Published in final edited form as:

Bone. 2012 February ; 50(2): 546–552. doi:10.1016/j.bone.2011.06.016.

Bone marrow fat has brown adipose tissue characteristics, which are attenuated with aging and diabetes

A. Krings^{1,*}, S. Rahman^{1,*}, S. Huang^{1,b}, Y. Lu¹, P.J. Czernik¹, and B. Lecka-Czernik^{1,2,3}

A. Krings: amreikrings@hotmail.de; S. Rahman: sima.rahman@rockets.utoledo.edu; S. Huang: doctorhsl@gmail.com; Y. Lu: Yalin.Lu@utoledo.edu; P.J. Czernik: piotr.czernik@utoledo.edu; B. Lecka-Czernik: beata.leckaczernik@utoledo.edu

¹Department of Orthopaedic Surgery, University of Toledo Health Sciences Campus, Toledo, OH 43614

²Department of Physiology and Pharmacology, University of Toledo Health Sciences Campus, Toledo, OH 43614

³Center for Diabetes and Endocrine Research, University of Toledo Health Sciences Campus, Toledo, OH 43614

Abstract

Fat occupies a significant portion of bone cavity however its function is largely unknown. Marrow fat expands during aging and in conditions which affect energy metabolism, indicating that fat in bone is under similar regulatory mechanisms as other fat depots. On the other hand, its location may determine specific functions in the maintenance of the environment for bone remodeling and hematopoiesis. We have demonstrated that marrow fat has a distinctive phenotype, which resembles both, white and brown adipose tissue (WAT and BAT, respectively). Marrow adipocytes express gene markers of brown adipocytes at levels characteristic for the BAT, including transcription factor Prdm16, and regulators of thermogenesis such as deiodinase 2 (Dio2) and PGC1 α . The levels of expression of BAT-specific gene markers are decreased in bone of 24 mo old C57BL/6 and in diabetic yellow agouti A^{vy/a} mice implicating functional changes of marrow fat occurring with aging and diabetes. Administration of antidiabetic TZD rosiglitazone, which sensitizes cells to insulin and increases adipocyte metabolic functions, significantly increased both, BAT (UCP1, PGC1 α , Dio2, β 3AR, Prdm16, and FoxC2) and WAT (adiponectin and leptin) gene expression in marrow of normoglycemic C57BL/6 mice, but failed to increase the expression of BAT, but not WAT, gene markers in diabetic mice. In conclusion, the metabolic phenotype of marrow fat combines both BAT and WAT characteristics. Decrease in BAT-like characteristics with aging and diabetes may contribute to the negative changes in the marrow environment supporting bone remodeling and hematopoiesis.

© 2011 Elsevier Inc. All rights reserved.

Corresponding Author: Beata Lecka-Czernik, Dept. Orthopaedic Surgery, Physiology and Pharmacology, Center for Diabetes and Endocrine Research, MS 1008, University of Toledo health Sciences Campus, 3000 Arlington Ave., Toledo, OH 43614, Phone: (419) 383-4140, Fax: (419) 383-2871, beata.leckaczernik@utoledo.edu.

*These authors contributed equally

^aCurrent Address: Maastricht University, Faculty of Health, Medicine and Life Science, PO Box 616, 6200 MD Maastricht, The Netherlands

^bCurrent Address: Department of Orthopaedic Surgery, Tongji Hospital and Medical College, Huazhong University of Science & Technology, 1095 Jiefang Avenue, Wuhan, China.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

bone; marrow fat; brown fat; white fat; adipogenesis; osteoblastogenesis

Introduction

Bone marrow provides an environment for controlling the maintenance of bone homeostasis, which is determined by autocrine, paracrine and endocrine activities of different cellular components. Amid advances in understanding the complexity of marrow environment and its role in the regulation of bone remodeling process, the role of fat, which is abundant marrow component in the adult bone, is still unclear in this process. Two types of fat tissues, white and brown adipose tissue (WAT and BAT, respectively), are relatively well understood with regards to their metabolic activities. The marrow fat or the yellow adipose tissue (YAT) constitutes a third category of fat tissue and its metabolic activity is largely unknown.

Fat plays an important role in the regulation of energy metabolism. It stores and releases energy under conditions of feeding and fasting, and regulates energy balance in peripheral tissues through its endocrine activities. Adipocytes accumulate energy in the form of lipids and burn it in the process of fatty acid β -oxidation. Moreover, energy balance is established through the production of adipokines, among them leptin and adiponectin, which regulate calorie intake and insulin sensitivity, respectively. The multiplex of fat functions is sequestered throughout different fat depots. Mitochondria-sparse WAT, constitutes ~10% of body weight in lean humans, and is represented by visceral and subcutaneous fat with a function in energy storage and regulation of insulin sensitivity and glucose metabolism in liver and muscle. Mitochondria-enriched BAT, is distributed in adult humans as discrete tissue deposits located in the neck, supraclavicular, paravertebral, and suprarenal regions (40) and is found abundantly in the scapulae of rodents. BAT, yielded by transcription factors Prdm16 and FoxC2 and co-activator PGC1 α , functions in adaptive thermogenesis by dissipating energy in the form of heat (11). This is mediated by uncoupling protein 1 (UCP1), which stimulates proton leak from the mitochondrial membrane to uncouple respiration from ATP synthesis to produce heat. BAT thermogenic activity is controlled by the central nervous system via catecholamines and β -adrenergic signaling, and deiodinase 2 (Dio2)-mediated thyroid hormone conversion from thyroxine (T4) to triiodothyronine (T3). Along with its role in adaptive thermogenesis, BAT also has a function in protecting against obesity, insulin resistance and diabetes (5, 8, 17, 18).

As demonstrated recently, BAT and WAT originate from different pools of mesenchymal precursors (32). In neonates, brown adipocytes originate from precursor cells, which express myogenic factor Myf5, and may also differentiate to muscle (32). On the other hand, the transcriptional regulator and tumor suppressor retinoblastoma protein pRb is involved in the lineage allocation of mesenchymal stem cells toward osteoblasts, and brown and white adipocytes (4, 10). Thus, a presence of pRb in early mesenchymal progenitors direct their differentiation towards osteoblasts, while an absence of pRb allows for commitment of the same progenitors to brown adipocyte lineage and their further differentiation under control of Prdm16. More interestingly, re-expression of pRb in cells already committed to brown adipocyte lineage converts them into adipocytes of white phenotype suggesting interconversion between white and brown phenotypes (4, 10). Indeed, BAT-like phenotype can be also induced in differentiated WAT suggesting a local function within WAT perhaps associated with on demand energy dissipation and not necessarily thermogenesis (31, 33, 39).

YAT, or yellow adipose tissue, bears its name due to a moderate number of mitochondria that gives it a yellowish appearance. It originates from the same marrow mesenchymal stem cells which can differentiate to osteoblasts, and in this respect it resembles WAT origin (1, 4). YAT accumulates in areas of trabecular bone of femur, tibia, and vertebrae and fills the entire marrow cavity by the 3rd decade of human life (24). Marrow fat may participate in lipid metabolism by clearing and storing circulating triglycerides, thereby providing a localized energy reservoir for emergency situations affecting, for example, osteogenesis (*e.g.*, bone fracture healing) (12). YAT responds to systemic changes in energy metabolism, which is demonstrated by changes in its volume with aging, estrogen deficiency, diabetes, TZD anti-diabetic therapy, caloric restriction and wasting diseases such as anorexia nervosa (2, 3, 6, 21, 34, 37). It is still unclear whether YAT constitutes a homogeneous population of WAT or BAT-like adipocytes or a heterogeneous population of both types of fat cells. Moreover, the metabolic role of this fat depot has yet been examined, although recent studies comparing gene expression profile of marrow fat and epididymal fat suggest that YAT possesses distinct phenotype and responds to aging differently than WAT (22). Here, we demonstrate that YAT has features of BAT-like tissue, which are attenuated with aging and diabetes.

Material and Methods

Animals

Non-diabetic C57BL/6 mice, adult (5 mo old) and old (26 mo old) males, were obtained from the colony maintained by the NIA under contractual agreement with Harlan Sprague Dawley, Inc. (Indianapolis, IN). Diabetic (A^{vy}/a phenotype) and non-diabetic (a/a phenotype) males of VY/WffC3Hf/Nctr- A^{vy} and VY/WffC3Hf/Nctr- a strains, respectively, were supplied from the colony maintained at the University of Toledo Health Sciences Campus (UT HSC). Genotype and phenotype of A^{vy}/a and a/a animals were described in details previously (7, 41). Briefly, A^{vy}/a mice are characterized by ectopic expression of agouti protein, due to continuous transcription of the agouti gene induced by a cryptic promoter in the intracisternal A particle (IAP) retrotransposon inserted in noncoding exon 2 of the agouti locus (7). In hypothalamic orexigenic neurons, agouti (Ag) protein binds to and represses the activity of MC4R, which regulates energy metabolism and satiety. Mice with A^{vy}/a phenotype (expressing Ag protein) develop obesity, hyperglycemia, hyperinsulinemia and insulin resistance by 8 week of age, whereas mice with a/a phenotype (non-expressing Ag protein) are lean, normoglycemic and insulin sensitive (41).

Animals were housed with free access to water and were maintained at a constant temperature, on a 12h light-dark cycle. The animal treatment and care protocols conformed to NIH Guidelines and were performed using a UT HSC Institutional Animal Care and Utilization Committee (IACUC) protocol.

For experiments testing rosiglitazone effects on gene expression profile in different fat depots, animals were fed for 4 weeks either diet supplemented with rosiglitazone maleate (Avandia, GlaxoSmithKline, King of Prussia, PA) at the dose of 20 mg/kg/day or non-supplemented diet, as described previously (20). At the end of experiment the following tissues were collected for RNA isolation: epididymal fat as a representative of WAT, interscapular fat as a representative of BAT, and a whole tibia bone as a representative of a tissue containing YAT. Tissues were homogenized in 1 ml of TRIzol (Invitrogen, Carlsbad, CA) and total RNA was extracted according to manufacturer's protocols.

Determination of marrow adipocyte number

Tibiae of experimental animals were decalcified in formic acid, embedded in paraffin and sectioned at 5 μ m. Histological sections were stained with hematoxylin and eosin. Fat cells identified as empty oval spaces were enumerated under magnification 20 $\times$ on five consecutive microscopic fields of the secondary spongiosa of the proximal tibia as described previously (30) and an average number of cells per high power field (20 $\times$ magnification) of 4 to 8 individual animals per group was calculated.

Analysis of gene markers expression by real-time PCR

One μ g of total RNA was digested with DNase I (Invitrogen) and converted to cDNA using the iScript cDNA synthesis kit (Biorad, Hercules, CA). Gene expression analysis was performed using real time PCR with Power SYBR Green detection system (Applied Biosystem, Foster City, CA), as previously described (14). A list of primers used in this analysis is provided in Table 1. Relative gene expression was measured by the comparative C_T method and normalized to the quantity of 18S RNA. In addition, bone samples were normalized to FABP4/aP2 expression levels in WAT and BAT to account for the differences in the fraction of adipocytes present in the analyzed specimen.

Statistical analysis

Gene expression analysis was performed on specimens derived from groups of animals, each consisting of 4 to 8 animals. Statistically significant differences between groups in each experiment were defined using one-way Anova (SPSS, Inc., Chicago, IL) after establishing homogeneity of variance and the normal distribution of the data. In all cases, $p < 0.05$ was considered significant. All values are presented as the Mean $\pm$ SD.

Results

YAT has phenotypic characteristics of BAT and WAT

In order to assess metabolic phenotype of marrow fat, we analyzed the relative expression of BAT- and WAT-specific gene markers in the tibia of 5 mo old C57BL/6 mice and compared the expression of these markers to the BAT and WAT derived from the same animals (Table 2). BAT-specific gene markers were represented by gene transcripts for regulators of adaptive thermogenesis and adrenergic response (UCP1, PGC1 α , Dio2, and β 3AR), and transcriptional regulators of BAT phenotype (Prdm16 and FoxC2) (29), whereas WAT-specific markers were represented by gene transcripts for two adipokines, which determine WAT endocrine function, adiponectin and leptin (38). Due to the complexity of extracting pure marrow adipose cells, YAT examination was performed on RNA isolated from the whole tibia. To account for differences in the contribution of mature adipocytes to the analyzed tissue samples, we normalized gene expression in bone samples to the expression of FABP4/aP2, which is relatively constant in differentiated adipocytes regardless of their origin. Table 2 lists the expression of tested markers in YAT and BAT relative to the expression in WAT. As expected, thermogenic markers UCP1, PGC1 α , and Dio2 were highly expressed in BAT reaching levels that exceeded WAT by 324-, 22-, and 88-fold, respectively. When compared to WAT, YAT showed elevated expression in Dio2 and PGC1 α , but not thermogenic marker UCP1 and adipocyte-specific beta-3-adrenergic receptor (β 3AR), which expression levels in YAT were even lower than in WAT. In addition, YAT appears to express relatively high levels of Prdm16 and FoxC2, two transcriptional regulators implicated in brown adipocyte differentiation. These results should be interpreted with caution, because some of these transcripts might be also expressed in other bone marrow cells. Thus, besides adipocytes Prdm16 is expressed in cells of myeloid lineage (25), FoxC2 is expressed in osteoblasts and endothelial cells (9, 16), and PGC1 α is

expressed in variety of cells where it controls glucose utilization and mitochondrial biogenesis (35). The expression of both adipokines, leptin and adiponectin, is lower in YAT than in WAT and BAT. Nevertheless, these data suggest that marrow adipose tissue might have properties of both brown and white adipose tissue.

Aging and diabetes decrease expression of BAT-like gene markers

There is an increasing evidence indicating that BAT function involutes with advancing age and with metabolic diseases such as diabetes (27). Therefore, we compared the expression of BAT-specific gene markers in bone of 5 mo and 26 mo old C57BL/6 mice (Fig. 1), and in bone of yellow agouti diabetic mice (A^{vy}/a strain) and their non-diabetic control (a/a strain) (Fig. 2) (41). As shown in Fig. 1, despite increased number of marrow adipocytes in bone of 26 mo old animals (Fig. 1A and B), the expression of BAT-specific transcriptional regulators, *Prdm16* and *FoxC2*, and genes involved in β -adrenergic signaling, $\beta 3AR$ and *Dio2*, was significantly lower than in 5 mo old animals (Fig. 1C). Similarly, although A^{vy}/a mice possess larger number of adipocytes in tibia bone than their non-diabetic a/a control (Fig. 2A), the expression of BAT markers in tibia of diabetic mice was significantly lower than in age-matched non-diabetic control (Fig. 2B). The expression of thermogenic activators, *UCP1* and *PGC1 α* , showed a tendency towards decrease in both aging and diabetes models, however did not reach statistical significance (data not showed). These data suggest that the metabolic status of YAT changes with alterations in systemic energy metabolism.

Rosiglitazone increases BAT-like phenotype in the bone of normoglycemic, but not in the bone of diabetic animals

It was previously demonstrated that TZDs, antidiabetic drugs and agonists for adipocyte-specific PPAR γ transcription factor, induce brown adipocyte phenotype in white adipocytes of subcutaneous origin (33, 39). It is well appreciated that TZDs, which sensitize cells to insulin, have a profound effect on systemic energy metabolism (13). Administration of rosiglitazone to animals and humans results in depot-specific changes in weight and volume of fat (20, 42). In normoglycemic C57BL/6 mice (Fig. 3A) and a/a mice (data not showed), rosiglitazone administration causes a decrease in the weight of WAT, an increase in the weight of BAT, and an increase in the number of marrow adipocytes in YAT (20), whereas in hyperglycemic and insulin resistant yellow agouti A^{vy}/a mice, rosiglitazone increases the weight of both WAT and BAT, and increases the number of adipocytes in the marrow (Fig. 3B). Similarly, TZDs significantly increase body weight of diabetic individuals, mainly due to increase in the weight of adipose tissue, which is considered a significant adverse effect of this therapy (42). Different pattern in fat accumulation in response to TZD treatment reflects difference in energy metabolism status between insulin sensitive (C57BL/6 and a/a) and insulin resistant (A^{vy}/a) mice.

To further assess YAT phenotype and its integration with energy metabolism system, we analyzed changes in the expression of adipocyte markers in tibia bone of normoglycemic (C57BL/6) and diabetic (A^{vy}/a mice) upon rosiglitazone treatment, and compared the expression of these markers in WAT and BAT of the same animals (Fig. 4, Suppl. Fig. 1 and 2). Rosiglitazone administration to normoglycemic C57BL/6 animals resulted in increased expression of BAT and WAT phenotype markers in bone (Fig. 4, graphs with gray bars). Rosiglitazone increased the expression of two markers of thermogenesis, *UCP1* and *PGC1 α* , and markers of brown adipocyte differentiation, *Prdm16* and *FoxC2*. Moreover, the expression of $\beta 3AR$ increased 9.5-fold in YAT upon rosiglitazone treatment suggesting upregulation of adipocyte-specific adrenergic signaling, which is necessary for thermogenesis and energy expenditure. Rosiglitazone also robustly increased expression of leptin and adiponectin (Fig. 4). In contrast, rosiglitazone effect on expression of these

markers in marrow fat derived from diabetic A^{vy}/a mice was very different (Fig. 4, graphs with black bars). Although rosiglitazone induced the expression of UCP1, however it failed to upregulate the expression of other markers of BAT phenotype. Similar to C57BL/6 non-diabetic animals, rosiglitazone upregulated the expression of leptin and adiponectin in marrow fat of A^{vy}/a mice. These results suggest that the phenotype of marrow adipocytes changes in diabetic conditions in a manner that renders cells less responsive to induction of BAT-like phenotype.

A comparison of the effect of rosiglitazone on bone adipocytes to the effect on BAT and WAT in the same animals shows interesting differences (Suppl. Fig. 1 and Fig. 2). In contrast to bone, the expression of FABP4/aP2 is not changed in WAT and BAT of C57BL/6 and A^{vy}/a mice indicating that in adult animals rosiglitazone did not induce *de novo* adipogenesis in these fat depots. However, it changes the metabolic profile of WAT in C57BL/6 mice, which is reflected by increased expression of UCP1 and PGC1 α , and decreased expression of leptin and FoxC2 (Suppl. Fig. 1). In WAT of A^{vy}/a mice, rosiglitazone robustly increased expression of UCP1 and β 3AR (Suppl. Fig. 2). Interestingly, although BAT weight increases in animals receiving rosiglitazone (Fig. 3A), changes in the expression of metabolic markers are not remarkable.

Taken together, these data suggest that bone marrow responds to rosiglitazone differently than WAT and BAT in the same animals, and that this response may include both, a direct effect on committed adipocyte and the effect on stem cells recruitment toward brown adipocyte lineage, which is reflected by increased expression of FABP4/aP2 and BAT-specific transcriptional regulators, Prdm16 and FoxC2.

Discussion

Our studies suggest that marrow fat has distinct phenotype, which consists of both, BAT and WAT characteristics. A relatively high expression of BAT-specific transcriptional regulators, Prdm16 and FoxC2, together with increase in their expressions in conditions which stimulate marrow adipocyte differentiation, indicates that YAT is under similar transcriptional control as BAT (15). Similarly, the expression of PGC1 α and Dio2 at the levels comparable to their expression in BAT, and significant upregulation of β 3AR and UCP1 by rosiglitazone, suggests YAT's role in energy dissipation. However, it is important to note that our analysis did not test the conditions of thermogenic stress stimulating sympathetic nervous system and β -adrenergic signaling and leading to UCP1 mediated thermogenesis. On the other hand, robust increase in the expression of leptin and adiponectin in bones of animals, which received rosiglitazone, indicates that marrow fat possesses also endocrine activity of WAT.

Importantly, BAT-like phenotype of marrow adipocytes may be attenuated with aging and diabetes, both conditions characterized by impaired systemic energy metabolism. Decrease in the expression of genes involved in the thermogenic response to adrenergic signaling and fatty acid oxidation suggests that with aging the phenotype of marrow adipocytes changes toward less efficient with respect to energy production. To further support this notion, we have demonstrated that adipocytes present in bone of diabetic animals do not respond to rosiglitazone in the same manner as adipocytes present in bone of non-diabetic animals. Although rosiglitazone increased number of adipocytes in the marrow of A^{vy}/a animals, which was associated with increased expression of UCP1, leptin and adiponectin, it failed to induce Prdm16 and FoxC2, β 3AR and PGC1 α gene expression. This indicates that diabetic conditions affect metabolic phenotype of marrow fat. It is important to notice that our observations are consistent with human studies, which showed that the function of brown fat, measured by ¹⁸F-glucose uptake after exposure to cold, declines with aging and diabetes

(27). In addition, recent studies of mitochondrial function in human marrow mesenchymal cells suggest that aging attenuates energy metabolism in the bone marrow by affecting mitochondrial biogenesis (28). These together suggest that energy-dissipating brown-like phenotype of marrow adipocytes may be regulated by the same mechanisms, which regulate systemic energy metabolism, and may be affected in conditions which impair this process.

Our observations pose an important question of physiological importance of brown fat phenotype in bone. Although there is a lack of solid experimental and clinical evidence to support the connection between metabolic status of bone marrow fat, which is largely unknown, and bone mass, one can speculate that a local decrease in the energy production (e.g. in the form of heat) may affect bone marrow environment supporting balanced bone remodeling. Indeed, a heterotopic bone formation in muscle injected with BMP2 is associated with accumulation of brown adipocytes expressing UCP1 (26). It is speculated that these adipocytes may function in recruitment of blood vessels, chondrocytes, and osteoblasts to the site of new bone formation. In support to the requirement of brown adipocytes for osteogenesis, injection of BMP2 into muscle of *Misty* mice, with genetic deficiency in brown adipocyte formation, switches phenotype of white to brown adipocytes at the site of bone formation (26). These data further corroborate our findings of increased expression, and perhaps activity, of brown fat markers in healthy animals with balanced bone remodeling, and decrease in these markers with aging and diabetes, conditions which lead to either unbalanced or attenuated bone remodeling (14, 19, 23, 36).

The observed mixed BAT/WAT phenotype of bone marrow fat may result from either mixed population of adipocytes representing each of the two phenotypes separately or distinct phenotype of marrow adipocytes, which combines both characteristics in one cell. Bone marrow consists of heterogeneous population of mesenchymal stem cells, which are at various stages of commitment to different lineages. Thus, it would not be surprising that Myf5⁺ precursors for brown adipocytes reside in the bone marrow in a significant number and can give rise to newly formed adipocytes of brown phenotype. On the other hand, it was demonstrated that adipocytes within WAT may acquire brown or brown-like phenotype (33, 39). We have confirmed in our models a possibility to induce brown phenotype within WAT, as we observed significant increase in UCP1 and PGC1 α expression in epididymal fat of rosiglitazone treated animals in the absence of upregulation of Prdm16 expression and even downregulation of FoxC2 expression. In contrast, activation of adipogenesis with rosiglitazone in bone marrow cells increases expression of all tested markers for brown phenotype, as well as markers of white phenotype, which probably reflexes a combination between residing adipocytes acquiring more metabolically efficient and/or BAT-like phenotype, and newly formed adipocytes from Myf5⁺ BAT precursors. More studies are needed to determine lineage identity of marrow adipocytes.

Our interpretation of presented studies may be limited by a use for gene expression analysis of RNA isolated from the whole tibia bone. This design was chosen for two reasons. First, an efficient isolation of pure population of adipocytes from the bone marrow poses technical difficulties due to their relatively dispersed localization in the bone cavity and embedment in the marrow extracellular matrix. Second, in the murine long bone large number of adipocytes is located in the region of epiphysis/metaphysis where they are juxtaposed to trabeculae (see Lecka-Czernik, this issue) and would be lost during bone marrow isolation by conventional flushing technique. Therefore, to have a representation of all adipocytes we decided to isolate RNA from the whole bone homogenate. Consequently, an interpretation of results of gene markers expression may be biased by the fact that the original cell population was a mixture of different types of cells. In addition, although some of the analyzed markers are unique for cells of adipocytic lineage, e.g. UCP1, β 3-AR, adiponectin, and leptin, others are less specific including Prdm16, FoxC2, Dio2 and PGC1 α (9, 15, 16,

25, 35). Moreover, the possibility exists that a skeletal localization of marrow adipocytes (e.g. axial vs. appendicular) may dictate their different metabolic profile. Nevertheless, and at least in respect to murine tibia bone, a correlative changes in the expression of several different markers, especially in conditions which favored adipocyte differentiation like rosiglitazone treatment, allows to believe that at least marrow adipocytes are not metabolically inert and have a tendency for changes in their metabolic phenotype with aging, diabetes, and rosiglitazone treatment.

Recent demonstration that the fate of mesenchymal stem cells toward osteoblasts, brown and white adipocytes is determined by pRb transcriptional regulator (4, 10) suggests considerable plasticity among these cell types. This creates a possibility to manipulate with osteoblast and brown adipocyte formation within a pool of marrow mesenchymal cells, which may open new therapeutic options for improvement of skeletal status during aging and in metabolic diseases, as well as for bone regenerative medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by funds from NIH/NIA AG 028935 and American Diabetes Association's Amaranth Diabetes Fund 1-09-RA-95.

References

1. Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells*. 2001; 19:180–92. [PubMed: 11359943]
2. Botolin S, McCabe LR. Bone loss and increased bone adiposity in spontaneous and pharmacologically induced diabetic mice. *Endocrinology*. 2007; 148:198–205. [PubMed: 17053023]
3. Bredella MA, Fazeli PK, Miller KK, Misra M, Torriani M, Thomas BJ, Ghomi RH, Rosen CJ, Klibanski A. Increased bone marrow fat in anorexia nervosa. *J Clin Endocrinol Metab*. 2009; 94:2129–36. [PubMed: 19318450]
4. Calo E, Quintero-Estades JA, Danielian PS, Nedelcu S, Berman SD, Lees JA. Rb regulates fate choice and lineage commitment in vivo. *Nature*. 2010; 466:1110–4. [PubMed: 20686481]
5. Cederberg A, Gronning LM, Ahren B, Tasken K, Carlsson P, Enerback S. FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell*. 2001; 106:563–73. [PubMed: 11551504]
6. Devlin MJ, Cloutier AM, Thomas NA, Panus DA, Lotinun S, Pinz I, Baron R, Rosen CJ, Bouxsein ML. Caloric restriction leads to high marrow adiposity and low bone mass in growing mice. *J Bone Miner Res*. 2010; 25:2078–88. [PubMed: 20229598]
7. Duhl DM, Vrieling H, Miller KA, Wolff GL, Barsh GS. Neomorphic agouti mutations in obese yellow mice. *Nat Genet*. 1994; 8:59–65. [PubMed: 7987393]
8. Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature*. 1997; 387:90–4. [PubMed: 9139827]
9. Estrach S, Cailleteau L, Franco CA, Gerhardt H, Stefani C, Lemichez E, Gagnoux-Palacios L, Meneguzzi G, Mettouchi A. Laminin-Binding Integrins Induce Dll4 Expression and Notch Signaling in Endothelial Cells. *Circ Res*. 2011 [Epub ahead of print].
10. Fernandez-Marcos PJ, Auwerx J. pRb, a switch between bone and brown fat. *Dev Cell*. 2010; 19:360–2. [PubMed: 20833358]
11. Gesta S, Tseng YH, Kahn CR. Developmental origin of fat: tracking obesity to its source. *Cell*. 2007; 131:242–56. [PubMed: 17956727]

12. Gimble JM, Zvonic S, Floyd ZE, Kassem M, Nuttall ME. Playing with bone and fat. *J Cell Biochem.* 2006; 98:251–66. [PubMed: 16479589]
13. Guan HP, Li Y, Jensen MV, Newgard CB, Steppan CM, Lazar MA. A futile metabolic cycle activated in adipocytes by antidiabetic agents. *Nat Med.* 2002; 8:1122–8. [PubMed: 12357248]
14. Huang S, Kaw M, Harris MT, Ebraheim N, McInerney MF, Najjar SM, Lecka-Czernik B. Decreased osteoclastogenesis and high bone mass in mice with impaired insulin clearance due to liver-specific inactivation to CEACAM1. *Bone.* 2010; 46:1138–45. [PubMed: 20044046]
15. Kajimura S, Seale P, Spiegelman BM. Transcriptional control of brown fat development. *Cell Metab.* 2010; 11:257–62. [PubMed: 20374957]
16. Kim SH, Cho KW, Choi HS, Park SJ, Rhee Y, Jung HS, Lim SK. The forkhead transcription factor Foxc2 stimulates osteoblast differentiation. *Biochem Biophys Res Commun.* 2009; 386:532–6. [PubMed: 19540201]
17. Kontani Y, Wang Y, Kimura K, Inokuma KI, Saito M, Suzuki-Miura T, Wang Z, Sato Y, Mori N, Yamashita H. UCP1 deficiency increases susceptibility to diet-induced obesity with age. *Aging Cell.* 2005; 4:147–55. [PubMed: 15924571]
18. Kopecky J, Clarke G, Enerback S, Spiegelman B, Kozak LP. Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *J Clin Invest.* 1995; 96:2914–23. [PubMed: 8675663]
19. Krakauer JC, McKenna MJ, Buderer NF, Rao DS, Whitehouse FW, Parfitt AM. Bone loss and bone turnover in diabetes. *Diabetes.* 1995; 44:775–82. [PubMed: 7789645]
20. Lazarenko OP, Rzonca SO, Hogue WR, Swain FL, Suva LJ, Lecka-Czernik B. Rosiglitazone induces decreases in bone mass and strength that are reminiscent of aged bone. *Endocrinology.* 2007; 148:2669–2680. [PubMed: 17332064]
21. Lecka-Czernik B. Bone Loss in Diabetes: Use of Anti-diabetic Thiazolidinediones and Secondary Osteoporosis. *Current Osteoporosis Reports.* 2010; 8:178–184. [PubMed: 20809203]
22. Liu LF, Shen WJ, Ueno M, Patel S, Kraemer FB. Characterization of Age-Related Gene Expression Profiling in Bone Marrow and Epididymal Adipocytes. *BMC Genomics.* 2011; 12 [Epub ahead of print].
23. Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. *Aging Cell.* 2004; 3:379–89. [PubMed: 15569355]
24. Moore SG, Dawson KL. Red and yellow marrow in the femur: age-related changes in appearance at MR imaging. *Radiology.* 1990; 175:219–23. [PubMed: 2315484]
25. Nishikata I, Nakahata S, Saito Y, Kaneda K, Ichihara E, Yamakawa N, Morishita K. Sumoylation of MEL1S at lysine 568 and its interaction with CtBP facilitates its repressor activity and the blockade of G-CSF-induced myeloid differentiation. *Oncogene.* 2011
26. Olmsted-Davis E, Gannon FH, Ozen M, Ittmann MM, Gugala Z, Hipp JA, Moran KM, Fouletier-Dilling CM, Schumara-Martin S, Lindsey RW, Heggeness MH, Brenner MK, Davis AR. Hypoxic adipocytes pattern early heterotopic bone formation. *Am J Pathol.* 2007; 170:620–32. [PubMed: 17255330]
27. Ouellet V, Routhier-Labadie A, Bellemare W, Lakhal-Chaieb L, Turcotte E, Carpentier AC, Richard D. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J Clin Endocrinol Metab.* 2010; 96:192–9. [PubMed: 20943785]
28. Pietila M, Palomaki S, Lehtonen S, Ritamo I, Valmu L, Nystedt J, Laitinen S, Leskela HV, Sormunen R, Pesala J, Nordstrom K, Vepsalainen A, Lehenkari P. Mitochondrial function and energy metabolism in umbilical cord blood- and bone marrow-derived mesenchymal stem cells. *Stem Cells Dev.* 2011 [Epub ahead of print].
29. Richard D, Picard F. Brown fat biology and thermogenesis. *Front Biosci.* 2011; 16:1233–60. [PubMed: 21196229]
30. Rzonca SO, Suva LJ, Gaddy D, Montague DC, Lecka-Czernik B. Bone is a target for the antidiabetic compound rosiglitazone. *Endocrinology.* 2004; 145:401–6. [PubMed: 14500573]

31. Schulz TJ, Huang TL, Tran TT, Zhang H, Townsend KL, Shadrach JL, Cerletti M, McDougall LE, Giorgadze N, Tchkonina T, Schrier D, Falb D, Kirkland JL, Wagers AJ, Tseng YH. Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc Natl Acad Sci U S A*. 2011; 108:143–8. [PubMed: 21173238]
32. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. *Nature*. 2008; 454:961–7. [PubMed: 18719582]
33. Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, Cohen P, Cinti S, Spiegelman BM. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J Clin Invest*. 2010; 121:96–105. [PubMed: 21123942]
34. Shen W, Chen J, Punyanitya M, Shapses S, Heshka S, Heymsfield SB. MRI-measured bone marrow adipose tissue is inversely related to DXA-measured bone mineral in Caucasian women. *Osteoporos Int*. 2007; 18:641–7. [PubMed: 17139464]
35. Soyak S, Krempler F, Oberkofler H, Patsch W. PGC-1alpha: a potent transcriptional cofactor involved in the pathogenesis of type 2 diabetes. *Diabetologia*. 2006; 49:1477–88. [PubMed: 16752166]
36. Syed FA, Hoey KA. Integrative physiology of the aging bone: insights from animal and cellular models. *Ann N Y Acad Sci*. 2010; 1211:95–106. [PubMed: 21062298]
37. Syed FA, Oursler MJ, Hefferanm TE, Peterson JM, Riggs BL, Khosla S. Effects of estrogen therapy on bone marrow adipocytes in postmenopausal osteoporotic women. *Osteoporos Int*. 2008; 19:1323–30. [PubMed: 18274695]
38. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem*. 2008; 77:289–312. [PubMed: 18518822]
39. Vernochet C, Peres SB, Davis KE, McDonald ME, Qiang L, Wang H, Scherer PE, Farmer SR. C/EBPalpha and the corepressors CtBP1 and CtBP2 regulate repression of select visceral white adipose genes during induction of the brown phenotype in white adipocytes by peroxisome proliferator-activated receptor gamma agonists. *Mol Cell Biol*. 2009; 29:4714–28. [PubMed: 19564408]
40. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P. Functional brown adipose tissue in healthy adults. *N Engl J Med*. 2009; 360:1518–25. [PubMed: 19357407]
41. Wolff GL, Roberts DW, Mountjoy KG. Physiological consequences of ectopic agouti gene expression: the yellow obese mouse syndrome. *Physiol Genomics*. 1999; 1:151–63. [PubMed: 11015573]
42. Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med*. 2004; 351:1106–18. [PubMed: 15356308]

Highlights

- In this study we examined phenotype of marrow fat by analysis of gene expression.
- The analysis included the expression of white fat and brown fat gene markers.
- Our studies show that marrow fat possesses brown adipocyte tissue characteristics.
- Brown phenotype of marrow fat is attenuated with aging and diabetes.

Figure 1.

Effect of aging on (A) – histological appearance of bone marrow in proximal tibia (vertical sections of undecalcified tibiae specimens were stained with Masson Trichrome and images were obtained under 4× magnification), (B) number of adipocytes, and (C) expression of BAT-specific gene markers in the tibia bone of 5 mo and 24 mo old C57BL/6 mice. Adipocyte number was quantified as described in Material and Methods and presented per high power field (AD/HPF) under 20x magnification. * $p < 0.05$

Figure 2.

Effect of diabetes on (A) adipocyte number and (B) expression of BAT-specific gene markers in the tibia bone of 4 mo old non-diabetic *a/a* and diabetic *A^{vy}/a* yellow agouti mice. Adipocyte number was quantified as described in Material and Methods and presented per high power field (AD/HPF) under 20x magnification. * $p < 0.05$

Figure 3.

The effect of rosiglitazone on fat content in tibia bone (adipocyte number per high power field [AD/HPF], magnif. 20x) and weights of epididymal WAT and interscapular BAT. Five months old non-diabetic C57BL/6 mice (A) and 4 mo old hyperglycemic and insulin resistant A^{vy/a} mice (B) received rosiglitazone (R) or regular chow (C) as described in Material and Methods. Gray bars - C57BL/6 mice; black bars - A^{vy/a} mice. * $p < 0.05$

Figure 4.

The effect of rosiglitazone administration on expression of adipocyte-specific gene markers in the tibia of 5 mo old non-diabetic C57BL/6 mice (grey bars) and 4 mo old hyperglycemic and insulin resistant A^{vy/a} mice (black bars). C-control; R-rosglitazone treated. * p < 0.05

Table 1

Oligonucleotide primers used for real time PCR analysis of gene expression

Gene name	Forward primer	Reverse primer
UCP1	GGATGGTGAACCCGACAAC	AACTCCGGCTGAGAAGATCTTG
PGC1 α	AACAAGCACTTCGGTCATCCCTG	TTACTGAAGTCGCCATCCCTTAG
Dio2	AAATGACCCCTTTGGTTTCC	TTCCCCATTATCCTTTCC
β 3AR	GGCACAGGAATGCCACTCCAAT	AGGAGGGGAAGGTAGAAGGAGAC
Prdm16	CCTAACTTTCCCCACTCCCTTA	GCTCAGCCTTGACCAGCAA
FoxC2	ACGAGTGCGGATTTGTAACC	ACAGTTGGGCAAGACGAAAC
Adipoq	GGC CGT TCT CTT CAC CTA CG	TGGAGGAGCACAGAGCCAG
Leptin	ATTTCACACACGCAGTCGGTAT	GGTGAAGCCCAGGAATGAAG
18S	TTCGAACGTCTGCCCTATCAA	ATGGTAGGCACGGCGACTA

Table 2

Relative expression of adipocyte-specific gene markers in WAT, BAT, and YAT of 5 mo old C57BL6 mice

Gene name	WAT	BAT	YAT	YAT/aP2
UCP1	1.0	324.4	0.02	0.37
PGC1 α	1.0	22.5	3.59	57.4
Dio2	1.0	87.7	14.8	236.9
Prdm16	1.0	14.4	1.02	16.5
FoxC2	1.0	0.30	0.46	7.41
β 3AR	1.0	0.77	0.01	0.06
Adipoq	1.0	0.29	0.03	0.39
Leptin	1.0	0.12	0.01	0.07

WAT - epididymal fat; BAT - interscapular fat; YAT – whole tibia; YAT/aP2 – YAT values normalized to the level of FABP4/aP2 expression in WAT and BAT.