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Uncoupling proteins: functional characteristics and role in the pathogenesis of obesity and Type II diabetes

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Abstract

Uncoupling proteins are mitochondrial carrier proteins which are able to dissipate the proton gradient of the inner mitochondrial membrane. This uncoupling process reduces the amount of ATP generated through an oxidation of fuels. The hypothesis that uncoupling proteins (UCPs) are candidate genes for human obesity or Type II (non-insulin-dependent) diabetes mellitus is based on the finding that a chemical uncoupling of the mitochondrial membrane reduces body adiposity, and that lower metabolic rates predict weight gain. It is straightforward to hypothesize that common polymorphisms of *UCP1*, *UCP2* and *UCP3* genes lower metabolic rate by a more efficient energy coupling in the mitochondria. Furthermore, genetically engineered mice over expressing different UCP homologues are lean and resistant to diet-induced obesity. The three uncoupling protein homologue genes *UCP1*, *UCP2*, and *UCP3* have been investigated for polymorphisms and mutations and their impact on Type II diabetes mellitus, obesity, and body weight gain or BMI. The main conclusion is that variation in the *UCP1*, *UCP2* or *UCP3* genes is not associated with major alterations of body weight gain. The contribution of *UCP* genes towards polygenic obesity and Type II diabetes is evaluated and discussed. [Diabetologia (2001) 44: 946–965]

Keywords Uncoupling proteins, Type II diabetes mellitus, obesity, genetics, body weight regulation, energy expenditure, metabolic rate, brown adipose tissue, white adipose tissue, reactive oxygen species, polymorphism, mutation, transgenics, gene knockout.

An enormous amount of data has been collected on the uncoupling proteins 2 and –3 (UCP2 and –3) since their discovery in 1997 and well over 400 publications with either UCP2 or UCP3 or both as a keyword currently exist in public bibliographic databases. This is the equivalent of two new papers a week on the subject during the last four years, and that is excluding those papers on UCP1 alone. Despite the intense focus on these proteins their function and role in metabolism is not clear. There have been numerous clues to the possible functions of these proteins, and many theories have been offered but it is still not known what these uncoupling protein homologues actually do [1–6].

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Abbreviations: UCP, Uncoupling protein; RMR, resting metabolic rate; BMR, basal metabolic rate; BAT, brown adipose tissue: ROS, reactive oxygen species; BMCP1, brain mitochondrial carrier protein 1; WAT, white adipose tissue; skm, skeletal muscle; LPS, lipopolysaccharide; PPAR, peroxisome proliferator activated receptor; 24h-EE, 24 hours energy expenditure; β3-AR, beta 3 adrenergic receptor; UTR, untranslated region; SMR, sleeping metabolic rate

Mitochondrial bioenergetics in brief

The mitochondrial oxidation of substrates results in reduced NADH and $FADH_2$, which deliver their electrons to the electron transport chain in the inner mitochondrial membrane. The chemiosmotic hypothesis proposes that oxidation is coupled by the elec-

tron transport chain to the pumping of protons into the mitochondrial intramembrane space [7]. This creates a pH-gradient (the proton motive force), and proton re-entry into the mitochondrial matrix is coupled to phosphorylation of ADP to ATP. Protons cannot enter the matrix through ATP synthase when ADP is not available. The steeper proton gradient in the absence of ADP inhibits the electron transport chain and the reduction of NAD^+ and FADH. As a result, substrate oxidation is closely coupled to energy needs.

However, mitochondria uses oxygen when ADP is not available (state 4 respiration), an indication that the coupling of substrate oxidation to ATP synthesis is imperfect. State 4 respiration is mostly due to uncoupling [8]. Uncoupling could, in theory, be due to proton leak (for example catalysed by an uncoupling protein) or to the slippage of the electron transport chain, so that it does not, for example, exclude a proton each time it transfers an electron.

Proton leaks and variations in energy expenditure

Proton leaks constitute a considerable part of the resting metabolic rate (RMR). An estimated 20–50 % of total energy expenditure is due to proton leaks with skeletal muscle as the main contributor [9, 10]. Energy expenditure in humans can be divided into the basal energy expenditure measured under resting conditions, the energy expenditure caused by physical activity and the adaptive energy expenditure (including thermogenesis) observed in the metabolism of substrates. Variations in the resting metabolic rate are due to several determinants, including body composition (fat vs fat free mass), concentrations of thyroid and steroid hormones, genetics as well as the activity of the sympathetic nervous system [11]. Variations in energy expenditure could be a source of body weight variation in human beings [12, 13]. What evidence is there that energy expenditure is an inherited trait? Several studies have addressed this question by computing the intrafamilial correlations for basal metabolic rate or 24-h energy expenditure giving heritabilities of 0.26 to 0.70 [13–16]. It is clear that variations in energy expenditure between subjects have genetic determinants.

Low energy expenditure could predict future weight gain. In infants, children and adults, lower energy expenditure rather than increased energy intake has been reported to predict later weight gain [17–19]. However, other reports have thrown doubt on the predictive value of resting metabolic rate (RMR) for weight gain [20, 21]. In one study, children of obese subjects had a lower RMR than the children of lean parents [22]. Only a slight imbalance between intake and expenditure (an estimated surplus of ~ 0.3 %) is necessary for a weight gain if it persists over years [23]. Increasing the energy expenditure by increasing the proton conductance of mitochondria has long been recognised as an effective way to obtain weight loss. This has been achieved by intake of the chemical uncoupler dinitrophenol, which was used in the 1930's (for a review see [24]).

The original uncoupling protein of brown fat: UCP1

Brown adipose tissue (BAT) is a thermogenic organ, present in almost all mammals where it is a major site of cold-induced non-shivering thermogenesis as well as contributing to diet-induced thermogenesis [25]. BAT is a metabolically active tissue, which consists of adipocytes rich in mitochondria and small lipid droplets (Fig. 1). This is in contrast to white adipose tissue (WAT), which contains large fat droplets and few mitochondria (Fig. 1). In large mammals, such as humans, BAT is mainly active in infancy after which it largely disappears.

Thermogenesis in BAT is due to uncoupling protein 1 (UCP1). The special function of BAT has made the underlying molecular mechanisms an issue of great interest, and UCP1 (previously just UCP or thermogenin) was purified as early as 1978 [26]. The amino acid sequence was determined and its coding DNA sequence cloned in 1985 [27]. UCP1 is a dimeric protein present in the inner mitochondrial membrane (Fig. 2A), and it dissipates the pH-gradient generated by oxidative phosphorylation, releasing chemical energy as heat. UCP1 is exclusively expressed in brown adipocytes, where the gene expression is increased by cold, adrenergic stimulation, β_3-agonists, retinoids and thyroid hormone [28] (Table 1).

UCP1 is activated by non-esterified fatty acids and inhibited by purine nucleotides [10]. Although coenzyme Q (ubiquinone) has been identified as a co-factor for the proton transport of UCP1 the mechanism by which UCP1 transports protons across the inner mitochondrial membrane is still to be determined [29, 30]. However, two models account for important facts concerning the kinetics and the regulation of UCP1 activity: the fatty acid protonophore (or flip-flop) model (Fig. 2B) [31] and the channel model (Fig. 2C) [32]. Both models predict a net transport of protons. The arguments for and against each mechanism have been reviewed elsewhere [10].

UCP2 and UCP3: homologues to UCP1

Uncoupling of the proton gradient of the mitochondria is thought to be important for energy dissipation as heat, maintenance of respiration, activation of substrate oxidation and prevention of reactive oxygen

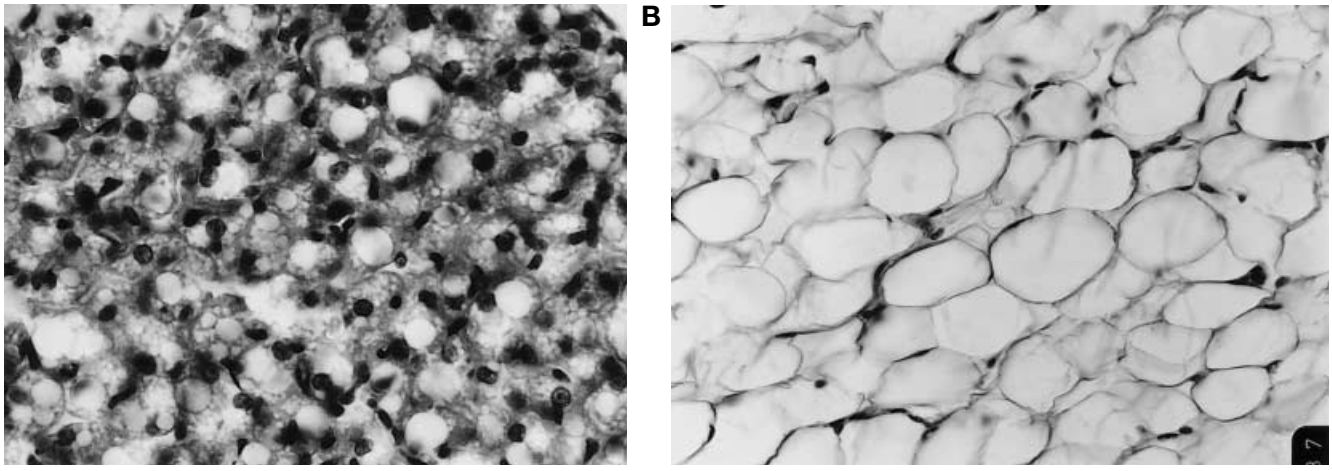


Fig. 1. A Brown adipose tissue. The lipid droplets are small and plentiful and the nuclei are large and placed centrally (haematoxylin-eosin, magnification × 580). **B** White adipose tissue. Cells contain one large lipid droplet and the nucleus is placed peripherally (haematoxylin-eosin, magnification × 295). From Histology, Munksgaard, with permission

species (ROS – oxygen radicals) [33,34]. In newborn children and rodents, UCP1 is a major contributor to the energy expenditure, but since human adults contain very little BAT, the contribution of UCP1 to whole body energy expenditure is probably less. Substantial proton leaks in mitochondria from tissues not expressing UCP1 could indicate that other proteins catalyse the transport of protons. Several independent groups subsequently identified uncoupling protein 2 – and shortly after UCP3 (UCP2 and UCP3) [35–39]. Later other homologues were identified (UCP4, UCP5/BMCP1) [40, 41]. Although UCP4 and UCP5/BMCP1 share homology with UCP1, –2 and –3, this homology is low (40 % amino acid homology) and comparable to the homology shared between UCP2/UCP3 and other mitochondrial transporters.

In contrast to UCP4 and UCP5, human UCP2 and UCP3 are both more closely related to UCP1. The similarity of amino acid sequence between human UCP2 and UCP3 is 71 % and the similarity to UCP1 is 55 % and 57 %, respectively [38]. The genomic structures of human *UCP2* and/or *UCP3* were found to be similar to each other and to *UCP1* (Fig. 3) [42–45]. The human *UCP3* gene gives rise to two main, alternative transcripts due to a polyadenylation site in intron 6, which terminates approximately 50 % of the transcripts. The first codon at the splice junction of exon 6/intron 6 is a stop codon. Therefore, human UCP3 exists as a long form called UCP3L and a short form called UCP3S. The latter lacks the last 37 C-terminal amino acids but is otherwise identical to UCP3L [44].

Tissue distribution of UCP2 and UCP3

UCP2 is expressed widely and in humans, high expression is seen in white adipose tissue. Other tissues (skeletal muscle, heart, placenta, liver, kidney, pancreas, brain, and cells of the immune system) express considerable amounts of UCP2 [35,36]. In mice, the tissue distribution of *UCP2* mRNA is even wider, with high expression also found in BAT, kidney, spleen and macrophages [35,36,46].

The tissue distribution of UCP3 is much narrower than UCP2. It is expressed, albeit abundantly, but only in skeletal muscle and brown adipocytes of humans and skeletal muscle and BAT of rodents [37,38,47]. This is interesting because of the important part skeletal muscle plays in the basal metabolic rate, which could, in part, be due to proton leaks caused by UCP3 and/or UCP2 [8].

Possible functional roles of uncoupling protein homologues: do proton leaks increase energy expenditure or prevent reactive oxygen species?

The precise role of the uncoupling protein homologues is not known but the body could have an energy-wasting system, inducible at times of positive energy balance, which could be composed of uncoupling protein homologues. Alternatively, the primary function of uncoupling protein homologues could be to act as inducible energy wasters. The energy-wasting process could also be a regulated means of thermogenesis in fever and inflammation [46, 48, 49].

A possible side effect of proton leaks is a higher flow rate through the electron-transport chain, and therefore a decrease in the half-life of radical intermediates such as the semi-quinone radical, which can donate its electron to oxygen and create reactive oxygen species (ROS). An uncoupling of oxidative phosphorylation to prevent free radical production when energy needs are low and ADP is not available

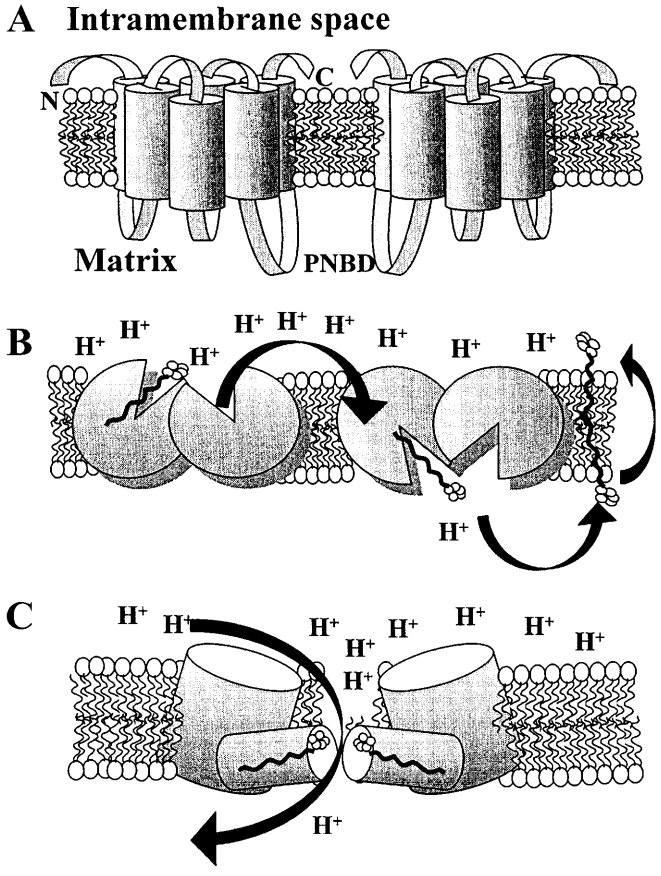


Fig. 2. **A** The three-dimensional structure of UCP1 is not known. However, UCP1 is a dimeric protein present in the mitochondrial membrane. Both the N- and C-terminal amino acids face the intramembrane space. The protein is believed to consist of 6 αα-helices connected by loops with the third matrix side loop containing the purine nucleotide-binding domain (PNBD), which is involved in regulation of proton translocating activity. Structures of UCP2 and UCP3 are believed to be similar because of the high sequence similarity (Adapted from reference 30). **B** In the fatty acid protonophore (or flip-flop) model, UCP1 is a carrier of fatty acid anions, which are transported by UCP1 from the matrix side to the intramembrane space. Here, the anion combines with a proton, becomes electrically neutral and flips back through the membrane and releases the proton in the matrix (31). **C** The channel model predicts a two-domain structure of UCP1 with a pore domain and a gating domain, which allows protons to pass through. Fatty acid carboxy groups participate in the proton transport by providing H^+-buffering capacity (32)

for phosphorylation would be advantageous. The cost of protecting from ROS is energy being wasted by uncoupling proteins as heat [2, 6]. The generation of ROS seems to be lower in the presence of UCPs. Fat oxidation increases ROS generation, and UCP2 mRNA is induced in fatty liver hepatocytes of *ob/ob* mice, where the proton permeability of liver mitochondria is increased and the ATP levels decreased [50, 51]. More direct evidence is obtained from UCP2 and UCP3 knock-out mice, which display increased amounts of ROS in macrophages and skeletal muscle, respectively [52, 53].

Evidence for uncoupling activity of UCP1, UCP2 and UCP3

It is generally accepted that UCP1 catalyses proton transport and is responsible for cold-induced thermogenesis in BAT [54,55]. Because of the similarity between UCP1, –2 and –3, it seemed probable that UCP2 and –3 would have the same function. However, the evidence of a proton leaking function for uncoupling activity of UCP2 and UCP3 is not clear.

UCP2 and UCP3 overexpressed in yeast were less regulated by nucleotides and NEFA than UCP1 [56–60]. This could be due to innate properties of UCP2 and UCP3 or to a lack of regulators normally present in the mitochondrial membrane. The absence of nucleotide regulated proton leak of BAT in UCP1 knock-out mice argues against the uncoupling activity of UCP2 and UCP3 [61]. Furthermore, the same conditions which up-regulate UCP2 or UCP3 expression do not change mitochondrial membrane potential [62]. However, the growth of UCP2 or UCP3 transformed yeast in aerobic conditions is severely stunted suggesting a reduced efficiency of oxygen dependent respiration and that UCP2 and UCP3 overexpressed in yeast depolarises the mitochondrial membrane [35, 36, 39, 59, 63]. Recent studies of UCP2 and UCP3 knock-out mice suggest that UCP2 and –3 do have uncoupling activity. The mitochondria were shown to have decreased state 4 respiration and were, therefore, more coupled [52, 53, 64]. Furthermore, in the presence of coenzyme Q, UCP2 and UCP3 were shown to have proton transport activity similar to that of UCP1 [65]. Because retinoids and coenzyme Q have been shown to increase UCP2 activity [66], model systems allowing reliable estimation of proton leak in the uncoupling protein homologues could soon be defined.

The short UCP3 isoform, UCP3S, appears less likely than UCP3L to uncouple in some, but not all, functional assays. There is evidence of similar uncoupling for UCP3S compared with UCP3L in studies on yeast mitochondrial membrane potential [59, 60, 67, 68] but UCP3S does not inhibit yeast growth to the same extent as UCP3L and therefore might have a smaller uncoupling function [60, 67]. A lower import rate of UCP3S to the mitochondria has also been reported [68].

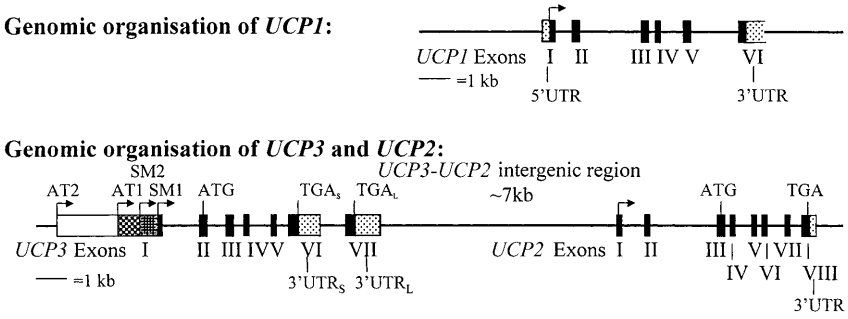


Fig. 3. Organisation of the *UCP1* gene and the UCP3-UCP2 genomic region in human beings. The chromosomal localisation of UCP1 in human beings is on chromosome 4 and human UCP2 and –3 are present on chromosome 11q13 with the transcription initiation of UCP2 placed only 7–8 kb downstream of the UCP3 stop codon. All three proteins have relatively few exons (6 to 8) and the coding sequence is confined to 6 exons in all of these. Exon 1 and 2 of UCP2 are untranslated and in human UCP3 there is at least one untranslated exon and multiple transcription initiation sites. *AT1* and *AT2*, adipose tissue transcription initiation sites 1 and 2,; *SM1* and *SM2*, skeletal muscle transcription initiation site 1 and 2. *Arrows* indicate the transcription initiation sites; ATG and TGA are the start and stop codons, respectively. TGA 'S' and 'L' denotes UCP3S and UCP3L stop codons. Database acc. no. are: UCP1: NT022840, X51952-X51955, UCP2: AF096289, UCP3: AF012196-AF012202

Transgenic approaches to elucidate function and importance of UCPs in body weight homeostasis

Transgenic and knock-out methods are increasingly common tools for investigating the impact of overexpression or the absence of single genes on the mouse phenotype. These models could be constructed in a site-specific manner to estimate the effect on individual tissues. UCP1 was removed through ablation of the brown fat depot (Table 1) [69], providing evidence that UCP1 had an important role in whole body energy homeostasis in mice. The UCP1 knock-out mouse, however, was not obese and merely showed sensitivity towards exposure to cold [70] but the respiration of BAT mitochondria of UCP1-/- mice was more coupled compared with wild type mice [71].

Another approach was to overexpress UCP1 in WAT mice who were expected to be lean due to constitutive expression of UCP1 [72]. However, the UCP1-WAT mice were similar to wild type mice until they were fed a diet high in saturated fat, for which they were obesity resistant. UCP1 is not massively overexpressed in WAT but does reduce the membrane potential of mitochondria [73]. UCP1 has also been ectopically expressed in skeletal muscle (UCP1-skm), which had a severe impact on the phenotype (Table 1) [74]. The UCP1-skm mice had an improved glucose tolerance, which was enhanced compared with wild type mice when on a high-fat diet. The phenotype of the UCP1-skm mouse is similar to the UCP3-skeletal muscle (UCP3-skm) overexpression model [75]. One difference between UCP1-skm and UCP3-skm mice was that the UCP3-skm transgenic strain massively overexpressed the protein, whereas low expression of UCP1 in skeletal muscle could induce the phenotype. These apparent differences in specific activity between UCP1 and UCP3 could be due to the absence

Table 1. Phenotypes of knock-out or transgenic mice models of *UCP1*, *UCP2* and *UCP3*

Mode	Molecular description	Phenotype	Reference
UCP-DTA	Ablation of BAT	Maturity-onset and diet-induced obesity. Hyperphagic	Lowell et al. 1993 [69]
UCP1-KO	Disruption of *UCP1* gene	Cold-sensitive, normal body weight homeostasis	Enerback et al. 1997 [70]
UCP2-KO	Disruption of *UCP2* gene	Normal body weight homeostasis. Increased coupling of mitochondria. Increased ROS-production. Immunity to toxoplasmosis infection. Hyperinsulinaemia due to increased insulin secretion	Arsenijevic et al. 2000 [52] Zhang et al. 2000 [76]
UCP3-KO	Disruption of UCP3 gene	Normal body weight homeostasis. Increased coupling of mitochondria. Increased ROS-production	Gong et al. 2000 [64], Vidal-Puig et al. 2000 [53]
UCP1-WAT	Transgenic expression of UCP1 in WAT	Sensitive to diet-induced obesity. Proton leak of WAT-mitochondria increased	Kopecky et al. 1995[72,73]
UCP1-skm	Low transgenic expression of UCP1 in skeletal muscle	Lean phenotype. Resistant to diet-induced obesity. Proton leak of skeletal muscle mitochondria increased	Li et al. 2000 [74]
UCP3-skm	High transgenic expression of UCP3 in skeletal muscle	Lean phenotype. Resistant to diet-induced obesity. Proton leak of skeletal muscle mitochondria increased	Clapham et al. 2000 [75]

DTA, diphtheria toxin A-chain; *BAT*, brown adipose tissue; *WAT*, white adipose tissue; *skm*, skeletal muscle

of inhibition of UCP1 or depletion of UCP3 activators in skeletal muscle. However, a probable explanation is that UCP1 is more effective in dissipating the mitochondrial proton gradient.

Although UCP3 null mice could be expected to become obese, two UCP3 knock-out mice, generated on different mouse strain backgrounds, did not become obese when placed on a normal or on a high fat diet [53, 64]. The UCP3 knock-out mice had increased energy coupling of their mitochondria, which was due to reduced proton leak [53, 64]. Because the formation of reactive oxygen species increased in UCP3 knock-out mice, UCP3 could have a role in apoptosis and ageing, though experiments still need to prove this hypothesis [6]. The UCP2 knock-out mouse is not obese or resistant to diet-induced obesity [52]. Furthermore, these mice have a normal body temperature regulation, indicating that UCP2 is not essential for maintaining body temperature or preventing obesity. The increase in ROS production by macrophages probably caused the observed resistance to toxoplasmosis. UCP2 knock-out mice with hyperinsulinaemia have also been reported [76].

In normal rat islets or insulinoma cells, adenovirus mediated overexpression of UCP2 inhibits glucose stimulated insulin secretion [77, 78] but in Zucker diabetic fatty rats, it had the opposite effect [79]. Because the amount of insulin is normal in islets overexpressing *UCP2* mRNA, UCP2 might not decrease biosynthesis of insulin and UCP2 might have a crucial role in the regulation of insulin secretion [77]. This data suggests UCP2 could have a regulatory function in the beta-cell and up regulation or down regulation of UCP2 activity could influence glucose homeostasis.

Regulation of UCP1, UCP2, and UCP3 messenger RNA expression

Almost all studies describing UCP2 and UCP3 expression have investigated mRNA content rather than protein content and so overlook possible post-transcriptional regulation. They have done this mainly due to difficulties in obtaining specific and sensitive antibodies for UCP2 and UCP3 [80, 81]. The amount of protein antigen seems to mirror the expression levels of mRNA, albeit with fewer fluctuations [80]. However, a recent study suggests that UCP2 mRNA expression does not predict the expression of UCP2 protein in vivo, because UCP2 is subject to translational regulation in addition to mRNA regulation [81]. Furthermore, mitochondria from tissues expressing increased amounts of UCP2 or UCP3 do not show increased proton leak [62]. Consequently, one must be careful in extrapolating from mRNA regulation to protein content or activity.

UCPs are regulated by a variety of stimuli (Table 2). In general *UCP1*, *UCP2* and *UCP3* mRNA are only weakly regulated by leptin, glucocorticoids and insulin [39, 82–85]. Both TNF-α and LPS strongly activate *UCP2* expression providing a link to the immune system [86]. Thyroid hormones consistently up-regulate *UCP* mRNA levels suggesting a thermogenic function for UCPs [87]. The β3-adrenergic regulation of *UCP* mRNA transcripts, observed in different tissues and in different animal models, could depend on different β3-agonists [39,88]. PPAR agonists (including TZDs) up-regulate *UCP2* mRNA expression in WAT [89–91] (Table 2). However, in human beings *UCP2* or *UCP3* expression in skeletal muscle and *UCP2* expression in WAT is not changed by troglitazone treatment [92].

The original brown fat uncoupling protein (UCP1) is strongly up-regulated by cold (Table 3), but the *UCP2* and *–3* transcripts are up-regulated a modest twofold by cold in a time-dependent fashion [93].

Endurance training could be expected to diminish *UCP2* and *UCP3* expression, in order to preserve energy for physical activity rather than thermogenesis. The effect of chronic exercise is to reduce *UCP2* and *UCP3* mRNA expression in skeletal muscle of rats [94]. In humans, humans who have undergone endurance training have similar or lower skeletal muscle content of UCP2 and –3 mRNA compared with subjects who have not [95] (Table 3).

In response to a diet with a high fat content a number of obesity resistant mice strains double their expression of *UCP2* mRNA in WAT, whereas obesity prone strains only increase *UCP2* mRNA slightly [35]. High fat feeding up-regulates *UCP3* in skeletal muscle but not in BAT [96] (Table 3). The increase of *UCP2* mRNA expression in WAT and *UCP3* in skeletal muscle of animals that are fed with high energy food, suggests that UCP2 and UCP3 could play a part in the mechanisms that counteract obesity. However, fasting or food-restriction consistently up-regulates *UCP3* in skeletal muscle of rodents, and down-regulates *UCP3* in BAT [97]. In human beings, both *UCP2* and *UCP3* are up-regulated in WAT in patients undergoing a short-term but not a long-term diet [85]. The plasma non-esterified fatty acid concentrations during fasting correlates with *UCP2* and *UCP3* expression in skeletal muscle of rodents and humans (Table 3) [84], indicating a role for UCP3 and/or UCP2 in lipid handling or substrate partitioning.

The induction by fasting and peroxisome proliferators indicates lipids are involved as fuel substrates [98]. However, up regulation of mRNA expression in response to thyroid hormone, cold, β3-adrenergic agonists and high-fat diets suggests an involvement in the regulation of energy expenditure. The hypothesis that UCP2 and UCP3 reduces the formation of reactive oxygen species fits with the induction of

Table 2. Regulation of *UCP* mRNA expression under various hormonal or metabolic influences. Both mRNA and protein levels measured for UCP1

	UCP1	*UCP2*				*UCP3*		
	BAT	BAT	WAT	Skeletal muscle	Other	BAT	WAT	Skeletal muscle
Leptin	↑	→	↑	→	–	↑	–	↑→
Insulin	↑	→	→	→	–	→	–	→
Glucocorticoids	↓	↓	–	↓	–	↓	–	↑/→
T3	↑	↑	↑	↑	–	↑	–	↑
$\beta\beta$3-adrenergic drugs	↑	→	↑	↑→	↑[a]	→	↑	↑→
PPAR stimulators	↑	–	↑→	–	↑[b]	–	↑	↑(↓)
TNF-$\alpha\alpha$	↑	↑	↑	↑	↑[a]	–	–	↑

[a] Liver
[b] Pancreatic islets

BAT, brown adipose tissue; *WAT*, white adipose tissue

Table 3. Regulation of *UCP* mRNA expression under different environmental and physiological influences[a]

	UCP1	*UCP2*				*UCP3*		
	BAT	BAT	WAT	Skeletal muscle	Other	BAT	Skeletal muscle	Other
Cold	↑	↑	–	↑[e]	↑[d]	↑	↑[e]	--
Chronic Exercise	→	→	→	↓→	↓[d]	↓	↓→	→[d]
High fat diet	↑	→	↑	→	↑[b]	→	↑→	→[b]
Starvation	↓	→↓	↑	↑→↓[f]	→[d]	↓	↑	→↑[b]
Free fatty acids	–	–	–	↑	–	–	↑	–

[a] Both mRNA and protein levels measured for UCP1
[b] Liver
[c] WAT
[d] Heart
[e] Time dependency of induction
[f] Conflicting data

UCP2 and UCP3 during cold, fasting and high fat feeding because these conditions require lipid oxidation and thus high activity of the respiratory chain. Interestingly, reduced ROS due to partial uncoupling by UCP2 or UCP3 could represent a link to the "thinness and longevity" phenomenon observed in rodents when diet restrictions increase their life span by up to 50 % [99, 100]. However, it cannot be excluded that essential thermogenesis is required by muscles during fasting, when physical activity is often reduced, and that UCP2 and UCP3 are up-regulated to serve that purpose.

Human studies of UCP expression in Type II diabetes and obesity

The *UCP1* mRNA in the intraperitoneal fat of obese subjects is 50 % lower than in normal weight subjects [101], although the amounts of brown adipocytes interspersed in the white fat depots in adult human beings' is low (approximately 1/200 white adipocytes) [102, 103].

UCP2 mRNA expression is also decreased in intraperitoneal WAT in obese subjects compared with their lean control subjects [104]. In subcutaneous fat, however, there is no difference between obese and lean subjects regarding *UCP2* expression [85, 104]. In skeletal muscle, *UCP2* mRNA expression is reported to be higher, equal or lower in obese human beings compared with lean subjects [85, 105, 106]. These studies comprised very few subjects and cannot therefore be considered representative.

In weight-reduced formerly obese subjects, *UCP3* mRNA expression in skeletal muscle seem to decrease below amounts in lean control subjects [107] perhaps explaining in part the reduced metabolic rate found in previously obese subjects [23]. When comparing obese and lean subjects, *UCP3* expression in skeletal muscle is generally not affected by obesity, and the expression of UCP3S and UCP3L are similar (Table 4) [85, 105, 107–109].

Both *UCP2* and *UCP3* skeletal muscle mRNA increased in a similar way in lean and obese subjects undergoing either fasting or low calorie diet [85–110]. These findings contrast with the regulation found in Type II diabetic subjects undergoing fasting, because fasting does not induce *UCP2* or *UCP3* mRNA expression in skeletal muscle of Type II diabetic patients [110]. In Type II diabetic subjects, the context of both *UCP2* and *UCP3* mRNA in skeletal muscle increases compared with weight-matched, glucose-tolerant subjects [110, 111]. However, a lower expression of *UCP3* mRNA in Type II diabetic patients has also been reported [112].

Table 4. Regulation of *UCP* mRNA expression in obesity and Type II diabetes in human patients (*WAT* white adipose tissue, *BAT* brown adipose tissue)

	UCP1	*UCP2*		*UCP3*
	WAT	WAT	Skeletal muscle	Skeletal muscle
Human subjects				
Obesity	↓	→[a]↓[b]	↑↓→	→
Type II diabetes	–	–	→↑	↓↑

[a] Intraperitoneal fat
[b] Subcutaneous fat

Studies of *UCP2* and *–3* mRNA expression in obese and Type II diabetic patients or both compared with control subjects have been difficult to reproduce (Table 4). This could be due to the small sample sizes but other factors such as different muscle groups investigated, sampling schemes and diet composition are also probable causes of the low reproducibility. Furthermore, there seems to be a high inter-individual variability in the mRNA expression of *UCP* homologues. Whether this is due to primary differences in the expression of *UCP2* or *–3* or to varying concentrations of circulating hormones and nutritional factors is not known.

Correlations between expression levels of *UCP2* and *UCP3* and sub-phenotypes of human obesity

Studies suggest that *UCP2* mRNA in WAT is positively correlated with BMI and RMR (adjusted for fat free mass) [85, 111, 113]. Changes in *UCP2* mRNA expression in WAT in subjects on a low calorie diet have been positively correlated with corresponding changes in 24h-EE [114]. However, other studies suggest no or negative correlations between *UCP2* mRNA expression in WAT and BMI, body fat content, concentrations of leptin, free fatty acids or insulin [114, 115]. In skeletal muscle, *UCP2* mRNA is positively correlated with BMI, body fat content and respiratory quotient, but other studies of skeletal muscle identify no such relationships [106, 111, 116, 117].

UCP3 expression in skeletal muscle is positively correlated to concentrations of non-esterified fatty acids [110, 117]. In addition, 24h-EE has been positively correlated with UCP3 expression (adjusted for both fat mass and fat free mass) and negatively correlated with BMI [116].

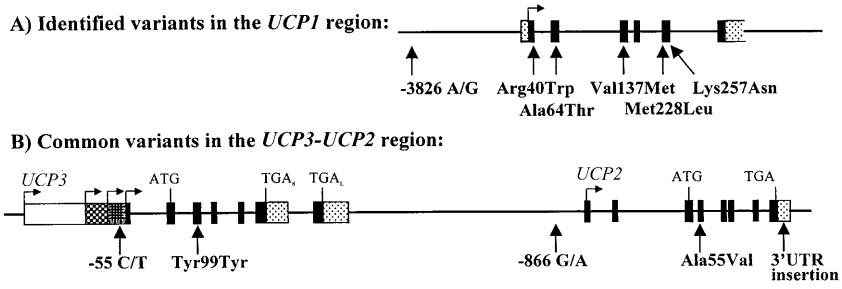
UCPs: candidate genes for obesity or Type II diabetes

Because UCP1, UCP2 and UCP3 have been found to decrease membrane potential and increase thermogenesis [118], all three genes are regarded as candidate genes for obesity and Type II diabetes. However, based on the expression pattern, *UCP2* and *UCP3* could be the more obvious candidate genes because of the large contribution of skeletal muscle to the basal metabolic rate, where *UCP2* and *UCP3* are co-expressed [8]. Mutations reducing the activity or expression of either protein could diminish regulated or basal energy expenditure by increasing the coupling of oxidative phosphorylation.

Nevertheless, certain observations speak against a role for UCP2 and –3 as proton translocators involved in regulation of energy expenditure. The tissue distribution does not quite match the proton leaks measured, because some tissues high in *UCP2* have a low proton leak and vice versa [10]. Moreover, the observation that starvation increases *UCP2* and *UCP3* mRNA and protein in WAT and skeletal muscle without increasing the proton leak argues against an uncoupling function of UCP2 and UCP3 [62]. As long as regulators of UCP2 and UCP3 activity have not been identified, care is needed when extrapolating from mRNA or protein expression to protein activity. There is no reason to think that UCP2 and UCP3 will be constitutively active when UCP1 activity is, in fact, highly regulated.

Data derived from overexpression of UCP1 and UCP3 in WAT and skeletal muscle indicate that a pharmacological increase of UCP activity could prevent or alleviate obesity in human subjects. The overexpression of UCP1 and UCP3 in WAT and skeletal muscle causes leanness. That lack of UCP1 and UCP3 in mice does not cause susceptibility to obesity could therefore be considered illogical. An explanation for this observation could be that redundancy in skeletal muscle where UCP2 and UCP3 are co-expressed. UCP2 is in fact up-regulated in UCP3 knock-out mice [64] and UCP1 is up-regulated in WAT of UCP2 knock-out mice [52] but it is not known if this causes the lack of obesity susceptibility in these mice is not known. Generation of UCP1, UCP2 and UCP3 triple knock-out mice will probably resolve this issue. In addition, the overexpression data in mice might arise from a pathological over loading of mitochondrial membranes. Because even a low UCP1 expression in skeletal muscle caused the lean phenotype, this is, however, not probable [74].

Even though the distantly related proteins, UCP4 and UCP5, are predominantly expressed in the brain, they could have a function or activity that compensates for the absence of UCP2 or UCP3. Both UCP4 and UCP5/BMCP1 have been shown to reduce mitochondrial membrane potential in yeast [40, 41, 119]. Furthermore, the mRNA regulation of UCP5 is consistent with its having a role in energy expenditure



C) Rare or less common variants in the *UCP3-UCP2* region:

	Missense:	Silent or intron:
UCP3:	Val9Met	Ala83Ala
	Arg70Trp	Val102Ile
	Gly84Ser	Exon 4, 5' of 5' splice site: 46 A/T, 47 A/G, 96 C/T, 143 A/G
	R143X	Exon 5, 5' of 5' splice site: 36 C/T
	Arg282Cys	Ile147Ile
	Arg308Trp	Tyr210Tyr
	Exon 6 G/A splice	Arg244Arg
UCP2:	Gly85Ser	-850 G/A, -337 C/G, -41 G/T, -28 insT, -5 insertion of 13 bp
	Ala232Thr	5'UTR: +45 C/T, +53 G/C, +123 C/T

Fig. 4. SNP-map of **A** the *UCP1* gene and **B** common SNPs in the *UCP3-UCP2* genomic region. **C** Table of less common variants identified in *UCP3* and *UCP2*. Genetic variants are from references cited in the text

because *UCP5* mRNA expression, in contrast to UCP2 and UCP3, decreases in liver but not in brain during fasting [119]. Thus, the influence of other UCP homologues on whole body energy homeostasis cannot be excluded.

The presence of mutations in the *UCP* genes or regulatory regions could, hypothetically, cause obesity – if function is decreased – or leanness – if function is increased – or contribute to development of Type II diabetes, either directly through the effect of beta-cells or as a consequence of obesity.

UCPs therefore probably have a function in regulating ROS, body weight and glucose homeostasis. Identifying mutations or polymorphisms in these genes and determining whether these are associated with obesity, Type II diabetes or a related phenotype will therefore throw light on the role of UCP2 or UCP3 in human obesity or Type II diabetes.

Genetic variation in the coding region of UCP1

Though there are no discernible brown fat depots in adult human beings, brown adipocytes expressing UCP1 are interspersed among white adipocytes [102, 120] and several SNPs are present in the human *UCP1* gene [121, 122] (Fig. 4). None of these are, however, associated with obesity or Type II diabetes, and none of the identified polymorphisms affect amino acid residues that have been identified as causing functional impairment of UCP1 when mutated [32]. It seems that variation in the coding region of *UCP1* though frequent, does not lead to obesity.

A possible synergistic effect of a *UCP1* promoter variant and a β3-Adrenergic receptor variant

The frequent ag substitution at position *–3826* upstream of the *UCP1* gene has been related for a long time to obesity and weight gain [123]. The functional areas of the human UCP1 promoter have only recently been investigated [124], and it appears that the *–3826* polymorphism is close to, but not directly in, the human enhancer region. Whether the substitution has a functional impact has still to be investigated. The discovery that the *β3-adrenergic receptor (β3-AR)* polymorphism *(Trp64Arg)* acted synergistically with the *–3826 UCP1 G-allele* to increase weight gain [125] sparked a number of studies (Table 5). The general consensus is that in cross-sectional studies there is no difference between the BMI of wild type and double heterozygotic subjects for the *UCP1* and *β3-AR* polymorphisms [125–131]. However, longitudinal studies indicate that there could be an increased risk of weight gain after follow-up or lower weight loss during low calorie dieting in double heterozygotic subjects [130–134]. A reduced BMR in the double heterozygotic subjects might explain this observation [132].

The *UCP1* gene is, at least in rodents, directly regulated by adrenergic stimuli acting in part through the β3-adrenergic receptor. Hence, a synergistic effect could occur if the function or expression of the *β3-AR* and *UCP1* genes were reduced. This would, in turn, result in an increased risk of weight gain over time or a reduced responsiveness towards weight loss. However, meta-analyses of the *Arg64* variant of the *β3-AR* have found no or very modest

Table 5. Studies of the synergistic effects of uncoupling protein 1 –3826 a/g and the ββ3 adrenergic receptor codon 64 Trp/Arg polymorphisms in obese, Type II diabetic or glucose tolerant control subjects[a]

Subjects (*n*)	Design	UCP1	ββ3-AR	Phenotype associated	Synergistic effect	Reference
123 (in families) French Canadian	12 year follow-up	✓*	NA	High weight gainers more frequently are UCP1 g/g *	NA	Oppert et al. 1994 [123]
238 French obese patients	9–41 year follow-up	✓	✓	High weight gainers more frequent in double heterozygotes*	+	Clement et al. 1996 [125]
163 French obese patients	Diet 3 months	✓*	✓	Weight loss lower in UCP1 g/g vs other*	–	Fumeron et al. 1996 [128]
85 Finnish obese patients	Diet 3 months + 1 year follow-up	✓	✓	Weight re-gain higher in double heterozygotes*	+	Fogelholm et al. 1998 [129]
170 Finnish obese patients	Cross-sectional	✓	✓	BMR lower in double heterozygotes*	+	Valve et al. 1998 [132]
153 Austrian obese patients	Cross-sectional and expression of UCP1	✓	✓	No associations to phenotypes. Expr. lower in UCP1 g/g	–	Esterbauer et al. 1998 [127]
264 Japanese metabolic	Cross-sectional	✓	✓	BMI higher in carriers of –3826 g (UCP1)	–	Hayakawa et al. 1999 [126]
379 young Danes	Cross-sectional	✓	✓	No associations to phenotypes	–	Urhammer et al. 2000 [130]
70 Finnish diabetic patients	10-year follow-up	✓	✓	Diabetic and control combined higher weight gain in double heterozygotes	–Diabetic; –control + combined	Sivenius et al. 2000 [133]
123 control subjects						
236 German obese patients	Cross-sectional			No associations to phenotypes	–Diabetic; –metabolic – control	Evans et al. 2000 [131]
381 German metabolic 198 control subjects						

*$p < 0.05$ with respect to weight gain, weight loss or other. Is there a synergistic effect of UCP1 and βb3-AR polymorphism? ($p < 0.05$ + : yes, -:no). Double heterozygotes are Trp64Arg of b3-AR and a/g –3826 of UCP1. Diabetic patients: Type II diabetic subjects. Metabolic: subjects with the metabolic syndrome, Control subjects: glucose tolerant control subjects

[a]Some studies are longitudinal follow-up studies; others are diet regimens with short-term follow-up. Unless noted, study groups are of normal weight. UCP1 or βb3-AR: Is the UCP1 –3826 or βb3-AR Trp64Arg polymorphism, respectively, investigated? (✓: yes, NA: not applicable)

direct effects on BMI [134] although the *Arg64* variant of the *β3-AR* has been shown to reduce function [135]. More proof is needed that the *UCP1* promoter polymorphism acts synergistically with the *β3-AR* variant because large studies with the power to detect even minor effects did not detect this *UCP1/β3-AR* synergism [130,131]. To determine synergistic effects of the rare polymorphism in the β3-AR with the *UCP1 –3826 a/g* variant, meta-analyses or multi-centre efforts are necessary to detect interactions between two or more genetic variants. An adequate study size is an ever-occurring problem in the genetics of polygenic disorders because the costs of recruiting and examining the subjects exceed the capacity of single research centres.

Linkage data from the UCP2-UCP3 chromosomal region

Evidence from mRNA expression studies in obesity-prone and obesity-resistant animals have shown differences in *UCP2* and *UCP3* regulation depending on nutrients [136]. The observed differences are probably genetically based and could be due to subtle differences in the regulatory regions of either *UCP2* or *UCP3*, although differences in trans-activating factors could also explain these observations.

UCP2 and *UCP3* are situated in close proximity to each other on chromosome 11q13, making it difficult for linkage studies to discern between linkages to either gene because the two genes are usually inherited as one block of DNA. In mice, quantitative traits linked to obesity have been mapped to the *UCP2* and *UCP3* region and in the GK rat, *UCP2* and *UCP3* map onto a region of glucose intolerance and adiposity [137–142]. However, genomic regions identified by linkage approaches tend to be rather large

Table 6. Summary of linkage studies containing markers in the UCP3-UCP2 region on chromosome 11q13[a]

Population	Markers	*n*	Phenotype	Lod/*p* value	Reference
French-Canadian Caucasians	3 in UCP2/3 region	640 (ext. fam)	RMR, fat mass, BMI	$p = 2 \cdot 10^{-5}$ RMR; $p = 0.02$ fat mass; no linkage to BMI	Bouchard et al. 1997 [148]
Utah Caucasians	7 in UCP2/3 region	618(ext. fam)	Type II diabetes, BMI, waist-hip ratio	No linkage	Elbein et al. 1997 [151]
African-Americans	2 in UCP3 gene	100–544 (sib-pairs)	BMI, % fat, RMR, leptin	No linkage	Chung et al. 1999 [164]
Mexican-Americans	~ 7 on chr 11 in total	458 (ext. fam)	BMI, fat mass, leptin, waist-hip ratio	Linkage exclusion	Comuzzie et al. 2000 [152]
Finnish Caucasians	Genome-wide scan	719 (sib-pairs)	Type II diabetes, fasting serum insulin	Linkage, Lod = 1.75 and Lod = 2.07	Ghosh et al. 2000 [150], Wata-nabe et al. 2000 [149]
French Caucasians	Genome-wide scan	514 (nucl. fam)	Obesity, BMI, leptin	No linkage	Hager et al. 1998 [146]
Americans	Genome-wide scan	513 (nucl. fam)	Obesity, BMI, % fat	No linkage	Lee et al. 1999 [147]

[a] *n* denotes number of subjects analysed; in the parentheses it is noted whether subjects are from extended families (ext. fam), nuclear families (nucl. fam) or are siblings (sib-pair). Markers used were multiallelic microsatellite markers except for Chung et al, [159], who used bi-allelic markers
RMR, resting metabolic rate; *% fat*, percentage body fat

and could contain hundreds of genes, many of which have a function which is still not known.

A number of studies investigating linkage of the human *UCP2* and *UCP3* region to obesity, Type II diabetes and quantitative intermediary phenotypes are listed in Table 6. The 11q21–23 region is linked to body fat, 24-h energy expenditure and BMI in obese and/or Type II diabetic Pima Indians [143–145]. However, this locus is probably too far from the *UCP2/UCP3* locus to be caused by variation in these genes. Other reports of genome-wide scans for obesity loci in human subjects have not identified the *UCP2/UCP3* region as one of the contributors to obesity or Type II diabetes [146, 147]. However, RMR has been linked to this locus in French Canadians [148]. Of note, the 11q13 region is weakly linked to Type II diabetes and values of fasting serum insulin in Finnish subjects [149, 150]. Variation in these two genes could alter sub-phenotypes of obesity or Type II diabetes, such as metabolic rate, energy expenditure or insulin secretion. Linkage studies suggest, however, the *UCP2/UCP3* region is not linked to overt obesity or Type II diabetes in human patients [151, 152]. A disadvantage of linkage studies is their low power to detect genes containing variants with moderate effects, so that linkage studies are most suited to identifying new genes causing major alterations of a given phenotype.

Impact of *UCP2* genetic variation on risk of obesity or Type II diabetes mellitus

The hypothesis that variation in the novel *UCP* homologues could contribute to obesity and Type II diabetes resulted in early investigations to identify genetic variation. In the *UCP2* coding region, two frequent polymorphisms exist: an alanine to valine substitution in codon 55, and a 45 bp insertion-polymorphism in the 3'untranslated region (UTR) (Fig. 4). No association studies have found differences in allele or genotype frequencies of the *Ala55Val* or the *3'UTR* insertion polymorphisms between obese and/or Type II diabetic subjects and control subjects (Table 7).

However, some studies have reported associations to sub-phenotypes related to obesity. The *3'UTR insertion* in heterozygous state was associated with higher values of SMR and 24-h EE and lower BMI, agreeing with a role of UCP2 in controlling energy expenditure in Pima Indians [153]. Moreover, the insertion homozygotic genotype was associated with increased BMI in South Indian females and increased values of serum leptin in British women [154] and in American children of different ethnic origin [155]. One study related the *Ala55Val* polymorphism to measures of energy expenditure [156], finding adjusted 24-h EE was lower in subjects carrying the *Val55-Val* genotype. However, numerous studies do not support a functional impact of the *3'UTR insertion* or the *Ala55Val* polymorphism in causing obesity or Type II diabetes (Table 6) [157–163].

The *3'UTR 45 bp insertion* could hypothetically exert an effect by altering mRNA stability. There is no difference, however, in *UCP2* mRNA expression between genotypes in skeletal muscle from Pima Indians [153] and in vitro stabilisation assays have not been published. The *Ala55Val* polymorphism could be evaluated for a functional impact in vitro in yeast heterologous systems.

Preliminary studies of the proximal *UCP2* promoter region identify a number of polymorphisms

Table 7. Summary of *UCP2* association and genotype-phenotype interaction studies in obese, Type II diabetic or glucose tolerant control subjects[a]

Population	Polymorphism	$n_{affected}$ (Allele frequency in %)	$n_{control}$ (Allele frequency in %)	Phenotypes	Reference
Danish Caucasians	Ala55Val	144 (48.3)	182 (45.6)	Biochemical and anthropometrical	Urhammer et al. 1997 [157]
Japanese	Ala55Val; Ala232Thr	210[b] (46.0); 210[b] (0.7)	218 (48.4); 218 (–)[d]	Biochemical and anthropometrical	Kubota et al. 1998 [159]
Swedish Caucasians	Ala55Val	110[e] (50)	90 (46.7)	RMR	Klannemark et al. 1998 [160]
Pima Indians	Ala55Val; 3'UTR ins-del	82 (57.3); 790 (50.2)	–; –	BMI* (3'ins); 24 h EE* (3'ins); SMR* (3'ins)	Walder et al. 1998 [153]
French Caucasians	Ala55Val; 3'UTR ins-del; Gly85Ser	72 (0.28) 483 (0.25) 72 (1.4)	36 (0.38) 113 (0.23) 120 (–)[d]	–	Otabe et al. 1998 [162]
German Caucasians	Ala55Val; 3'UTR ins	68; 68	104 (37.0[c]); 104 (29.0[c])	RMR	Tu et al. 1999 [161]
Danish Caucasians	3'UTR ins-del	744 (30.4)	791(29.6)	BMI, weight gain	Dalgaard et al. 1999 [158]
British and South Indians	3'UTR ins-del	91(25.9)	220 (18.2)	BMI*, Leptin*	Cassell et al. 1999 [154]
Japanese	Ala55Val; 3'UTR ins-del	100[b] (43.5) 99[b] (18.2)	120 (47.1); 120 (20.8)	Biochemical and anthropometrical	Shiinoki et al. 1999 [163]
Danish Caucasians	Ala55Val	–	60 (40.8)	BMI, 24 h EE*, SMR, fat oxidation*	Astrup et al. 1999 [156]
Americans (various ethnic origin)	Ala55Val; 3'UTR ins-del	68 (39.2); 68 (25.6)	–; –	BMI** (3'ins), body composition* (3'ins)	Yanovski et al. 2000 [155]

*$p < 0.05$ and **$p < 0.001$ between genotype groups for the particular phenotype

[a]The frequency of the valine 55 and 3' UTR (untranslated region) insertion is noted in parenthesis after the number of subjects. Unless noted, the affected subjects are obese. *EE*, energy expenditure; *SMR*, sleeping metabolic rate; *RMR*, resting metabolic rate

[b]Type II diabetic patients

[c]Data given only on combined allele frequency in both obese and control subjects (no association with obesity)

[d]A/T 232 and G/S85 not detected in control subjects

[e]Metabolic syndrome

(Fig. 4), but these do not seem to be associated with obesity or sub-phenotypes thereof in Danish Caucasian subjects (LT Dalgaard, O Pedersen, unpublished observations).

The functional impact of *UCP3* coding region polymorphisms and mutations

Although a number of different amino acid substitutions have been reported in the *UCP3* gene, most are rare [164–166] (Fig. 4). Amino acid polymorphisms in *UCP3* do therefore not make a large contribution to susceptibility to common obesity or Type II diabetes [43, 164]. However, the presence of rare but penetrating, variants could cause monogenic obesity. Rare mutations in *UCP3* have been identified: *Val9Met*, *Arg70Trp*, *Val102Ile*, *Arg143X*, *Arg282Cys*, *Arg308Trp* [164–166]. In addition, a mutation at the junction of exon 6 and intron 6, allowing only UCP3 short form to be translated from the allele (*exon 6 ivs + 1G > A*) was detected exclusively in subjects of African origin, with a prevalence of 0.1 of the minor allele (A). The *exon 6 ivs + 1G > A* polymorphism was associated with reduced fat oxidation and respiratory quotient in carriers and it has been suggested that UCP3 could participate in fuel partitioning [165]. However, other studies have found no evidence that this polymorphism causes obesity, altered fat oxidation or respiratory quotient [164].

In vitro studies of the *UCP3* variants show that the codon 9 methionine, isoleucine 102 and codon 143 stop versions of *UCP3* are not different from the wild type protein with regard to yeast growth properties or O_2-consumption [67, 168]. However, the mitochondrial membrane potential did not decrease to the membrane potential of UCP3L sample in the codon 143 stop codon construct, implying that this mutation does have a reduced function with regard to uncoupling of the membrane potential [168]. Neither the tryptophan 70 nor cysteine 282 variants uncouple

the mitochondria of yeast to nearly the same extent as UCP3L wild type does and these mutations therefore severely impair protein function. The *Arg308Trp* was not investigated in vitro [166]. The predicted functional effect of the *exon 6 ivs + 1G > A* polymorphism is an increased amount of UCP3S protein compared with UCP3L protein and presumably, slightly smaller proton leaks. The increased expression of UCP3S is confirmed by RNase protection analysis of skeletal muscle RNA from *exon 6 ivs + 1G > A*-carriers [164].

Functional impairment of UCP3 function by mutations is not well correlated to the clinical findings of massive obesity in the compound heterozygous subject carrying the codon 143 stop mutation and exon 6 ivs + 1G > A mutation or the co-segregation of obesity and the *Ile102Val* variant [165], none of which cause serious derangement of UCP3 protein function compared with the wild type [168]. These observations have been made in a sample of low-income, African-American patients among whom obesity is very prevalent, making it probable that the phenotype segregated with the polymorphic alleles by chance. Furthermore, the *Arg282Cys* variant was not associated with obesity despite serious functional derangement of the protein [164].

The paucity of common missense polymorphisms in the *UCP3* gene excludes the possibility that amino acid changes in UCP3 cause susceptibility to common obesity or Type II diabetes. In addition, there is no obvious correlation between the functional impairment of UCP3 and severity of obesity in subjects that mutations in UCP3 cannot be concluded to cause severe or monogenic obesity.

Investigations of the promoter region of UCP3 in obese, Type II diabetic and normal glucose-tolerant patients

The *UCP3 –55 c→t* transition is placed 55 bp upstream of the most commonly used transcription initiation site of skeletal muscle (SM1) [47] (Fig. 4). This polymorphism is potentially interesting because it is placed only 6 bp from the TATA box and 4 bp from a DR1 site. Mobility shift and reporter gene assays show that this DR1 site is part of a retinoic acid response element and that the sequence up to –61 bp is responsible for the MyoD dependent activity of the *UCP3* promoter [169].

The *c/c* genotype was found to be associated with lower expression of *UCP3* mRNA in skeletal muscle biopsies from Pima Indians [170], suggesting that the *–55 t* allele increases *UCP3* mRNA expression compared with the *c* allele. In Pima Indians, *UCP3* skeletal muscle mRNA is closely correlated with a sleeping metabolic rate. However, there was no association between *UCP3* mRNA expression and body fat content or BMI [116].

In a French cohort of morbidly obese subjects (predominantly females) the *–55 t/t* genotype was associated with increased BMI, although the allele and genotype frequencies were equal between the obese subjects and normal weight control subjects [171]. Control subjects carrying the *t/t* genotype were also slightly heavier than control subjects with other genotypes ($p = 0.09$). In another French population-based study the *–55 t/t* genotype was associated with dyslipidaemia (increased fasting total serum cholesterol, increased fasting serum LDL-cholesterol and fasting serum apolipoprotein B). It has been proposed that the t-allele protects against Type II diabetes, because its frequency was lower among French Type II diabetic patients compared with glucose-tolerant control subjects ($p = 0.03$) [172]. Among South Indian as well as British glucose-tolerant females and Type II diabetic females the *c/t* and *t/t* genotypes combined were associated with increased waist-to-hip ratio [173]. However, the *–55 c/t UCP3* polymorphism was not associated with obesity or alterations of BMI, waist-to-hip-ratio or fasting serum lipids between genotypes in Danish Caucasian subjects [174]. Thus, at least in some populations this UCP3 promoter polymorphism might be implicated in several phenotypes associated with Type II diabetes and the metabolic syndrome. The mechanisms of this genetic variant are still not known. To determine the possible physiological importance of this polymorphism, more studies, including functional studies and statistical meta-analyses are needed.

Conclusions and perspectives

The hypothesis that *UCP*s are candidate genes for human obesity is primarily based on the findings that the proteins act as uncouplers of the yeast mitochondrial membrane. As it is known that chemical uncoupling of the mitochondrial membrane reduces body adiposity, and because lower metabolic rates predicts of weight gain, the hypothesis that common polymorphisms of the *UCP* genes (coding or regulatory regions) cause lower metabolic rates due to more efficient energy coupling in the mitochondria seems reasonable. Furthermore, genetically engineered mice overexpressing UCP1 and UCP3 are lean and resistant to diet-induced obesity.

Using this hypothesis, investigators including ourselves, studied the coding regions of *UCP1*, *UCP2* and *UCP3* as well as the known regulatory regions for these genes. Prevalent polymorphisms were investigated in the coding, untranslated region and promoters and their impact on obesity, body weight gain, and BMI or Type II diabetes were estimated. Variation in the uncoupling protein genes has been found not to be associated with major alterations of

body weight, but it is more difficult to estimate the contribution of *UCP* genes towards polygenic obesity and Type II diabetes.

Studies of yeast and knock-out animals have established that the UCP2 and UCP3 have uncoupling activity. Their proton-leak properties, verified by in vitro studies, are not supported, however, by a direct relation between mRNA expression of UCP2 or UCP3 in various tissues and an increase in proton leak. This throws into doubt the hypothesis that lower proton leak and higher energy coupling leads to susceptibility towards obesity because UCP2 or UCP3 knock-out mice do not become obese.

A key finding on UCP3 from knock-out mice is that the mice show lower proton leak in their mitochondria. An increase in proton leak accompanied by the uncoupling of the mitochondrial membrane might, therefore, not result in reduced energy expenditure or reduced energy expenditure might not be a predictor of future weight gain. Reversing the chain of causality, the proton leak responsible for a portion of the basal metabolic rate probably does not occur through either UCP2 or UCP3, because a lack of either of these does not lead to a reduced metabolic rate. However, the *UCP2* and *UCP3* genes should not be dismissed until a double knock-out model is available or, even better, a triple knock-out mouse lacking UCP1, 2, and 3. It is clear, though, that studies investigating the nature of proton leaks across the inner mitochondrial membrane are needed to clarify the physiological consequences of increases or decreases in proton leaks. Furthermore, it is evident from transgenic overexpression in mice that UCPs have the ability to cause weight loss when activated making UCPs promising anti-obesity drug targets. Given the effect of overexpression of UCP3 and UCP1 in skeletal muscle and WAT, it is clear that these proteins influence metabolic rate.

Even though polymorphisms in the *UCP* genes have been shown to be associated with obesity, Type II diabetes or sub-phenotypes thereof, their impact on these phenotypes seems to be modest. This is characteristic of polygenetics, where many genes control the phenotype and the same phenotype could be caused by variants in different sets of genes. A major concern with genetic variants with a modest effect is that the population variance of the phenotype could obscure the observable effect of the variant. Therefore, large numbers of well-characterised patients have to be studied to detect the precise effect of a given variant. In addition, more stringent statistical significance criteria are needed for large-scale genome association studies to avoid false positive findings due to the huge number of polymorphisms in the human genome. However, more powerful computational tools will certainly permit the screening of the entire *UCP1* or *UCP2-UCP3* genomic region for polymorphisms in a large number of subjects and thus identify patterns of polymorphisms which could be associated with obesity or Type II diabetes. The next and indispensable step is to verify the functionality of associated variants in cell-based assays or other biochemical studies.

Sources. Current Contents, Medline and PubMed were searched for publications in English containing the words “uncoupling protein”, “UCP”, “UCP2”, “UCP3”, “thermogenesis”, “proton leak”, “brown adipose tissue”, “metabolic rate” alone or in conjunction with “obesity” and “diabetes”. A p value of less than 0.05 was considered to be statistically significant.

Note added in proof: UCP2 deficient mice secrete more insulin in response to glucose stimulus due to increased ATP levels in pancreatic islets [175]. Furthermore, the *866 g/a polymorphism* of the *UCP2* promoter was associated both increased transcriptional activity decreased BMI in middle-aged subjects [176].

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