AMPK/MITOCHONDRIA IN METABOLIC DISEASES

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Abstract

The obtaining of nutrients is the most important task in our lives. Energy is central to life's evolutions; this was one of the aspect that induced the selection of the more adaptable and more energetically profitable species. Nowadays things have changed in our modern society. A high proportion of people has access to plenty amount of food and the obesity appear as one of the pathological characteristics of our society. Energy is obtained essentially in the mitochondria with the transfer of protons across the inner membrane that produce ATP. The exactly regulation of the synthesis and degradation of ATP (ATP ↔ ADP + phosphate) is essential to all form of life. This task is performed by the 5' adenosine monophosphate-activated protein kinase (AMPK). mtDNA is highly exposed to oxidative damage and could play a central role in human health and disease. This high potential rate of abnormalities is controlled by one of the most complex mechanism: the autophagy. AMPK appears to be the key cellular energy sensor involved in multiple cellular mechanisms and is essential to have a good metabolic homeostasis to face all the aggression and start the inflammatory reaction. Therefore its disturbances have been related with multiple diseases. Recent findings support the role of AMPK in inflammation and immunity such as Metabolic Syndrome, Obesity and Diabetes. All these Metabolic Disorders are considered pandemics and they need an adequate control and prevention. One important way to achieve it is deepen in the pathogenic mechanisms. Mitochondria and AMPK are the key elements through which it happen, their knowledge and research allow us to a better management. The discovery and use of drugs that can modulate them is imperative to improve our way of manage the metabolic disorders.

Key words

AMPK, mitochondria, mtDNA, Metabolic Diseases, Metabolic Syndrome, Obesity, Diabetes, inflammation, inflammation treatment

INTRODUCTION

All forms of life need energy to survive. Energy is central to life's evolutions; we can only understand the properties of life if we bring energy to equation. The way the energy is produced give advantage to one organism against other one. During the evolution of all the form of lives in the earth, this was one of the aspect that induced the selection of the more adaptable and more energetically profitable species. The Homo Sapiens as an animal species has suffered famine period like other animal species, but we could survive. The obtaining of nutrients is the most important task in our lives. This was essential in the past because large periods of shortage nutrient were very common. But nowadays things have changed in our modern society. A high proportion of people has access to plenty amount of food. The way to deal with this time of plenty is consume the calories with exercise or accumulate the nutrients in adipose tissue. In a sedentary attitude of the population, the obesity appear as one of the pathological

characteristics of our society. Our entire metabolism, controlled by genes, need to be adapted to this abundance of nutrient but usually it cannot achieved. The breaks of the homeostasis and equilibrium in the metabolism involves most of the times to the development of metabolic disorder ([1]).

Essentially all living cells power themselves through the flow of protons (positively charge hydrogen atoms), in what amounts to a kind of electricity with protons in place of electrons. The energy we gain from burning food in respiration is used to pump protons across a membrane, forming a reservoir on one side of the membrane. The flow of protons back from this reservoir can be used to power work in the same way as a turbine in a hydroelectric dam. This transfer of protons across a membrane is known as chemiosmotic hypothesis ([2]). This use of proton gradients is universal across life on earth, proton power is as much an integral part of all life as the universal genetic code. This mechanism produce energy, but the requirements of energy to live is extremely high. The energy currency used by all living cells is a molecule called ATP (adenosine triphosphate). A single cell consumes around 10 million molecules of ATP every second. If we calculate 40 trillion cells contained in the human body we used about 60 to 100 kg per day, roughly our own body weight. ATP is usually split into two unequal pieces, ADP (adenosine diphosphate) and inorganic phosphate (PO_4^{3-}). The energy of respiration (the energy released from the reaction of food with oxygen) is used to make ATP from ADP and P_i. This energy comes from just one particular type of chemical reaction known as a redox reaction, in which electrons are transferred from one molecule to another. This takes place in the inner membrane of the mitochondria in the electron transport chain that comprise five protein-membrane-bound complexes. The electrons pass through this respiratory complexes, protons are ferried across the membrane, the enzyme ATP synthase is activated and ATP has been produced ([3]).

An appreciation of the rising global burden of chronic, non-communicable diseases and its influence in mortality and global health cost is a main concern in our society. It has been calculated that 35 million people died in 2005 from heart disease, stroke, cancer and other chronic diseases ([4]). Physicians and health managers have applied effective measures, including behavioral interventions and pharmaceutical treatment, in the prevention and management of chronic diseases, but these are neither widely used nor equitably distributed. Most of them has related with modern life style, mainly with sedentary way of living and obesity. Therefore, the most effective treatment is the prevention changing these parameters. But the results are not adequate, for instance overweight and obesity continues to grow its prevalence, even in children and adolescent ([5]). The only way to improve our knowledge of the mechanism of metabolic diseases is to deepen in the pathogenic mechanism involved in the energy production and its control. Energy production inside a cell is a very complex and accurate process that needs the proper function of the electron transport chain placed in the internal mitochondrial membrane. It is essential to attend all the environment requirements as soon as possible, with the exact amount that do not permit to waste energy.

MITOCHONDRIA

Mitochondria are ubiquitous in eukaryotes and are essential for survival. Their primary function is to support aerobic respiration and to provide energy substrates (such as ATP) for intracellular metabolic pathways. Mitochondria have also been shown to play an important role in iron and calcium homeostasis, amino acid, fatty acid and steroid hormone metabolism, and in cell

signaling, particularly in signaling for apoptotic cell death. Mitochondria host several metabolic pathways, including the Krebs cycle, β-oxidation, and lipid and cholesterol synthesis ([6]). Mitochondria are intracellular double membrane-bound structures. Although traditionally considered as small isolated organelles within the cell, it is more likely that mitochondria form a complex branching network. They derived from prokaryotic cells (bacteria) that were assimilated by another cell (archaea) through a process called endosymbiosis. Although these organelles usually retain some DNA, most of their original genome has moved to the host nucleus through endosymbiotic gene transfer ([7]). Human mtDNA is a circular double stranded molecule about 16·6 Kb long. It is much smaller than most nuclear genes. MtDNA codes for 22 transfer and 2 ribosomal RNAs, and for 13 proteins. Human mtDNA is extremely compact and contains virtually non-intronic (non-coding) regions. By encoding proteins of the respiratory chain, mtDNA allows individual mitochondria to respond, by gene expression, to changes in membrane potential. Although human mtDNA encodes the basic machinery for protein synthesis, it remains entirely dependent upon the nucleus for the provision of enzymes for replication, repair, transcription and translation. This dependency lies at the heart of several newly recognized human diseases that are characterized by secondary abnormalities of mtDNA.

There are good reasons to believe that genes affecting the mitochondria could play a central role in human health and disease. Most of the genes that have remained in the mitochondrion have been linked to a series of devastating diseases, indicating the importance of fully functional mitochondria to human health. Genes residing in the mitochondria pose a particular problem, in part because they are unusually prone to damage. Unlike nuclear genes, which are wrapped in protective proteins and stored safely away in the nucleus, mitochondrial genes are vulnerable to attack from highly reactive molecules called free radicals; these are generated during energy production. In mammals, the mutation rate of mitochondrial genes is 10 to 20 times higher than that of the nuclear genes. The idea that mutations in mitochondrial DNA could cause metabolic diseases, or even ageing, is widely accepted ([8]). These alterations range from changes to single DNA bases to deletions of large sections of the genome. Mitochondria that lose their genome (hydrogenosomes and mitosomes) lose the ability to synthesize ATP by chemiosmotic coupling ([9]). Respiration rates do correlate with the amount of mtDNA in the cell ([10]), and mutations that deplete mtDNA usually cause mitochondrial diseases ([6]). Oxidative phosphorylation is under tight control by the amount of mtDNA in the cell, and the full complement of mtDNA is necessary to maintain a normal energy production level ([11]). Mitochondria, along with their tiny genomes, are normally inherited only from the mother — they are present in huge numbers in the egg, whereas the handful in sperm is marked up for destruction in the fertilized egg. This gives at least some mitochondrial diseases a maternal-inheritance pattern. Even so, trying to spot mitochondrial diseases by looking to the mother can be grossly misleading, and has own played the importance of these organelles in disease. More than 80% of diseases known to be linked to faulty mitochondria don’t follow a maternal-inheritance pattern at all. Why not? At least partly because some mitochondrial diseases may be caused by mutations in the nuclear genes encoding mitochondrial proteins. So far, mutations in more than 30 nuclear genes have been shown to give rise to mitochondrial disease ([12]).

This high potential rate of abnormalities is controlled by one of the most complex mechanism: the autophagy. Autophagy is the most efficient mitochondrial turnover mechanism, providing for the complete removal of irreversibly damaged mitochondria (mitophagy). It is believed that mitochondria are normally replaced every 2–4 weeks in rat brain, heart, liver, and kidneys, although recent studies have shown that the turnover rate might be considerably higher. Mitophagy is particularly important for long-lived postmitotic cells, whose mitochondria have

pronounced oxidative damage ([13]), and also prevents an excessive accumulation of mitochondria. It should be mentioned that the changes in mitochondriogenesis are tissue specific ([14]), being more dramatic in the central nervous system, which is consistent with the generally higher susceptibility of neurons and other postmitotic cells to the aging process ([15]). Mitochondria are dynamic structures that show different morphologies: small spheres, short rods or long tubules that depend on cell type and also cell status. In most eukaryotic cells, mitochondria move along cytoskeletal tracks and their overall morphology depend on the balance between fusion and fission events. For example, an extent of fusion activities leads to interconnected mitochondrial networks, and on the contrary, an extent of fission events generates numerous different small spherical organelles ([16]). Mitochondrial membrane fusion/fission processes are clearly involved in mitochondria dynamics and that these events are undoubtedly critical for several cell functions and the balance cell life/cell death. Perturbations of mitochondrial dynamics can have tremendous consequences on cell metabolism and therefore on cell life/cell death ([17]). The frequent fusion/fission events undergone by the mitochondrial network appears clearly linked to the bioenergetic state of mitochondria ([18]) and involve a protein machineries and a group of lipids ([19]). Disturbed mitochondrial dynamics is involved in the most important chronic disease such as neurodegenerative disorders ([20]), aging process ([21]) and cardiovascular disease ([22]).

AMPK

The electron transport chain placed in the inner mitochondrial membrane has it main function producing ATP that is the battery of our cells. Therefore the exactly regulation of the synthesis and degradation of ATP (ATP ↔ ADP + phosphate) is essential to all form of life. This reaction is maintained by catabolism many orders of magnitude away from equilibrium, yielding a high ratio of ATP to ADP that is used to drive energy-requiring processes. ATP generation needs to remain in balance with ATP consumption, and regulatory proteins that sense ATP and ADP levels would be a logical way to achieve this. This task is performed by the 5' adenosine monophosphate-activated protein kinase (AMPK). It is a highly conserved sensor of cellular energy status, expressed in essentially all eukaryotic cells. AMPK is switched on by metabolic stresses and xenobiotic compounds that cause a cellular energy imbalance, which is detected as increases in the ratios of ADP-ATP and AMP-ATP ([23]). Because the energy status of the cell is a crucial factor in all aspects of cell function, it is not surprising that AMPK has many downstream targets whose phosphorylation mediates dramatic changes in cell metabolism, cell growth, and other functions. In general, AMPK switches on catabolic processes that provide alternative pathways to generate ATP, while switching off anabolic pathways and other processes consuming ATP, thus acting to restore cellular energy homeostasis. The kinase evolved in single-celled eukaryotes and is still involved in multicellular organisms in regulating energy balance in a cell-autonomous manner ([24]).

AMPK and its orthologues seem to exist universally as heterotrimeric complexes comprising a catalytic α-subunit and regulatory β- and γ-subunits. Each of these three subunits takes on a specific role in both the stability and activity of AMPK. Specifically, the γ subunit includes four particular Cystathionine beta synthase (CBS) domains giving AMPK its ability to sensitively detect shifts in the AMP-ATP ratio ([25]). In mammalian cells, AMPK is activated by various types of metabolic stresses (starvation for glucose or oxygen or addition of a metabolic poison, muscle contraction), drugs (metformin, phenformin, resveratrol, epigallocatechin, capsaicin, curcumin) and xenobiotics through the mechanisms described above, which involve increases in cellular

AMP, ADP or Ca^{2+}. These can now be regarded as the classical or 'canonical' AMPK activation mechanisms. However, recent work suggests that other stimuli activate AMPK via mechanisms that do not involve changes in the levels of AMP, ADP and Ca^{2+}, which can therefore be termed 'non-canonical' mechanisms such as those triggered by ROS and DNA damaging agents. AMPK acts as a metabolic master switch regulating several catabolic intracellular systems including the cellular uptake of glucose and fatty acids, the β-oxidation of fatty acids and the biogenesis of glucose transporter 4 (GLUT4) and mitochondrial biogenesis and mitophagy. Also conserves ATP by switching off almost all anabolic pathways, including the biosynthesis of lipids, carbohydrates, proteins and ribosomal RNA. It achieves this in part by phosphorylating and/or regulating enzymes or regulatory proteins that are directly involved in these pathways ([26]). AMPK appears to have evolved early in the evolution of unicellular eukaryotes as a signaling pathway that orchestrated responses to glucose starvation in a cell-autonomous manner. But also it is intriguing that hormones that modulate energy balance at the whole-body level, which clearly arose later during the evolution of multicellular organisms, appear to have adapted to interact with the AMPK system, especially in the hypothalamus. Adiponectin, leptin, ghrelin, insulin and triiodothyronine can influence the AMPK production in the hypothalamus ([27]). In mammals it also regulates metabolism and helps to maintain energy balance at the whole-body level. It does this by mediating effects of hormones and other agents acting on neurons in different hypothalamic regions, which regulate intake of food (and hence energy) and energy expenditure. AMPK also regulates diurnal rhythms of feeding and metabolism. By switching off biosynthetic pathways required for cell growth, AMPK activation exerts a cytostatic effect, helping to explain why its upstream activator, LKB1, is a tumor suppressor. Commensurate with its role in preserving cellular energy homeostasis, AMPK also downregulates ATP-requiring processes outside metabolism, including progress through the cell cycle (another potential tumor suppressor effect) and firing of action potentials in neurons ([28]). Some works demonstrate that the AMPK signaling pathway also plays a role in bone physiology. Activation of AMPK promotes bone formation in vitro and the deletion of α or β subunit of AMPK decreases bone mass in mice ([29]).

AMPK appears to be the key cellular energy sensor involved in multiple cellular mechanisms and is essential to have a good metabolic homeostasis to face all the aggression and start the inflammatory reaction. Therefore its disturbances have been related with multiple diseases. Age-related decrease of mitochondrial biogenesis is related, at least in part, to diminished AMPK activity ([30]). AMPK appears to be the key cellular energy sensor, linking decreased mitochondriogenesis to several aging associated changes, including insulin resistance and deficient lipid metabolism ([31]). AMPK activity and autophagy of monocytes were significantly decreased in Acute Coronary Syndrome patients due to a decrease in plaque vulnerability and subsequent plaque rupture ([32]). Recent findings support the role of AMPK in inflammation and immunity, providing the enticing prospect of new therapeutic approaches for inflammatory diseases. All of the AMPK activators have been reported to inhibit inflammatory responses in various model systems and AMPK-activating drugs do have anti-inflammatory actions in animal models ([33]). Tumor cells appear to be under selection pressure to downregulate AMPK, thus limiting its restraining influence on cell growth and proliferation. Paradoxically, however, a complete loss of AMPK function, which appears to be rare in human cancers, may be deleterious to survival of tumor cells. AMPK can therefore be either a friend or a foe in cancer, depending on the context ([34]).

METABOLIC SYNDROME AND OBESITY.

The metabolic syndrome (MetS) is a major and escalating public health and clinical challenge worldwide in the wake of urbanization, surplus energy intake, increasing obesity, and sedentary life habits. MetS confers a 5-fold increase in the risk of type 2 diabetes mellitus (T2DM) and 2-fold the risk of developing cardiovascular disease (CVD) over the next 5 to 10 years ([35]). Further, patients with the MetS are at 2- to 4-fold increased risk of stroke, a 3- to 4-fold increased risk of myocardial infarction (MI), and 2-fold the risk of dying from such an event compared with those without the syndrome ([36]) regardless of a previous history of cardiovascular events ([37]).

MetS is a state of chronic low-grade inflammation as a consequence of complex interplay between genetic and environmental factors. Insulin resistance, visceral adiposity, atherogenic dyslipidemia, endothelial dysfunction, genetic susceptibility, elevated blood pressure, hypercoagulable state, and chronic stress are the several factors that constitute the syndrome [Table 1].

Risk factor	Defining level
Abdominal obesity, given as waist circumference	Men >102 cm Women >88 cm
Triglycerides	≥150 mg/dL
HDL cholesterol	Men <40 mg/dL Women <50 mg/dL
Blood pressure	≥130/≥85 mm Hg
Fasting glucose	≥110 mg/dL

Table 1. Clinical identification of the metabolic syndrome.

There have been several definitions of MetS, but the most commonly used criteria for definition at present are from the World Health Organization (WHO) [Table 2].

Clinical measures	WHO (1998)
Insulin resistance	IGT, IFG, T2DM, or lowered insulin Sensitivity* **Plus any 2 of the following**
Body weight	Men: waist-to-hip ratio >90 cm; Women: waist-to-hip ratio >85 cm and/or BMI > 30 kg/m^2
Lipids	TGs ≥150mg/dL and/or HDL-C Men: <35 mg/dL Women: <39 mg/dL
Blood pressure	≥140/90mmHg
Glucose	IGT, IFG, or T2DM
Other	Microalbuminuria: Urinary excretion rate of >20mg/min or albumin: creatinine ratio of >30mg/g

Table 2. WHO clinical criteria for metabolic syndrome. *Insulin sensitivity measured under hyperinsulinemic euglycemic conditions, glucose uptake below lowest quartile for background population under investigation. BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; TGs: triglycerides; T2DM: type 2 diabetes mellitus; WC: waist circumference.

Hypercaloric diets initially result in obesity due to the storage of extra energy in the adipose tissue. However, the continuous caloric overload eventually results in the aberrant accumulation of lipids in non-adipose tissues ([38]). The direct pathological consequence of chronic hypercaloric diets is actually, a multi-systemic deterioration known as metabolic syndrome ([39]). Of note, the comorbidities associated with metabolic syndrome also overlap with some of the most important aging-associated diseases, namely diabetes, cardiovascular and cerebrovascular diseases, and cancer ([40]).

Inflammation in obesity and MetS.

Considering the "obesity epidemic" as mainly responsible for the rising prevalence of MetS due to the strong connection between this syndrome and, especially, abdominal obesity, it is vital to bear in mind that obesity is not only a risk factor, but also a disease in itself. Obesity contributes to hypertension, high serum cholesterol, low HDL cholesterol, and hyperglycemia, and it otherwise associates with higher CDV risk. Excess adipose tissue releases several products that apparently exacerbate these risk factors. They include free fatty acids (FFA), cytokines: interleukin-6 and Tumor Necrosis Factor-α (IL-6 and TNF-α), plasminogen activator inhibitor 1 (PAI-1), and adiponectin. A high plasma FFA level overloads muscle and liver with lipids, which is an event commonly found in abdominal obesity ([41]), enhancing insulin resistance ([42]). Evidence suggests that TNF-α induces adipocytes apoptosis ([43]) and promotes insulin resistance by the inhibition of the insulin receptor substrate 1 signalling pathway ([44]). Plasma TNF-α is positively associated with the body weight and triglycerides (TGs), while, a negative association exists between plasma TNF-α levels and High-density lipoprotein–cholesterol (HDL-C) ([43]). In addition, IL-6 has been shown to be positively associated with body mass index (BMI), fasting insulin, and the development of T2DM ([45]) and negatively associated with HDL-C ([46]).

Plasma PAI-1 levels are increased in abdominally obese subjects ([47]) and, an elevated PAI-1 contributes to a prothrombotic state, whereas low adiponectin levels that accompany obesity correlate with worsening of metabolic risk factors, since it regulates the lipid and glucose metabolism, increases insulin sensitivity, regulates food intake and body weight, and protects against a chronic inflammation ([48]). Adiponectin is seen to be "protective," not only in its inverse relationship with the features of MetS ([49]) but also through its antagonism of TNF-α action ([50]). Considering all these aspects, new therapies for metabolic diseases have been investigated.

Mitochondrial DNA alterations in obesity and MetS.

Obesity results from an energy surplus, grater energy intake than expenditure. Excess energy is stored as fat in white adipose tissue (WAT), dysfunction of which lies at the core of obesity and associated metabolic disorders. By contrast, brown adipose tissue (BAT) burns fat and dissipates chemical energy as heat. The development and activation of "brown-like" adipocytes, also known as beige cells, result in WAT browning and thermogenesis (heat production). Both "white" and "brown" adipose cells (colour is due to the quantity of mitochondria), play a crucial role in thermogenesis. "Brown-like" adipocytes, which have a rich sympathetic innervation, are present in rodents and infants, whereas in adults are usually interspersed with WAT. Due to the numerous mitochondria existing in BAT, these cells are an important heat source, generating more heat and less ATP than "white-like" adipocytes. This event occurs because of the presence of mitochondrial uncoupling proteins (UCP), as it has already been demonstrated in murine

models. Three isoforms have been identified, UCP1, 2 and 3, which are differently distributed in BAT.

These proteins "uncouple" oxidative phosphorylation, and those way mitochondria continues oxidizing substrates while producing minor quantities of ATP levels, which favours the net loss of energy in the form of heat. As one would expect, cold exposure or leptin administration increase UCP activity and quantity in BAT.

Noradrenalin is an hormone which acts on β_3-adrenergic receptors in BAT and boosts peroxisome proliferator-activated receptor gamma (PPAR-ϒ) activity, which actives, at the same time, the gene that encodes UCP-1. Curiously, β_3-adrenergic receptors expression is lower in mice genetically obese.

Until recently, studies on mtDNA biology in adipose tissue have been limited to the analysis of mitochondrial biogenesis in BAT. However, recent studies have highlighted the importance of mitochondrial biogenesis in WAT and the potential for mitochondrial alterations to disturb white adipocyte development and function. Studies by Corvera and collaborators ([51]) have shown that mitochondrial biogenesis is directly associated with white adipocyte differentiation; genetically obese mice (*ob/ob*) displayed impaired mitochondrial mass and function in white fat and thiazolidinediones, PPAR-γ activators that favour adipocyte differentiation, ameliorated these alterations ([52]). It has been also shown that white adipocyte differentiation is associated with increases in the relative abundance of mtDNA, and upregulation of components of the mtDNA replication and transcription machinery, such as TFAM (Mitochondrial Transcription Factor A) ([53]), and components of deoxynucleotide metabolism are required for mtDNA replication ([54]). Agents that promote white adipocytes differentiation in vitro, such as glitazones, also increase mtDNA levels in human adipocytes in vitro ([55]).

Studies of potential alterations in WAT mtDNA as they relate to obesity have focused on two aspects: changes in mtDNA levels that underlie obese phenotypes; and the occurrence of mutated, polymorphic, forms of mtDNA that are specifically associated with obesity. In experimental models of obesity, such as *ob/ob* or *db/db* mice, abnormally low levels of mtDNA have been reported ([56, 57]). As noted above for in vitro studies, treatment of obese mice with glitazones increases mtDNA levels in white fat ([57]). A study in which mice were treated with a diet enriched in polyunsaturated fatty acids identified mtDNA encoded transcripts and proteins among the most upregulated genes in WAT; this upregulation was associated with an enhancement of fatty acid oxidation in WAT ([58]).

In humans, the scenario appears to be more complex. It has been reported that mtDNA levels in adipose tissue are lowered in type 2 diabetic patients ([59]), and studies by Arner and collaborators ([60]) have confirmed that mtDNA levels are not associated with obesity per se, but rather with type 2 diabetes phenotypes. Moreover, mtDNA levels were found to be strongly related to lipogenesis in WAT, rather than to BMI.

The mechanism by which mtDNA copy number in white adipose tissue could affect lipogenesis rate remains to be established, but it stands in contrast to the expected relationship between mtDNA level variations and energy expenditure and fat oxidation. Moreover, in humans, as in rodents, pioglitazone treatment causes an increase in mtDNA levels in WAT of type 2 diabetes patients ([59]), but not in non-diabetic obese individuals ([61]). These findings highlight the potential role of mtDNA levels in WAT mass.

Homeostatic control of energy balance in obesity.

The arcuate nucleus (ARC) is a key hypothalamic nucleus that regulates appetite, eating behaviour and energy state ([62]). It receives afferent pathways from digestive tract and contains leptin; an adipokine involved in the regulation of satiety and energy intake ([44]), and other significant hormones receptors. A reduction in leptin levels activates orexigen neurons, leading to an increase in food intake, fat synthesis and storage (anabolism) and to a decrease in energy expenditure. In reverse, when increases in leptin levels take place, another group of neurons are activated, triggering an anorexigen effect and catabolism.

Energy balance depends directly on food intake, energy storage in adipose tissue and energy expenditure ([63]). For the majority of people, this process is closely connected by an homeostatic system which integrates different hormones, such as leptin ([64]), considered as an indicator of lipid reserves, since an augmentation in lipid deposits enhance leptin releasing in plasma by adipocytes. Ghrelin, which is released to the intestine during food intake and provide us the feeling of hunger, and cholecystokinin (CCK), secreted into the duodenum as a response to the process of food intake and digestion, providing satiety, as well.

Since homeostatic control in energy balance is extremely complex, it is not easy to precisely determine what does not work in obesity. In fact, when leptin was discovered ([65]), it was thought that an alteration in its kinetics would provide a simple explanation to this disease, but there is a notable variability in leptin sensibility among individuals and, some people seem to synthesize insufficient amounts of this hormone. However, plasmatic leptin usually reaches higher levels in obese than in normal weight people. What prevail in obese is a greater resistance to leptin and not an insufficient production of it ([66]). This resistance may obey to alterations in blood circulation leptin transport, in its transport to Central Nervous System (CNS) or in hypothalamic leptin receptors (like *db/db* mice).

In obesity, could be involved other alteration in mediators apart from leptin, for example TNF-α, cytokine that transmits information from adipose tissue to brain, which is increased in insulin resistant obese adipose tissue. Another common pathophysiological alteration in obesity is a decreasing insulin sensibility in skeletal muscle and adipose tissue.

Thus, events causing obesity depends on diet, exercise, social, economic and cultural factors, and genetic predisposition ([67]). Although other causes are related to alterations in leptin action or synthesis, thermogenesis decrease in adipocytes or a reduction in energy metabolic consumption. A key player at regulating energy balance is AMP-activated protein kinase (AMPK), at both cellular and whole-body levels, placing it at the centre stage in studies of obesity, diabetes and the metabolic syndrome ([68]).

AMPK: A master metabolic regulator.

Lipid metabolism controlled by AMPK.

AMPK plays a key role in lipid metabolism, being involved in Acetyl-Coa carboxylase (ACC) phophorylation and inactivation. ACC catalyse the transformation from Acetil-CoA into malonyl-CoA, which is the very first reaction in fatty acids biosynthesis in liver and adipose tissue. By inactivating ACC, AMPK is then, responsible for the inhibition of fatty acid synthesis in lipogenic tissues. AMPK also has long-term effects on transcriptional genes involved in lipogenesis, ACC

and fatty acid synthesis, interfering with the expression and the activity of transcriptional factors, such as sterol regulatory element binding protein 1c (SREBP1c) ([68, 69]) and carbohydrate response element binding protein (ChREBP) ([70]).

In addition, in both liver and striated muscle (skeletal and myocardial), malonyl-CoA produced by ACC plays a regulatory role. It blocks, in fact, fatty acids transport from cytosol to mitochondria by inhibiting carnitine-palmitil-transferase (CPT-1). AMPK activation in those tissues triggers a decrease in cytosolic concentration of malonyl-CoA, enabling that way the fatty acids penetration into the mitochondria and its consequent oxidation. By this mechanism, recent data reveal that the adipokines, leptin and adiponectin, stimulate fatty acid oxidation in both liver and skeletal muscle secondarily to the AMPK activation in these tissues ([71, 72]). So, the effect of lipid depletion on these tissues improves metabolic parameters in different insulin resistant rodent models. In fact, the accumulation of triglycerides in liver and skeletal muscle is linked to the pathophysiology of insulin resistance in human and animals, as well as lipid depletion in these tissues ameliorates insulin sensitivity (lipotoxicity concept). So here lies the metabolic interest of the AMPK activation on lipotoxicity reduction.

AMPK may be also involved in the hypothalamus satiety central control, this brain region, plays an essential role in energy homeostasis by controlling food intake and energy expenditure. Hypothalamic AMPK activity varies according to the nutritional status; AMPK is activated in fasting and inhibited in satiety period ([73]). At the same time, it is also interesting the existing relationship between the activity of hypothalamic AMPK and food intake in response to different hormones or metabolites known to be modulated by nutritional state. For example, ghrelin and endocannabinoids activate AMPK and induce food intake whilst insulin, glucose or leptin acts in the opposite manner ([74, 75]). Several studies have demonstrated that the variations in hypothalamic energetic state are able to directly modulate AMPK activity, what suggests that hypothalamic AMPK may be a therapeutic target in the numerous factors that affect eating behaviour. But, it is important to bear in mind that AMPK is differently regulated in the hypothalamus and in peripheral tissues. For example, leptin activates AMPK in skeletal muscle and inhibits it in the hypothalamus, what suggests that regulation mechanisms are not the same. Therefore, drawbacks in peripheral tissues must be taken into account when considering AMPK as a pharmacological inhibitor for the treatment of metabolic disorders.

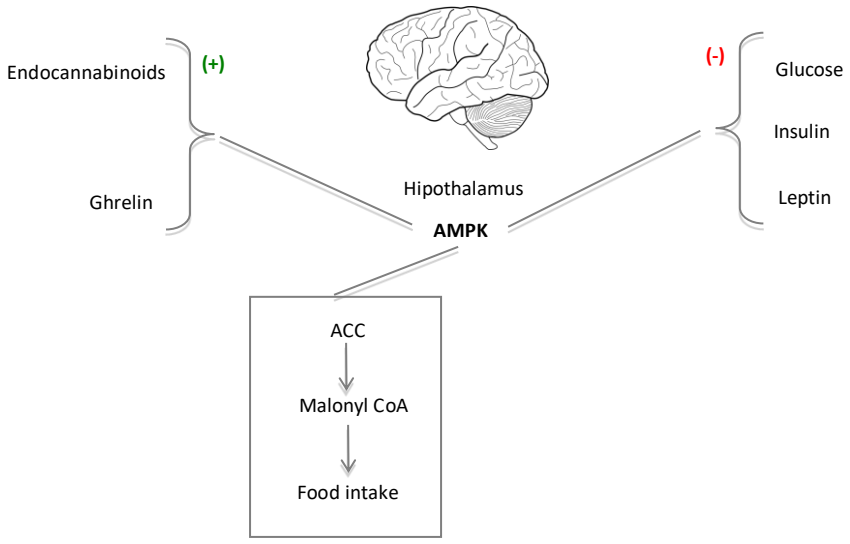

Figure 1. Hypothalamic AMPK regulation by hormonal and nutritional signals.

The obesity epidemic and its complications continue rising as a global health challenge, despite the increasing public awareness and the use of lifestyle and medical interventions, the main treatment for obesity consists on lifestyle modifications, based on a suitable diet and physical exercise. Since, the currently antiobesity drugs, such as Orlistat (lipase inhibitor) suffer from numerous disadvantages: gastrointestinal symptoms, elevated blood pressure, abdominal pain, dyspepsia, diarrhoea, flatulence, etc ([76]), the biomedical community is urged to develop new treatments for the metabolic diseases. AMP activated protein kinase (AMPK), as a master regulator of energy homeostasis, is a potential target for therapeutic agents that may meet this challenge. For these reasons, novel drugs activating AMPK may have potential for the treatment of obesity, T2DM and MetS.

Some compounds have already shown to have an effect on AMPK. 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) is a known activator of AMPK that induces allosteric changes in AMPK conformation and thereby leading to kinase activation. In 3T3L1 cells, AICAR inhibits adipocyte differentiation by downregulating key transcriptional factors such as Sterol regulatory element-binding protein 1(SREBP1), CCAAT-enhancerbinding proteins(C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ) which strictly regulates adipocyte differentiation. AICAR proved remarkably effective in maintaining body weight and epididymal fat content, improving insulin sensitivity and glucose tolerance in diet induced obese mice models. However in rats, the chronic administration of AICAR resulted in significant changes, in skeletal muscle that included an increase in GLUT4 and glycogen stores, and increased activity of hexokinase and mitochondrial oxidative enzymes ([77, 78]).

The potential to reduce hypertriglyceridemia and elevated storage of triglycerides by inhibiting triglyceride and fatty acid synthesis, and stimulating fatty acid oxidation and also the ability to lower blood glucose by activation of AMPK suggests that modulators of AMPK kinase activity might prove effective remedy for treating obesity and related metabolic disorders. Reports have also shown that certain strains of mouse that are resistant to diet induced obesity (mice over expressing uncoupling protein-1 in white adipocytes, stearoyl-CoA desaturase-1 knockouts and mice over expressing uncoupling protein-3 in skeletal muscle) exhibit increased basal level of AMPK activity ([79]). These findings have led to an intense interest in designing AMPK activators as potential therapies for type II diabetes and obesity.

Thus, AMPK plays a key role in regulating wide range of activities in lipid and glucose metabolism, it appears as a promising strategy for the treatment of obesity and related metabolic disorders.
Keeping in view the epidemic of obesity, the role of AMPK pharmacotherapy seems to be the most obvious tool to combat the disease and attempts to develop novel therapies via AMPK mediated mechanisms are worthy of pursuit.

DIABETES.

The incidence of diabetes is increasing worldwide approaching epidemic proportions. According to National Diabetes Statistics, the number of people diagnosed and undiagnosed diabetes in the United States reached 29,1 million people, which is 9,3% of the general population in 2012.

Diabetes is considered a metabolic disease that is characterized by high blood sugar levels over a prolonged period (hyperglycemia). We can differentiate three main types: type 1 which results from the pancreas failure to produce enough insulin, type 2 when cells fail to respond to insulin properly (insulin resistance), and gestational diabetes occurs when pregnant women without a previous history of diabetes develop a high blood sugar level.

Type 2 diabetes (T2DM) is the most common form of this pathology, and is directly related with obesity and metabolic syndrome. When obesity is established and body weight increases with age, a parallel state of chronic inflammation, characterised by an elevation of proinflammatory cytokines, can induce changes and switch the metabolic homeostatic set points, leading to T2DM ([80]). In T2DM, the major insulin-resistant organs include liver, muscle, and adipose tissue. In a state of insulin resistance, glucose uptake and utilization are dramatically decreased, and skeletal muscle becomes metabolically inflexible, unable to switch between glucose and fatty acid use. The main complications of diabetes are the microvascular and macrovascular complications such as retinopathy, nephropathy and cardiovascular diseases, which are mediated by inflammatory processes.

Inflammation in diabetes.

Nowadays, it is well known that inflammation plays a key role in the natural history of diabetes ([81]). Oxidative stress and inflammation are key players in insulin resistance progression and the establishment of T2DM. We know that inflammation is able to increase insulin resistance. The major cell involved in inflammation and insulin resistance is the adipocyte. Insulin regulates glucose uptake and triglyceride storage by adipocytes. The various adipocytokines, especially leptin, adiponectin, omentin, resistin, and visfatin contribute to beta cell dysfunction in the pancreas. Adipose tissue also secretes dipeptidyl peptidase-4 (DDP-4) which enhances the degradation of glucagon like peptide-1 (GLP-1) and has an insulinotropic effect on beta cells (insulin secreting cells) ([82]). Cytokines including tumour necrosis factor-alpha (TNF-α), interleukin beta (IL-1β), and interferon-gamma (IFN-ɣ) disrupt the regulation of intracellular calcium in the beta cells and hence insulin release. TNF-α acts on beta cells leading to their accelerated death ([83]).

Oxidative stress is another pathway that leads to inflammation through activation of cytokines ([84]). Pancreatic islets have low antioxidant defence and are hence vulnerable to oxidative stress. Marselli found differential regulation of oxidative stress genes in cells of T2DM subject compared with healthy controls ([85]).

An emerging body of evidence also suggests that insulin suppresses the inflammatory process not only through preventing hyperglycemia, but also by modulating key inflammatory molecules ([86]). With all this in mind, the search for anti-inflammatory therapies for diabetes was started.

Mitochondrion and Insulin Resistance.

The concept of insulin resistance was introduced like the previous step of T2DM. Although, it became evident that insulin resistance was not confined to T2DM, has been regarded as the centrepiece of the pathophysiologic mechanism of T2DM.

The relation between mitochondrial dysfunction and insulin resistance is known since 1960s. To demonstrate this relation was really important the assays of Jucker and his colleagues ([87]), who using a sophisticated nuclear magnetic resonance spectroscopy, they directly measured the mitochondrial function in the human liver and muscle. They showed that the insulin resistance in elderly people can be explained about 40% reduction in the mitochondrial oxidative phosphorylation capability compared to young people, and demonstrated that T2DM patients showed approximately 30% reduction in mitochondrial phosphorylation activity compared to the insulin sensitive control subjects ([88, 89]). A recent review, establish a direct relation between defective mitochondrial dysfunction, reduction on fatty acid oxidation and inhibition of glucose transport. This is the hallmark of insulin resistance and T2DM.The chronic production of excess ROS and inflammation result in mitochondrial dysfunction potentially inducing lipid accumulation and the endless vicious cycle of insulin resistance ([90]). Altered mitochondrial function is the major factor that leads to increased muscular lipid accumulation and decreased insulin sensitivity. Mitochondrial dysfunction could provide important implications for the diagnosis and treatment of T2DM and other related disease like obesity and metabolic syndrome ([91]).

Role of AMPK in Diabetes.

A number of physiological processes have been shown to stimulate AMPK, including conditions that lead to alterations of the intracellular AMP/ATP ratio (e.g., hypoxia, glucose deprivation) and calcium concentration, as well as the action of various hormones, cytokines, and adipokines. The activated form of the enzyme is responsible for metabolic changes via phosphorylation of various downstream substrates. The net effect is a change in local and whole-body energy utilization from an energy consuming state to an energy-producing state in order to restore energy balance.

We know AMPK plays a key role in the interconversion of glucose (the primary cellular energy substrate) and its storage forms, by affecting the transcription and translocation of the GLUT4 glucose transporter, glycogen synthesis, glycolysis and gluconeogenesis.

Beginning with mice clinical trials, Hardie was the first to relate a possible role of disturbed AMPK signalling in diabetes ([92]). After that, a growing body evidence has begun to validate this idea. For example, studies in AMPKα2 knockout mice observed hiperglycemia, glucose intolerance and increased hepatic glucose production ([93]). A recent review suggest that AMPK mediates glucose uptake and is complementary to insulin as well as possibly independent of this hormone, thereby implicating the kinase in diabetes pathophysiology ([26]).

AMPK is a key regulator of energy balance and plays many roles in human health and disease. Activation of AMPK by pharmacological agents holds a considerable potential to reverse the metabolic abnormalities associated with T2DM. So, AMPK could be a potential target for novel agents that may meet this global epidemic ([94]).

AMPK and inflammation: targets of treatment.

A growing body evidence is emerging to show that metabolic diseases are intimately related to chronic inflammation. The new pharmacological strategies are focus to reduce silent inflammation ([95]). The considered insulin sensitizers or glucose-lowering agents, appear to have greater anti-inflammatory activity than insulin-secreting agents ([96]).

Several glucose-lowering agents currently used as antidiabetic medications exert anti-inflammatory actions that may contribute to improved T2DM patients' outcomes. This effect may result from correction of hyperglycaemia, but may also be due to direct effects of the drug, independent of improvement of glucose control ([95]). This is demonstrated for metformin and thiazolidinediones (TZDs).

Metformin.

This is the first-choice drug for the management of T2DM. This biguanide acts as an AMP-activated protein kinase (AMPK) activator ([97]). Activation of AMPK has a number of potentially antiatherosclerotic effects, including reducing inflammatory cell adhesion to blood vessel endothelium, reducing lipid accumulation and proliferation of inflammatory cells caused by oxidized lipids, stimulation of gene expression responsible for cellular antioxidant defences and stimulation of enzymes responsible for nitric oxide formation ([98]).

Metformin can inhibit proinflammatory responses and cytokine-induced nuclear factor kappa B (NKkB) activation via AMPK activation in vascular endothelial cells ([99,100]), and also inhibit inflammatory responses via the AMPK-phosphatase and tensin homologue (PTEN) pathway in vascular smooth muscle cells ([101]). AMPK activity can also inhibit monocyte-to-macrophage differentiation ([102]), while other anti-inflammatory mechanisms have been proposed with lysosomes as a target of metformin ([103]).

Although, surveys with metformin in human showed less pronounced efficacy than TZDs in reducing various inflammatory markers ([104, 105]), it seems clear that metformin may confer benefits in chronic inflammatory diseases independent of its ability to normalize blood glucose. There is now growing interest in identifying and exploiting AMPK anti-inflammatory effects with the development of new compounds that are currently under investigation ([95]).

Thiazolidinediones.

TZDs or glitazones are a group of agonists of peroxisome proliferator-activated receptor gamma (PPARɣ). PPARs have been implicated as a molecular pathway in insulin resistance, T2DM and atherosclerosis ([106]). When rat muscle cells were isolated and incubated in culture medium containing TDZ for 15 min, significantly increased phosphorylation of AMPK ([107]), as well as the AMP/ATP ratio. It's suggested that TDZs can activate AMPK by a mechanism that is likely independent of PPARɣ-regulated gene transcription. However, the major effect of TDZs is likely to be on the release of adiponectin by adipocytes, leading to activation of AMPK in liver to reduce glucose production ([108]). Several studies have compared the anti-inflammatory effects of glitazones with other glucose-lowering agents and found glitazones to be superior ([109]).

Many others specific anti-inflammatory approaches have been investigated in recent years to treat T2DM and its associated vascular complications, but none has yet emerged for use in clinical practice ([110]).

CONCLUSIONS

Energy production and its control is a main task in every form of lives and is essential to have a proper homeostasis to adapt to the environment and to face every aggression. The Metabolic Disorders are considered pandemics and they need an adequate control and prevention. One important way to achieve it is deepen in the pathogenic mechanisms. Mitochondria and AMPK are the key elements through which it happen, their knowledge and research allow us to a better management. The discovery and use of drugs that can modulate them is imperative to improve our way of manage the metabolic disorders.

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