Role of Exercise in the Activation of Brown Adipose Tissue

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Abstract
Background: The energy-burning capacity of brown adipose tissue (BAT) makes it an attractive target for use in antiobesity therapies. Moreover, due to its ability to oxidize glucose and lipids, BAT activation has been considered a potential therapy to combat type 2 diabetes and atherogenesis. ***Summary:*** BAT is mainly regulated by the sympathetic nervous system (SNS); yet, recent findings have shown a group of novel activators that act independently of the stimulation of the SNS such as cardiac natriuretic peptides, irisin, interleukin-6, β-aminoisobutyric acid and fibroblast growth factor 21 that could influence BAT metabolism. Several strategies are being examined to activate and recruit BAT with no side effects. In this review, we postulate that exercise might activate and recruit human BAT through the activation of SNS, heart and skeletal muscle. ***Key Messages:*** Epidemiological and well-designed exercise-based randomized controlled studies are needed to clarify if exercise is able to activate BAT in humans.
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Introduction

The Problem

Obesity is considered to be a pandemic that has increased exponentially during the last decades. Currently, in the global scenario, prevalence of overweight and obesity is estimated to be as high as 36.9 and 38.0% in men and women, respectively [1]. Prevalence is higher even in developed countries [1]. It was estimated that in 2010, obesity caused 3.4 million of deaths worldwide [1]. Data from the European Health interview surveys (EU, Eurostat) indicate that more than half of the EU population is overweight or obese. Obesity is associated with a number of conditions and pathologies including insulin resistance, metabolic syndrome, cardiovascular diseases and type 2 diabetes. Data from the International Diabetes Federation indicate that 382 million people had diabetes in 2013, and this number is estimated to increase to 592 million by 2035 [2]. Diabetes caused 5.1 million deaths in 2013, and every 6 seconds a person dies from diabetes. Obesity and diabetes have an impact on society in terms of substantial direct and indirect costs, which have put a strain on healthcare and social resources. The obesogenic environment and behaviours (high-fat diets, physical in-

activity, etc.) are thought to be the main causes of the increasing levels of obesity and insulin resistance, which in turn leads to compensatory hyperinsulinaemia and ultimately type 2 diabetes. It is evident that our current knowledge and strategies are insufficient to combat the obesity and type 2 diabetes epidemic and new approaches have to be harnessed and exploited.

The Solution?

An ample amount of daily exercise improves physical and mental health of already healthy persons, increases the happiness levels in people, and makes them more productive [3]. It also prevents the development of many chronic diseases. Moreover, exercise is an excellent therapeutic intervention for controlling obesity, cardiovascular disease, type 2 diabetes, dementia, osteoporosis, depression, certain types of cancer and many other ailments [3]. In terms of efficacy, exercise can be as beneficial as the drugs that are prescribed for many of these diseases [3]; for example, obesity [4] or type 2 diabetes can be well controlled by regular exercise [5, 6]. However, the mechanisms that may mediate these effects are not fully understood for some of these pathologies.

The aim of the present review was to make a description of the recent knowledge related to the role of exercise in the activation and recruitment of brown adipose tissue (BAT) and its potential contribution to energy expenditure and to combat obesity, type 2 and related cardiovascular diseases.

White, Brown, and BRITE Adipose Tissue

In mammals, adipose tissue is found in 2 forms: white adipose tissue (WAT) and BAT. These 2 tissues have opposite roles in whole-body energy metabolism. WAT has the ability to store energy in the form of triacylglycerol and to release energy in the form of free fatty acids and glycerol, whereas BAT has the ability to dissipate energy in the form of heat by oxidation of glucose and lipids [7]. Brown adipocytes are thermogenic cells mainly regulated by the sympathetic nervous system (SNS) to defend body temperature when mammals are exposed to temperatures below thermoneutrality [7]. BAT is characterized by a light pink to dark red tone due to the high vascularization and the cytoplasm, which contains small fat-filled droplets and a large amount of mitochondria. High vascularization is necessary for nutrients and oxygen supply and for heat dissipation. The stored triacylglycerol depots are necessary for fast energy supply, and the SNS innervation is needed for fast activation of the tissue [8]. For maintenance of prolonged thermogenesis, the tissue receives substrates (fatty acids and glucose) from the circulation. Ultimately, heat production takes place through the uncoupling process, which is mediated by uncoupling protein 1 (UCP-1), a unique inner-membrane mitochondrial protein for BAT [7].

In humans it was for long believed that BAT was present only in newborns and was responsible for non-shivering thermogenesis [9]; yet, it was thought to be irrelevant in adults. However, serendipitously, radiologists using the radiotracer ^{18}F-fluorodeoxyglucose (^{18}F-FDG) in positron-emission tomography and computed tomography (PET/CT) to detect metabolically active tumours [10, 11] found competing areas with high rates of glucose uptake that were symmetrical in nature [12]. These areas were most commonly localized in the supraclavicular and neck regions [13–15].

The presence of active BAT in adult humans and its metabolic significance for human physiology was first claimed in 2007 [13] and finally recognized in 2009 [14, 16–18]. Currently, there is no doubt that this unique tissue exists and is thermogenically active in human adults. BAT activity seems to decrease with age [17, 19–21], is inversely correlated with body mass index (BMI) [17, 20] and visceral adiposity [18, 20] and is lower in men than in women [17, 20, 22], but gender differences have not been confirmed in other studies [23].

It is important to note, however, that the observed associations of BAT with BMI and sex should be interpreted cautiously when an individualized cooling protocol has not been carried out prior to the PET/CT scan [24]. BAT is the main contributor to non-shivering thermogenesis; so it is supposed to be fully activated when mammals are exposed to a temperature near the shivering threshold (i.e. the temperature when shivering starts). Lower temperatures are needed to induce shivering in males compared to females and in obese compared to lean individuals. Thus, the higher BAT activity obtained in females and in lean individuals could be biased by indoor temperatures, which may be low enough to induce a relevant non-shivering thermogenesis in females and in lean individuals but not in males or obese individuals. Future studies should assess BAT activity and mass after an individualized cooling protocol. This may include the determination of the shivering threshold of the individual, and prior to the PET/CT, the participant should be exposed to a cold environment relative to the temperature calculated at the shivering threshold, for instance, 3°C above the shivering threshold for 120 min [24]. The tempera-

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Table 1. Clinical implications of activating BAT

Stimulates basal metabolic rate
Stimulates meal-induced thermogenesis
Stimulates cold-induced thermogenesis
Increases the clearance of glucose and blood lipids
Improves cholesterol metabolism
Is positively associated with bone mineral density in women

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ture should be well regulated to avoid any shivering before the PET/CT. Findings from studies performing PET/CT after cold exposure have served to elucidate that BAT is highly prevalent in adult humans (near 100%) [14, 16, 25]. Moreover, Lee et al. [25] showed that even BAT-negative subjects on a PET/CT exploration possess a certain amount of BAT.

Recently, another type of cells called brown-in-white (BRITE) or 'beige' cells have been found in WAT of both rodents and humans [26]. BRITE cells possess a multilocular morphology, enriched mitochondria and express the brown adipocyte-specific UCP-1 [26–29]. They are peculiar in that they share characteristics with WAT and BAT, and their development is regulated by diverse factors in an endocrine, paracrine and autocrine fashion. The development of these thermogenically competent cells in WAT is greatly enhanced in response to chronic cold exposure or prolonged β-adrenergic stimulation, and the occurrence of these cells is associated with resistance to obesity, type 2 diabetes and other metabolic diseases.

Importance of Activating BAT

A potential clinical implication of activating BAT relates to the stimulation of resting energy expenditure, meal-induced thermogenesis [30] and cold-induced thermogenesis [31] (table 1). In humans, meal-induced thermogenesis seems to be higher in those possessing BAT, so that they convert a higher proportion of the calories in the meal directly to heat than do individuals with a less amount of BAT [32]. Vosselman et al. [33] reported an increased glucose uptake in BAT after consumption of a high-calorie, carbohydrate-rich meal in lean adult men, which indicates a role for BAT in reducing metabolic efficiency. Regarding, Kajimura and Saito [34] estimated a BAT-dependent energy expenditure of about 200–400 kcal/day under cold conditions.

The amount of active BAT in humans is rather heterogeneous. An estimate of 50 g of active BAT seems to be realistic in humans [15, 16, 30]. It has been estimated that 50 g of activated BAT might translate to ≈20% of resting energy expenditure [30]. Less optimistic approximations suggest that 50 g of activated BAT could amount to ≈5% of resting energy expenditure [15, 16]. A 5% chronic increase in resting energy expenditure turns to 75–100 kcal/day over the course of a year, which might translate to a loss of 4–4.5 kg of fat mass yearly [35]. Thus, even going by the less optimistic estimation, activated BAT might influence our propensity to become obese or reduce our body fat.

Beyond the negative energy balance, BAT activation could also exert beneficial metabolic effects. These effects derive from the ability of BAT to oxidize glucose and lipids, resulting in euglycaemic and hypolipidaemic effects [36]. If BAT is able to increase the clearance and oxidation of excess glucose and lipids, it might therefore delay the development of peripheral insulin resistance. Whether this is possible in humans remains to be elucidated. Moreover, more studies are needed to better understand if BAT activation is able to favour the restoration of peripheral insulin sensitivity, decrease the amount of insulin needed to maintain euglycaemia and prevent β-cell dysfunction [36]. Interestingly, a recent study showed that temperature-acclimated BAT is able to modulate insulin sensitivity in humans [37].

In animal models, cold-activated BAT potentially reduces plasma triacylglicerol levels [38, 39]. Moreover, Berbee et al. [40] showed in hyperlipidemic APOE*3-Leiden CETP mice that BAT activation by β3-adrenergic receptor stimulation not only increases energy expenditure but also decreases plasma triacylglycerols and cholesterol levels. They demonstrated that BAT activation enhances the selective uptake of fatty acids from triacylglycerols-rich lipoproteins into BAT, subsequently accelerating the hepatic clearance of the cholesterol-enriched remnants, and ultimately attenuating the atherosclerosis development [40]. Studies in humans are sparse and confined to one study that showed that improved cholesterol metabolism in human patients with hypercholesterolaemia, underscoring the potential of BAT activation as a possible anti-atherogenic treatment [41].

Finally, recent studies have suggested a link between BAT and bone metabolism [42], mainly in women [43, 44]. Lee et al. [43] reported a positive correlation of BAT volume with total and spine bone mineral density in women, independent of fat and lean body mass, which suggest a possible regulatory link between brown adipogenesis and bone density in humans. This finding concurs with another study that reported lower bone mineral density and BAT mass in women with anorexia nervosa [44].
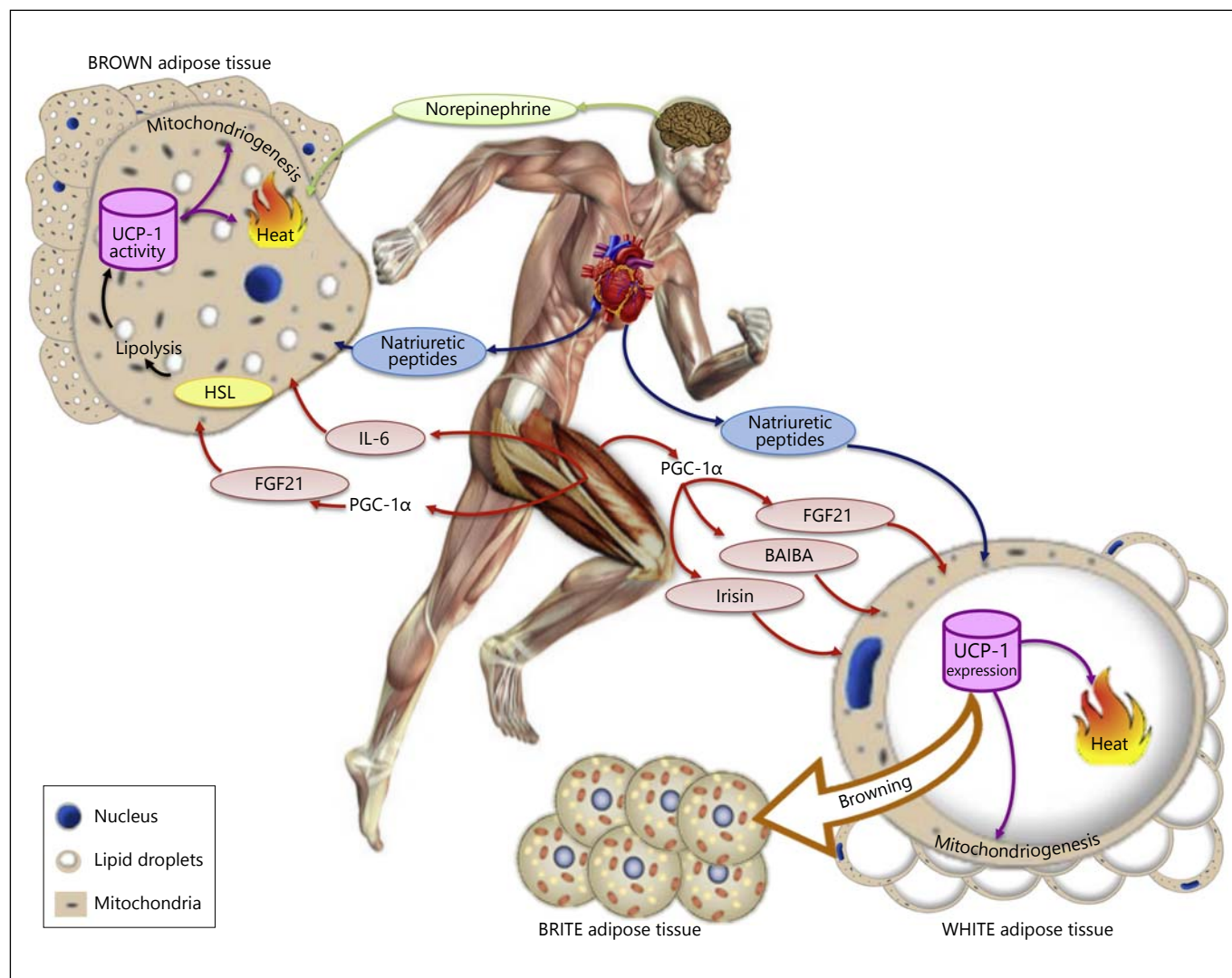
Fig. 1. Potential role of exercise in the activation of BAT.

Interestingly, studies conducted in animal models with defective brown adipogenesis also showed a reduced bone mass [45, 46]. Intervention studies are warranted to elucidate whether BAT activation induces improvement in bone mineral density, and whether BAT can be used as an anti-osteopenia and osteoporosis treatment.

Role of the SNS

Following the exposure to cold temperatures or acute food intake, the brain coordinates the activation of SNS. In the mature brown adipocytes, released norepinephrine bind to β-adrenergic receptors that are coupled with stimulatory G-proteins that activate adenylate cyclase; this in turn contributes to an activation of cAMP, protein kinase A, and p38MAPK, which subsequently activates lipolysis-stimulating enzymes, such as hormone-sensitive lipase, adipose triacylglycerol lipase and monoacylglycerol lipase [47] (fig. 1). The resulting increase in free fatty acids activates UCP-1. Norepinephrine also stimulates glucose uptake into brown adipocytes, and this is why BAT is visible to PET/CT scans. Exercise stimulates SNS and catecholamine release (epinephrine and norepinephrine) [48]. The duration and intensity of exercise are the main factors that are able to stimulate SNS and alter catecholamine responses to exercise [48]. It is therefore biologically plausible that exercise-induced adrenergic-

receptor stimulation has both acute (activation of UCP-1, stimulation of lipolysis) and chronic (UCP-1 gene transcription, mitochondrial biogenesis, hyperplasia of BAT, recruitment of brown adipocytes in WAT) effects on BAT (fig. 1).

Pathways Beyond the SNS

Interestingly, recent findings have shown a group of novel BAT activators that act independently of the stimulation of the SNS, such as cardiac natriuretic peptides, irisin, interleukin-6 (IL-6), β-aminoisobutyric acid (BAIBA) and fibroblast growth factor 21 (FGF21) that seem to be sensitive to exercise and that might influence BAT metabolism. This opens new horizons to study the potential effect of exercise-based therapeutic interventions.

Cardiac Natriuretic Peptides

Natriuretic peptides are hormones produced by the heart. Traditionally known actions of natriuretic peptides are natriuresis, diuresis, and vasodilation, which together serve to counteract the excessive cardiac wall stress. However, receptors for the natriuretic peptides are not restricted to the kidneys and vasculature, but fat tissue is also rich in the receptors that bind atrial natriuretic peptide and B-type natriuretic peptide, as well as the receptors that promote their clearance [49]. Natriuretic peptides increase cyclic GMP levels to activate cGMP-dependant protein kinase, which shares homology with PKA, activating p38MAPK [49]. These observed effects attributed to natriuretic peptides might be additive to, and perhaps synergistic with, those increases seen with classical β-adrenergic stimulation [50]. Bordicchia et al. [49] showed that in human adipocytes, natriuretic peptides induced lipolysis and UCP-1 expression, mitochondriogenesis, and increased uncoupled and total respiration [49]. Brain type natriuretic peptide treatment in mice enhanced energy expenditure and increased thermogenic protein levels in white and BAT [49].

Acute exercise increases the secretion of atrial and ventricular natriuretic peptides [51]. The stimulus for their secretion is the increase in heart rate as well as the stretch on atrial cardiomyocytes; therefore, atrial natriuretic peptides increase rapidly after the initiation of exercise [51, 52]. The effects of long-term exercise effects on atrial natriuretic peptides in adults and its role on human BAT activity and recruitment remain to be investigated. Moreover, whether the potential effects attributed to na-

triuretic peptides might be additive to, and perhaps synergistic with, those increases seen with classical β-adrenergic stimulation need to be studied.

FNDC5 Expression and Irisin Release

Skeletal muscle is an endocrine organ capable of communicating with other tissues through myokines, which are released into the circulation during exercise. Lin et al. [53] identified the transcriptional coactivator, peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), a molecule involved in the regulation of gene expression, that plays a critical role in the maintenance of glucose, lipid, and energy homeostasis. PGC-1α is induced in skeletal muscle by exercise and stimulates many of the best-known beneficial effects of exercise [54].

Bostrom et al. [55] showed that murine skeletal muscles, upon increased levels of PGC-1α, induce the expression of a protein called fibronectin type III domain containing 5 (FNDC5), which after cleavage is secreted into the blood stream as irisin. Irisin binds to the surface of white adipocytes, induces the expression of UCP-1, and triggers the transformation of white fat cells into BRITE cells [55]. This change was accompanied by an increase in total body energy expenditure, modest weight loss, and modest improvements in glucose intolerance. They also showed an increased *FNDC5* expression after 10 weeks of endurance exercise in obese male type 2 diabetic patients aged 53 years.

More recently, Lee et al. [56] showed in humans that circulating irisin increases 3-fold after 60 min of cycling at moderate intensity (40% VO$_2$max). Interestingly, they observed no increases in irisin plasma concentration after a maximal exercise test, suggesting that there might be a dose-response effect. In contrast, Timmons et al. [57] found no evidence that *FNDC5* expression was increased after exercise in humans; yet, they showed that a group of old active participants had a 30% greater *FNDC5* expression than sedentary controls. In addition to exercise, irisin appears to be influenced by a number of phenotypic traits including increased adiposity, lean mass and fasting plasma glucose that may partially explain the conflicting results emerged in human studies.

In summary, it seems that circulating irisin levels are upregulated after exercise in humans; yet, there are still some inconsistencies that warrant further investigation [58, 59]. Indeed, there are doubts on whether commercial methods are able to detect irisin as well as if irisin is really functional on humans. Most of the studies mentioned earlier have detected irisin by using the ELISAs kit.

Albrecht et al. [59] found no evidence for circulating irisin in human and several animal species when examining it by 4 different antibodies (three of them were used in corresponding ELISAs and the other one in western blot). All of these antibodies had prominent cross-reactions with non-irisin proteins in serum or plasma. However, they were the first to identify an irisin peptide at the correct size by mass spectrometry, which might be considered supporting evidence of the existence of irisin in humans. This serum irisin peptide has not been detected previously neither by western blot not by the ELISA kits, and this is the same peptide identified by Lee et al. [56]. Although irisin seems to have been definitely detected in humans, the low serum concentration of the detected irisin peptide could make it physiologically ineffective, and thus questioning its possible effect when it is released by exercise.

IL-6

IL-6 is a multifunctional proinflammatory cytokine produced by immune (e.g. T cells) and non-immune cells, mainly adipose tissue and skeletal muscle [60]. IL-6 acts on a wide range of tissues through the modulation of cell growth and differentiation [60]. For instance, in WAT, IL-6 increases lipolysis [61], whereas in skeletal muscle, it increases glycolysis and improves insulin sensibility [62]. Interestingly, although it is generally considered a proinflammatory hormone, it is known that the isoform released by skeletal muscle has anti-inflammatory effects [63]. Moreover, during the last decade, there has been some discussion about a possible role of IL-6 as an 'energy sensor' [64].

Exercise can promote IL-6 serum increases as significant as 100-fold [65]. Indeed, it seems that exercise intensity and duration, the form of muscular contraction (eccentric or concentric) and muscle damage are the main mechanisms that mediate the IL-6 response to acute exercise [64]. On the other hand, a relation between IL-6 and BAT metabolism has been documented. In rats, the overexpression of the IL-6 gene increases the thermogenic gene expression and elevates protein levels of UCP-1 in BAT, which is mediated by phosphorylation of the signal transducer and activator of transcription 3 (pSTAT3) [60, 66]. These molecular changes were accompanied by weight loss without modifying food intake and insulin resistance, or visceral adipose tissue reduction. However, it is to be noted that chronic central IL-6 stimulation (i.e. similar to the situation in the obese state) desensitized IL-6 signal transduction characterized by reversal of elevated pSTAT3 levels [66]. Stanford et al. [67] showed that

the beneficial effects of murine BAT transplantation into WAT depots (e.g. improvement of glucose homeostasis and insulin sensitivity, weight loss) were blunted when BAT came from IL-6 knockout mice. This suggests that IL-6 is indeed required to maintain the profound metabolic effects of BAT transplantations and suggests that BAT-derived IL-6 could be a key factor acting as an autocrine or paracrine agent.

Whether the observed relation between IL-6 and BAT activity in animal models is transferable to human physiology remains unclear. Moreover, taking into account that IL-6 is importantly regulated by exercising muscle and that BAT metabolism seems to be crucially mediated by IL-6, there is a need for human studies investigating the association between IL-6 increase in response to exercise and BAT activation and recruitment, and UCP-1 induction (fig. 1).

BAIBA

Early in 2014, Roberts et al. [68] reported that BAIBA levels in muscle cells are regulated by PGC-1α and increases the expression of brown adipocyte-specific genes. In addition, exposure of human-induced pluripotent stem cells to BAIBA during differentiation to mature white adipocytes resulted in the occurrence of a brown adipocyte-like phenotype. In fact, the effect of BAIBA on the expression of brown adipocyte-specific genes was reproduced in white adipocytes derived from human pluripotent cell lines. BAIBA also induced an increased expression of brown/BRITE adipocyte-specific genes in vivo and muscle specific PGC-1α expression. They also showed that 20 weeks of highly controlled endurance exercise training increased plasma BAIBA levels by 17%. BAIBA also decreased weight gain and improved glucose tolerance in mice. Furthermore, BAIBA increased the expression of the browning gene program through a specific PPARα-dependent mechanism on white adipocytes in vitro and in inguinal white fat depot of mice. Finally, BAIBA plasma concentrations were inversely correlated with cardiometabolic risk factors in a large human cohort study (Community-based Framingham Heart Study) and were increased during exercise training in subjects of the HERITAGE Family Study.

The expression studies carried out by Roberts et al. [68] suggest a role for PGC-1α expression in muscle with the production of BAIBA from valine. This is supported by the fact that skeletal muscle is a major site of branched-chain amino acid utilization. During exercise, catabolism of the branched-chain amino acids is elevated [69]. Furthermore, the expression of genes in the valine degrada-

Sanchez-Delgado/Martinez-Tellez/Olza/
Aguilera/Gil/Ruiz

tion pathway was found to be increased in the skeletal muscle of physically active members of twin pairs compared to their inactive co-twins [70]. However, Roberts et al. [68] did not attempt to evaluate the R and S enantiomers of BAIBA in plasma and they did not measure BAIBA in urine, neither in mice nor in humans. Indeed, it cannot be excluded that BAIBA might be produced by the degradation of thymine associated with the fibre cells muscle turnover.

FGF21

FGF21 is one of the 'hormone-like' members of the fibroblast growth family. It is mainly expressed by the liver, but also by other tissues such as the thymus, WAT and skeletal muscle [71, 72], as well as by BAT [73]. FGF21 can act on BAT as an autocrine, paracrine and endocrine agent, activating BAT thermogenesis and UCP-1 expression. On WAT, FGF21 induces the 'browning' process by enhancing adipose tissue PGC-1α protein levels [34, 74]. In humans, mild cold-exposure (19°C for 12 h) increased serum diurnal levels of FGF21 and it correlated to total energy expenditure [75]. More recently, Hanssen et al. [76] showed that circulating FGF21 levels were associated with BAT activity during acute cold exposure and that cold acclimation increased BAT activity in parallel with increased FGF21 levels in men.

It was speculated that exercise may increase the FGF21 levels and that some of the exercise-related metabolic improvements could be related to FGF21 induction [77]. Catoire et al. [78] found that FGF21 muscle expression was induced after 1 h one-leg cycling, while no variation was found in the non-exercising leg. Interestingly, Kim et al. [79] found no variation in serum FGF21 levels immediately after the exercise protocol but noticed variations after one hour of recovery. On the other hand, Cuevas-Ramos et al. [80] found no acute effect of exercise on serum FGF21 but an increase in concentration after 2 weeks of a combined training program. These findings contrast with those found by Scalzo et al. [81], who reported a decrease of FGF21 expression in muscle after 3 weeks of sprint interval training. Lee et al. [56] also failed to find any effect of exercise on FGF21, and they even observed a nonsignificant decrease in serum levels of FGF21. Additionally, a negative correlation has been described between cardiorespiratory fitness and FGF21 serum levels [82].

Several animal studies suggested that FGF21 induction by exercise could be dependent of the studied tissue. Kim et al. [79] found an increase in the hepatic FGF21 expression, but they did not find induction in skeletal muscle. It

has even been postulated that exercise benefits related to FGF21 are explained by an increased hepatic sensibility to FGF21. Thus, examining different tissues as well as studying different types and dose of exercise might explain the observed controversy. In addition, FGF21 follows an important circadian pattern [83], which could be a bias depending on the time of the day when the study is conducted. The evidence mentioned earlier suggests the need of well-designed studies able to elucidate the acute and chronic effect of exercise on FGF21 serum levels and FGF21 expression in several tissues, and whether it is related to changes in human BAT mass and activity, as well as in browning (fig. 1).

BAT Activity and Recruitment in Humans

Although there is evidence indicating that BAT activation is possible in humans, less data exist regarding the increase of BAT mass. An intervention study observed that BAT activity in morbidly obese participants was increased in 4 out of 10 patients after weight loss induced by bariatric surgery, indicating that recruitment can take place [84]. Yoneshiro et al. [85] showed that cold exposure at 17°C during 2-hour per day for 6 weeks resulted in an increase in BAT activity and cold-induced thermogenesis and a concomitant decrease in body fat mass in non-obese individuals with low BAT activity. Indeed, they observed that changes in BAT activity and body fat mass were negatively correlated. It would be of clinical interest to elucidate whether an exercise-based intervention is able to increase BAT activity in obese participants, as well as in normal-weight and overweight individuals and to determine their metabolic consequences in all three weight status categories. It is also of relevance to better understand whether BAT recruitment induces body fat mass reduction or whether body fat mass reduction induces BAT recruitment as a thermoregulatory mechanism.

Exercise, BAT and Browning in Animal Models

The potential influence of exercise training on BAT activity in animal models is not fully clear. In 2004, Cannon and Nedergaard [7] suggested that BAT is likely to be hypoactive during exercise, which was indeed confirmed by several studies carried out using animal models [86–90]. Segawa et al. [86] reported no effect on the thermogenic activity of BAT in rats after 9 weeks or running

training (5 days/week) [86], and Shibata and Nagasaka [88] reported that daily running for 5 weeks did not change the size of interscapular BAT. Similarly, Wickler et al. [89] showed that running on a treadmill 90 min/day for 6 weeks did not have any effect on resting oxygen consumption, norepinephrine-induced oxygen consumption, BAT, and brown fat blood flow.

In contrast, more recent studies have reported an increased metabolic activity of brown adipocytes and a weak activation of thermogenic program (UCP-1) after a 6-week exercise program in rats [91]. De Matteis et al. [91] showed an increased parenchymal vascularization of interscapular BAT, which concur with other studies that showed that a 9-week exercise training program increases angiogenesis in WAT [92]. They also showed that a 1-week running training program could be enough stimulus to browning the visceral fat [91]. These findings together with those reported by Bostrom et al. [55], and those reported by Slocum et al. [93] showed that exercising 60 min/day over 7 days upregulated mitochondrial UCP-1 in the BAT, and that this upregulation correlated with body weight loss in mice, are indicative of a potential role of exercise in BAT metabolism.

Exercise, BAT and Browning in Humans

To our knowledge, there is only one epidemiological study investigating the association between habitual physical activity and BAT. Dinas et al. [94] showed for the first time a promising association between self-reported habitual physical activity and BAT activity in a sample of 40 (14 females) patients with cancer. Their positive findings are of great importance and although their study design does not indicate any inference to any causal relationship, the results are informative and support evidence from exercise-based intervention studies conducted in animal models. Results are, however, limited due to the fact that they assessed habitual physical activity with a questionnaire, which has low accuracy [95]. Therefore, whenever possible, future epidemiological studies should determine the association between objectively measured physical activity and BAT in humans.

More recently, Vosselman [96] conducted a case-control study where they compared BAT activity as well as browning of subcutaneous abdominal WAT in male endurance-trained (i.e. runners, cyclist and swimmers with a VO₂max >55 ml/kg/min, and with a training experience of at least 2 years) with an age- and BMI-matched group of sedentary lean males. They observed that BAT activity was significantly lower in the endurance-trained group compared with their sedentary counterparts. Interestingly, they also observed that mRNA expression of *FNDC5* in skeletal muscle *(vastus lateralis)* was 1.6 times higher in the endurance-trained group, suggesting that long-term endurance exercise stimulates *FNDC5* expression. They did not show, however, that the higher *FNDC5* expression was associated with browning of subcutaneous abdominal WAT, which does not concur with the findings from animal models [55, 91–93, 97]. Moreover, Vosselman [96] did not detect mRNA *UCP-1* expression in WAT in either group, and found no differences between groups in mRNA expression of *PGC-1α*, *Cidea*, *TMEM26* or *CD137*. Norheim et al. [98] observed a little browning effect on selected browning genes (*UPC1*, *PRDM16*, *TBX1*, *TMEM26* and *CD137*) in subcutaneous abdominal WAT on 12 weeks of endurance and strength training in both normoglycaemic and normal weight participants and in pre-diabetic group [98]. Moreover, they detected that the mRNA expression of UCP-1 in subcutaneous fat tended to increase in both normoglycaemic and pre-diabetic groups after training, and significantly increased when data from both groups were combined (1.82-fold) [98]. They also observed that PGC-1α and FNDC5 mRNAs were not significantly enhanced in response to chronic training. Unfortunately, Norheim et al. [98] did not have data on BAT activity or mass before and after the exercise intervention. Lee et al. [56] reported an increase on irisin levels (3.1-fold) after one bout of moderate exercise intensity (60 min of cycling at 40% VO₂max), which was accompanied by an increased in vitro expression of FNDC5 and an increased BAT and BRITE gene expression in human adipocytes taken from the neck. The fact that some studies showed an expression of browning genes in WAT [56, 99] while others did not [96, 98] indicates that adipocytes from different locations might respond differently, and suggest that human BRITE cells might be located in specific anatomical depots such as the supraclavicular region [56]. The transforming growth factor β1 effector protein, mothers against decapentaplegic homolog 3 (SMAD3) might also partially explain the contradictory results. Tiano et al. [100] showed in animal models that SMAD3 negatively regulates irisin production and/or secretion from skeletal muscle. Future exercise-based intervention studies need to monitor, whenever possible, the browning of adipocytes from different fat depots. We also need to understand the role of SMAD3 in exercising the human skeletal muscle.

Sanchez-Delgado/Martinez-Tellez/Olza/
Aguilera/Gil/Ruiz

Conclusions

In summary, there is consensus on the presence of BAT in humans and its potential consequences on energy metabolism, glucose regulation and blood lipid balance. Agents able to stimulate BAT activity and to increase its thermogenic capacity are being examined in animal models as well as in humans. We suggest that exercise could exert a key role on BAT metabolism [101] (fig. 1). Exercise might activate and recruit human BAT through activation of SNS, heart and skeletal muscle; yet, a number of studies are needed to understand if exercise can play a key role in BAT metabolism, which type of exercise, if intensity matters, and how much time is needed to induce an effective BAT activation and recruitment. Data from case-control studies might help to explain if there are differences regarding types of exercise: for example, comparing endurance vs. strength phenotypes. Epidemiological studies determining the association of levels and patterns of physical activity with BAT might also bring some light. Whether exercise modulates the potential environmental effects on BAT needs to be investigated. Ultimately, well-designed, exercise-based randomized controlled studies will be able to clarify if exercise is able to activate or recruit BAT in humans.

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