Mini Review

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Osteocalcin as a potential risk biomarker for cardiovascular and metabolic diseases

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Abstract: Clear evidence supports a role for circulating and locally-produced osteocalcin (OC) in the pathophysiology of cardiovascular (CV) lesions and CV risk, also in combination with metabolic changes, including type 2 diabetes mellitus (T2DM). Reduced plasma OC levels are associated with greater incidence of pathological CV changes, like arterial and valvular calcification, coronary and carotid atherosclerosis and increased carotid intima-media thickness. The actual relationship between OC levels and incidence of major CV events is, however, still unclear. Moreover, reduced circulating OC levels have been mostly associated with insulin resistance, metabolic syndrome or T2DM, indicating relevant OC actions on pancreatic β-cells and insulin secretion and activity. Based on these observations, this review article will attempt to summarize the current evidence on the potential usefulness of circulating OC as a biomarker for CV and metabolic risk, also evaluating the currently open issues in this area of research.

Keywords: atherosclerosis; cardiovascular risk; metabolic syndrome; osteocalcin; type 2 diabetes mellitus.

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Introduction

Primary cardiovascular (CV) and metabolic prevention is nowadays a very relevant issue worldwide and the availability of novel and reliable predictive biomarkers, to be added and integrated with those already validated, is urgently required in order to better identify individuals at greater primary and secondary CV and metabolic risk. Indeed, a number of clinical trials, aimed at evaluating drug effects on CV endpoints, suggested that a relevant residual CV risk remains, in spite, for example, of low density lipoprotein-cholesterol (LDL-C) reduction well below specific cut-off values [1]. Identification and validation of circulating biomarkers, potentially useful in primary and secondary CV and metabolic risk assessment is thus necessary. Recent observations have shown reciprocal functional interactions between organs, such as the adipose tissue and the endocrine pancreas, controlling intermediary metabolism, and bone [2–4]. More specifically, adipose-borne leptin and adiponectin have been found to regulate bone turnover in a complex way, whereas osteocalcin (OC), a bone protein mainly expressed by osteoblasts, can promote insulin sensitivity and insulin secretion by pancreatic β-cells as well as their proliferation (Figure 1). In addition, measurement of plasma OC concentration has also been proposed as a potential biomarker of cardiometabolic risk, along with its established clinical significance as a biomarker of bone turnover [5]. Reduced plasma OC levels were indeed found to be associated with greater incidence of pathological CV changes, like arterial and valvular calcification [6, 7], carotid atherosclerosis [8], and increased carotid intima-media thickness [9]. Moreover, reduced circulating OC levels have consistently been associated with the presence of insulin resistance, metabolic syndrome and type 2 diabetes mellitus (T2DM) [10, 11], clearly linked to atherosclerosis development [8].

Despite this body of data, the actual relationship between OC levels and incidence of major CV events is still unclear, since some studies found no association of OC with stroke and myocardial infarction, at least in

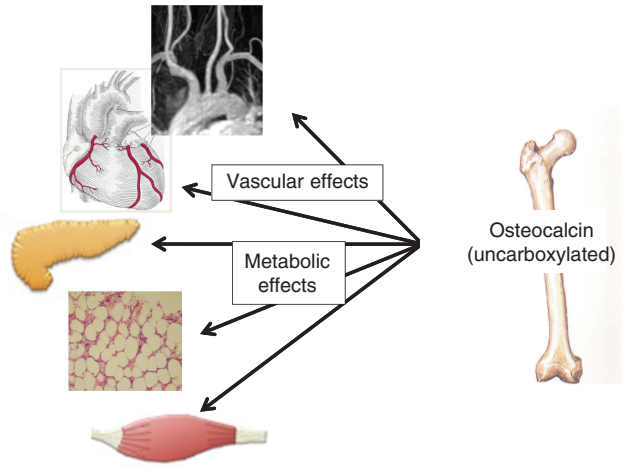

Figure 1: Osteocalcin is produced by bone osteoblasts and partly released into the bloodstream.
Circulating osteocalcin isoforms show a different extent of carboxylation and consequently different biological activities, including the regulation of vascular calcification and atherosclerosis and the modulation of insulin secretion by pancreatic β cells and insulin resistance in the adipose and muscle tissues.

specific ethnic cohorts [12], whereas others reported lower OC in younger subjects with premature myocardial infarction [13]. Based on these observations, the purpose of this review article is to summarize the current evidence on the potential usefulness of circulating OC as a biomarker for CV and metabolic risk. To this purpose, the PubMed and Embase databases were searched up to November 2015 for human studies addressing this topic.

Measurement of circulating osteocalcin concentrations

OC is the most important noncollagen protein in bone matrix, accounting for approximately 1% of the total protein in human bone. OC, the product of the bone γ-carboxyglutamate protein (*BGLAP*) gene, is a bone-specific protein of 46–50 aminoacid residues, that undergoes post-translational modifications by vitamin K-dependent γ-carboxylation of three glutamic acid residues [10]. Its production is dependent upon vitamin K and is stimulated by 1,25 dihydroxy vitamin D. Newly synthesized OC is partly incorporated into the bone matrix and partly released into the bloodstream. The extent of OC carboxylation results in circulating OC isoforms with different biological activities, ranging from bone mineralization, mainly by carboxylated OC (cOC) to insulin sensitization, mainly due to uncarboxylated OC (ucOC) [14]. The clinical use of plasma/serum OC concentration measurement is mainly useful in monitoring and assessing the effectiveness of antiresorptive therapy in patients treated for osteopenia, osteoporosis, Paget's disease, or other disorders in which OC levels are elevated. In addition, OC assay may be useful in the diagnosis of medical conditions associated with increased bone turnover, like bone cancer metastases, primary hyperparathyroidism and renal osteodystrophy. Immunochemical and chromatographic studies have demonstrated considerable heterogeneity for concentrations and molecular forms of circulating OC in normal individuals and in patients with osteoporosis, chronic renal failure, and Paget's disease. OC (1–49) and its fragments, including OC (1–43), are released into the blood stream. Serum OC (1–43) is also generated by the catabolic breakdown of OC (1–49) in the circulation, liver and kidney, as well as by degradation during storage of samples, because a labile six-amino acid C-terminal sequence, at room temperature, is cleaved off. Therefore, the measurement of the more stable N-terminal and mid-regional OC [N-MID OC, corresponding to OC (1–43/49)] is clinically more useful [15]. Different immunometric assays for the measurement of plasma/serum OC as well as ucOC have been developed. They include manually performed methods, such as immunometric approaches (ELISA, EIA), utilized in the past for clinical purposes. today mainly used in research studies and selectively able to detect the different OC molecular species, including ucOC. Nowadays, for clinical purposes, automated methods like 2-site immunometric (sandwich) assays using electrochemiluminescence (ECLIA) detection are preferred, since they are able to detect both the stable N-MID-fragment and intact OC and show a better precision and accuracy than manually performed assays [16]. OC reference values for adult subjects are around 8–42 ng/mL [17]. Elevated levels of OC generally indicate increased bone turnover. In patients taking antiresorptive agents, a decrease ≥20% from baseline OC level after 3–6 months of therapy suggests effective response to treatment [18]. Moreover, patients with diseases such as hyperparathyroidism, which can be cured, should have a reduction of OC to the reference range within 3–6 months after complete cure. In the case of assays making use of biotin-streptavidin interaction, to avoid assay interference from biotin in patients receiving therapy with high doses of this vitamin, it is appropriate to wait at least 8 h after the last biotin administration before collecting a blood specimen. Specific cautions in the interpretation of OC values include the following aspects. As OC is cleared by the kidneys, elevations may be observed in patients with impaired renal function without increased bone turnover. As OC is regulated by

1,25 dihydroxy vitamin D and vitamin K, serum OC may not reflect bone formation in patients treated with the former or antagonists of the latter.

Osteocalcin and cardiovascular risk

Different but related vascular lesions, ranging from vascular calcification to atherosclerotic plaques, are associated to the development of arterial diseases, leading to hypertension and ischemic organ damage, such as myocardial infarction and stroke. As a general statement, several, but not all, cross-sectional and longitudinal studies, mostly conducted in the Chinese and Korean populations, suggested the existence of a link between lower circulating OC levels and atherosclerosis development and CV disease (Tables 1 and 2). Among the different vascular pathological changes leading to CV disease, ectopic CV calcification appears related to a complex alteration of mineral metabolism at arterial and valvular sites, due to imbalance of circulating and local OC actions and CV mineralization, bearing similarities to osteogenesis in bone [44] and leading to reduced vessel elasticity and compliance. CV calcification has frequently been associated to genetic predisposition and aging [45] and, similarly to atherosclerosis, it may also be the consequence of low-grade chronic inflammation and endothelial dysfunction, promoting the differentiation of smooth muscle cells, from the arterial wall, to osteoblast-like cells [7]. Moreover, according to recent views, cells with an osteoblast-like phenotype may also derive from circulating stem cells [46]. These osteoblast-like subpopulations are able to form mineralized nodules and to express OC and other molecules able to promote calcification [7]. This latter process is thus the final consequence of an imbalance between pro-calcification agents and a series of inhibitors of arterial calcification, which include fetuin-A and adiponectin [45]. Relevant to this pathophysiological process, a recent cross-sectional study on 162 subjects reported that higher concentrations of serum ucOC and higher ucOC:OC ratio are associated to coronary artery calcification in men, independent of conventional CV risk factors [21]. Moreover, in a prospective cohort of 774 men followed for 10 years, higher baseline OC concentrations were associated with lower progression rate of abdominal aortic calcification and lower mortality [41], suggesting that OC level might be an independent indicator of CV risk. Moreover, in T2DM men, but not women, OC and ucOC levels were negatively associated with abdominal aortic calcification score [22].

Locally-produced OC may also play a relevant pathogenetic role also for atherosclerotic disease, although this cannot obviously be taken into consideration when measuring this marker in the peripheral circulation. Indeed, in addition to bone osteoblasts and the above-mentioned osteoblast-like cells in the vascular wall, other possible cellular sources of OC with potential CV impact appear to be also present in the circulation. Endothelial progenitor cells expressing OC are strongly associated with early and unstable coronary artery disease [47] and to severe calcific stenosis of the aortic valve [6]. In addition, platelets also express and release OC to a greater extent in patients with occlusive carotid artery disease, with a peculiar localization in atherosclerotic plaques, but not in normal tissue [48]. OC-expressing osteogenic monocytes in the coronary microcirculation are associated with specific features of patients with early coronary atherosclerosis and accumulate in the border region of plaques [49]. Interestingly, osteoclast-like cells have also been identified in atherosclerotic lesions, especially in association to extracellular matrix destruction, local mineral resorption and aneurysmal disease, thus suggesting a selective role in the pathogenesis of this latter vascular disease [7].

The relationship between lower circulating OC levels and atherosclerotic disease has been underlined by several clinical studies. A recent cross-sectional study conducted in 461 Chinese subjects showed an inverse relationship between OC levels, coronary artery disease and its severity, being OC lower in patients with diagnosed coronary heart disease (CHD), independently of metabolic markers [38]. The association of lower ucOC with coronary artery disease has also been observed in a case-control study conducted in Korean patients who underwent coronary artery bypass graft surgery [39].

Circulating OC levels also seem to be an independent risk factor for carotid atherosclerosis (Table 1). A negative relationship between OC concentrations and carotid intima-media thickness and presence of atherosclerotic plaques has been reported in two Chinese cohorts, one including 1319 postmenopausal women with subclinical atherosclerosis [9] and one with 1077 middle-age and elderly males, independent of altered glucose metabolism [19].

Cross-sectional studies showed that lower serum OC levels are associated with greater CV risk and CV disease incidence as well as to relevant glucometabolic changes or metabolic syndrome in a large middle-age and elderly Chinese population with self-reported CV disease [26] and in Chinese men [40]. Moreover, a recent case-control study in 302 subjects reported that in a large cohort of

Table 1: Clinical studies evaluating osteocalcin and cardiovascular and metabolic risk biomarkers.

Authors, year	Design	n	Biomarkers	Assay	Main outcome	References
Osteocalcin and C-IMT						
Yang et al., 2013	Cross-sectional	1319	Serum OC	ECLIA	Negative association of OC with C-IMT	[9]
Ma et al., 2014	Cross-sectional	1077	Serum OC	ECLIA	Negative association of OC with C-IMT	[19]
Luo et al., 2015	Cross-sectional	476	Serum OC	ECLIA	No association of OC with C-IMT (healthy subjects)	[20]
Osteocalcin and vascular calcification/atherosclerosis						
Choi et al., 2015	Cross-sectional	162	OC, ucOC	ELISA	Association of higher ucOC and ucOC:OC with greater coronary artery calcification	[21]
Ogawa-Furuya et al., 2013	Cross-sectional	118+100	OC, ucOC	IRMA, ECLIA	Negative association of OC/ucOC with abdominal aortic calcification in T2DM men	[22]
Holvik et al., 2014	Longitudinal	1319	Serum OC	IRMA	Association of higher OC with reduced (older men)/increased CVD risk (older women)	[23]
Reyes-Garcia et al., 2012	Cross-sectional	78	Serum OC	RIA	Positive association of OC with vascular atherosclerosis markers in T2DM patients	[24]
Prats-Puig et al., 2014	Cross-sectional	203	OC, ucOC	EIA	Association of ucOC with CVD risk markers in offsprings of families with MetS	[25]
Osteocalcin and glucose/lipid metabolism and fat mass						
Chen et al., 2013	Cross-sectional	783+946	Serum OC	RIA	Negative association of OC with glucose and lipid metabolism	[26]
Hwang et al., 2012	Cross-sectional	435	OC	IRMA	Association of serum OC with glucose tolerance and insulin sensitivity (men)	[27]
Kim et al., 2010	Cross-sectional	86	OC	ECLIA	Inverse association with visceral fat (obese/overweight men)	[28]
Luo et al., 2015	Cross-sectional	1481	OC	ECLIA	Inverse association of serum OC with visceral fat (postmenopausal women)	[29]
Pirila et al., 2014	Cross-sectional	155	Serum OC	ECLIA	No association of OC with gluco-lipidic markers (healthy young adults)	[30]
Gonzales-Garcia et al., 2015	Cross-sectional	136+531	OC	ECLIA	Inverse association of OC with T2DM risk in Latino adults	[31]
Diaz-Lopez et al., 2013	Prosp./case-con.	153+306	cOC, ucOC	ELISA	Low cOC and ucOC are strongly associated increased risk of incident diabetes	[32]
Hwang et al., 2012	Longitudinal	1229	OC	ELISA	No association of OC with development of T2DM (men)	[33]
Ngarmukos et al., 2012	Case-control	63+63	OC, ucOC	ECLIA, EIA	Association of reduced OC, but not ucOC, with subsequent development of T2DM	[34]
Kanazawa et al., 2011	Cross-sectional	289	OC, ucOC	RIA	Inverse association of ucOC with plasma glucose and fat mass in T2DM	[35]
Bulló et al., 2012	Cross-sec./longit.	79	OC, ucOC	ECLIA	Negative (OC) and positive (ucOC) association with HOMA-IR (elderly men)	[36]
Alfadda et al., 2013	Cross-sectional	203	OC, ucOC	ELISA,EIA	ucOC associated with HDL-C, OC negatively associated with triglycerides	[37]

C-IMT, Carotid intima-media thickness; CVD, cardiovascular disease; ECLIA, electrochemiluminescence immunoassay; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; HOMA-IR, homeostasis model assessment of insulin resistance; IRMA, immunoradiometric assay; MetS, metabolic syndrome; OC, osteocalcin; RIA, radioimmunoassay; ucOC, uncarboxylated osteocalcin; T2DM, type 2 diabetes mellitus.

Table 2: Clinical studies evaluating osteocalcin and cardiovascular disease.

Authors, year	Design	n	Biomarkers	Assay	Main outcome	References
Osteocalcin and cardiovascular disease						
Hwang et al., 2015	Longitudinal	1290	Serum OC	EIA	No association of OC with incident CVD	[12]
Goliasch et al., 2011	Case-control	302	Serum OC	ECLIA	Association of lower OC with premature myocardial infarction (young subjects)	[13]
Zhang et al., 2010	Cross-sectional	461	Serum OC	ECLIA	Association of lower OC with coronary heart disease	[38]
Kim et al., 2015	Case-control	61+61	OC, ucOC	ELISA	Association of lower ucOC with coronary heart disease	[39]
Bao et al., 2011	Cross-sectional	181	Serum OC	ECLIA	Association of lower OC with severity of coronary atherosclerosis/MetS	[40]
Osteocalcin and cardiovascular disease-related mortality						
Confavreux et al., 2013	Prospective	774	Serum OC	IRMA	Association of high OC with lower abdominal aortic calcification/mortality	[41]
Yeap et al., 2012	Prospective	3542	Serum OC	ECLIA	U shaped association of OC with all-cause/CVD-related mortality (older men)	[42]
Lerchbaum et al., 2013	Cross-sectional	2271	Serum OC	ECLIA	U shaped association of OC with fatal events in men at high CVD risk	[43]

C-IMT, Carotid intima-media thickness; CVD, cardiovascular disease; ECLIA, electrochemiluminescence immunoassay; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; HOMA-IR, homeostasis model assessment of insulin resistance; IRMA, immunoradiometric assay; MetS, metabolic syndrome; OC, osteocalcin; RIA, radioimmunoassay; ucOC, uncarboxylated osteocalcin; T2DM, type 2 diabetes mellitus.

patients with acute myocardial infarction at a young age, a rare disease with poor prognosis, circulating OC levels were decreased at both acute and stable (after 1 year) phases of disease [13]. Despite this body of data, the actual relationship between OC levels and incidence of major CV events remains unclear (Table 2). The complexity of such relationship is indeed underlined by studies indicating that not only reduced, but also elevated OC levels predict all-cause and CV disease-related mortality, thus following a U shaped relationship, with subjects at both ends of the distribution at increased risk, as shown for older men [42] and in a large cohort of men at high CV risk [43]. In a population-based longitudinal cohort study conducted in 1319 subjects, it was observed that higher OC was associated with reduced CV disease risk in older-old men and with increased CV disease risk in older-old women, emphasizing that gender differences may represent a further level of complexity in this issue [23]. Interestingly, it has been reported that not lower, but higher OC levels were associated with CHD, as well as with pathological intima-media thickness, carotid plaques and aortic calcifications in T2DM patients [24]. Conversely, no association of OC and CV disease occurrence has also been reported. Specifically, a large 8.7 year longitudinal (primarily retrospective) study in the Korean population reported that total OC was not associated with the development of CV disease, although OC values were inversely correlated with obesity and abnormal plasma glucose and lipids [12]. In addition, such relationship was also not found in 476 metabolically healthy Chinese subjects of both genders [20].

Osteocalcin, glucose and lipid metabolism

Accumulating experimental and clinical evidence suggests that serum OC levels are associated with favorable glycometabolic conditions, as elevated OC is associated with improved insulin secretion and sensitivity, as well as better glycemic control [50, 51]. Conversely, reduced OC levels have consistently been associated with the presence of insulin resistance, atherogenic dyslipidemia, metabolic syndrome or T2DM in different ethnic groups, as assessed in cross-sectional studies and meta-analises [10, 11, 26, 52] (Table 1). More specifically, in a large cross-sectional study on a Chinese cohort, OC levels were negatively correlated with fasting serum insulin, homeostasis model assessment of insulin resistance (HOMA-IR), triglycerides and total cholesterol, and positively to homeostasis model assessment of β-cell function. Moreover, in men, OC negatively correlated with body mass index, diastolic blood pressure, fasting plasma glucose and 2-h oral glucose tolerance test glucose after adjustment for age, whereas in post-menopausal women, OC correlated

negatively with waist-hip ratio, LDL-C and C-reactive protein, and positively with adiponectin, apparently an independent predictor of circulating OC levels [26]. Interestingly, OC levels maintain their association with improved glucose tolerance and insulin secretion and sensitivity, independent of plasma adiponectin level [27]. The negative correlation between abdominal obesity and serum OC was confirmed by other studies in different ethnic groups [28, 29].

As OC levels appear to be negatively related to age, it is interesting to report that the independent inverse correlation between OC and CV risk parameters, including lipid profile, fasting plasma glucose and insulin, is attenuated or lost in healthy young adults, when adjusted for fat mass or lifestyle factors, such as physical activity [30]. In another study, OC levels were inversely associated with T2DM in Latino adults, but not in children [31], whereas, in obese children, OC was lower and related to insulin resistance and leptin in both cross-sectional and longitudinal analyses [53]. Moreover, serum ucOC was found to be higher in children from families with metabolic syndrome and thus at potential CV risk [25]. In a cross-sectional study in young adults, OC and cOC, but not ucOC, were found to be inversely associated with obesity, insulin resistance, and cardiovascular risk factors [23].

As with the incidence of CV events, it is not clear whether lower OC levels predict the development of T2DM in the long-term, since related reports are inconsistent. A prospective, nested case-control study showed that serum cOC and ucOC were lower in diabetic subjects, compared to controls, and were associated with an increased risk of incident T2DM [32]. In agreement with most cross-sectional studies, conducted in subjects with impaired glucose tolerance or T2DM, in a large Korean male cohort, baseline lower serum total OC levels were associated with less favorable metabolic parameters [33]. However, a longitudinal evaluation of this population, over a more than 8 year follow-up, found that OC was not associated with the incidence of new T2DM, after adjustment for other risk factors [33]. This observation was also extended by the same authors to younger men and pre- and post-menopausal women. On the other hand, lower total OC was associated with T2DM development in a nested case-control study in men with a 10-year follow-up [34]. Taken together, these observations suggest that the association between OC levels and glucose tolerance in humans needs to be better clarified.

It is well established that the presence of impaired glucose tolerance or T2DM accelerates progression of atherosclerotic lesions. Indeed, lower OC levels have been shown in Chinese patients with both metabolic syndrome and severe coronary disease [40]. Other studies also reported a negative correlation between OC levels and parameters of atherosclerosis in T2DM patients [8]. Interestingly, in most studies, the association between lower OC and CV risk or damage is still present after adjustment for metabolic markers, suggesting that OC may affect CV risk through independent mechanisms. In this context, the study by Reyes-Garcia et al. should be mentioned again, as a relationship between higher OC levels and CV lesions and prevalence of coronary heart disease was actually observed in T2DM patients [24].

A limitation of several clinical studies is that they evaluated only total OC levels. Thus, it interesting to report observation on the association of cOC and ucOC with CV and metabolic markers. Specifically, in middle-aged male subjects, elevated levels of both cOC and ucOC were associated with improved glucose tolerance, and of ucOC with enhanced β-cell function, whereas cOC was associated with improved insulin sensitivity [35, 51]. Cross-sectional studies reported that OC and ucOC were found negatively and positively associated with HOMA-IR in elderly men at high cardiovascular risk, respectively [36] and that ucOC was negatively correlated with markers of insulin resistance, central obesity, and the presence of MetS in postmenopausal women [54]. In a cross-sectional study, OC and ucOC were significantly lower in patients with MetS, independent of body mass index, and ucOC was positively correlated with HDL-C, while OC was negatively correlated with serum triglycerides [37]. Thus, future studies should also address the actual individual contribution of cOC and ucOC to CV and metabolic health and disease in humans, in order to validate ucOC as a marker correlated with positive CV and metabolic outcomes.

OC has been also proposed to play a role in the development of non-alcoholic fatty liver disease (NAFLD), which is considered as the hepatic feature of the metabolic syndrome and related insulin resistance. In a cross-sectional study, lower OC levels were negatively associated with serum alanine transaminase and aspartate transaminase levels [55] and lower serum OC levels were reported in a large Chinese cohort of patients with NAFLD, compared to non-NAFLD subjects [56]. Another end-stage complication of the metabolic syndrome is chronic kidney disease, associated to deranged mineral, lipid and glucose metabolism and increased CV risk. In these patients, kidney, bone and metabolic alterations are interconnected with CV risk and disease, OC appears to have an altered metabolism and, together with other factors, may interfere with insulin concentrations and sensitivity [57].

Open issues and future perspectives

Ample evidence supports a role for serum and locally-produced OC in the pathophysiology of CV lesions and CV risk, also in combination with metabolic changes, including T2DM. To sum up, in some studies lower OC levels appear associated with greater CV risk, CV lesions and CV disease in an independent manner, although other reports do not support these findings. A partial explanation for such inconsistencies in the literature about OC and CV and metabolic diseases is given by the observation that serum OC levels are influenced by ethnicity, gender and menopausal status [58], and that in some studies a non-linear, but U shaped, association curve between OC and CV/metabolic endpoints was found. Moreover, vitamin D status/supplementation and the use of anti-vitamin K anticoagulant, which may per se affect circulating OC levels, are generally not taken into consideration in the available and cited clinical studies, indicating the need to include such parameters in future studies. These inconsistencies suggest caution relative to the potential usefulness of measuring OC as a biomarker of CV risk and indicate the need to establish specific gender and ethnic reference values.

Moreover, the circulating OC assay methods varied among the different reports, suggesting caution, in some cases, in the comparison of the results obtained.

In any case, on the basis of the reported information, it is possible to speculate that a positive modulation of serum OC concentrations might be of value in the prevention and treatment of vascular disease, although some open issues in this field are still present, as indicated below, and should be addressed by future studies.

1. The potential mechanisms underlying the association between serum OC and CV risk and disease remain unclear. On the one hand, since OC has been shown to promote pancreatic β-cell proliferation and insulin secretion and adiponectin release, thereby facilitating insulin sensitivity, lower OC levels may be associated to greater insulin resistance or T2DM and lower adiponectin, thus indirectly favoring CV damage. On the other hand, since the association of reduced OC with CV impairment is still present after adjustment for metabolic factors, lower OC may also directly and independently affect CV risk.
2. As above indicated, serum OC levels are influenced by a number of factors (ethnicity, gender, menopausal status, current drug treatment and vitamin D status, etc.) [58]. Specific reference values for total OC, cOC and ucOC need thus to be established, before proposing the evaluation of circulating OC for CV and metabolic risk assessment and follow-up.
3. In particular, interaction between OC and intermediary metabolism requires further studies, also evaluating whether drugs able to modulate OC expression and secretion may be of use or should be avoided in subjects at risk for T2DM or already with this disease. Well-known examples of drugs impairing glucose metabolism as well as OC expression/serum concentrations are glucocorticoids and thiazide diuretics. On the contrary, dipeptidyl peptidase-IV inhibitors, widely used to treat T2DM, increase the half-life of OC and, thereby, its serum levels, suggesting the possibility of a positive interaction between this hormone and glucagon-like peptide-1 to promote β-cell proliferation and insulin secretion [10]. Such a relationship appears, however, very complex, as, at least in vitro, the glucagon-like peptide-1 analog liraglutide has been shown to attenuate osteoblastic differentiation of vascular smooth muscle cells, reducing locally-produced OC and potentially counteracting arterial calcification [59].

In addition, if a causal relationship between lower circulating OC and atherosclerosis progression will be established, the above-reported observations may also suggest a potential role for OC as a novel therapeutical target for CV disease treatment.

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