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Nat Rev Endocrinol. Author manuscript; available in PMC 2020 May 01.

Published in final edited form as:
Nat Rev Endocrinol. 2019 November ; 15(11): 651–665. doi:10.1038/s41574-019-0246-y.

THE ROLE OF OSTEOBLASTS IN ENERGY HOMEOSTASIS

Naomi Dirckx[1], **Megan C. Moorer**[1,2], **Thomas L. Clemens**[1,2], **Ryan C. Riddle**[1,2]

[1]Department of Orthopaedic Surgery, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

[2]The Baltimore Veterans Administration Medical Center, Baltimore, MD, USA.

Abstract

Osteoblasts are specialized mesenchymal cells that synthesize bone matrix and coordinate the mineralization of the skeleton. These cells work in harmony with osteoclasts, which resorb bone, in a continuous cycle that occurs throughout life. The unique function of osteoblasts requires substantial amounts of energy production, particularly during states of new bone formation and remodeling. Over the last 15 years, studies have shown that osteoblasts secrete endocrine factors that integrate the metabolic requirements of bone formation with global energy balance through the regulation of insulin production, feeding behavior, and adipose tissue metabolism. In this article, we summarize the current understanding of three osteoblast-derived metabolic hormones (osteocalcin, lipocalin and sclerostin) and the clinical evidence that suggests the relevance of these pathways in humans, while also discussing the necessity of specific energy substrates (glucose, fatty acids and amino acids) to fuel bone formation and promote osteoblast differentiation.

INTRODUCTION

Bone is a dynamic tissue that is formed by osteoblasts and resorbed by osteoclasts in a continuous cycle occurring throughout life (the bone remodelling cycle), which adapts the structure of the skeleton for optimal function. During embryonic long bone development, a cartilage template of the skeleton is gradually replaced by bone produced by the combined actions of osteoblasts and osteoclasts. Subsequently, from infancy to puberty long bones grow in length via the proliferation of growth plate chondrocytes and increase in thickness via the deposition of new bone matrix on periosteal surfaces by osteoblasts. The concomitant resorption of bone on separate surfaces by osteoclasts (modelling) ensures that individual bones retain the shape that is most appropriate to their mechanical function. Of note, the overall rate of bone modellingdecreases dramatically after puberty, however, internal trabecular bone surfaces continue to be remodelled well into adulthood by the consecutive actions of osteoclasts and osteoblasts acting on the same bone surface. Moreover, remodelling is balanced in the youngwith the deposition of new bone equal to that which is

* rriddle1@jhmi.edu.

Competing interests
The authors declare no competing interests.

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resorbed. After the attainment of peak bone mass in the third decade of life, individuals show a declinein the rate of bone formation that leads to a negative bone balance. Over time, this negative bone balance contributes to the loss of bone mass and deterioration of bone strength, which in some individuals leads to osteoporosis with increased fracture risk[1–3].

Osteoblast precursors are recruited from the bone marrow to bone surfaces by cytokines and growth factors, like TGF-β and IGF-1[4], that are released from the bone matrix during the resorption phase of the bone remodelling cycle[5]. Subsequently, the precursor cells undergo several rounds of proliferation before maturing into the cuboidal, bone synthesising osteoblasts found at sites of active bone formation. During the differentiation process, osteoblasts first attain a polarized phenotype, thereby enabling cells to secrete bone matrix in a directional way. The initial bone matrix or osteoid consists of type I collagen and non-collagenous matrix proteins that compose the organic fraction of bone. With further differentiation, calcium and phosphate ions are secreted from osteoblasts to initiate the calcification process that is essential for the structural integrity of bone[2,6].

After replacing the packet of bone that has been resorbed by osteoclasts, most osteoblasts will undergo apoptosis, but a proportion of cells will become entrapped in the bone matrix and become terminally differentiated osteocytes. Of note, the functions of osteocytes are still being explored but the cell population is thought to survey bone health and control bone remodelling via the secretion of receptor activator of NFκB ligand (RANKL, an osteoclast differentiation and activation factorand direct communication via cell-cell contacts with other osteocytes and osteoblasts on the bone surface[7,8]. A second subset of osteoblasts dedifferentiates to form a quiescent population of bone-lining cellsthat protect against the re-initiation of the remodelling cycle while also serving as a source of rapidly activatable osteoblasts[9].

The synthetic phase of osteoblast differentiation requires substantial amounts of energy; therefore osteoblasts need considerable energy-generating capacity. Historical cytological and histochemical analyses of osteoblast structure performed in the 1950s demonstrated that osteoblasts accumulate mitochondria as they mature[6,10]. Later, more modern techniques, like Seahorse extracellular flux analysis, that allow for the assessment of mitochondrial function confirmed these findings and also demonstrated osteoblast metabolic plasticity[11] as well as increases in ATP production and mitochondrial transmembrane-potential[12] during differentiation. Much of this energetic capacity is probably required to drive extracellular matrix production in the osteoblast, as protein synthesis ranks among the most energy costly cellular processes[13,14]. Unsurprisingly, bone formation is in turn closely linked to metabolic status. For example, patients with anorexia nervosa and mouse models of caloric restriction show decreases in bone mass as compared to healthy individuals and control animals respectively, which are secondary to a lower rate of bone formation[15,16]. Furthermore, systemic dysregulation of energy storage and utilization in diabetes mellitus results in an imbalance in the bone remodelling cycle, leading to the development of skeletal frailty[17]. In addition, metabolic hormones including leptin, adiponectin and insulin (often dysregulated in diabetes mellitusstrongly influence skeletal development and remodeling[18–21].

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Historic studies conducted during the last century probed the intermediary metabolism of different subsets of bone cells to address mechanistic questions related to bone mineral ion mobilization.[22,23] The importance of these early studies has reemerged with the discovery that metabolic hormones regulate bone mass and that bone-derived factors influence adipose metabolism, insulin secretion and satiety. In this Review, we provide a comprehensive view of the endocrine functions of osteoblasts that enable this cell population to contribute to the regulation of systemic metabolism. Furthermore, we describe the experimental evidence that led to the discovery of osteocalcin, lipocalin and sclerostin as essential mediators of the endocrine functions of bone. As the evolution of the metabolic actions of osteoblasts was probably driven by the energetic costs of bone formation, we also highlight the utilization of glucose, fatty acids and amino acids by osteoblasts to generate ATP and the developmental pathways that govern their metabolism. Whenever possible, evidence that findings in mouse models are relevant to human physiology is emphasized.

OSTEOBLAST METABOLIC HORMONES

Osteoblasts have been discovered to produce several hormones with endocrine effects on metabolism. These endocrine factors include, but may not be limited to, osteocalcin, lipocalin and sclerostin.

Osteocalcin

Functions in bone mineralization.—Osteocalcin is encoded by *BGLAP* in humans and two homologs (*Bglap* and *Bglap2*) in mice[24,25]. This factor is the most abundant non-collagenous protein in the bone matrix and is produced almost exclusively by the osteoblast[26] Interestingly, osteocalcin was initially proposed to function as an inhibitor of bone matrix mineralization on the basis of its ability to inhibit the precipitation of calcium salts from solution[27] and the abundant expression at embryonic mineralizing sites[26]. Furthermore, observations in an osteocalcin-deficient ($Ocn^{-/-}$) mouse[28,29] appeared to support this idea as compared to wild type animals mutant mice showed an age-related increase in bone mass, with an increased mineral-to-matrix ratio and enlarged hydroxyapatite crystals. However, the hypothetical model of osteocalcin having an inhibitory role in mineralization was largely abandoned when a study showed that in vivo overexpression of osteocalcin did not inhibit bone formation[30]. A mechanical function of this factor, wherein matrix-bound osteocalcin and osteopontin bridge the interface between the mineral and organic constituents of bone, continues to be explored[31,32].

Metabolic functions.—A metabolic function for osteocalcin was first proposed in 2007, after a group reexamined the phenotypes of $Ocn^{-/-}$ mice and carried out a screen for osteoblast-restricted signalling cascades that affect whole-body metabolism[33]. The same research group had already demonstrated that the metabolic hormone leptin regulates bone mass[18], so it seemed reasonable to predict that bone would in turn produce a hormone that contributes to energy homeostasis. The 2007 study demonstrated that compared to wild type animals $Ocn^{-/-}$ mice are obese, have glucose-intolerance due to decreases in insulin production and β-cell proliferation, and are insulin-resistant[33]. An additional set of mutant mice that lacked *Esp* (which encodes osteotesticular protein tyrosine phosphatase (OST-

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PTP)) either globally or specifically in the osteoblast, developed the opposite metabolic phenotypes; these mice showed decreases in fat mass, hyperinsulinemia due to increased β-cell proliferation and enhanced insulin sensitivity. Of note, OST-PTP was shown to regulate the carboxylation of osteocalcin; the loss of OST-PTP from *Esp*-deficient mice decreased osteocalcin γ-carboxylation and resulted in greater serum abundance and bioavailability of osteocalcin which in turn stimulates insulin and adiponectin production[33]. Subsequent studies demonstrated that the administration of exogenous uncarboxylated osteocalcin enhanced glucose tolerance and insulin sensitivity in wild-type mice as well as those fed a high-fat diet[34,35]. In line with these observations, a further study reported that uncarboxylated osteocalcin administration increases human β-cell proliferation and insulin production in vitro as well as in vivo, after transplantation of human β-cells under the kidney capsule of nonobese diabetic-severe combined immunodeficient mice[36].

The regulation of insulin production and β-cell proliferation by osteocalcin led researchers to ask whether insulin in turn regulates osteoblast production of osteocalcin or its bioavailability[37]. The first evidence of the existence of a feed-forward, bone–pancreas endocrine loop was provided by two research groups that proposed separate, but non-exclusive mechanisms of action[20,21]. The first group focused on the anabolic actions of insulin in the skeleton and demonstrated that insulin is a potent stimulator of osteoblast differentiation and osteocalcin gene expression[21]. In this study, insulin treatment of cultures of primary calvarial osteoblasts or the mouse calvarial osteoblast cell line Mc3t3-E1 increased both osteocalcin mRNA levels and osteocalcin promoter activity as well as the binding of RUNX2 (a transcription factor initiating the osteogenic differentiation programme to regulatory elements in the promoter; this latter effect was at least partially related to the acute downregulation of the RUNX2 inhibitor TWIST2 by insulin. Importantly, this group also demonstrated that mice deficient in insulin receptor specifically in osteoblasts due to Ocn-Cre mediated recombination accumulated more body fat than wild type controls and developed hyperglycaemia secondary to hypoinsulinemia and impaired insulin sensitivity in addition to a low bone mass phenotype[21].Moreover, restoration of serum undercarboxylated osteocalcin levels, which were decreased in the osteoblast-specific insulin receptor deficient mice, improved insulin sensitivity[21].

A second research group using a osteoblast-specific Col1a1-Cre mediated recombination of the insulin receptor also demonstrated that insulin signalling regulates the carboxylation status and thus the bioavailability of osteocalcin as the mouse model exhibited impairments in glucose metabolism and decreased serum levels of undercarboxylated osteocalcin[20]. Since the loss of insulin receptor increased bone turnover and OST-PTP was not shown to interact with the cellular carboxylation machinery (however, OST-PTP does interact with the active insulin receptor), it was proposed that the acidic micro-environment of the osteoclastic resorption pit decarboxylates osteocalcin and then liberates the hormone from the bone matrix[20]. Subsequent studies identified FoxO1 (a transcription factor directly targeted by insulin signalling a) as a repressor of osteocalcin gene expression and bone turnover[38] and that the G protein coupled receptor Gprc6a acts as a receptor to mediate the endocrine action of osteocalcin in pancreatic β-cells[39,40]

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Effects on skeletal muscle metabolism.—In 2016, a second paradigm has emerged, wherein osteocalcin influences skeletal muscle metabolism and physiology. Skeletal muscle uses three well-established metabolic processes to power locomotion[41]. First, the initial burst of activity uses the small amount of ATP stored in muscle fibers. Second, myofibers utilize anaerobic processes to generate new ATP from the glycolytic metabolism of stored glycogen, as well as by phosphate donation to ADP from phosphocreatine. Finally, with prolonged exercise, glucose and fatty acids are metabolized via the tricarboxylic acid cycle and oxidative phosphorylation to generate ATP.

A major function of bone is to adapt to changes in the physical environment and the levels of circulating osteocalcin increase following exercise. In addition, bone mass, muscle mass and osteocalcin levels are known to decrease with age. As such, it was hypothesized that osteocalcin influences muscle function and metabolism during exercise. To examine this additional function, young (3-month) and old (10–15-month) mice were administered a single injection of uncarboxylated osteocalcin or were implanted with osteocalcin-releasing mini-pumps for 28 days and then challenged by treadmill running until exhaustion. In all cases, osteocalcin treatment increased exercise capacity and allowed old mice to run the same distance as untreated young mice[42,43]. Of note, a mouse model deficient in the osteocalcin receptor Gprc6a specifically in skeletal muscle and $Ocn^{-/-}$ mice showed a similar reduction in exercise capacity as compared to wild type controls. Moreover, muscle-specific Gprc6a-deficient mice were completely resistant to exogenous osteocalcin, suggesting a direct effect of osteocalcin signalling on myofiber function

The mitochondrial content of muscle fibers in $Ocn^{-/-}$ mice is similar to that of wild type littermates. Furthermore, no difference in mitochondrial respiration is evident in the muscle of muscle-specific Gprc6a-deficient mutants[42]. As such, one study explored whether osteocalcin influences the uptake of glucose and fatty acids in muscle and found that osteocalcin treatment of myoblasts increased the translocation of glucose transporter Glut4 to the plasma membrane in vitro and increased the expression of fatty acid transporting proteins Fatp1 and Cd36 in myotubes when osteocalcin was administered prior to exercise[42]. Moreover, both $Ocn^{-/-}$ mice and muscle-specific Gprc6a-deficient mice showed decreased breakdown of stored glycogen in myofibersin response to exercise as compared to wild type animals, whereas the muscle-specific Gprc6a mutants also exhibited decreased fatty acid uptake and catabolism in muscle[42]. Taken together, these findings suggest that osteocalcin increases exercise capacity by regulating nutrient uptake and ATP generation in skeletal muscle. Additional studies suggest that osteocalcin also increases expression of the myokine Il-6, which in turn completes an exercise induced feed-forward loop and instructs bone resorption and the further release of bone matrix-bound osteocalcin[42].

Osteocalcin actions in humans.—Serum osteocalcin levels decrease with advancing age in humans and continue to be used as a convenient biomarker of bone formation[26]. As osteocalcin is suggested to act as a hormone in mouse models, a rapidly growing number of studies have attempted to link serum abundance to metabolic functions in humans. Owing to the difficulties in assessing the serum abundance of undercarboxylated osteocalcin[33,44,45], the majority of studies have measured total serum osteocalcin. A general observation from these cross-sectional human studies is that there is a negative correlation between total

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serum osteocalcin levels and levels of blood glucose, fasting glucose and fasting insulin, as well as BMI and serum lipid levels[46–49]. Most notably, two large studies that each enrolled at least 2,400 individuals found associations between serum osteocalcin levels and risk factors [waist circumference, glucose levels, adipokine levels for the development of diabetes mellitus and metabolic syndrome[50,51]. A smaller number of studies have also drawn comparisons between serum undercarboxylated osteocalcin levels and diabetes risk factors. Although these studies have enrolled fewer numbers of participants, an association between serum osteocalcin and diabetes risk factors appears to be preserved[52–55].

It is important to consider that all of the aforementioned studies examining osteocalcin functions in humans are observational and do not prove causation. Indeed, one study attempted to interrogate the bone–pancreas endocrine loop by examining undercarboxylated osteocalcin in the serum of individuals who underwent a hyperinsulinemia-euglycemic clamp and saw no apparent relationship between the levels of insulin and osteocalcin[56]. Moreover, administration of anti-resorptive therapies (used to treat people at risk of fragility fractures), alendronate, zoledronic acid, or denosumab, did not increase diabetes mellitus incidence or fasting glucose[57]. These findings suggest that the mechanism of control over osteocalcin bioavailabilty in humans is different from that reported in the mouse[20]. To date, the most direct evidence for a relationship between osteocalcin and glucose homeostasis was reported in a 2012 study where the surgical removal of osteocalcin producing tumours from two patients substantially increased serum glucose levels[58]. Thus, more direct analyses will be needed to advance the idea that osteocalcin is a hormone in humans. For a more comprehensive analysis of the studies examining the association of serum osteocalcin with human metabolism, the reader is referred to a recent 2018 review[59].

Lipocalin

Evidence from animal models.—The first insights into an osteocalcin-independent effect of bone on energy homeostasis emerged in a study wherein mice deficient in osteoblasts due to an inducible CreER^{T2}-driven genetic system that activates the expression of diphtheria toxin A developed a metabolic phenotype that could not be fully rescued by systemic osteocalcin administration[60]. Notably, compared to wild type animals, osteoblast-deficient mice exhibited an increase in food intake (hyperphagia) that was not previously seen in osteocalcin-deficient animals[33] and was not affected by exogenous osteocalcin administration[60]. The underlying basis for the phenotype was revealed in a reassessment of mice with osteoblast-specific deletion of FoxO1(described earlier)[38,61] Interestingly, in addition to the increase in osteocalcin levels, osteoblast-specific FoxO1mutants also showed an increase in both the expression and circulating levels of lipocalin-2 (Lcn2), an adipokine involved in systemic glucose homeostasis and obesity[38,61–63]. Since expression levels of Lcn2 in bone are 10-fold higher than the levels seen in white adipose tissue (WAT), it has been proposed that Lcn2 could more aptly be referred to as an osteokine[63]. In accordance with this suggestion, WAT-specific deletion of Lcn2 in mice induced a 30% decrease in serum Lcn2 levels but produced no discernable metabolic phenotypes[63]. By contrast, osteoblast-specific Lcn2-deficient mice had almost a 70% decrease in serum Lcn2 levels compared to wild type animals and developed hyperphagia accompanied by glucose intolerance, insulin resistance and hypoinsulinemia due to decreased pancreatic β-cell mass.

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Interestingly, in osteoblast-specific Lcn2-deficient mice, no perturbations were observed in bone mass or osteocalin levels[63]. In this Lcn2 model, the increase in food intake precedes the changes in glycaemia and body weight. Moreover, a paired feeding system wherein food intake by the mutant mice was normalized to that of wild type controls the obese phenotype of osteoblast-specific Lcn2-deficient mice was partially improved, therefore the metabolic phenotype associated with Lcn2 deficiency was largely attributed to anorexigenic effects of Lcn2. However, the decrease in serum insulin levels in osteoblast-specific Lcn2-deficient mice was not rescued by paired feeding, suggesting that Lcn2 exerts direct effects on pancreatic β-cells, potentially inducing proliferation and insulin secretion[63].

Previous studies examining the importance of Lcn2 as a regulator of metabolism have showed mixed results in the beneficial influences on obesity, metabolic syndrome, glucose or lipid homeostasis and thermogenesis, ranging from very pronounced to rather minimal[64–69]. These discrepancies might be due to differences in genetic strategies used to generate the mouse models[63,70]. Nevertheless, the strength of this new Lcn2 paradigm is fueled by the observation that exogenous administration of Lcn2 to lean and obese mice decreases food intake and fat mass and improves glycaemic parameters[63]. Moreover, expression levels of Lcn2 in bone and the circulating serum levels were the highest 1–3 hours postprandially, which correlates with the suppression of food intake. Furthermore, restoring Lcn2 levels in fasting osteoblast-specific Lcn2 deficient mice corrected the hyperphagia within one hour upon refeeding, which suggests a role for Lcn2 in acute postprandial regulation of satiety[63]. Additionally, the same study delineated a neuroendocrine pathway by which Lcn2 suppresses feeding[63]. Of note, Lcn2 can cross the blood–brain barrier and intracerebroventricular injection evokes effects on feeding behavior that are similar to peripheral administration. In the CNS, Lcn2 binds the melanocortin 4 receptor (MC4R), which is essential to its function, in the paraventricular nucleus (PVN) of the hypothalamus, with an affinity similar to α melanocyte–stimulating hormone (αMSH); this pathway leads to increased cAMP production which is suggestive for receptor desensitization, and the expression of MC4R target genes to regulate appetite[63]. In summary, this study sheds new light on the skeleton's integration in the regulation of whole body energy homeostasis by controlling appetite.

Human studies.—Studies have been carried out that aim to identify associations between human LCN2 and metabolic functions that are comparable with the role of this factor in mouse models. Although contradictory with the above-mentioned mouse study, but suggestive for a compensatory mechanism, most of these studies indicate that the serum levels of LCN2 are increased in humans with obesity and insulin resistance but decrease again in long-term diabetics and the levels negatively correlate with body weight and glycaemic control[71–73]. In humans, the primary tissue of origin remains unknown, but increased LCN2 expression in adipose tissue and liver has been noted in obesity[74–76]. It is also possible that the initial homeostatic increase in Lcn2 seen after a meal in healthy individuals is lost in obesity, which is evidenced by a study demonstrating that serum LCN2 levels were increased within 30 minutes of a high fat meal in healthy woman but not in women with obesity[77]. Clearly, further work is required to better define the contribution of bone-derived LCN2 in human metabolism.

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Sclerostin

Paracrine actions in bone.—Sclerostin (encoded by *SOST*) is a secreted, cystine-knot glycoprotein with homology to the DAN domain family of bone morphogenetic protein antagonists[78]. The factor is best known as an antagonist of Wnt–β-catenin signaling and is a strong suppressor of bone mass accrual. The protein is primarily produced by bone matrix-embedded osteocytes, although low levels have been detected in the kidney and liver of mice[79] as well in the medial layer of major arteries during murine fetal development[80]. Importantly, genetic defects in the coding sequence of *SOST*[79,81], or a deletion of gene regulatory elements[82,83] that abolishes sclerostin production, underlie the rare bone diseases Sclerosteosis and van Buchem disease, respectively. Both conditions result in severe bone overgrowth, especially in the long bones, mandible and skull that lead to facial malformations, nerve entrapment and increased bone strength. In addition, Sclerosteosis is also associated with large stature and syndactyly (joined digits)[84].

Within the bone microenvironment, bone-forming osteoblasts and osteoprogenitor cells express the Wnt-coreceptors, low density lipoprotein receptor-related protein-5 (LRP5) and LRP6, which bind via β-propellor domains to secreted sclerostin. This interaction inhibits Wnt–β-catenin signalling by preventing the interaction of Wnt1 class ligands with LRP5 and LRP6 and the subsequent formation of the Wnt–Frizzled–LRP5–LRP6 signalling complex[85–87]. This signalling complex is required for the propagation of signals that lead to the stabilization of β-catenin, which is a transcription factor required for osteoblast commitment and function[88,89]. Notably, mice with germline deletions of *Sost*[90] or deletion of *Sost* specifically in cells of the osteoblast-lineage[91] recapitulate the bone-overgrowth phenotype that is evident in Sclerosteosis and van Buchem disease, which is secondary to substantial increases in osteoblast numbers and bone formation rate.

Potential endocrine actions.—Aside from its paracrine actions in bone, sclerostin protein is also present in the serum[92]. Although circulating levels in humans correlate with BMD and fracture risk[93,94], a growing body of evidence suggests that the sclerostin might also exert an endocrine function. For example, multiple studies have reported that serum sclerostin levels are increased in patients with type 2 diabetes mellitus (T2DM)[95–98], which is usually assumed to underlie the decrease in bone quality associated with T2DM[99,100]. Furthermore, serum sclerostin levels exhibit a positive correlation with BMI and fat mass in both patients with T2DM and healthy control individuals[101–104]. Moreover, in a cross-sectional study investigating 79 patients with impaired glucose regulation and 43 healthy control individuals, serum sclerostin levels were positively associated with fasting glucose production and insulin resistance in muscle, liver, and fat[105].

Motivated by these clinical associations, researchers examined the influence of sclerostin on metabolism using a sclerostin-deficient mouse model (*Sost*$^{-/-}$ mice) and an adeno-associated viral overexpression model[106]. In addition to the expected substantial increase in bone volume, *Sost*$^{-/-}$ mice developed a lean phenotype with statistically significant decreases in subcutaneous and visceral adipose tissue mass, considerable decreases in adipocyte hypertrophy and improved glucose handling[106]. By contrast, overexpression of sclerostin in the liver of post-pubertal mice produced the opposite effect, triggering a rapid

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increase in fat mass over an 8-week study[106]. Interestingly, the changes in body composition observed in these models is seemingly the result of the ability of sclerostin to direct adipocyte metabolism. For example, compared with wild type mice, the rate of de novo lipid synthesis was decreased, whereas fatty acid oxidation was increased in the white adipose tissue of *Sost*$^{-/-}$ mice. Moreover, in vitro studies using recombinant mouse sclerostin showed that it increased anabolic metabolism when administered to cultures of mouse adipocytes[106].

Sclerostin also regulates adipogenesis in the bone marrow, as evidenced by a study demonstrating that recombinant sclerostin induces marrow stromal cells to differentiate towards the adipocyte lineage and stimulates lipid accumulation in bone marrow adipogenic progenitor cells[107]. Importantly, the same study reported that *Sost*$^{-/-}$ mice as well as wild type mice treated with a sclerostin-neutralizing antibody showed decreased bone marrow adiposity. Similar to its effects on subcutaneous and visceral adiposity, the effects of sclerostin on marrow stromal cells were associated with changes in the expression of Wnt target genes[106,107]. Although additional studies are necessary to prove this in both adipose and bone marrow tissues, these data suggest that the antagonism of Wnt–β-catenin signalling is the primary mechanism by which sclerostin influences fat.

Targeting sclerostin therapeutically.—As sclerostin neutralizing antibodies enter the clinic for the treatment of postmenopausal osteoporosis[108,109] and potentially other conditions associated with skeletal frailty[110], it will be important to determine if this therapeutic paradigm will affect body composition and system metabolism. A preclinical assessment of the effect of sclerostin neutralization on body composition was performed via the weekly administration of anti-sclerostin antibodies to wild type mice fed a low or high fat diet for 8 weeks[106]. When compared to the vehicle-treated group, mice receiving the neutralizing antibody accumulated less body fat on each diet, which is compatible with the findings in *Sost*$^{-/-}$ mice. Furthermore, sclerostin neutralization inhibited the development of hyperglycaemia and hyperinsulinemia in mice fed a high fat diet[106]. These data suggest that sclerostin neutralizing therapeutics could have beneficial effects outside of the skeleton, but to the best of our knowledge, changes in systemic metabolism have not yet been examined in clinical trials.

INTERMEDIARY METABOLISM OF OSTEOBLASTS

The recognition of the skeleton as an important regulator of whole-body energy drew new attention to the cellular bioenergetics of osteoblastic cells[111–114]. Older literature, which has been largely forgotten by the field, contains descriptions of the oxidation of glucose, fatty acids and amino acids by skeletal explants or isolated osteoblasts. In the following section, we summarize the current view of the intermediary metabolism of the osteoblast, with specific attention to osteo-anabolic signalling pathways that govern fuel utilization.

Glucose

For more than 50 years, glucose has been recognized as a major fuel substrate for the osteoblast. In pioneering studies performed by William Neuman and colleagues, murine bone explants or calvarial cells were used to demonstrate that glucose is metabolized to

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lactate and citrate[22,23,115,116]. Contemporaneous studies confirmed these findings and indicated that citrate production in calvarial bone explants must be due to the metabolic activity of calvarial cells, as the amount of citrate released into the culture medium was greater than that initially present in the explant[117]. At the time of these early studies citrate was already known to accumulate within the bone matrix but its function was still unclear[118–120]. Since the bone-resorbing osteoclast had not yet been discovered, lactate and citrate were assumed to have a role in facilitating bone mineral release by lowering the local pH of bone matrix[22,121]. Today, a new role is attributed to citrate as a structural component of the hydroxyapatite crystals providing the strength and biomechanical properties of the bone[122].

Over the last few years, studies by several independent research groups have aimed to understand the linkage between glucose catabolism and bone anabolism, by using radio-labeled glucose analogs to examine glucose uptake in the skeleton of live mice[113,123,124]. For example, skeletal glucose uptake equals or even exceeds the uptake of typical metabolic organs such as liver, muscle and white adipose tissue and correlates with bone formation rate[124]. Although some controversy exists[123], glucose transport into the skeleton is at least partially regulated by insulin signalling, since mice fed a high fat diet and a mouse model with an osteoblast-specific deletion of the insulin receptor show decreased skeletal glucose uptake[124].

Glucose is transported into cells via facilitated diffusion through glucose transporters[125], which is a process that does not require energy[23]. Of note, 13 glucose transporters are known to exist and expression studies have detected the presence of three of these known transporters, Glucose transporter 1 (Glut1), Glut3 and Glut4, in osteoblastic cell lines and murine primary osteoblasts[123,126–130]. In osteoblasts, Glut1 seems to be the primary glucose transporter, and its role exceeds that of simple glucose diffusion. For example, Glut-1 mediated glucose-transport stimulates osteoblast differentiation and bone formation via at least two mechanisms: first by inhibiting AMP-activated protein kinase (AMPK)-dependent proteasomal degradation of RUNX2; second, by inducing mammalian target of rapamycin complex-1 (mTORC1)-mediated protein synthesis to facilitate the production of collagen matrix. Indeed, a mouse model with in Glut1 deficiency in osteolineage cells(encoded by *Slc2a1*) showed impaired osteoblast differentiation and reduced bone formation as compared with wild type animals[123].

Interestingly, in vitro assays using primary osteoblast culturessuggest that Glut4 (encoded by *Slc2a4*) is the only glucose transporter to show an increase in expression during osteogenic differentiation and that this transporter is required in osteoblasts for insulin-stimulated glucose uptake[130]. Moreover, Glut4-deficient primary osteoblast cultures showed a decrease in osteoblast performance established by reduced expression of osteocalcin and diminished calcium incorporation in extracellular matrix. However, a mouse model, with conditional deletion of *Slc2a4* in mature osteoblasts did not cause an obvious skeletal defect. Interestingly, osteoblast-specific Glut4-deficient mice developed hyperinsulinemia and insulin resistance on a normal chow diet[130], thereby providing evidence that osteoblasts have a role in insulin-stimulated glucose disposal. Of note, the contributions of Glut3 to bone development have not been studied in vivo. This particular protein is often referred to as the

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'neuronal' glucose transporter[131,132], thus its expression by calvarial osteoblasts might be due to their development derivation from the neural crest.

Once glucose is acquired by cells of the osteoblast lineage, the catabolic pathway that it follows is seemingly highly dependent on the stage of differentiation. As noted earlier, the transition from an uncommitted mesenchymal stem cell to a proliferating osteoblastic progenitor and finally to a polarized, highly synthetic mature osteoblast probably requires dramatic shifts in bioenergetic programming. An elegant study using in vitro primary mouse calvarial osteoblasts revealed a correlation between the rate of osteoblastic oxygen consumption and the degree of cellular differentiation[11]. In this work, mouse osteoblastic progenitor cells were shown to generate ATP mainly through glycolysis, whereas oxidative phosphorylation increases after the induction of osteogenic differentiation in order to meet the high energetic demands of matrix production. After mineralization, the primary cultures returned to mainly glycolytic metabolism. It is tempting to speculate that this increase in glycolysis is associated with an increase in the number of osteocytes within the culture. Little is known about the bioenergetic properties of osteocytes, which are the most abundant cell type in bone however, these long-lived descendants of the osteoblast can acidify their microenvironment to promote lacunar bone resorption[133]. Interestingly, human mesenchymal stem cells seem to have a nearly identical bioenergetic profile to that seen in mouse cells during osteogenic differentiation and show concurrent increases in mitochondrial biogenesis and oxidative phosphorylation[134,135].

Control of osteoblast glucose usage by Wnt.—Wnt signaling is known to contribute to the regulation of bone mass accrual via direct effects on bone cells as well as indirectly via the actions of this pathway in other tissues[136,137]. Of note, in vitro work in ST2 cells (a cell line that models mouse bone marrow stromal cells) showed that Wnt3a and Wnt10b, but not Wnt5a, act through the Wnt co-receptor, Lrp5, to increase the abundance of key enzymes involved in glycolysis (that is, Hk2, Pfk1, Pfkfb3, Ldha and Pdk1) via a post-transcriptional mechanism[138]. The increase in glycolytic enzyme expression was mediated by activation of mTORC2 and AKT, but was independent of β-catenin signaling. Importantly, germline $Lrp5^{-/-}$ mice and osteoblast-specific Lrp5-deficient mice both had lower bone mass and decreased levels of glycolytic enzymes than wild type animals. By contrast, mice with a mutation in *Lrp5* ($Lrp5^{A124V}$ mice) that is associated with high bone mass had elevated levels of glycolytic enzymes[138]. The enhancement of aerobic glycolysis by Wnt–mTORC2 signalling in osteoprogenitor-like ST2 cells suggests that Warburg metabolism is important for early osteoblast differentiation. Thus, osteoprogenitors resemble cancer cells in a way that they utilize Warburg-like metabolism for rapid proliferation and to generate the required intermediates for lipid and nucleotide assembly[139].

Wnt-regulated changes in metabolism also potentially regulate osteoblast performance via epigenetic regulation of gene expression. For example, treating ST2 cells with Wnt3a to activate Wnt signalling led to a greater number of downregulated genes than upregulated genes[140]. Since β-catenin primarily functions as a transcriptional activator, Wnt signalling was predicted to contribute to the regulation of histone modification. Indeed, levels of histone acetylation were decreased in ST2 cells after Wnt3a treatment, which was an effect associated with a decrease in nuclear acetyl-CoA (the substrate for histone

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acetyltransferases) levels as well as citrate (the precursor for acetyl-CoA synthesis)[140]. Molecularly, Wnt-signaling acts to suppress adipogenic gene expression and favor the attainment of an osteoblastic phenotype in a bipotent progenitor cell by the upregulation of PDK1 (a kinase involved in the regulation of the oxidative decarboxylation of pyruvate into acetyl-CoA) and thereby inhibiting pyruvate flux into the tricarboxylic acid cycle and reducing the nuclear acetyl-coA availability . These studies uncovered new mechanisms of how Wnt-signalling favors osteogenic differentiation on a posttranslational and epigenetic level by modulating osteogenic cell metabolism and nutrient usage[140].

Control of osteoblast glucose usage by parathyroid hormone.—Parathyroid hormone (PTH) is a key regulator of serum calcium levels and forms the basis for the most widely used anabolic therapies for osteopenia and osteoporosis. This factor has long been recognized as a strong inducer of glycolysis in osteoblastic cells but the molecular mechanism was not known[115,116,121,141]. In osteolineage cells, the induction of insulin-like growth factor-1 (IGF1) expression is a well-established response to PTH through Gαs-mediated cyclic adenosine monophosphate (cAMP) production and both IGF1 and the IGF1 receptor (IGF1R) are required for the osteoanabolic actions of PTH[142,143]. As such, it was proposed that IGF1-signalling might contribute to the regulation of glycolysis[114]. Similar to Wnt stimulation[138], PTH increased the protein levels of glycolytic enzymes (Hk2, Ldha, and Pdk1) in the Mc3t3-E1 osteoprogenitor cell line, which increased glucose consumption and was dependent upon the activation of cAMP production, IGF1R and PI3K-AKT-mTORC2 signalling[114]. Additionally, in vivo studies in a mice demonstrated that the co-administration of dichloroacetate (a Pdk1 inhibitor promoting pyruvate flux into the TCA cycle) and PTH reverses the anabolic effect of PTH on trabecular bone formation and restores cellular metabolism[114]. These findings suggest that the anabolic actions of PTH are mediated through IGF1-induced glycolysis and osteoblast function

Interestingly, the induction of IGF1 expression by PTH and subsequent metabolic reprogramming to enhance aerobic glycolysis is seemingly only directly related to bone formation, since markers of osteoclastic bone resorption were still increased by PTH in dichloroacetate-treated mice. However, it is important to note that in these studies dichloroacetate was administered systemically, so the influence of alterations in systemic metabolism cannot be ruled out. Moreover, extracellular fluxed analysis in vitro revealed a paradoxical increase in O_2 consumption in PTH-treated osteoblasts, despite a concurrent decrease in glucose oxidation occurring in the same cells, which suggests that the oxidation of other substrates is also under the control of PTH.

Control of osteoblast glucose usage by Notch.—Notch signalling is a negative regulator of bone formation by contributing to the regulation of cell fate specification. In mammals, Notch signalling pathways are initiated by a family of four conserved Notch transmembrane receptors (Notch1–4) and its ligands (Jagged1 and –2 and Delta-like 1, –3, and –4) inducing proteolysis of the receptors by the γ-secretases which results in the release of the Notch intracellular domain (NICD) and Notch-mediated gene transcription[144]. The function of Notch in bone is evidenced by studies showing that the conditional deletion of the receptor Notch2 in early mesenchymal progenitor cells using the Prx-Cre driver strain

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results in increased bone mass[145] while sustained activation of Notch2 signaling in osteoblasts induces osteopenia in mice[146]. It was proposed that one of the mechanisms by which Notch exerts its effects on bone is through the suppression of glycolysis in early osteolineage cells and a subsequent reduction in osteoblast differentiation[147]. Indeed, upon Notch2 activation, NICD forms a transcriptional complex and induces transcriptional activation of as of yet unidentified repressors that decrease the expression of both glycolytic enzymes and mitochondrial respiration proteins. Additionally, NICD-mediated suppression of the reactive oxygen species-producing mitochondrial complex-1 led to a reduction in AMPK activity and further suppression of glycolysis in ST2 cells[147]. Finally, evidence suggests that PTH inhibits Notch signalling in osteoblast- and osteocyte-enriched cultures, which raises the possibility that these two pathways might coordinately influence osteoblast metabolism[148].

Control of osteoblast glucose usage by hypoxia-inducible factor.—The hypoxia-inducible factors (HIFs) are evolutionarily conserved regulators of the cellular response to decreases in the concentration of molecular oxygen, however, HIF expressioncan also be induced in response to growth factor and other anabolic signalling[149]. The Hif-1α and Hif-2α subunits, which have overlapping but non-redundant functions, are constitutively expressed in most tissues but are recognized by prolyl hydroxylase enzymes and the von Hippel-Lindau protein to initiate rapid proteasomal degradation at normoxia. When oxygen tension drops below 5%, the α-subunits are stabilized and pair with Hif-1β to induced the expression of genes involved in the regulation of angiogenesis and cellular metabolism among others. During embryonic bone development or fracture healing processes, HIFs act to coordinate the coupling of angiogenesis and osteogenesis; in a mouse model, the ablation of the von Hippel-Lindau disease tumor suppressor (Vhl, a suppressor of HIF under normoxic conditions) in mature osteoblasts led to substantial increases in bone volume and skeletal vascularization by the actions of vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis and target gene of HIF[150]. Other studies suggest that in addition to increased vascularization, the regulation of glucose metabolism by HIFs contributes to bone formation[151]. Indeed, a mouse model with induced overexpression of a stabilized form of Hif1α (the oxygen sensitive subunit of HIF1) in osterix-expressing osteoprogenitor cells (osterix is a osteogenic transcription factor). produced a high bone mass phenotype. Intriguingly, the genetic ablation of VEGF to inhibit the increase in vascularization was not sufficient to reverse the HIF-induced increase in bone formation[151]. Since Hif1α activation is known to increase glycolysis[152], a metabolic mechanism of action was suggested for the high bone mass phenotype[151]. Indeed, protein levels of glycolytic enzymes (Hk2, Pdk1 and Ldha) were increased in the skeletal tissue of osteoblast-specific Hif1α-overexpressing mutant mice and the application of glycolysis inhibitor dichloroacetate preventing the increase in bone volume enforced the hypothesis that HIF1α -induced glycolysis supports bone formation[151]. Interestingly, another mouse model with osteolineage-specific Vhl deletion, a negative regulator of the hypoxia signaling pathways, showed increases in osteoblastic HIF-signaling, bone volume and osteogenic glucose consumption. However, the high bone mass phenotype observed in this mouse model could not be solely attributed to increased glycolysis as pharmacological inhibition of PDK1 with dichloroacetate (restoring the cellular metabolism) was not able to prevent the development

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of the high bone mass phenotype. Furthermore Vhl-deletion reduces osteogenic differentiation and increased the pool of osteoblast progenitors in vivo [113]. The difference between the osteoblast-specific stable Hif1a overexpressing mouse model and the osteoblast-specific Vhl-deficient mouse model could be explained by the overexpression of Hif2α in the Vhl model, which has been shown to play a protective role against bone resorption[153]. Due to the broad spectrum of signaling cascades affected by Hif-signaling, the interpretation of both of these studies requires care and additional work is necessary.

Fatty acids

The juxtaposition of trabecular bone surfaces and bone marrow fat, which can occupy ~70% of the available bone marrow volume in healthy adults[154], suggests that fatty acids liberated from stored triglycerides in marrow might be an important energy source the energetic demands of bone formation. Indeed, the oxidation of fatty acids yields far more energy than that provided by glucose or amino acid metabolism. Historic studies from the laboratory of Herbert Fleisch demonstrated that osteoblasts could oxidize fatty acids in vitro and that fatty acid utilization was under hormonal control (stimulated by vitamin D and diminished by insulin)[154,155]. In these studies, palmitate oxidation was predicted to yield an energy amount that is comparable to 40–80% of the energy that osteoblasts derive from glucose utilization. Over the last decade, tracing studies have enabled the comparison of skeletal lipid uptake with that of other tissues. Using fluorescent or radiolabeled chylomicron remnants, one study demonstrated that skeletal uptake of postprandial lipoproteins was second only to the liver (which is the most lipid-avid tissue) and that the uptake between cortical bone and bone marrow were comparable[156]. Similar results were obtained by administering radiolabeled Bromo-palmitate (an analog that cannot be oxidized) to wild type C57Bl/6 mice by gavage, which suggests that the skeleton is a major contributor to the clearance of lipids from the circulation[157].

When enriched to the diet of growing and developing mice and rats, polyunsaturated fatty acids have positive effects on osteoblast performance and reduced the risk of osteoporosis[158]. Furthermore, L-carnitine oral supplementation in ovariectomized rats (a model of osteoporosis) is protective against bone loss[159]. In addition, etomoxir (an inhibitor of fatty acid oxidation) administration[112,160] and the depletion of lipoproteins from the cell culture medium[161] have detrimental effects on in vitro osteoblast mineralization and cell growth. Taken together, these findings suggest that fatty acid utilization is key to the maintenance of normal bone architecture.

To more directly test the hypothesis that fatty acids are key for healthy bone homeostasis, one group examined the skeletal phenotype of mice deficient in carnitine palmitoyltransferase 2 (Cpt2, an obligate enzyme in mitochondrial long chain fatty acid oxidation) specifically in osteoblasts and osteocytes[157]. Interestingly, male mutants showed only a transient defect in trabecular bone mass owing to dramatic increases in glucose utilization by bone, however, female mutants showed less metabolic flexibility and had more substantial defects in bone structure. Estrogen is known to favor fatty acid utilization at the expense of glucose metabolism in a number of tissues[162–164] and might contribute to the sexual dimorphism in the skeletal phenotypes of this model. Of note, this study also

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demonstrated that although osteoblasts accumulate lipid droplets in a similar manner to most other cells[160], it is mostly the acquisition of exogenous fatty acids that fuels bone formation, rather than the usage of intracellular stores, as the osteoblast-specific ablation of Atgl (which catalyzes the first step in energy production from endogenous triacylglycerol hydrolysisdid not impact bone mass accrual[157]. The transporters that mediated uptake of fatty acids by the osteoblast are not known, but both apolipoprotein E[165] and CD36[166], which are known to be important in other tissues, are expressed in bone.

Fatty acid utilization by the osteoblast was shown to be under the control of Wnt–Lrp5 signalling by both in vitro and in vivo studies in mice[112]. Although the specific ablation of either Lrp5 or Lrp6 in osteoblasts and osteocytes reduces bone mass in mice[167], Lrp5 mutants develop an increase in fat mass and an increase in serum triglyceride levels, which suggests a defect in fatty acid catabolism. Indeed, the expression of several enzymatic mediators of β-oxidation were decreased in Lrp5-deficient primary mouse osteoblasts and increased in primary osteoblasts that express a variant of Lrp5 ($Lrp5^{G171V}$) associated with high bone mass or following Wnt10b stimulation[112]. Follow-up studies using mice with osteoblast-specific β-catenin-deficiency indicated that Wnt-signalling regulates fatty acid catabolism via the canonical Wnt–β-catenin pathway[168]. As Wnt-induced glycolysis was reported to be regulated by non-canonical mTOR activation and protein synthesis[138], it seems that pathways downstream of Wnt signalling can differentially influence fuel utilization.

Of note, PTH signalling is suggested as a regulator of fatty acid utilization. This hormone induces lipolysis in adipocytes[169] and has been shown to reduce bone marrow adiposity when administered to mice[170,171]. In vitro work using an elegant co-culture system containing adipocytes and osteoblast progenitors demonstrated that after lipolysis, fatty acids are transferred from adipocytes to cells of the osteoblast lineage[171]. Although additional studies will be necessary, the transferred fatty acids could be speculated to be metabolized as an energy source.

Amino acids

Sufficient dietary protein intake is closely associated with skeletal health and osteoblasts express the specialized amino acid receptors and transporters that enable the adjustment of cellular bioenergetics according to fluctuations in amino acid availability[172]. Amino acids, classified as ketogenic or glucogenic, are building blocks that can be used by osteoblasts for protein synthesis or metabolized to generate ATP. Of note, ketogenic amino acids are metabolized to acetyl-coA or acetoacetate, whereas glucogenic amino acids are converted into pyruvate or TCA cycle intermediates (such as oxaloacetate, α-ketoglutarate, fumarate or succinyl-CoA).

Pioneering studies by Albert Fischer in 1948 uncovered a requirement for several amino acids [to enable proper osteoblast proliferation[173], whereas later studies reported the presence of the specialized amino acid transport systems (system A, system L, and system ASC) to be required for amino acid transport in bone[174–176]. Although clear mechanistic data are lacking, early studies examined the regulation of amino acid transport in osteoblasts and suggested roles for cAMP[177] and several hormones and growth factors including thyroid

hormone[178], insulin and IGF1[179,180]. At the transcriptional level, amino acid transport is regulated by activating transcription factor-4 (ATF4), which is activated by the unfolded protein response or by amino acid depletion and is recognized as a marker of osteogenic differentiation[181,182]. Importantly, in maturing osteoblasts, ATF4 provides a link between amino acid transport and collagen production, thereby ensuring an equilibrium between synthetic processes and demand. Indeed, in a mouse model that mimics Coffin-Lowry syndrome, a disease characterized by a defect in the RSK kinase that activates ATF4, skeletal defects could be rescued by feeding the mice a high-protein diet[181,182].

Glutamine is the most abundant amino acid in the bloodstream[183] and is well-investigated in cancer biology. In malignant cells, PDK1 mediates the switch to aerobic glycolysis and decreases the flux of pyruvate to the TCA cycle. For these cells to avoid detrimental depletion of TCA cycle intermediates (cataplerosis), the reliance on glutamine as an alternative substrate for the TCA cycle gains importance. The conversion of glutamine to glutamate by glutaminase (GLS) and subsequently to α-ketoglutarate replenishes the TCA cycle (anaplerosis); this fact renders glutamine an ideal bio-energetic substrate for cells that are highly reliant on glycolysis as well as an anabolic agent by providing intermediates for active biosynthesis[184,185]. Importantly, the glycolytic nature of osteoblasts suggests that glutamine probably contributes to the regulation of osteoblast differentiation and matrix mineralization[186,187]. Indeed, a recent 2019 study demonstrated that mice that lack GLS in limb bud mesenchyme using the Prx-Cre driver strain and in leptin receptor-positive skeletal stem cells develop a low bone mass phenotype due to alterations in mesenchymal progenitor cell proliferation and lineage specification[188].

Wnt3a stimulation during osteoblast differentiation has been shown to increase aerobic glycolysis and consequently induces rapid and abundant glutamine consumption by increased mTORC1-driven GLS activity, in order to prevent cataplerosis[189]. Indeed, stable isotope labelling experiments in osteoblasts revealed that glutamine was converted into citrate by oxidation in the TCA-cycle. Besides its role in providing ATP, in vitro studies using ST2 cells showed that increased glutamine catabolism also initiates the activation of general control nonderepressible 2 (Gcn2), which is a sensor of amino acid deficiency[189]. By phosphorylating eukaryotic translation initiation factor-2α, Gcn2 stimulates a transcriptional program that increases protein biosynthesis through induction of ATF4 in ST2 cells[189]. Confirming these in vitro findings, deletion of Gcn2 in a mouse model that also expresses the high-bone mass allele of Lrp5 ($Lrp5^{A124V}$), normalized osteoblast numbers and bone mass[189]. Thus, this data supports the notion that glutamine is not solely an important energy source but also a gatekeeper for amino acid influx and adequate protein synthesis.

Intriguingly, age-related bone loss has also been found to be associated with decreased glutamine anaplerosis and diminished expression of both GLS and estrogen-related receptor α (ERRα)[190]. These proteins show increased expression during osteoblast differentiation, with ERRα inducing transcription of GLS in an mTOR-dependent manner. Of note, glutamine, through its conversion to glutathione, also contributes to the maintenance of redox balance in osteogenic cells. For example, in mouse periosteum-derived cells (which aremultipotent mesenchymal progenitor cells forming a membrane surrounding the bone),

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hypoxia induces GLS-mediated synthesis of glutathione from glutamine, which to neutralizes reactive oxygen species and increases cell survival in stressed conditions at the expense of anaplerosis[191].

CONCLUSIONS

The application of advanced genetic approaches in mouse models, together with modern biochemical methods for quantitating fuel substrate metabolism, has enabled the discoveries summarized in this review. Taken together, these studies define a new conceptual role for the skeleton in global metabolism. In this model, osteoblasts communicate with other organ systems to coordinate bone formation with the overall energy balance. From an evolutionary perspective, it is reasonable to speculate that the endocrine actions of bone-derived osteocalcin, lipocalin, and sclerostin probably evolved in response to the energetic demands of a constantly remodeling skeleton. In this regard, it should not be surprising that the locally and hormonally controlled signalling events that are essential for driving bone formation also program the intermediary metabolism of the osteoblast. The coupling of these metabolic programs could enable osteoblasts to optimize fuel consumption according to different functional demands encountered during different stages of life and in pathophysiological situations, such as fracture repair.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the work by other investigators that has not be cited in this manuscript because of space limitations. Work in the author's laboratories is supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (DK099134, R.C.R) and the Biomedical Laboratory Research and Development Service of the Veterans Affairs Office of Research and Development (BX003724, R.C.R; BX001234, T.L.C.). T.L.C is also the recipient of a Senior Research Career Scientist Award from the Department of Veterans Affairs.

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