Metabolic regulation of skeletal cell fate and function in physiology and disease

Nick van Gastel[1,2,*] & Geert Carmeliet[3,*]

[1]de Duve Institute, Université Catholique de Louvain, Brussels, B-1200, Belgium

[2]Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Harvard University, Cambridge, MA 02138, USA

[4]Laboratory of Clinical and Experimental Endocrinology, Department of Chronic Diseases, Metabolism and Ageing, KU Leuven, Leuven, B-3000, Belgium

*Corresponding author

Abstract

The skeleton is diverse in its functions, which include mechanical support, movement, blood cell production, mineral storage and endocrine regulation. This multifaceted role is achieved by an interplay of osteoblasts, chondrocytes, bone marrow adipocytes and stromal cells, all generated from skeletal stem cells. Emerging evidence shows the importance of cellular metabolism in the molecular control of the skeletal system. The different skeletal cell types not only have distinct metabolic demands relating to their particular function, but are also impacted by microenvironmental constraints. Specific metabolites control skeletal stem cell maintenance, direct lineage allocation and mediate cellular communication. Here, we discuss recent findings on the role of cellular metabolism in determining skeletal stem cell fate, coordinating osteoblast and chondrocyte function and organizing stromal support of haematopoiesis. We also consider metabolic dysregulation in skeletal ageing and degenerative diseases, and provide an outlook on how the field may evolve in the coming years.

Main

Skeletal development and maintenance are orchestrated by the action of key cell types that differ in their location and function (Figure 1). Osteoblasts are the bone-forming cells, laying down a characteristic collagen-rich matrix known as osteoid, which is mineralized to give bone its strength[1, 2]. During the ossification process a subset of osteoblasts become embedded in the bone matrix, after which they are referred to as osteocytes[1, 3]. The second major skeletal cell type are chondrocytes, which form the different types of cartilage. Hyaline cartilage is found in the articular surfaces of bones, growth plates, ribs, nose, larynx and trachea, fibrous cartilage makes up the intervertebral discs and menisci and elastic cartilage the external ear and epiglottis[4]. Chondrocytes also play a key role in the endochondral ossification process during bone development and fracture repair, where a hyaline cartilage intermediate is formed that is later replaced by bone through the combined action of osteoclasts, bone-degrading cells of haematopoietic origin, and osteoblasts[5, 6].

In the bone marrow cavity, two additional mature skeletal cell types are found: marrow adipocytes and haematopoiesis-supportive stromal cells. Marrow adipose tissue has unique features that distinguish it from the better characterized extramedullary white, brown and beige adipose deposits[7]. Bone marrow adipocytes contribute to local and systemic metabolic processes including haematopoiesis, osteogenesis and energy metabolism, and undergo changes as a function of age and diet. Haematopoiesis-supportive stromal cells, also referred to as mesenchymal stromal cells, are a population of fibroblastic cells that supply essential cytokines for blood cell production[8, 9]. This population of cells remains poorly characterized and may contain subpopulations of cells with distinct functions in haematopoietic support[10-12].

The different mature skeletal cell types originate from a common precursor cell known as the skeletal stem cell (SSC)[13-15]. While the skeletal system resembles the hierarchical structure of other stem cell-driven tissues such as the haematopoietic system in many ways – with a self-renewing stem cell

residing at the apex that progressively gives rise to multipotent progenitor cells, committed progenitor cells and mature cells – it harbours three distinct stem cell pools, with SSCs found in the growth plate, the periosteum and the bone marrow[13-15]. Certain aspects are shared between the different SSC pools, such as the ability to self-renew and the expression of certain cell surface markers, but the differentiation potential of the distinct SSC pools varies and appears to be linked to the specific requirement of the locations in which they are found[13-15]. The SSCs found in the bone marrow share many characteristics with haematopoiesis-supportive stromal cells, and it remains unclear whether these are the same cells or distinct populations that share markers. In Figure 1, we list the most commonly used markers for mouse and human SSCs and their progeny. For a more detailed overview of SSC biology and the current mouse models used for genetic manipulation of SSCs, we refer the reader to a number of excellent recent review articles[13-15].

The rapid expansion of markers and tools to identify, isolate and genetically alter different skeletal cell populations has tremendously increased our insight in the molecular pathways that govern their formation, maintenance and function. The concept that skeletal cell biology is controlled not only by cytokine and hormone signalling but also by metabolic pathways has gained traction over recent years. Metabolic programs depend on the functions a cell has to fulfil and may thus be different in proliferating progenitors versus matrix-producing differentiating cells. In addition, the supply of oxygen and nutrients is related to the density and type of vasculature in the microenvironment, as well as the metabolic status of surrounding cell types. Skeletal tissue is characterized by low oxygen levels (1,5-4%)[16], with regional differences, whereas insight in local nutrient availability is largely lacking. Metabolic pathways are therefore adapted to sustain bioenergetic, biosynthetic and redox homeostasis and to allow epigenetic modifications. Although knowledge is still limited, metabolic pathways in skeletal cells are likely controlled by cell-intrinsic factors and are modified by growth factors and hormones. In the following sections, we summarize current findings on the metabolic regulation of skeletal cells and look towards the potential future impact of this research area.

Metabolic control of skeletal stem and progenitor cells

Stem cells are characterized by their ability to self-renew (*i.e.* give rise to at least one new stem cell during cell division) and their multipotency (*i.e.* have several different mature cell types as progeny). This process is under a tight molecular control and it is now appreciated from different stem cell fields that metabolic pathways play a key role in this decision-making process[17]. Our understanding of the metabolic regulation of SSCs is still very limited (Figure 2), and most findings come from culture-expanded bone marrow cells selected by adherence to plastic, which likely represent a mixture of true SSCs, skeletal progenitor cells and haematopoiesis-supportive stromal cells, or heterogeneous periosteal cell cultures established through plastic adherence and containing both SSCs and osteogenic progenitor cells. Given their heterogeneous nature, we refer to these cells as bone marrow stromal cells (BMSCs) or periosteum-derived cells (PDCs).

Cultured human BMSCs are highly glycolytic[18]. Glycolysis provides some energy in the form of ATP, but it also supplies proliferating cells with carbon for biomass generation[19]. Indeed, glucose can contribute to the pentose phosphate pathway (PPP) and the serine synthesis pathway. The PPP generates precursors for nucleotide biosynthesis and reducing molecules for anabolism and to counter oxidative stress, whereas the serine synthesis pathway contributes to nucleotide, amino acid and lipid biosynthesis[19]. However, the role of these pathways in SSCs has not yet been studied.

The low oxygen tensions in the bone marrow (1-2%[16]) may induce the glycolytic phenotype, as *in vitro* expansion of BMSCs at ambient oxygen levels (20-21%) progressively increases mitochondrial glucose oxidation, associated with increased oxidative stress and cellular senescence[18]. In both BMSCs and PDCs the transcription factor hypoxia-inducible factor 1α (HIF-1α) plays a key role in regulating the glycolytic phenotype[20, 21]. In addition, loss of HIF-1α severely compromises survival and bone formation by mouse PDCs upon transplantation *in vivo*[21], underscoring the importance of HIF-1α for PDC function in oxygen- and nutrient-deficient conditions. Of note, in both bone marrow and periosteum, SSCs and

skeletal progenitors are found in perivascular locations under homeostatic conditions[13-15, 22], raising the question whether and under which conditions SSCs in their native niches depend on HIF-1α stabilization and glycolysis. In addition, the role of glucose oxidation in SSCs, especially in *in vivo* settings, remains unclear.

When glucose is primarily used in glycolysis for bioenergetics or biosynthesis, other nutrients can fuel the mitochondrial tricarboxylic acid (TCA) cycle to supplement cellular needs. The TCA cycle provides reducing equivalents (NADH, $FADH_2$) for oxidative phosphorylation as well as metabolic intermediates for biosynthetic processes and cellular signalling[23]. Besides glucose-derived pyruvate, several other metabolites can fuel the TCA cycle either by providing acetyl-CoA for oxidation (fatty acids, ketone bodies) or as anaplerotic substrates (glutamine, odd-chain fatty acids)[23]. Little is known about the role of fatty acid oxidation (FAO) in SSCs. While human BMSCs perform FAO in culture, this pathway is responsible for less than 0.5% of ATP production[24]. Our recent findings confirm this observation, showing that mouse bone marrow SSCs and PDCs express genes encoding essential FAO enzymes, such as carnitine palmitoyltransferase 1a (*Cpt1a*), and perform FAO in culture[25]. However, SSC viability is relatively unaffected by loss of CPT1a both *in vitro* and *in vivo*[25], indicating that their metabolism does not rely heavily on FAO.

In contrast to fatty acids, human BMSCs in culture can oxidize the ketone body acetoacetate in the TCA cycle with high efficiency to generate ATP, producing much lower levels of reactive oxygen species (ROS) compared to glucose oxidation[26]. It remains to be confirmed whether ketone body oxidation by SSCs also occurs *in vivo*, and whether this pathway for example provides a nutrient source for SSCs during periods of fasting when levels of ketone bodies in the blood rise[27]. Ketone bodies also have important signalling roles, including epigenetic regulation and post-translational protein modification, further warranting a more detailed study of this metabolic pathway in SSCs.

Amino acid metabolism plays a major role in cellular energetics and biosynthesis, especially in proliferating cells[28]. In line with this, a recent report has shown that the amino acid sensor EIF2AK4/GCN2 is required to support the robust proliferative capacity of mouse SSCs during bone homeostasis[29]. While the role of most individual amino acids in SSCs remains unstudied, emerging evidence from both *in vitro* and *in vivo* studies shows the importance of glutamine for SSC function. Human and mouse BMSCs as well as mouse PDCs take up glutamine in culture[30-33]. Depletion of extracellular glutamine, inhibition of the enzyme glutaminase (GLS), which converts glutamine into glutamate with the release of ammonia, or inhibition of glutamate transaminases, which link glutamine catabolism to both the TCA cycle and amino acid synthesis, all strongly reduce colony formation and proliferation of mouse BMSCs[33]. Cultured BMSCs and PDCs use glutamine to fuel the mitochondrial TCA cycle, amino acid synthesis and glutathione biosynthesis[31-33]. Interestingly, conditional deletion of *Gls* in SSCs in mice using $Prrx1^{cre}$ does not impact the number of bone marrow SSCs, while deletion using $Lepr^{cre}$ does strongly decrease SSC number[33]. However, given that $Lepr^{cre}$ also recombines in the hypothalamus and in limbic and cortical brain regions[34], and complete GLS-deficient mice have severe neural phenotypes[35], the SSC phenotype of $Lepr^{cre}Gls^{lox/lox}$ mice is hard to interpret. Thus, considering the lack of SSC number alterations in $Prrx1^{cre}Gls^{lox/lox}$ mice, SSCs do not appear to require GLS for their maintenance or proliferation *in vivo* as they do *in vitro*. A similar differential dependency on GLS *in vivo* versus *in vitro* has been described by us and others in different cancer models[36-38], underscoring the importance of examining the metabolism of cells in their native environment.

Nutritional and metabolic requirements of mature bone and bone marrow cells

Osteoblasts and the high anabolic demand of bone formation

The process of bone formation by osteoblasts involves the production and secretion of large amounts of collagen type 1-rich matrix, which later becomes mineralized[1, 2]. Protein synthesis is a metabolically demanding process, and the high rate of matrix synthesis requires osteoblasts to produce large

amounts of energy as well as amino acids. Matrix-producing osteoblasts often localize close to blood vessels, suggesting ample supply of oxygen and nutrients[39, 40]. Most studies thus far have focused on glucose metabolism (Figure 2). *In vivo* tracing studies in mice using radio-labelled glucose analogues have revealed that skeletal glucose uptake equals or exceeds that of metabolic organs such as liver, muscle and white adipose tissue[41, 42]. Osteoblastic glucose uptake can even impact whole-body glucose homeostasis. A recent study showed how stabilization of HIF-1α in osteoblasts dramatically increased their glucose uptake, causing hypoglycaemia and improved glucose tolerance[41]. Osteoblasts express the glucose transporters GLUT1, GLUT3 and GLUT4[43-45]. Glucose uptake by GLUT1 is necessary for proper osteoblast differentiation and bone formation through a mechanism that involves AMPK-dependent regulation of RUNX2, the master osteogenic transcription factor, and mTORC1[44]. In contrast, GLUT4 mediates insulin-stimulated glucose uptake by mature osteoblasts and osteocytes *in vitro*, but is dispensable for bone development and homeostasis *in vivo*[43], while the role of GLUT3 in osteoblasts remains unknown.

In culture, most of the glucose taken up by osteoblasts is metabolized to lactate even in the presence of ambient oxygen levels, a process known as aerobic glycolysis[46]. In mature osteoblasts aerobic glycolysis can account for up to 80% of ATP production[47]. It is estimated that under these conditions only 15-20% of glucose carbon goes to the mitochondrial TCA cycle or to synthetic processes[48, 49]. Recent findings show that one important fate of glucose carbon entering into the mitochondria in osteoblasts is the generation of malate, integral to the malate-aspartate shuttle that regenerates cytosolic NAD^+ to maintain glycolysis[47].

Glycolysis in osteoblasts is regulated by numerous factors. Forced activation of glycolysis in osteoblasts by overexpressing a stabilized form of HIF-1α or deletion of the von Hippel–Lindau protein increases osteoblast number and bone mass in mice, supporting the importance of this pathway for osteoblast function[41, 50]. In contrast, Notch signalling, important for SSC maintenance[51], suppresses glycolysis to restrict osteoblast differentiation[52]. Moreover, anabolic stimuli such as parathyroid hormone (PTH) or

WNT promote bone formation in part by further increasing glycolysis at the expense of glucose oxidation[49, 53, 54]. Both PTH and WNT increase levels of glycolytic enzymes through activation of mTORC2. In parallel to stimulating glycolysis, HIF-1α and PTH signalling also promote glycogen synthesis in immature osteogenic cells such as PDCs and calvarial pre-osteoblasts[32, 55]. Glycogen granules decrease in size as the cells become mature osteoblasts[56, 57], suggesting that the glycogen stores are used to support increasing bioenergetic or biosynthetic demands during bone formation.

Interestingly, PTH also increases glucose flux through the PPP[49]. Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme in the PPP, is highly expressed by osteoblasts and its activity increases upon osteoblast activation in response to mechanical loading or during bone fracture healing[58, 59]. Studies from the 1980s suggested that G6PD provides NADPH necessary to sustain the vitamin K-cycle which is crucial for matrix calcification by osteoblasts[59, 60], but this concept awaits validation by modern genetic and metabolic techniques.

Besides the dependency on glycolysis, a strong surge in oxygen consumption linked to increased ATP production has been described during the *in vitro* osteogenic differentiation of both mouse and human BMSCs[20, 61, 62]. Deletion of the transcription factor PPAR-γ co-activator 1α (PGC-1α), a master regulator of mitochondrial biogenesis and oxidative metabolism, in SSCs (using *Prrx1*-Cre or *LepR*-Cre mice, both giving similar results) reduces osteogenic differentiation and induces bone loss[63], although it is not known whether and how metabolic pathways are changed. Concomitant with the increase in oxidative phosphorylation, osteogenic differentiation of BMSCs is paired with an increase in antioxidative capacity[61]. Accordingly, osteoblasts, but not BMSCs, are sensitive to inhibition of oxidative phosphorylation or increasing oxidative stress[61, 64]. The increase in oxygen consumption and mitochondrial number during osteogenic differentiation may be transient, as recently shown in human adipose tissue-derived stromal cells[65]. This could explain the apparent contradiction in literature, with some studies showing decreasing oxygen consumption after osteogenic differentiation[47].

The reasons for the increased mitochondrial metabolism during osteoblast differentiation are a focus of ongoing research, although an increased need for energy and biosynthetic intermediates during matrix production and mineralization are likely involved. However, recent findings also point to alternative roles for mitochondrial activity in osteoblasts. Shares *et al.* found that hyperactivation of oxidative phosphorylation during osteogenic differentiation of mouse BMSCs increases mitochondrial citrate production, which after conversion in the cytosol to acetyl-CoA stimulates β-catenin acetylation[66]. Acetylation of β-catenin, the downstream effector of WNT signalling, leads to its activation, nuclear translocation and promotion of osteogenic differentiation. Similarly, in human BMSCs induced to undergo osteogenic differentiation, citrate exiting from mitochondria may be converted to α-ketoglutarate in the nucleus, which is a co-factor of histone demethylases, leading to transcriptional activation of osteogenesis-related genes[65]. Citrate produced by osteoblasts is also incorporated into the structure of the hydroxyapatite nanocrystal/collagen complex, which is essential for the biomechanical properties of bone[67], although the metabolic source of this citrate remains unknown. Intriguingly, WNT stimulation of the mouse ST2 skeletal progenitor cell line promotes osteogenesis in part by limiting glucose oxidation, thus decreasing nuclear citrate and acetyl-CoA levels, which in turn lead to a reduction in histone acetylation and overall gene expression[68]. As with oxygen consumption and mitochondrial number[65], a dynamic and stage-specific regulation of citrate levels, compartmentalization and metabolic fate may be key for correct osteogenic differentiation and function.

While osteoblasts have the ability for glucose oxidation, glucose does not appear to be a major fuel for mitochondrial TCA cycle activity[25, 48, 49], suggesting a role for other nutrients. FAO has long been known to play an important role in osteoblast energy metabolism. Older biochemical studies showed that around 40% of ATP production in osteoblasts can come from the oxidation of fatty acids[69, 70]. More recent studies confirm that osteoblasts express the requisite receptors and catabolic enzymes to metabolize fatty acids, and tracing studies show that the skeleton avidly takes up lipids from the circulation, second only to the liver[25, 71, 72]. We recently described that while lipid deprivation or loss of the key FAO enzyme CPT1a does not affect SSC survival, it does prevent differentiation to the

241 osteoblast lineage[25]. Kim *et al.* further showed that loss of CPT2 in mature osteoblasts reduces bone
242 formation by interfering with proper osteoid mineralization[71]. The effects were exacerbated in the
243 presence of oestrogen, which promotes FAO at the expense of glycolysis[71]. In addition, deletion of
244 PPAR-δ, a key transcriptional regulator of FAO, in osteoprogenitors (using *Runx2*-Cre mice) impairs
245 osteogenic differentiation and mineralization and reduces bone mass[73]. Oxidation of fatty acids by
246 osteoblasts is further stimulated by WNT signalling, with activation of β-catenin inducing the expression
247 of key FAO enzymes[74].
248
249 A high rate of FAO in osteoblasts may explain the apparent paradox of aerobic glycolysis occurring in
250 cells with a highly active TCA cycle. Dunham *et al.* calculated that in bone tissue the amount of acetyl-
251 CoA that could theoretically be produced by FAO and glucose oxidation would far exceed what could
252 be accommodated by the TCA cycle activity[70]. They proposed that the acetyl-CoA produced directly by
253 FAO within the mitochondria close to the site of most of the TCA cycle enzymes might be favoured,
254 especially since the conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase is readily
255 inhibited by acetyl-CoA. Since accumulation of pyruvate would block the biosynthetic activity of
256 glycolysis and the PPP, conversion of pyruvate to lactate allows for maximal metabolic activity. It will
257 be of interest to test this 40-year-old hypothesis *in vivo* using the mutant mouse models and metabolic
258 tracing techniques that are now available. In addition, the exact role of FAO in osteoblasts, being energy
259 production, biosynthesis or epigenetic regulation, remains to be determined.
260
261 Besides fatty acids, TCA cycle activity in the osteoblast is also fuelled by glutamine. Ablation of GLS in
262 the skeletal lineage (using *Prrx1*-Cre mice) reduces osteoblast development and bone formation[33]. Both
263 mouse and human BMSCs increase glutamine consumption and metabolism during osteogenic
264 differentiation *in vitro*[30, 33], and glutamine withdrawal or inhibition of GLS prevents osteogenic
265 differentiation[33]. The exact metabolic fate of glutamine in osteoblasts remains to be determined. As with
266 glycolysis and FAO, glutamine catabolism in osteoblasts is increased in response to WNT signalling to

support increased bone formation[75]. However, while WNT stimulates glycolysis in osteoblasts through mTORC2[54] and FAO through β-catenin[74], glutaminolysis is promoted via mTORC1 activation[75].

Nutritional restrictions of the unique chondrocyte microenvironment

Cartilage is one of the only avascular tissues in the human body, creating unique challenges regarding nutrient availability and metabolic waste removal (Figure 2). Diffusion is the main mode of solute mobility in cartilage matrix, which contains up to 80% water, meaning that small molecules such as glucose or amino acids may be readily available to matrix-embedded chondrocytes while larger molecules such as lipids are scarcer[25, 76, 77]. The absence of blood vessels in cartilage can create hypoxic environments, as elegantly shown by several groups in the foetal growth plate which contains a central hypoxic region, whereas the late hypertrophic chondrocytes at the border with the highly vascularized primary spongiosa are not hypoxic[78-81].

The presence of hypoxia in cartilage leads to HIF-1α stabilization, which promotes chondrogenesis by increasing expression of the master chondrogenic transcription factor SOX9, enhances chondrocyte survival and supports anaerobic glycolysis by regulating the transcription of key glycolytic enzymes[78, 80-82]. Anaerobic glycolysis is the main fate of glucose in cartilage[83, 84], but up to 20% of glucose may pass through the PPP[85, 86]. Chondrocytes depend on GLUT1 for glucose uptake and their proper functioning, and this transporter is under control of bone morphogenetic protein signalling, a key pathway for cartilage development[87]. We recently found that although mouse growth plate chondrocytes in culture generate the majority (>60%) of their ATP through glycolysis, they also require glucose oxidation to avoid energy distress[88]. Excessive HIF-1α stabilization in PHD2-deficient chondrocytes decreases glucose oxidation, leading to skeletal dysplasia by reducing proliferation and interfering with collagen biosynthesis[88].

Glucose may also fuel glycogen synthesis in chondrocytes, and normal hyaline cartilage contains glycogen in all its cells. The distribution of glycogen particles in the epiphyseal growth plate increases

294 with the degree of maturation of the chondrogenic cells[56, 89]. Glycogen stores appear to be used
295 predominantly during periods of rapid growth[56]. Accordingly, disruption of autophagy, which prevents
296 glycogenolysis, induces apoptosis in the proliferating zone of the growth plate and causes growth
297 retardation[90].
298
299 In contrast to osteoblasts, FAO does not seem to play a major role in chondrocytes. While epiphyseal
300 chondrocytes contain low levels of FAO enzymes[70], only a very small fraction of their ATP is generated
301 by FAO[88]. Mouse chondrocytes do not depend on FAO for their development or survival, and excess
302 extracellular lipids prevent the differentiation of SSCs towards chondrocytes both *in vitro* and *in vivo* by
303 decreasing *Sox9* expression[25]. SOX9 in turn acts as a metabolic regulator in chondrocytes by
304 suppressing FAO, but the exact molecular mechanism for this regulation remains unknown[25]. Since
305 chondrocytes exist in an environment with limited access to circulating lipids, they may depend on *de*
306 *novo* lipogenesis for their supply of fatty acids, as is seen in cancer[91]. While this concept has not yet
307 been studied in detail, one study shows that loss of ELOVL6, an enzyme involved in fatty acid elongation
308 during *de novo* lipogenesis, interferes with proper chondrocyte proliferation and differentiation[92].
309
310 SOX9 also promotes glutamine metabolism in chondrocytes by increasing glutamine consumption and
311 levels of GLS[31]. However, glutamine catabolism does not support ATP generation in chondrocytes.
312 Instead, we found that glutamine controls chondrogenic gene expression epigenetically through
313 glutamate dehydrogenase-dependent acetyl-CoA synthesis, necessary for histone acetylation[31]. In
314 addition, transaminase-mediated aspartate synthesis fuelled by glutamine supports chondrocyte
315 proliferation and matrix synthesis, while glutamine-derived glutathione synthesis avoids harmful ROS
316 accumulation[31]. Others have shown that glutamine is also required by chondrocytes as a nitrogen donor
317 for glycosaminoglycan synthesis[93]. Excessive HIF-1α stabilization increases glutamine metabolism, but
318 has to be avoided as increased glutamine-derived α-ketoglutarate production stimulates, as a cofactor,
319 collagen hydroxylases and this posttranslational modification of collagen leads to a less resorbable
320 chondrocyte matrix and disturbed endochondral ossification[88].

Taken together, current evidence shows that chondrocytes exhibit metabolic adaptations allowing them to proliferate and synthetize large amounts of matrix under limiting nutritional conditions. Being dependent on diffusion alone, glucose and glutamine appear to be the preferred substrates for chondrocyte metabolism, although potential roles for other small molecular weight nutrients able to diffuse through the unique cartilage matrix await further exploration.

The peculiar metabolic properties of marrow adipocytes

Adipose tissue plays a prominent role in whole body metabolism due to its role in lipid synthesis, storage and release (Figure 2). Bone marrow adipose tissue (MAT) is distinct in origin and characteristics from white and brown adipose tissue, but its exact function in the bone marrow microenvironment is still the subject of active research. MAT exists in two district populations: “constitutive” MAT (cMAT), found in the distal skeleton, important during early vertebrate development and “regulated” MAT (rMAT), found scattered throughout the skeleton, that may influence haematopoiesis[94]. Several studies show that marrow adipocytes and osteoblasts develop from a common progenitor, leading to the general notion that formation of adipocytes occurs at the expense of osteoblast differentiation[7]. However, marrow adipocytes also release a myriad of factors that can impact osteoblast function in both negative and positive ways[95]. In addition, PTH stimulation induces marrow adipocytes to release free fatty acids which are taken up by osteoblasts[96], showing that the relation between these two cell types is complex.

During adipogenesis, skeletal progenitors exhibit a strong increase in oxidative metabolism, characterized by increased mitochondrial biogenesis, oxidative phosphorylation and generation of ROS[97, 98]. In contrast to osteogenesis, where ROS appears to be mostly harmful, endogenous ROS generated from the mitochondrial ETC complex III is required to initiate adipogenesis[98]. Lipid stores formed during adipogenic differentiation are generated using both exogenous fatty acids as well as fatty acids synthetized *de novo* using mainly glucose but also acetate, pyruvate and glutamine as substrates[99, 100]. Some level of lipid synthesis appears to be required for adipogenic differentiation, as

deletion of the lipogenic enzyme ATP citrate lyase (ACLY) prevents adipogenesis even in the presence of exogenous lipids[101]. In pre-adipocytes, ACLY-dependent production of acetyl-CoA supports histone acetylation, particularly favouring expression of genes involved in glucose uptake, glucose metabolism and fatty acid synthesis, and thus coordinates nuclear activity with cellular metabolic state[101].

Compared to other adipose tissue deposits, marrow adipocytes harbour some unique metabolic properties. Under caloric restriction, bone marrow adipocytes do not decrease in size compared to white adipocytes[102]. Marrow adipocytes preserve their triglyceride stores and do not release free fatty acids, but appear to be an important source of cholesterol and related metabolites under these conditions[102]. However, lipolysis in MAT appears to be site- and treatment-specific. In contrast to cMAT, rMAT does undergo lipolysis in response to caloric restriction and β3-adrenergic agonist treatment, while both bone marrow adipose deposits respond to the adenylyl cyclase activator forskolin[103]. In addition, researchers found that cMAT contains more unsaturated lipids than rMAT[94], suggesting that metabolic heterogeneity may exist within the MAT. The physiological relevance of these observations, and the importance of metabolic communication between marrow adipocytes and different skeletal and haematopoietic cell types remains to be explored.

Metabolic support of haematopoietic cells by bone marrow stromal cells

The specific metabolic requirements of haematopoiesis-supportive stromal cells have not yet been studied. As mentioned, it remains unclear whether bone marrow SSCs and haematopoiesis-supportive stromal cells are distinct cell populations, and most metabolic studies have been performed on heterogeneous BMSC cultures. In this section, we instead focus on metabolic support of haematopoietic and leukemic cells by BMSCs given recent discoveries in this area (Figure 3).

BMSCs are characterized by expression of CD73, an ecto-5′-nucleotidase that catalyses the last step in the conversion of extracellular ATP into adenosine[11]. Extracellular ATP is seen as a danger signal that promotes proliferation and migration and influences differentiation of haematopoietic stem cells

(HSCs), while adenosine signalling enhances proliferation of committed haematopoietic progenitors but does not impact more primitive cell populations[104, 105]. In accordance, CD73 knockout mice engrafted with wildtype bone marrow cells exhibit reduced generation of B cells and particularly myeloid cells, while T cell and HSC numbers were similar to wildtype recipients[11]. Whether extracellular adenosine is also taken up and metabolized by haematopoietic cells is unknown.

Another important metabolic role for cells of the bone marrow microenvironment may be the control of amino acid availability, possibly to maintain bone marrow amino acids at levels optimal for haematopoiesis. We recently showed that the amino acid composition of bone marrow plasma is very different from that of the peripheral blood, with bone marrow containing substantially higher levels of aspartate, glutamate, asparagine, alanine and proline but lower levels of glutamine and tryptophan[38]. Bone marrow endothelial cells, osteoblasts and BMSCs also secrete a plethora of both essential and non-essential amino acids[38, 106, 107]. While the roles for these secreted amino acids in haematopoiesis are still largely uncharacterized, one study has provided some first insights in the importance of valine, which is essential for HSC maintenance and proliferation and is secreted by endothelial and stromal cells in the bone marrow microenvironment. However, the relative importance of niche-derived versus direct diet-supplied valine for HSCs remains to be determined[106].

In addition to metabolites, BMSCs can also transfer organelles to metabolically influence haematopoietic cells. BMSCs have been shown to secrete exosomes, a type of membrane-bound extracellular vesicles, packed with metabolites associated with immunomodulation[108]. Additionally, in response to increasing ROS levels during acute bacterial infection, mitochondria are transferred from BMSCs, but not osteoblasts, to HSCs through connexin 43-type gap junctions[109]. This process facilitates a rapid shift from glycolysis to oxidative phosphorylation in HSCs, followed by leukocyte expansion. A similar mitochondrial transfer occurs between BMSCs and acute myeloid leukaemia (AML) cells, and may play a role in protecting AML cells from chemotherapy[110].

The importance of metabolic crosstalk with stromal cells for leukaemia growth and chemoresistance development is underscored by several other studies. Zhang *et al.* described how BMSCs import cystine and convert it to cysteine, which is then released into the microenvironment for uptake by chronic lymphoid leukaemia cells promoting glutathione synthesis, cell survival and drug resistance[107]. We recently showed that BMSCs convert glutamine into aspartate, used by AML cells for pyrimidine generation and protecting them from chemotherapy-induced cell death[38]. AML cells also induce bone marrow adipocytes to release fatty acids, which supports AML cell proliferation and survival by fuelling FAO[111]. It will be of interest for future studies to investigate what exact role these different forms of stromal metabolic support observed in leukaemia play during normal haematopoiesis.

Running low on fuel: cell metabolism in skeletal ageing and degeneration

Ageing exerts profound effects on skeletal cell function and is an important risk factor for the development of degenerative skeletal diseases such as osteoporosis and osteoarthritis[112]. Several metabolic changes have been found in aged skeletal cells that may contribute to the initiation and progression of these diseases (Figure 4). On the other side of the balance, promoting specific metabolic pathways can enhance cell fitness and prevent skeletal degeneration, or benefit regenerative strategies.

One important skeletal cell population impacted by ageing are BMSCs, which show a reduction in SSC frequency and loss of osteogenic potential at the expense of adipogenic differentiation[63, 113]. Aged BMSCs display reduced mitochondrial content and an abnormal ultrastructure, resulting in lower oxygen consumption, reduced ATP synthesis and increased ROS generation during osteogenic differentiation[113]. While the mechanism of this compromised mitochondrial function is not fully understood, it may involve reduced levels of the key mitochondrial metabolite NAD^+, as often seen in aged cells, leading to decreased activity of Sirtuins, NAD^+-dependent protein deacetylases involved in mitochondrial metabolism and oxidative stress regulation[114]. Accordingly, administration of nicotinamide

427 mononucleotide, a key NAD^+ intermediate, promotes BMSC expansion, enhances osteogenesis,
428 reduces adipogenesis and protects bone from aging and irradiation-induced damage in mice[115].
429
430 Other mechanisms may also contribute to reduced mitochondrial activity in aged BMSCs. Activity of
431 PGC-1α decreases with age via p53 signalling in response to genomic stress, impacting BMSC fate
432 decisions and the bone-fat balance[63, 116]. Aging also reduces activity of GLS linked to reduced
433 oestrogen-related receptor alpha signalling, thus suppressing glutamine anaplerosis and osteogenic
434 differentiation of BMSCs[117]. Kynurenine, an oxidized metabolite of the essential amino acid tryptophan
435 that increases in abundance with age, also impacts mitochondrial metabolism in BMSCs and
436 osteoblasts, leading to reduced osteoblastic numbers, bone loss and osteoporosis[118, 119].
437
438 Taken together, current evidence indicates that aging reduces mitochondrial metabolism and increases
439 oxidative stress in BMSCs, impacting bone formation both at the level of SSC cell fate decisions and
440 osteoblast functioning. Similar effects of age on periosteal SSC number and osteogenic capacity have
441 been described, compromising their ability to orchestrate fracture healing[120], but whether they undergo
442 the same metabolic changes as their bone marrow counterparts remains to be determined.
443
444 Another skeletal tissue where aging has a large impact is articular cartilage, where age is the main risk
445 factor for the development of osteoarthritis (OA)[112]. Advanced age can trigger OA by altering
446 functionality of major homeostatic mechanisms including cellular metabolism. Dysregulation of
447 mitochondrial activity, associated with increased ROS generation, elevated oxidative stress, decreased
448 mitochondrial ATP generation and upregulation of glycolysis, is known to occur in OA and has been
449 reviewed in detail previously[121]. Inflammatory stimuli may further enhance the metabolic reprogramming
450 of articular chondrocytes by increasing glycolysis and reducing oxidative phosphorylation[122].
451
452 Accordingly, targeting dysfunctional mitochondria appears a promising therapeutic strategy in OA.
453 Trehalose for example ameliorates oxidative stress-mediated mitochondrial dysfunction and ER stress

by stimulating autophagy, providing protection from OA development in mice[123]. Pharmacological stabilization of HIF-1α is another strategy to alleviate OA, and seems to work by enhancing mitophagy[124]. Interestingly, inhibition of the glycolytic enzyme lactate dehydrogenase A (LDHA) can also reduce ROS generation as well as the catabolic response in both mouse and human articular chondrocytes, and protects from OA in mouse models[122].

Recent findings show that altered activity of other metabolic processes may also contribute to the pathogenesis of OA. For example, OA chondrocytes have increased levels of cholesterol because of enhanced uptake, upregulation of cholesterol hydroxylases and increased production of oxysterol metabolites[125]. Overexpression of cholesterol hydroxylases in mouse joint tissues can cause experimental OA, whereas knockout of these enzymes abrogates experimentally-induced disease development. Fatty acid metabolism changes in OA as well. Excess saturated fatty acid intake induces cartilage degradation and subchondral bone changes in rats[126], while loss of PPAR-δ in chondrocytes prevents disease development in an injury-induced OA model[127]. We recently described how high levels of extracellular fatty acids prevent chondrogenic commitment of PDCs by suppressing activity of FOXO and SOX9 transcription factors[25]. Both FOXO and SOX9 levels are decreased in OA[128, 129], suggesting that changes in local fatty acid levels, possibly linked to increased inflammation or angiogenesis[130], may play a key role in OA progression.

Future perspectives

The last five years have witnessed a great expansion of investigations into the metabolic regulation of skeletal cells, driven by technological advances and a renewed interest in cellular metabolism. Yet, our insight in the metabolic pathways controlling skeletal cell fate and function remains limited. As in other areas of study, the majority of studies on skeletal cell metabolism are performed *in vitro*, and there is a clear need for more *in vivo* validation. As highlighted by recent findings on cancer cell metabolism, metabolic dependencies that exist *in vitro* are not always recapitulated *in vivo*[36-38]. If we aspire as a field

to translate findings on skeletal cell metabolism into new therapies, studying cells in their native microenvironment will be essential (Figure 5). Genetic mouse lines with conditional alleles of many metabolic enzyme-encoding genes are available, and will be key tools in this effort. The number of skeletal cell type-specific, Cre recombinase-expressing lines has equally expanded over recent years, even though development of more specific lines or inducible variants would be welcome[14, 131, 132]. In situations where *in vitro* cultures of skeletal cells are required, the aim should be to recapitulate the metabolic characteristics of the *in vivo* microenvironment as close as possible, including the use of physiological oxygen tensions[133] and appropriate cell culture media[134].

Particularly when it comes to immature cells, a better differentiation between SSCs, skeletal progenitors and haematopoiesis-supportive stromal cells, both in mouse and human studies, would greatly improve our understanding of the metabolic control of cell fate. Unravelling the metabolic differences between quiescent and proliferating SSCs, and between SSCs found at different locations, would also be of interest considering efforts to improve cell expansion for tissue engineering applications[18].

One methodological advance that would allow a better understanding of skeletal cell metabolism *in vivo* is the development of protocols for the isolation of cells with preservation of their metabolic profile, which would permit direct metabolic comparison of different skeletal cell types through metabolomics or stable isotope tracer analysis. In haematology and immunology, where cells are more easily accessible, these methods have generated unprecedented insight in the metabolic programs controlling cell behaviour in their native environment[38, 135, 136]. Although not yet at the resolution of individual cell types, a recent example shows the feasibility and value of performing *in vivo* stable isotope tracing in whole bone tissue in mice[47]. Other technologies such as mass cytometry now allow simultaneous analysis of cellular identity and metabolic enzyme protein levels at the individual cell level[137, 138], while continuously-improving metabolic imaging methods can provide spatial information at the nanoscale[137, 139]. We anticipate that these new technological developments will facilitate important discoveries in skeletal cell metabolism in the coming years.

Finally, increasing evidence points to an important role of the skeletal system as an endocrine organ[46, 140]. Nevertheless, the interplay between intermediary metabolism in skeletal cells and whole-body metabolism remains largely unexplored. It will be of considerable interest to better understand how metabolic conditions that are known to impact bone health, such as obesity, diabetes, starvation, hormone deficiencies and vitamin shortages, alter skeletal cell metabolism, and whether pharmacological targeting of the dysregulated metabolic pathways in bone cells can restore their function. On the other hand, a few recent examples highlight how glucose uptake in osteoblasts can affect global glucose metabolism[41, 43]. Whether there are other ways in which metabolic programs in skeletal cells regulate whole-body metabolism remains an open question for now.

References

1. Capulli, M., Paone, R. & Rucci, N. Osteoblast and osteocyte: games without frontiers. *Arch. Biochem. Biophys.* **561**, 3-12 (2014).
2. Rodan, G.A. Introduction to bone biology. *Bone* **13 Suppl 1**, S3-6 (1992).
3. Bonewald, L.F. The amazing osteocyte. *J. Bone Miner. Res.* **26**, 229-238 (2011).
4. Krishnan, Y. & Grodzinsky, A.J. Cartilage diseases. *Matrix Biol.* **71-72**, 51-69 (2018).
5. Hallett, S.A., Ono, W. & Ono, N. Growth Plate Chondrocytes: Skeletal Development, Growth and Beyond. *Int. J. Mol. Sci.* **20** (2019).
6. Roberts, S.J., van Gastel, N., Carmeliet, G. & Luyten, F.P. Uncovering the periosteum for skeletal regeneration: the stem cell that lies beneath. *Bone* **70**, 10-18 (2015).
7. Bukowska, J. *et al.* Bone Marrow Adipocyte Developmental Origin and Biology. *Curr. Osteoporos. Rep.* **16**, 312-319 (2018).
8. Crane, G.M., Jeffery, E. & Morrison, S.J. Adult haematopoietic stem cell niches. *Nat. Rev. Immunol.* **17**, 573-590 (2017).
9. Hoggatt, J., Kfoury, Y. & Scadden, D.T. Hematopoietic Stem Cell Niche in Health and Disease. *Annu. Rev. Pathol.* **11**, 555-581 (2016).
10. Baryawno, N. *et al.* A Cellular Taxonomy of the Bone Marrow Stroma in Homeostasis and Leukemia. *Cell* **177**, 1915-1932 e1916 (2019).
11. Severe, N. *et al.* Stress-Induced Changes in Bone Marrow Stromal Cell Populations Revealed through Single-Cell Protein Expression Mapping. *Cell Stem Cell* **25**, 570-583 e577 (2019).
12. Tikhonova, A.N. *et al.* The bone marrow microenvironment at single-cell resolution. *Nature* **569**, 222-228 (2019).
13. Ambrosi, T.H., Longaker, M.T. & Chan, C.K.F. A Revised Perspective of Skeletal Stem Cell Biology. *Front. Cell Dev. Biol.* **7**, 189 (2019).
14. Matsushita, Y., Ono, W. & Ono, N. Skeletal Stem Cells for Bone Development and Repair: Diversity Matters. *Curr. Osteoporos. Rep.* (2020).
15. Serowoky, M.A., Arata, C.E., Crump, J.G. & Mariani, F.V. Skeletal stem cells: insights into maintaining and regenerating the skeleton. *Development* **147** (2020).
16. Spencer, J.A. *et al.* Direct measurement of local oxygen concentration in the bone marrow of live animals. *Nature* **508**, 269-273 (2014).
17. Chandel, N.S., Jasper, H., Ho, T.T. & Passegue, E. Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. *Nat. Cell Biol.* **18**, 823-832 (2016).
18. Salazar-Noratto, G.E. *et al.* Understanding and leveraging cell metabolism to enhance mesenchymal stem cell transplantation survival in tissue engineering and regenerative medicine applications. *Stem Cells* **38**, 22-33 (2020).
19. Lunt, S.Y. & Vander Heiden, M.G. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu. Rev. Cell Dev. Biol.* **27**, 441-464 (2011).
20. Shum, L.C., White, N.S., Mills, B.N., Bentley, K.L. & Eliseev, R.A. Energy Metabolism in Mesenchymal Stem Cells During Osteogenic Differentiation. *Stem Cells Dev.* **25**, 114-122 (2016).
21. Stegen, S. *et al.* Adequate hypoxia inducible factor 1alpha signaling is indispensable for bone regeneration. *Bone* **87**, 176-186 (2016).
22. Tournaire, G. *et al.* Nestin-GFP transgene labels skeletal progenitors in the periosteum. *Bone* **133**, 115259 (2020).
23. Martinez-Reyes, I. & Chandel, N.S. Mitochondrial TCA cycle metabolites control physiology and disease. *Nat. Commun.* **11**, 102 (2020).
24. Fillmore, N. *et al.* Effect of fatty acids on human bone marrow mesenchymal stem cell energy metabolism and survival. *PLoS One* **10**, e0120257 (2015).
25. van Gastel, N. *et al.* Lipid availability determines fate of skeletal progenitor cells via SOX9. *Nature* **579**, 111-117 (2020).

26. Board, M. *et al.* Acetoacetate is a more efficient energy-yielding substrate for human mesenchymal stem cells than glucose and generates fewer reactive oxygen species. *Int. J. Biochem. Cell Biol.* **88**, 75-83 (2017).
27. Newman, J.C. & Verdin, E. beta-Hydroxybutyrate: A Signaling Metabolite. *Annu. Rev. Nutr.* **37**, 51-76 (2017).
28. Kurmi, K. & Haigis, M.C. Nitrogen Metabolism in Cancer and Immunity. *Trends Cell Biol.* **30**, 408-424 (2020).
29. Hu, G. *et al.* The amino acid sensor Eif2ak4/GCN2 is required for proliferation of osteoblast progenitors in mice. *J. Bone Miner. Res.* (2020).
30. Chen, Y. *et al.* miR-206 inhibits osteogenic differentiation of bone marrow mesenchymal stem cells by targetting glutaminase. *Biosci. Rep.* **39** (2019).
31. Stegen, S. *et al.* Glutamine Metabolism Controls Chondrocyte Identity and Function. *Dev. Cell* (2020).
32. Stegen, S. *et al.* HIF-1alpha Promotes Glutamine-Mediated Redox Homeostasis and Glycogen-Dependent Bioenergetics to Support Postimplantation Bone Cell Survival. *Cell Metab.* **23**, 265-279 (2016).
33. Yu, Y. *et al.* Glutamine Metabolism Regulates Proliferation and Lineage Allocation in Skeletal Stem Cells. *Cell Metab.* **29**, 966-978 e964 (2019).
34. DeFalco, J. *et al.* Virus-assisted mapping of neural inputs to a feeding center in the hypothalamus. *Science* **291**, 2608-2613 (2001).
35. Masson, J. *et al.* Mice lacking brain/kidney phosphate-activated glutaminase have impaired glutamatergic synaptic transmission, altered breathing, disorganized goal-directed behavior and die shortly after birth. *J. Neurosci.* **26**, 4660-4671 (2006).
36. Davidson, S.M. *et al.* Environment Impacts the Metabolic Dependencies of Ras-Driven Non-Small Cell Lung Cancer. *Cell Metab.* **23**, 517-528 (2016).
37. Tardito, S. *et al.* Glutamine synthetase activity fuels nucleotide biosynthesis and supports growth of glutamine-restricted glioblastoma. *Nat. Cell Biol.* **17**, 1556-1568 (2015).
38. van Gastel, N. *et al.* Induction of a Timed Metabolic Collapse to Overcome Cancer Chemoresistance. *Cell Metab.* **32**, 391-403 (2020).
39. Kristensen, H.B., Andersen, T.L., Marcussen, N., Rolighed, L. & Delaisse, J.M. Increased presence of capillaries next to remodeling sites in adult human cancellous bone. *J. Bone Miner. Res.* **28**, 574-585 (2013).
40. Prisby, R. *et al.* Intermittent PTH(1-84) is osteoanabolic but not osteoangiogenic and relocates bone marrow blood vessels closer to bone-forming sites. *J. Bone Miner. Res.* **26**, 2583-2596 (2011).
41. Dirckx, N. *et al.* Vhl deletion in osteoblasts boosts cellular glycolysis and improves global glucose metabolism. *J. Clin. Invest.* **128**, 1087-1105 (2018).
42. Zoch, M.L., Abou, D.S., Clemens, T.L., Thorek, D.L. & Riddle, R.C. In vivo radiometric analysis of glucose uptake and distribution in mouse bone. *Bone Res.* **4**, 16004 (2016).
43. Li, Z. *et al.* Glucose Transporter-4 Facilitates Insulin-Stimulated Glucose Uptake in Osteoblasts. *Endocrinology* **157**, 4094-4103 (2016).
44. Wei, J. *et al.* Glucose Uptake and Runx2 Synergize to Orchestrate Osteoblast Differentiation and Bone Formation. *Cell* **161**, 1576-1591 (2015).
45. Zoidis, E., Ghirlanda-Keller, C. & Schmid, C. Stimulation of glucose transport in osteoblastic cells by parathyroid hormone and insulin-like growth factor I. *Mol. Cell. Biochem.* **348**, 33-42 (2011).
46. Dirckx, N., Moorer, M.C., Clemens, T.L. & Riddle, R.C. The role of osteoblasts in energy homeostasis. *Nat. Rev. Endocrinol.* **15**, 651-665 (2019).
47. Lee, W.C., Ji, X., Nissim, I. & Long, F. Malic Enzyme Couples Mitochondria with Aerobic Glycolysis in Osteoblasts. *Cell Rep.* **32**, 108108 (2020).
48. Borle, A.B., Nichols, N. & Nichols, G., Jr. Metabolic studies of bone in vitro. I. Normal bone. *J. Biol. Chem.* **235**, 1206-1210 (1960).

49. Esen, E., Lee, S.Y., Wice, B.M. & Long, F. PTH Promotes Bone Anabolism by Stimulating Aerobic Glycolysis via IGF Signaling. *J. Bone Miner. Res.* **30**, 2137 (2015).
50. Regan, J.N. *et al.* Up-regulation of glycolytic metabolism is required for HIF1alpha-driven bone formation. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 8673-8678 (2014).
51. Hilton, M.J. *et al.* Notch signaling maintains bone marrow mesenchymal progenitors by suppressing osteoblast differentiation. *Nat. Med.* **14**, 306-314 (2008).
52. Lee, S.Y. & Long, F. Notch signaling suppresses glucose metabolism in mesenchymal progenitors to restrict osteoblast differentiation. *J. Clin. Invest.* **128**, 5573-5586 (2018).
53. Chen, H. *et al.* Increased glycolysis mediates Wnt7b-induced bone formation. *FASEB J.* **33**, 7810-7821 (2019).
54. Esen, E. *et al.* WNT-LRP5 signaling induces Warburg effect through mTORC2 activation during osteoblast differentiation. *Cell Metab.* **17**, 745-755 (2013).
55. Schmid, C., Steiner, T. & Froesch, E.R. Parathormone promotes glycogen formation from [14C]glucose in cultured osteoblast-like cells. *FEBS Lett.* **148**, 31-34 (1982).
56. Schajowicz, F. & Cabrini, R.L. Histochemical studies on glycogen in normal ossification and calcification. *J. Bone Joint Surg. Am.* **40-A**, 1081-1092 (1958).
57. Scott, B.L. & Glimcher, M.J. Distribution of glycogen in osteoblasts of the fetal rat. *J. Ultrastruct. Res.* **36**, 565-586 (1971).
58. Dodds, R.A., Ali, N., Pead, M.J. & Lanyon, L.E. Early loading-related changes in the activity of glucose 6-phosphate dehydrogenase and alkaline phosphatase in osteocytes and periosteal osteoblasts in rat fibulae in vivo. *J. Bone Miner. Res.* **8**, 261-267 (1993).
59. Dodds, R.A., Catterall, A., Bitensky, L. & Chayen, J. Effects on fracture healing of an antagonist of the vitamin K cycle. *Calcif. Tissue Int.* **36**, 233-238 (1984).
60. Dodds, R.A., Catterall, A., Bitensky, L. & Chayen, J. Abnormalities in fracture healing induced by vitamin B6-deficiency in rats. *Bone* **7**, 489-495 (1986).
61. Chen, C.T., Shih, Y.R., Kuo, T.K., Lee, O.K. & Wei, Y.H. Coordinated changes of mitochondrial biogenesis and antioxidant enzymes during osteogenic differentiation of human mesenchymal stem cells. *Stem Cells* **26**, 960-968 (2008).
62. Guntur, A.R., Le, P.T., Farber, C.R. & Rosen, C.J. Bioenergetics during calvarial osteoblast differentiation reflect strain differences in bone mass. *Endocrinology* **155**, 1589-1595 (2014).
63. Yu, B. *et al.* PGC-1alpha Controls Skeletal Stem Cell Fate and Bone-Fat Balance in Osteoporosis and Skeletal Aging by Inducing TAZ. *Cell Stem Cell* **23**, 193-209 (2018).
64. Pan, J.X. *et al.* APP promotes osteoblast survival and bone formation by regulating mitochondrial function and preventing oxidative stress. *Cell Death Dis.* **9**, 1077 (2018).
65. Morganti, C. *et al.* Citrate Mediates Crosstalk between Mitochondria and the Nucleus to Promote Human Mesenchymal Stem Cell In Vitro Osteogenesis. *Cells* **9** (2020).
66. Shares, B.H., Busch, M., White, N., Shum, L. & Eliseev, R.A. Active mitochondria support osteogenic differentiation by stimulating beta-catenin acetylation. *J. Biol. Chem.* **293**, 16019-16027 (2018).
67. Franklin, R.B., Chellaiah, M., Zou, J., Reynolds, M.A. & Costello, L.C. Evidence that Osteoblasts are Specialized Citrate-producing Cells that Provide the Citrate for Incorporation into the Structure of Bone. *Open Bone J.* **6**, 1-7 (2014).
68. Karner, C.M. *et al.* Wnt Protein Signaling Reduces Nuclear Acetyl-CoA Levels to Suppress Gene Expression during Osteoblast Differentiation. *J. Biol. Chem.* **291**, 13028-13039 (2016).
69. Adamek, G., Felix, R., Guenther, H.L. & Fleisch, H. Fatty acid oxidation in bone tissue and bone cells in culture. Characterization and hormonal influences. *Biochem. J.* **248**, 129-137 (1987).
70. Dunham, J. *et al.* Aerobic glycolysis of bone and cartilage: the possible involvement of fatty acid oxidation. *Cell Biochem. Funct.* **1**, 168-172 (1983).
71. Kim, S.P. *et al.* Fatty acid oxidation by the osteoblast is required for normal bone acquisition in a sex- and diet-dependent manner. *JCI Insight* **2** (2017).
72. Niemeier, A. *et al.* Uptake of postprandial lipoproteins into bone in vivo: impact on osteoblast function. *Bone* **43**, 230-237 (2008).

73. Muller, D.I.H. *et al.* PPARdelta-mediated mitochondrial rewiring of osteoblasts determines bone mass. *Sci. Rep.* **10**, 8428 (2020).
74. Frey, J.L. *et al.* Wnt-Lrp5 signaling regulates fatty acid metabolism in the osteoblast. *Mol. Cell. Biol.* **35**, 1979-1991 (2015).
75. Karner, C.M., Esen, E., Okunade, A.L., Patterson, B.W. & Long, F. Increased glutamine catabolism mediates bone anabolism in response to WNT signaling. *J. Clin. Invest.* **125**, 551-562 (2015).
76. Torzilli, P.A., Arduino, J.M., Gregory, J.D. & Bansal, M. Effect of proteoglycan removal on solute mobility in articular cartilage. *J. Biomech.* **30**, 895-902 (1997).
77. Torzilli, P.A., Grande, D.A. & Arduino, J.M. Diffusive properties of immature articular cartilage. *J. Biomed. Mater. Res.* **40**, 132-138 (1998).
78. Amarilio, R. *et al.* HIF1alpha regulation of Sox9 is necessary to maintain differentiation of hypoxic prechondrogenic cells during early skeletogenesis. *Development* **134**, 3917-3928 (2007).
79. Maes, C. *et al.* Soluble VEGF isoforms are essential for establishing epiphyseal vascularization and regulating chondrocyte development and survival. *J. Clin. Invest.* **113**, 188-199 (2004).
80. Robins, J.C. *et al.* Hypoxia induces chondrocyte-specific gene expression in mesenchymal cells in association with transcriptional activation of Sox9. *Bone* **37**, 313-322 (2005).
81. Schipani, E. *et al.* Hypoxia in cartilage: HIF-1alpha is essential for chondrocyte growth arrest and survival. *Genes Dev.* **15**, 2865-2876 (2001).
82. Maes, C. *et al.* VEGF-independent cell-autonomous functions of HIF-1alpha regulating oxygen consumption in fetal cartilage are critical for chondrocyte survival. *J. Bone Miner. Res.* **27**, 596-609 (2012).
83. Bywaters, E.G.L. Metabolism of Cartilage. *Nature* **138**, 30-31 (1936).
84. Kunin, A.S. & Krane, S.M. The effect of dietary phosphorus on the intermediary metabolism of epiphyseal cartilage from rachitic rats. *Biochim. Biophys. Acta* **107**, 203-214 (1965).
85. Hough, S., Russell, J.E., Teitelbaum, S.L. & Avioli, L.V. Regulation of epiphyseal cartilage metabolism and morphology in the chronic diabetic rat. *Calcif. Tissue Int.* **35**, 115-121 (1983).
86. Silverton, S.F., Matsumoto, H., DeBolt, K., Reginato, A. & Shapiro, I.M. Pentose phosphate shunt metabolism by cells of the chick growth cartilage. *Bone* **10**, 45-51 (1989).
87. Lee, S.Y., Abel, E.D. & Long, F. Glucose metabolism induced by Bmp signaling is essential for murine skeletal development. *Nat. Commun.* **9**, 4831 (2018).
88. Stegen, S. *et al.* HIF-1alpha metabolically controls collagen synthesis and modification in chondrocytes. *Nature* **565**, 511-515 (2019).
89. Daimon, T. The presence and distribution of glycogen particles in chondrogenic cells of the tibiotarsal anlage of developing chick embryos. *Calcif. Tissue Res.* **23**, 45-51 (1977).
90. Horigome, Y. *et al.* Loss of autophagy in chondrocytes causes severe growth retardation. *Autophagy* **16**, 501-511 (2020).
91. Daniels, V.W. *et al.* Cancer cells differentially activate and thrive on de novo lipid synthesis pathways in a low-lipid environment. *PLoS One* **9**, e106913 (2014).
92. Kikuchi, M. *et al.* Crucial Role of Elovl6 in Chondrocyte Growth and Differentiation during Growth Plate Development in Mice. *PLoS One* **11**, e0159375 (2016).
93. Handley, C.J., Speight, G., Leyden, K.M. & Lowther, D.A. Extracellular matrix metabolism by chondrocytes. 7. Evidence that L-glutamine is an essential amino acid for chondrocytes and other connective tissue cells. *Biochim. Biophys. Acta* **627**, 324-331 (1980).
94. Scheller, E.L. *et al.* Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues. *Nat. Commun.* **6**, 7808 (2015).
95. Li, Y., Meng, Y. & Yu, X. The Unique Metabolic Characteristics of Bone Marrow Adipose Tissue. *Front. Endocrinol. (Lausanne)* **10**, 69 (2019).
96. Maridas, D.E. *et al.* Progenitor recruitment and adipogenic lipolysis contribute to the anabolic actions of parathyroid hormone on the skeleton. *FASEB J.* **33**, 2885-2898 (2019).
97. Tencerova, M. *et al.* Metabolic programming determines the lineage-differentiation fate of murine bone marrow stromal progenitor cells. *Bone Res.* **7**, 35 (2019).

98. Tormos, K.V. *et al.* Mitochondrial complex III ROS regulate adipocyte differentiation. *Cell Metab.* **14**, 537-544 (2011).
99. Collins, J.M. *et al.* De novo lipogenesis in the differentiating human adipocyte can provide all fatty acids necessary for maturation. *J. Lipid Res.* **52**, 1683-1692 (2011).
100. Suchacki, K.J. *et al.* Bone marrow adipose tissue is a unique adipose subtype with distinct roles in glucose homeostasis. *Nat. Commun.* **11**, 3097 (2020).
101. Wellen, K.E. *et al.* ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* **324**, 1076-1080 (2009).
102. Attane, C. *et al.* Human Bone Marrow Is Comprised of Adipocytes with Specific Lipid Metabolism. *Cell Rep.* **30**, 949-958 e946 (2020).
103. Scheller, E.L. *et al.* Bone marrow adipocytes resist lipolysis and remodeling in response to beta-adrenergic stimulation. *Bone* **118**, 32-41 (2019).
104. Hofer, M. *et al.* The pharmacological activation of adenosine A1 and A 3 receptors does not modulate the long- or short-term repopulating ability of hematopoietic stem and multipotent progenitor cells in mice. *Purinergic Signal.* **9**, 207-214 (2013).
105. Rossi, L., Salvestrini, V., Ferrari, D., Di Virgilio, F. & Lemoli, R.M. The sixth sense: hematopoietic stem cells detect danger through purinergic signaling. *Blood* **120**, 2365-2375 (2012).
106. Taya, Y. *et al.* Depleting dietary valine permits nonmyeloablative mouse hematopoietic stem cell transplantation. *Science* **354**, 1152-1155 (2016).
107. Zhang, W. *et al.* Stromal control of cystine metabolism promotes cancer cell survival in chronic lymphocytic leukaemia. *Nat. Cell Biol.* **14**, 276-286 (2012).
108. Showalter, M.R. *et al.* Primed mesenchymal stem cells package exosomes with metabolites associated with immunomodulation. *Biochem. Biophys. Res. Commun.* **512**, 729-735 (2019).
109. Mistry, J.J. *et al.* ROS-mediated PI3K activation drives mitochondrial transfer from stromal cells to hematopoietic stem cells in response to infection. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 24610-24619 (2019).
110. Moschoi, R. *et al.* Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. *Blood* **128**, 253-264 (2016).
111. Shafat, M.S. *et al.* Leukemic blasts program bone marrow adipocytes to generate a protumoral microenvironment. *Blood* **129**, 1320-1332 (2017).
112. Grote, C., Reinhardt, D., Zhang, M. & Wang, J. Regulatory mechanisms and clinical manifestations of musculoskeletal aging. *J. Orthop. Res.* **37**, 1475-1488 (2019).
113. Bellantuono, I., Aldahmash, A. & Kassem, M. Aging of marrow stromal (skeletal) stem cells and their contribution to age-related bone loss. *Biochim. Biophys. Acta* **1792**, 364-370 (2009).
114. Neri, S. & Borzi, R.M. Molecular Mechanisms Contributing to Mesenchymal Stromal Cell Aging. *Biomolecules* **10** (2020).
115. Song, J. *et al.* Nicotinamide mononucleotide promotes osteogenesis and reduces adipogenesis by regulating mesenchymal stromal cells via the SIRT1 pathway in aged bone marrow. *Cell Death Dis.* **10**, 336 (2019).
116. Sui, B., Hu, C. & Jin, Y. Mitochondrial metabolic failure in telomere attrition-provoked aging of bone marrow mesenchymal stem cells. *Biogerontology* **17**, 267-279 (2016).
117. Huang, T. *et al.* Aging Reduces an ERRalpha-Directed Mitochondrial Glutaminase Expression Suppressing Glutamine Anaplerosis and Osteogenic Differentiation of Mesenchymal Stem Cells. *Stem Cells* **35**, 411-424 (2017).
118. Kondrikov, D. *et al.* Kynurenine inhibits autophagy and promotes senescence in aged bone marrow mesenchymal stem cells through the aryl hydrocarbon receptor pathway. *Exp. Gerontol.* **130**, 110805 (2020).
119. Pierce, J.L. *et al.* Kynurenine suppresses osteoblastic cell energetics in vitro and osteoblast numbers in vivo. *Exp. Gerontol.* **130**, 110818 (2020).
120. Hadjiargyrou, M. & O'Keefe, R.J. The convergence of fracture repair and stem cells: interplay of genes, aging, environmental factors and disease. *J. Bone Miner. Res.* **29**, 2307-2322 (2014).
121. Mobasheri, A. *et al.* The role of metabolism in the pathogenesis of osteoarthritis. *Nat. Rev. Rheumatol.* **13**, 302-311 (2017).

122. Arra, M. *et al.* LDHA-mediated ROS generation in chondrocytes is a potential therapeutic target for osteoarthritis. *Nat. Commun.* **11**, 3427 (2020).
123. Tang, Q. *et al.* Trehalose ameliorates oxidative stress-mediated mitochondrial dysfunction and ER stress via selective autophagy stimulation and autophagic flux restoration in osteoarthritis development. *Cell Death Dis.* **8**, e3081 (2017).
124. Hu, S. *et al.* Stabilization of HIF-1alpha alleviates osteoarthritis via enhancing mitophagy. *Cell Death Dis.* **11**, 481 (2020).
125. Choi, W.S. *et al.* The CH25H-CYP7B1-RORalpha axis of cholesterol metabolism regulates osteoarthritis. *Nature* **566**, 254-258 (2019).
126. Sekar, S. *et al.* Saturated fatty acids induce development of both metabolic syndrome and osteoarthritis in rats. *Sci. Rep.* **7**, 46457 (2017).
127. Ratneswaran, A. *et al.* Peroxisome proliferator-activated receptor delta promotes the progression of posttraumatic osteoarthritis in a mouse model. *Arthritis Rheumatol.* **67**, 454-464 (2015).
128. Akasaki, Y. *et al.* Dysregulated FOXO transcription factors in articular cartilage in aging and osteoarthritis. *Osteoarthritis Cartilage* **22**, 162-170 (2014).
129. Zhong, L., Huang, X., Karperien, M. & Post, J.N. Correlation between Gene Expression and Osteoarthritis Progression in Human. *Int. J. Mol. Sci.* **17** (2016).
130. Ashraf, S., Mapp, P.I. & Walsh, D.A. Contributions of angiogenesis to inflammation, joint damage, and pain in a rat model of osteoarthritis. *Arthritis Rheum.* **63**, 2700-2710 (2011).
131. Elefteriou, F. & Yang, X. Genetic mouse models for bone studies--strengths and limitations. *Bone* **49**, 1242-1254 (2011).
132. Stiers, P.J., van Gastel, N., Moermans, K., Stockmans, I. & Carmeliet, G. Regulatory elements driving the expression of skeletal lineage reporters differ during bone development and adulthood. *Bone* **105**, 154-162 (2017).
133. Ast, T. & Mootha, V.K. Oxygen and mammalian cell culture: are we repeating the experiment of Dr. Ox? *Nat. Metab.* **1**, 858-860 (2019).
134. Lagziel, S., Gottlieb, E. & Shlomi, T. Mind your media. *Nat. Metab.* (2020).
135. Agathocleous, M. *et al.* Ascorbate regulates haematopoietic stem cell function and leukaemogenesis. *Nature* **549**, 476-481 (2017).
136. Ma, E.H. *et al.* Metabolic Profiling Using Stable Isotope Tracing Reveals Distinct Patterns of Glucose Utilization by Physiologically Activated CD8(+) T Cells. *Immunity* **51**, 856-870 e855 (2019).
137. Hartmann, F.J. *et al.* Single-cell metabolic profiling of human cytotoxic T cells. *Nat. Biotechnol.* (2020).
138. Levine, L.S. *et al.* Single-cell metabolic dynamics of early activated CD8 T cells during the primary immune response to infection. *bioRxiv*, 2020.2001.2021.911545 (2020).
139. Narendra, D.P. & Steinhauser, M.L. Metabolic Analysis at the Nanoscale with Multi-Isotope Imaging Mass Spectrometry (MIMS). *Curr. Protoc. Cell Biol.* **88**, e111 (2020).
140. DiGirolamo, D.J., Clemens, T.L. & Kousteni, S. The skeleton as an endocrine organ. *Nat. Rev. Rheumatol.* **8**, 674-683 (2012).

Acknowledgements

N.v.G. is supported by a Young Investigator Award of Alex's Lemonade Stand Foundation for Childhood Cancer and Tap Cancer Out, and by funding from the de Duve Institute. G.C. receives funding from FWO G0B3418 and G0C5120, KUL-C24/17/077.

Contributions

N.v.G. and G.C. conceived the idea, wrote and revised the manuscript, and approved the final version; N.v.G. prepared the figures.

Ethics declarations

Competing Interests

The authors declare no competing interests.

Figure Legends

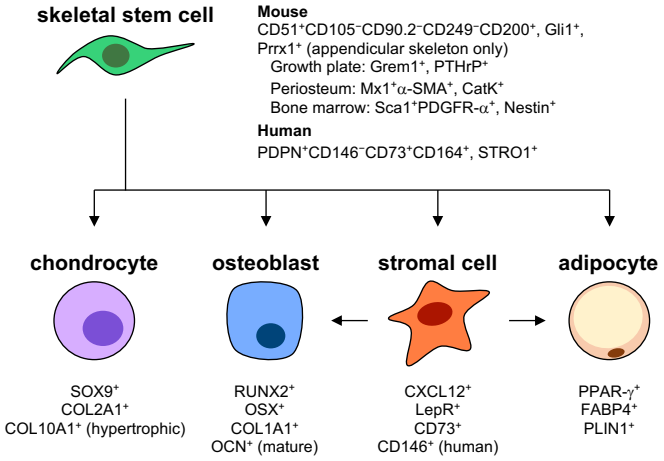
Fig. 1: Overview of the main skeletal cell types. Skeletal stem cells residing at the apex of the skeletal system give rise to the different types of mature skeletal cells. Haematopoiesis-supportive stromal cells (or a subpopulation of them) also have stem/progenitor cell characteristics and contribute to osteoblasts and adipocytes in the bone marrow. The most commonly-used markers for identification and/or isolation are listed for each cell type[1, 5, 6, 7, 10-15].

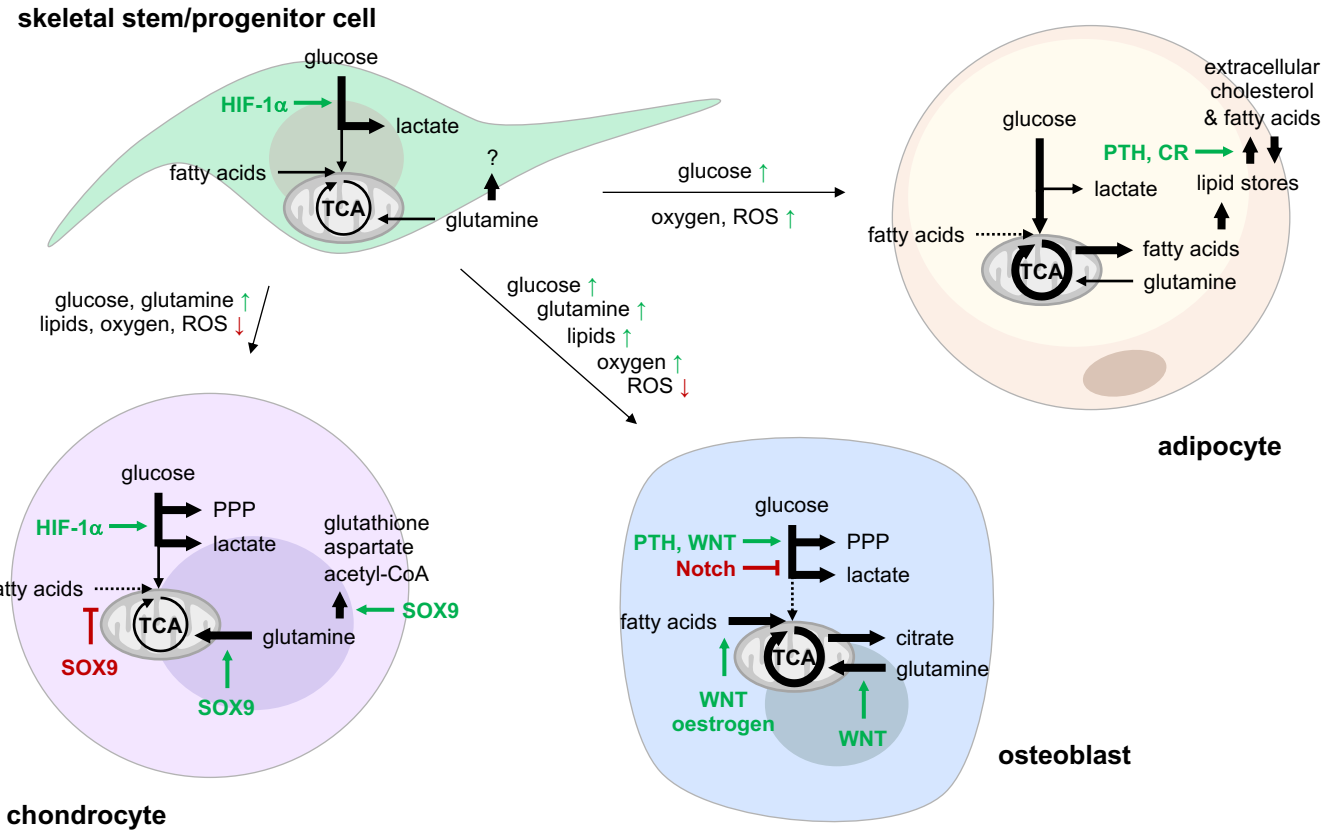
Fig. 2: Current understanding of skeletal cell metabolism. Recent years have witnessed a large increase in studies into the metabolic control of skeletal cell behaviour, although most research efforts thus far have focused on central carbon metabolism and particularly on glucose. These discoveries have been enabled by the ever-growing availability of mouse models for genetic deletion of metabolic enzymes, as well as technological advances in metabolic profiling (metabolomics), metabolic pathway analysis (stable isotope tracing) and real-time assessment of cell metabolism (extracellular oxygen/pH flux analysis, fluorescent probes). CR, caloric restriction; HIF-1a, hypoxia-inducible factor 1a; PPP, pentose phosphate pathway; PTH, parathyroid hormone; ROS, reactive oxygen species; SOX9, SRY-box transcription factor 9; TCA, tricarboxylic acid cycle.

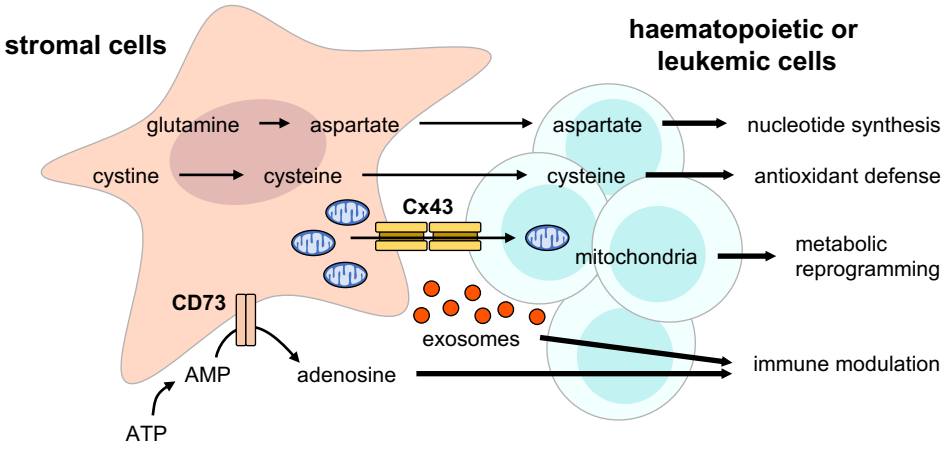
Fig. 3: Metabolic communication between stromal and blood cells in the bone marrow niche. The importance of metabolic support of haematopoietic or leukemic cells by bone marrow stromal cells is increasingly being recognized, with stromal cells synthetizing and transferring essential metabolites as well as organelles. Skeletal cells may also play a key role in shaping the metabolic composition of the bone marrow microenvironment and maintaining nutrient levels optimal for haematopoiesis. AMP, adenosine 5'-monophosphate; ATP, adenosine 5'-triphosphate; Cx43, connexin 43.

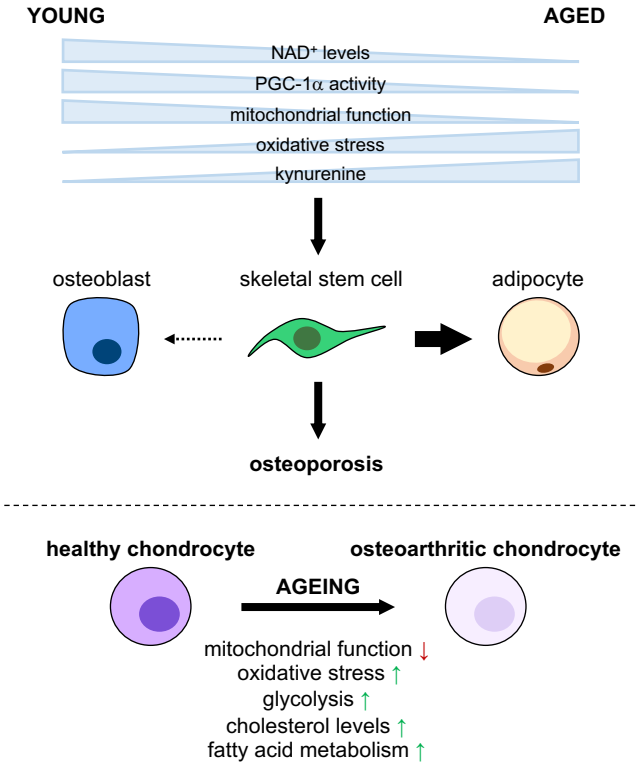
Fig. 4: Metabolic effects of ageing on skeletal cells. A hallmark of ageing in skeletal cells is mitochondrial dysfunction, which decreases energy production and increases oxidative stress. In skeletal stem cells in the bone marrow, this metabolic change induces a shift from osteogenesis to adipogenesis, leading to osteoporosis. In articular cartilage, metabolic reprogramming leads to increased inflammation, loss of chondrocyte properties and osteoarthritis.

Fig. 5: Moving towards a more comprehensive understanding of skeletal cell metabolism. A convergence of new methodologies and state-of-the-art technologies can propel the field of skeletal cell metabolism research. The growing array of metabolic analysis techniques with continuously-increasing resolution will enable a better understanding of the metabolic programs controlling the fate and function of skeletal cells in their native microenvironment.

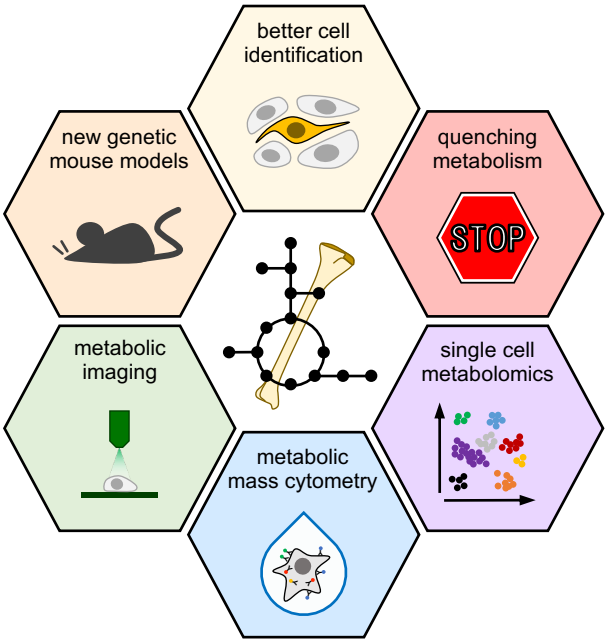

skeletal stem cell
Mouse
CD51+CD105−CD90.2−CD249−CD200+, Gli1+,
Prrx1+ (appendicular skeleton only)
Growth plate: Grem1+, PTHrP+
Periosteum: Mx1+α-SMA+, CatK+
Bone marrow: Sca1+PDGFR-α+, Nestin+
Human
PDPN+CD146−CD73+CD164+, STRO1+
chondrocyte
osteoblast
stromal cell
adipocyte
SOX9+
COL2A1+
COL10A1+ (hypertrophic)
RUNX2+
OSX+
COL1A1+
OCN+ (mature)
CXCL12+
LepR+
CD73+
CD146+ (human)
PPAR-γ+
FABP4+
PLIN1+


skeletal stem/progenitor cell
glucose
HIF-1α
lactate
fatty acids
TCA
?
glutamine
glucose ↑
oxygen, ROS ↑
extracellular
cholesterol
& fatty acids
glucose
PTH, CR
lactate
lipid stores
fatty acids
fatty acids
TCA
glutamine
adipocyte
glucose, glutamine ↑
lipids, oxygen, ROS ↓
glucose ↑
glutamine ↑
lipids ↑
oxygen ↑
ROS ↓
glucose
HIF-1α
PPP
lactate
glutathione
aspartate
acetyl-CoA
fatty acids
TCA
SOX9
glutamine
SOX9
SOX9
chondrocyte
glucose
PTH, WNT
Notch
PPP
lactate
fatty acids
TCA
citrate
glutamine
WNT
oestrogen
WNT
osteoblast


stromal cells
haematopoietic or leukemic cells
glutamine
aspartate
aspartate
nucleotide synthesis
cystine
cysteine
cysteine
antioxidant defense
Cx43
mitochondria
metabolic reprogramming
CD73
exosomes
immune modulation
AMP
adenosine
ATP


YOUNG
AGED
NAD+ levels
PGC-1α activity
mitochondrial function
oxidative stress
kynurenine
osteoblast
skeletal stem cell
adipocyte
osteoporosis

healthy chondrocyte
osteoarthritic chondrocyte
AGEING
mitochondrial function ↓
oxidative stress ↑
glycolysis ↑
cholesterol levels ↑
fatty acid metabolism ↑


better cell identification
new genetic mouse models
quenching metabolism
STOP
metabolic imaging
single cell metabolomics
metabolic mass cytometry