptin (*ob*) in 1994 (2, 159). This vast topic has been reviewed extensively elsewhere (10, 46, 47, 90–92, 139, 152) so is summarized only briefly here.

The hypothalamus (in particular the arcuate nucleus, ARC) regulates feeding behavior (feeding initiation, termination, and frequency) by integrating afferent signals that indicate long-, medium-, and short-term nutritional status and then relaying efferent signals via connections with other parts of the brain, most notably the paraventricular hypothalamic nucleus, which in turn regulates activity of the autonomic nervous system and the pituitary and thyroid glands (56). The hypothalamus receives indicators of long-term nutritional status directly from white adipocytes in the form of leptin. Circulating leptin levels are proportional to adiposity, thus leptin acts as a gauge of fat reserves. Leptin receptors are found in two groups of hypothalamic neurons that exert opposing effects on feeding behavior. Put simply, leptin inhibits the activity of cells that stimulate feeding behavior and simultaneously stimulates the activity of cells that inhibit feeding.

Hypothalamic neurons that stimulate appetite express neuropeptide Y (NPY) and Agouti-related protein (AgRP), whereas neurons that suppress appetite express α-melanocyte-stimulating hormone α-MSH [or, more correctly, express pro-opiomelanocortin (POMC), which is post-translationally cleaved to give α-MSH] and cocaine- and amphetamine-related transcript (CART) (139). The genes encoding these molecules and their receptors have been cloned, enabling production of mouse models and studies of polymorphisms in human populations, and allowing investigation of their potential role in obesity (7) and their evaluation as drug targets (28). Targeted disruption of the genes encoding these neuromodulators and their receptors has varying degrees of impact on body weight in mouse models, presumably reflecting varying levels of redundancy in these signaling systems. For instance, *NPY*$^{-/-}$ mice have no apparent body weight phenotype (50), whereas knockout mice lacking the α-MSH receptor, MCR4, are as morbidly obese as leptin-deficient *ob/ob* mice (77). In contrast with the leptin signaling system, in which mutations are very seldom the cause of obesity (53), mutations in such genes may contribute to body weight dysregulation in a significant proportion of the population. For instance, around 5% of severe human obesity is attributable to loss-of-function mutations in *MCR4* (54), and a particular *MCR4* polymorphism is associated with an 18% lower risk of obesity (156).

Two significant issues are emerging from these types of studies. First, many mutations in this hypothalamic system of appetite regulation also alter energy expenditure in a concerted manner, e.g., mutation in the leptin receptor or *MCR4* genes results in both increased energy intake and decreased energy expenditure. Second, follow-up studies have shown that phenotypic expression of such mutations is dependent on genetic background and other factors, such as age. For example, *NPY*$^{-/-}$ mice have no apparent body weight phenotype, but *NPY* ablation ameliorates obesity in *ob/ob* mice (50, 51). Similarly, AgRP-deficient mice are not, as might be expected, lean, but show an age-dependent lean phenotype resulting from increased energy expenditure (119, 151).

Since obesity is a polygenic disorder, components of hypothalamic signaling may prove to be valid therapeutic targets for particular subsets of obese individuals, depending on precise combinations of polymorphisms. Furthermore, given the homeostatic mechanisms to resist changes in body weight by compensatory changes in appetite, the demonstrably close relationship between regulation of appetite and of energy expenditure in the hypothalamus makes it a suitable system for effective therapeutic regulation (150). It may be no coincidence that sibutramine, which is thought to influence ARC signaling, both decreases food intake and increases thermogenesis (66, 137).

The hypothalamus also receives short-term indicators of nutritional status from the gut (107), where nutrient-sensitive cells release

potent peptide regulators of meal size. Gut peptides such as choleocystokinin, ghrelin, and glucagon-like peptide-1 (GLP-1) act directly on the hypothalamus via receptors or indirectly by altering circulating levels of hormones such as leptin (34, 143), integrating short- and long-term signals of nutritional status. The location of the ARC near the median eminence, a highly vascularized structure with fenestrated endothelial cells, may mean that the extracellular concentration of glucose in this hypothalamic region is more similar to that in the plasma. Glucose-sensitive neurons are present in the ARC, and these may contribute moment-to-moment information on nutritional status (148).

New components of this brain-gut-adipocyte axis are regularly identified. Of particular interest are endocannabinoids, which mediate pleasure-related aspects of eating (113). Cannabinoid receptors are expressed in the hypothalamus, and mice lacking the cannabinoid receptor CB1 eat less and are lean (121). Rimonabant is a cannabinoid receptor antagonist that promotes weight loss. In mice, rimonabant suppresses appetite for a limited period but also exerts a weight-loss effect lasting many days after eating has returned to normal (37). It increases basal oxygen consumption in mice while stimulating glucose uptake by skeletal muscle, leading to speculation that it stimulates thermogenesis (95). Recent research into effects of rimonabant on adipose tissue of treated mice suggests that this drug stimulates thermogenesis in brown adipose tissue and β-oxidation in white adipose tissue. It is intriguing that increased levels of mRNA encoding the adenine nucleotide translocase (which is largely responsible for basal mitochondrial proton leak) were also found in the white adipocytes of treated mice (79).

OTHER POSSIBLE EFFECTS OF DECREASED COUPLING EFFICIENCY

Although simple chemical uncouplers may more directly achieve the same ends as current antiobesity drugs, the fatal consequences of initial and more recent illicit experiments with DNP may account for the apparent wariness of the pharmaceutical industry in developing uncouplers as antiobesity therapeutics. As we have mentioned, it may be possible to identify uncouplers that have a relatively wide therapeutic window and may therefore avoid the potential overdose dangers and side effects of uncouplers. However, even these compounds may have undesirable side effects due to the role of mitochondria in diverse functions such as immune responses and glucose homeostasis (see below).

To evaluate chemical uncouplers as potentially useful antiobesity drugs, we must consider all the physiological functions of protonmotive force in addition to the obvious ones of ATP synthesis and metabolite transport. Production of ROS is favored at high protonmotive force and therefore uncouplers may have beneficial effects in reducing ROS-mediated damage. However, uncouplers may disrupt physiological processes in which ROS or high protonmotive force are useful. As further described below, ROS destroy pathogens in immune responses, and high-protonmotive force (via cellular ATP concentrations) is a signal in pancreatic β-cell function and glucose homeostasis.

Immune System

Macrophages generate superoxide to destroy pathogens (108), and UCP2 is highly expressed in tissues of the immune system, including macrophages (55). The role of UCP2 in modulating macrophage ROS production has been investigated using *Ucp2*$^{-/-}$ mice infected with the intracellular protozoan parasite *Toxoplasma gondii*. Infection with *T. gondii* is lethal in wild-type mice, whereas *Ucp2*$^{-/-}$ mice are completely resistant. Resistance is associated with increased ROS generation by *Ucp2*$^{-/-}$ macrophages and improved elimination of *T. gondii* tachyzoites and *Salmonella typhimurium* cells by *Ucp2*$^{-/-}$ macrophages in vitro (6). Kizaki et al. (84) have reported that UCP2 protein concentration is downregulated in a macrophage cell line in response

GSIS: glucose-stimulated insulin secretion

to lipopolysaccharide (a component of bacterial cell walls). These studies show that UCP2 (presumably by regulating mitochondrial coupling efficiency) has an important role in macrophage function. Although they have not been followed up extensively, the studies imply that chemical uncoupler-based antiobesity therapies may compromise pathogen destruction in immune responses.

Glucose Homeostasis

Mitochondrial coupling efficiency is pivotal in glucose homeostasis. Specifically, it modulates glucose-stimulated insulin secretion (GSIS) in the β-cells of the pancreas. In the current consensus model of GSIS, β-cells oxidize glucose to generate ATP by oxidative phosphorylation, which stimulates insulin secretion (reviewed in 42, 132). The molecular device linking glucose metabolism to insulin exocytosis is the K_{ATP} channel of the β-cell plasma membrane, a potassium channel that closes in response to glucose-stimulated intracellular increases in ATP and decreases in ADP concentration.

In healthy individuals, blood glucose concentration is stable at approximately 5 mM. According to the consensus GSIS model, under conditions of low (<5 mM) plasma glucose (i.e., minimal ATP production), K_{ATP} channels maintain high plasma membrane K^+ conductance, clamping the plasma membrane potential above the threshold for voltage-dependent calcium channel opening in the resting β-cell (**Figure 2a**). When the extracellular glucose concentration exceeds 5 mM (e.g., after a meal), sufficient ATP is generated to close K_{ATP} channels, which initiates membrane depolarization and opens voltage-dependent calcium channels. The resultant calcium influx, via poorly understood mechanisms, stimulates insulin secretion (**Figure 2b**). Given the central role of ATP in β-cell glucose sensing, it is not surprising that mitochondria are instrumental in GSIS (72, 100, 101). In fact, 1% of all type 2 diabetes is thought to be caused by defects in mitochondrial DNA (149). Mitochondrial coupling efficiency in β-cells therefore deserves attention as a regulator of insulin secretion in the context of diabetes.

Diabetes is a disease of insufficiency of insulin action. Type 1 diabetes is an autoimmune disease in which pancreatic β-cells are selectively destroyed by the immune system, resulting in an absolute deficiency of insulin (104). By contrast, individuals with type 2 diabetes typically have higher levels of plasma insulin than do nondiabetics. Type 2 diabetes results from a "deafening" in peripheral tissue (particularly liver and skeletal muscle) to the insulin signal (155), coupled with an inability of the pancreatic β-cells to compensate by increasing the GSIS insulin peak above background (i.e., β-cell dysfunction; 123) (57).

Obesity is the main risk factor for type 2 diabetes (87), which accounts for 90%–95% of all diabetes (106) and is the world's most common metabolic disorder (97). Many of the detrimental effects of obesity on health can be attributed to the strong causal relationship between adiposity and type 2 diabetes (160). Therefore, the treatment of type 2 diabetes represents a means of ameliorating obesity-associated disorders.

Zhang et al. (157) showed that UCP2 activity negatively regulates GSIS by demonstrating that GSIS in isolated *Ucp2*$^{-/-}$ islets is three times that in wild-type islets, correlating with increased islet ATP content. This result is supported by studies in clonal β-cells, where knockdown of UCP2 using siRNA lowers mitochondrial proton conductance, raises coupling efficiency, and enhances insulin secretion (1). Recent studies suggest that inhibiting UCP2 activity may ameliorate obesity-associated type 2 diabetes. For instance, UCP2 protein is upregulated in the islets of diabetic *ob/ob* mice, and the diabetes of these mice is significantly relieved by UCP2 ablation (157); reduction of UCP2 expression using siRNA improves insulin secretion in a model of lipid-induced β-cell dysfunction (154); *Ucp2*$^{-/-}$ mice are resistant to becoming glucose intolerant after high-fat feeding; and GSIS is not impaired in isolated *Ucp2*$^{-/-}$ islets following chronic exposure to fatty acids in vitro (82, 83).

Human islets express UCP2 (33); therefore, UCP2 antagonists may provide antidiabetic therapies (63, 64). Purine nucleoside di- and triphosphates inhibit UCP2 activity in vitro, but small molecules suitable for attenuating UCP-mediated uncoupling in vivo have proved elusive. However, genipin, a constituent of green tea, inhibits UCP2 activity in isolated mitochondria and ameliorates obesity-induced dysfunction in β-cells (158). Anecdotally, green tea lowers plasma glucose in diabetic mice, albeit in the absence of increased plasma insulin (144).

Excess superoxide may contribute to β-cell dysfunction in diabetes via UCP2 activation. If so, antioxidants may increase mitochondrial coupling efficiency and thereby improve β-cell function. Krauss et al. (86) demonstrated that superoxide activates UCP2 in mouse islets, that this attenuates GSIS, and that endogenous UCP2 activation is prevented by Mn-superoxide dismutase overexpression or by the superoxide dismutase mimetic MnTBAP. These results suggest that antioxidants may have therapeutic value in treating type 2 diabetes.

Given that UCP2 activity impairs β-cell function, why has it persisted throughout evolution? ROS levels are elevated in *Ucp2*$^{-/-}$ islets (82, 86), and slightly higher rates of β-cell apoptosis are seen in *Ucp2*$^{-/-}$ mice (83), supporting the preventative antioxidant hypothesis. However, *Ucp2* ablation does not adversely affect islet insulin content or β-cell size or number (83), and no detrimental effects have been reported on the health of *Ucp2*$^{-/-}$ mice. Moreover, this is an unappealing explanation for the persistence of UCP2 in β-cells, given their low antioxidant status and the protonmotive force-dependent mechanism of glucose sensing (89). An intriguing possibility is that UCP2 participates in a superoxide-dependent signaling mechanism that regulates GSIS, and low antioxidant defenses increase the sensitivity of response. Perhaps, just as brown fat cells have hijacked the ancestral function of UCP1 in thermogenesis, β-cells have hijacked UCP2 as a signaling mechanism in GSIS, providing a further layer in the control of insulin secretion.

Signal Transduction in Other Cells

The function of UCP2 in modulating ATP-dependent signal transduction via changes in mitochondrial efficiency may not be limited to β-cells, since other nutrient-sensitive cell types involved in regulating energy intake, energy expenditure, and glucose homeostasis possess the necessary machinery for such a system.

Intestinal L-cells secrete glucagon-like peptide-1 in response to glucose through K_{ATP} channel closure, although the physiological significance is unclear since glucose is unlikely to reach the distal intestine to stimulate these cells (65). Glucose-sensitive cells of the hypothalamus also express K_{ATP} channels (99), and UCP2 mRNA is very highly expressed in the ARC (124) and so may sense glucose in a similar manner to β-cells (in fact β-cells, being electrically active secretory cells, are rather similar to neurons) (136). Very recently, it has been reported that thyroid hormones regulate activity of UCP2 in hypothalamic neurons expressing NPY and AgRP (39) and that POMC neurones respond to glucose in a UCP2-dependent manner (114). Mitochondrial coupling efficiency, in addition to influencing obesity directly by modulating energy expenditure, may play a role in the central homeostatic signaling systems that regulate body weight. Modulation of the uncoupling activity of UCPs may not only affect nutrient sensing in the brain directly, but may also be an indirect way to alter peripheral coupling efficiency through poorly characterized signaling pathways.

CONCLUSIONS

It is clear that variation in coupling efficiency provides one potential explanation for the differences in weight loss achieved by different individuals on weight-loss programs. Whether such differences in coupling efficiency are significant, and their contribution relative to other

mechanisms of energy dissipation, remains to be established. Pharmacological manipulation of energy expenditure and perhaps coupling efficiency may already be a small part of the mechanism of current antiobesity drugs, specifically sibutramine and rimonabant. Historically, chemical uncoupling by dinitrophenol was very effective at causing weight loss in humans by decreasing coupling efficiency, with an efficacy exceeding that of modern drugs. Perhaps safer chemical uncouplers can be found that possess the advantages of DNP without suffering from its disadvantages. Exploiting natural uncoupling pathways to lower coupling efficiency remains an exciting possibility for the treatment of obesity. In principle, this could be achieved by amplifying brown adipose depots, perhaps by recruiting pre-existing white adipocytes, and by switching on the activity of UCP1 in the recruited tissue, but formidable practical problems in achieving such amplification and activation remain. Up-regulating the expression and activity of UCP3 in skeletal muscle to achieve the same end remains an attractive possibility. Altering coupling efficiency in hypothalamic neurons may be an indirect way to achieve weight loss through perturbations in the pathways that regulate satiety, appetite, exercise, and peripheral energy expenditure.

However, direct general alterations in peripheral coupling efficiency may have too great a cost in compromising normal bioenergetic function in the major and minor energy-consuming organs. Widespread disturbances in ATP production, calcium homeostasis, metabolite transport, and ROS metabolism are not desirable, as indicated by the profound and widespread pathological effects of natural dysfunction of mitochondria, or the increasing recognition that bioenergetic insufficiency may play a significant role in conditions such as developmental abnormalities in the brain, stroke, aging, and the diseases of aging. Tissue-specific alterations in coupling efficiency may burn fat stores and lower ROS production and thus may be successful in brown adipose tissue or skeletal muscle, but when carried out in other tissues they may also have specific negative effects, such as compromising immune function or decreasing insulin secretion in pancreatic β-cells. These potential problems will have to be avoided or overcome before pharmacologically decreasing coupling efficiency to treat obesity can become a practical possibility.

SUMMARY POINTS

1. Coupling efficiency is the proportion of oxygen consumption used to make ATP and do useful work. High coupling efficiency may lead to fat deposition; low coupling efficiency may decrease fat stores.
2. Coupling efficiency is generally less than 100% in cells and organs; submaximal coupling efficiency can contribute to both obligatory and facultative energy expenditure.
3. The main mechanism of submaximal coupling efficiency is a futile cycle of proton pumping and proton leak across the mitochondrial inner membrane.
4. Basal proton conductance is catalyzed primarily by the adenine nucleotide translocase but can be mimicked by chemical uncouplers.
5. Inducible proton conductance is catalyzed by specific uncoupling proteins. UCP1 in brown adipose tissue (and UCP3 in skeletal muscle) can lower coupling efficiency.
6. There are both opportunities and pitfalls of targeting these processes to decrease coupling efficiency and increase energy expenditure as a treatment for obesity.

FUTURE ISSUES

Further research is needed on:

1. Chemical uncouplers having large dynamic ranges, including safety and antiobesity efficacy in experimental animals.
2. Application of novel spectroscopic methods (e.g., magnetic resonance, optical) to assess uncoupling in human tissues in vivo.
3. Biological factors underlying interindividual variation in weight gain and loss during positive and negative energy balance.
4. Functions of the uncoupling proteins, particularly UCP2 and UCP3.
5. Recruitment and activation of brown adipose tissue in adulthood.

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DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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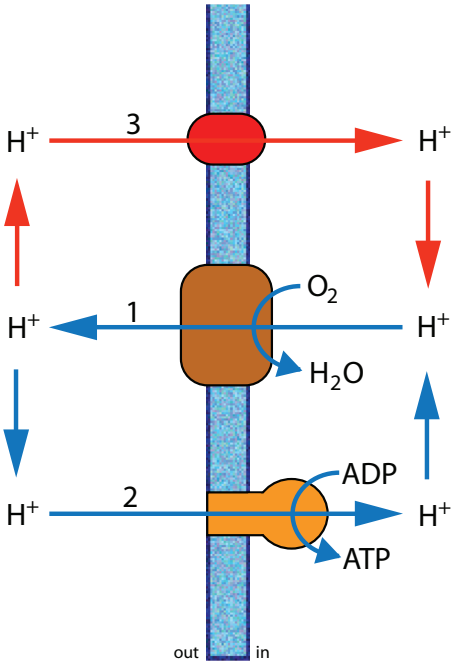

Figure 1

Coupling efficiency of oxidative phosphorylation: Reaction 1 denotes the electron transport chain in the mitochondrial inner membrane, reducing oxygen to water using electrons derived from reduced substrates and pumping protons out from the mitochondrial matrix to set up a protonmotive force. Reaction 2 is the adenosine triphosphate (ATP) synthase that allows the protonmotive force to drive protons back to the matrix, causing synthesis of ATP and coupling oxidation to phosphorylation. Reaction 3 represents the basal and inducible proton-conductance pathways that dissipate protonmotive force without ATP synthesis, producing heat. Coupling efficiency is high when reaction 3 is much slower than reaction 2, and low when reaction 3 becomes significant relative to reaction 2.

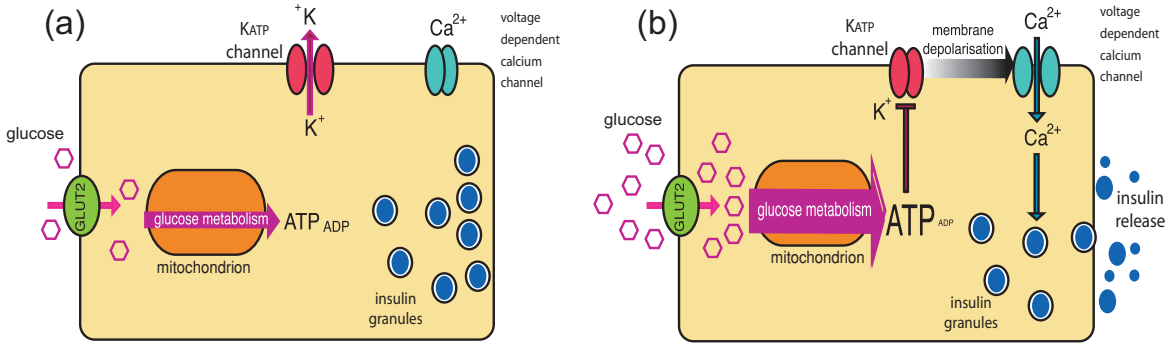

Figure 2

Consensus model of β-cell glucose-stimulated insulin secretion (GSIS). (*a*) Plasma glucose is low (<5 mM), and "resting" concentrations of ATP and ADP permit K_{ATP} channels to remain open. The plasma membrane is freely permeable to potassium ions, and the plasma membrane potential is clamped above the threshold value required to close voltage-gated calcium channels. (*b*) Plasma glucose concentration is high (5–25 mM), and glucose is transported into the cell by a glucose transporter (GLUT2). The resultant increase in glucose metabolism raises ATP and lowers ADP concentrations, closing K_{ATP} channels. The decrease in potassium permeability depolarizes the plasma membrane and opens voltage-dependent calcium channels. Calcium influx triggers insulin secretion.

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Errata

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