



# Osteokines and the vasculature: a review of the *in vitro* effects of osteocalcin, fibroblast growth factor-23 and lipocalin-2

Sophie A. Millar, Susan I. Anderson and Saoirse E. O'Sullivan

Division of Graduate Entry Medicine and Medical Sciences, School of Medicine, Royal Derby Hospital, University of Nottingham, Derby, United Kingdom

## ABSTRACT

Bone-derived factors that demonstrate extra-skeletal functions, also termed osteokines, are fast becoming a highly interesting and focused area of cross-disciplinary endocrine research. Osteocalcin (OCN), fibroblast growth factor-23 (FGF23) and lipocalin-2 (LCN-2), produced in bone, comprise an important endocrine system that is finely tuned with other organs to ensure homeostatic balance and health. This review aims to evaluate *in vitro* evidence of the direct involvement of these proteins in vascular cells and whether any causal roles in cardiovascular disease or inflammation can be supported. PubMed, Medline, Embase and Google Scholar were searched for relevant research articles investigating the exogenous addition of OCN, FGF23 or LCN-2 to vascular smooth muscle or endothelial cells. Overall, these osteokines are directly vasoactive across a range of human and animal vascular cells. Both OCN and FGF23 have anti-apoptotic properties and increase eNOS phosphorylation and nitric oxide production through Akt signalling in human endothelial cells. OCN improves intracellular insulin signalling and demonstrates protective effects against endoplasmic reticulum stress in murine and human endothelial cells. OCN may be involved in calcification but further research is warranted, while there is no evidence for a pro-calcific effect of FGF23 *in vitro*. FGF23 and LCN-2 increase proliferation in some cell types and increase and decrease reactive oxygen species generation, respectively. LCN-2 also has anti-apoptotic effects but may increase endoplasmic reticulum stress as well as have pro-inflammatory and pro-angiogenic properties in human vascular endothelial and smooth muscle cells. There is no strong evidence to support a pathological role of OCN or FGF23 in the vasculature based on these findings. In contrast, they may in fact support normal endothelial functioning, vascular homeostasis and vasodilation. No studies examined whether OCN or FGF23 may have a role in vascular inflammation. Limited studies with LCN-2 indicate a pro-inflammatory and possible pathological role in the vasculature but further mechanistic data is required. Overall, these osteokines pose intriguing functions which should be investigated comprehensively to assess their relevance to cardiovascular disease and health in humans.

Submitted 21 February 2019

Accepted 17 May 2019

Published 24 July 2019

Corresponding author

Sophie A. Millar,  
stxsamil@nottingham.ac.uk

Academic editor

Maria Deli

Additional Information and  
Declarations can be found on  
page 14

DOI 10.7717/peerj.7139

© Copyright  
2019 Millar et al.

Distributed under  
Creative Commons CC-BY 4.0

OPEN ACCESS

**Subjects** Cell Biology, Anatomy and Physiology, Cardiology, Diabetes and Endocrinology

**Keywords** Osteocalcin, Fibroblast growth factor 23, Lipocalin-2, Endothelial cells, Vasculature, Vascular smooth muscle cells, Osteokines

## INTRODUCTION

The role of the skeleton has greatly evolved from its premise of being a functional tissue providing primary protection, support, and haematopoietic maintenance. It is now recognised as an endocrine organ providing exciting opportunities for cross-disciplinary research. One candidate thread of enquiry is the skeleton's interaction with the vascular system. Bone is a highly vascularised tissue, which is important for growth, remodelling and repair. The endothelial cells lining blood vessels have an important influence on bone cells, often in a paracrine manner, establishing a 'bone-vascular axis' ([Brandi & Collin-Osdoby, 2006](#)). This traditionally viewed axis is also evolving to reflect that on the other hand, secreted, circulating bone-derived factors have direct effects on vascular cells themselves.

Recent research has demonstrated that three 'osteokines', namely osteocalcin (OCN), fibroblast growth factor-23 (FGF23) and lipocalin-2 (LCN-2), comprise an important endocrine system that is finely tuned with other organs to ensure homeostatic balance and health ([Han et al., 2018](#)). The endocrine functions of OCN have been extensively reviewed elsewhere ([Ducy, 2011](#); [Li et al., 2016](#); [Karsenty, 2017](#); [Han et al., 2018](#)). Briefly, OCN has been reported to play an active role in the regulation of energy metabolism by improving glucose tolerance and insulin sensitivity and insulin production ([Lee et al., 2007](#); [Oury et al., 2011](#); [Oury et al., 2013](#)). Elsewhere, OCN<sup>-/-</sup> male mice were observed to be poor breeders due to decreased testosterone production and thus has been linked to fertility ([Oury et al., 2011](#)). Interestingly, these OCN<sup>-/-</sup> mice also displayed behaviours associated with anxiety and depression and it was demonstrated that OCN can cross the blood-brain-barrier and enhance monoamine neurotransmitter synthesis, drawing a link between OCN and cognition ([Oury et al., 2013](#)). FGF23 is well characterised as the regulator of phosphate, parathyroid hormone (PTH), and vitamin D in the body which has been recently reviewed elsewhere ([Richter & Faul, 2018](#)). Briefly, in the kidneys, FGF23 increases phosphate excretion in urine, thus lowering serum concentrations. FGF23 is secreted by bone following stimulation by increased circulating vitamin D and extracellular phosphate. FGF23 in turn also suppresses 1,25(OH)<sub>2</sub>D<sub>3</sub> production. Thus, a regulatory feedback loop exists between the kidneys and bone through FGF23. In the kidneys, FGF23 also increases reabsorption of calcium and sodium ([Andrukhova et al., 2014a](#); [Andrukhova et al., 2014b](#)), while in the parathyroid gland FGF23 suppresses PTH production. The endocrine role of LCN-2 was an interesting discovery through the use of mice lacking LCN-2 in osteoblasts (Lcn2<sup>osb-/-</sup>). These mice displayed a decrease in insulin sensitivity and secretion, through direct activity on pancreatic β-cells, and decreased glucose tolerance ([Mosialou et al., 2017](#)). It has also been shown that LCN-2 crosses the blood brain barrier and activates melanocortin 4 receptor-dependent appetite signalling in the hypothalamus ([Mosialou et al., 2017](#)).

While these investigations have drawn evidence for the skeletal control of energy metabolism, mineral homeostasis and much more, the effects of these hormones on the vasculature itself has also gathered interest. Conflicting observational data exists between OCN, FGF23 and LCN-2 and markers of cardiovascular disease in humans ([Dalal et al., 2011](#); [Wang, 2012](#); [Millar et al., 2017](#)), which are discussed further within this review.

In order to assess the relevance of these osteokines to cardiovascular health, we aimed to provide an analysis of the direct effects of OCN, FGF23 and LCN-2 on micro- and macro-vascular cells *in vitro*, and to highlight gaps in the literature which need addressing, for use by clinicians and researchers.

## SURVEY METHODOLOGY

To retrieve articles concerning the exogenous administration of OCN, FGF23 and LCN-2 to micro- or macro-vascular cells, the following search terms were used for database searching (PubMed, Embase, Medline, Google Scholar): fibroblast growth factor 23, bone gamma-carboxyglutamic acid protein, osteocalcin, lipocalin 2, LCN-2, neutrophil gelatinase-associated lipocalin, NGAL, vascular cell, vascular endothelial cell, and vascular smooth muscle cell. Additionally, the reference lists of relevant review articles and included articles were hand-searched for further studies. Searches were carried out up until 20/09/2018. Retrieved articles were included if they described the exogenous addition of OCN, FGF23 or LCN-2 to vascular cells *in vitro* i.e., studies that examined the endogenous over-expression of these osteokines were excluded, as the results would not be deemed to be bone-derived. Similarly, passive reporting of osteokines e.g., expression studies, were not evaluated as this approach excludes the possibility of bone-derived effects and we were interested in clarifying any direct, causal effects of ‘circulating’ osteokines. Only peer-reviewed, full-text articles were included, not conference abstracts or articles not in English.

## OSTEOCALCIN

Osteocalcin (OCN) has been pioneered as a skeletal hormone for over a decade now, solidifying a role in influencing energy metabolism ([Lee et al., 2007](#)). The mature OCN is a 46 or 49 amino acid protein in mice and humans respectively, predominantly produced by osteoblasts, and is the most abundant, non-collagenous protein in the mineralised matrix of bone ([Hauschka, Lian & Gallop, 1975](#); [Price et al., 1976](#); [Celeste et al., 1986](#); [Hauschka et al., 1989](#)). Due to the presence of 3 glutamic acid residues which may undergo carboxylation, OCN is present in various forms. These include undercarboxylated OCN with 1 or 2 glutamic acid residues (unOCN), carboxylated OCN with no glutamic acid residues (cOCN), or uncarboxylated OCN (ucOCN) with 3 glutamic acid residues. Carboxylation induces a conformational change resulting in a high affinity for calcium ions. Thus, OCN is also referred to as bone  $\gamma$ -carboxyglutamic acid-containing protein (BGLAP) and binds to hydroxyapatite crystal lattices present in bone extra-cellular matrix ([Hauschka et al., 1989](#)). unOCN and ucOCN have less affinity to hydroxyapatite and are more readily released into the circulation. These forms have been the most widely studied and are considered to be the ‘active’ forms of OCN ([Plantalech et al., 1991](#); [Cairns & Price, 1994](#); [Ferron et al., 2010](#)). Despite the abundance of OCN in bone, its exact role and function in bone reformation has not been clearly defined. However, serum concentrations of OCN may be used as a marker of bone turnover as it is widely accepted to be released from mature osteoblasts during bone formation ([Bellows, Reimers & Heersche, 1999](#)).

## Circulating concentrations of OCN

There is currently no consensus on which OCN fragments to measure, and there are no international or national standards (Lee, Hodges & Eastell, 2000). Total OCN concentrations have been reported by Hannemann et al. by age and gender (Hannemann et al., 2013). However, reported serum ucOCN and unOCN concentrations in studies have largely varied, for example between 0.1 and 0.3 ng/mL, and up to 21.4 ng/mL or 33.0 ng/mL (Plantalech et al., 1991; Binkley et al., 2000; Iki et al., 2012). OCN concentrations increase with age but can be reduced by supplementation with vitamin K (Knapen, Hamulyak & Vermeer, 1989; Plantalech et al., 1991; Knapen et al., 1993). cOCN concentrations have also not been defined and have been reported to lie around 8–10 ng/mL or higher e.g., 23.9 ng/mL (Luukinen et al., 2000; Tsukamoto et al., 2000; Sugiyama & Kawai, 2001). To add to the complexity of measuring circulating OCN, only 25% is found intact, and further additional forms are also present including N-terminal, mid-region, mid-region-C-terminal, and C-terminal fragments released by proteolytic cleavage (Garnero et al., 1994).

## OCN and the vasculature

A number of cross-sectional studies have highlighted associations between OCN concentrations and atherosclerosis and calcification, however, we found that a meta-analysis of these data could not provide a conclusive role as study results were mixed and conflicting with a number of limitations (Millar et al., 2017). Analysing the direct interaction of OCN and its forms with vascular cells may elucidate whether OCN is a mediator or marker of vascular calcification or atherosclerosis, and whether it functions to affect vascular cells independently of its previously noted metabolic influences. Cross-sectional evidence also exists regarding a role for OCN in inflammation, with some population data highlighting an inverse association between OCN concentrations and systemic inflammatory makers such as C-reactive protein and interleukin-6 (Pittas et al., 2009; Chen et al., 2013; Lucey et al., 2013; Sarkar & Choudhury, 2013; Liao et al., 2015). A recent summary of *in vivo* ucOCN treatments in mice and rats concluded that ucOCN protects vascular function and protects against markers that are commonly associated with the development of atherosclerosis (Tacey et al., 2018). However, these findings are concomitant with improved metabolic status, and as such, the establishment of direct effects of OCN and its forms on vascular cells needs confirmation. The following section aims to summarise *in vitro* investigations on the exogenous addition of OCN to vascular endothelial or smooth muscle cells (see Table 1).

## OCN in human and animal cells

Jung et al. (2013) reported an increase in Akt and eNOS phosphorylation and nitric oxide (NO) production in a PI3-kinase dependent manner in human aortic endothelial cells (HAECs) when treated with ucOCN (0.3–30.0 ng/mL) (Jung et al., 2013). They also demonstrated an anti-apoptotic effect of ucOCN, in which 30 ng/mL pre-treatment prevented linoleic acid induced apoptosis, also via the PI3K/Akt pathway. Similarly, another study showed an increase in eNOS phosphorylation in HAECs after 30 min incubation with ucOCN (25 and 100 ng/mL) (Kondo et al., 2016). Interestingly, eNOS phosphorylation

**Table 1** Summary of studies investigating *in vitro* effects of osteocalcin on human and animal vascular cells.

Study	Cell type	Type of OCN	Concentration	Results	Conclusions
<i>Idelevich, Rais &amp; Monsonego-Ornan (2011)</i>	Murine VSMCs	Total OCN	Unknown	OCN was shown to be a glucose metabolism-modulating factor, through HIF-1 $\alpha$ .	OCN is involved in glucose-metabolism
<i>Zhou et al. (2013)</i>	Murine VECs and VSMCs	ucOCN	5 ng/mL	ucOCN protected against tunicamycin induced ER stress and autophagy, and improved insulin signalling in an Akt/mTOR/NF $\kappa$ B pathway.	Protective effect of ucOCN and improved insulin signalling
<i>Jung et al. (2013)</i>	Human AECs	ucOCN	0.3–30 ng/mL	Increased eNOS and NO, and prevented linoleic acid induced apoptosis in PI3-K/Akt dependent manner.	Protective effect of ucOCN
<i>Dou et al. (2014)</i>	HUVECs	Total OCN	10–150 ng/mL	>30 ng/mL OCN increased eNOS and Akt phosphorylation in a time- and dose-dependent manner.	Potential protective effect of total OCN
<i>Kondo et al. (2016)</i>	Human AECs	ucOCN cOCN	25 and 100 ng/mL	ucOCN increased eNOS phosphorylation after 30 min but cOCN had no effect.	Potential protective effect of ucOCN
<i>Guo et al. (2017)</i>	HUVECs	ucOCN	5 ng/mL	In insulin-resistant cells (induced by tunicamycin) ucOCN improved insulin signal transduction via PI3-K/Akt/NF $\kappa$ B pathways.	ucOCN is involved in insulin signalling

**Notes.**

Abbreviations: OCN, osteocalcin; VECs, vascular endothelial cells; VSMCs, vascular smooth muscle cells; AECs, aortic endothelial cells; HUVECs, human umbilical vein endothelial cells; ucOCN, uncarboxylated OCN; cOCN, carboxylated OCN; ER, endoplasmic reticulum; mTOR, mechanistic target of rapamycin; NF $\kappa$ B, Nuclear factor- $\kappa$ B; HIF-1 $\alpha$ , hypoxia inducible factor 1 –  $\alpha$ ; PI3-K, phosphoinositide 3-kinase; eNOS, endothelial nitric oxide synthase.

however was not affected by the same concentrations of cOCN (*Kondo et al., 2016*). Total OCN (>30.0 ng/mL) was reported to increase Akt and eNOS phosphorylation in human umbilical vein endothelial cells (HUVECs) in a time- and dose-dependent manner (*Dou et al., 2014*). Another study in HUVECs, in which insulin resistance was induced by tunicamycin, ucOCN (5 ng/mL) improved insulin signal transduction via PI3K/Akt/NF- $\kappa$ B pathways (*Guo et al., 2017*). Similarly, mouse vascular endothelial cells (VECs) and smooth muscle cells (VSMCs) were treated with 5 ng/mL ucOCN which protected against tunicamycin induced endoplasmic reticulum (ER) stress and autophagy, and improved insulin signalling in an Akt/mTOR/NF- $\kappa$ B pathway (*Zhou et al., 2013*).

*Idelevich, Rais & Monsonego-Ornan (2011)* importantly identified that OCN may indeed be an active player in vascular calcification, and not merely a product of differentiated VSMCs to an osteogenic phenotype. They demonstrated that overexpression of OCN stimulated glucose utilisation through activation of HIF-1 $\alpha$  and promoted mineralisation and osteogenic differentiation in mouse vascular smooth muscle cells (MOVAS). Silencing of OCN RNA suppressed these effects. However, this does not distinguish an effect of osteoblast derived, circulating OCN, but rather locally produced effects from overexpression. However, when total OCN was exogenously added to wild-type MOVAS, OCN was shown to be a glucose metabolism-modulating factor, through HIF-1 $\alpha$ . No

investigations have been conducted in human vascular smooth muscle cells to date. Ultimately, further investigation is needed into the potential direct influence of OCN on vascular calcification.

Overall, these studies support a hypothesis that OCN, ucOCN particularly, is directly vasoactive, and can increase nitric oxide via the PI3K/Akt/eNOS signalling pathway, which ultimately influences vasodilation. Additionally, further atheroprotective effects are demonstrated through protecting against high fatty acid induced apoptosis and improving insulin signalling. Although *Jung et al. (2013)* who used relatively physiological concentrations demonstrated a potential positive role of ucOCN in HAECs (increasing NO production and thus improving endothelial function), Idelevich et al. in contrast propose that OCN stimulates mineralisation and differentiation of mice VSMCs. It remains to be seen whether these disparities are due to differing effects of forms of OCN within different cell types and species, incubation timings or origin and concentrations of OCN, and whether OCN displays various functionalities that cannot be simply categorised into a good or bad role within the vasculature. The direct role of OCN within inflammation in the vasculature has also not been investigated. These limited studies and abundant questions signal there is need to clarify the role of physiologically relevant concentrations of OCN in human vascular cells. Moreover, the effects of both unOCN and cOCN, and potentially other OCN fragments, must be distinguished and deserves greater focus. Particularly as unOCN is more likely to be in circulation than fully non carboxylated OCN.

## FIBROBLAST GROWTH FACTOR 23

Osteocytes, which are the most abundant bone cell type, are the predominant secretors along with osteoblasts of fibroblast growth factor 23 (FGF23). FGF23 is the first described bone hormone and original phosphatonin (*Bonewald & Wacker, 2013; Bonnet, 2017*). FGF23 is a 251 amino acid protein in humans regulated at gene and protein level (*Bonewald & Wacker, 2013*). FGF23 is proteolytically cleaved upon secretion. Susceptibility to cleavage is reduced by O-glycosylation by polypeptide N-acetylgalactosaminyltransferase 3 which allows more intact, biologically active FGF23 to be secreted (approximately 32 kDa) (*Erben, 2017*). On the other hand, phosphorylation of FGF23 promotes cleavage resulting in C-terminus and N-terminus fragments, whose biological activity is unclear. Soluble or transmembrane  $\alpha$ -Klotho is believed to be the cofactor required to allow for activation of FGF receptors by FGF23, though in some tissues FGF23 can have klotho independent effects (*Urakawa et al., 2006; Chen et al., 2018; Richter & Faul, 2018*). Within bone, the actions of locally produced FGF23 by osteocytes, as well as osteoblasts, have not been fully elucidated. Within mice, FGF23 may be a regulator of bone mineralisation in an autocrine/paracrine manner through inhibiting tissue non-specific alkaline phosphatase (TNAP). This results in increased pyrophosphate concentrations, likely in a klotho independent manner due to low concentrations of klotho within bone (*Murali et al., 2016; Erben, 2017*).

### Circulating concentrations of FGF23

FGF23 reaches its target organs through the circulation. Similar to OCN, there is complexity in measuring FGF23 as there are various presenting forms. Intact FGF23 (iFGF23) is the

most commonly used measurement, while C-terminus FGF23 (cFGF23) assays can also be used which measure both iFGF23 and cFGF23 (Smith, 2014). iFGF23 is also subject to *ex vivo* degradation and exhibits diurnal variation, while cFGF23 concentrations can be influenced by iron levels, fibrous dysplasia or familial tumoral calcinosis (Wolf, Koch & Bregman, 2013; Smith, 2014). In adults, mean cFGF23 has been reported at 49.0 RU/mL, and iFGF23 at 26.1 pg/mL, but high intra-individual variability exists which limits its application as a diagnostic or management tool (Smith et al., 2012). Paediatric reference values have been published for cFGF23 only (22.0–91.0 RU/ml) (Fischer et al., 2012). In patients with chronic kidney disease (CKD), circulating FGF23 is increased with decreasing renal function, and can reach up to 1000 times higher than normal levels (Larsson et al., 2003; Fliser et al., 2007; Gutierrez et al., 2008; Isakova et al., 2011).

### **FGF23 and the vasculature**

A number of epidemiological studies in humans have reported associations between increased circulating FGF23 concentrations and vascular dysfunction (Mirza et al., 2009a), vascular calcification (Roos et al., 2008; Desjardins et al., 2012) and increased risk of cardiovascular disease (Dalal et al., 2011). FGF23 concentrations have been positively correlated with left ventricular hypertrophy (LVH), which is particularly apparent in CKD patient cohorts (Hsu & Wu, 2009; Mirza et al., 2009b). Other studies have not shown an association between FGF23 and vascular calcification or atherosclerotic events in CKD patients (Seiler et al., 2014; Sarmiento-Dias et al., 2016). A recent meta-analysis of prospective studies concluded that the relationship between FGF23 and cardiovascular disease risk is unlikely to be causal (Marthi et al., 2018). An established line of thought is that increased FGF23 may follow, as opposed to initiate, cardiovascular dysregulation (Stohr et al., 2018). It is likely that negative effects of high FGF23 are due to dysregulated phosphate metabolism. However, the research surrounding FGF23 is constantly evolving as the investigation into  $\alpha$ -klotho dependent and independent effects continues, as well as the potential effects of locally produced FGF23 within the cardiovascular system. FGF23 levels have also been associated with inflammation in CKD patients and in other inflammatory disorders (David, Francis & Babitt, 2017). The following section reviews the direct effects of exogenously added FGF23 on vascular cells in order to provide insight into the possible bone-derived effects as FGF23 travels through the circulation to reach its canonical target organs (see Table 2).

### **FGF23 in human and animal cells**

In recent years, literature has emerged directed at elucidating the direct effects of FGF23 in the vasculature. In human aortic smooth muscle cells (HASMCs), 10 ng/mL of exogenous FGF23 has been shown to increase phosphorylation of ERK (extracellular signal-regulated kinase) after 10 min in serum-starved cells, which in turn increased production of Early Growth Response Protein-1 (EGR-1) (Nakahara et al., 2016). Also in human VSMCs, 10 ng/mL of FGF23 increased hydrogen peroxide production and did not induce nitric oxide (NO) production on its own, however when soluble klotho and phosphate were also added, NO was increased (Six et al., 2014). In the context of calcification, 5 ng/mL of

**Table 2** Summary of studies investigating *in vitro* effects of FGF23 on human and animal vascular cells.

Study	Cell type	Context	Concentration	Results	Conclusions
<i>Lim et al. (2012)</i>	Human ASMCs	Calcification	5 ng/mL	FGF23 stimulated proliferation and phosphorylation of ERK and AKT in a klotho dependent fashion. Pre-treatment with FGF23 and calcitriol followed by treatment with calcification media inhibited development of calcification, in a klotho dependent fashion.	Vitamin D receptor activators can restore Klotho expression and unmask FGF23 anti-calcific effects. Klotho is required for vascular FGF23 signalling.
<i>Zhu et al. (2013)</i>	Murine VSMCs	Calcification	10 and 50 ng/mL	FGF23 reduced calcium deposition and increased phosphorylation of ERK1/2 (but not AKT). Concomitant exposure to FGF-23 and an ERK1/2 inhibitor (PD98059; 10 $\mu$ M) increased calcification.	FGF23 signalling is likely to be a protective mechanism in calcification.
<i>Lindberg et al. (2013)</i>	Bovine VSMCs	Calcification	0.125–2 ng/mL	FGF23 did not modify calcification.	No support for a role of FGF23 in vascular calcification.
<i>Scialla et al. (2013)</i>	Human VSMCs, murine VSMCs	Calcification	1, 2, 10, 20 and 50 ng/mL	FGF23 had no effect on phosphate uptake or phosphate-induced calcification even in the presence of soluble klotho. FGF23 did not induce phosphorylation of ERK or FRS2 $\alpha$ .	No support for a role of FGF23 in vascular calcification
<i>Silswal et al. (2014)</i>	MurineAECs	Normal	9 ng/mL	FGF23 increased superoxide levels compared with vehicle which was inhibited by pre-treatment with tiron.	FGF23 may reduce vasorelaxation by decreasing NO.
<i>Six et al. (2014)</i>	Human VSMCs, HUVECs	Normal	10 ng/mL	H <sub>2</sub> O <sub>2</sub> concentrations increased with FGF23 and klotho in HVSMCs but not in HUVECs. FGF-23 augmented H <sub>2</sub> O <sub>2</sub> concentration induced by phosphate. FGF23 did not increase NO production in HUVECs, but FGF23, klotho and phosphate together, did.	Klotho deficiency may be deleterious as appears protective against increased ROS production from FGF23/phosphate.
<i>Nakahara et al. (2016)</i>	Human ASMCs	Normal	10 ng/mL	FGF23 increased phosphorylated ERK, which subsequently increased EGR-1 expression.	FGF23 increased phosphorylated ERK expression and EGR-1 in HASMCs.

*(continued on next page)*

Table 2 (continued)

Study	Cell type	Context	Concentration	Results	Conclusions
<i>Richter et al. (2016)</i>	Human CAECs	Normal	10 ng/mL	FGF23 increased activation of FGFR1. Klotho levels were unaffected. FGF23 enhanced Klotho release and increased levels of ADAM17. FGF23 increased NO through Akt and eNOS. Cytosolic ROS increased via Nox2 and ROS detoxification via SOD2 and CAT. Blocking Klotho resulted in enhanced ROS formation and reduced NO availability.	Excess FGF23 may promote oxidative stress and endothelial dysfunction.
<i>Chung et al. (2017)</i>	Human AECs and Human BMECs	Normal	0.5–100 ng/mL	FGF23 stimulated cell proliferation, eNOS, and NO production in AECs. High phosphate mitigated these effects which could not be rescued by soluble Klotho. Adding soluble $\alpha$ -Klotho following $\alpha$ -Klotho knockdown also did not rescue EC resistance to FGF23. None of these effects were observed in HBMECs.	FGF23 promoted proliferation and phosphorylation of eNOS. AECs were only responsive to FGF23 in the presence of $\alpha$ -Klotho synthesized by ECs. HBMECs did not respond to FGF23.
<i>Verkaik et al. (2018)</i>	Human MVECs	Normal	Approx. 3.2 ng/mL	30 min treatment with FGF23 did not increase ERK phosphorylation.	FGF23 did not induce signal transduction through ERK.

**Notes.**

Abbreviations: FGF-23, fibroblast growth factor 23; VSMCs, vascular smooth muscle cells; AECs, aortic endothelial cells; ASMCS, aortic smooth muscle cells; HUVECs, human umbilical vein endothelial cells; OPG, osteoprotegerin; MVECs, microvascular endothelial cells; BMECs, brain microvascular endothelial cells; CAECs, coronary artery endothelial cells; FRS2 $\alpha$ , FGF receptor substrate 2 $\alpha$ ; EGR-1, early growth response protein-1; NO, nitric oxide; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species; Nox2, NADPH oxidase 2; SOD2, superoxide dismutase 2; CAT, catalase; FGFR1, fibroblast growth factor receptor 1.

FGF23 increased HASMC proliferation after 24 h in cells cultured in calcification media, and increased phosphorylation of ERK and AKT in a klotho-dependent manner (*Lim et al., 2012*). 24 h pre-treatment with FGF23 and calcitriol, followed by culture in calcification media, deterred calcification in HASMCs in a klotho dependent fashion, but this effect was not seen with FGF23 alone (without calcitriol) (*Lim et al., 2012*). *Scialla et al. (2013)* did not find any evidence for a pro-calcific effect of FGF23 in HASMCs (1–50 ng/mL), nor an increase in ERK phosphorylation (*Scialla et al., 2013*). Reflecting results shown in human VSMCs, FGF23 alone (10 and 50 ng/mL) reduced calcium deposition in the context of calcification in murine VSMCs through ERK signalling (*Zhu et al., 2013*), whereas in bovine VSMCs 0.2–2.0 ng/mL FGF23 did not modify calcification (*Lindberg et al., 2013*). In a study with murine VSMCs no effect of FGF23 on calcification was observed (1–50 ng/mL) (*Scialla et al., 2013*).

In microvascular endothelial cells, FGF23 did not increase ERK phosphorylation, nor in human umbilical vein endothelial cells did FGF23 increase hydrogen peroxide production (*Six et al., 2014; Verkaik et al., 2018*). In HAECs, FGF23 (50 and 100 ng/mL) increased proliferation after 48 h, while 10 ng/mL stimulated phosphorylated eNOS and NO production (*Chung et al., 2017*). These results were no longer observed following  $\alpha$ -klotho knockdown and could not be rescued by addition of soluble klotho, indicating

that these effects were dependent on localised endogenous  $\alpha$ -klotho production in HAECs. Interestingly, no increase in proliferation, nor eNOS phosphorylation or NO production were observed in the same conditions in human brain microvascular cells ([Chung et al., 2017](#)). In coronary artery endothelial cells (HCAECs), [Richter et al. \(2016\)](#) demonstrated that 10 ng/mL of FGF23 increased expression of the FGF receptor-1, increased eNOS phosphorylation and NO in an Akt-dependent manner, increased ROS production and ROS detoxification, and additionally increased secretion of soluble klotho via upregulation of ADAM17 ([Richter et al., 2016](#)). In blocking klotho, the authors further demonstrated an upregulation of ROS and decrease in NO. In murine aortic endothelial cells, 9 ng/mL FGF23 significantly increased superoxide levels ([Silswal et al., 2014](#)).

These collated results begin to untie whether FGF23 may have any causal effects within the context of cardiovascular pathologies. A few conclusions can be made including: FGF23 initiates direct effects on vascular cells including SMCs and ECs but actions may be tissue specific; FGF23 appears to increase eNOS phosphorylation and NO production likely in an Akt dependent manner, and predominantly in a klotho-dependent manner (perhaps restricted to locally produced klotho and not systemic circulating klotho); however, FGF23 also appears to increase ROS and oxidative stress, which is counteracted by klotho; within calcification models, FGF23 increased proliferation of SMCs, but does not appear to increase calcification and indeed may be anti-calcific. Bringing these results together paints a conflicting but potential positive role of FGF23 in vascular function. The results presented encourages the hypothesis that the associations and correlations between FGF23 and CVD are most likely due to deleterious effects of altered phosphate and mineral metabolism and indeed that FGF23 levels likely follow, as oppose to direct, cardiovascular disturbances. It is a complex area, with further discrepancies such as variable physiological and disease states, deficiency of klotho, origin of secretion of FGF23 and klotho, and many more factors leaving the role of FGF23 in the vasculature not a simple question to be answered. Further confirmative examinations are necessary and investigations into whether N- and C-terminal fragments of FGF23 are additionally biologically active and if so, how they differ from intact FGF23. Interestingly, any direct pro- or anti-inflammatory properties of FGF23 on vascular cells have not yet been explored despite accumulating evidence in other tissues for a role of FGF23 in inflammation ([David, Francis & Babitt, 2017](#)).

## LIPOCALIN-2

Lipocalin-2 (LCN-2), also referred to as neutrophil gelatinase-associated lipocalin (NGAL) or 24p3, is the most recently identified osteokine. However, it is debatable whether LCN-2 can be strictly described as such as it is secreted by a wide variety of cell types, and has indeed been more widely described as an adipokine to date. Despite this, it has recently been reported to be produced in bone ten-fold more than in white fat, at least in mice, and it remains to be reported whether this is also the case in humans ([Mosialou et al., 2017](#)). As such, LCN-2 is predominantly an osteoblast and adipocyte derived 25 kDa secreted glycoprotein which acts as a lipid chaperone positioning itself as a key pro-inflammatory link between obesity and associated metabolic disorders and vascular disease ([Kjeldsen](#)

*et al.*, 1993; Wang, 2012). It exists *in vivo* as a monomer, homodimer, or heterodimer by forming a complex with matrix metalloproteinase-9 (MMP-9) which stabilizes MMP-9 by preventing autodegradation (Kjeldsen *et al.*, 1993; Yan *et al.*, 2001; Chakraborty *et al.*, 2012). LCN-2 has been identified to mediate an innate immune response to bacteria by sequestering iron and induces apoptosis in many cell types (Devireddy *et al.*, 2001; Flo *et al.*, 2004).

### **Circulating concentrations of LCN-2**

LCN-2 is increased in obese individuals, which is presumed derived from increased adipose tissue and also increased expression from the liver, but may also be due to changes in skeletal homeostasis which is also altered in obesity (Wang *et al.*, 2007; Luo *et al.*, 2016). It has been suggested that LCN-2 can be used as a biomarker for early renal injury (Mishra *et al.*, 2004; Mishra *et al.*, 2005). For example, in children undergoing surgery, those who did not subsequently develop acute renal injury had LCN-2 serum concentrations <50 ng/mL, but 50% of those who did develop acute renal injury has concentrations >50 ng/mL (Mishra *et al.*, 2005). In a study of 1,203 Chinese obese and non-obese men, LCN-2 serum concentrations were in the range 29.3–53.5 ng/mL (Luo *et al.*, 2016). In patients with Type 2 Diabetes, serum levels were significantly higher in those with subclinical atherosclerosis than those without (112.9 ng/mL versus 77.2 ng/mL) (Xiao *et al.*, 2013). LCN-2 serum concentrations are also higher in patients with metabolic syndrome compared to those without (83.2 ng/mL versus 67.5 ng/mL) (Wang *et al.*, 2007).

### **Lipocalcin-2 and the vasculature**

LCN-2 is a novel, debatable osteokine. Examining its effects on vascular cells when originating from osteoblasts presents a difficult task, requiring for example tissue specific knock-down studies. Further validation to characterise LCN-2 as an osteokine with more convincing human data is primarily needed. As LCN-2 has been associated with a stage-dependent contribution to atherosclerosis, promoting lipotoxicity within the vasculature in obese states, and causing endothelial dysfunction and cardiovascular complications, it is important to establish whether bone-derived LCN-2 is a key mediator that could be therapeutically targeted (Wang, 2012; Amersfoort *et al.*, 2018). LCN-2 is also proposed as a sensitive marker for cardio-renal disease in patients with acute heart failure (Mishra *et al.*, 2005; Alvelos *et al.*, 2011).

Most studies to date have focused on observational and epidemiological data on the involvement of LCN-2 in obesity and diabetic associated vascular complications. It is currently unfounded to speculate whether these effects are influenced by bone derived LCN-2. The limited investigations on the direct exogenous addition of this interesting protein in vascular cells are summarised below and in Table 3.

### **LCN-2 in human and animal cells**

Wang and colleagues, interested in investigating pulmonary hypertension, demonstrated that in human pulmonary artery smooth muscle cells (HPASMCs), LCN-2 at 10 and 20 ng/mL increased proliferation after 24 hrs (Wang *et al.*, 2015). LCN-2 promoted the activity of the PI3-K pathway by increasing Akt phosphorylation (3–30 ng/mL) after 24

**Table 3** Summary of studies investigating *in vitro* effects of LCN-2 on human and animal vascular cells.

Study	Cell type	Concentration	Results	Conclusions
<i>Lee et al. (2011)</i>	Rat BMVECs	10,000 ng/mL	LCN-2 increased expression of chemokine CXCL10	LCN-2 may promote chemoattractants and neuroinflammation
<i>Wang et al. (2014)</i>	Human PASMCs	10 ng/mL–100 ng/mL	Decreased serum deprivation induced apoptosis and H <sub>2</sub> O <sub>2</sub> induced apoptosis with LCN-2. LCN-2 decreased the cleavage and activity of caspase-3, and expression of Bax (apoptotic protein). LCN-2 increased expression of SOD1/2 and decreased intracellular ROS.	LCN-2 appears protective against apoptosis and decreased ROS
<i>Wu et al. (2015)</i>	Rat BECs	500–2,000 ng/mL	Enhanced Matrigel tube formation with LCN-2 and migration of cells via iron and ROS related pathways	LCN-2 may contribute to neurovascular recovery
<i>Wang et al. (2015)</i>	Human PASMCs	3–30 ng/mL	LCN-2 increased proliferation, at least in part via PI3-K signalling pathway	LCN-2 increased HPASMC proliferation
<i>Eilenberg et al. (2016)</i>	HUVECs, Human CASMCs	200 ng/mL, 500 ng/mL or 1,000 ng/mL	Increased secretion of inflammatory markers IL-8, IL-6 and MCP-1 dose-dependently with LCN-2	LCN-2 may be pro-inflammatory
<i>Wang et al. (2017)</i>	Human PASMCs	10 ng/mL	LCN-2 increased ER stress and proliferation via increased intracellular iron levels	LCN-2 increased HPASMC proliferation and ER stress

**Notes.**

Abbreviations: LCN-2, lipocalin-2; BMVECs, brain microvascular endothelial cells; BECs, brain endothelial cells; HUVECs, human umbilical vein endothelial cells; CASMCs, coronary artery smooth muscle cells; PASMCs, pulmonary artery smooth muscle cells; ROS, reactive oxygen species; IL-8, interleukin-8; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; SOD1/2, superoxide dismutase 1/2; ER, endoplasmic reticulum.

hrs, which was abrogated by an Akt inhibitor (*Wang et al., 2015*). This inhibitor also partly prevented the LCN-2 induced increase in proliferation. Interestingly, this group previously showed in the same cell type that 10 ng/mL LCN-2 decreased serum deprivation induced apoptosis after 24 hrs compared to control, and furthermore decreased the susceptibility of HPASMCs to H<sub>2</sub>O<sub>2</sub> induced apoptosis (*Wang et al., 2014*). They further showed that LCN-2 was decreasing the cleavage and activity of caspase-3, and decreasing expression of Bax, an important pro-apoptotic factor. Finally, they confirmed LCN-2 (3–100 ng/mL for 24 hrs) increased the expression of superoxide dismutase 1 and 2 (SOD1 and SOD2) dose-dependently and 10 ng/mL decreased intracellular reactive oxygen species (ROS) both with and without H<sub>2</sub>O<sub>2</sub>. This group went on to show that LCN-2 (10 ng/mL) in fact promoted endoplasmic reticulum (ER) stress and proliferation within HPASMCs via increased intracellular iron levels (*Wang et al., 2017*).

HUVECs and human coronary artery smooth muscle cells (HCASMCs) treated with LCN-2 (200 ng/mL, 500 ng/mL or 1 μg/mL) significantly increased secretion of inflammatory markers interleukin-8 (IL-8), IL-6 and monocyte chemoattractant protein-1 (MCP-1) in a dose-dependent manner (*Eilenberg et al., 2016*). Within the central nervous

system, LCN-2 (10  $\mu\text{g}/\text{mL}$ ) was shown to increase mRNA expression of C-X-C motif chemokine 10 in mice brain microvascular endothelial cells (BMVECs), providing preliminary evidence that LCN-2 may act as a chemoattractant inducer and promote neuroinflammation ([Lee et al., 2011](#)). In another study in rat brain endothelial cells, LCN-2 (0.5–2  $\mu\text{g}/\text{mL}$ ) enhanced angiogenesis, shown through Matrigel tube formation and scratch migration, via iron and ROS related pathways ([Wu et al., 2015](#)).

Despite the clinical associations between LCN-2 and vascular disturbances, the mechanisms at a cellular level by physiological concentrations of exogenous LCN-2 have been largely disregarded and research is warranted. The investigations to date have largely focused on LCN-2 in the context of pulmonary hypertension and the use of HPASMCs. Within this cell type LCN-2 is shown to have anti-apoptotic and pro-proliferative effects, while also decreasing ROS but promoting ER stress. It has also been demonstrated that LCN-2 may be pro-inflammatory in a number of cells types, which may be viewed as beneficial in the acute inflammatory response ([Castellheim et al., 2009](#)). Concentrations of LCN-2 used in the studies reviewed have been quite varied (3  $\text{ng}/\text{mL}$ –10  $\mu\text{g}/\text{mL}$ ). It is clear there is a large scope of research yet to be completed in this area to inform researchers and clinicians about any potential contribution of LCN-2 in vascular physiological and pathophysiology.

## SUMMARY AND CONCLUSIONS

The effects of bone-derived factors on systemic health is an emerging, interesting branch of endocrinology research. As conflicting epidemiological reports have linked these bone-derived factors with multiple vascular pathologies such as atherosclerosis and calcification, there is interest in their therapeutic target potential. In order to derive any causal effects, it is important to review the basic *in vitro* science that has been carried out to date. Thus, this review aimed to collate studies investigating the *in vitro* effects of OCN, FGF23 and LCN-2 to clarify whether there is support for a basic biological or pathological role within the vasculature. Overall, the evidence base points to direct vasoactive properties of the osteokines OCN, FGF23 and LCN-2 across human and animal cells. Both OCN and FGF23 have been demonstrated to be anti-apoptotic and increase eNOS phosphorylation and NO production through Akt signalling. Taken together, these findings suggest a role for these osteokines in supporting normal vascular functionality. OCN has been implicated in improving insulin signalling, and ensuing protectiveness against ER stress. OCN may be involved in calcification but further research is warranted, while there is no evidence at least of a pro-calcific effect of FGF23 *in vitro*. FGF23 and LCN-2 have been shown to increase proliferation in some cell types and increase and decrease ROS respectively. LCN-2 has been shown to have anti-apoptotic effects and may increase ER stress as well as have pro-inflammatory properties. LCN-2 has also been demonstrated to promote angiogenesis. There is no strong evidence to suggest a pathological role of OCN or FGF23 in the vasculature, however further research with LCN-2 needs to be completed in order to better understand its role. Despite the topical area, a number of preliminary questions remain to be answered. For example, the role of OCN and FGF-23 in inflammation, the

role of OCN and LCN-2 in calcification, as well as their cellular receptors and related downstream signalling pathways in vascular cells. Various cleaved forms of the circulating proteins should also be investigated to assess their biological activity. It is clear that further, human data should be generated to understand the roles and mechanisms of actions of these osteokines in physiological and pathophysiological states.

## ADDITIONAL INFORMATION AND DECLARATIONS

### Funding

This work was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/M008770/1]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Grant Disclosures

The following grant information was disclosed by the authors:  
Biotechnology and Biological Sciences Research Council: BB/M008770/1.

### Competing Interests

The authors declare there are no competing interests.

### Author Contributions

- Sophie A. Millar conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Susan I. Anderson and Saoirse E. O’Sullivan authored or reviewed drafts of the paper, approved the final draft.

### Data Availability

The following information was supplied regarding data availability:  
This was a literature review.

## REFERENCES

- Alvelos M, Pimentel R, Pinho E, Gomes A, Lourenco P, Teles MJ, Almeida P, Guimaraes JT, Bettencourt I. 2011.** Neutrophil gelatinase-associated lipocalin in the diagnosis of type 1 cardio-renal syndrome in the general ward. *Clinical Journal of the American Society of Nephrology* **6**:476–481 DOI [10.2215/CJN.06140710](https://doi.org/10.2215/CJN.06140710).
- Amersfoort J, Schaftenaar FH, Douna H, Van Santbrink PJ, Kroner MJ, Van Puijvelde GHM, Quax PHA, Kuiper J, Bot I. 2018.** Lipocalin-2 contributes to experimental atherosclerosis in a stage-dependent manner. *Atherosclerosis* **275**:214–224 DOI [10.1016/j.atherosclerosis.2018.06.015](https://doi.org/10.1016/j.atherosclerosis.2018.06.015).
- Andrukhova O, Slavic S, Smorodchenko A, Zeitz U, Shalhoub V, Lanske B, Pohl EE, Erben RG. 2014a.** FGF23 regulates renal sodium handling and blood pressure. *EMBO Molecular Medicine* **6**:744–759 DOI [10.1002/emmm.201303716](https://doi.org/10.1002/emmm.201303716).

- Andrukhova O, Smorodchenko A, Egerbacher M, Streicher C, Zeitz U, Goetz R, Shalhoub V, Mohammadi M, Pohl EE, Lanske B, Erben RG. 2014b.** Fgf23 promotes renal calcium reabsorption through the Trpv5 channel. *EMBO Journal* **33**:229–246 DOI [10.1002/emboj.201284188](https://doi.org/10.1002/emboj.201284188).
- Bellows CG, Reimers SM, Heersche JN. 1999.** Expression of mRNAs for type-I collagen, bone sialoprotein, osteocalcin, and osteopontin at different stages of osteoblastic differentiation and their regulation by 1, 25 dihydroxyvitamin D3. *Cell and Tissue Research* **297**:249–259 DOI [10.1007/s004410051353](https://doi.org/10.1007/s004410051353).
- Binkley NC, Krueger DC, Engelke JA, Foley AL, Suttie JW. 2000.** Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. *American Journal of Clinical Nutrition* **72**:1523–1528 DOI [10.1093/ajcn/72.6.1523](https://doi.org/10.1093/ajcn/72.6.1523).
- Bonewald LF, Wacker MJ. 2013.** FGF23 production by osteocytes. *Pediatric Nephrology* **28**:563–568 DOI [10.1007/s00467-012-2309-3](https://doi.org/10.1007/s00467-012-2309-3).
- Bonnet N. 2017.** Bone-derived factors: a new gateway to regulate glycemia. *Calcified Tissue International* **100**:174–183 DOI [10.1007/s00223-016-0210-y](https://doi.org/10.1007/s00223-016-0210-y).
- Brandi ML, Collin-Osdoby P. 2006.** Vascular biology and the skeleton. *Journal of Bone and Mineral Research* **21**:183–192 DOI [10.1359/JBMR.050917](https://doi.org/10.1359/JBMR.050917).
- Cairns JR, Price PA. 1994.** Direct demonstration that the vitamin K-dependent bone Gla protein is incompletely gamma-carboxylated in humans. *Journal of Bone and Mineral Research* **9**:1989–1997.
- Castellheim A, Brekke OL, Espevik T, Harboe M, Mollnes TE. 2009.** Innate immune responses to danger signals in systemic inflammatory response syndrome and sepsis. *Scandinavian Journal of Immunology* **69**:479–491 DOI [10.1111/j.1365-3083.2009.02255.x](https://doi.org/10.1111/j.1365-3083.2009.02255.x).
- Celeste AJ, Rosen V, Buecker JL, Kriz R, Wang EA, Wozney JM. 1986.** Isolation of the human gene for bone gla protein utilizing mouse and rat cDNA clones. *EMBO Journal* **5**:1885–1890 DOI [10.1002/j.1460-2075.1986.tb04440.x](https://doi.org/10.1002/j.1460-2075.1986.tb04440.x).
- Chakraborty S, Kaur S, Guha S, Batra SK. 2012.** The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. *Biochimica et Biophysica Acta/General Subjects* **1826**:129–169.
- Chen L, Li Q, Yang Z, Ye Z, Huang Y, He M, Wen J, Wang X, Lu B, Hu J, Liu C, Ling C, Qu S, Hu R. 2013.** Osteocalcin, glucose metabolism, lipid profile and chronic low-grade inflammation in middle-aged and elderly Chinese. *Diabetic Medicine* **30**:309–317 DOI [10.1111/j.1464-5491.2012.03769.x](https://doi.org/10.1111/j.1464-5491.2012.03769.x).
- Chen G, Liu Y, Goetz R, Fu L, Jayaraman S, Hu MC, Moe OW, Liang G, Li X, Mohammadi M. 2018.** alpha-Klotho is a non-enzymatic molecular scaffold for FGF23 hormone signalling. *Nature* **553**:461–466 DOI [10.1038/nature25451](https://doi.org/10.1038/nature25451).
- Chung CP, Chang YC, Ding Y, Lim K, Liu Q, Zhu L, Zhang W, Lu TS, Molostvov G, Zehnder D, Hsiao LL. 2017.** alpha-Klotho expression determines nitric oxide synthesis in response to FGF-23 in human aortic endothelial cells. *PLOS ONE* **12**:e0176817 DOI [10.1371/journal.pone.0176817](https://doi.org/10.1371/journal.pone.0176817).

- Dalal M, Sun K, Cappola AR, Ferrucci L, Crasto C, Fried LP, Semba RD. 2011.** Relationship of serum fibroblast growth factor 23 with cardiovascular disease in older community-dwelling women. *European Journal of Endocrinology* **165**:797–803 DOI [10.1530/EJE-11-0577](https://doi.org/10.1530/EJE-11-0577).
- David V, Francis C, Babitt JL. 2017.** Ironing out the cross talk between Fgf23 and inflammation. *American Journal of Physiology-Renal Physiology* **312**:F1–F8 DOI [10.1152/ajprenal.00359.2016](https://doi.org/10.1152/ajprenal.00359.2016).
- Desjardins L, Liabeuf S, Renard C, Lenglet A, Lemke HD, Choukroun G, Druke TB, Massy ZA. 2012.** Fgf23 is independently associated with vascular calcification but not bone mineral density in patients at various Ckd stages. *Osteoporosis International* **23**:2017–2025 DOI [10.1007/s00198-011-1838-0](https://doi.org/10.1007/s00198-011-1838-0).
- Devireddy LR, Teodoro JG, Richard FA, Green MR. 2001.** Induction of apoptosis by a secreted lipocalin that is transcriptionally regulated by Il-3 deprivation. *Science* **293**:829–834 DOI [10.1126/science.1061075](https://doi.org/10.1126/science.1061075).
- Dou J, Li H, Ma X, Zhang M, Fang Q, Nie M, Bao Y, Jia W. 2014.** Osteocalcin attenuates high fat diet-induced impairment of endothelium-dependent relaxation through Akt/eNOS-dependent pathway. *Cardiovascular Diabetology* **54**(6):1291–7 DOI [10.1186/1475-2840-13-74](https://doi.org/10.1186/1475-2840-13-74).
- Ducy P. 2011.** The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. *Diabetologia* **54**:1291–1297 DOI [10.1007/s00125-011-2155-z](https://doi.org/10.1007/s00125-011-2155-z).
- Eilenberg W, Stojkovic S, Piechota-Polanczyk A, Kaun C, Rauscher S, Groger M, Klinger M, Wojta J, Neumayer C, Huk I, Demyanets S. 2016.** Neutrophil gelatinase-associated lipocalin (NGAL) is associated with symptomatic carotid atherosclerosis and drives pro-inflammatory state in vitro. *European Journal of Vascular and Endovascular Surgery* **51**:623–631 DOI [10.1016/j.ejvs.2016.01.009](https://doi.org/10.1016/j.ejvs.2016.01.009).
- Erben RG. 2017.** Pleiotropic actions of FGF23. *Toxicologic Pathology* **45**:904–910 DOI [10.1177/0192623317737469](https://doi.org/10.1177/0192623317737469).
- Ferron M, Wei J, Yoshizawa T, Ducy P, Karsenty G. 2010.** An ELISA-based method to quantify osteocalcin carboxylation in mice. *Biochemical and Biophysical Research Communications* **397**:691–696 DOI [10.1016/j.bbrc.2010.06.008](https://doi.org/10.1016/j.bbrc.2010.06.008).
- Fischer DC, Mischek A, Wolf S, Rahn A, Salweski B, Kundt G, Haffner D. 2012.** Paediatric reference values for the C-terminal fragment of fibroblast-growth factor-23, sclerostin, bone-specific alkaline phosphatase and isoform 5b of tartrate-resistant acid phosphatase. *Annals of Clinical Biochemistry* **49**:546–553 DOI [10.1258/acb.2012.011274](https://doi.org/10.1258/acb.2012.011274).
- Fliser D, Kollerits B, Neyer U, Ankerst DP, Lhotta K, Lingenhel A, Ritz E, Kronenberg F, Kuen E, Konig P, Kraatz G, Mann JF, Muller GA, Kohler H, Riegler P. 2007.** Fibroblast growth factor 23 (Fgf23) predicts progression of chronic kidney disease: the Mild to Moderate Kidney Disease (Mmkd) Study. *Journal of the American Society of Nephrology* **18**:2600–2608 DOI [10.1681/ASN.2006080936](https://doi.org/10.1681/ASN.2006080936).

- Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, Akira S, Aderem A. 2004. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestering iron. *Nature* 432:917–921 DOI 10.1038/nature03104.
- Garnero P, Grimaux M, Seguin P, Delmas PD. 1994. Characterization of immunoreactive forms of human osteocalcin generated in vivo and in vitro. *Journal of Bone and Mineral Research* 9:255–264.
- Guo Q, Li H, Xu L, Wu S, Sun H, Zhou B. 2017. Undercarboxylated osteocalcin reverts insulin resistance induced by endoplasmic reticulum stress in human umbilical vein endothelial cells. *Scientific Reports* 7:46.
- Gutierrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, Smith K, Lee H, Thadhani R, Juppner H, Wolf M. 2008. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *New England Journal of Medicine* 359:584–592 DOI 10.1056/NEJMoa0706130.
- Han Y, You X, Xing W, Zhang Z, Zou W. 2018. Paracrine and endocrine actions of bone—the functions of secretory proteins from osteoblasts, osteocytes, and osteoclasts. *Bone Research* 6:Article 16 DOI 10.1038/s41413-018-0019-6.
- Hannemann A, Friedrich N, Spielhagen C, Rettig R, Ittermann T, Nauck M, Wallaschofski H. 2013. Reference intervals for serum osteocalcin concentrations in adult men and women from the study of health in Pomerania. *BMC Endocrine Disorders* 13:11 DOI 10.1186/1472-6823-13-11.
- Hauschka PV, Lian JB, Cole DEC, Gundberg CM. 1989. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiological Reviews* 69:990–1047 DOI 10.1152/physrev.1989.69.3.990.
- Hauschka PV, Lian JB, Gallop PM. 1975. Direct identification of the calcium-binding amino acid, gamma-carboxylglutamate, in mineralized tissue. *Proceedings of the National Academy of Sciences of the United States of America* 72:3925–3929 DOI 10.1073/pnas.72.10.3925.
- Hsu HJ, Wu MS. 2009. Fibroblast growth factor 23: a possible cause of left ventricular hypertrophy in hemodialysis patients. *American Journal of the Medical Sciences* 337:116–122 DOI 10.1097/MAJ.0b013e3181815498.
- Idelevich A, Rais Y, Monsonego-Ornan E. 2011. Bone Gla protein increases Hif-1alpha-dependent glucose metabolism and induces cartilage and vascular calcification. *Arteriosclerosis, Thrombosis, and Vascular Biology* 31:e55–e71.
- Iki M, Tamaki J, Fujita Y, Kouda K, Yura A, Kadowaki E, Sato Y, Moon JS, Tomioka K, Okamoto N, Kurumatani N. 2012. Serum undercarboxylated osteocalcin levels are inversely associated with glycemic status and insulin resistance in an elderly Japanese male population: Fujiwara-kyo Osteoporosis Risk in Men (Formen) Study. *Osteoporosis International* 23:761–770 DOI 10.1007/s00198-011-1600-7.
- Isakova T, Xie H, Yang W, Xie D, Anderson AH, Scialla J, Wahl P, Gutierrez OM, Steigerwalt S, He J, Schwartz S, Lo J, Ojo A, Sondheim J, Hsu CY, Lash J, Leonard M, Kusek JW, Feldman HI, Wolf M. 2011. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *Jama* 305:2432–2439 DOI 10.1001/jama.2011.826.

- Jung CH, Lee WJ, Hwang JY, Lee MJ, Seol SM, Kim YM, Lee YL, Park JY. 2013.** The preventive effect of uncarboxylated osteocalcin against free fatty acid-induced endothelial apoptosis through the activation of phosphatidylinositol 3-kinase/Akt signaling pathway. *Metabolism: Clinical and Experimental* **62**:1250–1257 DOI [10.1016/j.metabol.2013.03.005](https://doi.org/10.1016/j.metabol.2013.03.005).
- Karsenty G. 2017.** Update on the biology of osteocalcin. *Endocrine Practice* **23**:1270–1274 DOI [10.4158/EP171966.RA](https://doi.org/10.4158/EP171966.RA).
- Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N. 1993.** Isolation and primary structure of Ngal, a novel protein associated with human neutrophil gelatinase. *Journal of Biological Chemistry* **268**:10425–10432.
- Knapen MH, Hamulyak K, Vermeer C. 1989.** The effect of vitamin K supplementation on circulating osteocalcin (bone Gla protein) and urinary calcium excretion. *Annals of Internal Medicine* **111**:1001–1005 DOI [10.7326/0003-4819-111-12-1001](https://doi.org/10.7326/0003-4819-111-12-1001).
- Knapen MH, Jie KS, Hamulyak K, Vermeer C. 1993.** Vitamin K-induced changes in markers for osteoblast activity and urinary calcium loss. *Calcified Tissue International* **53**:81–85 DOI [10.1007/BF01321883](https://doi.org/10.1007/BF01321883).
- Kondo A, Kawakubo-Yasukochi T, Mizokami A, Chishaki S, Takeuchi H, Hirata M. 2016.** Uncarboxylated osteocalcin increases serum nitric oxide levels and ameliorates hypercholesterolemia in mice fed an atherogenic diet. *Electronic Journal of Biology* **13**.
- Larsson T, Nisbeth U, Ljunggren O, Juppner H, Jonsson KB. 2003.** Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. *Kidney International* **64**:2272–2279 DOI [10.1046/j.1523-1755.2003.00328.x](https://doi.org/10.1046/j.1523-1755.2003.00328.x).
- Lee AJ, Hodges S, Eastell R. 2000.** Measurement of osteocalcin. *Annals of Clinical Biochemistry* **37**(Pt 4):432–446 DOI [10.1177/000456320003700402](https://doi.org/10.1177/000456320003700402).
- Lee S, Kim JH, Seo JW, Han HS, Lee WH, Mori K, Nakao K, Barasch J, Suk K. 2011.** Lipocalin-2 is a chemokine inducer in the central nervous system: role of chemokine ligand 10 (CXCL10) in lipocalin-2-induced cell migration. *Journal of Biological Chemistry* **286**:43855–43870 DOI [10.1074/jbc.M111.299248](https://doi.org/10.1074/jbc.M111.299248).
- Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, Mckee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. 2007.** Endocrine regulation of energy metabolism by the skeleton. *Cell* **130**:456–469 DOI [10.1016/j.cell.2007.05.047](https://doi.org/10.1016/j.cell.2007.05.047).
- Li J, Zhang H, Yang C, Li Y, Dai Z. 2016.** An overview of osteocalcin progress. *Journal of Bone and Mineral Metabolism* **34**:367–379 DOI [10.1007/s00774-015-0734-7](https://doi.org/10.1007/s00774-015-0734-7).
- Liao M, Huang L, Mao Y, Jiang Y, Yao Z, Lin X, Lu Z, Wu C, Qin X, Zhang H, Mo Z. 2015.** Serum osteocalcin is associated with inflammatory factors in metabolic syndrome: a population-based study in chinese males. *Mediators of Inflammation* **2015**:Article 683739.
- Lim K, Lu TS, Molostvov G, Lee C, Lam FT, Zehnder D, Hsiao LL. 2012.** Vascular Klotho deficiency potentiates the development of human artery calcification and

- mediates resistance to fibroblast growth factor 23. *Circulation* **125**:2243–2255 DOI [10.1161/CIRCULATIONAHA.111.053405](https://doi.org/10.1161/CIRCULATIONAHA.111.053405).
- Lindberg K, Olauson H, Amin R, Ponnusamy A, Goetz R, Taylor RF, Mohammadi M, Canfield A, Kublickiene K, Larsson TE. 2013.** Arterial klotho expression and FGF23 effects on vascular calcification and function. *PLOS ONE* **8**:e60658 DOI [10.1371/journal.pone.0060658](https://doi.org/10.1371/journal.pone.0060658).
- Lucey AJ, Paschos GK, Thorsdottir I, Martinez JA, Cashman KD, Kiely M. 2013.** Young overweight and obese women with lower circulating osteocalcin concentrations exhibit higher insulin resistance and concentrations of C-reactive protein. *Nutrition Research* **33**:67–75 DOI [10.1016/j.nutres.2012.11.011](https://doi.org/10.1016/j.nutres.2012.11.011).
- Luo Y, Ma X, Pan X, Xu Y, Xiong Q, Xiao Y, Bao Y, Jia W. 2016.** Serum lipocalin-2 levels are positively associated with not only total body fat but also visceral fat area in Chinese men. *Medicine (Baltimore)* **95**:e4039 DOI [10.1097/MD.0000000000004039](https://doi.org/10.1097/MD.0000000000004039).
- Luukinen H, Kakonen SM, Pettersson K, Koski K, Laippala P, Lovgren T, Kivela SL, Vaananen HK. 2000.** Strong prediction of fractures among older adults by the ratio of carboxylated to total serum osteocalcin. *Journal of Bone and Mineral Research* **15**:2473–2478 DOI [10.1359/jbmr.2000.15.12.2473](https://doi.org/10.1359/jbmr.2000.15.12.2473).
- Marthi A, Donovan K, Haynes R, Wheeler DC, Baigent C, Rooney CM, Landray MJ, Moe SM, Yang J, Holland L, Di Giuseppe R, Bouma-De Krijger A, Mihaylova B, Herrington WG. 2018.** Fibroblast growth factor-23 and risks of cardiovascular and noncardiovascular diseases: a meta-analysis. *Journal of the American Society of Nephrology* **29**:2015–2027 DOI [10.1681/ASN.2017121334](https://doi.org/10.1681/ASN.2017121334).
- Millar SA, Patel H, Anderson SI, England TJ, O’Sullivan SE. 2017.** Osteocalcin, vascular calcification, and atherosclerosis: a systematic review and meta-analysis. *Frontiers in Endocrinology* **8**:Article 183 DOI [10.3389/fendo.2017.00183](https://doi.org/10.3389/fendo.2017.00183).
- Mirza MA, Larsson A, Lind L, Larsson TE. 2009a.** Circulating fibroblast growth factor-23 is associated with vascular dysfunction in the community. *Atherosclerosis* **205**:385–390 DOI [10.1016/j.atherosclerosis.2009.01.001](https://doi.org/10.1016/j.atherosclerosis.2009.01.001).
- Mirza MA, Larsson A, Melhus H, Lind L, Larsson TE. 2009b.** Serum intact FGF23 associate with left ventricular mass, hypertrophy and geometry in an elderly population. *Atherosclerosis* **207**:546–551 DOI [10.1016/j.atherosclerosis.2009.05.013](https://doi.org/10.1016/j.atherosclerosis.2009.05.013).
- Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, Ruff SM, Zahedi K, Shao M, Bean J, Mori K, Barasch J, Devarajan P. 2005.** Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet* **365**:1231–1238 DOI [10.1016/S0140-6736\(05\)74811-X](https://doi.org/10.1016/S0140-6736(05)74811-X).
- Mishra J, Mori K, Ma Q, Kelly C, Barasch J, Devarajan P. 2004.** Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. *American Journal of Nephrology* **24**:307–315 DOI [10.1159/000078452](https://doi.org/10.1159/000078452).
- Mosialou I, Shikhel S, Liu JM, Maurizi A, Luo N, He Z, Huang Y, Zong H, Friedman RA, Barasch J, Lanzano P, Deng L, Leibel RL, Rubin M, Nickolas T, Chung W, Zeltser LM, Williams KW, Pessin JE, Kousteni S. 2017.** MC4R-dependent suppression of appetite by bone-derived lipocalin 2. *Nature* **543**:385–390 DOI [10.1038/nature21697](https://doi.org/10.1038/nature21697).

- Murali SK, Roschger P, Zeitz U, Klaushofer K, Andrukhova O, Erben RG. 2016.** Fgf23 regulates bone mineralization in a 1, 25(OH)<sub>2</sub>D<sub>3</sub> and klotho-independent manner. *Journal of Bone and Mineral Research* **31**:129–142 DOI [10.1002/jbmr.2606](https://doi.org/10.1002/jbmr.2606).
- Nakahara T, Kawai-Kowase K, Matsui H, Sunaga H, Utsugi T, Iso T, Arai M, Tomono S, Kurabayashi M. 2016.** Fibroblast growth factor 23 inhibits osteoblastic gene expression and induces osteoprotegerin in vascular smooth muscle cells. *Atherosclerosis* **253**:102–110 DOI [10.1016/j.atherosclerosis.2016.08.010](https://doi.org/10.1016/j.atherosclerosis.2016.08.010).
- Oury F, Khrimian L, Denny CA, Gardin A, Chamouni A, Goeden N, Huang YY, Lee H, Srinivas P, Gao XB, Suyama S, Langer T, Mann JJ, Horvath TL, Bonnin A, Karsenty G. 2013.** Maternal and offspring pools of osteocalcin influence brain development and functions. *Cell* **155**:228–241 DOI [10.1016/j.cell.2013.08.042](https://doi.org/10.1016/j.cell.2013.08.042).
- Oury F, Sumara G, Sumara O, Ferron M, Chang H, Smith CE, Hermo L, Suarez S, Roth BL, Ducy P, Karsenty G. 2011.** Endocrine regulation of male fertility by the skeleton. *Cell* **144**:796–809 DOI [10.1016/j.cell.2011.02.004](https://doi.org/10.1016/j.cell.2011.02.004).
- Pittas AG, Harris SS, Eliades M, Stark P, Dawson-Hughes B. 2009.** Association between serum osteocalcin and markers of metabolic phenotype. *Journal of Clinical Endocrinology and Metabolism* **94**:827–832 DOI [10.1210/jc.2008-1422](https://doi.org/10.1210/jc.2008-1422).
- Plantalech L, Guillaumont M, Vergnaud P, Leclercq M, Delmas PD. 1991.** Impairment of gamma carboxylation of circulating osteocalcin (bone gla protein) in elderly women. *Journal of Bone and Mineral Research* **6**:1211–1216.
- Price PA, Otsuka AA, Poser JW, Kristaponis J, Raman N. 1976.** Characterization of a gamma-carboxyglutamic acid-containing protein from bone. *Proceedings of the National Academy of Sciences of the United States of America* **73**:1447–1451 DOI [10.1073/pnas.73.5.1447](https://doi.org/10.1073/pnas.73.5.1447).
- Richter B, Faul C. 2018.** FGF23 Actions on Target Tissues—With and Without Klotho. *Frontiers in Endocrinology* **9**:Article 189.
- Richter B, Haller J, Haffner D, Leifheit-Nestler M. 2016.** Klotho modulates Fgf23-mediated NO synthesis and oxidative stress in human coronary artery endothelial cells. *Pflugers Archiv. European Journal of Physiology* **468**:1621–1635 DOI [10.1007/s00424-016-1858-x](https://doi.org/10.1007/s00424-016-1858-x).
- Roos M, Lutz J, Salmhofer H, Luppia P, Knauss A, Braun S, Martinof S, Schomig A, Heemann U, Kastrati A, Hausleiter J. 2008.** Relation between plasma fibroblast growth factor-23, serum fetuin-A levels and coronary artery calcification evaluated by multislice computed tomography in patients with normal kidney function. *The Clinical Endocrinology* **68**:660–665 DOI [10.1111/j.1365-2265.2007.03074.x](https://doi.org/10.1111/j.1365-2265.2007.03074.x).
- Sarkar PD, Choudhury AB. 2013.** Relationships between serum osteocalcin levels versus blood glucose, insulin resistance and markers of systemic inflammation in central Indian type 2 diabetic patients. *European Review for Medical and Pharmacological Sciences* **17**:1631–1635.
- Sarmiento-Dias M, Santos-Araujo C, Poinhos R, Oliveira B, Silva IS, Silva LS, Sousa MJ, Correia F, Pestana M. 2016.** Fibroblast growth factor 23 is associated with left ventricular hypertrophy, not with uremic vasculopathy in peritoneal dialysis patients. *Clinical Nephrology* **85**:135–141.

- Scialla JJ, Lau WL, Reilly MP, Isakova T, Yang HY, Crouthamel MH, Chavkin NW, Rahman M, Wahl P, Amaral AP, Hamano T, Master SR, Nessel L, Chai B, Xie D, Kallem RR, Chen J, Lash JP, Kusek JW, Budoff MJ, Giachelli CM, Wolf M. 2013. Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. *Kidney International* **83**:1159–1168 DOI [10.1038/ki.2013.3](https://doi.org/10.1038/ki.2013.3).
- Seiler S, Rogacev KS, Roth HJ, Shafein P, Emrich I, Neuhaus S, Floege J, Fliser D, Heine GH. 2014. Associations of Fgf-23 and sKlotho with cardiovascular outcomes among patients with CKD stages 2–4. *Clinical Journal of American Society of Nephrology* **9**:1049–1058 DOI [10.2215/CJN.07870713](https://doi.org/10.2215/CJN.07870713).
- Silswal N, Touchberry CD, Daniel DR, McCarthy DL, Zhang S, Andresen J, Stubbs JR, Wacker MJ. 2014. FGF23 directly impairs endothelium-dependent vasorelaxation by increasing superoxide levels and reducing nitric oxide bioavailability. *American Journal of Physiology, Endocrinology and Metabolism* **307**(5):E426–E436.
- Six I, Okazaki H, Gross P, Cagnard J, Boudot C, Maizel J, Druke TB, Massy ZA. 2014. Direct, acute effects of Klotho and Fgf23 on vascular smooth muscle and endothelium. *PLOS ONE* **9**:e93423 DOI [10.1371/journal.pone.0093423](https://doi.org/10.1371/journal.pone.0093423).
- Smith ER. 2014. The use of fibroblast growth factor 23 testing in patients with kidney disease. *Clinical Journal of American Society of Nephrology* **9**:1283–1303 DOI [10.2215/CJN.10941013](https://doi.org/10.2215/CJN.10941013).
- Smith ER, Cai MM, McMahon LP, Holt SG. 2012. Biological variability of plasma intact and C-terminal Fgf23 measurements. *Journal of Clinical Endocrinology and Metabolism* **97**:3357–3365 DOI [10.1210/jc.2012-1811](https://doi.org/10.1210/jc.2012-1811).
- Stohr R, Schuh A, Heine GH, Brandenburg V. 2018. Fgf23 in cardiovascular disease: innocent bystander or active mediator? *Frontiers in Endocrinology* **9**:Article 351 DOI [10.3389/fendo.2018.00351](https://doi.org/10.3389/fendo.2018.00351).
- Sugiyama T, Kawai S. 2001. Carboxylation of osteocalcin may be related to bone quality: a possible mechanism of bone fracture prevention by vitamin K. *Journal of Bone and Mineral Metabolism* **19**:146–149 DOI [10.1007/s007740170034](https://doi.org/10.1007/s007740170034).
- Tacey A, Qaradakh T, Brennan-Speranza T, Hayes A, Zulli A, Levinger I. 2018. Potential role for osteocalcin in the development of atherosclerosis and blood vessel disease. *Nutrients* **10**:Article 1462.
- Tsukamoto Y, Ichise H, Kakuda H, Yamaguchi M. 2000. Intake of fermented soybean (natto) increases circulating vitamin K2 (menaquinone-7) and gamma-carboxylated osteocalcin concentration in normal individuals. *Journal of Bone and Mineral Metabolism* **18**:216–222 DOI [10.1007/s007740070023](https://doi.org/10.1007/s007740070023).
- Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. 2006. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* **444**:770–774 DOI [10.1038/nature05315](https://doi.org/10.1038/nature05315).
- Verkaik M, Juni RP, Van Loon EPM, Van Poelgeest E, Kwekkeboom RFJ, Gam Z, Richards WG, Ter Wee PM, Hoenderop JGJ, Eringa EC, Vervloet MG. 2018. FGF23 impairs peripheral microvascular function in renal failure. *The American Journal of Physiology-Heart and Circulatory Physiology* **315**(5):H1414–H1424.

- Wang G, Liu S, Wang L, Meng L, Cui C, Zhang H, Hu S, Ma N, Wei Y. 2017.** Lipocalin-2 promotes endoplasmic reticulum stress and proliferation by augmenting intracellular iron in human pulmonary arterial smooth muscle cells. *International Journal of Biological Sciences* **13**:135–144 DOI [10.7150/ijbs.17758](https://doi.org/10.7150/ijbs.17758).
- Wang G, Liu X, Meng L, Liu S, Wang L, Li J, Cui C, Meng J, Hu S, Wei Y. 2014.** Up-regulated lipocalin-2 in pulmonary hypertension involving in pulmonary artery SMC resistance to apoptosis. *International Journal of Biological Sciences* **10**:798–806 DOI [10.7150/ijbs.9470](https://doi.org/10.7150/ijbs.9470).
- Wang G, Ma N, Meng L, Wei Y, Gui J. 2015.** Activation of the phosphatidylinositol 3-kinase/Akt pathway is involved in lipocalin-2-promoted human pulmonary artery smooth muscle cell proliferation. *Molecular and Cellular Biochemistry* **410**:207–213 DOI [10.1007/s11010-015-2553-5](https://doi.org/10.1007/s11010-015-2553-5).
- Wang Y. 2012.** Small lipid-binding proteins in regulating endothelial and vascular functions: focusing on adipocyte fatty acid binding protein and lipocalin-2. *British Journal of Pharmacology* **165**:603–621 DOI [10.1111/j.1476-5381.2011.01528.x](https://doi.org/10.1111/j.1476-5381.2011.01528.x).
- Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J, Tso AW, Chow WS, Wat NM, Xu JY, Hoo RL, Xu A. 2007.** Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. *Clinical Chemistry* **53**:34–41.
- Wolf M, Koch TA, Bregman DB. 2013.** Effects of iron deficiency anemia and its treatment on fibroblast growth factor 23 and phosphate homeostasis in women. *Journal of Bone and Mineral Research* **28**:1793–1803 DOI [10.1002/jbmr.1923](https://doi.org/10.1002/jbmr.1923).
- Wu L, Du Y, Lok J, Lo EH, Xing C. 2015.** Lipocalin-2 enhances angiogenesis in rat brain endothelial cells via reactive oxygen species and iron-dependent mechanisms. *Journal of Neurochemistry* **132**:622–628 DOI [10.1111/jnc.13023](https://doi.org/10.1111/jnc.13023).
- Xiao Y, Xu A, Hui X, Zhou P, Li X, Zhong H, Tang W, Huang G, Zhou Z. 2013.** Circulating lipocalin-2 and retinol-binding protein 4 are associated with intima-media thickness and subclinical atherosclerosis in patients with type 2 diabetes. *PLOS ONE* **8**:e66607.
- Yan L, Borregaard N, Kjeldsen L, Moses MA. 2001.** The high molecular weight urinary matrix metalloproteinase (MMP) activity is a complex of gelatinase B/MMP-9 and neutrophil gelatinase-associated lipocalin (NGAL), modulation of MMP-9 activity by NGAL. *Journal of Biological Chemistry* **276**:37258–37265 DOI [10.1074/jbc.M106089200](https://doi.org/10.1074/jbc.M106089200).
- Zhou B, Li H, Liu J, Xu L, Zang W, Wu S, Sun H. 2013.** Intermittent injections of osteocalcin reverse autophagic dysfunction and endoplasmic reticulum stress resulting from diet-induced obesity in the vascular tissue via the NFkappaB-p65-dependent mechanism. *Cell Cycle* **12**:1901–1913 DOI [10.4161/cc.24929](https://doi.org/10.4161/cc.24929).
- Zhu D, Mackenzie NC, Millan JL, Farquharson C, Macrae VE. 2013.** A protective role for Fgf-23 in local defence against disrupted arterial wall integrity? *Molecular and Cellular Endocrinology* **372**:1–11 DOI [10.1016/j.mce.2013.03.008](https://doi.org/10.1016/j.mce.2013.03.008).