High-density lipoprotein (HDL) metabolism and bone mass

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Abstract

It is well appreciated that high-density lipoprotein (HDL) and bone physiology and pathology are tightly linked. Studies, primarily in mouse models, have shown that dysfunctional and/or disturbed HDL can affect bone mass through many different ways. Specifically, reduced HDL levels have been associated with the development of an inflammatory microenvironment that affects the differentiation and function of osteoblasts. In addition, perturbation in metabolic pathways of HDL favors adipoblastic differentiation and restrains osteoblastic differentiation through, among others, the modification of specific bone-related chemokines and signaling cascades. Increased bone marrow adiposity also deteriorates bone osteoblastic function and thus bone synthesis, leading to reduced bone mass. In this review, we present the current knowledge and the future directions with regard to the HDL–bone mass connection. Unraveling the molecular phenomena that underline this connection will promote the deeper understanding of the pathophysiology of bone-related pathologies, such as osteoporosis or bone metastasis, and pave the way toward the development of novel and more effective therapies against these conditions.

Key Words

- ▶ adipose tissue
- ▶ apolipoprotein
- ▶ bone formation and resorption
- ▶ cholesterol
- ▶ skeletal biology

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Introduction

Recent advances in the field of lipoproteins highlight a multifunctional role of high-density lipoprotein (HDL) in health and disease (Constantinou *et al.* 2015). HDL has been for decades an intriguing lipoprotein that attracted the attention of biomedical community, mainly because of its important role in atheroprotection (Constantinou *et al.* 2015). Indeed, the inverse correlation between HDL cholesterol (HDL-C) levels and the risk for developing coronary heart disease (Gofman *et al.* 1954, Havel *et al.* 1955, Miller *et al.* 1975, Tsompanidi *et al.* 2010, Kypreos *et al.* 2013, Karavia *et al.* 2014) suggested that high HDL-C levels in plasma are protective against the

development of atherosclerosis. As a result, the majority of studies in the literature focused on the understanding of HDL-C levels in human pathology, a simplified approach to HDL that dates back to the early days when little was known about HDL structure and function. However, more recent data from experimental mice and clinical trials indicated that HDL particle functionality, as determined by its apolipoprotein (apo) and lipid content, is far more important in atheroprotection than HDL-C levels alone (Tsompanidi *et al.* 2010, Karavia *et al.* 2014, Constantinou *et al.* 2015). Even though HDL is usually referred to as the ‘good cholesterol’, certainly it is far more

than just 'cholesterol'. HDL is rather a macromolecular assembly of proteins and lipids synthesized in the circulation as a result of a concerted action of apolipoproteins, lipid transporters and plasma enzymes. The main lipid cargo of mature HDL particles is esterified cholesterol. However, other lipids (phospholipids, sphingolipids, ceramides and so forth) are also part of HDL lipidome (Filou *et al.* 2016).

Though HDL was originally brought under the spotlight due its importance in protection from atherosclerosis, more recent findings propose a multifunctional role of HDL in numerous other biological processes including inflammation, oxidative stress, nitric oxide production and regulation of plasma glucose homeostasis (Karavia *et al.* 2014). In addition to atherosclerosis and coronary heart disease, recent data indicate that HDL may play a pivotal role in the pathology and treatment of other diseases including morbid obesity, non-alcoholic fatty liver disease, type 2 diabetes mellitus, obstructive pulmonary disease and numerous other diseases of the central nervous system (Constantinou *et al.* 2015). Recently, we identified a novel role of HDL in the pathogenesis of degenerative and metabolic bone diseases using experimental mouse models (Triantaphyllidou *et al.* 2013, Blair *et al.* 2016a), suggesting that low and dysfunctional HDL may contribute to an increased prevalence of these diseases by influencing molecular processes associated with bone synthesis and catabolism. Here, we review all new information pertinent to the effects of HDL and its major apolipoproteins on bone metabolism and function.

Principles of HDL biogenesis and metabolism

Recent data indicate that HDL is a mixture of lipoprotein particles with densities in the range of 1.063–1.21 g/mL, and depending on their lipid composition, these particles may assume a discoidal or spherical geometry. Mature spherical HDL particles contain approximately 45–55% apoproteins, 26–32% phospholipids, 15–20% esterified cholesterol, 3–5% free cholesterol and approximately 5% triglycerides (Tsompanidi *et al.* 2010). The main protein component of HDL is apolipoprotein A1 (APOA1) that plays a key role in the biogenesis and functions of HDL (Zannis *et al.* 2004). However, studies in mice showed that other apolipoproteins such as apolipoprotein E (APOE) (Kypreos & Zannis 2007) and apolipoprotein CIII (APOCIII) (Kypreos 2008) are also capable of promoting the *de novo* biogenesis of HDL in a pathway similar to the

one for the formation of APOA-I-containing HDL. Notably, APOA1-containing HDL appears to be structurally and functionally distinct from APOE-containing HDL particles (Filou *et al.* 2016).

Studies in cell cultures, as well as studies in experimental mouse models, showed that biogenesis of classical APOA1-containing HDL involves the lipid transporters ATP-binding cassette A1 (ABCA1) and G1 (ABCG1) and the plasma enzyme lecithin:cholesterol acyl transferase (LCAT) (Soutar *et al.* 1975, Chroni *et al.* 2003, Fitzgerald *et al.* 2004). In the early steps of HDL formation, lipid-free APOA1 that is secreted at about 70% by the liver (Timmins *et al.* 2005) and 30% by the intestine (Brunham *et al.* 2006) interacts functionally with a dimeric form of the lipid transporter ABCA1 to acquire phospholipid and cholesterol (Nagata *et al.* 2013), thus forming a minimally lipidated APOA1. Through a series of intermediate steps that involve ABCG1, minimally lipidated APOA1 gradually forms discoidal HDL particles, which are then converted into spherical particles by plasma enzyme lecithin:cholesterol acyl transferase (LCAT) (Soutar *et al.* 1975). APOA1 on both discoidal and spherical HDL particles is then capable of interacting with scavenger receptor class B type I (SRBI) present on the surface of cells (Krieger 2001, Liu *et al.* 2002, Van Eck *et al.* 2005) to deliver cholesterol esters to the cell. Studies in mice support that the interactions of APOA1 on HDL with cell-surface SRBI are important for the atheroprotective functions of HDL (Trigatti *et al.* 2003). Additional steps in the metabolism of HDL involve its further processing by plasma enzyme cholesteryl ester transfer protein an enzyme that mediates the exchange of HDL cholesteryl esters with VLDL (very low-density lipoprotein) triglycerides. This step contributes to the reduction of HDL particle size, making it a more suitable substrate for SRBI, and the eventual catabolism of HDL cholesteryl esters by the LDL receptor through receptor-mediated uptake of cholesteryl ester-rich LDL. Additional processing of HDL in circulation involves the hydrolysis of its phospholipids and triglycerides by various lipases (lipoprotein lipase, hepatic lipase and endothelial lipase) and the transfer of phospholipids from VLDL/LDL to HDL by phospholipid transfer protein (Zannis *et al.* 2004).

Proteomic analyses revealed a diversity in HDL proteome that depending on the method of HDL isolation, includes more than 85 different proteins (Karlsson *et al.* 2005a,b, Heller *et al.* 2005, Rezaee *et al.* 2006, Vaisar *et al.* 2007, Shah *et al.* 2013), in addition to APOA1, APOE and APOCIII. Moreover, these studies showed that the plasma abundance of HDL-associated proteins is insufficient

Osteoblasts are metabolically active cells, and as such, they possess large nucleus containing 2–4 nucleoli, very rich rough endoplasmic reticulum and Golgi apparatus. The name ‘osteoblast’ has its origins from the Greek words ‘*osteo*’-‘*ὀστό*’ that means bone and ‘*blast*’-‘*βλασταίνω*’ that means ‘been born’. These cells are responsible for the production of the extracellular matrix that is composed mainly of collagen type 1, with smaller amounts of specialized proteins including osteocalcin (Blair *et al.* 2007). Osteoblasts are under the tight control of paracrine, autocrine and endocrine signals. In fact, virtually all common signal transduction pathways converge on osteoblasts regulating their homeostasis (de Gorter & ten Dijke 2013).

Osteoblast, the bone-forming cell and the 'osteoblast family'

Bone is a form of specialized connective tissue that performs several essential functions including mechanical support for soft tissues, protection of internal organs, calcium homeostasis and support of haematopoiesis. The maintenance of bone mass in mammals depends upon the fine balance between bone synthesis and resorption, two vital tasks accomplished by osteoblasts and osteoclasts, respectively. Continuous bidirectional cross-talk between osteoblasts–osteoclasts is coordinated in time and space through a process that is fundamental for bone homeostasis and is called ‘bone remodeling’ (Fig. 1). This process is under the strict control of autocrine, paracrine and endocrine signals and is essential for the adaptation of bone to the mechanical loading and for the repair after damage (Sims & Gooi 2008). Most importantly,

New osteoblasts differentiate from mesenchymal stem cells in a skillfully coordinated fashion at the bone surfaces. Key transcription factors for osteoblast differentiation are osterix and RUNX2; the former is often used to regulate cre recombinase for osteoblast-specific gene expression (Blair *et al.* 2008). The molecular pathways that are responsible for osteoblast differentiation, proliferation and ultimately apoptosis are complex (de Gorter & ten Dijke 2013). Among the most thoroughly studied and potent regulators of osteoblast differentiation are the mitogen-activated kinases (MAPK), the Wnt/ β -catenin, the bone morphogenic proteins (BMP) and transforming growth factor- β (TGF β), the hedgehog, the Notch, the insulin-like growth factor 1 (IGF1), the fibroblast growth factor (FGF) and the calcium signal transduction pathways that ultimately converge on RUNX2, which is the master regulator of osteoblast differentiation (Blair *et al.* 2016b). The osteoblasts are connected through tight junctions, and gap junctions consisted mainly of connexin-43. The tight junctions exclude low molecular matrix materials, whereas bone is alive. Notably, however, this is not the case for dead bone, which is immediately permeable (Eberhardt *et al.* 2001). The most common bone formation defects, usually include mutations in type I collagen (osteogenesis imperfecta) (Forlino & Marini 2016). There are many causes of low or high bone mass that are secondary to defects in specific factors, including serum lipids and lipoprotein receptors, as discussed elsewhere in this review.

Except from the bone-forming cells, the '*osteoblast family*' also includes the bone lining cells and the osteocytes. *Bone lining cells* are bone surface-residing cells that are spindle shaped and metabolically and functionally quiescent. These cells separate bone matrix from the tissue extracellular fluid, creating a type of a tight 'epithelium'. The separation of matrix and extracellular space is an essential feature of bone, which is often not appreciated. Under the influence of specific signals, bone lining cells can regain their bone-forming capacity and produce bone. As osteoblastic function progresses and bone matrix expands, surface osteoblasts are eventually incorporated into the matrix that they have produced being transformed to *osteocytes*, the third member of the '*osteoblast family*' (Bonewald 2011). Osteocytes are permanent components of living bone and the most abundant cells in human skeleton. Indeed, in the fully mature skeleton, osteocytes compose the 90–95% of the total number of bone cells, which is enormous given that osteoblasts constitute only the 4–6% (Bonewald 2011). As these cells are entrapped in mineralized bone matrix, they

harbor a delicate network of cell membrane processes to communicate with each other and with the bone surface cells. These processes contain connexin-43 and run in small channels, the canaliculi, which permeate the extracellular matrix (Blair *et al.* 2016b). For years, it was believed that osteocytes are terminally differentiated cells with a small impact on skeletal metabolism. Nevertheless, it is now clear that the osteocyte network is surprisingly active, serving as an endocrine unit with key roles in bone remodeling and homeostasis (Bonewald 2011).

One of the most important functions of skeleton is to receive and respond to mechanical stimuli, primarily related to gravity and movement. Osteocytes are the most mechanosensitive cells of bone. Indeed, their large number and the perfectly developed cell–cell and cell–surface communications render them capable of receiving mechanical cues, which, in turn, are converted to biochemical and ultimately molecular response, through a finely tuned but extremely complex process called mechanotransduction (Papachristou *et al.* 2009, Thompson *et al.* 2012). Another worth-mentioning function of osteocytes is the production of sclerostin (Delgado-Calle *et al.* 2016), a protein that inhibits further growth of bone. Other important signals produced by osteocytes and osteoblasts are mediated by fibroblast growth factor 23 (FGF23), a specialized fibroblast growth factor family protein, which responds to 1,25-dihydroxyvitamin D3 and reduces phosphate reabsorption in the kidney (Huang *et al.* 2013).

Osteoclasts, the bone-degrading cells

Osteoclasts are multinucleated cells derived from monocyte family precursors (Blair *et al.* 2008), in sharp contrast to osteoblast that are of mesenchymal origin. Osteoclast precursors are mononucleated cells that during the process of differentiation and under the influence of specific factors that will be mentioned briefly later in this review, fuse forming the mature multinucleated cells, capable of bone resorption (Fig. 1). The name '*osteoclast*' originates from the Greek words '*osteo*'-'*ὀστό*' that means bone and '*clast*' that means cut/destroy. As their name defines, the main function of these cells is the degradation of bone extracellular matrix. To accomplish this function, osteoclasts adhere tightly to bone surfaces through specialized integrin (mainly $\alpha_v\beta_3$) adhesions. Acid-secreting H⁺-ATPase, which resides at their apical surfaces, facilitates bone mineral resorption by adding acid to it, solubilizing phosphate and calcium (Blair *et al.* 1989). The acid secretion is supported by chloride–proton

exchange and chloride channels (Schlesinger *et al.* 1997, Palagano *et al.* 2015). Osteoclasts secrete acid proteinases, including cathepsins B and K and matrix metalloproteinases (MMP) –9 and –13 that degrade collagen. Removal of bone components is achieved mainly by vacuolar translocation (Blair *et al.* 2008). As with other macrophage family cells, osteoclasts are dependent upon the macrophage colony-stimulation factor-1 (CSF1), via the tyrosine kinase receptor c-fms, for survival and differentiation (Pixley & Stanley 2004, Blair *et al.* 2005). CSF1 is produced by mesenchymal cells including osteoblasts and stromal cells and is responsible for the differentiation proliferation and survival of the committed cells and their progression to committed pre-osteoclasts (Fig. 1). Osteoclast precursors also express the receptor RANK (receptor activator for nuclear factor κ B) that regulates the maturation of mono- and multi-nucleated pre-osteoclasts through its binding with RANK ligand (RANKL). RANKL is a type II homotrimeric transmembrane protein produced by osteoblast and stromal cells and is also secreted by activated T cells (Martin & Sims 2015). In addition to RANKL, osteoblasts and stromal cells also produce and secrete osteoprotegerin (OPG), a soluble RANK decoy receptor, which prevents RANK from acting at distant sites as a scavenger and pharmacologically is a useful inhibitor of osteoclast formation (Boyle *et al.* 2003, Martin & Sims 2015). Increased RANKL/OPG ratio promotes osteoclast formation and thus bone resorption (Fig. 1).

Primary osteoclast defects are rare, usually including defects in acid secretion, acid proteinases and cellular adhesion (Blair *et al.* 2004).

There is a tremendous amount of additional information on osteoclast differentiation, too much for a general review. Molecules including pituitary hormones, sex steroids, glucocorticoids, specific cytokines and growth factors are implicated in the regulation of bone formation and degradation (Blair *et al.* 2016b); nevertheless, our appreciation of the overall physiological control of bone homeostasis is still vague.

Obesity, bone marrow fat, HDL and bone metabolism

The past few years there is gradually increasing volume of data documenting a strong connection between fat and bone metabolism (Papachristou & Blair 2016). Indeed, it has been demonstrated that disturbances in lipid metabolic pathways differentially affect bone cells resulting in the development of skeletal pathologies.

In this vein, a large number of epidemiological and animal studies have attempted to explore the association between obesity and bone mass. Notably, however, the results are contrasting, and it is not clear whether obesity has a positive or negative effect on bone mass. As a matter of fact, several, mainly older studies, suggested that obesity in terms of increased body weight has a protective role against bone loss and osteoporosis development (Reid *et al.* 1994, Reid 2008). This notion was mainly supported by the fact that increased body weight results in augmented mechanical stimulation on bone, which promotes osteoblast activation and bone formation, whereas on the other hand, it inhibits osteoclastogenesis and bone resorption (Papachristou *et al.* 2009). From a metabolic standpoint, obesity is associated with the secretion of the pancreatic hormones insulin, resistin and amylin, as well as with increased estrogen levels, molecules that serve as critical regulators of bone metabolism (Barsh *et al.* 2002). In addition, visceral adipocytes can secrete adipokines, and in particular leptin, adiponectin and resistin that also variably affect bone cells (Barsh *et al.* 2002). However, more recent studies have shown that the incidence of osteoporosis and bone fractures is increased in obese individuals compared to that in individuals within normal weight (Cao 2011). In this line, a more recent study on healthy premenopausal women showed that central adiposity is strongly associated reduced bone quality, stiffness and bone formation rate. In addition, epidemiological studies in humans propose that fat mass determines the quality of bone in a manner that is independent of body weight, in postmenopausal women (Reid *et al.* 1994). One possible explanation for this is that the local phenomena that take place within bone marrow have strong effect on bone cell functions, and thus, on bone mass, that possibly overcomes the impact of bone-acting circulating hormones. Indeed, conditions that are characterized by increased visceral adiposity are associated with increased bone marrow fat and reduced bone mass (Ng & Duque 2010) (Fig. 2). The increased bone marrow adiposity that is observed in these conditions is primarily attributed to the fact that osteoblasts and bone marrow adipocytes that have common progenitor, the mesenchymal stem cell, represent the two sides of the same coin. Therefore, increased bone marrow adiposity is accompanied by decreased number of osteoblasts and vice versa (Reid 2008).

The reduced bone mass observed in obese individuals is also linked to the fact that obesity elicits low-grade inflammatory response, primarily mediated by the pro-inflammatory cytokines tumor necrosis factor- α (TNF α),

HDL and Bone Mass

Figure 2

This diagram shows the molecular mechanisms that underline the effect of impaired and/or dysfunctional HDL on bone mass. The red arrows represent the 'positive' effects, the blue arrows represent the 'negative' effects and the green arrows are indicative of 'no' or 'undefined' effect. ANXA2, Annexin-2; BM-ADC, bone marrow adipocytes; CLCX12, CXC chemokine ligand 12; Coll, collagen; IL, interleukin; MCP, macrophages; MSC, mesenchymal stem cells; OC, osteocalcin; OCL, osteoclast; OBL, osteoblast; ON, osteonectin; OPN, osteopontin; RANK, receptor activator for nuclear factor κ B; RANKL, RANK Ligand; TNF α , tumor necrosis factor alpha.

interleukine-6 (IL6) and IL1 β (Okin *et al.* 2013) and affects bone formation–resorption equilibrium and hence bone mass. Activated T lymphocytes can directly bind macrophages, triggering the production and secretion of the aforementioned cytokines. Under normal conditions, HDL inhibits the T lymphocyte–macrophage interaction preventing the initiation of the inflammatory response (Burger *et al.* 2002). It is now well appreciated that inflammation has a strong impact on bone remodeling differentially affecting the function of both the osteoblasts and the osteoclasts and thus possesses a central role in the development of bone-related metabolic pathologies, such as osteoporosis (Redlich & Smolen 2012). Osteoblastic differentiation and activation are under the strict control of the Wnt/ β -catenin signaling axis. Briefly, this axis is activated when extracellular Wnt binds to a specific co-receptor complex composed of a member the Frizzled family of receptors (FZD) and the extracellular domain of the LDL receptor-related proteins (LRPs) -5 and -6 (in vertebrates) (Pandur & Kuhl 2001). Among the common substrates of the Wnt/ β -catenin cascade, which is referred as the 'canonical Wnt pathway', are RUNX2, COX2, parathyroid hormone (PTH) and Indian hedgehog (IHH), crucial regulators of osteoblastic physiology. In an inflammatory background, TNF α , IL6 and IL1 β activate Dickkopf, sclerostin and secreted frizzled-related proteins (sFRP), which impede the formation of the Wnt–LRPs–FZD assembly and hence suppress the anabolic downstream effects of the Wnt/ β -catenin pathway on osteoblasts (Clevers 2006). Another mechanism that is responsible for the inflammation-related bone loss is the

activation of the IL6-JAK-STAT ((JUN N-terminal kinase)/ (signal transducers and activators of transcription)) signal transduction pathway that restrains the activation of the mitogen-activated protein kinase (MAPK)–AP1 signaling cascade, which in turn regulates the expression of the osteoblastogenic factors RUNX2, Osterix, Collagen type 1, Osteocalcin, PTHrP and OPG (Krum *et al.* 2010, Redlich & Smolen 2012). A similar effect is evoked via the activation of the TNF-SMAD ubiquitylation regulatory factor (SMURF) 1/2 cascade that also deteriorates the expression of osteoblast regulators (Kaneki *et al.* 2006).

The effect of low-grade inflammation is also pronounced on osteoclasts. More specifically, activated T lymphocytes express RANKL and therefore have the ability to directly bind and activate osteoclast precursor cells, via the RANK-RANKL axis (Devlin *et al.* 1998, Ma *et al.* 2004). In addition, pro-inflammatory molecules secreted by the bone marrow adipocytes can enhance osteoclasts differentiation and activation in a RANK/RANKL-independent fashion (Kobayashi *et al.* 2000). Inflammation is also associated with the production of the monocyte chemoattractant protein 1 (MCP1), a pro-inflammatory cytokine that is also implicated in the transcription of osteoclast-specific genes, primarily through the JAK-STAT signaling cascade (Redlich & Smolen 2012). Nevertheless, less is known about the direct effect of HDL on osteoclasts physiology, a research field that is under scrutiny (Fig. 2).

In an effort to shed light into the effect of diet-induced obesity on bone mass, a recent study on adult mice that were fed high-fat diet for 11 weeks uncovered

that bone responds to increased body weight in a bimodal fashion. Initially, obesity has an anabolic effect on bone due to enhanced mechanical loading and/or secretion of adipokines or growth factors that augment bone synthesis. Remarkably, however, after prolonged insult with high-fat diet animals enter a second, catabolic phase characterized by reduced bone mass, probably related with diet-induced alterations in metabolic profile of these obese mice (Lecka-Czernik *et al.* 2015).

The effect of the components of the HDL metabolic pathways on cartilage and bone

HDL and cartilage homeostasis

A considerable volume of epidemiological studies has uncovered a strong connection between osteoarthritis (OA) and pathological conditions such as hypertension, dyslipidemia, coronary heart disease and type 2 diabetes. Therefore, it not surprising that several authors suggest that OA should be considered a facet of metabolic syndrome (Velasquez & Katz 2010).

In symphony with the clinical data, molecular studies have shown that perturbations in lipid metabolic pathways are strongly related to the pathobiology of OA. Indeed, a sophisticated comparative proteome analysis on hypertrophied OA chondrocytes revealed that pathways regulating lipid metabolism, prostaglandin synthesis, glutathione metabolism and metabolism through the cytochrome p450, display differences between normal and hypertrophic OA chondrocytes (Tsolis *et al.* 2015). Moreover, gene expression analyses on human-derived OA chondrocytes uncovered disturbed expression levels of genes that regulate cholesterol influx and efflux and lipid metabolism (Tsolis *et al.* 2015). It has also been documented that the expression of a set of genes that regulate cholesterol efflux, namely ABCA1, APOA1 and LXR α is significantly suppressed in chondrocytes derived from OA in comparison to normal cartilage (Tsezou *et al.* 2010). Notably, after treatment with the LXR agonist TO-901317, the OA chondrocytes displayed augmented ABCA1 and APOA1 expression and cholesterol efflux (Tsezou *et al.* 2010). This finding is in line with a previous study by Collins-Racie and coworkers having examined 48 nuclear receptors and found that the expression levels of 23 of them had significant differences between normal and OA human cartilage samples (Collins-Racie *et al.* 2009). Interestingly, the mRNA of the nuclear receptors LXR α and LXR β and their heterodimeric partners retinoid

X receptor (RXR) α and RXR β , as well as the LXR target genes ABCG1 and apolipoproteins D and E were greatly reduced in the OA compared to those in the normal cartilage, a finding proposing that the HDL metabolic pathway is deregulated in OA. Their finding that the LXR signaling alterations are restored with the use of the LXR agonist TO-901317 suggests LXR signaling modulation could add to the therapeutic armamentarium against OA (Collins-Racie *et al.* 2009).

Driven by the aforementioned data, and based on previous results of our research team on the role of the lipoprotein transfer system and HDL metabolic pathways in the pathogenesis of diet-induced obesity and non-alcoholic fatty liver disease (Karagiannides *et al.* 2008, Karavia *et al.* 2012), we were tempted to further explore the role of HDL in the pathobiology of OA. For this purpose, we applied histological, histomorphometrical and molecular/biochemical methodologies and found that after the consumption of Western-type diet both LCAT- and APOA1-deficient mice developed OA, in contrast to the control groups (Triantaphyllidou *et al.* 2013). This suggests that alterations in HDL biogenesis and maturation predispose to the development of OA in mice after chronic insult with Western-type diet. Notably, we also observed that in sharp contrast to the LCAT $^{-/-}$ mice, the APOA1 $^{-/-}$ were not quite as obese, an observation that raises the challenging possibility that altered HDL metabolism may have a direct destructive effect on articular cartilage that is independent of body weight and significantly contributes to the development of OA (Triantaphyllidou *et al.* 2013).

HDL and bone homeostasis

Recent data propose that there is an association between serum HDL levels and bone mass (Papachristou & Blair 2016). However, the results that have been generated from epidemiological studies on humans are contradicting. Indeed, even though a considerable volume of these studies have shown that increased HDL is associated with better bone quality and reduced risk of osteoporosis, others support a negative relation between serum HDL levels and bone mass (Jeong *et al.* 2010, Ackert-Bicknell 2012, Li *et al.* 2015). It is believed that factors such as genetic background, age, dietary habits and metabolic status are responsible for this inconsistency. Therefore, research groups that work on this field have recruited experimental animal models in an effort to illuminate the molecular mechanisms that link HDL and bone homeostasis,

as will be described in the following sections of the present review article.

The role of Scarb1 Several recent studies have investigated the possible connection between HDL and bone metabolism. Toward this direction the group of Moreau has extensively studied the role of the principal HDL receptor, the scavenger receptor class B, type I (SRBI), in the regulation of bone mass. SRBI is the product of the *Scarb1* gene and binds HDL with high affinity, facilitating the transportation of cholesterol from peripheral organs to the liver (Acton *et al.* 1996), a process called reverse cholesterol transport.

In an elegant study, this group examined the phenotypic characteristics of femora obtained from *Scarb1*-deficient mice, in comparison to their wild-type counterparts (Martineau *et al.* 2014a,b). Serologic analysis revealed that the *Scarb1* knockout mice had significantly elevated both total plasma and HDL cholesterol. Dynamic and static (μ CT-based) histomorphometry uncovered that *Scarb1* deficiency was followed by increased bone volume and elevated osteoblast number and function, particularly in the trabecular bone of 2-month-old female mice femora. Their *in vitro* approaches on MSC obtained from knockout (KO) and wild-type mice were in symphony with their histological and histomorphometrical results, further supporting the osteoblastogenic behavior of the *Scarb1*-null mice. SRBI regulates the uptake of cholesterol at the adrenal glands, and thus, *Scarb1*-deficient mice are characterized by reduced glucocorticoid but elevated adrenocorticotrophic hormone (ACTH) levels (Martineau *et al.* 2014a,b). Given that high concentrations of ACTH are anabolic for osteoblasts (Isales *et al.* 2010), this mechanism may possibly explain the high bone mass phenotype observed in the *Scarb1* KO mice. Nevertheless, other mechanisms such as intrinsic cellular alterations of the canonical and non-canonical Wnt pathways that are not directly related to plasma HDL levels may explain the increased bone mass of *Scarb1* KO mice (Martineau *et al.* 2014a,b), further adding to the complexity of the relationship between HDL and bone metabolism.

The ‘enigmatic’ role of APOE ApoE is a 34.2kDa glycoprotein produced by the liver and other peripheral organs. Its main functions are atheroprotection and maintenance of plasma lipid homeostasis as it mediates the cellular uptake of chylomicron remnants, very low-density lipoprotein and low-density lipoprotein and their clearance from the circulation. Notably, APOE

is also implicated in the *de novo* biosynthesis of HDL (Kypreos & Zannis 2007). In a relatively recent study, investigating the genetic connection between HDL and bone mineral density (BMD), Ackert-Bicknell reported that APOE, along with PPAR γ , ESR1 and IL6, belongs to a set of genes that regulate both HDL and BMD (Ackert-Bicknell 2012). Nevertheless, the role of APOE in bone pathophysiology still remains quite puzzling. Indeed, back in 2005, Schilling and coworkers, first published the results of a very interesting research study on the effects of APOE deficiency on bone mass. With the use of histological, histomorphometrical, biomechanical and *in vitro* approaches, the authors showed that the *ApoE*-null mice exhibit augmented osteoblastic function and increased rate of bone synthesis (Schilling *et al.* 2005). Previous *in vitro* and experimental animal-based studies have documented that APOE deficiency is associated with reduced uptake of vitamin K-containing lipoproteins by osteoblasts and that exogenous administration of APOE increases the uptake of chylomicron remnants that contain vitamin K (Newman *et al.* 2002, Niemeier *et al.* 2005). Taking into account that vitamin K is essential for osteocalcin carboxylation, the authors correctly surmised that impaired APOE results in enhanced bone mass, paralleling the phenotype of the osteocalcin-depleted mice (Niemeier *et al.* 2005). Nonetheless, this mechanism does not explain the whole phenotype of APOE deficiency as studies in mouse models have shown that shortage of osteocalcin is associated with augmented fat mass (Oldknow *et al.* 2015), in contrast to APOE deficiency that is characterized by reduced body fat content and smaller adipocytes (Huang *et al.* 2006). To further add to the complexity of the role of APOE in bone biology, a more recent study by the same group showed that the bone mass of APOE-null mice is substantially reduced after consumption of diabetogenic high-fat diet for 16 weeks. These knockout mice also have an interesting metabolic profile, characterized by normal weight and lowered levels of serum insulin, glucose and leptin (Bartelt *et al.* 2010). The aforementioned findings reinforce the prevailing notion that body weight *per se* cannot define the quality of bone; rather, it appears that the fine balance between local and systemic metabolic pathways is responsible for the determination of bone mass.

As mentioned previously, osteoblasts and lipoblasts originate from the same precursor, the bone marrow mesenchymal stem cell. Interestingly, APOE-deficient mice challenged with high fat display both reduced bone mass and bone marrow adiposity (Bartelt *et al.* 2010). This observation allows drawing the conclusion that lack

of APOE possibly halts bone marrow MSC maturation in early stages and thus affects both lipoblastic and osteoblastic lineage, through unidentified mechanisms. More recently, Dieckman and coworkers performed biochemical, histomorphometrical and genetic analyses on transgenic mice and demonstrated that human APOE2 has the most significant effect on bone turnover, in comparison to the other natural human isoforms, namely APOE3 and APOE4. They also presented data introducing the APOE ϵ 2 allele as a latent risk factor for reduced trabecular bone mass and vertebrae fractures in humans (Dieckmann *et al.* 2013).

The role of APOE in the function of osteoclasts is also poorly understood. The studies of Bartelt and coworkers and Schilling and coworkers demonstrated that shortage of APOE does not influence the number or the function of osteoclasts in mice fed both high-fat or chow diet (Schilling *et al.* 2005, Bartelt *et al.* 2010). In sharp contrast, a more recent *in vitro* report on bone marrow-derived macrophages showed that APOE has an inhibitory effect on osteoclasts, which is mediated by c-FOS-, NFATc1- and NF- κ B-related signaling cascades (Kim *et al.* 2013).

In aggregate, the current literature indicates that APOE serves as a potent regulator of mesenchymal stem cells and therefore affects bone mass; nevertheless, its role on osteoclastic function warrants further investigation.

The role of APOA1 APOA1 is a key molecule in the regulation of HDL biogenesis. Its role in the regulation of bone mass is graphically presented in Fig. 3. We have recently documented that in addition to its role in atheroprotection, APOA1 is also implicated in the

development of non-alcoholic fatty liver disease as well as in the pathogenesis of osteoarthritis in mice (Karavia *et al.* 2012, Trantaphyllidou *et al.* 2013). To further explore whether HDL possesses a causative role in the regulation of bone mass, we studied the involvement of LCAT and APOA1 in the function of bone cells. For this purpose, we used a 12-week-old *Lcat* and *ApoA1* KO male mice. Static and dynamic histomorphometrical analyses showed that the bone quality as well as the rate of new bone formation and the number of tartrate-resistant acid phosphatase (TRAP) positive cells were similar between the *Lcat* KO and the wild-type animals. Importantly, however, the *ApoA1*^{-/-} mice had significantly reduced bone mass in comparison with their wild-type counterparts, implying that it is the impaired synthesis and not the incomplete maturation of HDL that affects bone mass. Bone quality studies including Raman spectroscopy and three-point bending test revealed that these mice had a remarkable reduction of cross-linked collagen, which insinuates a significant deterioration of the biomechanical properties of femora obtained from these mice. Dynamic histomorphometry showed a significant reduction in calcein-labeled and double-labeled surfaces, but no differences in the TRAP-positive cells between the two groups. Through a series of *in vitro* and molecular experiments on mesenchymal stem cells, osteoblasts and osteoclasts isolated and cultured from femora of the two mouse groups, we uncovered that the osteoblast-related factors and signaling axes, RUNX2, osterix, COLLa1 and RANKL, were significantly impaired in the *ApoA1* KO compared to those in the wild-type mice. On the contrary, genes that regulate osteoclastic differentiation and function namely TRAP (*Acp5*),

Figure 3

Diagram depicting the molecular changes observed in the *ApoA1*-knockout mice (B) in comparison to their wild-type counterparts (A). All the molecular alterations are described in detail in the text. Note that the increased or decreased expression levels are represented by larger or smaller boxes. For example in the *ApoA1*-deficient mice, the expression of CLCX12 is significantly decreased (X0.3), whereas the expression levels of its CXCL12 receptor CXCR4 is greatly elevated (X3). APOA1, Apolipoprotein A-1; CLCX12, CXC chemokine ligand 12; CXCR4, CXC Receptor 4; LBL, lipoblast; MSC, mesenchymal stem cells; OBL, osteoblast; WT, wild type.

cathepsin K (*Ctsk*) and RANK (*Tnfrsf11a*) were unaffected (Blair *et al.* 2016a). These interesting findings indicate that the reduced bone mass observed in the *ApoA1* KO mice is attributed primarily to suppressed osteoblastic bone synthesis and not to increased osteoclastic bone degradation.

It is well appreciated that bone marrow adiposity possesses an essential role in the regulation of osteoblasts and osteoclasts, and consequently, bone mass (Lecka-Czernik & Stechschulte 2014, Scheller *et al.* 2016). Histological examination of *ApoA1* KO mice femora revealed that bone marrow of these animals have significantly elevated number of lipoblasts. In addition to the enhanced adiposity, the mesenchymal stem cells derived from the *ApoA1*-deficient mice exhibit increased expression levels of the lipoblast master regulators PPAR γ and CEBPa (Blair *et al.* 2016a). Taking under consideration our finding that the number of bone marrow total mesenchymal stem cell population of both mouse groups was similar, we hypothesize that APOA1 may perform as a modulator of the mesenchymal stem cells pools within bone marrow, tipping the balance toward osteoblastogenesis (Blair *et al.* 2016a). The mechanisms that underline this phenomenon remain insufficiently elucidated.

One the most potent regulators of mesenchymal stem cell homing and bone synthesis is the CXC chemokine ligand 12 (CLCX12) that binds primarily to the receptor CXC receptor 4 (CXCR4) and is regulated, at least in part, by Annexin-2 (ANXA2) (Jung *et al.* 2011). We have very recently reported that APOA1 deficiency greatly disturbs the ANXA2–CLCX12–CXCR4 connection, providing a plausible mechanistic explanation for the impaired osteoblastic function observed in the *ApoA1* KO mice (Blair *et al.* 2016a). It should also be borne in mind that the CLCX12–CXCR4 axis is also crucial for the regulation of osteoblastic bone marrow niche that participates in an array of processes, including hematopoietic stem cell (HSC) homing and mobilization and bone metastasis (Greenbaum *et al.* 2013, Kfoury & Scadden 2015). Taken together, these reports fuel the attractive hypothesis that APOA1 may have a role in the pathobiology of HSC dormancy and/or motivation, and thus, it may be involved in the development of hematopoietic neoplasms. Moreover, given that reduced HDL levels are associated to increased metastasis rate (Pan *et al.* 2012), we advance the intriguing theory that perturbations in APOA1 may be implicated in the pathogenesis of bone metastases through alterations in signaling cascades and molecular axes, such as ANXA2–CLCX12–CXCR4 and

RANK–RANKL, which modify the microenvironment of the osteoblastic niche. Definitely, further studies are essential for the substantiation of these hypotheses.

Conclusions and future perspectives

As research on HDL progresses, new data in the literature strongly support a causative role of dysfunctional HDL with a number of metabolic disorders, including bone metabolic diseases (Constantinou *et al.* 2015, Papachristou & Blair 2016). Nonetheless, several critical issues should be addressed to unfold the ‘mysteries’ that underline the bone–HDL connection. First, the involvement of additional key regulators of the lipoprotein metabolism, such as ABCA1, LDLR and SRB1 in bone biology should be extensively assessed. The use of genetically modified mice is the best approach toward the salvation of these issues. Second, the role of bone marrow adiposity must be thoroughly examined. It is known that elevated marrow adiposity variably affects bone cells and is involved in the pathogenesis of bone-related pathologies such as osteoporosis and bone metastases. Moreover, novel data suggest that bone marrow microenvironment and the osteoblastic niche in particular are greatly influenced by HDL status. Taking into account that osteoblastic niche regulates the fine balance between dormancy and motivation of the HSCs and plausibly participates in the pathogenesis of hematopoietic neoplasias, it would be very intriguing to unveil the molecular cobblestone that link HDL metabolism to the development of these diseases. Finally, it should be kept in mind that HDL efficacy does not rely solely on the HDL-C levels, but also on the HDL particle functionality, which is defined by HDL apolipoprotein and lipid content (Filou *et al.* 2016). For this reason, the in-depth appreciation of the structure–function association of HDL will pave the way not only toward the thorough understanding of the mechanisms that link HDL and bone mass, but also toward the development of effective pharmaceuticals that will target HDL functionality. It is our expectation that the identification of surrogate markers of HDL functionality may prove invaluable for predicting the risk for developing bone metabolic disorders.

Obviously, many ‘rivers need to be crossed’ to achieve a proper understanding of the molecular basis of the complex HDL–bone interactions. However, it is now clear that the functional crosstalk between HDL and bone metabolism determines the beginning of a beautiful friendship that can potentially set the basis for

the development of novel HDL-directed pharmaceuticals for the treatment of bone pathologies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

- Ackert-Bicknell CL 2012 HDL cholesterol and bone mineral density: is there a genetic link? *Bone* **50** 525–533. (doi:10.1016/j.bone.2011.07.002)
- Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH & Krieger M 1996 Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* **271** 518–520. (doi:10.1126/science.271.5248.518)
- Barsh GS & Schwartz MW 2002 Genetic approaches to studying energy balance: perception and integration. *Nature Reviews Genetics* **3** 589–600. (doi:10.1038/nrn902)
- Bartelt A, Beil FT, Schinke T, Roeser K, Ruether W, Heeren J & Niemeier A 2010 Apolipoprotein E-dependent inverse regulation of vertebral bone and adipose tissue mass in C57Bl/6 mice: modulation by diet-induced obesity. *Bone* **47** 736–745. (doi:10.1016/j.bone.2010.07.002)
- Blair HC, Teitelbaum SL, Ghiselli R & Gluck S 1989 Osteoclastic bone resorption by a polarized vacuolar proton pump. *Science* **245** 855–857. (doi:10.1126/science.2528207)
- Blair HC, Borysenko CW, Villa A, Schlesinger PH, Kalla SE, Yaroslavskiy BB, García-Palacios V, Oakley JI & Orchard PJ 2004 In vitro differentiation of CD14 cells from osteopetrotic subjects: contrasting phenotypes with TCIRG1, CLCN7, and attachment defects. *Journal of Bone and Mineral Research* **19** 1329–1338. (doi:10.1359/JBMR.040403)
- Blair HC, Robinson LJ & Zaidi M 2005 Osteoclast signalling pathways. *Biochemical and Biophysical Research Communications* **328** 728–738. (doi:10.1016/j.bbrc.2004.11.077)
- Blair HC, Schlesinger PH, Huang CL & Zaidi M 2007 Calcium signalling and calcium transport in bone disease. *Subcell Biochemistry* **45** 539–562. (doi:10.1007/978-1-4020-6191-2_21)
- Blair HC, Zaidi M, Huang CL & Sun L 2008 The developmental basis of skeletal cell differentiation and the molecular basis of major skeletal defects. *Biological Reviews Cambridge Philosophical Society* **83** 401–415. (doi:10.1111/j.1469-185X.2008.00048.x)
- Blair HC, Kalyvioti E, Papachristou NI, Tourkova IL, Sygdelos SA, Deligianni D, Orkoulas MG, Kontoyannis CG, Karavia EA, Kypreos KE, et al. 2016a Apolipoprotein A-I regulates osteoblast and lipoblast precursor cells in mice. *Laboratory Investigation* **96** 763–772. (doi:10.1038/labinvest.2016.51)
- Blair HC, Larrouette QC, Li Y, Lin H, Beer Stoltz D, Liu L, Tuan RS, Robinson LJ, Schlesinger PH & Nelson DJ 2016b Osteoblast differentiation and bone matrix formation in vivo and in vitro. *Tissue Engineering Part B* [in press]. (doi:10.1089/ten.TEB.2016.0454)
- Bonewald LF 2011 The amazing osteocyte. *Journal of Bone and Mineral Research* **26** 229–238. (doi:10.1002/jbmr.320)
- Boyle WJ, Simonet WS & Lacey DL 2003 Osteoclast differentiation and activation. *Nature* **423** 337–342. (doi:10.1038/nature01658)
- Brunham LR, Kruit JK, Iqbal J, Fievet C, Timmins JM, Pape TD, Coburn BA, Bissada N, Staels B, Groen AK, et al. 2006 Intestinal ABCA1 directly contributes to HDL biogenesis in vivo. *Journal of Clinical Investigation* **116** 1052–1062. (doi:10.1172/JCI27352)
- Burger D & Dayer JM 2002 High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmunity Reviews* **1** 111–117. (doi:10.1016/S1568-9972(01)00018-0)
- Cao JJ 2011 Effects of obesity on bone metabolism. *Journal of Orthopaedic Surgery and Research* **6** 30. (doi:10.1186/1749-799X-6-30)
- Chroni A, Liu T, Gorshkova I, Kan HY, Uehara Y, von Eckardstein A & Zannis VI 2003 The central helices of ApoA-I can promote ATP-binding cassette transporter A1 (ABCA1)-mediated lipid efflux. Amino acid residues 220–231 of the wild-type ApoA-I are required for lipid efflux in vitro and high density lipoprotein formation in vivo. *Journal of Biological Chemistry* **278** 6719–6730. (doi:10.1074/jbc.M205232200)
- Clevers H 2006 Wnt/b-catenin signaling in development and disease. *Cell* **127** 469–480. (doi:10.1016/j.cell.2006.10.018)
- Collins-Racie LA, Yang Z, Arai M, Li N, Majumdar MK, Nagpal S, Mounts WM, Dorner AJ, Morris E & LaVallie ER 2009 Global analysis of nuclear receptor expression and dysregulation in human osteoarthritic articular cartilage: reduced LXR signaling contributes to catabolic metabolism typical of osteoarthritis. *Osteoarthritis Cartilage* **17** 832–842. (doi:10.1016/j.joca.2008.12.011)
- Constantinou C, Karavia EA, Xepapadaki E, Petropoulou PI, Papakosta E, Karavryaki M, Zvintzou E, Theodoropoulos V, Filou S, Hatziri A, et al. 2015 Advances in high-density lipoprotein physiology: surprises, overturns, and promises. *American Journal of Physiology: Endocrinology and Metabolism* **310** E1–E14. (doi:10.1152/ajpendo.00429.2015)
- de Gorter DJJ & ten Dijke P 2013 Signal transduction cascades controlling osteoblast differentiation. In *Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, edn 8, pp 15–24. Ed CJ Rosen. Ames, IA, USA: John Wiley & Sons, Inc. (doi:10.1002/9781118453926.ch2)
- Delgado-Calle J, Sato AY & Bellido T 2016 Role and mechanism of action of sclerostin in bone. *Bone* **96** 29–37. (doi:10.1016/j.bone.2016.10.007)
- Devlin RD, Reddy SV, Savino R, Ciliberto G & Roodman GD 1998 IL-6 mediates the effects of IL-1 or TNF, but not PTHrP or 1,25(OH)₂D₃, on osteoclast-like cell formation in normal human bone marrow cultures. *Journal of Bone and Mineral Research* **13** 393–399. (doi:10.1359/jbmr.1998.13.3.393)
- Dieckmann M, Beil FT, Mueller B, Bartelt A, Marshall RP, Koehne T, Amling M, Ruether W, Cooper JA, Humphries SE, et al. 2013 Human apolipoprotein E isoforms differentially affect bone mass and turnover in vivo. *Journal of Bone and Mineral Research* **28** 236–245. (doi:10.1002/jbmr.1757)
- Eberhardt AW, Yeager-Jones A & Blair HC 2001 Regional trabecular bone matrix degeneration and osteocyte death in femora of glucocorticoid-treated rabbits. *Endocrinology* **142** 1333–1340. (doi:10.1210/endo.142.3.8048)
- Filou S, Lhomme M, Karavia EA, Kalogeropoulou C, Theodoropoulos V, Zvintzou E, Sakellaropoulos GC, Petropoulou PI, Constantinou C, Kontush A, et al. 2016 Distinct roles of apolipoproteins A1 and E in the modulation of high-density lipoprotein composition and function. *Biochemistry* **55** 3752–3762. (doi:10.1021/acs.biochem.6b00389)
- Fitzgerald ML, Morris AL, Chroni A, Mendez AJ, Zannis VI & Freeman MW 2004 ABCA1 and amphipathic apolipoproteins form high-affinity molecular complexes required for cholesterol efflux. *Journal of Lipid Research* **45** 287–294. (doi:10.1194/jlr.M300355-JLR200)

- Forlino A & Marini JC 2016 Osteogenesis imperfecta. *Lancet* **387** 1657–1671. (doi:10.1016/S0140-6736(15)00728-X)
- Gofman JW, Glazier F, Tamplin A, Strisower B & De Lalla O 1954 Lipoproteins, coronary heart disease, and atherosclerosis. *Physiological Reviews* **34** 589–607.
- Greenbaum A, Hsu YM, Day RB, Schuettelpelz LG, Christopher MJ, Borgerdting JN, Nagasawa T & Link DC 2013 CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. *Nature* **495** 227–230. (doi:10.1038/nature11926)
- Havel RJ, Eder HA & Bragdon JH 1955 The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *Journal of Clinical Investigation* **34** 1345–1353. (doi:10.1172/JCI103182)
- Heller M, Stalder D, Schlappritzi E, Hayn G, Matter U & Haerberli A 2005 Mass spectrometry-based analytical tools for the molecular protein characterization of human plasma lipoproteins. *Proteomics* **5** 2619–2630. (doi:10.1002/pmic.200401233)
- Huang X, Jiang Y & Xia W 2013 FGF23 and phosphate wasting disorders. *Bone Research* **1** 120–132. (doi:10.4248/BR201302002)
- Huang ZH, Reardon CA & Mazzon T 2006 Endogenous ApoE expression modulates adipocyte triglyceride content and turnover. *Diabetes* **55** 3394–3402. (doi:10.2337/db06-0354)
- Isales CM, Zaid M & Blair HC 2010 ACTH is a novel regulator of bone mass. *Annals of the New York Academy of Sciences* **1192** 110–116. (doi:10.1111/j.1749-6632.2009.05231.x)
- Jeong IK, Cho SW, Kim SW, Choi HJ, Park KS, Kim SY, Lee HK, Cho SH, Oh BH & Shin CS 2010 Lipid profiles and bone mineral density in pre- and postmenopausal women in Korea. *Calcified Tissue International* **87** 507–512. (doi:10.1007/s00223-010-9427-3)
- Jung Y, Shiozawa Y, Wang J, Patel LR, Havens AM, Song J, Krebsbach PH, Roodman GD & Taichman RS 2011 Annexin-2 is a regulator of stromal cell-derived factor-1/CXCL12 function in the hematopoietic stem cell endosteal niche. *Experimental Hematology* **39** 151–166. (doi:10.1016/j.exphem.2010.11.007)
- Kaneki H, Guo R, Chen D, Yao Z, Schwarz EM, Zhang YE, Boyce BF & Xing L 2006 Tumor necrosis factor promotes Runx2 degradation through up-regulation of Smurf1 and Smurf2 in osteoblasts. *Journal of Biological Chemistry* **281** 4326–4333. (doi:10.1074/jbc.M509430200)
- Karagiannides I, Abdou R, Tzortzopoulou A, Voshol PJ & Kypreos KE 2008 Apolipoprotein E predisposes to obesity and related metabolic dysfunctions in mice. *FEBS Journal* **275** 4796–4809. (doi:10.1111/j.1742-4658.2008.06619.x)
- Karavia EA, Papachristou DJ, Liopeta K, Triantafyllidou IE, Dimitrakopoulos O & Kypreos KE 2012 Apolipoprotein A-I modulates processes associated with diet-induced nonalcoholic fatty liver disease in mice. *Molecular Medicine* **18** 901–912. (doi:10.2119/molmed.2012.00113)
- Karavia EA, Zvintzou E, Petropoulou PI, Xepapadaki E, Constantinou C & Kypreos KE 2014 HDL quality and functionality: what can proteins and genes predict? *Expert Review of Cardiovascular Therapy* **12** 521–532. (doi:10.1586/14779072.2014.896741)
- Karlsson H, Leanderson P, Tagesson C & Lindahl M 2005a Lipoproteomics I: mapping of proteins in low-density lipoprotein using two-dimensional gel electrophoresis and mass spectrometry. *Proteomics* **5** 551–565. (doi:10.1002/pmic.200300938)
- Karlsson H, Leanderson P, Tagesson C & Lindahl M 2005b Lipoproteomics II: mapping of proteins in high-density lipoprotein using two-dimensional gel electrophoresis and mass spectrometry. *Proteomics* **5** 1431–1445. (doi:10.1002/pmic.200401010)
- Kfoury Y & Scadden DT 2015 Mesenchymal cell contributions to stem cell niche. *Cell Stem Cell* **16** 239–253. (doi:10.1016/j.stem.2015.02.019)
- Kim WS, Kim HJ, Lee ZH, Lee Y & Kim HH 2013 Apolipoprotein E inhibits osteoclast differentiation via regulation of c-Fos, NFATc1 and NF- κ B. *Experimental Cell Research* **319** 436–446. (doi:10.1016/j.yexcr.2012.12.004)
- Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, Nakagawa N, Kinoshita M, Yamaguchi K, Shima N, et al. 2000 Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL–RANK interaction. *Journal of Experimental Medicine* **191** 275–286. (doi:10.1084/jem.191.2.275)
- Krieger M 2001 Scavenger receptor class B type I is a multiligand HDL receptor that influences diverse physiologic systems. *Journal of Clinical Investigation* **108** 793–797. (doi:10.1172/JCI14011)
- Krum SA, Chang J, Miranda-Carboni G & Wang CY 2010 Novel functions for NF κ B: inhibition of bone formation. *Nature Reviews Rheumatology* **6** 607–611. (doi:10.1038/nrrheum.2010.133)
- Kypreos KE 2008 ABCA1 promotes the de novo biogenesis of apolipoprotein CIII-containing HDL particles in vivo and modulates the severity of apolipoprotein CIII-induced hypertriglyceridemia. *Biochemistry* **47** 10491–10502. (doi:10.1021/bi801249c)
- Kypreos KE & Zannis VI 2007 Pathway of biogenesis of apolipoprotein E-containing HDL in vivo with the participation of ABCA1 and LCAT. *Biochemical Journal* **403** 359–367. (doi:10.1042/BJ20061048)
- Kypreos KE, Gkizas S, Rallidis LS & Karagiannides I 2013 HDL particle functionality as a primary pharmacological target for HDL-based therapies. *Biochemical Pharmacology* **85** 1575–1578. (doi:10.1016/j.bcp.2013.03.004)
- Lecka-Czernik B & Stechschulte LA 2014 Bone and fat: a relationship of different shades. *Archives of Biochemistry and Biophysics* **561** 124–129. (doi:10.1016/j.abb.2014.06.010)
- Lecka-Czernik B, Stechschulte LA, Czernik PJ & Dowling AR 2015 High bone mass in adult mice with diet-induced obesity results from a combination of initial increase in bone mass followed by attenuation in bone formation; implications for high bone mass and decreased bone quality in obesity. *Molecular and Cellular Endocrinology* **410** 35–41. (doi:10.1016/j.mce.2015.01.001)
- Li S, Guo H, Liu Y, Wu F, Zhang H, Zhang Z, Xie Z, Sheng Z & Liao E 2015 Relationship of serum lipid profiles and bone mineral density in postmenopausal Chinese women. *Clinical Endocrinology* **82** 53–58. (doi:10.1111/cen.12616)
- Liu T, Krieger M, Kan HY & Zannis VI 2002 The effects of mutations in helices 4 and 6 of ApoA-I on scavenger receptor class B type I (SR-BI)-mediated cholesterol efflux suggest that formation of a productive complex between reconstituted high density lipoprotein and SR-BI is required for efficient lipid transport. *Journal of Biological Chemistry* **277** 21576–21584. (doi:10.1074/jbc.M112103200)
- Ma T, Miyanishi K, Suen A, Epstein NJ, Tomita T, Smith RL & Goodman SB 2004 Human interleukin-1-induced murine osteoclastogenesis is dependent on RANKL, but independent of TNF- α . *Cytokine* **26** 138–144. (doi:10.1016/j.cyto.2004.02.001)
- Martin TJ & Sims NA 2015 RANKL/OPG; critical role in bone physiology. *Reviews in Endocrine and Metabolic Disorders* **16** 131–139. (doi:10.1007/s11154-014-9308-6)
- Martineau C, Kevorkova O, Brissette L & Moreau R 2014a Scavenger receptor class B, type I (Scarb1) deficiency promotes osteoblastogenesis but stunts terminal osteocyte differentiation. *Physiological Reports* **2** e12117. (doi:10.14814/phy2.12117)
- Martineau C, Martin-Falstrault L, Brissette L & Moreau R 2014b The atherogenic Scarb1 null mouse model shows a high bone mass phenotype. *American Journal of Physiology: Endocrinology and Metabolism* **306** E48–E57. (doi:10.1152/ajpendo.00421.2013)
- Miller GJ & Miller NE 1975 Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet* **1** 16–19. (doi:10.1016/S0140-6736(75)92376-4)
- Nagata KO, Nakada C, Kasai RS, Kusumi A & Ueda K 2013 ABCA1 dimer-monomer interconversion during HDL generation revealed by single-molecule imaging. *PNAS* **110** 5034–5039. (doi:10.1073/pnas.1220703110)
- Newman P, Bonello F, Wierzbicki AS, Lumb P, Savidge GF & Shearer MJ 2002 The uptake of lipoprotein-borne phyloquinone (vitamin K1) by osteoblasts and osteoblast-like cells: role of heparan sulfate proteoglycans and apolipoprotein E. *Journal of Bone and Mineral Research* **17** 426–433. (doi:10.1359/jbmr.2002.17.3.426)

- Ng A & Duque G 2010 Osteoporosis as a lipotoxic disease. *BoneKey* **7** 108–123. (doi:10.1138/20100435)
- Niemeier A, Kassem M, Toedter K, Wendt D, Ruether W, Beisiegel U & Heeren J 2005 Expression of LRP1 by human osteoblasts: a mechanism for the delivery of lipoproteins and vitamin K1 to bone. *Journal of Bone and Mineral Research* **20** 283–293. (doi:10.1359/JBMR.041102)
- Okin PM, Hille DA, Wiik BP, Kjeldsen SE, Lindholm LH, Dahlöf B & Devereux RB 2013 In-treatment HDL cholesterol levels and development of new diabetes mellitus in hypertensive patients: the LIFE Study. *Diabetic Medicine* **30** 1189–1197. (doi:10.1111/dme.12213)
- Oldknow KJ, MacRae VE & Farquharson C 2015 Endocrine role of bone: recent and emerging perspectives beyond osteocalcin. *Journal of Endocrinology* **225** R1–R19. (doi:10.1530/JOE-14-0584)
- Palagano E, Blair HC, Pangrazio A, Tourkova I, Strina D, Angius A, Cuccuru G, Oppo M, Uva P, Van Hul W, *et al.* 2015 Buried in the middle but guilty: intronic mutations in the *tcirg1* gene cause human autosomal recessive osteopetrosis. *Journal of Bone and Mineral Research* **30** 1814–1821. (doi:10.1002/jbmr.2517)
- Pan B, Ren H, He Y, Lv X, Ma Y, Li J, Huang L, Yu B, Kong J, Niu C, *et al.* 2012 HDL of patients with type 2 diabetes mellitus elevates the capability of promoting breast cancer metastasis. *Clinical Cancer Research* **18** 1246–1256. (doi:10.1158/1078-0432.CCR-11-0817)
- Pandur P & Kuhl M 2001 An arrow of wingless to take-off. *Bioassays* **23** 207–210. (doi:10.1002/1521-1878(200103)23:3<207::AID-BIES1029>3.0.CO;2-0)
- Papachristou DJ & Blair HC 2016 Bone and high-density lipoprotein: beginning of a beautiful friendship. *World Journal of Orthopedics* **7** 74–77. (doi:10.5312/wjo.v7.i2.74)
- Papachristou DJ, Papachroni KK, Basrda EK & Papavassiliou AG 2009 Signaling networks and transcription factors regulating mechanotransduction in bone. *Bioessays* **31** 794–804. (doi:10.1002/bies.200800223)
- Pixley FJ & Stanley ER 2004 CSF-1 regulation of the wandering macrophage: complexity in action. *Trends in Cell Biology* **14** 628–638. (doi:10.1016/j.tcb.2004.09.016)
- Redlich K & Smolen JS 2012 Inflammatory bone loss: pathogenesis and therapeutic intervention. *Nature Reviews Drug Discovery* **11** 234–250. (doi:10.1038/nrd3669)
- Reid IR 2008 Relationships between fat and bone. *Osteoporosis International* **19** 595–606. (doi:10.1038/nrd3669)
- Reid IR, Ames RW, Evans MC, Sharpe SJ & Gamble GD 1994 Determinants of the rate of bone loss in normal postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **79** 950–954. (doi:10.1210/jc.79.4.950)
- Rezaee F, Casetta B, Levels JH, Speijer D & Meijers JC 2006 Proteomic analysis of high-density lipoprotein. *Proteomics* **6** 721–730. (doi:10.1002/pmic.200500191)
- Scheller EL, Cawthorn WP, Burr AA, Horowitz MC & MacDougald OA 2016 Marrow adipose tissue: trimming the fat. *Trends in Endocrinology and Metabolism* **27** 392–403. (doi:10.1016/j.tem.2016.03.016)
- Schilling AF, Schinke T, Münch C, Gebauer M, Niemeier A, Priemel M, Streichert T, Rueger JM & Amling M 2005 Increased bone formation in mice lacking apolipoprotein E. *Journal of Bone and Mineral Research* **20** 274–282. (doi:10.1359/JBMR.041101)
- Schlesinger PH, Blair HC, Teitelbaum SL & Edwards JC 1997 Characterization of the osteoclast ruffled border chloride channel and its role in bone resorption. *Journal of Biological Chemistry* **272** 18636–18643. (doi:10.1074/jbc.272.30.18636)
- Shah AS, Tan L, Long JL & Davidson WS 2013 Proteomic diversity of high density lipoproteins: our emerging understanding of its importance in lipid transport and beyond. *Journal of Lipid Research* **54** 2575–2585. (doi:10.1194/jlr.R035725)
- Sims NA & Gooi JH 2008 Bone remodeling: multiple cellular interactions required for coupling of bone formation and resorption. *Seminars in Cell and Developmental Biology* **19** 444–451. (doi:10.1016/j.semcdb.2008.07.016)
- Soutar AK, Garner CW, Baker HN, Sparrow JT, Jackson RL, Gotto AM & Smith LC 1975 Effect of the human plasma apolipoproteins and phosphatidylcholine acyl donor on the activity of lecithin: cholesterol acyltransferase. *Biochemistry* **14** 3057–3064. (doi:10.1021/bi00685a003)
- Thompson WR, Rubin CT & Rubin J 2012 Mechanical regulation of signaling pathways in bone. *Gene* **503** 179–193. (doi:10.1016/j.gene.2012.04.076)
- Timmins JM, Lee JY, Boudyguina E, Kluckman KD, Brunham LR, Mulya A, Gebre AK, Coutinho JM, Colvin PL, Smith TL, *et al.* 2005 Targeted inactivation of hepatic *Abca1* causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. *Journal of Clinical Investigation* **115** 1333–1342. (doi:10.1172/JCI200523915)
- Triantaphyllidou IE, Kalyvioti E, Karavia E, Lilis I, Kypreos KE & Papachristou DJ 2013 Perturbations in the HDL metabolic pathway predispose to the development of osteoarthritis in mice following long-term exposure to western-type diet. *Osteoarthritis Cartilage* **21** 322–330. (doi:10.1016/j.joca.2012.11.003)
- Trigatti BL, Krieger M & Rigotti A 2003 Influence of the HDL receptor SR-BI on lipoprotein metabolism and atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology* **23** 1732–1738. (doi:10.1161/01.ATV.0000091363.28501.84)
- Tsezou A, Iliopoulos D, Malizos KN & Simopoulou T 2010 Impaired expression of genes regulating cholesterol efflux in human osteoarthritic chondrocytes. *Journal of Orthopaedic Research* **28** 1033–1039. (doi:10.1002/jor.21084)
- Tsolis KC, Bei ES, Papathanasiou I, Kostopoulou F, Gkretsi V, Kalantzaki K, Malizos K, Zervakis M, Tsezou A & Economou A 2015 Comparative proteomic analysis of hypertrophic chondrocytes in osteoarthritis. *Clinical Proteomics* **12** 12. (doi:10.1186/s12014-015-9085-6)
- Tsomanidi EM, Brinkmeier MS, Fotiadou EH, Giakoumi SM & Kypreos KE 2010 HDL biogenesis and functions: role of HDL quality and quantity in atherosclerosis. *Atherosclerosis* **208** 3–9. (doi:10.1016/j.atherosclerosis.2009.05.034)
- Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, *et al.* 2007 Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. *Journal of Clinical Investigation* **117** 746–756. (doi:10.1172/JCI26206)
- Van Eck M, Pennings M, Hoekstra M, Out R & van Berkel TJ 2005 Scavenger receptor BI and ATP-binding cassette transporter A1 in reverse cholesterol transport and atherosclerosis. *Current Opinion in Lipidology* **16** 307–315. (doi:10.1097/01.mol.0000169351.28019.04)
- Velasquez MT & Katz JD 2010 Osteoarthritis: another component of metabolic syndrome? *Metabolic Syndrome and Related Disorders* **8** 295–305. (doi:10.1089/met.2009.0110)
- Zannis VI, Kypreos KE, Chroni A, Kardassis D & Zanni EE 2004 Biophysical analysis of apolipoprotein E3 variants linked with development of type III hyperlipoproteinemia. In *Molecular Mechanisms of Atherosclerosis* pp 111–174. Ed J Loscalzo. New York, NY, USA: Taylor & Francis.

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