Serum total osteocalcin level as a vascular marker in elderly patients with metabolic syndrome

Marwa A. Saad[a], Nany H. Al-Gaiar[a], Marwa H. Mahmood[b], Amr M. Al-Abd[c]

[a]Departments of Internal Medicine, Geriatric Unit, [b]Clinical Pathology, [c]Diagnostic Radiology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Correspondence to Marwa A. Saad, MD, Internal Medicine, Geriatric Unit, Internal Medicine Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
Tel: 01222571192; fax: 002/033252668
e-mail: drmarwasaad74@gmail.com

Received 11 November 2015
Accepted 03 January 2016

Egyptian Journal of Obesity, Diabetes and Endocrinology
2015, 1:109–114

Metabolic syndrome (MetS) is a major public health problem and a clinical challenge worldwide. Several epidemiological studies have confirmed the increased risk for cardiovascular diseases (CVD) in individuals with MetS. Total osteocalcin (TOC) is a bone-derived, noncollagenous protein that was recently recognized as a hormone-regulating energy metabolism factor. Importantly, osteocalcin expression has been described as having a role in calcifying vascular smooth muscle cells. We aimed in the present study to analyze the correlation between serum levels of TOC and vascular calcification in elderly persons with MetS. Seventy-four elderly men aged 65 years or older were included in the present study and divided into two groups. Group I comprised 40 patients who satisfied at least three criteria for MetS according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) definition, and group II comprised 34 age-matched healthy men who served as the control group. BMI was calculated, blood samples were taken for lipid profile analysis, and total osteocalcin (OCN) levels were evaluated using enzyme-linked immunosorbent assay kits. Carotid Doppler B mode ultrasonography was performed for all participants. Patients with MetS exhibited significantly higher BMIs, waist circumference, fasting blood sugar, triglycerides, blood pressure, total cholesterol, and lower high-density lipoprotein-cholesterol compared with controls. Patients with MetS had significantly lower levels of TOC compared with controls. Also, patients with MetS had significantly higher intima–media thickness and a higher number of carotid plaques compared with controls. TOC was significantly negatively correlated with parameters of carotid atherosclerosis. It is also negatively correlated with dyslipidemic parameters. Its correlation with components of MetS did not reach statistical significance. We concluded that serum osteocalcin levels were negatively correlated with carotid atherosclerosis in patients with MetS. This may reflect the role of osteocalcin as a circulating endocrine factor that regulates glucose metabolism and thereby cardiovascular risk in patients with MetS. Prospective studies are needed to assess the time course and relevance of serum osteocalcin in the development of atherosclerosis in patients with MetS.

Keywords:
cardiovascular risk, carotid atherosclerosis, metabolic syndrome, osteocalcin

Egyptian Journal of Obesity, Diabetes and Endocrinology 1:109–114

2356-8062

Introduction

Metabolic syndrome (MetS) is a major public health problem and clinical challenge worldwide. MetS is a constellation of interconnected physiological, biochemical, clinical, and metabolic factors that directly increase the risk for atherosclerotic cardiovascular diseases, type 2 diabetes mellitus, and all-cause mortality [1]. Its main components are dyslipidemia, elevation of arterial blood pressure, and dysregulated glucose homeostasis; abdominal obesity and insulin resistance have gained increasing attention as the core manifestations of the syndrome. Recently, other abnormalities such as chronic proinflammatory and prothrombotic states, nonalcoholic fatty liver disease, and sleep apnea have been added to the entity of the syndrome, making its definition even more complex. Dysfunctional adipose tissue plays an important role in the pathogenesis of obesity-related insulin resistance [2]. Both adipose cell enlargement and infiltration of macrophages into adipose tissue result in the release of proinflammatory cytokines and promote insulin resistance [3]. Insulin resistance appears to be the primary mediator of MetS [4]. The distribution of adipose tissue appears to affect its role in MetS. Visceral or intra-abdominal fat correlates with inflammation, whereas subcutaneous fat does not. There are a number of potential explanations for this, including experimental observations that omental fat is more resistant to insulin and may result in a higher concentration of toxic free fatty acids in the portal circulation [5]. Abdominal fat is known to produce potentially harmful levels of cytokines, such as tumor necrosis factor, adiponectin, leptin, resistin, and plasminogen activator inhibitor [6].

Besides the many components and clinical implications of MetS, there is still no universally accepted pathogenic mechanism or clearly defined diagnostic criteria. Furthermore, there is still debate as to whether this entity represents a specific syndrome or is a surrogate of combined risk factors that put the individual at particular risk [7]. The most commonly used criteria for definition at present are from the WHO [8], the European Group for the study of Insulin Resistance (EGIR) [9], the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) [10], American Association of Clinical Endocrinologists (AACE) [11], and the International Diabetes Federation (IDF) [12]. Currently, the two most widely used definitions are those of the NCEP-ATP III and IDF focusing specifically on waist circumference, which is a surrogate measure of central obesity. In contrast, the AACE, WHO, and EGIR definitions are all largely focused on insulin resistance [7]. Clearly, the prevalence of MetS varies and depends on the criteria used in different definitions, as well as on the composition (sex, age, race and ethnicity) of the population studied [13]. No matter which criteria are used, the prevalence of MetS is high and increasing in all western societies, probably as a result of the obesity epidemic [14]. According to National Health and Examination Survey (NHANES) 2003–2006 [15], ~34% of people studied met the NCEP-ATP III revised criteria for MetS. The prevalence of MetS increases with age, with about 40% of people older than 60 years meeting the criteria [16].

Several epidemiological studies have confirmed the increased risk for CVD in individuals with MetS, independently of the diagnostic criteria used [17,18]. Overall, a range of 1.5–3 times greater risk for CVD and coronary heart diseases (CHD) mortality has been found in several prospective studies (REFS), whereas a recent meta-analysis showed that MetS was associated with a two-fold increase in cardiovascular outcomes and a 1.5-fold increase in all-cause mortality [19].

The pathophysiology is very complex and has been only partially elucidated. Most patients are older, obese, and sedentary, and have a degree of insulin resistance. Stress can also be a contributing factor. The most important factors are genetics [20], aging, diet (particularly sugar-sweetened beverage consumption) [21], sedentary behavior [22], low physical activity [23], disrupted chronobiology/sleep [24], mood disorders/psychotropic medication use [25], and excessive alcohol use [26].

Osteocalcin is a noncollagenous, 49-amino-acid glutamate-rich polypeptide bone matrix protein with a molecular weight of about 5800 kDa. Osteoblasts produce osteocalcin and incorporate it into the bone matrix. Osteocalcin is released into the circulation from the matrix during bone resorption and therefore is considered a marker of bone turnover rather than a specific marker of bone formation [27]. Osteocalcin has raised much attention as a hormone-regulating glucose metabolism factor and fat mass. Recently, osteocalcin has been recognized as a bone-derived hormone to regulate energy metabolism. Osteocalcin knockout mice exhibited glucose intolerance, increased fat mass, insulin resistance, decreased expression of insulin target genes in liver and muscle, and decreased adiponectin gene expression in adipose tissue [28], whereas administration of recombinant osteocalcin increased insulin secretion, decreased blood glycemia, and weakened the development of obesity [29]. Osteocalcin undergoes γ-carboxylation. The g-carboxylated form binds hydroxyapatite and is abundant in bone extracellular matrix. In contrast, the undercarboxylated circulating form has been implicated as a novel hormone and positive regulator of glucose homeostasis. Importantly, osteocalcin expression has been described in calcifying vascular smooth muscle cells, although the physiological significance of this observation has remained unclear. Osteocalcin is considered a novel regulator of osteochondrogenic differentiation of pathologically mineralizing vascular smooth muscle cells [30], which provides the first evidence that osteocalcin may be an active player in vascular calcification, with its presence in the calcified vasculature, and potentially in circulation, activating novel signaling pathways that promote mineralization. Pathological mineralization of the vasculature has a detrimental effect on cardiovascular function and is associated with increased mortality in patients with aging, atherosclerosis, type 2 diabetes, and chronic kidney disease [31].

The relationship between total osteocalcin (TOC), MetS, and atherosclerosis has been explored in recent studies [32,33]. We aimed in the present study to analyze the correlation between serum levels of OCN and vascular calcification in elderly persons with MetS.

Materials and methods

The current study included 74 elderly men aged 65 years or older who were recruited either from the geriatric outpatient clinic or from among hospitalized patients. Participants were divided into two groups. Group I comprised 40 patients who satisfied at least three criteria of the MetS, and group II comprised 34 healthy men who served as the control group. We used the definition of MetS according to the NCEP-ATP III [34]; fasting blood glucose level of at least

110 mg/dl, blood pressure of at least 130/85 mmHg, triglycerides of at least 150 mg/dl, high-density lipoprotein-cholesterol (HDL-ch) less than 40 mg/dl, and waist circumference greater than 102 cm for men. The exclusion criteria included the presence of any significant liver disease, renal failure requiring renal replacement therapy, and the intake of lipid-lowering drugs or any drugs that could influence bone metabolism.

The study was approved by the ethics committee of the faculty of medicine, Alexandria University. All study participants were given a detailed prescription of the study, its purpose, and benefits, and an informed written consent was obtained.

Anthropometric measurements (including height and weight) were taken, and the BMI was calculated. The cutoff values of BMI were as follows: underweight: <18.5; normal: 18.5–24.9; overweight: 25.0–29.9; obese: >30; and morbidly obese: ≥40 [35].

Blood samples were obtained from all participants after an overnight fast for determination of total serum cholesterol, HDL-ch, serum triglycerides, and fasting blood glucose.

Serum TOC was measured using an N-MID osteocalcin enzyme-linked immunosorbent assay kit (Elecsys, Roche Diagnostic Ltd, Switzerland, Switzerland).

Carotid Doppler B mode ultrasonography was performed for all participants using an Acuson Sequoia 512 at the Diagnostic Radiology Department, Faculty of Medicine, Alexandria University. Intima–media thickness (IMT) of the common carotid artery, carotid bulb, and internal carotid arteries was assessed. IMT was defined as the distance between the lumen–intima interface and the media–adventitia interface [36]. Common carotid artery IMT was defined as the mean of the maximum IMT in both right and left sides of the common carotid artery. The plaque of the carotid artery (common carotid artery, carotid bulb, and internal carotid artery) is defined as a localized protrusion of the internal part of the vessel wall into the lumen of 50% of the surrounding IMT value. Plaque presence was defined as at least one plaque in any of the carotid arteries [37].

Data were fed into a computer and analyzed using the IBM SPSS software package, version 20.0 (USA). Qualitative data were described using number and percentage. Quantitative data were described as range (minimum and maximum), mean, SD, and median. Comparison between different groups regarding categorical variables was tested using the χ^2-test. The distributions of quantitative variables were tested for normality. For normally distributed data, comparison between two independent populations was made using the independent *t*-test. Correlations between two quantitative variables were assessed using Pearson's coefficient. For abnormally distributed data, comparison between two independent populations was made using the Mann–Whitney test. Correlations between two quantitative variables were assessed using Spearman's coefficient. Significance of the obtained results was judged at the 5% level.

Results

Seventy-four elderly men participated in the current study and were divided into two groups according to the presence or absence of MetS, which was determined as per the NCEP-ATP III criteria. Group I comprised 40 men (62.5%) with MetS. Their mean age was 70.70 ± 4.98 years. These men were compared with 34 age-matched men (37.5%) without MetS, who served as the control group. Their mean age was 70.76 ± 4.59 years. There was no statistically significant difference between the two groups ($P = 0.954$).

Group I patients exhibited significantly higher BMI, compared with group II, with a mean of 30.90 ± 1.68 kg/m^2 in group I and 26.33 ± 2.0 kg/m^2 in group II ($P < 0.001$).

Group I patients had significantly higher waist circumference, fasting blood sugar, triglycerides, blood pressure, and lower HDL-ch compared with group II individuals. Also, total cholesterol was significantly higher in group I patients than in group II individuals (Table 1).

Patients of group I had significantly lower levels of TOC compared with individuals in group II (Table 1).

Group I had significantly higher IMT compared with group II ($P<0.001$). Also group I patients had significantly higher plaque numbers compared with group II ($P < 0.001$).

Levels of osteocalcin were negatively correlated with IMT of the carotid artery and with the number of carotid plaques in patients with MetS ($P < 0.001$) (Table 2).

Serum TOC was negatively correlated with systolic and diastolic blood pressure, waist circumference, fasting blood sugar, and triglycerides, although the

Table 1 Comparison between the two studied groups on the basis of different parameters

Study variables	Group I (MetS) (n = 40)	Group II (controls) (n = 34)	P
Age	70.70 ± 4.98	70.76 ± 4.59	0.954
Systolic	154.37 ± 7.78	132.79 ± 6.30	<0.001*
Diastolic	92.50 ± 4.24	78.09 ± 4.27	<0.001*
FBG	140.82 ± 32.54	103.18 ± 4.12	<0.001*
Normal	6 (15.0%)	34 (100.0%)	<0.001*
Abnormal	34 (85.0%)	0 (0.0%)	
TG	153.87 ± 21.75	107.35 ± 14.86	<0.001*
Normal	13 (32.5%)	34 (100.0%)	<0.001*
Abnormal	27 (67.5%)	0 (0.0%)	
HDL	47.30 ± 7.25	60.65 ± 6.28	<0.001*
Normal	6 (15.0%)	34 (100.0%)	<0.001*
Abnormal	34 (85.0%)	0 (0.0%)	
Total cholesterol	200.0 (88.0–240.0)	102.0 (89.0–188.0)	<0.001*
Normal	18 (45.0%)	34 (100.0%)	<0.001*
Abnormal	22 (55.0%)	0 (0.0%)	
WC	103.78 ± 6.60	88.94 ± 2.81	<0.001*
Normal	16 (40.0%)	34 (100.0%)	<0.001*
Abnormal	24 (60.0%)	0 (0.0%)	
BMI	30.90 ± 1.68	26.33 ± 2.0	<0.001*
Normal	0 (0.0%)	10 (29.4%)	<0.001*
Overweight	14 (35.0%)	23 (67.6%)	
Obese	26 (65.0%)	1 (2.9%)	
TOC	5.79 ± 1.77	30.20 ± 8.69	<0.001*
Normal	0 (0.0%)	33 (97.1%)	<0.001*
Abnormal	40 (100.0%)	1 (2.9%)	
IMT	1.07 ± 0.16	0.70 ± 0.07	<0.001*
Carotid plaques [n (%)]			
0	5 (12.5)	31 (91.2)	<0.001*
1	23 (57.5)	3 (8.8)	
2	12 (30.0)	0 (0.0)	

FBG, fasting blood glucose; HDL, high-density lipoprotein; IMT, intima–media thickness; MetS, metabolic syndrome; TG, triglyceride; TOC, total osteocalcin; WC, waist circumference; Statistically significant at $P \leq 0.05$; *Highly significant.

Table 2 Correlation between total osteocalcin and carotid atherosclerosis

Carotid atherosclerosis parameters	TOC			
	Group I (MetS)		Group II (controls)	
	r	P	r	P
IMT	−0.899*	<0.001	−0.779*	<0.001
Carotid plaques	−0.630*	<0.001	−0.460*	0.006

IMT, intima–media thickness; MetS, metabolic syndrome; r, Pearson's coefficient; TOC, total osteocalcin; *Statistically significant at $P \leq 0.05$.

correlation was significant only with triglyceride levels. No significant correlation was found between TOC and HDL-ch (Table 3).

TOC was negatively correlated with triglycerides and total cholesterol levels (P = 0.005 and 0.020 respectively) and positively correlated with HDL-ch, although the correlation was statistically insignificant (P = 0.474) (Table 4).

Discussion

The prevalence of MetS has been increasing worldwide, most probably because of the increased prevalence of high-fat diets combined with the general decrease in physical activity [38]. Thus, MetS has recently become a major public health issue around the world, because it is associated with a doubling of cardiovascular event risk and a 10-year cardiovascular mortality. The goal of identifying MetS is to prevent the occurrence of these diseases. To investigate whether the presence of MetS increases the relationship between TOC and the prediction of CVD risk, we divided our participants (based on the NCEP-ATP III guidelines) into those with and those without MetS. We detected a highly significant difference between the two groups. Patients with MetS had a higher BMI, waist circumference, total cholesterol level, triglyceride level, and lower HDL-ch level. Also TOC was significantly lower in patients with MetS in comparison with those without MetS. These differences point toward an association between TOC and the dyslipidemia that is found in patients with MetS. In our study, serum osteocalcin correlated negatively with triglycerides and total cholesterol. It is well established that elevated serum total cholesterol was an independent risk factor for the development of CVD. Given that serum low osteocalcin levels were significantly associated with total cholesterol levels, it is plausible to consider osteocalcin as a promising candidate for risk assessment and a potential intervention target for CVD. An Australian study [39] showed that serum osteocalcin levels predicted all-cause and CVD-related mortality in community-dwelling older men.

Our study showed that the level of TOC was significantly lower in patients with MetS. Serum osteocalcin was negatively correlated with some components of the MetS – namely, blood pressure and waist circumference – although that did not reach statistical significance. Recent studies have demonstrated that serum TOC levels are associated with MetS. Saleem *et al.* [40] determined that serum TOC is negatively associated with MetS in both blacks and non-Hispanic whites. Oosterwerff *et al.* [41] found that plasma TOC was inversely associated with MetS in a community-dwelling cohort of older persons in the Netherlands, and reported that the subjects with the lowest quartile of TOC concentrations had an ~3.7-fold higher risk for MetS than did the subjects with the highest quartile. Also, Yeap *et al.* [42] reported that men with lower serum TOC concentrations have a higher risk for MetS. Moreover, Alfadda *et al.* [43] studied 203 patients with and without MetS. MetS was defined on the basis of the NCEP-ATP III criteria. They stated that TOC was significantly lower

Table 3 The correlation between OCN and components of metabolic syndrome

MetS variables	OCN			
	Group I (MetS)		Group II (controls)	
	r	*P*	*r*	*P*
Systolic	−0.127	0.434	−0.196	0.266
Diastolic	−0.102	0.532	−0.120	0.501
FBG	−0.050	0.760	−0.128	0.470
TG	−0.469*	0.005	0.042	0.815
HDL	0.116	0.474	0.322	0.064
WC	−0.029	0.858	−0.273	0.118

FBG, fasting blood glucose; HDL, high-density lipoprotein; MetS, metabolic syndrome; *r*, Pearson's coefficient; TG, triglyceride; WC, waist circumference; *Statistically significant at $P \leq 0.05$; .

Table 4 The correlation between OCN and dyslipidemic parameters

Lipid profile	TOC			
	Group I (MetS)		Group II (controls)	
	r	*P*	*r*	*P*
TG	−0.469*	0.005	0.042	0.815
Total cholesterol	−0.366*	0.020	0.057	0.725
HDL	0.116	0.474	0.322	0.064

HDL, high-density lipoprotein; MetS, metabolic syndrome; *r*, Pearson's coefficient; TG, triglyceride; TOC, total osteocalcin; *Statistically significant at $P \leq 0.05$.

in patients with MetS compared with those without MetS, independent of BMI. In patients with MetS, TOC was significantly and negatively correlated with serum triglycerides, as detected in our study.

In a study by Bezerra dos Santos *et al.* [44], the mean osteocalcin was significantly lower in patients with MetS and decreased significantly with the increase in the number of criteria for the diagnosis of MetS. Serum osteocalcin was lower in patients with BMI at least 25 and fasting plasma glucose (FPG) at least 100 mg/dl, and in hypertensive and diabetic patients, and was inversely associated with BMI, waist circumference, FPG, and systolic blood pressure.

There is evidence to show the influence of bone proteins on cardiovascular diseases [45]. During atherogenesis, bone matrix proteins, including osteocalcin, may have a regulatory role in the atherosclerotic calcification process [46]. Recent evidence suggests that osteoblast-like cells are present in the vasculature and capable of calcifying vascular cells. Furthermore, paracrine regulators of bone metabolism, such as osteocalcin, are also present in atherosclerotic arteries. Thus, the vascular microenvironment possesses mechanisms similar to those in bone tissues to maintain mineral homeostasis [47]. Osteocalcin-knockout mice develop extensive calcification of arteries that rapidly becomes lethal, suggesting that osteocalcin has an antimineralization role in the artery. In humans, osteocalcin was detected in human carotid arteries in endarterectomy samples. Thus, osteocalcin could play a pivotal role not only in bone mineralization but also in vascular wall calcification [48]. However, at present, little is known about whether serum osteocalcin secreted from osteoblasts in bone or osteoblast-like cells in vessels could actually modulate atherosclerosis. Thus, further studies are needed to clarify the pathophysiological processes underlying the relationship between serum osteocalcin level and atherosclerosis parameters.

In our study, serum osteocalcin was negatively correlated with carotid atherosclerosis, IMT and carotid plaques. In accordance with our results a Japanese study found that serum osteocalcin was negatively associated with IMT of the common carotid artery in type 2 diabetic men [48]. Bao *et al.* [49] observed that serum levels of osteocalcin were inversely associated with the MetS and the severity of coronary artery disease in Chinese men.

Conclusion

Our study indicated that serum osteocalcin levels were negatively correlated with carotid atherosclerosis in patients with MetS. This may reflect the role of osteocalcin as a circulating endocrine factor that regulates glucose metabolism and thereby cardiovascular risk in patients with MetS. Prospective studies are needed to assess the time course and relevance of serum osteocalcin in the development of atherosclerosis in patients with MetS.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Grundy SM, Cleeman JI, Daniels SR, *et al.* Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005; 112:2735–2752.

2 Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. Physiol Behav 2008; 94:206–218.

3 Gustafson B, Hammarstedt A, Andersson CX, Smith U. Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. Arterioscler Thromb Vasc Biol 2007; 27:2276–2283.

4 Lann D, LeRoith D. Insulin resistance as the underlying cause for the metabolic syndrome. Med Clin North Am 2007; 91:1063–1077.

5 Després JP, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E, *et al.* Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. Arterioscler Thromb Vasc Biol 2008; 28:1039–1049.

6 Türkoglu C, Duman BS, Günay D, Cagatay P, Ozcan R, Büyükdevrim AS. Effect of abdominal obesity on insulin resistance and the components

of the metabolic syndrome: evidence supporting obesity as the central feature. Obes Surg 2003; 13:699–705.

7 Kassi E, Pervanidou P, Kaltsas G, Chrousos G. Metabolic syndrome: definitions and controversies. BMC Med 2011; 9:48.

8 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15:539–553.

9 Balkau B, Charles MA. Comment on the provisional report from the WHO consultation: European Group for the Study of Insulin Resistance (EGIR). Diabet Med 1999; 16:442–443.

10 Cleeman JI. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). J Am Med Assoc 2001; 285:2486–2497.

11 Einhorn D, Reaven GM, Cobin RH, Ford E, Ganda OP, Handelsman Y, *et al*. American College of Endocrinology position statement on the insulin resistance syndrome. Endocr Pract 2003; 9:237–252.

12 International Diabetes Federation The IDF consensus worldwide definition of the metabolic syndrome

13 Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, *et al*. The metabolic syndrome. Endocr Rev 2008; 29:777–822.

14 Hollman G, Kristenson M. The prevalence of the metabolic syndrome and its risk factors in a middle-aged Swedish population – mainly a function of overweight? Eur J Cardiovasc Nurs 2008; 7:21–26.

15 Ervin RB. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States, 2003–2006. Natl Health Stat Report 2009; 13: 1–7.

16 Ford ES, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among US adults. Diabetes Care 2004; 27:2444–2449.

17 Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K, Pyorala K. Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. Arch Intern Med 2004; 164:1066–1076.

18 McNeill AM, Rosamond WD, Girman CJ, Golden SH, Schmidt MI, East HE, *et al*. The metabolic syndrome and 11-year risk of incident cardiovascular disease in the atherosclerosis risk in communities study. Diabetes Care 2005; 28:385–390.

19 Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, *et al*. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol 2010; 56:1113–1132.

20 Pollex RL, Hegele RA. Genetic determinants of the metabolic syndrome. Nat Clin Pract Cardiovasc Med 2006; 3:482–489.

21 Malik VS, Popkin BM, Bray GA, Després JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care 2010; 33:2477–2483.

22 Edwardson CL, Gorely T, Davies MJ, Gray LJ, Khunti K, Wilmot EG, *et al*. Association of sedentary behaviour with metabolic syndrome: a meta-analysis. PLoS One 2012; 7:e34916.

23 Katzmaryk PT, Leon AS, Wilmore JH, Skinner JS, Rao DC, Rankinen T, Bouchard C. Targeting the metabolic syndrome with exercise: evidence from the HERITAGE family study. Med Sci Sports Exerc 2003; 35: 1703–1709.

24 He D, Xi B, Xue J, Huai P, Zhang M, Li J. Association between leisure time physical activity and metabolic syndrome: a meta-analysis of prospective cohort studies. Endocrine 2014; 46:231–240.

25 Vancampfort D, Correll CU, Wampers M, Sienaert P, Mitchell AJ, De Herdt A, *et al*. Metabolic syndrome and metabolic abnormalities in patients with major depressive disorder: a meta-analysis of prevalences and moderating variables. Psychol Med 2013; 21:1–12.

26 Vancampfort D, Vansteelandt K, Correll CU, Mitchell AJ, De Herdt A, Sienaert P, *et al*. Metabolic syndrome and metabolic abnormalities in bipolar disorder: a meta-analysis of prevalence rates and moderators. Am J Psychiatry 2013; 170:265–274.

27 Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J. The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. Osteoporos Int 2000; 11 Suppl 6:2–17.

28 Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, *et al*. Endocrine regulation of energy metabolism by the skeleton. Cell 2007; 130:456–469.

29 Ferron M, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci USA 2008; 105:5266–5270.

30 Idelevich A, Rais Y, Monsonego-Ornan E. Bone Gla protein increases HIF-1alpha-dependent glucose metabolism and induces cartilage and vascular calcification. Arterioscler Thromb Vasc Biol 2011; 31:e55–e71.

31 Sage AP, Tintut Y, Demer LL. Regulatory mechanisms in vascular calcification. Nat Rev Cardiol 2010; 7:528–536.

32 Ma H, Lin H, Hu Y, Li X, He W, Jin X, *et al*. Serum levels of osteocalcin in relation to glucose metabolism and carotid atherosclerosis in Chinese middle-aged and elderly male adults: the Shanghai Changfeng Study. Eur J Intern Med 2014; 25:259–264.

33 Liu JJ, Toy WC, Wong MD, Tan CS, Tavintharan S, Wong MS, *et al*. Elevated undercarboxylated and reduced carboxylated osteocalcin are associated with metabolic syndrome in middle age Asian females. Exp Clin Endocrinol Diabetes 2013; 121:329–333.

34 National Institutes of Health. Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Executive Summary. Bethesda, MD: National Institutes of Health, National Heart, Lung and Blood Institute; 2001.

35 World Health Organization. Obesity preventing and managing the Global Epidemic: report of a WHO consultation on obesity. Geneva: World Health Organization; 1998.

36 Li Q, Yang Z, Lu B, Wen J, Ye Z, Chen L, *et al*. Serum uric acid level and its association with metabolic syndrome and carotid atherosclerosis in patients with type 2 diabetes. Cardiovasc Diabetol 2011; 10:72.

37 Chen L, Yang Z, Lu B, Li Q, Ye Z, He M, *et al*. Serum CXC ligand 5 is a new marker of subclinical atherosclerosis in type 2 diabetes. Clin Endocrinol (Oxf) 2011; 75:766–770.

38 Misra A, Khurana L. Obesity and the metabolic syndrome in developing countries. J Clin Endocrinol Metab 2008; 93:S9–S30.

39 Yeap BB, Chubb SA, Flicker L, McCaul KA, Ebeling PR, Hankey GJ, *et al*. Associations of total osteocalcin with all-cause and cardiovascular mortality in older men. The Health In Men Study. Osteoporos Int 2012; 23:599–606.

40 Saleem U, Mosley TH Jr, Kullo IJ. Serum osteocalcin is associated with measures of insulin resistance, adipokine levels, and the presence of metabolic syndrome. Arterioscler Thromb Vasc Biol 2010; 30:1474–1478.

41 Oosterwerff MM, van Schoor NM, Lips P, *et al*. Osteocalcin as a predictor of the metabolic syndrome in older persons: a population-based study. Clin Endocrinol (Oxf) 2013; 78:242–247.

42 Yeap BB, Chubb SA, Flicker L, McCaul KA, Ebeling PR, Hankey GJ, *et al*. Associations of total osteocalcin with all-cause and cardiovascular mortality in older men. The Health In Men Study. Osteoporos Int 2012; 23:599–606.

43 Alfadda AA, Masood A, Shaik SA, Dekhil H, Goran M. Association between osteocalcin, metabolic syndrome, and cardiovascular risk factors: role of total and undercarboxylated osteocalcin in patients with type 2 diabetes. Int J Endocrinol 2013; 2013:197519.

44 Bezerra dos Santos Magalhães K, Magalhães MM, Diniz ET, Lucena CS, Griz L, Bandeira F. Metabolic syndrome and central fat distribution are related to lower serum osteocalcin concentrations. Ann Nutr Metab 2013; 62:183–188.

45 Reyes-Garcia R, Rozas-Moreno P, Jimenez-Moleon JJ, Villoslada MJ, Garcia-Salcedo JA, Santana-Morales S, Muñoz-Torres M. Relationship between serum levels of osteocalcin and atherosclerotic disease in type 2 diabetes. Diabetes Metab 2012; 38:76–81.

46 Dhore CR, Cleutjens JP, Lutgens E, Cleutjens KB, Geusens PP, Kitslaar PJ, *et al*. Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol 2001; 21:1998–2003.

47 Sheng L, Cao W, Cha B, Chen Z, Wang F, Liu J. Serum osteocalcin level and its association with carotid atherosclerosis in patients with type 2 diabetes. Cardiovasc Diabetol 2013; 12:22.

48 Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Kurioka S, Yano S, Sugimoto T. Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. J Clin Endocrinol Metab 2009; 94:45–49.

49 Bao Y, Zhou M, Lu Z, Li H, Wang Y, Sun L, *et al*. Serum levels of osteocalcin are inversely associated with the metabolic syndrome and the severity of coronary artery disease in Chinese men. Clin Endocrinol (Oxf) 2011; 75:196–201.