The role of the uncoupling protein 1 (UCP1) on the development of obesity and type 2 *diabetes mellitus*

Papel da proteína desacopladora 1 (UCP1) no desenvolvimento da obesidade e do diabetes melito tipo 2

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SUMMARY

It is well established that genetic factors play an important role in the development of both type 2 *diabetes mellitus* (DM2) and obesity, and that genetically susceptible subjects can develop these metabolic diseases after being exposed to environmental risk factors. Therefore, great efforts have been made to identify genes associated with DM2 and/or obesity. Uncoupling protein 1 (UCP1) is mainly expressed in brown adipose tissue, and acts in thermogenesis, regulation of energy expenditure, and protection against oxidative stress. All these mechanisms are associated with the pathogenesis of DM2 and obesity. Hence, *UCP1* is a candidate gene for the development of these disorders. Indeed, several studies have reported that polymorphisms -3826A/G, -1766A/G and -112A/C in the promoter region, Ala64Thr in exon 2 and Met299Leu in exon 5 of *UCP1* gene are possibly associated with obesity and/or DM2. However, results are still controversial in different populations. Thus, the aim of this study was to review the role of UCP1 in the development of these metabolic diseases. Arq Bras Endocrinol Metab. 2012;56(4):215-25

SUMÁRIO

Está bem estabelecido que fatores genéticos têm papel importante no desenvolvimento do diabetes melito tipo 2 (DM2) e obesidade e que indivíduos suscetíveis geneticamente podem desenvolver essas doenças metabólicas após exposição a fatores de risco ambientais. Assim, grandes esforços têm sido feitos para a identificação de genes associados ao DM2 e/ou à obesidade. A proteína desacopladora 1 (UCP1) é principalmente expressa no tecido adiposo marrom e atua na termogênese, regulação do gasto energético e proteção contra o estresse oxidativo, mecanismos associados tanto à patogênese do DM2 como à obesidade. Portanto, *UCP1* é um gene candidato para o desenvolvimento dessas doenças. De fato, diversos estudos relataram que os polimorfismos -3826A/G, -1766A/G e -112A/C na região promotora, Ala64Thr no éxon 2 e Met299Leu no éxon 5 do gene *UCP1* estão possivelmente associados à obesidade e/ou ao DM2. Entretanto, os resultados são ainda controversos em diferentes populações. Então, o objetivo deste estudo foi revisar o papel da UCP1 no desenvolvimento dessas doenças metabólicas. Arq Bras Endocrinol Metab. 2012;56(4):215-25

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INTRODUCTION

Type 2 *diabetes mellitus* (DM2) and obesity are common, multifactorial conditions for which susceptibility is determined by the joint actions of genetic and environmental factors (1). The prevalence of obesity and DM2 is increasing worldwide at an alarming rate, and both traits are associated with increased morbidity and mortality (2,3). The dramatic increase in the

prevalence of these disorders over the past two decades is mostly likely due to changes in diet and physical activity (4). However, it is believed that these environmental changes would only lead to DM2 and/or obesity under a permissible genetic background (1). Therefore, great efforts have been made to identify genes associated with these disorders, and a number of studies have been focused on the genes related to energy expenditure, such as those encoding adrenergic receptors and mitochondrial uncoupling proteins (UCPs) (5).

Uncoupling protein 1 (UCP1) plays important roles in metabolic and energy balance and regulation, cold- and diet-induced thermogenesis, and in decreasing the production of reactive oxygen species (ROS) by mitochondria, which are mechanisms associated with the pathogenesis of obesity and/or DM2 (6,7). Thus, the aim of the present study was to review the role of UCP1 in relation to the development of these conditions.

Mitochondrial respiratory chain

Mitochondria are essential organelles in all eukaryotic cells and are involved in many processes that are crucial for cell survival and functioning, including energy production, redox control, calcium homeostasis, and certain metabolic and biosynthetic pathways. In addition, mitochondria are the main sources of ROS and often play a key role in physiological cell death mechanisms (7,8).

The main source of cell energy is the synthesis of ATP from ADP and inorganic phosphate (Pi) by oxidative phosphorylation (OXPHOS) carried out in the mitochondrial respiratory chain (MRC) (7). The MRC is located in the inner mitochondrial membrane, and is constituted by four multienzymatic complexes, an oligomeric protein complex (ATP-synthase), and two proteins responsible for electron transport, coenzyme Q (CoQ), and cytochrome c (Figure 1). OXPHOS involves the coupling of electron transport, by means of the complexes I-IV of the MRC, to the active pumping of protons across the inner mitochondrial membrane, and ATP formation by ATP-synthase (8).

Oxidation of reduced nutrient molecules by means of cellular metabolism yields electrons in the form of reduced hydrogen carriers (NADH and $FADH_2$), which donate electrons to the MRC. The movement of electrons through the MRC is driven by a redox potential that is found across the chain. Complexes I, III, and IV pump protons across the inner membrane as electrons pass down the MRC. This produces an electrochemical potential difference across the inner membrane, known as proton-motive force, consisting mostly of an electrochemical gradient (membrane potential) and a chemical gradient (pH difference). The energy that is conserved in the proton gradient across the inner membrane is used by ATP-synthase to synthesize ATP as protons are transported back from the intermembrane space into
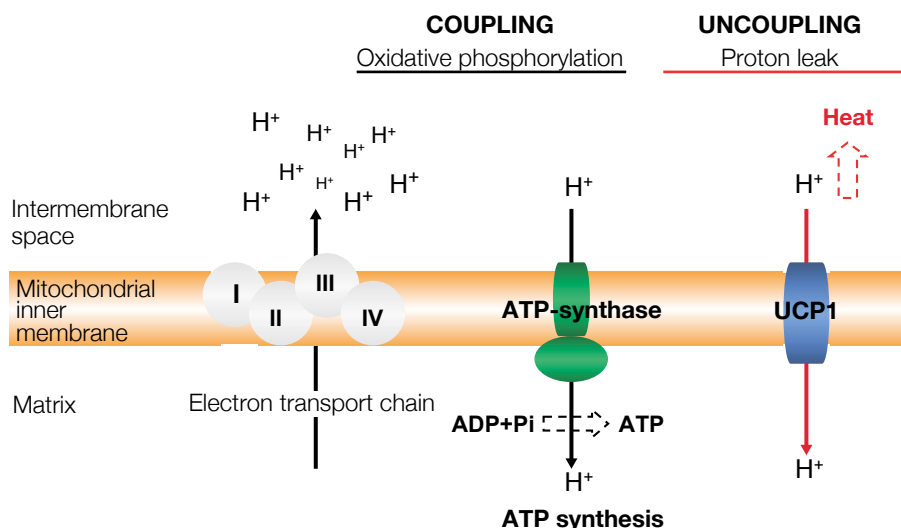
Figure 1. UCP1 location and function in the mitochondrial respiratory chain (MRC). Numbers I-IV corresponds to the MRC complexes. ATP-synthase is the fifth complex of the MRC. During respiration, protons are pumped through the MRC complexes, and a proton gradient is generated. The energy of the proton gradient drives the synthesis of ATP by the ATP-synthase complex. UCP1 catalyzes a regulated re-entry of protons into the matrix, uncoupling the MRC and, consequently, reducing ATP synthesis and generating heat.

the mitochondrial matrix. The final destination of the electrons is the generation of molecular oxygen, which is reduced to water by complex IV, in the last step of the MRC. Therefore, the process of substrate oxidation and oxygen reduction is also called respiration (8).

The coupling of respiration to ATP synthesis is not 100% efficient, and some of the energy is dissipated as heat. Partial uncoupling of respiration from ATP synthesis, also known as proton leak, can be mediated by UCPs and by other mitochondrial inner membrane proteins, such as adenine nucleotide translocase (ANT); which prevents the inhibition of MRC by excessive levels of ATP (6,9).

Although OXPHOS constitutes a vital part of cellular metabolism, the MRC is probably the most important site of ROS production (9). ROS correspond to a variety of molecules and free radicals (chemical species with one unpaired electron) derived from the metabolism of molecular oxygen. Superoxide anion (O_2^-) is the precursor of most ROS and a mediator in oxidative chain reactions (10). Dismutation of O_2^- (either spontaneously or by a reaction catalyzed by superoxide dismutases) produces hydrogen peroxide (H_2O_2) which, in turn, may be fully reduced to water or, in the presence of ferrous or cuprous ions, may form the highly reactive hydroxyl radical (^+OH) (7,8). ROS normally exist in all aerobic cells in balance with biochemical antioxidants. Oxidative stress occurs when this critical balance is disrupted because of excess ROS, depletion of antioxidants, or both. This stress causes damage to cellular macromolecules, such as nucleic acids, proteins, lipids, and structural carbohydrates (11). Moreover, oxidative stress can also lead to cell death by necrosis or apoptosis, mechanisms involved in the pathogenesis of ageing and some disorders, such as DM2 and its chronic complications (11).

Uncoupling of the mitochondrial respiratory chain, changes in energy expenditure, and adaptive thermogenesis

Total body energy expenditure represents the conversion of oxygen and food (or storable forms of energy) to carbon dioxide, water, heat and "work" on the environment (10). Energy expenditure in humans can be subdivided into: 1) basal energy expenditure or resting metabolic rate (RMR), measured under resting conditions and required for normal cell functioning; 2) energy expenditure resulting from physical activity; and 3) energy expenditure attributed to adaptive thermogenesis (Figure 2) (7,10).

Uncoupling (proton leak) of the MRC constitutes a considerable part of the RMR (7). Approximately 20%-50% of total energy expenditure is due to proton leaks, with the skeletal muscle as the main contributor (10). Variations in the RMR are due to several determinants, including body composition (fat *vs*. fat-free mass), con-
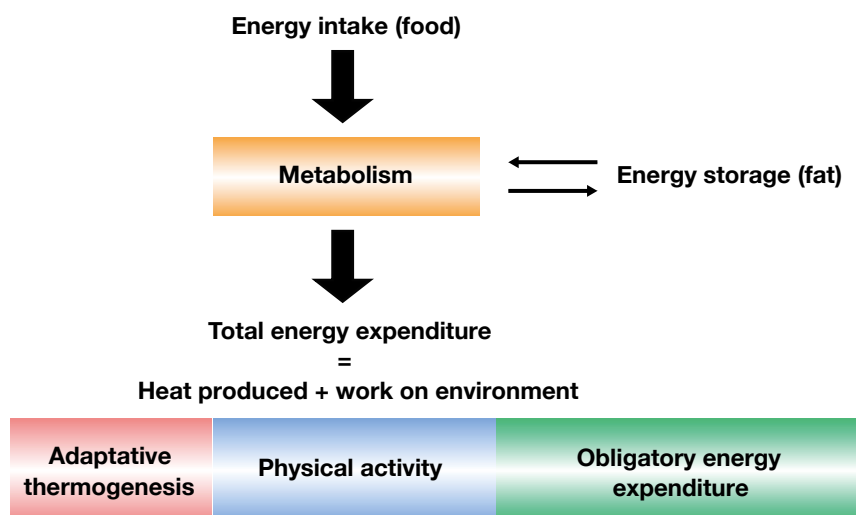
Figure 2. A thermodynamic perspective of energy expenditure. Energy enters the body as food and leaves it as heat and "work". Energy can also be obtained from fat storage. Total energy expenditure can be divided into: obligatory energy expenditure or resting metabolic rate, measured under resting conditions and required for cell functioning; energy expenditure resulting from physical activity (variable); and energy expenditure attributed to adaptive thermogenesis (variable, regulated by the brain), which is defined as non-shivering heat production in response to environmental temperature or diet. Adaptive thermogenesis mainly occurs in brown adipocytes mitochondria and skeletal muscles. Adapted from Lowell and Spiegelman (10).

centrations of steroid and thyroid hormones, genetic factors, and the activity of the sympathetic nervous system (7). It is known that low energy expenditure could predict future weight gain, and that only a slight imbalance between energy intake and energy expenditure is necessary for weight gain, if it persists for several years (10,11). Thus, increasing the energy expenditure by increasing proton leak in mitochondria has been recognized as an effective way to achieve weight loss (7).

Brown adipose tissue (BAT) is found in newborns, rodents and hibernating mammals, and is the main site of adaptive thermogenesis, which is defined as non-shivering heat production in response to environmental temperature or diet (10,12). As a result, thermogenesis in BAT has important roles in thermal and energetic balance and, when deficient, may lead to obesity (10). BAT is a metabolically active tissue, which consists of adipocytes rich in mitochondria and numerous small lipid droplets, and is heavily innervated by sympathetic nerves (7). This tissue differs from the white adipose tissue (WAT), which contains large lipid droplets and few mitochondria (7,12).

In fetuses and newborns, BAT has traditionally been regarded as occurring in specific depots, such as axillary, interscapular, perirenal, and periadrenal ones (10). At birth, human newborns have considerable amounts of BAT, corresponding to 1%-5% of total body weight. This amount takes care of heat generation for the body when the skeletal muscles are yet not able to make any controlled movements and thus, produce heat (10). Children have highly active functional BAT until 13-15 years of age; but, until a few years ago, it was thought that the quantity of BAT declined after puberty, and was rare in adults. Nevertheless, nowadays it is known that BAT can be found in adults in the presence of catecholamine-secreting tumors, such as pheochromocytomas and paragangliomas (13). Besides, some recent studies have shown that BAT in adults is highly active both functionally and metabolically, especially after chronic exposure to cold (14).

In 1978, Himms-Hagen and Desautels (15) showed that BAT metabolism played a role in the development of obesity, and that obese mice had a defect in the mechanisms necessary for the activation of BAT thermogenesis. After this pioneering work, many studies have also shown that defective BAT thermogenesis is involved in the development of obesity in most rodent models, and activation of BAT thermogenesis reduces weight gain in these animals (7,10). Studies

in humans show that although the amount of BAT is reported to be decreased in healthy adults, it still responsible for 1%-2% of the energy expenditure, preventing weight gain of 1-2 kg a year (16,17). Interestingly, when healthy men that have BAT are exposed to cold (19°C), they have a 30% increase in energy expenditure compared to thermoneutrality (27°C), in contrast to those men with almost no BAT, who did not show any increase in cold-induced energy expenditure (18).

It has been suggested that development of ectopic BAT within the WAT may play an important role in preventing obesity (9). In agreement with this hypothesis, transgenic mice overexpressing UCP1 in their skeletal muscle or WAT develop a resistance to diet-induced obesity and DM2, and have a marked stimulation of fatty acid oxidation in muscles (7). In addition, Tiraby and cols. (19) reported that the adenovirus-mediated expression of human PGC-1α (PPARγ-coactivator-1α) increased the expression of UCP1, respiratory chain proteins, and fatty acid oxidation enzymes in human subcutaneous white adipocytes. Changes in the expression of other genes were also consistent with brown adipocyte mRNA expression profile. The authors concluded that human white adipocytes can therefore acquire typical features of brown fat cells following proper stimulation (19). These data indicate that moderate induction of UCP1 in WAT may be used to increase metabolic energy expenditure in obese subjects. Thus, specific uncoupling of adipocyte mitochondria remains an attractive target for the development of anti-obesity drugs (6,9).

Mitochondrial uncoupling proteins (UCPs)

UCPs 1, 2, 3, 4, and 5 are members of an anion-carrier protein family, and are located in the inner mitochondrial membrane (20). These proteins have similarities in their structures, but different tissue expression in mammals. The original UCP, UCP1, is mainly expressed in BAT (7). UCP2 is widely distributed, whereas UCP3 is mainly restricted to the skeletal muscle, and UCP4 and 5 are mainly expressed in the brain (7,8,20).

Over the last few years, several studies have shown that UCPs decrease metabolic efficiency by uncoupling substrate oxidation in mitochondria from ATP synthesis by the MRC. This is thought to be accomplished by promoting net translocation of protons from the intermembrane space, across the inner mitochondrial membrane, to the mitochondrial matrix, thereby dissipating

the potential energy available for ATP synthesis, and consequently, decreasing ATP production (6,8). This uncoupling effect then leads to homologue- and tissue-specific functions, such as thermogenesis and energy expenditure (UCP1), regulation of free-fatty acids (FFAs) metabolism (UCP2 and UCP3), reduction in ROS formation (UCP1-3 and UCP5), and regulation of ATP-dependent processes (UCP2) (6,8,20).

Uncoupling protein 1 (UCP1)

Thermogenesis in BAT is due to UCP1, also called thermogenin or SLC25A7 (7). In 1985 the coding DNA sequence of *UCP1* was cloned, and its amino acid sequence was determined (5). The *UCP1* gene covers a 9kb region on chromosome 4 (region 4q28-q31), and contains 6 exons and 5 introns (Figure 3) (7). UCP1 is a 33-kDa dimeric protein that dissipates the pH gradient generated by OXPHOS (Figure 1), releasing chemical energy as heat (7,9). *UCP1* gene expression is increased by cold, adrenergic stimulation, β3-agonists, retinoid and thyroid hormones, and cAMP (6,7,9). Its expression is activated by non-sterified fatty acids and inhibited by purine nucleotides (GDP, ATP and ADP) (6,20). Many studies based on the use of drugs that activate the β3-adrenergic receptor (β3-AR) confirmed that the sympathetic nervous system was the main trigger of UCP1 activation and induction (6). Moreover, uncoupling of the MRC by means of UCP1 action is only observed when cells are properly stimulated, for example, by norepinephrine (6,20). Norepinephrine stimulation of β3-AR results in three joint processes: 1) activation of p38 mitogen-activated protein kinase (MAPK) pathways that upregulate UCP1 synthesis; 2) activation of protein kinase A (PKA)-mediated pathways that initiate lipolysis and release of acute regula-

tors of UCP1, such as FFAs; and 3) inhibition of lysosomal pathways that degrade UCP1 (6). In addition, it is well known that PGC-1α plays a pivotal role in the regulation of *UCP1* gene expression after adrenergic stimulation, by means of a MAPK pathway that is associated with the JNK-interacting protein (JIP) family of scaffold proteins (6,7).

The uncoupling activity of UCP1 is explained by its ability to transport protons across the inner mitochondrial membrane, in particular when FFAs bind to the protein. However, although CoQ has been described as a cofactor essential for its activity, the precise mechanism by which FFAs regulate transport through UCP1 is still a matter of intense debate (6,9). The main proposed mechanisms include the fatty acids protonophore (or *flip-flop*) model, and the channel (or *proton buffering*) model. In the *flip-flop* model, UCP1 is a carrier of fatty acid anions, which are transported by this protein from the matrix side to the intermembrane space. In this model, each fatty acid anion combines with a proton, becomes electrically neutral and flips back through the membrane, releasing the proton in the matrix (6). The channel model predicts a two-domain structure of UCP1 with a pore domain and a gating domain, which allows protons to pass through the UCP1. In this model, fatty acid carboxyl groups are involved in the proton transport by providing H^+ buffering capacity (6). The arguments for and against each model were reviewed by Brand and cols. (21), in 1999.

Transgenic and knockout rodent models are common approaches to investigate the impact of overexpression or absence of a particular gene on the mouse phenotype. UCP1-knockout mice did not become obese, and merely showed an increased sensitivity to
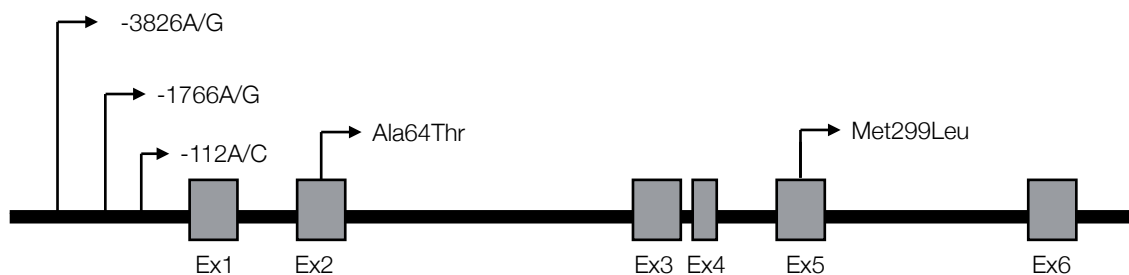
Figure 3. Map of *UCP1 locus* on chromosome 4 (region 4q28-q31). The six exons (boxes) are numbered from left to right according to the transcriptional region. The vertical arrows show the main common polymorphisms associated with obesity or type 2 *diabetes mellitus*. Figure adapted from Jia and cols. (5).

cold exposure (7). On the other hand, transgenic mice with increased UCP1 expression in WAT were obesity-resistant after being fed a diet rich in saturated fat (7). UCP1 has also been ectopically expressed in skeletal muscle of mice, and these animals showed improved glucose tolerance after being fed a high-fat diet, when compared with wild-type mice (7). In humans, UCP1 expression in the intraperitoneal fat of obese subjects is 50% lower than in normal weight subjects, in spite of the fact that the amount of BAT interspersed in WAT depots in adult individuals is relatively low (approximately 1 brown adipocyte/200 white adipocytes) (12,20). Until a few years ago, it was believed that UCP1 was expressed exclusively in BAT; but it has been recently reported that UCP1 mRNA expression and/or protein were also observed in WAT of mouse and humans, mammalian pancreatic islets, human skeletal muscle, bovine retina, human longitudinal smooth muscle layers, and rat and mouse thymocytes (22-26). However, the physiological role of UCP1 in these tissues is still a matter of debate (7). As already mentioned, uncoupling of the MRC due to UCP1 activity allows a more rapid flux of electrons through the inner mitochondrial membrane, reducing membrane potential and, consequently, decreasing ROS production (6,7,20). Therefore, the main role of UCP1 in these other tissues seems to be protection against oxidative stress (27,28). Superoxide anions could activate UCPs through lipid peroxidation products, such as 4-hydroxy-2-nonenal (HNE), which is a marker of oxidative stress and a direct activator of UCP1 (28,29). On the other hand, superoxide dismutase enzymes inhibit UCP1 activity (27). Interestingly, Cui and cols. (25) reported that endothelial cells from bovine retina incubated with high levels of glucose increased UCP1 and UCP2 expression, which protected them from ROS damage caused by glucotoxicity, suggesting a protective role of these UCPs in the pathogenesis of diabetic retinopathy, a chronic diabetic complication.

UCP1 gene polymorphisms associated with obesity and/or type 2 diabetes mellitus

Because UCP1 has been found to decrease membrane potential and increase energy expenditure, UCP1 gene is regarded as a candidate gene for obesity, DM2 or related-traits (7). For that reason, the relationship between UCP1 locus and susceptibility to these disorders has been investigated in a number of genetic studies, with particular attention being paid to -3826A/G, -1766A/G, and -112A/C polymorphisms in the promoter region; Ala64Thr polymorphism in exon 2; and Met299Leu polymorphism in exon 5 of the UCP1 gene (Figure 3). Results of these studies have been variable (Table 1): while some of them showed an association of one or more of these polymorphisms with obesity, DM2, body fat accumulation, body mass index (BMI), or other characteristics of metabolic syndrome (17,22,30-50), other studies were unable to find any association between these polymorphisms and these characteristics (51-61).

Most association studies about the effects of UCP1 gene polymorphisms focused on -3826A/G (rs1800592) polymorphism. The -3826G allele has been associated with reduced UCP1 mRNA expression in intraperitonial adipose tissue of obese subjects, indicating that this polymorphism has functional importance (62). Several independent studies support the association between the -3826G allele and obesity, BMI or other obesity-related parameters (35,36,40-43,46,63,64). Additionally, other studies indicate that the -3826G allele might be associated with reduced HDL-cholesterol levels (22,33,37,44), increased triglycerides (37,43), or LDL-cholesterol levels (39,44), and increased systolic and/or diastolic blood pressure (35,44).

Conversely, studies on the association of the effects of UCP1 gene polymorphisms on DM2 have shown controversial results: a few studies reported an association between the -3826G allele and DM2, insulin resistance (IR) or increased insulin or glucose levels (37,41), whereas other studies indicated that this polymorphism is not associated with these characteristics (32,36,40,52,54-56,59,60). A number of studies analyzed the association between other UCP1 gene polymorphisms and DM2. Mori and cols. (38) reported that the C allele of -112A/C polymorphism and Leu allele of Met299Leu polymorphism were associated with susceptibility to DM2 in Japanese subjects. A recent study showed that the -3826A/-112A/Met229 UCP1 haplotype was associated with increased risk for DM2 in Indian subjects (50). In addition, the study of Fukuyama and cols. (32) indicated that the -112A/C polymorphism was associated with both increased insulin resistance and hepatic lipid content in Japanese subjects with DM2.

Table 1. Studies of the association between *UCP1* gene polymorphisms and obesity, type 2 *diabetes mellitus* or related-traits

Polymorphism	Population and design	Characteristics	Results	Ref.
-3826A/G	Colombian (455 patients with DM2 and 449 non-diabetic controls)	DM2	Association between the A allele and DM2 (OR = 0.78, P = 0.02)	(47)
-3826A/G	Korean (40 obese women). Randomized clinical trial of low-calorie meals (white *vs.* mixed rice), in a 6-week follow-up	Weight, BMI, lipid profile, and blood glucose	A/A genotype was associated with significant weight loss in the mixed rice group	(48)
-3826A/G	Japanese (32 obese women). Low-calorie diet intervention in a 2-month follow-up	Obesity- and lipid-related parameters	The degree of reduction in the HDL levels was significantly smaller in G allele carriers than in A/A carriers	(33)
-3826A/G	239 African-Americans and 583 Hispanics	BMI, WHR, lipid profile, blood glucose, IR, and AIRg	A/A genotype was associated with AIRg in African-Americans and HDL levels in Hispanics	(22)
-3826A/G	Chinese (127 obese and 257 non-obese subjects)	Obesity	No association	(51)
-3826A/G	Swedish (292 obese and 481 non-obese women)	IR and obesity	No association	(52)
-3826A/G	Czech (295 DM2 patients, 113 offspring of DM2 patients, and 120 healthy adults)	Anthropometric parameters, lipid profile, and blood glucose	No association with DM2. In the offspring of DM2 patients, the A/G genotype was associated with higher BMI and subcutaneous fat mass compared with A/A carriers	(63)
-3826A/G	Spanish (159 obese and 154 non-obese subjects)	MetS related-traits	Within the obese group, the G allele was associated with greater BMI, greater percentage of body fat and higher DBP and SBP values than A/A carriers	(35)
-3826A/G	Spanish (160 men and 172 women with and without obesity)	BMI, WHR, insulin, blood glucose, leptin, and lipid profile	The G allele was more frequent in obese than in non-obese women (0.31 *vs.* 0.17, P = 0.008)	(36)
-3826A/G	Polish (118 obese subjects)	Lipid profile, blood glucose, insulin, and leptin	G/G genotype carriers had higher triglyceride levels and decreased HDL and insulin levels than A allele carriers	(37)
-3826A/G	German (154 obese and 154 non-obese subjects)	Obesity	No association	(53)
-3826 A/G	Danish (379 subjects)	Obesity, WHR, IR, blood glucose, and lipid profile	No association	(54)
-3826A/G	Finnish (70 DM2 patients and 123 non-diabetic subjects), in a 10-year follow-up	BMI, blood glucose, insulin, and BP	No association	(55)
-3826A/G	Turkish (271 obese and non-obese subjects)	BMI, BP, blood glucose, and lipid profile	G/G genotype carriers showed BMI-associated increases of cholesterol levels that were more marked than in A allele carriers	(39)
-3826A/G	214 Japanese men	BMI and IR	BMI was higher in subjects with the G allele *vs.* those without it	(40)
-3826A/G	Indian (89 DM2 patients and 100 non-diabetic controls)	DM2	No association	(56)
-3826A/G	Japanese (251 men)	BMI, blood glucose, and lipid profile	Men with the A/G genotype had higher BMI than those with the A/A genotype	(42)
-3826A/G	Japanese (182 postmenopausal and 99 premenopausal women), in a 4-year follow-up	BMI and lipid profile	In the premenopausal women, G allele carriers had higher BMI than A/A genotype carriers. In the postmenopausal women, the 4-year change in triglyceride levels was higher in G allele carriers than in non-carriers	(43)
-3826A/G	Korean (190 obese subjects)	Lipid, blood glucose, and BP	The G allele was associated with higher DBP and LDL levels and with lower HDL levels compared with A/A genotype carriers	(44)

Continuation

Table 1. Studies of the association between *UCP1* gene polymorphisms and obesity, type 2 *diabetes mellitus* or related-traits

Polymorphism	Population and design	Characteristics	Results	Ref.
-3826A/G	Swedish (674 obese and 311 non-obese subjects)	Obesity	No association	(61)
-3826A/G	Finish (170 obese women). Treatment with low-calorie diet, in a 12-week follow-up	Weight loss and RMR	No association	(57)
-3826A/G	Australian (526 obese or overweight women)	BMI, DM2, blood glucose, lipid profile, insulin	The G allele was associated with higher BMI and glucose levels than A/A genotype carriers	(41)
-3826A/G	French (238 morbidly obese and 91 non-obese subjects)	Obesity and weight gain	The G allele was associated with high weight gain during adult life (OR = 1.4, P = 0.02)	(46)
-3826 A/G	Finish (170 obese subjects)	RMR	No association	(58)
-3826 A/G	German (236 morbidly obese and 198 non-obese subjects)	Obesity and DM2	No association	(59)
-3826 A/G	German (1,020 subjects)	BMI, DM2, blood glucose, and lipid profile	No association	(60)
-3826A/G -412A/C	Korean (367 women)	Body fat distribution	Alleles -3826G and -412C were individually associated with larger areas of abdominal subcutaneous fat. The haplotype [GC] enhanced the significance of this association	(64)
-3826A/G Ala64Thr	German (162 subjects)	BMI and WHR	The 64Thr allele was significantly associated with higher WHR	(30)
-3826A/G -112A/C	Japanese (93 DM2 patients)	DM2-related clinical characteristics	IR and hepatic lipid content were significantly greater in -112C allele carriers than in non-carriers.	(32)
Met229Leu -112A/C	Japanese (320 DM2 patients and 250 non-diabetic controls)	DM2	Leu229 and -112C allele frequencies were higher in DM2 patients than in the control group	(38)
-1176A/G	Korean (387 women)	BMI, WHR, percentage of body fat	WHR, body fat mass and percentage of body fat were significantly higher in G allele carriers compared with A/A genotype carriers	(31)
Met229Leu Ala64Thr	German (293 obese and 134 non-obese children)	Obesity	Thr/Thr genotype was associated with risk for obesity	(17)
-3826A/G -1776A/G Ala64Thr	Korean (453 overweight women)	Body fat distribution	The haplotype [GAA] was associated decreased abdominal fat tissue area, body fat mass and WHR	(45)
-3826A/G -112A/C Met229Leu	Indian (812 DM2 patients and 990 non-diabetic subjects)	DM2	Association between the -3826A/ -112A / Met229 haplotype and risk for DM2 (OR = 1.82, P = 0.009)	(50)

AIRg: acute insulin response to glucose; BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; DM2: type 2 *diabetes mellitus*; IR: insulin resistance; MetS: metabolic syndrome; WHR: waist-to-hip ratio; : RMR: resting metabolic rate.

Similar to UCP1, β3-AR is expressed in BAT and WAT and plays an important role in the induction of lipolysis and in the regulation of energy homeostasis (5). In addition, it is the main adrenoreceptor that stimulates UCP1 expression (6). The Trp64Arg polymorphism in the β*3-AR* gene has been associated with weight gain and other obesity-related indexes, as well as with insulin resistance in different populations [which were reviewed in (5)]. Interestingly, some studies have showed that a synergistic effect between the -3826A/G polymorphism (*UCP1* gene) and the Trp64Arg polymorphism (β*3-AR* gene) is associated with an increased tendency for weight gain (46), lower RMR (58), resistance to weight loss (57), or subsequent weight-maintenance after a low-calorie diet (57). In contrast, other studies did not find any influence of the interaction between these two polymorphisms on the resistance to a low-calorie diet (65), BMI and triglyceride

levels (40,43) or several metabolic parameters related to obesity and DM2 (60). Ethnical and age differences, as well as environmental factors and a synergistic effect with other genes might explain the controversial results among different investigations (5).

In brief, studies on these associations cited here indicated that the -3826A/G polymorphism contributes to the susceptibility to obesity. On the other hand, results reported by other studies on the effects of -3826A/G polymorphism and other *UCP1* gene polymorphisms on lipid profile, blood pressure or DM2 are still inconclusive.

CONCLUSION

Several studies have contributed to the understanding of the mechanisms underlying BAT function and UCP1 activity in this tissue. Interestingly, recent studies have shown that UCP1 can also be detected in pancreatic islets, WAT, skeletal muscle, longitudinal smooth muscle layers, retina, and thymus. However, the physiological functions of UCP1 in these tissues are not as well established as in BAT, and future studies will determine the role of UCP1 in these tissues.

Obesity and DM2 are multifactorial diseases associated with both genetic and environmental factors. Knowledge on factors associated with these disorders will allow us to better understand them, and may provide us with more effective approaches to treatment and prevention. UCP1 plays important roles in thermogenesis, regulation of energy expenditure, and in decreasing oxidative stress, which are mechanisms associated with the pathogenesis of obesity and DM2. Thus, *UCP1* gene is an excellent candidate for these disorders. Indeed, numerous studies strongly suggest that the *UCP1* -3826A/G polymorphism is associated with obesity. Further studies are required to investigate *UCP1* gene polymorphism in different populations in order to confirm the association between these polymorphisms and DM2, and also to elucidate the molecular mechanisms of association between *UCP1* polymorphisms and obesity, DM2, and related-phenotypes.

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