REVIEW ARTICLE

Molecular Regulators of Metabolism and Cardiometabolic Disease

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Abstract

BACKGROUND: The mechanisms that are responsible for energy management in cells in an organism require a complex network of transcription of factors and cofactors.

CONTENT: All living systems must maintain a tight equilibrium between energy intake, storage and expenditure for optimal performance. This tight equilibrium must be both robust and flexible to allow for adaptation to every situation such as exercise or rest and famine or feast. Organisms rely on finely tuned and complex signaling network to confront with all the possibilities. Metabolic imbalance can cause dysfunction and perturbation of these networks, which if uncorrected will induce disease such as obesity and diabetes mellitus.

SUMMARY: During the last decades the understanding of the transcriptional regulation of diverse metabolic pathways has contributed to the elucidation of mechanisms of metabolic control and to a better knowledge of the pathogenesis of metabolic diseases.

KEYWORDS: AMPK, SIRT1, PGC-1α, FGF21, mTORC1

Indones Biomed J 2012; 4 (3): 129-142

Abstrak

LATAR BELAKANG: Mekanisme yang bertanggung jawab untuk mengatur energi dalam sel di seluruh organisme membutuhkan jaringan yang kompleks dari faktor transkripsi dan kofaktor.

ISI: Semua sistem yang hidup harus menjaga keseimbangan yang ketat antara asupan, penyimpanan dan pengeluaran energi untuk kinerja yang optimal. Keseimbangan yang ketat ini harus baik, kuat serta fleksibel untuk memungkinkan adaptasi dalam setiap situasi, seperti keadaan olahraga atau istirahat dan kondisi lapar atau kenyang. Organisme mengandalkan sinyal dari sistem jaringan yang ada dan kompleks untuk menghadapi segala kemungkinan. Ketidakseimbangan metabolisme dapat menyebabkan disfungsi dan gangguan jaringan ini, yang apabila tidak dikoreksi akan menyebabkan penyakit seperti obesitas dan diabetes mellitus.

KESIMPULAN: Selama dekade terakhir pemahaman tentang regulasi transkripsi beragam jalur metabolisme telah berkontribusi pada penjelasan mekanisme kontrol metabolik dan untuk pengetahuan yang lebih baik tentang patogenesis penyakit metabolik.

KATA KUNCI: AMPK, SIRT1, PGC-1α, FGF21, mTORC1

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Introduction

Metabolism is broadly defined as the sum of biochemical processes in a living organism that either produce or consume energy. More than 8,700 reactions and 16,000 metabolites are now annotated in The Kyoto Encyclopedia Genes and Genome. Core metabolism is the pathway involving metabolism of carbohydrates, fatty acids and amino acids, which are essential for energy homeostasis and macromolecular synthesis in human. This metabolism can be classified into three classes: anabolism, catabolism, and waste disposal. Anabolism is the set of metabolic pathways that construct more complex macromolecules from smaller units or simple molecules, this process need some energy. Catabolism is the set of metabolic pathways that degrade molecules into smaller units and release energy. Waste disposal is that mechanism that helps eliminate the toxic wastes produced (1).

Metabolism can not be viewed only as a self-regulating network that operates independently on other biological systems. Rather, metabolism impacts or is impacted by virtually every other cellular process, so there is no space in biological research that is totally free from the influence of metabolism. Recent work has identified numerous regulatory mechanisms, which either link cell signaling to the orchestration of metabolic pathways or enable cells to sense fuel availability and transmit the information through signaling networks (1).

AMP-activated Protein Kinase (AMPK) and Silent Information Regulator T1 (SIRT1) are metabolic sensor and gatekeeper for activity of the master regulator of mitochondria. PGC-1α plays an important role in regulatory network for metabolic homeostasis (2). Fibroblast growth factor 21 (FGF21) has been identified as a potent metabolic regulator that regulates energy homeostasis in adipocyte through activation of AMPK and SIRT1, resulting in enhanced mitochondrial oxidative function (3).

Dynamic mechanisms also sense cellular energy status and regulate the balance between anabolism and catabolism. PI3K/Akt/mTOR pathway promotes anabolism and suppresses catabolism, AMPK does the reverse. Serin-threonin kinase is a "fuel sensor" activated during compromised bioenergetic states such as acute nutrition deprivation and hypoxia (4). By phosphorylating a number of key targets AMPK inactivates energy-consuming, growth promoting pathways like protein and lipid synthesis, and activates catabolism of fatty acids and other fuels. This enables the cell to rebalance energy supply with demand (1).

Deacylation reactions also regulate metabolism. A class of deacetylases, the situins, comprises nicotinamide adenine dinucleotide (NAD)-dependent deacetylases whose targets include histone and metabolic enzymens. The sirtuins are key evolutionary conserved factors linking caloric restriction to longevity. Over expression of sirtuins in model systems ameliorates a variety of age-related phenotypes, including cancer, diabetes and neurodegeneration (5). AMPK was found to enhance NAD^+-dependent type III deacylase sirtuin 1 (SIRT1) activity by increasing cellular NAD^+ levels, resulting in modulation of the activity of downstream SIRT1 targets (6). AMPK has been shown to play important role in the therapeutic benefits of metformin (7,8), thiazolidinediones (9) and exercise (10,11), all corner stones in the management of type 2 diabetes and metabolic syndrome. Activation of AMPK maintains energy balance by switching on catabolic pathways, enhancing oxidative metabolism, and mitochondrial biogenesis (3).

These players explain many of the beneficial effects of physical activity and dietary intervention against type 2 diabetes and other metabolic disorder. Therefore understanding on the mechanism by which they act can guide us to identify and improve preventive and therapeutic strategies for metabolic disease (2).

AMP-activated Protein Kinase (AMPK)

Living cells use ATP and ADP in a manner similar to the chemicals in a rechargeable battery. Most cellular processes require energy and are driven (directly or indirectly) by the hydrolysis of ATP to ADP and phosphate (or, less frequently, to AMP and pyrophosphate), thus "flattening the battery." In heterotrophic organisms, the battery is recharged by catabolism; i.e. the oxidation of reduced carbon compounds of organic origin, such as glucose. In most cells (especially quiescent cells), oxidation of glucose usually proceeds completely to carbon dioxide via the process of oxidative phosphorylation. Under these conditions, most ATP synthesis occurs at the inner mitochondrial membrane (4). This means any rise in the ADP/ATP ratio, which signifies falling energy status, causes the adenylate kinase reaction to be displaced toward ATP and AMP production. Thus, falling cellular

energy is associated with increases not only of ADP but also of AMP. It seems logical that protein sensing cellular energy status should monitor either the ADP/ATP or AMP/ATP ratio or both (12).

In most of eukaryotic cells, the principal energy sensor seems to be AMPK (7). In support of this, increasing ADP/ATP and AMP/ATP ratio during stresses such as muscle contraction (13), ischemia in cardiac muscle (14) or treatment of hepatocytes with metformin (15) are larger in cells or tissue which AMPK or its essential activating up stream kinase liver kinase B1 (LKB1 also known as STK (11) have been knocked out (12).

AMPK exist as an obligate heterotrimer, containing a catalytic subunit (α), and two regulatory subunits (β and γ). AMPK is hypothesized to be activated by two-pronged mechanism (16). Under lowered intracellular ATP levels, AMP or ADP can directly bind to the γ regulatory subunits of AMPK, leading to conformational change that promote AMPK phosphorylation and also protect AMPK from dephosphorylation to ensure it remains activated. For AMPK activation, phosphorylation is required of Thr 172 in the activation loop of AMPK and serin/threonine kinase LKBI directly mediates this event (17).

AMPK is crucial cellular energy sensor that regulates metabolic energy balance at the whole-body level. Once activated by falling energy status, it promotes ATP production by increasing the activity or expression of protein involved in catabolism while conserving ATP by switching off biosynthetic pathways (12).

As befits its role in maintaining energy homeostasis, AMPK switches on catabolic pathways that generate ATP, while switching off anabolic pathways that consume ATP. Examples of catabolic pathways that are up-regulated include glucose uptake (via activation of both GLUT1 (18), and GLUT4 (19,20), glycolysis (via phosphorylation and activation of two of four isoforms of 6-phosphofructo-2-kinase, which synthesizes the glycolytic activator fructose-2,6-bisphosphate) (21,22), fatty acid uptake (via translocation of the fatty acid transporter FAT/CD36) (23), and fatty acid oxidation (via phosphorylation of the ACC2 isoform of acetyl-CoA carboxylase, thus lowering malonyl-CoA, an inhibitor of fatty acid uptake into mitochondria (4).

AMPK activation also inhibits many anabolic pathways acutely via direct phosphorylation of key metabolic enzymes. Thus, it inhibits fatty acid synthesis

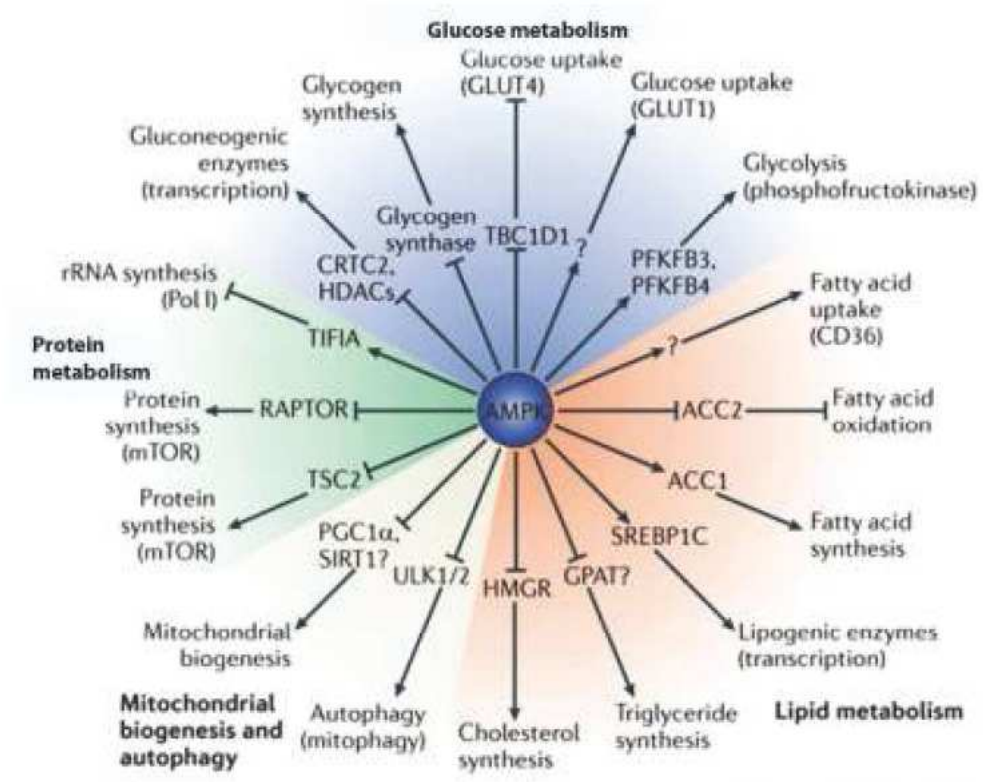

Figure 1. Effects of activation of AMPK on cellular metabolism (Adapted with permission from Hardie DG, *et al.* Ref # 12 Nature Publishing Group 2012)

by phosphorylation of ACC1, isoprenoid synthesis by phosphorylation of HMG-CoA reductase, triglyceride and phospholipid synthesis by inactivation of glycerol phosphate acyl transferase (7,24). AMPK down-regulates expression of enzymes of fatty acid synthesis at the transcriptional level by a mechanism involving phosphorylation of the transcription factor SREBP-1c, inhibiting its proteolytic processing to the active, nuclear form (8,25). It also represses transcription of mRNAs encoding enzymes involved in gluconeogenesis, such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, apparently via multiple mechanisms (4).

In addition, a particularly important target of AMPK is mTOR complex-1 (TORC1), which is switched off by direct phosphorylation of both its upstream regulator, TSC2, and the TORC1 subunit Raptor (26). TORC1 is stimulated by amino acids and by growth factors that activate the Akt and Raf–MEK–Erk pathways, and it promotes both the initiation and elongation of translation via phosphorylation of 4EBP1 and p70 S6 kinase (27).

Mitochondrial biogenesis is another crucial process activated by AMPK, which in the longer term generates increased capacity for oxidative catabolism of both glucose and fatty acids (12). The 'master regulator of mitochondrial biogenesis is peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), a co-activator that enhance the activity of several transcription factors acting on nuclear-encoded mitochondrial genes. AMPK directly phosphorylates PGC1α, which has been proposed to cause activation of its own transcription via a positive feedback loop (28,29).

During fasting and after exercise, skeletal muscle efficiently switches from carbohydrate to lipid as the main energy source to preserve glycogen stores and blood glucose levels for glucose-dependent tissues such as brain or red blood cells. Skeletal muscle cells sense this limitation in glucose availability and transform this information into transcriptional and metabolic adaptations. AMPK acts as the prime initial sensor that translates this information into SIRT1-dependent deacetylation of the transcriptional regulators PGC-1a and FOXO1, culminating in the transcriptional modulation of mitochondrial and lipid utilization genes (30).

In general, activation of AMPK acts to maintain cellular energy stores, switching on catabolic pathways that produce ATP, mostly by enhancing oxidative metabolism and mitochondrial biogenesis, while switching off anabolic pathways that consume ATP (31).

Sirtuins Family

Sirtuins belong to the class III protein deacetylase family, which are the only histone deactyalses (HDACs) that require NAD for their enzymatic activity. NAD is involved in many enzymatic reactions and an important co-factor for the electron transport chain. Owing to the characteristic NAD requirement for their enzymatic reaction, the activity of sirtuins is directly linked to the metabolic state in the cell (32).

Of the mammalian sirtuins, SIRT1, 2, 3, 4, 5, and 6 have been shown to have this activity (33). Some SIRT family members (e.g., SIRT4 and SIRT6) also have ADP-ribosyltransferase activity (33-35). In mammals, the Sir2 orthologue SIRT1 is primarily a nuclear protein in most cell types and has evolved to deacetylate transcription factors and cofactors that govern many central metabolic pathways. Targets of SIRT1 include transcriptional proteins that are important in energy metabolism such as nuclear receptors, peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), and forkhead box subgroup O (FOXO) (36-39). SIRT1 also regulates components of the circadian clock, such as BMAL1 and PER2, which underscores the interconnectedness of protein acetylation, metabolism, circadian rhythm, and aging (40,41). SIRT1 is also closely coupled to AMP-kinase activity in a mutually enforcing mechanism that adjusts cellular physiology for conditions of energy limitation (6)

One early indication that SIRT1 might be important in diseases of metabolism was the finding that the protein could influence differentiation and fat accumulation in the 3T3-L1 adipose-cell line and in primary preadipocytes in rats (42). In a second case, calorie restriction triggered a SIRT1–PGC-1α–dependent increase in muscle mitochondrial biogenesis (43) and the activation of fatty acid oxidation by SIRT1 and peroxisome-proliferator–activated receptor α (PPAR-α) (44), which together favor insulin sensitivity and evidently a slower rate of aging-related decline. In liver, SIRT1 has been found to govern two pathways with opposing effects on gluconeogenesis. On the one hand, the activation of PGC-1α and FOXO1 appears to favor glucose production (37), whereas the deacetylation and destabilization of the cyclic AMP response-element–binding (CREB) coactivator CRCT2 would suppress it (45). The relative importance of each pathway may switch as a function of the duration of fasting to fine-tune the magnitude of glucose production over time (5).

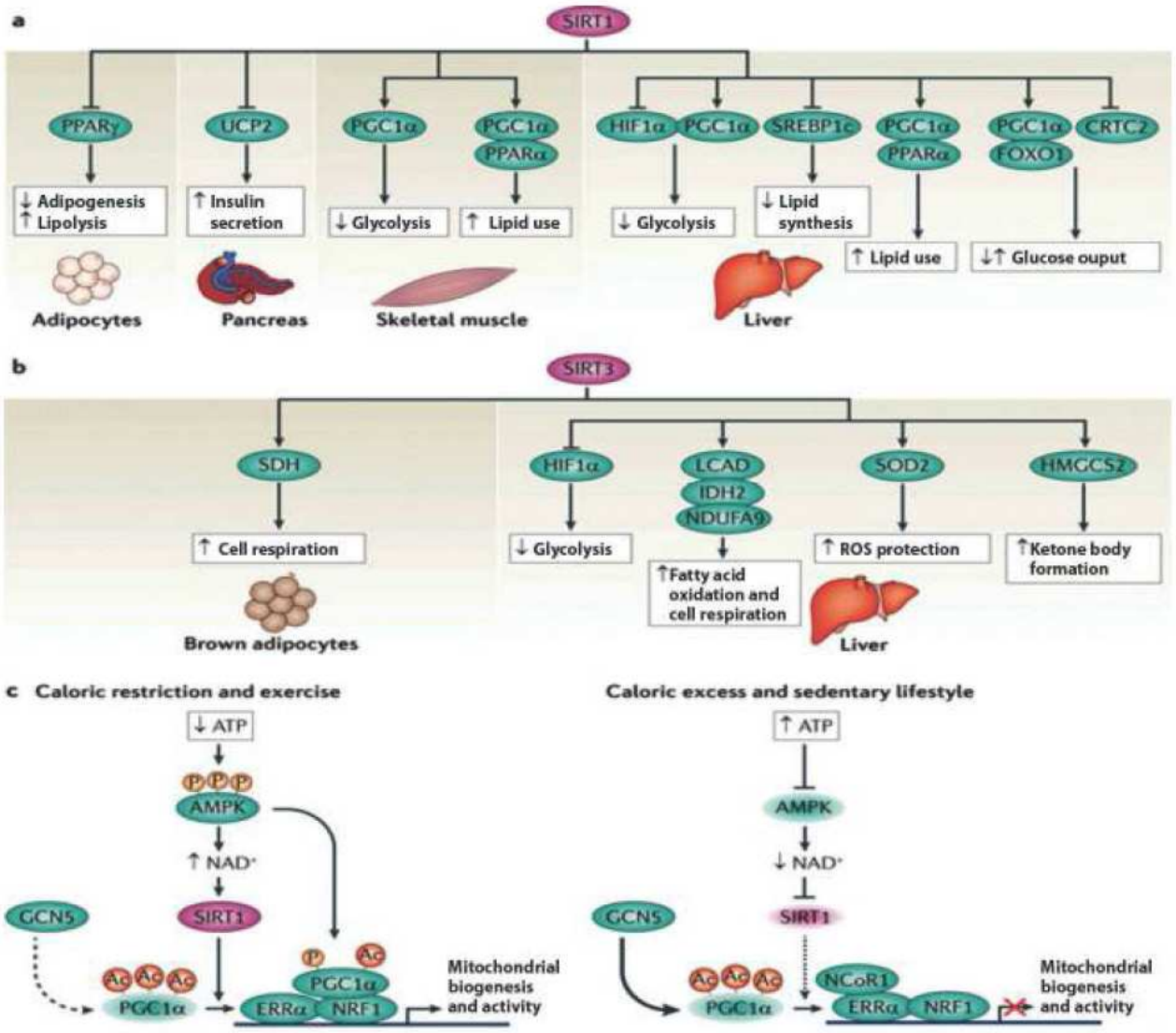

Figure 2. Sirtuin mediated metabolic responses in several tissues during different physiological challenges (Adapted with permission from Houtkooper RH, *et al.* Ref # 46 Nature Publishing Group 2012)

Deacetylation of PGC-1α by SIRT1 not only controls gluconeogenesis, but also fatty acid oxidation in coordination with peroxisome proliferator-activated receptor alpha (PPARα) (44). Recent studies have shown SIRT1 to regulate fatty acid oxidation in the liver, sense nutrient availability in the hypothalamus, influence obesity-induced inflammation in macrophages, and modulate the activity of circadian clock in metabolic tissues. The activity of SIRT1 also appears to be under control of AMPK and adiponectin (47).

Mitochondria play critical roles in energy production, metabolism, apoptosis, and intracellular signaling (48-50). These highly dynamic organelles have the ability to change their function, morphology and number in response to physiological conditions and stressors such as diet, exercise, temperature, and hormones (51). Proper mitochondrial function is crucial for maintenance of metabolic homeostasis and activation of appropriate stress responses. Not surprisingly, changes in mitochondrial number and activity are implicated in aging and age-related diseases, including diabetes, neurodegenerative diseases, and cancer (48). One of the principal bioenergetic functions of mitochondria is to generate ATP through the process of oxidative phosphorylation (OXPHOS), which occurs in the inner-mitochondrial membrane (52).

Three sirtuins, SIRT3, SIRT4 and SIRT5, are located in the mitochondria, dynamic organelles that function as the primary site of oxidative metabolism and play crucial roles in apoptosis and intracellular signaling. Recent findings have shed light on how the mitochondrial sirtuins function in the control of basic mitochondrial biology, including energy production, metabolism, apoptosis and intracellular signaling (52).

Several lines of evidence suggest a strong interplay between metabolism and the circadian clock (53-55). The dominance of feeding cycles as a Zeitgeber for peripheral clocks implies that the circadian clock plays an important role in nutrient processing and energy homeostasis (41). NAD biosynthesis is linked to the circadian clock cycle because nicotinamide phosphoribosyl transferase (Nampt) is regulated by a complex consisting of the circadian locomotor output cycle kaput (CLOCK), brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1 (BMALI1) and SIRT1 and in turn regulates SIRT1 activity through NAD (56). Thus SIRT1 functions as an enzymatic rheostat of circadian function, transduces signals originated by cellular metabolites to the circadian clock (40).

A number of studies have revealed that SIRT1 mediates different stress responses including inflammation, hypoxic stress, heat shock and genotoxic stress, and inflammation in particular is a highly important cause of aging and aging related diseases (57). During hypoxia, NAD level gradually decreases and subsequently SIRT1 is deactivated. Therefore, it has been speculated that SIRT1 triggers a switch from HIF2α to HIF1α activation to coordinate metabolism, vascular formation, and hypoxic stress responses (58).

The anti inflammatory effect of sirtuins may be much broader, since both SIRT1 and SIRT6 repress the activity of the major proinflammatory transcription factor, nuclear factor κB (57,59). SIRT1 appears to possess cardiovascular protective properties beyond those deriving solely from metabolic fitness (5). The earliest connection between SIRT1 and endothelial cells was the finding that SIRT1 deacetylates and activates endothelial nitric oxide synthase (eNOS) (60). The activation of eNOS and repression of AT1 suggest that SIRT1 activity ought to curb high blood pressure (5).

Interestingly, calorie restriction is known to protect against atherosclerosis (61), and many of the physiological effects of calorie restriction are blunted in eNOS –/– mice. These findings indicate that SIRT1 helps facilitate the favorable effect of calorie restriction on cardiovascular function by its effects on eNOS, AT1, and perhaps other targets (5). SIRT1 deacetylates and activates the nuclear receptor liver X receptor (LXR), which up-regulates

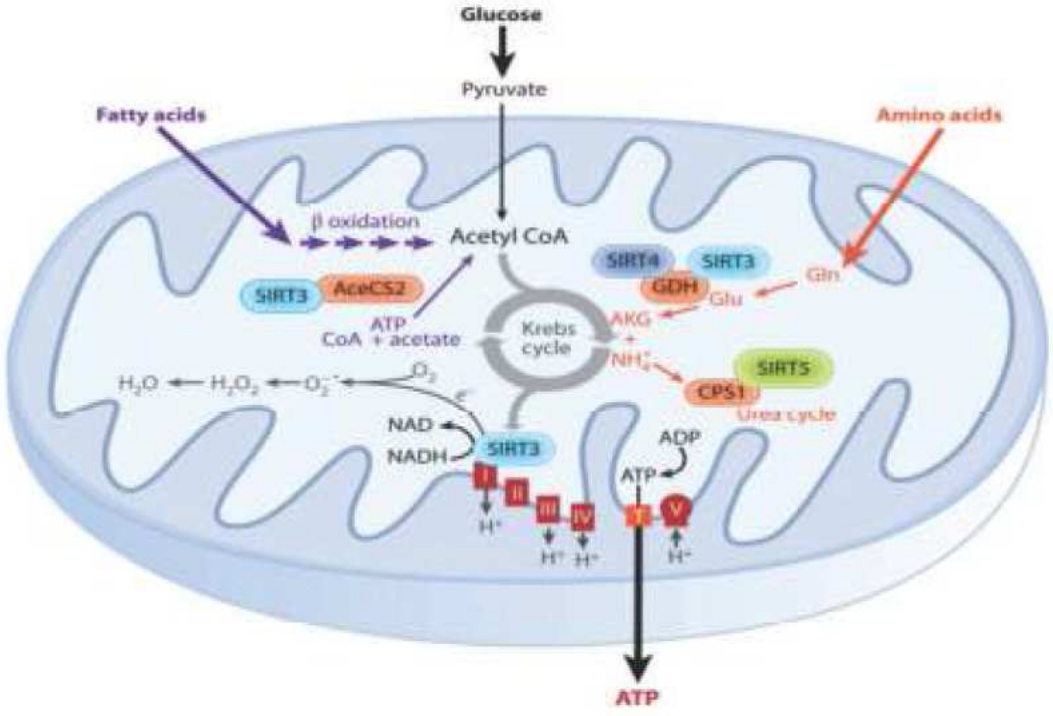

Figure 3. Network of mitochondrial sirtuins (SIRT3-5) (Adapted with permission from Haigis MC, *et al.* Ref # 35 Annual Reviews Inc 2012).

the ATP-binding cassette transporter A1 to facilitate reverse cholesterol transport from peripheral tissues (36). LXR and FXR activation by SIRT1 has the potential to increase the production of high-density lipoprotein (HDL) cholesterol and protection against atherosclerosis by facilitating cholesterol removal (5).

In conclusion, sirtuins are a unique class of proteins that link protein acetylation to metabolism and exert profound effects on mammalian physiology and diseases of aging. The development of drugs that target sirtuins to treat these diseases is ongoing (5).

Peroxisome Proliferator-activated Receptor γ coactivator 1α (PGC-1α)

Metabolic equilibrium is maintained in the cell by an intricate regulatory circuitry, which is controlled to a large extent by transcriptional mechanisms (62,63). These pathways imply many transcription factors that directly contact DNA and execute major changes in gene expression and transcriptional co-regulators, which are responsible for the fine-tuning of the transcriptional response (64).

The role of co-regulators in metabolic control is perhaps nowhere better shown than with peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (65), the master regulator of mitochondrial biogenesis and energy expenditure. Several metabolic functions have been attributed to PGC-1α. In brown adipose tissue (BAT), PGC-1α acts as a cold-inducible protein that controls adaptive thermogenesis. Fasting induces hepatic PGC-1α expression, thereby increasing gluconeogenesis, whereas in skeletal and cardiac muscle exercise increases PGC-1α–mediated mitochondrial biogenesis and respiration (64). Thus, PGC-1α expression seems finely tuned to reflect cellular energy needs, with conditions of increased energy demands inducing its expression. PGC-1α performs all these tasks by regulating the activity of a large number of transcription factors, including, among others peroxisome proliferator-activated receptor (PPAR) γ (65), PPARα (66), estrogen receptor–related α (ERRα) (67), FoxO1 (68), hepatocyte nuclear factor 4α (HNF4α) (69), and nuclear respiratory factor 1 (NRF1) (70). By regulating the transcriptional activities of these proteins, PGC-1α modulates a number of genes involved in metabolic pathways as gluconeogenesis and fatty acid synthesis and oxidation or glycolysis (64).

Posttranslational mechanisms, equally important as the transcriptional mechanisms, also extensively regulate PGC-1α. To date, phosphorylation, ubiquitination, methylation, acetylation, and GlcNAcylation or PGC-1α have all been described. The discovery of PGC-1α and the pleiotropic and the robust effects it has on metabolic homeostasis unveiled how the PGC-1α cofactor network is central to the regulation of mitochondrial biogenesis and function, thereby having an effect on whole-body energy expenditure. Dysfunction of these pathways through abnormal PGC-1α activity has a profound effect on general metabolism and, if uncorrected, could predispose and contribute to metabolic diseases such as obesity, the metabolic syndrome, and type 2 diabetes (64).

Fibroblast Growth Factor (FGF) Family

The Fibroblast Growth Factor (FGF) family comprises at least 22 members that involve in development, differentiation, cell survival and growth, wound healing and tumor formation (71,72). Recent data shows that they may play important roles in defining and regulating functions of some endocrine-relevant tissues and organs, as well as modulating various metabolic processes. For example, FGF-10 is implicated in the differentiation processes in white adipose tissue (73) and pancreas (74,75), while FGF-16 (76) is considered to be a specific factor for brown adipocytes. Another recently characterized molecule, FGF-19 (77,78), has been shown to cause resistance to diet-induced obesity and insulin desensitization and to improve insulin, glucose, and lipid profiles in diabetic rodents. FGF-19 can be considered as a regulator of energy expenditure (79,80).

FGF21 is expressed predominantly in white adipose tissue, liver, and pancreas (81), with most circulating FGF21 originating from the liver. Because its production is induced in murine livers by starvation (82,83), and because many of its actions, induction of gluconeogenesis, fat oxidation, and ketogenesis, coupled to a state of torpor (82-84), mimic the effects of fasting, FGF21 has been considered a possible starvation signal. Recent studies have shown that feeding can promote FGF21 synthesis in white adipose tissue (85,86). Most likely, the activation of the transcription factor peroxisome proliferator–activated receptor-γ (PPARγ) accounts for this up-regulation, as PPARγ agonists [thiazolidinediones (TZDs)] induce

FGF21 expression in white fat. Interestingly, FGF21 controls the sumoylation of PPARγ, thereby preventing its inhibition (81,85,87).

Therapeutic administration of FGF-21 reduced plasma glucose and triglycerides to near normal levels in both ob/ob and db/db mice. These effects persisted for at least 24 hours after cessation of FGF-21 administration. Importantly, FGF-21 did not induce mitogenicity, hypoglycemia, or weight gain at any dose tested in diabetic or healthy animals or when overexpressed in transgenic mice (88). Another twist in the unfolding roles of FGF21 is in browning of white fat in response to cold or adrenergic stimulation (89). FGF21 is linked with increased thermogenic activity (90).

It is now clear that there are two distinct types of brown adipose cells. One is the classical brown fat that arises from a myf5, muscle-like cell lineage (92,93). The other, UCP1-positive, brown fat-like cells can emerge in most white fat white adipose tissue [WAT]) depots upon prolonged cold exposure or b-adrenergic receptor activation (94-96). These brown fat cells, which are not derived from a myf5-positive lineage, are designated beige or brown-in-white (brite) cells (97,98).

To promote browning of white fat, FGF21 enhances PGC-1α activity, potentially through inducing its post translational modification (89). The pathways through which FGF21 activates PGC-1α and browning has not yet been known, but the signaling pathway involving the enzymes AMPK and SIRT1 warrant scrutiny. Activation of AMPK and deacetylation of PGC-1α by SIRT1 are essential for FGF21 to trigger PGC-1α activity to enhance mitochondrial function (3). The action of FGF21 on hepatic lipid metabolism also requires PGC-1α (84), further suggesting that activation of the AMPK-SIRT1 signaling axis may be a general downstream feature linking FGF21 and PGC-1α activity (91).

Human data have establish FGF21 as a starvation hormone; the amount of circulating FGF21 display no apparent circadian variation or a feeding fasting cycle, and prolonged fasting (7 days) increase plasma FGF21 concentration (99). But circulating FGF21 concentration also increases in overweight patients with various features of the metabolic syndrome (100), potential hinting at the existence of an obesity induce FGF21 resistance state (101), although this is debated (102).

It seems unlikely that FGF21 resistance has a major contributory role toward the development of obesity. Nevertheless, FGF21 resistance likely contributes significantly toward the complications associated with weight gain and obesity, such as glucose intolerance and elevated circulating NEFAs. Nevertheless, FGF21 resistance can be added to the hormone-resistant states observed in obesity. Understanding the mechanisms of this process could represent a new target for therapeutic treatment of obesity and obesity-related diseases (101). Thus FGF21 has become particularly interesting because its endocrine actions endow it with potential therapeutic uses (91).

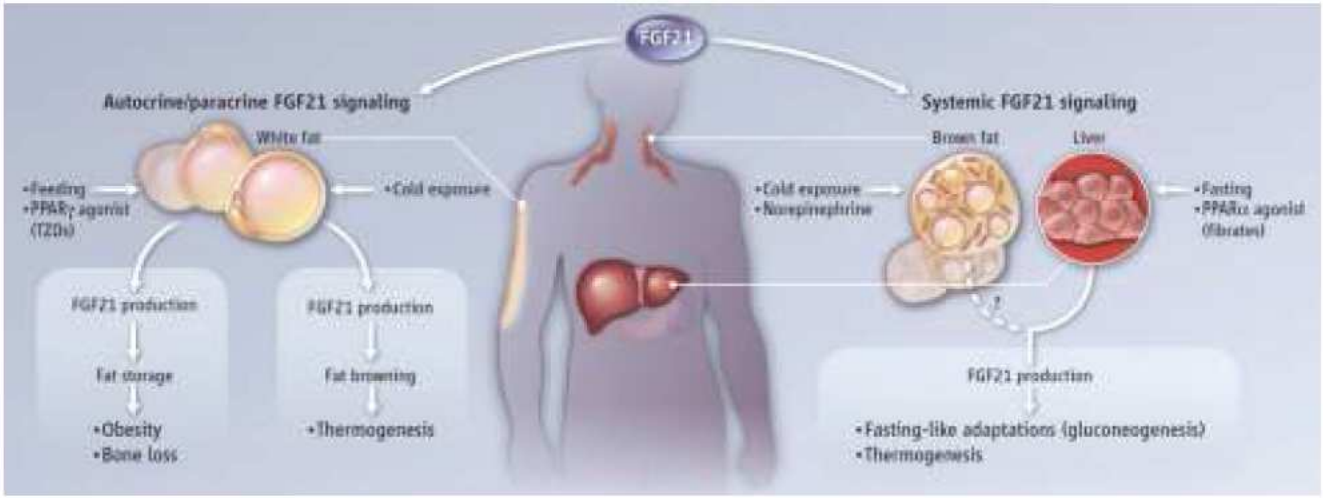

Figure 4. FGF21 Actions (Adapted with permission from Cantó C, *et al.* Ref # 91 The American Association for the Advancement of Science 2012).

Mammalian Target of Rapamycin Comolex 1 (mTORC1)

The mammalian target of rapamycin (mTOR) pathways is the latest intracellular fuel sensing mechanism to be implicated in the regulation of energy balance (103-105). mTOR is actually the catalytic subunit of two distinct multiprotein complexes, respectively named mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is included as the main subject of this review because of its recently established role in the regulation of energy balance and peripheral metabolism (106).

mTOR is highly conserved serine-threonine kinase, which in peripheral cells is known to integrate nutrient signal with hormonal signals to control cell growth and proliferation (107). Cellular ATP levels increase mTOR activity, mTOR kinase itself serves as a cellular ATP sensor (108). mTOR thus work as a critical checkpoint by which cells sense and decode change in energy status (107). Recent studies have demonstrated that within the central nervous system and in particular the hypothalamus, mTORC1 represents an essential intracellular target for the actions of hormones and nutrients on food intake and body weight regulation (103,106).

Insulin and leptin require an intact PI3K/Akt signaling in the arcuate nucleus (ARC) to reduce food intake (109). The fact that mTORC1 is a downstream target of the PI3K/Akt pathway leads us to hypothesize that hypothalamic mTORC1 signaling might be required for leptin-induced anorexia. The mTORC1 pathway is clearly present in every organ, where it displays tissue specific functions. Thus, apart from its role in the CNS and particularly hypothalamus, it would be appropriate at this point to briefly mention its function in metabolically relevant tissues, such as the skeletal muscle, the adipose tissue, the pancreas and the liver (106).

mTORC1 is arguably one of the most important nutrient-regulated intracellular signaling pathway in the muscle. It is responsible for protein synthesis in the muscle tissue, where it is activated through nutrients, insulin, growth factors and exercise (110). Available evidence also

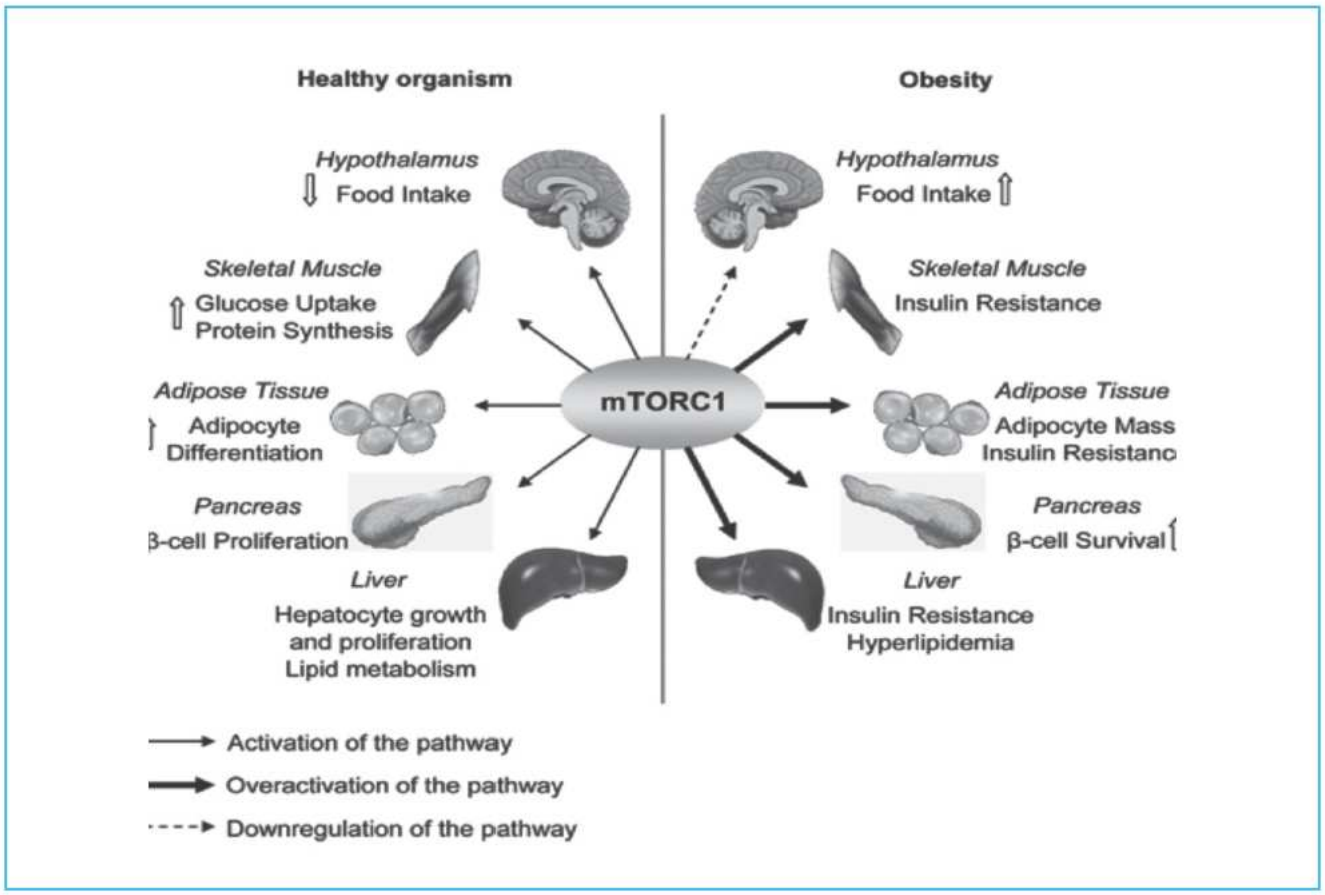

Figure 5. Regulation and function of the mTORC1 pathway in different organs in a healthy organism and in obese organism (Adapted with permission from Catania C, *et al.* Nature Publishing Group 2012).

supports a role of the mTORC1 pathway in the muscle glucose uptake and insulin sensitivity (111,112). In particular, mTORC1 may alter insulin action in response to chronic increase of fatty acid availability (111).

In vitro experiments have demonstrated that mTORC1 is essential for the differentiation and maintenance of white adipocytes. Indeed, mTORC1 activation is necessary for insulin or nutrient (amino acid) to induce adipogenesis and expression of SREBP1 and PPARγ, which are master transcriptional regulators of adipocyte differentiation and lipid homeostasis. In diet induced obesity, overactivity of the mTORC1 signaling favors the expansion of the WAT mass, leading to adpocyte insulin resistance through elevated phosphorylation of IRS1 (113-115).

In diet-induced obesity, overactivity of the mTORC1 signaling favors the expansion of the WAT mass, leading to adipocytes insulin resistance through elevated phosphorylation of IRS1 (116). *In vivo* overactivity of mTORC1 signaling, due to deletion of either TSC2 (a negative regulator of mTORC1) or loss of LKB1 (a positive regulator of AMPK) in β-cell mass and improves glucose tolerance (117,118). Conversely rapamicyn treatment leads to inhibition of β-cell proliferation and apoptosis (119,120), and worsens hyperglycemia in obese animals by inhibiting glucose-stimulated insulin secretion (121,122).

As expected, once activated, mTORC1 signaling has a positive effect on hepatocyte growth, protein synthesis and cell cycle and hence contributes to liver regeneration (123). Furthermore, as already reported for other tissue, either amino acids or insulin can stimulate hepatic mTORC1 signaling (124). Hence mTORC1 signaling is actually a critical mediator of cellular adaptation to nutrient overload and increased insulin demand during diet-induced obesity, which is further confirmed by genetically inducing mTORC1 overactivity within β-cells (117,118,125).

The mechanistic mTOR signaling pathway senses and integrates a variety of environmental cues to regulate the organism's growth and homeostasis. The pathway regulates many major cellular processes and is implicated in an increasing number of pathological conditions, including cancer, obesity, type 2 diabetes, and neurodegeneration. There are significant ongoing efforts to pharmacologically target the pathway (113).

Calorie Restriction Mimetic: Resveraltrol (RSV)

Although short-term dietary restriction has metabolic effects in humans such as lowered metabolic rate (126), improved insulin sensitivity (127,128) and reduced cardiovascular risk factors (61), eating less for the sake of creating a desirable metabolic profile is unlikely to gain widespread compliance. As such, the focus has been on the development of calorie restriction mimetics that evoke some of the benefits of calorie restriction without an actual reduction in calorie intake. In that respect, sirtuins are considered an important molecular target (129).

Resveratrol, a natural polyphenolic compound present in various dietary components such as mulberries, peanuts, grapes, and red wine, was identified as the most potent activator of SIRT1 (130). Recently, however, it was shown that resveratrol may not activate SIRT1 directly (131,132), but rather exerts its effects on SIRT1 through activation of AMPK (6,30,133-136). In muscle, resveratrol activated AMPK, increased SIRT1 and PGC-1a protein levels, increased citrate synthase activity without change in mitochondrial content and improved muscle mitochondrial respiration on a fatty acid-derived substrate. Furthermore, resveratrol elevated intramyocellular lipid levels and decreased intrahepatic lipid content, circulating glucose, triglycerides, alanine-aminotransferase, and inflammation markers. Systolic blood pressure dropped and HOMA index improved after resveratrol intake (137).

Treatment of mice with RSV significantly increased their aerobic capacity as evidenced by their increased running time and consumption of oxygen in muscle fibers. RSV's effects were associated with an induction of genes for oxidative phosphorylation and mitochondrial biogenesis and were largely explained by an RSV-mediated decrease in PGC-1α acetylation and an increase in PGC-1α activity. This mechanism is consistent with RSV being a known activator of the protein deacetylase, SIRT1, and by the lack of effect of RSV in SIRT1-/- MEFs. Importantly, RSV treatment protected mice against diet-induced-obesity and insulin resistance (138). Thus resveratrol is a natural compound that affects energy metabolism and mitochondrial function and serves as a calorie restriction mimetic, at least in animal models of obesity (137).

Conclusions

Considering the urgent issue modern societies are facing about how to efficiently tackle obesity and type 2 diabetes, intense research is required to elucidate the biological mechanism underlying the nutrient sensing, regulation of energy balance, and peripheral metabolism. Thus, it has become essential to understand the mechanism that regulates energy balance and the peripheral metabolism to identify possible therapeutic targets that may help halt obesity and its disastrous metabolic consequences.

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