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REVIEW

Brown adipose tissue and bone

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Brown adipose tissue (BAT) is capable of transforming chemically stored energy, in the form of triglycerides, into heat. Recent studies have shown that metabolically active BAT is present in a large proportion of adult humans, where its activity correlates with a favorable metabolic status. Hence, the tissue is now regarded as an interesting target for therapies against obesity and associated diseases such as type 2 diabetes, the hypothesis being that an induction of BAT would be beneficial for these disease states. Apart from the association between BAT activity and a healthier metabolic status, later studies have also shown a positive correlation between BAT volume and both bone cross-sectional area and bone mineral density, suggesting that BAT might stimulate bone anabolism. The aim of this review is to give the reader a brief overview of the BAT research field and to summarize and discuss recent findings regarding BAT being a potential player in bone metabolism.

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INTRODUCTION AND SEARCH STRATEGY

Obesity and related metabolic diseases such as type 2 diabetes constitute a great problem worldwide. Several anti-obesity drugs have been approved in previous years, but many of them have been withdrawn from the market due to safety issues. Today, very few effective therapies are available and the scientific community is struggling to find new promising targets for anti-obesity drugs. So far most efforts have focused on reducing the intake of calories, either by inducing satiety or by reducing the caloric uptake. Less attention has been paid to the other alternative to reduce stored energy levels, which is to increase the expenditure of ingested calories. In the 1930s, a small lipophilic molecule called 2,4-dinitrophenol was a popular, effective and widely used drug for losing weight.[1] 2,4-Dinitrophenol increases energy expenditure through thermogenesis by disrupting the proton gradient across the inner mitochondrial membrane of cells, thereby uncoupling cellular respiration from ATP production. Unfortunately 2,4-dinitrophenol had to be removed from the market due to its narrow therapeutic index with risk of serious side effects.[2] However, the fact that uncoupling of oxidative phosphorylation can induce rapid loss of weight is conceptually important, and if such energy-expending processes could be stimulated in a more controlled way it might pose a new avenue when developing novel therapies against obesity.

Brown and beige adipocytes are thermogenic cells that are rich in mitochondria and express uncoupling protein 1 (UCP1), a transmembrane protein exclusively found in these cell types. UCP1 is localized in the inner mitochondrial membrane of the cells and similar to 2,4-dinitrophenol, activated UCP1 uncouples oxidative phosphorylation by short circuiting the proton gradient over the inner mitochondrial membrane, resulting in generation of heat. Hence, brown and beige adipocytes have the ability to, in a controlled way, transform chemically stored energy in the form of triglycerides into heat. It is well established that mice with an increased amount of brown adipose tissue (BAT) are protected against the negative effects of a high-fat diet, as they maintain a healthy metabolic phenotype even after the increased caloric load.[3–5] Despite the encouraging data from the animal studies, BAT has not been considered being a road worth exploring when

developing new therapies against obesity, as human adults, the main target group for such therapies, were thought to lack significant amounts of the tissue. Although it has been recognized that human infants have substantial amounts of BAT, which is believed to be essential for their thermoregulation, it was assumed that the tissue regressed as we age and that adults essentially lacked the tissue.[6] This view changed about 5 years ago when several studies showed that a high proportion of adults retain significant amounts of metabolically active BAT, as determined by positron emission tomography/computed tomography (PET/CT) using 2-deoxy-2-[^{18}F]-fluoro-D-glucose (^{18}F-FDG) as a tracer.[7–11] The main locations of BAT are in the neck region, where the supraclavicular depot is the most prominent one, along the spine and around the kidneys.[6] Since the discovery of metabolically active BAT in adult humans, the tissue is regarded as a highly interesting target for anti-obesity drugs and is now subject to intense research, the hypothesis being that an increased amount or activity of the tissue would be beneficial in the battle against obesity and related diseases such as type 2 diabetes. Importantly, the idea that the presence of metabolically active BAT would be beneficial for a healthy metabolic phenotype also in humans is supported by several studies showing that the presence of BAT is associated with a low body mass index, low total adipose tissue content and a lower risk of type 2 diabetes.[8,11–14]

Interestingly, apart from the association between BAT and a healthy metabolic phenotype, there is also growing evidence suggesting a connection between the presence of BAT and bone anabolism. Recent human studies have shown positive correlations between BAT volume and both bone cross-sectional area and bone density.[15–18] Hence, therapies aiming at expanding or activating BAT to treat metabolic disease might also prove to be beneficial for the skeletal structure.

In this review, we will summarize and discuss recent work related to BAT and how the tissue might influence bone structure. For the review, we selected full papers published in English. PubMed was searched in November 2014, using the search terms 'brown adipose tissue' (8125 articles retrieved) and 'brown adipose tissue' AND 'bone' OR 'brown fat' AND 'bone' (207 articles retrieved).

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The retrieved articles were manually scanned and evaluated for their relevance for this review. References cited in identified articles were examined separately and used for further leads. In total, 57 articles were finally included in this review.

BAT AND THERMOGENIC ADIPOCYTES

The typical BAT organ, the so-called interscapular BAT depot, is located between the shoulder blades of smaller mammals including human infants,[19] and serves to protect the organism in a cold environment by means of non-shivering thermogenesis. The tissue is largely built by classical brown adipocytes, is densely innervated and has a rich blood supply. In response to cold stimuli, the thermogenic capacity of the tissue is triggered by efferent sympathetic nerve signals from hypothalamus. The norepinephrine released from the nerve endings activates β-adrenergic receptors on the brown adipocytes, thereby inducing lipolysis of stored triglycerides into glycerol and free fatty acids.[20] Apart from being the main substrate for the thermogenesis, the released free fatty acids also serve as direct activators of UCP1.[21] Upon activation by cold, the perfusion of BAT also increases markedly,[22,23] serving to supply the tissue with oxygen for thermogenesis and to spread the generated heat to the rest of the body.

Adipocytes, as chondrocytes, bone and skeletal muscle cells derive from the mesoderm. For a long time brown adipocytes and the energy-storing white adipocytes were believed to originate from a common progenitor cell.[24] However, this view changed when Seale et al.[25] by a lineage tracing approach, showed that classical brown adipocytes, but not white adipocytes, develop from progenitors that at some time point have expressed the myogenic marker gene Myf5. The authors also demonstrated that the transcriptional regulator PR-domain zinc-finger protein 16 (PRDM16) controls a bidirectional cell fate switch between brown adipocytes and skeletal muscle cells, as overexpression of the protein in skeletal myoblasts induced brown adipogenesis, whereas knockdown of the protein in brown preadipocytes induced a skeletal muscle cell phenotype. Importantly, the authors also found that the UCP1-expressing beige adipocytes (also referred to as inducible brown adipocytes or brite adipocytes) that appear in white adipose tissue (WAT) after treatment with the β3-adrenergic receptor agonist CL316 243, a situation mimicking prolonged cold exposure, descend from progenitors that, similar to white adipocytes, have never expressed Myf5. Hence, despite their many similarities, classical brown and beige adipocytes appear to derive from different progenitors, suggesting that they might be recruited by different cues. Thus the two cell types might represent two distinct potential targets for therapies aiming at expanding adipose tissue thermogenic capacity to combat obesity.

The origin of the beige adipocytes is still unclear. One idea, although still a bit controversial, is that these cells originate from mature white adipocytes through transdifferentiation in response to cold or β3-adrenergic receptor agonists.[26] However, there is growing evidence that most of the beige adipocytes arise by de novo adipogenesis from precursor cells rather than from mature adipocytes.[27] Recent data suggest that beige and white adipocytes develop from different sets of progenitor cells.[28] By using clonal cell lines derived from the stromal vascular fraction (enriched in precursor cells) of subcutaneous inguinal WAT of mice, the WAT depot in which beige adipocytes are most abundant, they identified two unique types of progenitor cells having the potential to differentiate into beige and white adipocytes, respectively. After differentiation of the beige progenitor cells into mature adipocytes, they presented with a gene expression signature different from both white and classical brown adipocytes. Under basal conditions, the expression of thermogenic genes such as Ucp1 was not significantly different between the cells differentiated into beige and white adipocytes, and it was much lower than that in classical brown adipocytes.

However, after stimulation with a β-adrenergic receptor agonist or forskolin (an activator of adenylyl cyclase mimicking β-adrenergic signaling), the beige adipocytes, but not the white adipocytes, had the ability to induce a thermogenic gene program and Ucp1 expression levels similar to that in classical brown adipocytes. Hence, at least in subcutaneous WAT, beige and white adipocytes might derive from different progenitors. Lee et al.[29] recently showed, using a lineage tracing approach, that in response to β-adrenergic signaling, beige adipocytes in abdominal WAT arise from a bipotential precursor cell expressing platelet-derived growth factor receptor α, CD34 and Sca-1. Interestingly, in response to a high-fat diet these progenitor cells instead differentiated into white adipocytes. Hence, the bipotential precursor cell appears to have the capacity to induce energy-consuming beige adipogenesis in response to cold stimuli, and energy-storing white adipogenesis in response to a high caloric load.

During the last years, the knowledge of factors affecting brown and beige adipogenesis has increased substantially, and important transcriptional regulators and secreted factors recruiting classical brown and beige adipocytes have been identified. Although an extensive portrayal of these factors is beyond the scope of this review, some of them will be briefly mentioned below. For the interested reader, there are several excellent reviews available on the subject.[30–34]

Marrow adipose tissue (MAT) is a third type of adipose tissue. The function of this tissue, also called yellow adipose tissue, is largely unknown, and a detailed characterization of its adipocytes is still lacking. The idea that brown adipocytes might be present in MAT was raised in the mid 90s, when it was shown that bone marrow contains adipocyte precursor cells having the ability to differentiate into multilocular adipocytes expressing Ucp1.[35] This idea is also supported by the fact that so-called hibernomas, rare and benign tumors consisting of brown adipocytes, can develop in bone marrow.[36,37] Recently, it was shown that MAT has a distinct phenotype resembling both WAT and BAT.[38] The authors showed that MAT expresses several BAT-selective genes including Prdm16, Dio2 and Pgc1α at similar levels as in the interscapular BAT depot. However, the expression level of the thermogenic effector gene Ucp1 was very low, possibly due to the presence of unstimulated beige or beige-like adipocytes in the MAT. Interestingly, the expression of the BAT-selective genes was decreased in bone of older as well as diabetic mice, two states associated with impaired bone remodeling. Owing to the difficulties of isolating a pure population of adipocytes from bone marrow, it is still not clear whether MAT is built by a single adipocyte population with characteristics of both white and brown/beige adipocytes or a heterogeneous adipocyte population containing both white and brown/beige adipocytes. Further studies are warranted to determine the identity of the adipocytes in MAT.

THE CONNECTION BETWEEN BAT AND BONE

Several studies have suggested a possible link between BAT and bone metabolism. For example, the transcriptional regulator and tumor suppressor retinoblastoma-associated protein (pRb), has been shown to be an important switch with the potential of directing mesenchymal stem cells toward either an osteoblast or adipocyte lineage. Calo et al.[39] showed that pRb directs common mesenchymal precursor cells into an osteoblastic cell fate while inhibiting their differentiation into adipocytes, and that loss of pRb in these precursors greatly increased the presence of BAT at the expense of calcified bone.

Interestingly, brown adipocytes have also been connected to bone formation in a process known as heterotopic ossification, a pathological condition in which bone forms in nonskeletal tissues, often as a result of traumatic injury. Olmsted-Davis et al.[40] have proposed that aberrant expression of bone morphogenetic proteins (BMPs) in soft tissues leads to the recruitment of brown

adipocytes that in turn induce local hypoxia, an environment promoting differentiation of stem cells into chondrocytes and subsequent heterotopic bone formation. Whether thermogenic adipocytes are present in MAT and have a similar role in normal bone formation is presently not known.

As implicated in the introduction, there is now growing evidence for human BAT affecting bone structure in a positive way. Bredella et al.[16] showed that young women with detectable cold-activated BAT (along the neck, and in the supraclavicular and paravertebral regions), as determined by ^{18}F-FDG-PET/CT, have a higher total bone mineral density (BMD), and that there is a positive correlation between BAT volume and BMD. Lee et al.[17] found a similar positive correlation between BAT volume and BMD in a group of healthy women, but not in men, suggesting the possibility of a gender-specific connection between BAT and bone. However, such gender-specific connection was not found in a cross-sectional study by Ponrartana et al.[18] that showed a significant correlation between BAT volume and femoral cortical bone area and cross-sectional area (CSA) in children and teenagers regardless of sex. Notably, the authors of the study did not exclude the possibility that the association between BAT and bone could, at least in part, be mediated by skeletal muscle, as the predictive power of the model increased by adding skeletal muscle as an independent variable while significantly decreasing the contribution of BAT. A late study by Bredella et al.[15] also shows a positive correlation between BAT volume and total femoral cortical bone area and CSA in adults. In addition, the results from the study imply a positive correlation between BAT volume and thigh muscle CSA, again suggesting that the involvement of BAT in bone formation might, at least partly, be mediated by skeletal muscle. The authors also found a positive correlation between BAT volume and thigh muscle CSA. This is in line with the results of a previous study showing that children and teenagers with detectable BAT, as determined by ^{18}F-FDG-PET/CT, have a significantly greater muscle volume than individuals with no detectable BAT.[41]

In addition to the human studies mentioned above, a couple of animal studies have recently evaluated the effects of BAT on bone structure. The forkhead transcription factor FOXC2 has previously been shown to induce a BAT-like phenotype of otherwise WAT depots when specifically overexpressed in adipocytes of mice.[4] The induction of the presumably beige adipocytes is accompanied by protection against obesity and diet-induced insulin resistance in response to a high-fat diet.[4,42] Using this mouse strain as a model for increased 'BAT content', Rahman et al.[43] recently showed that these mice have significantly higher trabecular bone mass, due to increased bone formation associated with high bone turnover, as compared with wild-type mice, suggesting that BAT is anabolic for the skeleton. Interestingly, the authors also shed some light on a potential mechanism by which BAT stimulates this effect. One potential mechanism for the interaction between BAT and bone is that the two tissues communicate via secreted factors. The authors evaluated the gene expression levels of some known regulators of bone anabolism (Wnt10b (wingless-type MMTV integration site family, member 10B),[44,45] Igfbp2 (insulin-like growth factor binding protein 2),[46] Igf1 (insulin-like growth factor 1),[47] Bmp4,[48] Adipoq (adiponectin)[49] and Angpt2 (angiopoietin 2)) in both adipose tissue and bone marrow adipocytes with elevated FOXC2 levels. They found that the expression levels of the genes encoding the endocrine factors ADIPOQ, IGF1 and IGFBP2, as well as the paracrine factors WNT10B, BMP4 and ANGPT2 were all upregulated in both adipose tissue and bone marrow adipocytes with elevated FOXC2 levels. Further they showed that conditioned media from cells overexpressing FOXC2 had a pro-osteoblastic effect on recipient preosteoblastic cells, an effect that was inhibited by immunodepletion of IGFBP2 and WNT10B from the media. Hence, IGFBP2 and WNT10B constitute

two potential factors acting as messengers between brown/beige adipocytes and bone, inducing an anabolic skeletal phenotype.

Other mechanisms that would help explaining the positive correlation between metabolically active BAT and bone mass have been proposed. It is known that bone density is partly regulated by sympathetic activity triggered by leptin, the hormone released from adipose tissue in response to increased energy storage in the tissue, and it has been shown that leptin deficiency results in low sympathetic tone accompanied by high bone mass.[50] In line with this, β-adrenergic receptor antagonists increase bone mass, whereas β-adrenergic receptor agonists decrease bone mass. In addition, mice deficient in Adrb2, the gene encoding the β2-adrenergic receptor, have increased bone formation.[51] With this information in mind, Motyl and Rosen[52] hypothesized that 'increased sympathetic activity, due to cold stimulus and/or impaired response to cold by BAT, can cause bone loss'. Recently, the authors tested their hypothesis by analyzing the skeletal phenotype of Misty mice, a mouse strain harboring a deleterious mutation in the Dock7 gene resulting in defective BAT.[53,54] In line with their hypothesis, the authors found that Misty mice have accelerated age-dependent trabecular bone loss that can be inhibited by the β-adrenergic receptor antagonist propranolol.[55] On the basis of these results, the authors concluded that BAT function is involved in skeletal metabolism in part through modulating the activity of the sympathetic nervous system.

Apart from these two suggested mechanisms by which BAT can positively affect bone structure, by blunting cold-induced increases in adrenergic tone to bone, and by secreting factors affecting bone structure, respectively, one can envision additional mechanisms for the interaction. For instance, one can speculate that the positive effects of BAT on bone structure are indirectly mediated by the improved metabolic status associated with active BAT. Both in animals and humans, increased BAT activity is associated with improved glucose metabolism, and protection against type 2 diabetes, a disease state linked with an increased risk of fractures.[56,57] It is therefore likely that an improved metabolic status, mediated by BAT, would be positive on bone metabolism and reduce the risk of fractures. Hence, as a bonus, BAT-inducing therapies aiming at expanding or activating BAT to treat metabolic disease might also turn out to improve skeletal status.

CONCLUSIONS

Recent studies have suggested that BAT activity has positive effects on bone structure. The notion that BAT function would either directly or indirectly influence skeletal metabolism is intriguing. So far, only a few studies dedicated to explore the role of BAT on bone metabolism in humans have been published, and to get a better understanding of the interaction between the two tissues additional studies are clearly needed. In addition, valuable information can also be gained from further animal studies. Today, several mouse models for increased and decreased body BAT content are available, and these models will definitely be important tools when deciphering potential interactions between BAT and bone in the coming years.

CONFLICT OF INTEREST

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DISCLAIMER

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