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Review Article

Thermogenesis and Obesity; A Brief Review and rs104894319 Polymorphism in Venezuelan Population

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Abstract

Currently, obesity is an important public health problem in the world, which is considered as a multifactorial and chronic disease, with many situations conditioning the development of this pathology. Therefore, obesity is the result of the interaction between lifestyle (excessive of energy-dense food intake and sedentariness) and genetic predisposition. Some genetic variations have been linked to obese phenotype, affecting metabolic pathways related to energy production at the mitochondrial level, such as mutations in uncoupling proteins (UCPs). Besides of updating key concepts about thermogenesis and its relationship to obesity, we report experimental results in a couple of Venezuelan obese subjects (among n = 95 analyzed individuals) with the pathogenic polymorphism in exon 4 of the UCP3 gene (C→T mutation at −427 bp), named rs104894319. UCPs are proteins located in the mitochondrial inner membrane that carry out an important role in adaptive thermogenesis, a process that takes place during electron transport chain and oxidative phosphorylation when ATP is produced, but instead of using protons (H^+) to generate the electrochemical gradient in ATP synthase, the UCPs redirect H^+ to dissipate that energy as heat. In fact, high capacity to produce heat of brown adipose tissue (BAT) is due to this uncoupling process, as well as during browning process that may suffer white adipose tissue (WAT) in the conversion to beige/brite adipose tissue. This might result after adaptation mechanisms from nutritional intervention, supplementation or physical exercise practice, which in turn may be used as a complementary strategy to deal with obesity nowadays.

Keywords: *White Adipose Tissue; Brown Adipose Tissue; Uncoupling Protein; Mitochondria; Genetic Variation; Nutrition; Physical Activity*

Introduction

Obesity is a multifactorial disease characterized by an expansion of lipid storage in the form of triacylglycerides in adipose tissue (mainly in White Adipose Tissue, WAT). According to WHO, around 650 million of adults were obese in 2016. The fact sheet literally states *"...overweight and obesity are linked to more deaths worldwide than underweight. Globally there are more people who are obese than underweight - this occurs in every region except parts of sub-Saharan Africa and Asia".* Thus, this pathology must be addressed from an anatomic, neurophysiologic, psycho-social, metabolic and genetic point of view, since medical staff would seize an integral approach during individual treatment in each patient [1,2].

Obesity is one the biggest public health problems, due to obese people show serious limitations in their biological and psychosocial functions which may evoke in less life expectancy in comparison with normal-weight people. Furthermore, obese people trend to develop

chronic non-communicable diseases (NCDs), such as arterial hypertension, type 2 diabetes, cardiovascular disease, etc [2]. Thus, two well-established criteria to diagnose and evaluate progress of obesity are body mass index (BMI: kg/m^2) [class I obesity (BMI 30.0 - 34.9), class II obesity (BMI 35.0 - 39.9), and class III obesity (BMI ≥ 40.0)] [3]; and waist-hip ratio (W/H) [men (> 0.90), and women (> 0.85)] [4]. Moreover, an obese patient might be diagnosed with metabolic syndrome if fulfill minimum three of the following [5]; abdominal circumference (> 102 cm in men, and > 88 cm in women), serum HDL (< 40 mg/dL in men, and < 50 mg/dL in women), triacylglycerides (> 150 mg/dL), basal plasma glucose (> 100 mg/dL), and arterial pressure (> 130/85 mmHg).

There are many differences of distribution and composition of adipose tissue. A very important type is Brown Adipose Tissue (BAT), whose color is due to huge number of mitochondria and its subsequent high vascularization (respiration rate of mitochondria requires a constant oxygen supply, this leads a high perfusion rate in circulation). BAT is located in specific zones, such as interscapular, cervical, axillary region, and surrounding thymus, heart, kidney, among others (Table 1) [6]. This spread distribution responds to the need of energy transfer from BAT to other tissues and main blood vessels by contact or convection [7].

Visceral brown fat
• Perivascular: Aorta, common carotid artery, brachiocephalic artery, paracardial mediastinal fat, epicardial coronary artery and cardiac veins, internal mammary artery, and intercostal artery and vein • Periviscus: Heart, trachea and major bronchi at lung hilum, esophagus, greater omentum, and transverse mesocolon • Around solid organs: Thoracic paravertebral, pancreas, kidney, adrenal, liver, and hilum of spleen
Subcutaneous brown fat
• Between anterior neck muscles and supraclavicular fossa • Under the clavicles • In the axilla • Anterior abdominal wall • Inguinal fossa

Table 1: *Distribution of human BAT. Taken from: Sacks., et al (2013).*

In fact, a common mechanism to increase body temperature resides in the own mitochondria, in a process called thermogenesis. These power centrals provide with heat when is necessary through uncoupling proteins (UCPs), which serve as proton carriers from mitochondrial inner membrane (MIM) to matrix and thereby shunt energy from electron transport chain during ATP synthesis. UCP1 is exclusively present in BAT; however, new isoforms of UCPs have been reported in different tissues, which highlight the relevance of this process in cellular energy homeostasis. Hence, in this review we link new advances in thermogenesis to obesity diagnosis, development and treatment from a nutrition and physical activity insight, as well as we report the first experimental results of the pathogenic polymorphism C427T (rs104894319) in UCP3 gene in Venezuelan population by Restriction Fragment Length Polymorphism (RFLP).

Subjects and Methods

Narrative Review

A literature search using several databases (PubMed/MEDLINE, EMBASE, Google Scholar, and Sci Search) was conducted to identify publications that evaluated the relationship between thermogenesis and obesity, nutrition, and exercise. 52 selected publications met inclusion criteria (published in the past 10 years, experimental or nonexperimental design, human or mechanistic models). Publications before 2007 form the basic body of the text.

Population

The study sample, recruited from Dr. Félix Gómez Endocrine-Metabolics Research Center, Faculty of Medicine at Universidad del Zulia (Maracaibo, Venezuela), comprised 60 women and 35 men (n = 95). The mean (SD) age of all sample was 45.2 (11.4) years, with BMI (SD) of 35.5 (5.9) kg/m^2, and abdominal circumference (SD) of 109.8 (14.2) cm. Each individual signed an informed consent. All subjects were tested for rs104894319 polymorphism in UCP3 gene by RFLP.

Genetic analysis

DNA Extraction: Genomic DNA was extracted from peripheral blood leukocytes through Salting Out as described Miller., *et al* (1988).

DNA Amplification: To amplify the target DNA in the exon 4 of UCP3 gene we used the oligonucleotide primers 5*-ATGAGGAGGCTCTGAGTGGA-3* and 5*-TCAGAATCACTGGAACACGC-3*. PCR was carried out in a volume of 25 µL containing 3 µL genomic DNA (≈250 ng/µL), 0.25 µL of each primer (5 pmol/L), 0.3 µL Taq-Polymerase (5 IU/µL; Promega, USA), 0.5 µL of dNTPs (20 mmol/L each), 5 µL of buffer recommended by the supplier, 1.5 µL of $MgCl_2$ (2.5 mmol/L), and 14.2 µL of H_2O. After denaturation for 5 min at 96°C 35 cycles were

carried out, each cycle consisting of 30s at 95°C, 45 s at 61.2°C and 60s at 72°C. The last synthesis step was extended to 10 minutes. The amplified exon was checked by horizontal electrophoresis (BIO-RAD®) in agarose gel (1%) and visualized in UV light using Digit Doc-It system. Positive amplification generated a unique DNA fragment of 436bp in length.

RFLP-analysis: 0.2 µL of the PCR-products from above were digested in a volume of 20 µL with 5 µL of AvaII (4 IU/µL; Promega, USA) under the appropriate buffer conditions (37°C, 2 - 3h). The fragments were resolved on a 4% non-denaturing polyacrylamide gel. C/C allele was characterized by 50, 184 and 202 bp fragments; T/T allele by 50 and 386 bp fragments; and C/T allele by 50, 184, 202 and 386 pb fragments (Figure 1).

Figure 1: *Ava II restriction site.*

Thermogenesis; Molecular Mechanisms

UCPs belong to the solute carrier family 25 (SLC25), which are located at MIM and transport H+ directly from intermembrane space to matrix. Initially, its name came from *thermogenin* or UCP1 (SLC25A7), an isoform that is only expressed in BAT [8], but in recent years four other isoenzymes have been found in human. UCP2 (SLC25A48) is expressed in several tissues, for example skin, muscle, pancreas, adipose tissue, etc. UCP3 (SLC25A9) is found in cardiac and skeletal muscle mainly [9]. Finally, UCP4 (SLC25A27) and UCP5 (SLC25A14, also called *brain mitochondrial carrier protein-1*) are expressed in central nervous system [10]. It is probable that more than 29 members of this UCPs family exist, although most of them have not been identified [11]. Even though action mechanisms of UCP isoforms have several points in common (i.e. C- and N-terminal chains are found in intermembrane space), its biological role seems to be different according to expression tissue [8], and H^+ transport mechanism remains unclear though [12]. After stimulation, UCPs allow the passive movement of H^+ from intermembrane space to mitochondrial matrix. This process releases the oxidation energy as heat and decrease ATP synthesis rate [13]. Interestingly, these proteins possess an allosteric regulation site for nucleotides (ADP, GDP, etc.), which down-regulates UCPs activity after binding [14].

WAT can suffer a process of "browning", where it becomes the so called beige or brite adipose tissue and acquires an increased proportion of adipocytes with high mitochondrial density. Nowadays, the known mediators of this phenomenon include interleukin-6 (IL-6), fibroblast growth factor 21 (FGF21), bone morphogenic proteins (BMPs), and irisin (cleavage product from fibronectin type III domain containing 5, FNDC5). Interestingly, some of these molecules can be secreted by BAT itself (called batokines), which indicates autocrine effects [15]. Recent studies have shown that not only WAT but also BAT are also regulated by circadian proteins [15,16], possibly via circadian repressors such as REV-ERBα (inhibits UCPs activity) or PER2/PPARα (activates UCPs activity) [16-18].

Importance of Fatty Acid Interaction in UCP Function

The presence of fatty acids has shown to activate UCP, via two possible mechanisms. First, Ježek., *et al.* [11] proposed that UCP family can also transport anions outside the mitochondria, such as fatty acids derivatives, in order to allow them to protonate and get back to cytoplasm. In this sense, UCP would prevent fatty acid accumulation in mitochondria [19]. On the other hand, fatty acids might work as allosteric activators of UCP and in this way participate during H^+ transport. This mechanism has been studied in depth by Berardi and Chou [20], where by using nuclear magnetic resonance and mutagenesis described a very important flippase activity that is necessary for H^+ translocation. Basically, UCPs can also catalyze the flipping of ionized fatty acids (ASO-) in the mitochondrial membrane after electrostatic interaction at the peripheral site, which indirectly allow for sustained shuttling of H+ by fatty acids (Figure 2). In addition, this study showed the interesting negative regulation of nucleoside diphosphates (ADP, GDP, etc.), since these molecules can bind to UCPs cavity and allosterically displace fatty acids from peripheral site and thus prevent H^+ transport [20].

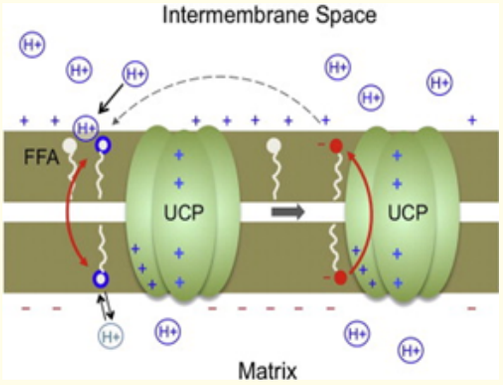

Figure 2: *Model for Flippase Activity of UCPs during H^+ transport. First, protonated or unionized free fatty acid (FFA) readily undergo flip-flop that carries H+ across the lipid bilayer. Upon releasing the H^+ to the matrix, the ionized fatty acid is recruited to the peripheral binding site of UCP2 via electrostatic interaction. Second, binding fatty acid causes minor structural change in UCP2 which allows the acidic head group of fatty acid to enter the cavity of UCP2. Third, upon entering the cavity, the acidic head group of fatty acid is retained by the strong positive charge potential of the cavity while being driven across the UCP2 by the strong membrane potential. Importantly, GDP binding inside the cavity can allosterically displace fatty acid from its binding site, which prevents fatty acid flipping by UCP2 and thus makes H^+ shuttling by fatty acid unsustainable. Taken from: Berardi & Chou (2014).*

Body Temperature Control; Non-Shivering Thermogenesis

As mentioned before, UCP1 is only expressed in BAT adipocytes, where is in charge of uncoupling oxidation-reduction reactions of cellular respiration by carrying protons to mitochondrial matrix; therefore, dissipates proton motive force (Δp), increases oxygen consumption, and consequently augments heat production in a process known as adaptive thermogenesis [8,9]. Mammals rely on two mechanisms to increase heat production in response to low-temperature conditions: shivering and non-shivering thermogenesis. The last seems to be controlled by UCPs since their activity and expression are upregulated after cold exposition [21]. This adaptation mechanism of non-shivering thermogenesis is under control of hypothalamus, which via sympathetic activation induces noradrenaline secretion, activation of AMPc signaling pathway, higher transcription/expression of certain genes/proteins (i.e. UCP1), and a subsequent mitochondrial biogenesis and size [22].

Cross-talking mechanisms mediated by cytokines and hormones might have an active role stimulating UCP1. For example, it seems that leptin might influence gene expression of UCP1 [23], and it is well known that thyroid hormone actives UCP1, but its presence is not fully necessary [24]. In addition, the myokine irisin may affect considerably UCPs activation and perhaps regulates mitochondrial adaptation processes associated to physical exercise [25]. Pharmaceutical products can also stimulate UCPs activation [8]; however, some authors have failed replicating these results [21].

Oxidative Stress and Lipotoxicity

UCP2 is also abundant in immune system and its main role seems to be linked to electric gradient reduction in MIM, via dissipating H^+ accumulation and avoiding generation of reactive oxygen species (ROS) [26]. This isoform is present in pancreatic beta cells as well, where increased expression is associated with suppression of glucose-induced insulin secretion [19]. The mechanism of glucose-induced insulin secretion is based on fast modification of redox potential ($NADH/NAD^+$), which evokes the augmentation in mitochondrial Δp, the increase in the ATP/ADP ratio, and closure of the ATP-sensitive K^+ channels. The resulting decrease in K^+ permeability leads to depolarization and subsequent opening of Voltage-dependent Calcium Channels (VDCCs), which finally stimulates exocytosis of insulin-containing granules [9]. It is important to note the key role of ROS in insulin secretion process [27].

On the other hand, although UCP3 are highly expressed in muscle tissue, it does not contribute importantly to heat generation in cold environment; however, it has great impact in basal thermogenesis and other specific conditions [8,9]. Indeed, its main function would be associated to oxidative stress protection, since might mitigate ROS emission from electron transport chain due to the ability to reduce Δp

[22,28]. For instance, this electrochemical potential has been reported to be important in superoxide production [29]. It is interesting to note that either ROS or their derivatives have shown to activate UCP3, which creates an important negative-feedback loop that controls ROS production [30]. Finally, UCP3 is also thought to protect against mitochondrial lipotoxicity by means of lipid hydroperoxides (LOOH) exportation, which might reduce their accumulation in mitochondrial matrix and avoid damage of mt-DNA, enzymes, and integral membrane components [31]. Even though amino acid sequence and expression tissue for UCP4 and UCP5 are different from UCP3, these isoforms would share the same biological function [10].

Creatine Metabolism and UCP1-Independent Thermogenesis

Creatine (Cr) and phosphocreatine (PCr) are important components of the Creatine Kinase (CK) system, which serves to improve the thermodynamic efficiency of ATP hydrolysis by maintaining low intracellular ADP concentration and high ATP/ADP ratios at those subcellular sites where CK is functionally coupled to ATP-requiring processes. This energy transfer circuit involves several components of the cell (i.e. cytosol, mitochondrial reticulum, ion pumps, etc.), and is based on two cytoplasmic and two mitochondrial CK (CKMT) isozymes [32]. Importantly, Cr increases mitochondrial ATP production as well as increasing flux of substrate through the respiratory chain.

CK activity and characteristic genes of Cr metabolism are coordinately elevated by cold-exposure in beige/brite adipocytes [33]. In fact, as Kazak., *et al.* [34] stated, "reduction of Cr level in mice significantly blunts β3-adrenergic mediates increases in whole body oxygen consumption, suggesting that this pathway plays a critical natural role in beige/brite fat thermogenesis". Recently, Bertholet and collaborators [35] used patch-clamping and bioenergetics analyses that allowed characterizing two types of beige/brite adipocytes; UCP1 positive and UCP1 negative (although UCP1 was not detectable on the protein level in UCP1-negative adipocytes specifically in visceral fat, *Ucp1* mRNA was upregulated in these cells as compared to WAT). As a matter of fact, UCP1-negative adipocytes were classified as beige/brite fat due to physio-metabolic similarities with the classic UCP1-positive beige adipocytes (i.e., enhanced mitochondrial biogenesis, activated thermogenic gene program, multilocularity, etc.). Interestingly, authors also found higher expression of *Ckmt2* in UCP1-negative adipocytes, which highlighted the role of Cr metabolism in their thermogenesis. This coincides with the recent results of [36], where genetic-induced Cr depletion (AGAT knock-out, limiting enzyme in Cr production) showed a significant reduction in thermic effect of food and evokes an obese phenotype in mice. In this sense, CK system provide an alternative mechanism of heat production (recently called creatine-driven thermogenesis) that coexist with UCP1-dependent thermogenesis in some adipocytes, but it represents the unique mitochondrial thermogenic pathway in UCP1-negative adipocytes. Furthermore, UCP1-negative adipocytes are unable to exhibit a rapid adaptive thermogenic response, perhaps because creatine-driven thermogenesis is more responsible for the slow modulation of basal metabolic rate (BMR) in WAT [35]. More research is needed.

Nutrition and Exercise in Thermogenesis

Total energy expenditure encompasses three components; BMR, physical activity-related energy expenditure (including non-exercise- and exercise-related activity thermogenesis; NEAT and EAT respectively), and thermic effect of food (TEF, also known as diet-induced thermogenesis). The last refers to the increase in energy expenditure that accompany food intake, and is measured as the amount of energy spent in digesting, absorbing and metabolizing/storing the nutrients that are not oxidized plenty [37].

Intraindividual variation of TEF is so big that it can fluctuate between 6 and 125% depending on the method used; however, it has been estimated that average value is 10% when there is an energy balance [38]. In fact, under the state of overfeeding or high-fat diets (HFD), it has been shown both in mice and human that energy expenditure can be raised to avoid the increase in body mass [39,40]. TEF has two components; a mandatory that is associated with digestion and enzymatic reactions of macronutrients (fat 0 - 3%, carbohydrates 5 - 10%, and proteins 20 - 30%) [41], and a facultative or adaptive component that refers to postprandial increase in energy expenditure above processing energy of food, which seems to be influenced by sympathetic nervous system via unclear mechanisms that postulate UCPs and BAT [42]. Actually, an increase in 1% of dietary protein leads to an augmentation in TEF of 0.22 ± 0.42%. Alcohol can also increase TEF about 7 - 9% when around 20% of caloric intake comes for an alcoholic beverage; however, this does not give any extra benefits. Conversely, an increase in dietary protein not only has been shown to augment TEF but also enhance satiety, a key aspect in regulation of body mass [38], and is recommended during caloric restriction diet in obese and overweight people [43,44].

Additionally, some dietary ingredients can increase TEF, but specially enhance browning process in WAT. It is well known that browning is stimulated by exercise and physical activity (which also promotes mitochondrial biogenesis), cold, and β-adrenergic agonists; but recently, several food-derived molecules have been studied because of their potential to promote thermogenesis and accelerate browning (i.e., capsaicin, capsaicinoids, ginger extract, catechins, piperine, theaflavins, olive-derived polyphenols, hydroxycitric acid, siberian ginseng, etc.) [42]. Consequently, many "thermogenic supplements" are distributed and sold claiming to reduce body weight and promote fat loss, but neither dosages nor active compounds are based on scientific evidence, with no consideration of the insignificant effect seen in practice. In table 2 we summarize several dietary ingredients that are under research because of their thermogenic potential (induce considerable changes in energy expenditure) and safety if consumed in recommended doses; nonetheless, it is necessary to clarify that they are not able to solve obesity/overweight by their own but might provide some advantages in long term treatments.

Food or Supplement	Recommended Dosage and Active Molecule	Possible Mechanism	Reference
Green Tea (*Camelia sinensis)*	6 - 8 mg of Green Tea with 50% of Epigalocatechin-3-gallate (EGCG) per kg of body mass	- Thermogenic and antioxidant properties due to polyphenols and catechins - Seems to increase fat oxidation under resting and post-exercise - Prolongs catecholamine-induced lipolysis during exercise probably by inhibition of catechol-o-methyltransferase (COMT) - Xanthine derivatives can act as phosphodiesterase (PDE) inhibitors	[45-50]
Chilli Pepper (*Capsicum*)	2 - 10 mg Capsaicinoids per kg of body mass	- Thermogenic and anti-obesity potential - Increases resting energy expenditure - Activates sympathetic nervous system and BAT via Transient Receptor Potential Vanilloid 1 (TRPV1) and probably Melastatin 8 Channel (TRPM8) - Stimulates lypolisis through lipases activation	[49-55]
Anhydrous Caffeine	2 - 4 mg Caffeine (1,3,7-trimethylxanthine) per kg of body mass	- Thermogenic and anti-obesity potential - Increases BMR and promotes weight loss - Activates BAT - PDE inhibition	[50,56-59]
Black Piper *(Piper Nigrum)* and Ginger (*Zingiber officinale*)	20 mg of Piperine and 2 - 3 g of Ginger (Gingerol and Paradol)	- Thermogenic potential - Activates sympathetic nervous system via Transient Receptor Potential Vanilloid 1 (TRPV1) - Piperine might inhibit COMT and PDE - Ginger enhances TEF and satiety. Also might prevent NCDs	[49,60-63]

Table 2: *Nutritional Ingredients or Molecules with Thermogenic and Anti-obesity Potential.*

In short, there are some easily and safely applicable dietary strategies that can be taken into account to support and strengthen fat loss programs in order to improve body composition and health [64]; however, with current evidence is still weak the desirable effect regarding magnitude of required weight reduction.

In recent years, it has been pointed out that muscles play a fundamental role as a metabolic, endocrine, and thermogenic organ. Particularly, many important proteins released from skeletal muscle (known as myokines) have been linked specifically to thermogenesis, such as irisin and interleukyne-6 (IL-6). In response to exercise, peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is able to activate FNDC5. After cleavage from this last, irisin is secreted into blood increasing thermogenesis by browning of subcutaneous WAT [65]. On the other hand, SLN serves as a modulator of sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) and heat production from muscle tissue (Figure 3) [66].

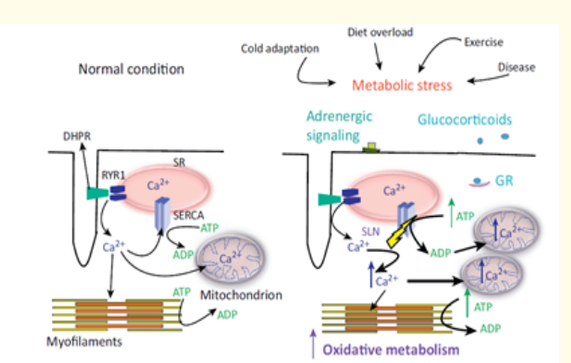

Figure 3: *Sarcolipin Promotes Oxidative Metabolism under Conditions of Increased Energy Demand. Many different pathophysiological states increase energy demand in muscle. In addition, several cytokines and neurohormonal mechanisms (including glucocorticoids and adrenergic signaling) are activated to orchestrate muscle metabolism. Sarcolipin (SLN) expression is also upregulated under these high-energy demand conditions. Higher SLN/SERCA level leads to higher cytosolic $Ca2^+$ and facilitates its uptake into mitochondria, which serves as a trigger for oxidative metabolism. In addition $Ca2^+$-signaling pathways activate increased transcription of genes involved in oxidative metabolism. Abbreviations: DHPR, dihydropyridine receptor; GR, glucocorticoid receptor; RYR1, ryanodine receptor 1. Taken from: Pant., et al. (2016).*

Muscle tissue might be an important component of facultative diet-induced thermogenesis in humans, since SLN can play a key role in metabolism and energy expenditure. In this sense, it has been found in mice that SLN increases fat oxidation and, in certain extent, might protect against diet-induced obesity [67]. For example, it has shown that overexpression of SLN in rodents improves skeletal muscle performance during prolonged physical activity. These findings make necessary to delve into human studies that allow recognizing SLN influence in thermogenesis, energy metabolism and body composition.

Genetic Disposition to Obesity; rs104894319 Polymorphism in Venezuelan Population

Nowadays, several genetic variations associated to the obese phenotype have been studied. Polymorphisms are variations in a specific nucleotide of the DNA sequence between individuals of a population, and correspond to one of the multiple phenomenon's that trigger obesity. Most relevant obesity-associated genes are FTO, MC4R, APOA2, UCP3, FAIM2, SH2B1, PLIN1, and BDNF [68].

In particular, UCP3 gene is located on chromosome 11: 74,000,281-74,009,435 reverse strand (Ensembl ID: ENSG00000175564), and is composed of seven exons. Some studies have shown an association between UCP3 gene polymorphisms with overweight and obesity [69]; however, one of the most important is the well-described and pathogenic polymorphism rs104894319 (also known as C427T or R143X). rs104894319 consists in a missense mutation (C427T) in exon 4 that introduce a premature stop codon at residue 143 (R143X). This mutation produces a truncated protein with an altered transmembrane domain, which might affect the ability to undergo thermogenesis in adipose tissue and diminish energy expenditure, among other new proposed roles. In this study, we provide with the first characterization of rs104894319 polymorphism in obese subjects from Maracaibo, Venezuela. Data characterization of study group is shown in table 3.

Characterization Variables	Women (n = 60) $\bar{x}$ ± DE	Men (n = 35) $\bar{x}$ ± DE	Total $\bar{x}$ ± DE
Age (years)	45,8 ± 13,1	44,0 ± 11,2	45,2 ± 11,4
Body Mass (kg)	87,8 ± 18,2	103,8 ± 25,9	93,7 ± 22,6
Height (m)	1,6 ± 0,7	1,7 ± 0,7	1,62 ± 0,9
Body Mass Index (kg/m^2)	35,5 ± 5,3	35,4 ± 5,1	35,5 ± 5,9
% body fat mass	44,9 ± 3,5	33,9 ± 9,4	40,6 ± 8,4
Abdominal circumference (cm)	106,8 ± 9,9	114,8 ± 18,6	109,8 ± 14,2
Basal glycemia (mg/dl)	105,9 ± 29,5	108,7 ± 39,3	107,1 ± 33,3
Insulin (mU/L)	21,6 ± 12	20,6 ± 12,2	21,2 ± 12,1
Total cholesterol (mg/dl)	207,7 ± 54,5	197,6 ± 51,8	203,9 ± 53,5
Triacylglycerides (mg/dl)	174,2 ± 155	173,4 ± 145	173,9 ± 148,3
LDL-c (mg/dl)	129,2 ± 35,5	123,4 ± 35,8	127 ± 35,6
HDL.-c (mg/dl)	43,6 ± 10,8	39,5 ± 7,2	42,1 ± 9,8
Systolic Arterial Pressure (mmHg)	122,3 ± 19,3	128,1 ± 19,1	124,4 ± 16,9
Diastolic Arterial Pressure (mmHg)	80,3 ± 11	85,1 ± 14	82,1 ± 12,3

Table 3: *Anthropometrical and biochemical parameters in the study group. Values are means ± Standard Deviation.*

After amplification of DNA fragment from exon 4 of UCP3 gene, we analyzed the C→T mutation at −427 bp by RFLP with AvaII in all patients. PCR product of 436 pb contained two recognition sequences for the restriction enzyme AvaII, which meant three fragments would be generated (50, 184, and 202 bp). rs104894319 mutation eliminated one restriction site, so only two fragments were generated (50, 386 bp). After RFLP analysis, a heterozygote mutation was found in two patients (Figure 4).

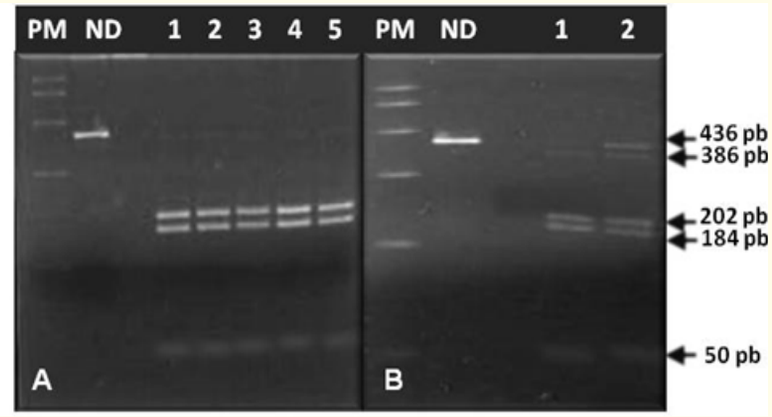

Figure 4: *RFLP-analysis with AvaII on DNA fragment from exon 4 of UCP3 gene. A, Restriction pattern in homozygotic patients with normal C/C allele. B, Restriction pattern in heterozygotic patients with one normal and one mutant C/T allele. Note that 436 bp fragment corresponds to non-digested portions by AvaII.*

Both patients exhibited multiple metabolic alterations, with diagnosed metabolic syndrome. Patient 1 was a 44-year-old woman with a BMI of 36 kg/m^2, hypercholesterolemia (269 mg/dL), hypertriglyceridemia and arterial hypertension (PAS: 175 mmHg, PAD: 110 mmHg). Patient 2 was a 42-year-old man with a BMI of 31.97 kg/m^2, hypertriglyceridemia (188 mg/dL), reduced HDL-c (38 mg/dL), hyperglycemia (128 mg/dL) and hypertension.

Previously, in a 16-year-old with morbid obesity (BMI: 51.8 kg/m^2) and type II diabetes, Argyropoulos., *et al.* [70] found compound heterozygosity for the rs104894319 mutation in exon 4. Pedigree analysis and DNA sequence determination of family members showed that this mutation was transmitted to the compound heterozygous proband from the grandmother, through the mother, in typical Mendelian fashion (OMIM Database; 602044 Gene Description).

Later, mutagenesis assays in yeast showed that mutated versions of UCP3 exhibited reduced uncoupling activity, which highly impacts on membrane potential and might be implicated in predisposition to metabolic diseases [22]. This suggests an important role of UCP3 in metabolic disorders in obese individuals, as we reported; however, it is important to note that the primary role of UCP3 is not uncoupling, which open possibility for other non-described functions in human.

Conclusions

Obesity is a world health problem characterized by an abnormal and excessive accumulation of fat that may cause serious physiologic and metabolic issues. The main cause of obesity is the hypercaloric nutrition and lack of physical activity. OMS recommendations at the individual level encompass limit energy intake, increase consumption of dietary fiber, and engage in regular physical activity. In fact, changing lifestyle by practicing exercise or including some food ingredients during caloric restriction (green tea, capsicum, ginger, etc.) may stimulate the process of browning in WAT in obese population. Nowadays, it is not known if these beige/brite adipocytes are generated through transdifferentiation of white adipocytes or by *de novo* differentiation and precursors maturation [7]. BAT is the effector organ of non-shivering thermogenesis (both cold and diet-induced), so it may promote negative energy balance by utilizing large quantities of glucose and lipids from the circulation [15]. This process of heat generation can be either UCP-dependent or UCP-independent (recently known as creatine-driven thermogenesis). While UCP-dependent thermogenesis involves flipping of fatty acids to transport H+ to mitochondrial matrix (this uncouples electron transport chain and transform chemical energy into heat), some UCP-negative beige/brite adipocytes rely only on CK system to dissipate energy and stimulate mitochondrial respiration (Figure 5). Even though diet can be improved with ideal dosage of food ingredients that can activate BAT, the net effect on weight loss is still debatable, but is clear that development of more strategies for obesity treatment is urgent [55]. Finally, RFLP-analysis with AvaII allowed detection of a heterozygotic C/T mutation in exon 4 of UCP3 gene in two obese patients from Maracaibo, Venezuela. This is the first report of polymorphism rs104894319 characterization in this population, which coincides with clinical significance (pathogenic) found around the world.

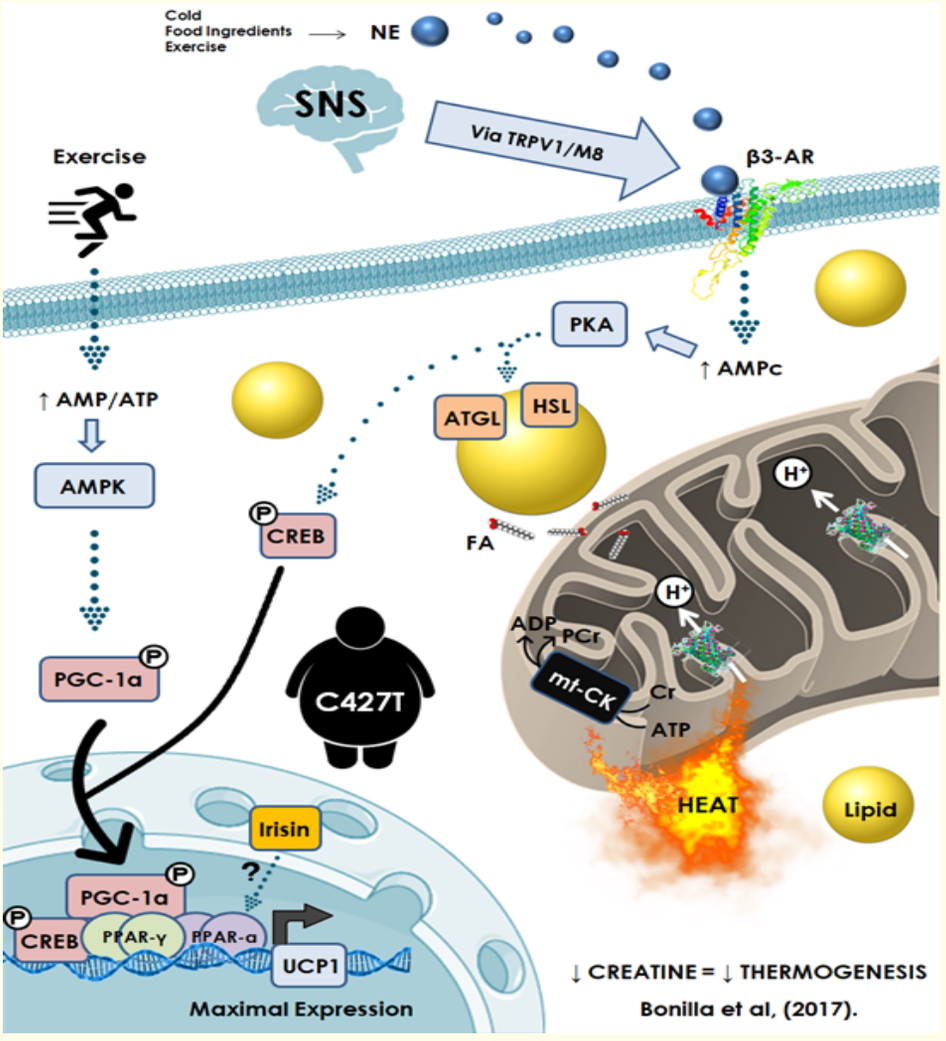

Figure 5: *Esquematic representation of thermogenesis and obesity. Main signaling pathways of factors that affect thermogenesis are represented. Maximal expression of UCP1 is driven by transcription factors and mediator proteins that bind to promoter region. For further mechanistic explanations see previous sections. Illustrated proteins represent β3-AR (UniProtKB ID: P13945) and UCP1 (UniProtKB ID: P25874). Abbreviations: Adipose triglyceride lipase (ATGL), Hormone-sensitive lipase (HSL), cAMP response element-binding (CREB), peroxisome proliferator-activated receptor gamma (PPAR-γ) and alpha (PPAR-α), AMP-activated protein kinase (AMPK), protein kinase A (PKA), norepinephrine (NE).*

Author Contributions

D.B. and E.M. conceived the project. E.M. performed genetic analysis experiments. D.B., E.M., A.P., L.C., and J.P. performed narrative review. D.B., E.M., A.P., L.C., and J.P. wrote the manuscript. M.K., S.V., F.M., J.P., D.K. supervised. D.B., A.P., L.Q., A.B., J.P., M.K., and J.L. discussed the results and commented on the manuscript.

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