"Deconstructing metabolic homeostasis"

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1. Introduction

There is an undeniable inner beauty in how organisms at all levels of the evolutionary scale have found ways to translate changes in environmental conditions into fine metabolic adjustments. To do so, organisms must sense environmental conditions and trigger intracellular signalling and metabolic pathways, which act as a coordinated network to translate this message and generate an integrated response that allows proper adaptation. A paradigmatic and illustrative example of these coordinated actions can be found in the regulation of energetic metabolism. Energy homeostasis requires the coordinated regulation of energy intake, storage and expenditure. In healthy individuals, fluctuations in any of these processes are normally counterbalanced by regulation of the other two. In contrast, abnormalities in the equilibrium of the caloric equation lead to metabolic malfunctions.

Over the past 50 years, the prevalence of a cluster of interrelated metabolic diseases, including obesity, insulin resistance and type 2 diabetes has increased in dramatic proportions. Among the different diseases related to metabolic dysfunctions that concern our modern society, type 2 diabetes (T2DM) is rapidly becoming a world wide epidemic (1). To date, only 1-2% of the incidence of type 2 diabetes can be attributed to genetic mutations. The other 98% is related to environmental factors, mostly physical inactivity and excessive caloric intake, which lead to misbalance of energy homeostasis (2).

2. Preventing metabolic disease by modifying lifestyle

In obesity, and all its derived metabolic and cardiovascular complications, we face a basic defect in the energy balance, where energy intake exceeds energy expenditure. Therefore, the most obvious way to compensate this balance would be to decrease energy (caloric) intake or to increase energy expenditure. Energy expenditure is composed by an invariable component, which is our basal metabolic rate, and a variable energy expense, which depends on our amount of physical activity. Consistently, it is intuitive to conclude that physical activity might be linked to susceptibility to metabolic disease.

The view that a healthy lifestyle is directly linked to the rising incidence of insulin resistance and type 2 diabetes is not new. Thirty years

ago, it was noted that the incidence of diabetes was reported to be greater in groups of people who migrate from their native land to a modern (Western) environment (3, 4), coupled with the finding that rural dwellers have a lower prevalence of diabetes than their urban counterparts (5-7). In these investigations, differences in the prevalence of diabetes were attributed to differences in the level of habitual physical activity. Cross-sectional and retrospective epidemiological studies have subsequently provided direct evidence that a lack of physical activity is strongly associated with impaired glucose tolerance. For example, individuals with type 2 diabetes are less active (8, 9) and report less physical activity over their lifetime than persons without diabetes (8). Cross-sectional studies conducted in individuals without type 2 diabetes have also demonstrated that after an oral glucose tolerance test (OGTT), blood glucose and insulin concentrations remain significantly higher in less-active individuals compared to more-active individuals (8, 10-14). The results of these investigations strongly consolidate 'environmental' factors rather than 'genetic' changes as the primary causative factor for the current diabesity epidemic.

Then, can a modification in lifestyle prevent the insulin resistant state that precedes type 2 diabetes? The answer is 'yes.' Amongst others, perhaps the most impressive results in support of the case for lifestyle interventions were reported by the Diabetes Prevention Program Research Group (15), where > 3000 non-diabetic subjects aged > 25 years, with a body mass index (BMI) of > 24 and elevated fasting and post-load plasma glucose concentrations were randomly assigned to placebo (no intervention), drug (850 mg twice daily of metformin, the drug most widely prescribed against type 2 diabetic patients), or lifestyle modification. The goals for the lifestyle intervention were to achieve and attain a reduction in body mass (BM) of 7% of initial BM and to engage in at least 150 min/week of moderate-intensity physical activity. The duration of the average follow-up was 2.8 years. Compared with placebo the incidence of diabetes was reduced by 31% with metformin and 58% with the lifestyle intervention, with the magnitude of improvements similar in both men and women, and across racial and ethnic groups. As intended, the subjects who participated in the lifestyle intervention were also characterized by a significantly greater loss in BM (5.6 vs 0.1 and 2.1 kg for lifestyle intervention, placebo and

metformin, respectively). Taken collectively, the results of this clinical trial and other similar studies (16, 17) strongly support the hypothesis that it is possible to achieve a sustained improvement in insulin sensitivity by a healthy lifestyle and moderate weight loss (Figure 1).

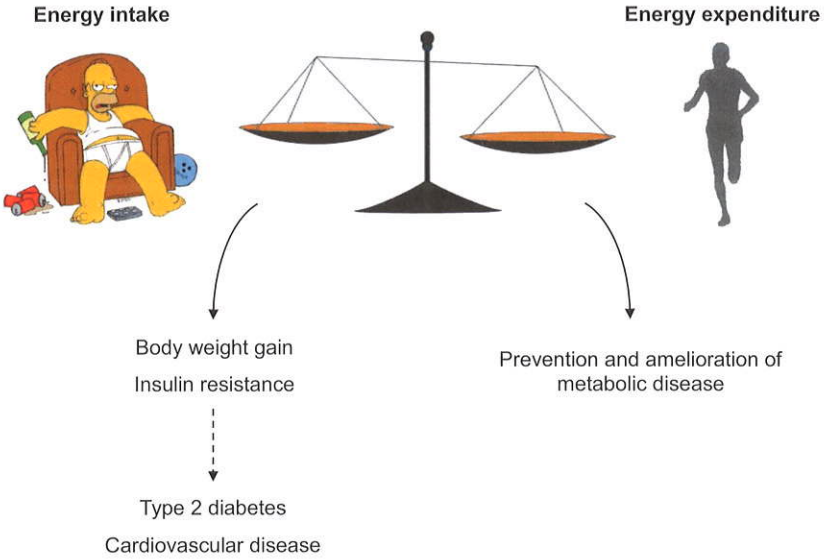

Figure 1. Lifestyle conditions metabolic homeostasis. Whole body energy homeostasis is composed by two different components: energy intake and energy expenditure. Their balance is mandatory to prevent the development of metabolic diseases.

Independent evidence on how modifying lifestyle and the energy balance can impact on global health and even on lifespan comes from the studies on caloric restriction (CR). CR is usually defined as a moderate (normally, 20-40%) reduction in caloric intake as compared with an ad libitum diet, without compromising the maintenance of all essential nutrients. Around 70 ago, McCay and colleagues published a milestone report showing that CR increases maximal longevity in rats (18). From then on, multiple lines of evidence indicate that the effects of CR on lifespan extension stretch all along the evolutionary scale. Up to a 50% increase in maximum lifespan has been reported in caloric restricted yeast, rotifers, spiders, worms, flies, fish, mice and rats (19). There are

two ongoing studies on the effects of CR in Rhesus monkeys. While the final outcome and conclusions of these experiments may still take a few decades to be realized, current data already indicate that caloric restricted monkeys are protected from many age-associated pathophysiological changes, such as the development of insulin resistance and type 2 diabetes (20, 21), atherosclerosis (22), and reductions in their basal metabolic rate (23), body temperature (24), oxidative damage (25) and senescence of the immune system (26). Despite only having very preliminary evidence based on surrogate measures on how CR may impact on human longevity, most available data sets indicate that CR exerts similar adaptive responses in humans as in laboratory animals, reducing the risk of developing age-associated pathological complications, including obesity and insulin resistance.

3. What is the molecular basis for metabolic disease?

Evidence gathered during the last decade indicates that alterations in lipid metabolism may have a central role in the onset of T2DM. First, insulin resistant states are commonly linked with a decreased efficiency to use fatty acids as an energy source in skeletal muscle (27-29). This, in turn, redirects the fatty acid flux toward storage, leading to the increased ectopic lipid deposition. Evidence for this has been obtained in a wide array of experimental models of human insulin resistance (30-32), to the point that intramuscular triglyceride accumulation has been recognized as one of the most consistent markers of whole-body insulin resistance (33). Together, these observations led to the hypothesis that defects in the ability to use fat mass as an energetic substrate (lipid oxidative function) and the subsequent decrease in energy expenditure may contribute to the metabolic dysfunctions observed both in insulin resistant states, T2DM and aging. Fatty acids, the building blocks of triglyceride, are oxidized in a specific cellular compartment named mitochondria. The number and size of mitochondria per cell is variable, depending on the tissue and circumstance, and they constitute the main energy factories of the cell. Confirming that the oxidative capacity of the tissues might be compromised in pathophysiological states, two seminal studies by Gerald Shulman's lab demonstrated that two different populations with high susceptibility to

develop T2DM, i.e. lean, healthy offspring of type 2 diabetic parents and an elderly population, displayed impaired mitochondrial function in skeletal muscle (34, 35). Subsequent studies reporting altered mitochondrial function in T2DM patients further confirmed these observations (36, 37).

At the molecular level, the oxidative dysfunction displayed in T2DM subjects may find an explanation in a coordinated decrease in the expression of genes involved in lipid oxidation and mitochondrial metabolism in skeletal muscle, as demonstrated by gene-clustering approaches (38, 39). Furthermore, changes in gene expression are also correlated with changes in muscle oxidative phenotype (35, 40). Hence, obese and diabetic individuals display lower ratios of type I muscle fibers, which display high mitochondrial content and oxidative rates, relative to type IIb fibers, which have a glycolytic nature. The latter observation is important since insulin sensitivity is positively correlated with the oxidative capacity of the muscle (41). Considering the major role of muscle in whole-body glucose disposal (42), increased glycolytic muscle mass may contribute into decreased whole-body insulin sensitivity. For these reasons, it seems logical that regulation of mitochondrial oxidative capacity may hold promise as a preventive and therapeutic strategy to reduce the burden of T2DM and its associated diseases.

4. The resveratrol studies

All the studies described above clearly show that the roots of metabolic disease are, in the vast majority of cases, derived from a disequilibrium in the energy intake vs. energy expenditure balance and that lifestyle modifications can, to a great extent, prevent and ameliorate metabolic disease. However, this idea is not easy to put into practice. Indeed, western societies are not prone to easily change their habits in terms of diet or physical activity. For this purpose, there has been a strong pharmacological interest in understanding the molecular basis by which physical activity and CR prevent metabolic disease and in developing compounds that could artificially promote similar effects.

In 2006, two key reports indicated that resveratrol (Rsv), a natural compound derived from the skin of red grapes, could promote lifespan

in high-fat fed rodents and prevent against metabolic disease (43, 44). Until then, Rsv was known for its anticancerous and antioxidant properties. In lower eukaryotes (yeast and worms), Rsv promoted lifespan extension to the same extent as calorie restriction (45). Therefore, it was long speculated that Rsv could act as a "calorie restriction" mimetic.

What are the effects of Rsv in mammals? The study performed by Lagouge et al. (2006) in our lab demonstrated that Rsv increased the amount of mitochondria in key tissues such as skeletal muscle and brown adipose tissue. This allowed an increased use of fat as energy substrate, which rendered the animals leaner and healthier. The higher mitochondrial metabolic capacity provided by Rsv also led to other interesting phenotypes. For example, mice fed with Rsv had a higher endurance performance and could run twice as much as their control littermates. Subsequent studies by other laboratories have shown that the ability of Rsv to increase mitochondrial metabolism does not only prevent against metabolic disease, but also against oxidative damage and cancer (46). It is important to note that also calorie restriction and physical activity prevents oxidative stress and cancer susceptibility, further supporting the concept that Rsv could be a natural compound with the ability to mimic the beneficial effects of lifestyle interventions.

5. Developing ideas to find the molecular basis for resveratrol action

The astonishing results provided by the Rsv studies prompted the question of how did Rsv work at the molecular level. In 2003, it was proposed that Rsv could directly interact and activate SIRT1 (47). SIRT1 is an enzyme that mediates NAD^+-dependent deacetylation of target substrates, mostly gene transcription factors and metabolic enzymes (48). Interestingly, SIRT1 is one of the mammalian homologs of Sir2, a protein previously described to strongly affect lifespan in yeast and worms, and one of the most likely candidates mediating the effects of CR on lifespan in these organisms (45). However, previous to the Rsv studies, a number of labortatories indicated that, while SIRT1 is generally more active in cells after Rsv treatment, the activation of SIRT1 was not direct (49, 50).

In early 2007, professor Johan Auwerx and myself speculated that the question of how Rsv and CR work should actually be expanded to how do energy stresses like exercise promote beneficial effects. Indeed, on a superficial level, many would consider intuitive to make the statement that exercise is a good thing. However, still to these days, the answer to the question of how exactly at the mechanistic level is exercise beneficial for human health is not obvious.

Upon such questions, we reasoned that both CR and exercise constitute energy stresses, led by the limiting nutrient availability and by an increased energy demand, respectively. Therefore, we concluded that it could be likely that the beneficial effects rendered by both interventions could be mediated by a convergent cellular sensor of energy stress. Expanding this hypothesis, we speculated that whatever Rsv did, it should converge at some point in the activation of this "energy sensor", explaining why it promotes similar beneficial effects on health.

6. AMP-activated protein kinase comes in

Given the rational above described, we became interested in the AMP-activated protein kinase (AMPK). The AMPK is a conserved fuel-gauge that has probably played a major role in the maintenance of intracellular energy balance during eukaryotic evolution. Mammalian AMPK is a Ser/Thr kinase that is directly activated by alterations in the cellular AMP/ATP ratio. Hence, perturbations in this ratio due to either defects in energy production or increased energy consumption will activate the kinase. Once activated, AMPK switches on catabolic pathways to produce ATP while simultaneously shutting down energy-consuming anabolic processes. In order to perform these actions, AMPK can quickly regulate metabolic enzymes through direct phosphorylation, but, additionally, AMPK also has long-term effects at the transcriptional level in order to adapt gene expression to energy demands. Hence, upon energy deficiency, AMPK will enhance the expression of genes related to glucose transport and glycolysis (51, 52) and mitochondrial respiration (53) while down-regulating lipid synthesis genes (54).

The above paragraph clearly indicates that AMPK is a very likely candidate to explain Rsv action. Therefore we decided to test whether Rsv

could activate AMPK. Confirming our expectations, treatment of muscle cells with Rsv increased AMPK activity within a few minutes (55). Importantly, the activity of SIRT1, the previously speculated candidate target for Rsv, took 4-6 hours to increase (55), indicating that, unlike AMPK, the activation of SIRT1 is a really distal effect. It is also important to mention that Rsv only activated AMPK in living cells, but not in test tube using the isolated proteins, indicating that the activation was not direct (44). The question was then, how does resveratrol activate AMPK? The answer relies in studies indicating that resveratrol binds and mildly inhibits the ATP synthase complex in the mitochondria (56). Therefore, Rsv treatment rapidly but mildly lowers ATP synthesis, creating a minor energy stress, unable to compromise cell survival, but enough to activate energy stress response. This observation was recently confirmed by a report showing that AMPK forms unable to detect changes in AMP/ATP levels do not become active upon Rsv treatment (57). From these experiments we concluded that Rsv potently and rapidly activates AMPK activation, and that AMPK activation constitutes the most proximal signalling event described to date upon Rsv treatment. Importantly, exercise also activates AMPK, further reinforcing the idea that AMPK could constitute the nodal link by which energy stresses promoted beneficial effects on health.

7. Downstream of AMPK (I): SIRT1

The next step, however, was to understand how AMPK activation translated into beneficial health effects. It was long know that AMPK activation promoted mitochondrial biogenesis, which could explain it. But how did AMPK increase mitochondrial number? The first key observation we made was that not only Rsv, but also any other AMPK activator we tested in the laboratory increased, in a matter of hours, SIRT1 activity (55). This happened not only in cell based tests, but also in mice. For example, when we pharmacologically activated AMPK on mice, we had a consequent increase in SIRT1 activity in a matter of hours (55). These findings were also expanded to physiological situations in experiments where muscle AMPK was activated by exercising mice until exhaustion. A single bout of exercise was enough to concomitantly increase SIRT1 activity a few hours after the cessation of

exercise (55). These results proved that activation of AMPK is systematically followed by the activation of SIRT1 in cells and tissues. This hypothesis was then fully confirmed by two different genetic approaches. First, we used a constitutively active form of AMPK to demonstrate that SIRT1 could get active in response to AMPK activation in the absence of stress or pharmacological agents (55). For our second approach, we collaborated with the laboratory of Prof. Juleen R. Zierath in the Karolinska Institute (Stockholm, Sweden), and used transgenic mouse lacking the g3 subunit of AMPK, which is the predominant one in glycolytic skeletal muscle. Our results showed that while energy stresses such as fasting and exercise led to SIRT1 activation in glycolytic skeletal muscle, this did not happen in mice lacking the AMPKg3 subunit (58). Furthermore, SIRT1 was not activated in response to Rsv in muscles from AMPKg3 knock-out mice (58). All the above findings clearly indicate that activation of SIRT1 is a downstream event of AMPK activation.

As mentioned above, SIRT1 is an enzyme that mediates NAD^+-dependent deacetylation of target substrates. The Km of SIRT1 for NAD^+ is around the physiological intracellular levels of NAD^+, meaning that an increase in intracellular NAD^+ levels would allow higher SIRT1 activity (59). Importantly, both physiological and pharmacological activation of AMPK were always followed by an increase in intracellular NAD^+ at timings that perfectly matched those of SIRT1 activation (55). Therefore, changes in NAD^+ constituted a plausible mechanism by which AMPK increases SIRT1 activity. We subsequently identified two different strategies by which AMPK influences NAD^+ homeostasis. First, AMPK activation promotes a rapid shift to the use of fat as major energy source and NADH reoxidation to NAD^+ in the mitochondria. Consolidating this hypothesis, the blockage of fatty acid oxidation was enough to prevent AMPK-induced increases in NAD^+ levels (55). On a longer time frame, Vittorio Sartorelli's (60) and our lab (55) reported that AMPK increases the expression of an enzyme called Nampt, which resynthesizes NAD^+ from its breakdown product, nicotinamide, further sustaining the AMPK on NAD^+ levels.

Next, we aimed to test whether the activation of SIRT1 by AMPK participated in the actions of AMPK on mitochondrial biogenesis. To do that, we generated cellular models in which SIRT1 was either sub-

stantially reduced (knock-down) or completely ablated (knock-out). In both models, the ability of AMPK to increase the expression of genes related to fat oxidation or mitochondrial enzymes was largely blunted (55), indicating that SIRT1 is a critical mediator of the metabolic effects of AMPK. To further sustain our findings we evaluated oxygen consumption (as a readout of mitochondrial metabolism) and lipid oxidation in these models. In both cases, the deficiency of SIRT1 critically impaired the action of AMPK (55). More precisely, AMPK activation shifted glucose for fat as main fuel for mitochondrial oxidation. In the absence of SIRT1, AMPK was unable to shift substrates (55). The ability to shift from glucose to fat is crucial in several physiological scenarios, such as fasting and exercise. During fasting or energy deficits, the limiting glucose availability must be compensated by an increase in mitochondrial fatty acid oxidation in skeletal muscle and other peripheral tissues to preserve blood glucose levels to supply glucose-dependent tissues, such as the brain or red blood cells. Similarly, after exercise, skeletal muscle relies on fat oxidation in order to fully restore glycogen stores. The lack of this metabolic flexibility to correctly adapt to energy demands and nutrient availability constitutes a burden on energy homeostasis, and is speculated to be one of the major causes ultimately leading to metabolic disease (33).

8. Downstream of AMPK (II): PGC-1α and FOXOs

Finally, how does activation of the AMPK/SIRT1 axis translate into changes in the gene expression of mitochondrial and lipid oxidation genes? SIRT1 is an enzyme that catalyzes the deacetylation of a number of transcriptional regulators. Amongst them, we initially became interested in the peroxisome proliferator-activated receptor gamma (PPARg) coactivator-1a (PGC-1α). PGC-1α was originally cloned as a cold-inducible coactivator of PPARg in brown adipose tissue (61), but it has emerged as a potent coactivator of a plethora of transcription factors impacting on whole body energy expenditure (see (62) for review). The beauty in PGC-1α is that, once active, it coordinates the expression of genes related to lipid oxidation and mitochondrial metabolism. Illustrating this concept, muscle-specific PGC-1α transgenic animals

display increased mitochondrial number and function, as well as a higher relative amount of oxidative fibers (63). Conversely, mice with a muscle-specific deletion of PGC-1α show abnormal glucose homeostasis linked to a moderate reduction in the number of oxidative fibers, decreased endurance capacity and mitochondrial gene expression (64). Altogether, these data provide compelling evidence that PGC-1α is a key regulator of mitochondrial biogenesis.

Interestingly, PGC-1α activity is crucially regulated by its acetylation status. When highly acetylated, the activity of PGC-1α is rather low. However, upon deacetylation, PGC-1α ability to bind and coactivate transcription factors related to energy metabolism is dramatically increased (65). To date, SIRT1 is the only enzyme described to date with the ability to deacetylate PGC-1α. As expected from the fact that AMPK activates SIRT1, AMPK activation also culminated in PGC-1α deacetylation, and therefore, transcriptional activation of genes related to mitochondrial and lipid metabolism. Furthermore, we demonstrated that, in models of defective AMPK activity, PGC-1α activation upon energy stress is strongly compromised (58), disabling cells and animals to adapt to environmental nutrient availability and energy requirements. Also confirming our observations, it has been recently described that the action of AMPK on these genes is blunted in mice lacking PGC-1α (66).

However, it must be mentioned that the impact of AMPK/SIRT1 on transcriptional regulators is not limited to PGC-1α. We have also reported that the AMPK/SIRT1 axis of action also impacts on the forkhead family of transcripton factors (FOXOs). Upon AMPK activation, FOXO1 and FOXO3 become deacetylated by SIRT1 (55, 58). FOXOs are critical regulators not only of lipid metabolism but also of anti-oxidant protection. Consequent with this role, AMPK activation, as happens with Rsv, is linked to an increase in the expression of detoxification enzymes, like superoxide dismutase 1 and 2 (SOD1-2) and catalase. Another interesting point on FOXOs is that, like SIRT1 and Rsv, its activity is crucially linked to lifespan extension in lower mammals (67). Moreover, and unlike in mammals, yeast and worms do not have PGC-1α, and, in this organisms, FOXO acts as the main transcriptional regulator of the adaptations aimed to shift between carbohydrate and lipid fuels (67).

9. Phosphorylation and deacetylation – the double hit hypothesis

While performing our research on the AMPK/SIRT1/PGC-1α axis, one manuscript from Bruce Spiegelman's lab described that AMPK could phosphorylate and activate PGC-1α (68). This work was apparently at odds with ours, as such direct phosphorylation would negate the role for SIRT1 in the axis of action. However, it inspired us to dig deeper and understand how all these pieces could fit together.

To solve this apparent discrepancy, we used mutant forms of PGC-1α where the Ser^{538} and Thr^{177} residues phosphorylable by AMPK were mutated to alanine. This PGC-1α mutant, therefore, cannot be phosphorylated by AMPK. To our surprise, when this mutant form of PGC-1α was expressed, AMPK could not trigger PGC-1α deacetylation and remained inactive. We initially wondered whether the expression of this mutant would be affecting the ability of AMPK to increase NAD^+ or SIRT1 activity. However, none of these were the cases, as NAD^+ increased normally upon exposure to AMPK agonists, and SIRT1 activity was higher in the cells, as could be manifested by the deacetylation of other substrates, such as FOXOs. Consequently, these results indicate that phosphorylation of PGC-1α is necessary to target it for deacetylation. To fully test whether the lack of activity of the mutant was due to the defective phosphorylation *per se* or because it could not be deacetylated, we used a mutated form of PGC-1α where the 13 acetylable lysine residues of PGC-1α are converted to arginine, making it impossible to acetylate it, or, in other words, being a constitutively deacetylated mutant. We then took this "non-acetylable" (NA) mutant form of PGC-1α and, additionally, mutated the two AMPK phosphorylable residues to alanine, generating a "non-phosphorylable"-"non acetylable" (NP-NA) mutant. If phosphorylation determined activation of PGC-1α, this (NP-NA) mutant would be inactive. In contrast, if deacetylation was the main driver of activity, the NP-NA mutant would be active. Our results indicated that the NP-NA mutant was as strongly active as the NA mutant, concluding that deacetylation is the main driver of PGC-1α activity, while phosphorylation acts as a mechanism to target PGC-1α for deacetylation.

When thinking a little deeper on the implications of the above results, one finds out in fact a beautiful model of action. It is somehow intuitive, but generally eluded, that SIRT1 is a deacetylase that can target a large

variety of proteins, from histones to transcription factors to metabolic enzymes. Considering how tightly cellular functions and gene expression are coordinated, it would be extremely unlikely that, upon SIRT1 activation, SIRT1 unspecifically deacetylated all possible substrates. Of note, many SIRT1 substrate proteins regulate functions unrelated to energy homeostasis or AMPK action. Therefore, there must be some way by which AMPK indicates SIRT1 who to deacetylate at a given moment. Our results on PGC-1α indicate that phosphorylation on substrates can be a way to make a substrate "recognizable" for SIRT1, and, therefore, a way to specify SIRT1 action. Consequently, we deciphered

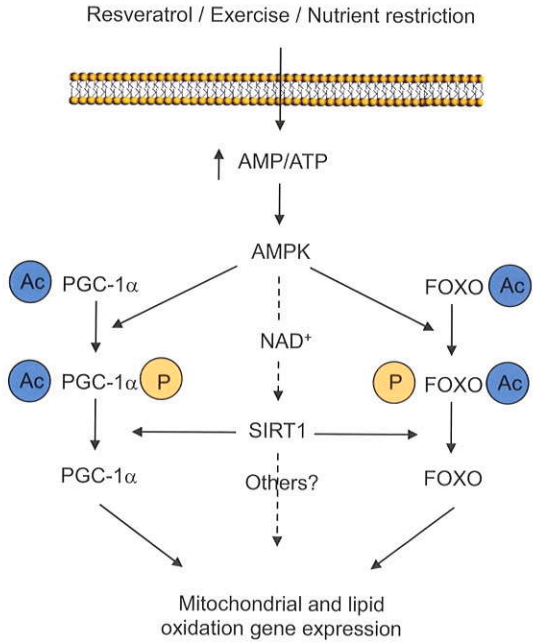

Figure 2. Translating energy stress into gene expression. Scheme illustrating the convergent actions of diverse forms of energy stress (exercise, nutrient deprivation) and drugs, such as resveratrol or metformin. All these inputs converge in the activation of AMPK, which triggers an increase in NAD^+, in turn activating SIRT1. Activated AMPK also phoshorylates PGC-1α and primes it for subsequent deacetylation by SIRT1. The impact of AMPK and SIRT1 on the acetylation status of PGC-1α and other transcriptional regulators, such as the FOXO family of transcription factors, will then modulate mitochondrial function and metabolism.

the cascade of sequential events by which energy stress increases mitochondrial biogenesis and oxidative stress resistance, namely: changes in AMP/ATP ratio are sensed by AMPK, which, from one side, activates SIRT1 by increasing NAD^+ and, from the other, phosphorylates PGC-1α. The phosphorylation of PGC1a allows recognition by SIRT1, which then deacetylates PGC-1α. Once deacetylated, PGC-1α can then coactivate the transcription factors regulating mitochondrial and lipid oxidation genes promoters (Figure 2).

10. Implications and future perspectives

In the work described above, we identify how energy stresses derived from low nutrient availability (fasting or glucose limitations) or high energy consumption (exercise) are sensed and translated into the activation of specific gene expression programs aimed to adapt to these circumstances. After the publication of this work in two seminal papers (55, 58), a number of different laboratories have not only confirmed our findings, but actually also identified the AMPK/SIRT1/PGC-1α axis as the key molecular mechanisms by which hormones like leptin (69), adiponectin (70) and fibroblast growth factor 21 (71) control mitochondrial gene expression. Therefore, the implications of our findings expand beyond energy stress and constitute a key mechanism by which also different hormones control metabolism.

Both AMPK and SIRT1 are highly conserved enzymes. That means that they exist in all eukaryotic organisms to day. Consequently, the axis of action we described constitutes a fundamental regulatory pathway conserved in organisms and aimed to respond to changes in nutrient availability. As mentioned above, PGC-1α is not present in lower eukaryotes. However, other transcription factors related to energy metabolism do. We have previously mentioned that FOXOs are also deacetylated by SIRT1 upon AMPK activation. In parallel to our investigations, another lab reported that FOXOs can be directly phosphorylated by AMPK (72). The double effect of AMPK on FOXOs biology suggests that, as with PGC-1α, there might exist a double hit on them, in which AMPK phosphorylation might target FOXO for deacetylation.

On a pharmaceutical edge, AMPK activation probably explains the beneficial actions of some anti-diabetic drugs, principally metformin,

the most widely prescribed anti-diabetic drug. It is surprising that, given the promising perspectives of using AMPK activators on the battle against metabolic disease, very little was known on how AMPK regulated gene expression (Figure 3). Our work constitutes one of the first detailed analyses on how AMPK regulates transcriptional events. Additionally, the identification of the downstream events of the pathway also provides a number of novel targets and strategies to develop new pharmacological agents.

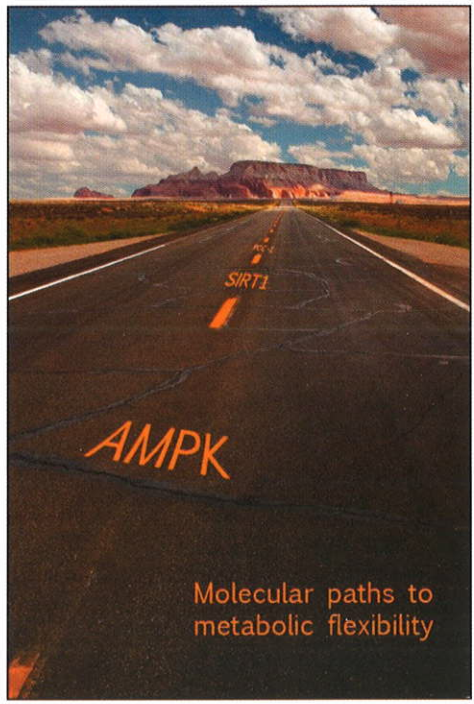

Figure 3. AMPK-SIRT1-PGC-1α conform a molecular path whose activation allows a flexible response to an ever-changing nutrient environment. AMPK is activated upon a number of metabolic stresses and leads to SIRT1 activation, which allows transcriptional adaptation through the coactivator PGC-1α. The road symbolizes the hierarchical activation of these proteins during the adaptation and how further downstream events linking this path to its final goal (transcriptional changes on mitochondrial genes- the mountain) are yet to be fully explored/perceived. (Road picture kindly provided by Roger Orpinell)

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