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# **Lifestyle and Aging Effects in the Development of Insulin Resistance — Activating the Muscle as Strategy Against Insulin Resistance by Modulating Cytokines and HSP70**

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Additional information is available at the end of the chapter

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## **Abstract**

This chapter discusses about subclinical processes related to insulin resistance development that worsen the muscle metabolic functions, generated by factors such as lifestyle (bad quality food intake and sedentary behavior) and aging. Also discussed are the effects of regular physical exercise as a strategy to prevent the metabolic impairment in organisms, approaching since muscle subclinical molecular processes to the whole body's integrative physiology. Insulin resistance development includes modification in the pattern of inflammatory cytokines, heat shock proteins, tissue-specific defects in insulin action and signaling, oxidative stress and ectopic lipid deposition. The exercise is a known modulator of all parameters listed above and has important role in the regulation of "immune-metabolic" homeostasis from the muscle to the whole body. This chapter aims to present a new molecular approach related to the control of metabolism and encourage scientists and students to propose new strategies against insulin resistance and diabetes type 2 developments.

**Keywords:** Insulin resistance, exercise, heat shock proteins, cytokines, oxidative stress

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## **1. Introduction**

Metabolism can be defined as the sum of all reactions that occur in the whole body, from cells to complex system, with a multi-organ talk about energy transfer, signaling, and then regula-

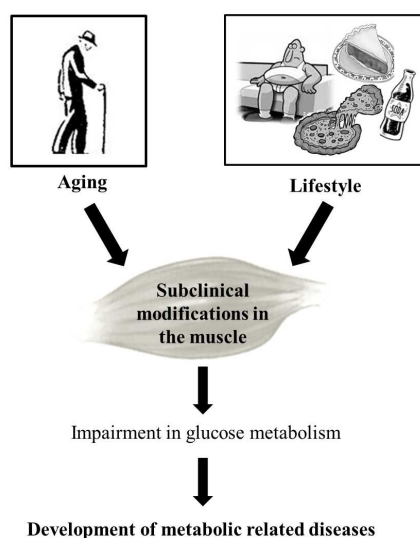
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tory pathways of metabolic status. One of the most important parameters of human metabolic homeostasis is glycemia that results from the availability and the utilization of nutrient sources. In this way, the cellular uptake and glycogenesis in skeletal muscle fibers have important implications in the regulation of blood glucose. This metabolic process was regulated by insulin that acts on muscle promoting the translocation of glucose transporter-4 (GLUT4), which is the most abundant insulin-dependent transporter in the cell membranes of the skeletal muscle, heart muscle, and adipose tissue, which leads to the uptake of glucose into the cell.

In this scenario, the muscle can be considered an important organ in glucose metabolism regulation, functioning at the same time as locus of start- and end-point of metabolic disorders related to glucose metabolism, and also the key organ of intervention strategies against insulin resistance. This chapter proposes an overview about effects of physical activity and exercise in the muscle as a strategy against insulin resistance. The physiological approach in this chapter is based on two major signaling pathways and biomarkers of muscle function, as well as its interaction with other cells and tissues—the cytokines and heat shock proteins (HSPs).

Skeletal muscle contains anatomic and physiological characteristics that represent different possibilities and functions in terms of metabolic properties and also in contractility and mitochondrial activity. There are three important factors that influence these characteristics: age, the dietary behavior, and the levels of physical activity. Together, these factors determine the muscle health status and capacity of physical performance, then, consequently, the whole body homeostasis. Aging, high caloric diet consumption, and sedentary behavior leads the progression of the muscle dysfunction that culminates in the loss of metabolic homeostasis, promoting dyslipidemia and glycemic alterations.

However, these outcomes are presented in the established metabolic disease, while many subclinical processes precede the onset of disease. Subclinical modifications also accompanied the undesired progression of metabolic disease, increased the incidence of comorbidities and increased hospital admissions. These silent subclinical effects, such as inflammation, oxidative stress, and molecular alterations, decreased gradually the individual health status and decreased the quality of life (Figure 1).



**Figure 1.** Muscle as the target of subclinical modification related to metabolic disease development.

Thus, in this chapter we first discussed factors that worsen the muscle's subclinical metabolic functions, such as lifestyle (bad quality food intake and sedentary behavior) and aging; second we discussed about the effects of exercise as a strategy to prevent metabolic impairment, in the maintenance of muscle health, and to improve body homeostatic control, from the muscle's subclinical molecular processes to the whole body.

## 2. Lifestyle

A high dietary fat intake and low levels of physical activity characterizes much of the overall lifestyle. Surplus of fat intake is stored in many human tissues and these intracellular lipids serve as a rapidly available energy source during, for example, physical activity. Mainly in the sedentary condition, lipid excess leads to the development of modern diseases such as obesity and insulin resistance [1].

The consumption of high-fat diets (HFD) is associated with an excessive storage of fatty acids in the skeletal muscle [1]. The human (and also laboratory animals) body are composed of several muscles that contain slow-twitch (type I) fibers, which contain a high number of mitochondria and use oxidative metabolism as an energy source, and fast-twitch (type II) fibers, which generate energy mainly through glycolysis. High intake of hypercaloric or high-fat diets promotes a series of structural and metabolic changes that affect muscle capacity. An inadequate diet, such as HFD, induces muscle adaptations at molecular levels, promoting an increase in the proportion of oxidative fibers (type I fiber) by increasing the levels of the myosin heavy chain, slow fiber type protein, complexes of the oxidative phosphorylation, and the mitochondrial membrane composition. However, despite the increased oxidative fibers proportion, these modifications are insufficient to prevent impairments in oxidative metabolism [2]. The long-term HFD consumption promotes a decrease in the muscle mass and an increase in muscle triglyceride accumulation in parallel to the increased expression of biomarkers of mitochondrial metabolism such as succinate dehydrogenase complex subunit-myocytes and within the fascia surrounding skeletal muscle (increase in the intra- and enzyme of  $\beta$ -oxidation), and the phosphorylation of acetyl-CoA carboxylase (ACC) (regulation of lipid synthesis). These alterations contribute to the morphological impairment known as myosteatosis or the ectopic skeletal muscle adiposity that represents fat infiltration within myocytes and within the fascia surrounding skeletal muscle (increase in the intra- and intermuscular fat content, respectively) [3]. Mounting evidence indicates that elevated intramyocellular lipid deposition is associated with diminished insulin sensitivity in the skeletal muscle, promoting insulin resistance. Since fiber type I have a higher capacity for "fat burning", studies have reported a negative association between adiposity and the relative percentage of type I fibers. In other words, more muscle oxidative capacity results in less adiposity [4]. The increase in type I fiber proportion represent an attempt to restore the energy homeostasis between the source and energy demand. If this adaptive response is insufficient, the human body is susceptible to metabolic dysfunction.

Insulin resistance in the skeletal muscle in humans is associated with decreased oxidative capacity of ATP synthesis and also related to the decrease of many genes expression. Genes that control mitochondrial activity, including peroxisome proliferator-activated receptor

gamma coactivator 1-alpha (PGC-1 $\alpha$ ), may indeed play a crucial role in the development of mitochondrial dysfunction, insulin resistance, and diabetes mellitus type 2 (T2DM) through the western lifestyle that is rich in hypercaloric or high-fat diets. Three days of HFD reduces PGC-1 $\alpha$  protein levels by approximately 20% in humans and 40% in C57B1/6J mice after three weeks on an HFD treatment [5]. Sparks et al. [5] emphasized that HFDs in both humans and mice were associated with the reduction in the expression of genes involved in electron transport chain, nuclear genes encoding mitochondrial proteins (e.g., mitochondrial carrier proteins), and those involved in mitochondrial biogenesis (e.g., PGC1 and PGC), supporting the hypothesis that HFDs or high-fat flux explain the reduction in oxidative phosphorylation pathway (OXPHOS) genes seen in aging, the prediabetic state, and in overt diabetes.

Ciapaite et al. [6] suggest that the consumption of unhealthy obesogenic HFDs in combination with a sedentary lifestyle may create a vicious cycle by impairing skeletal muscle function and decreasing exercise potential, which may lead to further aggravation of obesity and skeletal muscle dysfunction. The adaptation response to dietary lipid overload occurs by fiber-type-specific mechanisms, leading to differential impairment of fast-twitch and slow-twitch skeletal muscle contractile function. Fast-twitch fibers suffered impairment in mitochondrial ATP production and Ca<sup>2+</sup> homeostasis, and slow-twitch fibers have changed the sarcomere composition and force production. Together, changes in both types of fiber related to the consumption of HFD affect the functionality and muscular performance [6].

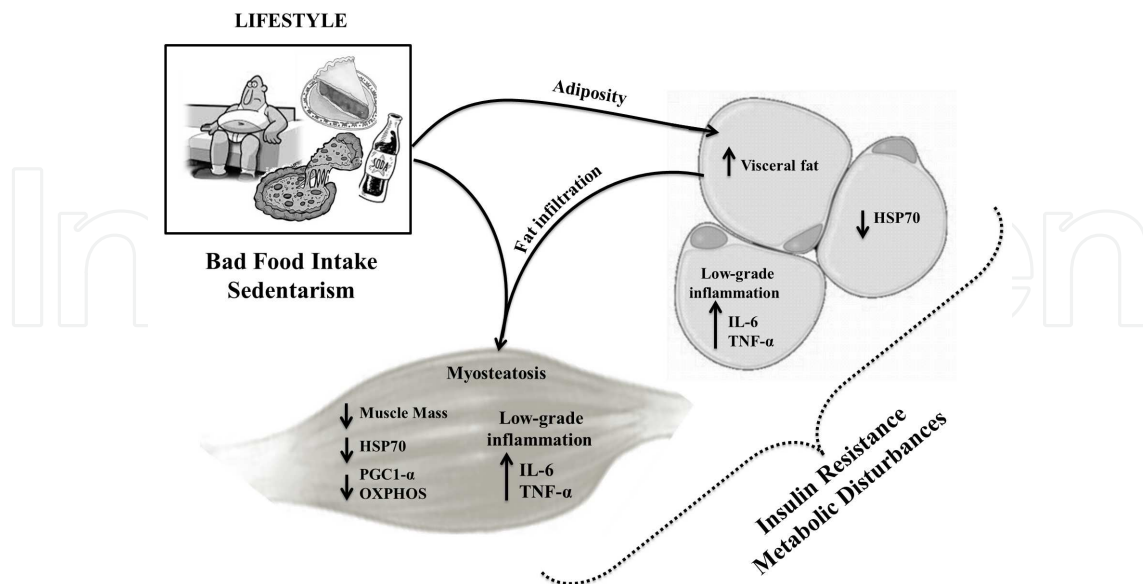
Muscle metabolic function can also be impaired by increasing visceral fat accumulation. This fat is more lipolytic (rapidly turned over) than subcutaneous fat and less sensitive to the anti-lipolytic effect of insulin. As abdominal fat develops in obesity related T2DM, the adipocytes release non-esterified fatty acids, many inflammatory products and reactive oxygen species (ROS). When non-esterified fatty acids accumulate in cells they undergo  $\beta$ -oxidation, forming acetyl-CoA that enters the Krebs cycle. The excessive amount of free radical formed in this situation requires a protective response against oxidative stress—decreased entry of glucose into the cell to avoid more free radical formation by glucose metabolism. Thus, indirectly, whole body adiposity inhibit the phosphorylation of tyrosine in insulin receptor substrate 1 (IRS-1) as a 'protective mechanism' that down-regulates insulin sensitivity in the muscle [7]. In the same direction, fat accumulation inside muscle cells may lead to the entry of fatty acids into the mitochondria where they are prone to ROS production [1]. Considering that the muscle tissue plays a key role in the regulation of metabolism, especially concerning glucose levels, disturbances on functionality and in the redox state in the muscle might be related to diabetogenic effects.

Adipose tissue insulin resistance and dysfunctional lipid storage in adipocytes are sentinel events in the progression toward metabolic dysregulation with obesity [8]. Many of the complications of obesity are due to a chronic subclinical inflammation. Gene expression profiling of the obese phenotype revealed a differential regulation of many pro-inflammatory genes. In part, a significantly higher number of macrophages are present in obese adipose tissue explain the increase in pro-inflammatory status [9]. Thus, a major determinant for many obesity-induced implications is the low-grade inflammation of the enlarged adipose tissue and the persistent release of inflammatory adipokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6) [10, 11]. The basis for this view is that increased circulating levels of several

markers of inflammation, both pro-inflammatory cytokines and acute-phase proteins, are elevated in the obese [11].

The most crucial step in insulin signal transduction, the phosphorylation of the IRS-1 can be blunted by both TNF- $\alpha$  and free fat acids conferring insulin resistance in target tissues. Also, several serine kinases are involved in this impaired insulin signaling as c-jun amino terminal kinase (JNK) that is potently induced by TNF- $\alpha$  and free fat acids. Interestingly, increased intramyocellular lipid levels is correlated to insulin resistance with no significant changes in TNF- $\alpha$ , IL-6 or adiponectin concentrations, suggesting that a dysregulation in muscular fatty acid oxidation per se may mediate insulin resistance by mitochondrial defect in oxidative phosphorylation [9]. Thus, systemic inflammation may participate in insulin resistance development but muscle metabolism impairment can be crucial to T2DM installation.

Conditions of tissue stress, such as oxidative stress, inflammation, and molecular alterations, could develop initial compensatory responses of cytoprotection, such as the expression of HSP70. The severity of the metabolic state (measured by glycemia, glucose intolerance, obesity, or insulin resistance) promotes modification in the muscle and adipose HSP70 expression. The initial impairment and moderate glucose intolerance promotes an increase in HSP70 content in adipose tissue and no modification in the muscle [12] as an initial adaptative response, while obesity plus T2DM have an decrease in both muscle and adipose HSP70 content [13]. Since HSP70 expression can inhibit nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and JNK dependent mechanisms that promote insulin resistance, blunted heat shock response can be interpreted as an additional silent effect of HFD consumption and sedentarism. These results support the hypothesis that an increase in visceral fat, closely associated with the lifestyle (high-fat intake and sedentarism) promotes subclinical effects that is associated with the development of muscle insulin resistance [14] (Figure 2).



**Figure 2.** Lifestyle promotes molecular and metabolic alterations in muscle and adipose tissue, promoting insulin resistance.

Another fact is that increased extracellular HSPs levels (mainly 70kDa isoforms as eHSP72) are correlated with oxidative damage and stress in diabetes and in obesity. Moreover, the content of the plasma eHSP72 is higher in T2DM obesity compared to DM or only obese subjects, suggesting eHSP70 levels as biomarker of glucose homeostasis unbalance [15]. Together, eHSP70 and pro-inflammatory cytokines represent a link between metabolic and immune related events. During severe stress response that can cause insulin resistance, we can observe the action of inflammatory cytokines such as IL-1 and TNF- $\alpha$  [16]. Under hypoglycemic conditions, as a part of the homeostatic stress response, HSP70 is secreted to the bloodstream and may be purely a danger signal to all the tissues of the body for the enhancement of immune and metabolic surveillance state or actively participate in glycemic control under stressful situations [17]. Additionally, eHSP70 can bind receptors in immune cells that induce pro-inflammatory cytokine release.

### 3. Aging

Aging can be understood as a natural result between destructive processes that act on cells and organs over a lifetime and the responses that promote homeostasis, vitality, and longevity [18]. Aging is associated with the decline of a number of physiological systems, such as when they reach critical levels of functionality. The time of this decline depends on variables such as genetics, development, metabolism, and lifestyle [19].

The sarcopenia in the elderly is a significant public health problem because it leads to gradual slowing of movement, increasing the risk of injury, and loss of independence. The mass and muscle strength are lost due to a decline in neuromuscular transmission, the structure, function, metabolism, and muscle performance. Muscle, bone, and their metabolic needs must be considered as a single entity. Muscle strength, energy balance, and bone health influence the ability to perform physical tasks of daily life and the ease with which these tasks can be performed determines the degree of independence that an individual can keep up with advancing age [19].

Cross-sectional studies indicate that muscle mass decreases with aging in the early third decade [20] and that approximately 10%–15% of the mass is lost between the ages of 20 and 50. In women with menopause, muscle mass decreases at an accelerated rate of approximately 1% per year, affecting muscle strength and bone integrity, with a negative impact on activities of daily living [21]. In addition, older, postmenopausal women are more likely to develop abdominal adiposity and highest lipase activity, which is associated with increased systemic levels of inflammatory cytokines and free fatty acids [22].

Similar to what happens in obese individuals, elderly individuals also exhibit elevated levels of inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 (IL-1), TNF- $\alpha$  and C-reactive protein (CRP) in the extracellular space (e.g., serum and plasma). This pro-inflammatory profile has no acute characteristic such as after acute traumas (sepsis, stroke, e.g.), but is a low-grade chronic inflammation present in elderly individuals that has been defined as "inflammaging." Epidemiological studies have shown that "inflammaging" is a risk factor in

the accelerated decline of muscle mass and strength, and these changes in muscle performance can be a critical step in mediating the causal link between "inflammaging" and disability [23].

The pathogenesis of T2DM is established by a vicious cycle of metabolically induced inflammation, impaired insulin responsiveness, and loss of homeostatic signaling. Approach in metabolic, pro-inflammatory cytokines may influence the state of the insulin response and can lead to insulin resistance in acute or chronic form. Adipocytes and macrophages secrete inflammatory cytokines (e.g., TNF- $\alpha$ ) that activate the serine-threonine kinases JNK and inhibitor of  $\kappa$ B kinase (IKK- $\beta$ ) in insulin sensitive organs, such as liver, skeletal muscle, and adipose tissue. JNK and IKK- $\beta$  both impair the function of the insulin receptor and interfere with downstream signaling [24].

Cytokines released into the bloodstream can bind to receptors and activate intracellular signaling pathways, thus facilitating the activation of phosphorylation of JNK and inhibiting the signal transduction of insulin. JNK is also activated by stress and fatty acids [25]. Situations of inflammatory stress can also affect the process of regulating the availability of non-insulin dependent glucose as the activation of AMP-activated protein kinase (AMPK) enzyme sensitive to intracellular energy status [25, 26]. This pathway is frequently studied in the animal model of insulin resistance (or in some cases T2DM) by high fat diet protocols (Goettems-Fiorin et al., manuscript in preparation). These studies of insulin resistance show that several structural and metabolic changes in the muscle are correlated with increased levels of fasting glucose and/or response to glucose intolerance during glucose tolerance test (GTT).

Aging and tissue degeneration also involve the accumulation of damage to cellular macromolecules. Chemical damage due to oxidative stress, glycation, and the addition of sugar residues has the capacity to modify both DNA and proteins. Situations of inflammatory stress, as described above, are able to activate the expression of genes that perform the cytoprotection in many tissues, especially when we talk about the muscle tissue. The muscle, which is rich in protein chains, uses the expression of HSPs to repair the damage that can be induced by both the consumption of high-fat diets and aging.

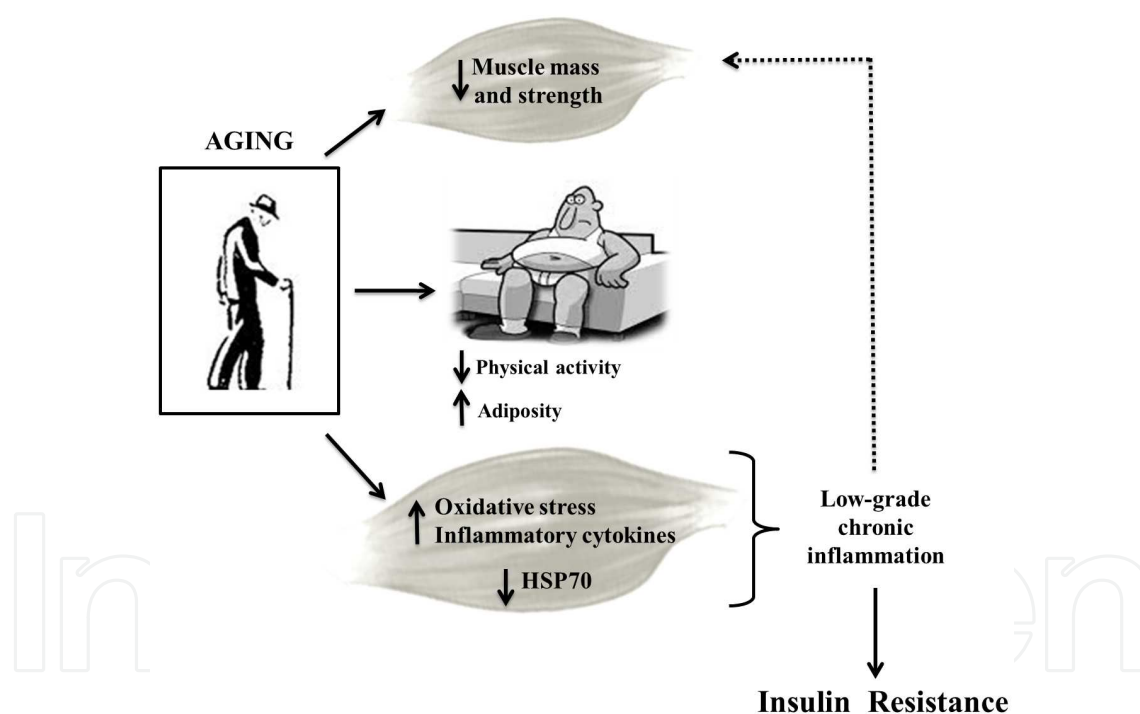
It has been assumed that HSPs, particularly 70kDa (HSP70) levels, generally decrease during normal aging processes. As HSP70 functions to chaperone cytosolic proteins to allow appropriate refolding or degradation by ubiquitination pathways, these reductions in HSP70 have been implicated in the aging process, as cells accumulate oxidation products without adequate cellular protection [27]. Age-dependent decline in the heat shock response is also observed in muscle tissues. In these tissues, vigorous contraction leads to the induction of HSP that is cytoprotective in nature. These effects are severely blunted in the muscles of older animals and aging humans, suggesting that decline in muscle mass and force generation may be related to loss of HSP expression [18].

The leak of HSP70 expression in muscles of older subjects represents a failure to exercise adaptation, in terms of both structural and metabolic characteristics. Loss of heat shock factor 1 (HSF1) activity and expression, the transcription factor of HSP70, which is at the same time the result and consequence of obesity and aging, induce an impairment of muscle mass maintenance and insulin signaling (Figure 3). The relevance of HSP70 expression in the muscle



can be identified since the pro-inflammatory mediators JNK and NF- $\kappa$ B can be inhibited by the HSP70 expression, which is low in age, T2DM, obesity, and finally in sedentary subjects, suggesting that active muscle can be a non-pharmacological option to prevention or repair this “meta-inflammation” process that harm the human health.

Older adults with diabetes are vulnerable to accelerated loss of lean body mass. Declines in lower extremity muscle mass in particular, can be associated with decreased muscle strength and lead to muscle weakness, poor lower-extremity performance, and mobility loss, all of which have been reported in persons with diabetes [28]. Aging also produces muscle changes such as decrease in type II fibers, decline in oxidative capacity of type IIa and I fibers, and forming a favorable development of metabolic alterations. During aging, changes in the morphological structure of the skeletal muscle and also in the number and function of mitochondria are observed in the decline in this tissue. Studies have consistently shown that PGC-1 $\alpha$ , a transcription factor that promotes mitochondrial biogenesis, decline with aging and in many age-related chronic diseases suggesting that such decline may explain the progressive mitochondrial dysfunction with aging [23].



**Figure 3.** Aging alterations in the muscle related to insulin resistance.

As previously described, both the lifestyle and aging promote body changes, which may be directly related to skeletal muscle dysfunctions. Changes in composition and functional capacity of type 1 and type 2 fibers, which reduce the mass and muscle strength, occur due to molecular disorders affecting the muscle, whether caused by the high consumption of HFD and physical inactivity or by aging. Myosteatosis, reduction of PGC-1 $\alpha$  development of a low-grade inflammatory with increased expression of pro-inflammatory cytokines such as TNF-

$\alpha$  and IL-6, and reduction of HSPs, represent the body's response to aging is quite similar to lifestyle (hipercaloric food intake and sedentarism). The term "inflammaging", described in several studies highlights the low-grade inflammatory condition in the aging process, with an increase of pro-inflammatory cytokines (IL-1, IL-6 and TNF- $\alpha$ ) that promotes the activation of JNK and IKK- $\beta$  in the muscle interfering with the sensitivity to insulin (Figure 3). Thus, many subclinical process of insulin resistance development occurs in the muscle and activating this organ can be an important strategy to obtain a healthy aging process.

#### 4. Exercise

Human lifestyle changed from daily strong physical activity patterns of hunter-gatherer societies (about 1,000–1,500 kcal/day with 3–4 h/day of moderate-to-vigorous physical activities) to digital industrialized society with dramatic reductions in daily activity levels. Physical inactivity is a "modern" human behavior that increases the risk of many adverse health conditions, including the development of diseases, such as T2DM and metabolic syndrome, decreasing the life expectancy. Although nowadays health professionals and population has knowledge about the necessity of exercise practice) to avoid many diseases, the definition of sedentarism still lacks a consensual definition [29, 30].

From ancient physicians, including Hippocrates and Plato, scientists believed in the benefits of physical activity for human and animal health. Classical epidemiological studies, as performed by Morris et al. [31], showed comparative evidences about the risk of sedentary routine to the cardiovascular system. However, in quantitative analyses, many protocols and descriptions were used in the sedentarism research field to classify an inactive person. "Active" definition may be based on total energy expenditure since an increase in daily energy expenditure (aprox.150 kcal) can promote health benefits. Other definition is about accumulative activities during one week, since less than 30 minutes of moderate-intensity physical activity represent also a cardiovascular risk factor. Recently, it was estimated that physical inactivity causes 7% of the burden of disease from T2DM and causes 9% of premature mortality, or more than 5 million of deaths [30]. If the percentage of inactivity decreases at least 10% in the world's population, it is estimated that around more than one million deaths could be averted per year.

A recent meta-analysis has reported that exercise training is associated with a decline in glycosylated hemoglobin (HbA1c) levels. This effect is presented in aerobic, resistance, or combined aerobic and resistance training modes and also is compared to reductions achieved by commonly used oral antidiabetic medications. Three times a week of an exercise program with more than 30 minutes and at moderate intensity (above 50%  $VO_2$ ) is sufficient to reduce 10% in HbA1c levels mainly in insulin resistance subjects, according to the Diabetes Association in the past decades [32]. However, recent data suggest that structured exercise training of more than 150 minutes per week is associated with greater HbA1c declines than that of 150 minutes or less per week and that physical activity advice is also associated with lower HbA1c, but only when combined with dietary advice [33]. Current opinion appointed that the volume of exercise training (and not the type or intensity of exercise) is a major determinant of glycemic

control in patients with T2DM. Reduction in HbA1c is associated with exercise frequency in supervised aerobic training, and with weekly volume of resistance exercise in supervised combined training promotes better results in the reduction of HbA1c [34]. Furthermore, a dose response can be estimated to exercise benefits with longer life expectancy for those who accumulate more than 450 minutes of exercise per week [35].

For elderly individuals, combined training (resistance and aerobic) performed twice a week promotes similar muscular adaptation (strength and thickness) and some similar cardiovascular adaptations when compared to three times per week, suggesting that it is applicable as an exercise prescription for aged people to improve the adherence to a health life style. [36]. Regular exercise can block the aging-associated increase in sympathetic nervous system activity to peripheral tissues (probably an adaptation to improve energy consumption). Chronically augmented sympathetic stimulation promotes reductions in the peripheral blood flow and vascular conductance and thus can also contribute to the metabolic dysfunction, by increasing glucose intolerance and insulin resistance. Each exercise session possibly substitutes the necessity of an increase in the sympathetic activity induced by aging and then stimulates thermogenesis to prevent increasing adiposity [37]. In this way, daily physical activity is able to promote weight loss without caloric restriction and also can reduce obesity, particularly abdominal obesity, and insulin resistance in men. Exercise programs without weight loss reduce abdominal fat and prevent further weight gain, preventing insulin resistance [38].

The epidemiologic and clinical trials results listed above exist because exercise represents a physical stress that challenges human body homeostasis. In response to this stressor, autonomic nervous system and the hypothalamic-pituitary-adrenal axis are known to react and to participate in the maintenance of homeostasis. It is well known that exercise induces several changes on both immune system [39, 40] and endocrine system. Cytokines may be modulated by the secretion of hormones from the hypopituitary-hypothalamus axis and in an integration cross-talk, hypopituitary-hypothalamus axis is also modulated by immunologic status by secretion of cytokines, showing an important neuroendocrine-immune loop in the human body [41, 42]. Skeletal-muscle fibers also can produce several hundred secreted factors, including proteins, growth factors, cytokines, and metalloproteinases, with such secretory capacity increasing during muscle contractions or after exercise training. Muscle-derived molecules exerting either paracrine or endocrine effects are termed "myokines" and are strong candidates to make up a substantial fraction of the exercise "polypill" effect to the whole body [37].

These signaling actors can be influenced by multiple factors including mode, duration, and intensity of exercise. Changes are proportional to exercise intensity and duration of exercise, although the effect of intensity is more marked, as showed by impaired immune functions and high secretion of hormones after an acute bout of vigorous exercise [39]. Some adaptations from regular training appears to be related to modified circulating hormones, as cortisol, insulin and glucagon, and also by alterations in the pro/anti-inflammatory cytokine balance (IL-2/IL-10 ratio and more th1/th2 lymphocytes cytokines ratio).

Thus, chronic moderate exercise improves immune functions [43] by the induction of an anti-inflammatory environment with each bout of exercise promoted. The IL-6 is expressed in high

amounts in active muscle and is the first cytokine released into the circulation during exercise, followed by increased anti-inflammatory cytokines as IL-10 [44, 45]. Also, each exercise session promotes an increase in ROS production that result in improved antioxidant defense in the muscle, plasma, and other tissues [37]. In parallel, some studies demonstrated that exercise is a physiological stimulus that promotes an increase in the muscle HSP70 expression and eHSP70 concentration (plasma), influenced by the intensity [46] and duration [47]. Increased intracellular HSP70 expression in leukocytes can be associated to less TNF- $\alpha$  plasma concentration after an exercise session [48]. Thus, regular exercise can shift the whole body from a pro-oxidant and pro-inflammatory state to an equilibrium induced by molecular, redox, hormonal, and “immune-metabolic” adaptations.

Associated to the progression of cardiovascular disease was observed higher levels of inflammatory markers such as IL-6 and CRP that are related to reductions in nitric oxide (NO) concentrations caused by reduced eNOS activity [49]. These extracellular pro-inflammatory signaling is increased in obese and type two diabetic people. Curiously, higher eHSP70 content in the bloodstream is also observed in these individuals, connecting immune-inflammatory events that promote a pro-atherogenic profile. eHSP70 levels were associated with multiple biomarkers of the acute-phase reaction, inflammation, and endothelial-cell activation, indicating the presence of a complex stress response that involves immune-metabolic signaling by eHSP70, by interaction of eHSP70 with cell surface receptors in immune cells. The release of these proteins promotes the bind to Toll-like receptor 4 (TLR4)/CD14 receptors, resulting in endothelial cells expressing adhesion molecules in smooth muscle cells leading to proliferation, and in macrophages inducing a range of proinflammatory cytokines [50].

The elevation of eHSP70 levels could be an important integrative response, from/to immunologic/metabolic center of homeostasis control in response against physiological disorder or disease [14, 17, 51, 52]. Whereby healthy people have low plasmatic levels of eHSP70, the association of these proteins with illness, disease progression, and mortality were hypothesized, as well as longevity and health parameter status were attributed to this protein [53]. Interestingly, in the last years, from Walsh et al. (2001) [54] to present [55-57], some studies investigated eHSP70 concentration in response to exercise. However, eHSP70 released from immune cells during exercise can be an immune signal from the periphery to the central nervous system (e.g. hypothalamus) leading to the “fatigue sensation/behavior”. In other words, after eHSP70 concentrations had reached a critical level (not known yet), higher exercise loads or duration would be dangerous to the whole body [51]. Then, eHSP70 can be a signal to impose fatigue sensation and shutdown of exercise, avoid a pro-inflammatory state to maintaining homeostatic, metabolic, and hemodynamic equilibrium.

In the bloodstream, eHSP70s and cytokines might participate in the physiologic responses of physical exercise, as chemical messengers released during an effort. Increased levels of eHSP70 in the plasma during exercise may participate in the fatigue sensation, also acting as a danger signal from the immune system [51]. On the other hand, intracellular HSP70 (iHSP70) synthesis is necessary for homeostasis maintaining in the muscle and other tissues. In the intracellular

millie, these proteins have molecular chaperone action of such proteins, limiting protein aggregation, facilitating protein refolding, and maintaining structural function of proteins. iHSP70 have further been demonstrated to provide cytoprotection by anti-apoptotic mechanisms, inhibiting gene expression and regulating cell cycle progression [58]. Intracellularly activated HSP70 are anti-inflammatory by avoiding protein denaturation and excessive NF- $\kappa$ B activation that may be damaging to the cells. Thus, iHSP70 act as a suppressor of NF- $\kappa$ B pathways (inhibiting TNF- $\alpha$  expression), an important anti-inflammatory role of HSP70 family proteins [51].

The chaperone function of iHSP70 is more than microscopic measurements of laboratory research field. Muscle disuse result in muscular atrophy that is represented by the decrease in muscle mass, fiber cross sectional area, and total myofibrillar protein content. In this situation, contractile protein breakdown exceeds protein synthesis. Moreover, in atrophied muscle occurs an increase in the proportion of fibers containing the fast myosin heavy chain by the transformation from the slow myosin heavy chain (MyHC-I/ $\beta$ ) to the fast myosin heavy chain (MyHC-II $d/x$ ). As early as 18 hours and as late as 18 days after muscle disuse, it is possible to measure a decrease in iHSP70 in the soleus muscle [59]. Interestingly, previous heat treatment is a strategy to induce iHSP70 expression in the muscle and this molecular adaptation results in the maintenance of muscle mass during a 7-day period of immobilization [60]. In this way, iHSP70 expression appears to have no full protective effect on muscle mass, fiber cross sectional area, and total myofibrillar protein content, but prevents the decrease of MyHC-I/ $\beta$  and the increase of MyHC-II $d/x$  induced during the atrophy process [61]. These evidences suggest that HSP70 can inhibit a key signaling pathway for atrophy in muscle cells preventing the muscular atrophy.

Heat treatment also has been tested in humans. Short wave diathermy therapy is a clinical strategy that means to increase deep heating of tissues with higher water content. This strategy may promote a 58% increase of HSP70 expression in *vastus lateralis* [62]. It is possible that previous heat treatments cannot reduce markers of muscle damage but is able to reduce muscular pain, preserve strength, and improve range of motion following eccentric contractions. Curiously, there is a gender difference in heat shock response in both basal and induced by exercise iHSP70 levels, with men showing low pre-exercise levels and an attenuated iHSP70 response. The gender difference may be explained by the effects of estrogen modulation in heat shock response [62].

If the disused muscle is in trouble, the reuse of the musculature may represent many stages of soreness. After immobilization, the reload process to the muscle implies in newest molecular adaptations. If less required muscle is submitted to a challenge, the iHSP70 expression increased greatly (~200%) in the first two weeks of reload process and the return to basal levels (above disuse levels) early as eight weeks [59, 63]. This effect is accompanied by an increase in the percentage of slow type I MHC fibers (MyHC-I/ $\beta$ ). Although many factors appear to be related to the down- and upregulation of iHSP70, the expression of this protein is closely related to the morphological and functional changes of muscle cells.

Exercise-induced expression of iHSP70 in the skeletal muscle has a major role in restoring muscle metabolic functionality as it provides cytoprotection to damaged cells. An iHSP70 inducing ability has been shown as a result in different protocols of exercise, including eccentric, concentric not damaging, and aerobic or resistive, all of which are capable to induce intramuscular HSP70 expression [64, 65]. Although time and intensity of the physical effort are determinant factors to increase of intramuscular iHSP70, its rise may be detectable just 2 h after the onset of an acute exercise session, when HSPA1A mRNA expression peaks [54]. Moreover, exercise-induced iHSP70 presents a time and intensity dependence [46].

Since iHSP70 family members promote the facilitation of protein transport into the mitochondria, allowing and improving structural integrity of the organelle during fast energy flow, iHSP70 content has been correlated with an increase in the oxidative capacity of muscle cells. Several studies have demonstrated the relationship between high iHSP70 levels in the skeletal muscle and increased activity of mitochondrial enzymes after a short training period [66]. On the other hand, decreased mitochondrial function is known to be associated with the accumulation of intramyocyte triglycerides (and its byproducts), insulin resistance and diabetes. For this additional reason, exercise-induced iHSP70 expression can lead to improvements in metabolite oxidation and, consequently, insulin sensitivity.

Thus, exercise training or regular physical activity tend to reduce the stressful impact of each exercise session and improve glucose uptake and storage, antioxidant capacity, associated with the increase in the iHSP70 content in muscle [67, 68]. In fact, increased iHSP70 expression has been demonstrated to participate in cell signaling that prevents insulin resistance [67]. Since iHSP70 family members promote the facilitation of protein transport into the cytoplasm environment and help the cell to maintain the structural integrity of the organelles and proteins involved in cross-bridge contraction, iHSP70 can help prevent the loss of muscle mass, the decrease in the type II fiber content, and the loss of metabolic capacity as oxidative metabolism and thus improving glucose usage by the active muscle [66], which is important both in obese and aging conditions.

Exercise also promotes several redox related adaptations. The increase in ROS levels and adenosine monophosphate (AMP), signaling the activation of the enzyme AMPK to stimulate the catabolism of substrates in producing new ATP [69]. In this way, AMPK in skeletal muscles contributes to the maintenance of normoglycemia by two mechanisms—most glucose uptake occurs by increasing the translocation of GLUT4 and by reducing peripheral insulin resistance. In addition, this enzyme acts on hypothalamic functions by modulating the events related to hunger and satiety. Thus, plays a key role in the regulation of cellular metabolism and in the maintenance of energy homeostasis [70].

During a moderate aerobic exercise session the skeletal muscle is able to uptake glucose without insulin signaling and increase fatty acid mobilization to use as an energy source. Belonging to the family of enzymes activated by cellular stress caused primarily by ATP depletion, AMPK is responsible for the regeneration of ATP levels via oxidation of fatty acids and glucose [71]. When the AMPK is activated, it is capable of inhibiting both enzymes, the 3-hydroxy-3-methylglutaryl CoA reductase (HMGCoA reductase) and ACC. In the liver, the inactivation of the malonyl CoA ACC concentrations is insufficient to inhibit CPT1. Thus, there

is a predominance of the  $\beta$ -oxidation of fatty acids synthesis, and the production of energy outweighs the expense [72, 73]. Considering intervention strategies for T2DM, exercise improves muscular oxidative capacity and, consequently, insulin sensitivity, up regulating the expression of PGC-1 $\alpha$  in healthy subjects. In many rodent models, the increase of the OXPHOS is coordinated by PGC-1 $\alpha$ , which increases the transcriptional activity of PPAR $\gamma$ , reducing the effects that promote insulin resistance.

The predominant oxidative metabolism in type I fibers, which are modified by the action of aerobic training at moderate or high intensity, may be a key point to glucose metabolism regulation. There is an increase in the number of mitochondria and GLUT4 vesicles, thus promoting the acceleration of oxidative metabolism with increased ROS production accompanied by increased antioxidant defense capacity. Increase in antioxidant enzyme activity observed in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, and also in glutathione (GSH) content, represent oxidative stress preventive adaptations induced by exercise training. The redox protection has direct impact in the maintenance of insulin sensitivity in the muscle [74, 75]. These changes may be triggered or stimulated by increased HSP70 expression [76].

Regular exercise promotes the acute increase of blood flow and shear stress and, in turn, improves the NO bioavailability, hence increasing the endothelium-dependent vasodilatation. This NO effect occurs in parallel to the decrease in pro-inflammatory biomarkers after exercise training. This improvement in NO and decrease in pro-inflammatory markers could represent one of the most important mechanisms of cardioprotection induced by regular exercise that prevent comorbidities associated to T2DM. However, the exercise intensity seems to be a crucial variable to future studies. iHSP70 expression depends on exercise intensity (level of physical challenge, measured by workload or time), while many other adaptations could not be influenced by exercise training intensity. We believe that exercise can induce both intracellular and extracellular HSP70, but promotes equilibrium in HSP70 signaling to the whole body in T2DM [77, 78].

## 5. Conclusion

Exercise is a known modulator of all parameters listed above: glycemia, eHSP70, iHSP70, IL-6, TNF- $\alpha$ , and ROS. Intracellular and extracellular HSP70 have different roles in the regulation of “immune-metabolic” homeostasis as well as cytokines. Equilibrium is also obtained by exercise in redox state by improving antioxidant defenses in the muscle and between pro/anti-inflammatory cytokines production. Reduction or prevention of obesity by active muscles can be considered key to the process. By metabolic and anti-inflammatory effects, exercise represents a good and safe strategy against insulin resistance induced by age and lifestyle factors (Figure 4).

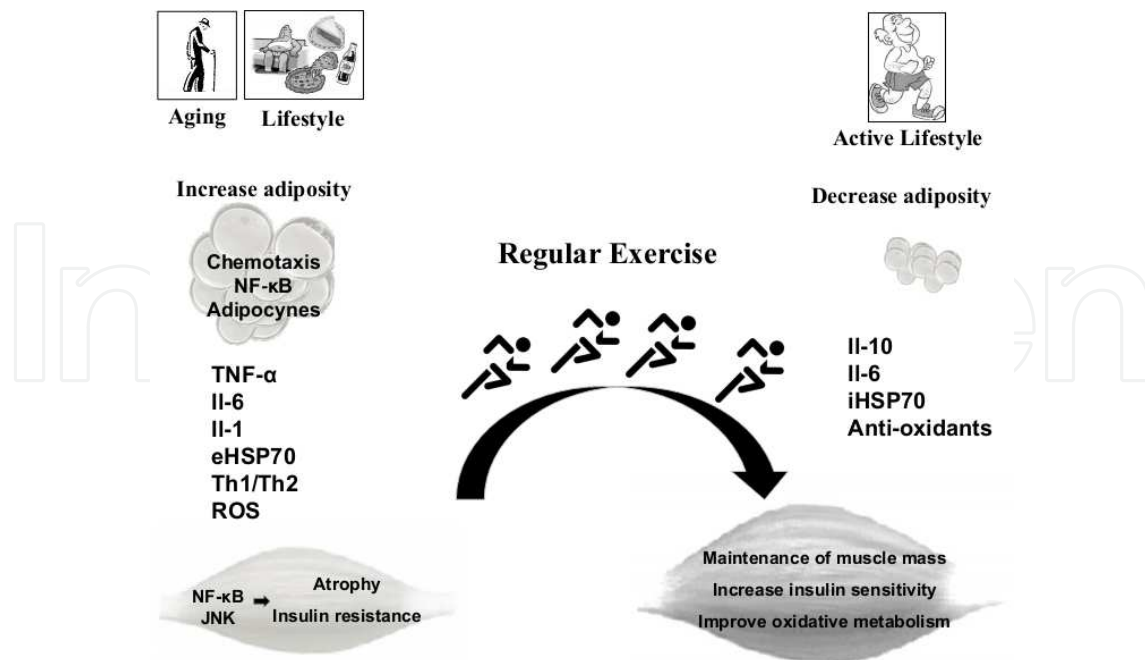


Figure 4. Exercise as a strategy against muscle insulin resistance induced by aging and lifestyle.

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