Mitochondrial function, energy expenditure, aging and insulin resistance

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**SUMMARY**
Mitochondria are the cells’ powerhouse that produce the ubiquitous energy currency (ATP) by consuming oxygen, producing water and building up the proton motive force. Oxygen consumption is a classical means of assessing energy expenditure, one component of energy balance. When energy balance is positive, weight increases. This is observed during the dynamic phase of obesity, and during body composition changes associated with aging. Whether intrinsic defaults in mitochondria occur is the matter of this review. Indeed, the ratio of ATP over oxygen consumed, which is not fixed, is one way of regulating heat release and ATP flux, but can also be the consequence of environmental conditions of mitochondrial work. For example, various hormones (T3, glucocorticoids), changes in lipid membrane composition, changes in food intake and exercise, and various drugs, can modify the ratio of ATP over oxygen consumed. Aging and insulin resistance are other regulators of this ratio. Finally there is a rising body of evidence linking diabetes to mitochondrial functions.

**Key-words:** Mitochondrial function · Energy expenditure · Ageing · Insulin resistance.

**Résumé**
Fonction mitochondriale, dépense énergétique, vieillissement et insulinorésistance
Les mitochondries sont les centrales cellulaires qui produisent la forme ubiquitaire de l’énergie utilisable (ATP) en consommant l’oxygène, en produisant de l’eau et en constituant le gradient de protons. La mesure de la consommation globale d’oxygène est une méthode classique pour évaluer la dépense énergétique, une des composantes du bilan énergétique. Lorsque la balance énergétique est positive, le poids augmente. Ceci est observé au cours de la phase dynamique de l’obésité ainsi que lors des modifications corporelles liées à l’âge. Existe-t-il des altérations intrinsèques de la mitochondrie, ceci est le propos de cette revue. Naturellement, le ratio ATP/consommation globale d’oxygène est variable, il s’agit d’un moyen pour réguler la production de chaleur (thermogenèse) et le flux global d’ATP, mais il peut également être la conséquence des facteurs environnementaux sur le métabolisme mitochondrial. Ainsi, des hormones (T3, glucocorticoïdes), des modifications de la composition lipidique membranaire, des changements dans les prises alimentaires ou l’activité physique, divers médicaments, peuvent modifier le ratio ATP/consommation globale d’oxygène. Le vieillissement et l’insulinorésistance sont également des facteurs modifiant ce ratio. Enfin, il existe actuellement un nombre croissant de données qui relient diabète et fonctions mitochondriales.

**Mots-clés :** Fonction mitochondriale · Dépense énergétique · Vieillissement · Insulinorésistance.

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Obesity is considered as an epidemic, with population weight and body mass index (BMI, the ratio of weight in kg to squared height in m²) increasing with time. This can be viewed as the result of a positive energy balance if the thermodynamic principles are considered the primam movens of weight and body composition changes. Indeed, according to these principles that were set by Lavoisier at the end of the 18th century, energy can neither be created nor destroyed and we have been continuously claiming that weight gain was the result of either or both a reduction in energy expenditure or an increase in energy intake.

An alternative point of view is to consider that changes in body composition are preceding changes in energy intake and energy expenditure. Then, energy imbalance is not the culprit but the victim of physiological, pathological or environmental events. The maintenance of a positive energy balance is merely maintaining weight and BMI at too high values, and regulatory processes are switched on to prevent further weight gain. Mitochondrial dysfunction could be one of those candidates that could explain changes in energy expenditure, body composition, energy intake, without affecting the energy balance in the first hand.

Mitochondria produce most of the energy required by cells. Mitochondria could be considered as the cells’ slave, simply responding to cell demand. When metabolic disarray occurs there are often alterations in mitochondrial functions. It is not yet clear whether then mitochondria are the culprit or the victim of the metabolic disarray. This ambivalence has received considerable attention. This review brings some arguments to tie together aging, insulin resistance and mitochondrial function.

Energy expenditure is decreased in elderly people

Healthy aging is that occurring naturally as age increases without interference with diseases. It is accompanied by large changes in body composition. Weight tends to increase until 75 yrs of age, then decreases. Because of reduction in height, BMI increases, and values above 25 kg/m² are common, meeting the criteria for overweight and obesity. However, if fat mass is increased as in obese adults, fat free mass is decreased. Both longitudinal and cross-sectional studies show that fat free mass decline is mainly the consequence of muscle mass loss (sarcopenia) and marginally of bone loss [1]. Muscle mass loss occurs from 35 yrs onward in men and from 45-50 yrs of age in women. It is associated with a reduction in functional capacities that are far more important than changes in mass would predict. Organ weight tends to remain stable or to increase [2]. Sarcopenia is due to deconditioning (the effect of the reduction in physical activity), to the repeated episodes of metabolic aggression (stress, diseases, drugs...) and a subclinical chronic inflammation. Fat mass gradually increases as does fatness. This concerns subcutaneous fat but also perivisceral fat as evidenced by the increase in waist circumference, without change in hip circumference [3].

Resting energy expenditure is the largest component of daily energy expenditure. Expressed in absolute values (MJ/d), resting energy expenditure decreases with age at a rate of about 2-4% per decade between 30 and 80 yrs [data acquired from both cross-sectional and longitudinal studies, review in [2]]. The decline starts at 30 yrs, accelerates from 40 yrs onward in men and from 50 yrs onward in women. This decline is mainly attributed to the loss of fat-free mass. However, an increasing amount of data argues that there is a genuine age-related metabolic defect, which is independent of changes in body composition [4-6]. After differences in body composition are taken into account, women expend about 11% less energy than men [7]. Although fat-free mass remains an important determinant of resting metabolic rate in the elderly [7], the percentage of variance explained is usually smaller than in young pairs. Several studies have shown that other parameters do influence the variability of resting energy expenditure. Fat mass becomes a significant contributor [8]. It is not yet possible to tell whether it is the mass of fat that is a determinant or whether some products released by adipose tissue (adipokines) influence energy expenditure. The level of usual physical activity is also an important determinant of total (TEE) and resting (REE) energy expenditure [4, 5, 7].

Diet-induced thermogenesis (DIT) is the amount of energy expended during the processing of food into nutrient stores. There is no clear evidence that diet-induced thermogenesis is altered with age. DIT represents a small proportion of TEE (5-10%), and any age-related change in DIT is unlikely to modify significantly the energy requirements [2].

It is widely accepted that there is an age-related reduction in physical activity, and of the energy expended for physical activity [9, 10]. It appears that the energy expended for a specific movement is hardly affected by age except for walking [7, 11]. Therefore, the reduction in energy expenditure is the result of a reduction of the time spent in physical activity. Therefore, daily energy expenditure is reduced with aging [12]. However, the figure is far more complex when centenarians are considered. Rizzo et al. [13] have shown that energy expenditure is increased in a centenarian group compared to the 66-94 yrs group. This is a unique observation which needs to be reproduced but it raises some questions about the energy metabolism of people able to live healthy for that long.

Total energy expenditure therefore declines with age. A little less than 50% of the reduction is attributed to reduced REE, while the remaining is associated with reduced physical activity. However, it is impossible to provide numerical values since TEE depends on body mass, fat free mass and physical activity.
Mitochondria produce ATP

Cellular ATP (adenosine triphosphate) is produced either by anaerobic glycolysis or by mitochondrial oxidative phosphorylation. This represents a huge difference in efficiency since anaerobic glycolysis yields 2 moles ATP per mole glucose, while under aerobic conditions, glycolysis produces 38 moles ATP. Mitochondrial respiration can therefore be viewed as an improvement in energy conversion. Mitochondria are the site of about 90% of oxygen consumption of the body. Figure 1 describes these reactions. Reducing equivalents produced by the Krebs cycle or coming from β-oxidation of fatty acids (NADH or FADH₂) are oxidized by the respiratory chain. Reduced equivalents provide electrons to the first two complexes of the respiratory chain located on the inner mitochondrial membrane. Electron transfer along the respiratory chain complexes builds up the proton motive force, by pumping protons through complexes I, III and IV of the respiratory chain. Electrons are finally accepted by oxygen to finally produce water. This proton motive force is the force driving F1-ATP synthase and the conversion of ADP (adenosine diphosphate) to ATP [14]. The generation of ATP therefore consumes oxygen. This is the origin of the terms mitochondrial respiration (oxygen consumption) and oxidative phosphorylation (OXPHOS).

In the preceding paragraph, oxidation is coupled to phosphorylation. We could dream of an organism where this coupling is so tight that there is no waste of energy. This would be economically an advantage (making ATP from the lowest resource). To give an example, in muscle three quarter of the potential energy contained in substrates is wasted as heat. Here we are obsessed by the fear of food shortage (2-3 millions year of human evolution) and by the necessary improvement or optimum of productivity.

Uncoupling is the concept by which some of the proton motive force is wasted and the ATP production is far from ideal. The proton motive force is either used to fuel the regeneration of ATP from ADP through ATP synthase or is wasted through leaks. Proton leaks play a key role in mitochondrial energetics. When protons leak through the membrane, heat is released. Proton leaks are either promoted by uncoupling proteins or by intrinsic characteristics of the internal membrane, like its lipid composition [14]. The obvious interest is for hibernating animals. They require less ATP than when moving, but do need heat to stay alive. This is accomplished by UCP1 in brown adipose tissue. In non hibernating mammals energy dissipation through uncoupling is estimated to account for 20-25% of the basal metabolic rate [15]. If more evolved organisms are prepared to pay a very high energy price to maintain this phenomenon, it must have some highly beneficial advantages. Among these advantages is thermogenesis, a safety valve for the avoidance of a too high membrane potential that would disrupt the membranes, an ability to continue carbon metabolism when ATP demand is low [16].

Regulation of energy production is driven by ATP needs, and it is assumed that the efficiency of the conversion of energy of reducing equivalents into ATP is not fixed. There is therefore scope for energy wastage or saving, and different amounts of oxygen can be consumed to produce a defined quantity of ATP. Propagated to the whole body level, it means that the quantity of ATP required for achieving a function may imply the oxidation of varying quantities of energy substrates (glucose and fatty acids) because of adaptive changes in energy production by mitochondria. Depending on the challenge, the oxidative capacity can slightly increase or can vary over an order of magnitude, the mitochondria reacting accordingly with either subtle changes in the activity of OXPHOS, with an increased biosynthesis of some of the OXPHOS subunits, or with an increase in the number and size of the organelles [17]. Therefore, mito-

Figure 1
Mitochondrial respiratory chain (Q: quinone; C: cytochrome). Reducing equivalents (NADH...) produced by the Krebs cycle or coming from beta-oxidation of fatty acids are oxidized by the respiratory chain. This consumes oxygen (O₂ which accepts the electrons) to finally produce water, while protons are pumped through complexes I, III and IV of the respiratory chain to create a proton gradient. This proton motive force is the force driving F1-ATP synthase (complex V) and the conversion of ADP to ATP. If the number of protons pumped per electron transferred changes, or if part of the proton gradient is diverted away from ATP synthase, the ATP yield per mole oxygen consumed decreases (P:O₂ ratio). It ensues that since the ATP demand drives mitochondrial respiration, more substrate is used and more oxygen is consumed at the whole body level.
Mitochondria are the primary targets for the regulatory agents affecting ATP yield, and such major regulators are steroid and thyroid hormones [17]. Thyroid hormones affect both the nuclear and mitochondrial genome, and both mitogenesis and mitochondrial respiration [18-20]. The mechanisms involved in the regulation by glucocorticoids are far less well understood [21-25].

What happens to mitochondria during aging?

Liver and the digestive tract account for about 25-30% of resting energy expenditure while muscle takes 20 more percent [15]. Therefore, the aging-induced changes in those two organs are potentially important to explain changes in energy expenditure at the whole body level. Liver size hardly changes with age while muscle mass decreases [1].

It is known that changes in those gene expressions associated with mitochondrial functions are observed with aging [26] especially muscle gene expression for subunits of ATP synthase and NADP transhydrogenase [27-28]. At one extremity of the aging process, there is a maturation profile for most of the steps leading to ATP generation in the rat liver, *i.e.* activities of complexes of the respiratory chain and respiration increase between 2 and 5 weeks of age [29]. Between 80 and 180 days of age, rats decrease respiration, adenosine nucleotide transporter (ANT) and COX (complex IV) content. Only palmitate-induced but not basal proton leak increase with age, without apparent change in the efficiency of ATP production [30]. But, it has to be noted that these data were obtained in very young rats and adults.

At the other extremity of the aging process (transition between adult to old animals), oxidative capacity is reduced with aging in muscle and heart [31, 32], even in humans [33, 34]. More importantly, in *vivo* muscle ATP content is reduced in old humans compared to younger pairs [33]. Mitochondrial density may also be decreased in muscle [34]. In contradiction with these results, muscle oxygen consumption in Wistar rats is slightly higher in very old (33 months) than in youngest rats [35]. Therefore, there may be arguments to indicate that the production of energy in the liver is less efficient in old animals. By virtue of the size of the muscle compartment, this may play a key role in the whole body energy budget. However, more work is needed, especially in humans, to unravel the influence of aging on mitochondrial function.

Very few data are pertinent to the liver. In very old mice (30 months) compared to 3 month old pairs, oxygen consumption is reduced by 15%, proton leak is increased and the fraction of oxygen consumption attributed to ATP production is reduced while that affected to proton leak is enlarged [14].

Aging is associated with changes in the lipid composition of the inner mitochondrial membrane with an increase in the proportion of C22:4 and C22:5 fatty acids and a decrease in the ratio of C18:2 (linoleic) and C18:1 fatty acids [36, 37]. It is suggested that the decreased ratio in linoleic acid reduces respiration [38] while it influences protein leak [39, 40].

A case for oxidative stress

According to the oxidative stress theory of aging, progressive declines in physiological function can be the result of the accumulation of oxidative damages caused by reactive oxygen species (ROS). Between 0.2 and 2% of oxygen taken up by cells is converted into ROS and mitochondria are the main site for ROS production [14]. ROS production is increased when the electron carriers in the initial steps of respiratory chain harbor excess electrons. Electrons residing in the electron carriers can be donated directly to oxygen to generate superoxide anion, which will be converted into $\text{H}_2\text{O}_2$ by peroxide dismutases. Iron-sulfur centers in mitochondrial enzymes are particularly sensitive to ROS inactivation. Hence, the mitochondria are the primary target for cellular oxidative damage.

The rate of ROS production is affected substantially by energy expenditure. ROS production is highest when the ATP demand is low and the proton motive force is high. If the mitochondria are tightly coupled, most of the energy of the proton motive force is used by ATP synthase to produce ATP, and very little energy leaks as heat. In the presence of excess calories or little expenditure through exercise, the electrochemical gradient is not dissipated, and membranes tend to hyperpolarize. The respiratory chain continues to draw on the excess calories and electron carriers become maximally occupied with electrons, tending to spill over to O$_2$. In individuals who exercise, electrons flow through the respiratory chain to sustain the proton motive force and the electron carriers retain few electrons, which limits ROS production.

ROS are harmful for mitochondria (membranes, protein), for mitochondrial DNA (which has lower repair capacities than nuclear DNA) and for other components of the cytosol and of the nucleus. Mitochondrial membranes are highly sensitive to oxidative damage, especially their long chain polyunsaturated fatty acid components. Peroxidation of these fatty acids and production of highly reactive aldehyde species result in secondary detrimental effects [14]. Mitochondria harbor a specific phospholipid, cardiolipin that is highly unsaturated and very sensitive to oxidative damage. Both the quantity and the fatty acid composition of cardiolipin influence OXPHOS efficiency, by influencing the activity of the adenine nucleotide transporter (ANT) and that of COX. Furthermore, in vitro studies have shown that ANT and ATP synthase are highly sensitive to oxidative stress.

Relationship between insulin resistance, diabetes, the metabolic syndrome and mitochondrial function

Insulin resistance is a key feature of type 2 diabetes and is the *primum movens* of the metabolic syndrome [41]. It is also a trait of aging, both because of reduced physical activity [42] and increase in visceral fat (see above the changes in body composition).
The relationship between diabetes and insulin resistance and mitochondrial alterations in genome and functions is rather complex. On one hand, mutations in either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) can be associated with diabetes. A classical example is the A3243G mutation which, when present at low level, results in diabetes with or without deafness. On the other hand, metabolic abnormalities associated with insulin resistance can be associated with mitochondrial dysfunctions. Wallace has reviewed extensively this subject [26].

The special case of MODY

Maturity onset of diabetes in the young (MODY) is an early onset of the autosomal dominant form of type 2 diabetes that is associated with mitochondrial dysfunction. MODY is the result of mutations in glucokinase. MODY 1 is due to mutations in the hepatocyte nuclear factor (HNF-1α), MODY 3 to mutations in the hepatocyte nuclear factor (HNF-4α), and MODY 4 to mutations in insulin promoter factor (IPF)-1. Glucokinase is thought to be the glucose sensor. HNF-1α is a transcription factor and mutations in its gene are associated with diabetes. HNF-1α is also important in regulating the expression of GLUT-2 transporters. HNF-4α is an upstream regulator of HNF-1α.

A mitochondrial defect at the origin of β-cell failure

There are arguments to suggest that mitochondrial defects affect insulin secretion. When the affinity for ATP of the ATP dependent K⁺ channel is reduced it results in a severe reduction in serum insulin, and hyperglycaemia. Inactivation of T�m in β-cells, mitochondrial transcription factor, results in increased blood glucose and apoptosis of β-cells. Thus mitochondrial ATP production is critical in the signaling system of β-cells.

Mitochondrial dysfunctions and the metabolic disorders

A higher incidence of metabolic syndrome was found in individuals with mtDNA mutations, some of the mutations being associated with reduced mitochondrial ATP production [26]. As the age of the onset of the proband increases, the probability that the mother is the affected parent also increases, reaching a ratio of 3:1 at 46 years. It is usual to consider that the mother transmits mitochondrial abnormalities because spermatozoo contribute very little to the egg mitochondrial equipment.

Furthermore mitochondrial functions and gene expression are often down-regulated during type 2 diabetes. Insulin resistant offspring of type 2 diabetic patients display impaired mitochondrial energetic [43]. Similar findings are observed in insulin resistance associated with age [44]. It also seems that insulin directly stimulates mitochondrial respiration [45, 46]. Finally, he level of PPARγ-coactivator-1, a major regulator of mitochondrial biogenesis and fat oxidation is altered in diabetes [27].

Wallace [26] proposes a very elegant theory to unify all these findings. There may be an energetic interplay between the body’s various organs. The organs can be divided in four categories: energy-utilizing tissues (skeletal muscle, heart, kidney and brain), energy-storage tissues (brown and white adipose tissue), energy-homeostasis tissue (liver) and some energy-sensing tissues like pancreatic α-β-cells. All these organs interact to coordinate the utilization and storage of energy, based on the availability of the calories in the environment. During the time of plenty, and mainly the growing season, when plants accumulate starch, blood glucose concentration may slightly rise (plasma glucose could be a surrogate for monitoring plant calorie abundance). Energy-sensing β-cells respond by secreting insulin. The insulin signal informs energy-utilizing tissues (muscle and heart) to down-regulate mitochondrial energy utilization, since food seeking behavior is not pressing. It also informs energy storing tissues to store the excess calories as fat.

When plant calories become limiting, mild to severe calorie restriction occurs, insulin secretion declines and the α-cells secrete glucagon. Their hormonal message informs energy-utilizing tissues to up-regulate OXPHOS, allowing food seeking capacity. They also mobilize energy storages so that triglycerides are used to fuel increased mitochondrial OXPHOS. At the same time, signals inform the liver to make up glucose. This model is based on recent advances in transcriptional regulation of mitochondrial gene expression.

These observations link diabetes and mitochondrial disorders. It is then tempting to associate these defects in metabolic disorders such as those of the metabolic syndrome. In individuals with a defect in OXPHOS, the capacity of energy utilizing cells to oxidize fuels and to generate ATP is reduced. In face of a high calorie diet, tissue utilization of fuels is reduced, resulting in a higher glycaemia, the initiation of the insulin resistance cascade, leading to diabetes. β-cells respond by increasing insulin secretion. The presence of high calorie diet induce a high ROS production by the respiratory chain which damages further mitochondrial function, creating a vicious circle. However, this is not supported by strong evidence yet.

Conclusion

Mitochondria are the cell’s energy (ATP) powerhouse, which produce energy by consuming oxygen. The ratio of ATP produced to oxygen consumed varies and can be viewed as a regulatory mechanism, which has long been regarded as a means to generate heat. Today, an increasing amount of data suggests that is not only the case. This regulatory mechanism can be used to meet a very high demand in ATP and can regulate ROS production. ROS production is the “evil face” of the increased efficiency of OXPHOS as compared to simple glycolysis.
Changes in lipid mitochondrial membrane composition and variations in metabolite concentrations can affect ATP production in various cells. It is obvious that ATP shortage will affect key functions (for example exocytosis of insulin from β-cell which requires energy).

Aging and various situations of insulin resistance probably involve mitochondrial dysregulation.

We are now entering a new age, that of mitochondrial medicine. When these relationships will be perfectly understood, it will be time for therapeutic trials where changes in nutrition (quality of the lipid ingested to change the lipd membrane composition) and drugs will be used to target changes in mitochondrial functions.

References