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INDEPENDENT AND ADDITIVE EFFECTS OF CYTOKINE PATTERNS AND THE METABOLIC SYNDROME ON ARTERIAL AGING IN THE Sardinia Study

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Abstract

Objective—Metabolic syndrome (MetS) and its components accelerate age-associated increases in arterial stiffness and thickness. We investigated whether specific proinflammatory cytokines contribute to arterial aging, independent of age, sex, MetS, and other traditional CV risk factors.

Research Design and Methods—MetS components (ATP III criteria) and arterial properties were assessed in 6,148 subjects, aged 14–102 in Sardinia, Italy. Common carotid artery (CCA) diameter, intima-media thickness (IMT), and aortic pulse wave velocity (PWV), adiponectin, leptin, high-sensitivity C reactive protein (hsCRP), Monocyte Chemoattractant Protein 1 (MCP1), and interleukin 6 (IL6) were measured.

Results—While cytokine levels – except for MCP1 – were significantly higher (lower for adiponectin) in MetS than in control subjects, and the increased PWV and CCA IMT with aging were associated with MetS, this association was independent of cytokine levels ($p < 0.001$ for both PWV and CCA IMT). Specific cytokines, however, were significantly associated with arterial stiffness (higher leptin – $p < 0.001$ - and higher hsCRP – $p < 0.001$) or thickness (lower adiponectin

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– $p < 0.05$ - and higher IL6 – $p < 0.001$) – independent of age, sex, MetS and other traditional CV risk factors. The co-occurrence of both MetS and higher cytokines levels was associated with greater increases in arterial stiffness and thickness.

Conclusion—While MetS and specific cytokine patterns associated with arterial aging, the increases in arterial stiffness and thickness are greater when both MetS and higher cytokine levels are present, suggesting a possible synergistic effect of MetS and inflammation on the arterial wall.

Keywords

Cytokines; metabolic syndrome; aging; large artery properties; arterial stiffness; carotid intima media thickness

INTRODUCTION

The metabolic syndrome (MetS) has been established as a significant risk factor for new onset diabetes mellitus and cardiovascular events (1), even in older subjects in whom MetS confers a 38% higher risk of myocardial infarction and stroke (2). We have previously reported that the MetS is accompanied by accelerated central arterial aging (3–4), namely with increased arterial stiffness and thickness. Additionally, specific clusters of the components of the MetS are associated with a higher odds of having extremely stiff or thick arteries (4).

A growing body of literature suggests that diverse inflammatory and adipose tissue related cytokines correlated with arterial damage (5) and, thus, with endothelial dysfunction (6) and increased arterial stiffness and thickness (7–9). Cytokine levels have been reported to be increased (decreased for adiponectin) in subjects with diabetes mellitus (10), and a proinflammatory status accompanies the presence of altered MetS components.

The aims of the present study were to determine: a) whether MetS (and specific clusters of its components) and proinflammatory cytokines are associated with arterial stiffness and thickness independent of each other; after controlling for age, sex, traditional CV risk factors; and b) the simultaneous occurrence of MetS and higher proinflammatory cytokine levels increase the likelihood for greater arterial stiffness or thickness.

SUBJECTS AND METHODS

Study population

The SardiNIA Study investigates the genetics of complex traits/phenotypes, including CV risk factors and arterial properties, in a Sardinian founder population (11–12). Over a 3-yr period, from November 2001 to December 2004, all residents aged 14 years and older in 4 towns of Sardinia Region, Italy were invited to participate in the Study. The response rate was 60%, resulting in enrolment of 6148 participants aged 14–102 years old. Participants came to the clinic after fasting overnight, provided an informed consent, and after donating a blood sample, underwent a detailed medical history and full medical examination, including blood pressure and anthropometric measurements, a 12-lead resting EKG, measurements of arterial structure and function, and personality testing.

Variables Measured

Blood pressure—Blood pressure determinations were performed in the morning, with subjects in the seated position, and following a five minute quiet resting period. Blood pressure was measured in both arms with a mercury sphygmomanometer using an appropriately sized cuff. Values for systolic blood pressure (SBP) and diastolic blood pressure (DBP) were defined by Korotkoff phase I and V, respectively. The average of second and third measurements on both the right and left arm were used in the analysis. Pulse pressure was computed as $PP = (SBP - DBP)$; mean BP was computed as $MBP = DBP + (PP / 3)$.

Anthropometry—Height, weight and waist circumference were determined for all participants. Body mass index (BMI) was calculated as body weight (kg) / height (m)².

Fasting plasma lipids and glucose—Blood samples were drawn from the antecubital vein between 7 and 8 AM after an overnight fast. Subjects were not allowed to smoke, engage in significant physical activity or take medications prior to the collection of the samples. Plasma triglycerides and total cholesterol, and fasting glucose were determined by commercially available kit (12). LDL-cholesterol concentrations were estimated by the Friedewald formula.

Arterial structure and function

Aortic PWV was measured as previously described from simultaneous records of common carotid and femoral arteries flow waves (12).

The IMT was measured on the right common carotid artery (CCA) at end diastole by use of a linear-array 5- to 7.5-MHz transducer (HDI 3500-ATL Ultramark Inc) as previously described (12). Measurement was obtained from 5 contiguous sites at 1-mm intervals, and the average of the 5 measurements was used for analyses. All the measurements were performed by a single reader (AS). CCA systolic and diastolic diameter (d and D, respectively) were identified via ECG gating and measured similarly to IMT. CCA cross-sectional area (CSA) was calculated, as:

$$CCA\ CSA = \rho * (\pi Re^2 - \pi D^2)$$

where ρ is the arterial wall density ($\rho = 1.06$), $Re = CCA\ IMT + CCA\ D$ (4).

Definition of the Metabolic Syndrome

The Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) (1) defined the MetS as an alteration in three or more of the following five components: abdominal obesity ($W > 102$ cm for men or > 88 cm for women), high triglycerides ($T \geq 150$ mg/dl), low HDL cholesterol ($H < 40$ mg/dl for men or < 50 mg/dl for women), elevated blood pressure (systolic or diastolic) ($B \geq 130/85$ mmHg), and elevated fasting glucose ($G \geq 110$ mg/dl).

Cytokine assays

Serum levels of hsCRP were measured by the high sensitivity Vermont assay (University of Vermont, Burlington), an enzyme-linked immunosorbent assay calibrated with WHO Reference Material. The lower detection limit of this assay is 0.007 mg/l, with an inter-assay coefficient of variation of 5.14%. Leptin (Human Serum Adipokine -panel B; Lincoplex kit: Cat. # HADK2-61K-B) and adiponectin (Human Serum Adiponekine - Panel A; Lincoplex kit: Cat. # HADK1-61K-A) were measured with a multiplex testing Luminex Model no. Luminex 200 IS Serial No. LX10006265401. Serum levels of IL-6 and MCP-1 were measured by Quantikine High Sensitive Human Immunoassays (R&D Systems, Inc.), according to the manufacturer's instructions. This method employs solid-phase ELISA techniques. For IL-6, the lower detection limit is 0.039 pg/ml. The intra-assay coefficients of variation (CVs) were 6.9% to 7.8% over the range 0.43–5.53 pg/ml. For MCP-1, the lower detection limit is 5.0 pg/ml. The intra-assay coefficients of variation (CVs) were 4.7% to 7.8% over the range 76.7 – 1121 pg/ml.

Statistical analysis

Data are presented as mean $\pm$ SD unless otherwise specified. All analyses were performed using the SAS package for Windows (9.1 Version Cary, NC). Differences in mean values for each of the measured variables among groups were tested by ANOVA, followed by Bonferroni's test for multiple comparisons. An ANCOVA was used to assess differences in mean values of arterial properties in subgroups of subjects stratified according to the presence of MetS and/or extreme values of individual cytokines, after controlling for age, sex, individual components of MetS, and LDL cholesterol.

Univariate and multivariable linear regression analyses for continuous variables, or logistic regression for categorical variables, were conducted to detect associations of covariates and arterial structure or function. These models were constructed with arterial stiffness or thickness, alternatively, as dependent variables. The model fit for logistic regression was verified using the Hosmer and Lemeshow goodness of fit test. A two-sided p value <0.05 indicated statistical significance.

RESULTS

Cytokine levels in MetS and control subjects

Cytokine levels, except for MCP1, differed between control subjects and MetS ($p<0.0001$), after controlling for age and sex. Specifically, adiponectin levels were lower, whereas leptin, hsCRP, and IL6 levels were dramatically higher in MetS than in control subjects (Table 1). Differences in MetS components and arterial wall characteristics are listed in Supplement Table 1. These differences are similar to those noted previously (4). In univariate analysis, cytokines were significantly correlated with MetS and its components (Supplement Table 2). For some cytokines the correlation was stronger with lipids (adiponectin), or with elevated blood pressure (MCP1, IL6), or adiposity (leptin). Additionally, the prevalence of subjects in the upper quartile (lower quartile for adiponectin) for cytokines increased as the number of altered MetS components increased (Supplement Figure 1). Any altered component of MetS was associated with a higher prevalence of extreme cytokine values.

Cytokine levels and arterial PWV and IMT

Univariate analyses showed that all cytokines were significantly and positively associated with PWV, PWV/MBP, CCA IMT, and CCA CSA (Supplement Table 3). These associations remained highly significant even after adjustment for age and sex. The association of “proinflammatory” cytokines and stiffer and thicker arteries was further investigated by comparing PWV and CCA IMT values in subjects with varying numbers of cytokines with extreme values. As shown in Supplement Table 4, after controlling for age, sex, and traditional CV risk factors, having 3 or more cytokines with extreme values was associated with significantly stiffer and thicker arteries. Out of all the combinations of 3 MetS components tested, some specific clusters of altered cytokines were preferentially associated with increased arterial thickness and stiffness (Supplement Table 4 right columns).

Concurrent vs. independent associations of MetS and cytokines and arterial stiffness and thickness

Are MetS and cytokine associations with accelerated arterial aging independent of each other?

Multiple regression models were constructed with arterial stiffness or thickness, alternatively, as dependent variables. The first set of models included age, sex, LDL cholesterol levels, and individual components of the MetS as covariates; in the second set of models, full cytokine panel was added as a covariate. In Model 1, age, sex, MetS ($p < 0.0001$), together with waist circumference, SBP, DBP, and fasting glucose levels, were all significantly associated with PWV (Table 2 Model 1). Addition of the full panel of cytokine panel to the multivariable regression model (Model 2) did not change the impact of the above mentioned significant variables on PWV (Table 2 Model 2). Leptin ($p < 0.001$) and hsCRP ($p < 0.0001$) levels emerged as additional significant determinants of arterial stiffness, independent of MetS and its components. Similar results were observed when PWV was normalized for MBP, although the model ($R^2=0.38$) was lower than that observed for “absolute” PWV.

Age, sex, MetS ($p < 0.0001$) together with waist circumference, SBP, DBP, HDL and LDL cholesterol, and triglyceride levels were all significantly associated with CCA IMT (Table 2, Model 1). Addition of the full panel of cytokine levels to the multivariable regression model 1, attenuated the impact of waist, HDL and LDL cholesterol levels on CCA IMT levels. Adiponectin ($p < 0.05$) and IL6 ($p < 0.0001$) levels emerged as significant determinants of CCA IMT, independent of MetS and its components (Table 2 Model 2). Similar results were observed when CCA IMT was expressed as CSA – thus accounting for arterial diameter, and the model R^2 (0.51) was higher than that observed for CCA IMT ($r^2=0.48$). When secondary analyses were run with further adjustment for smoking status, the results remained virtually unchanged for both PWV and CCA IMT.

Thus, multiple regression analysis models above confirmed that the association of MetS and arterial stiffness and thickness is independent of cytokine levels. Yet, specific inflammatory cytokines play and independently associated with these arterial properties.

To test for a possible interaction between cytokines and MetS to accelerate increasing arterial stiffness or thickness, we performed an ANCOVA analysis with age, sex, levels in LDL cholesterol and MetS components, and cytokine interaction terms between MetS and cytokine. The interaction terms between MetS and leptin or hsCRP (for PWV) and or adiponectin or IL6 (for CCA IMT) were significant ($p < 0.001$). To better visualize the interaction between MetS and specific cytokines on arterial stiffness and thickness, average levels of PWV and CCA IMT in four subgroups defined by the presence/absence of MetS and by the presence/absence of individual cytokine levels in the upper quartile (the lower for adiponectin) are presented in Figure 1. Higher leptin was associated with higher PWV in both subjects with or without MetS ($p < 0.001$) (Figure 1 top left panel). A higher hsCRP level was associated with a higher PWV in control ($p < 0.001$) but not MetS subjects (Figure 1 top right panel). With regard to CCA IMT, low adiponectin was associated with higher IMT only in subjects with MetS ($p < 0.001$) (Figure 1 bottom left panel), whereas higher IL6 level was accompanied by higher CCA IMT in control, but not in MetS subjects (Figure 1 bottom right panel). Thus, the co-occurrence of both MetS and specific higher cytokine levels was associated with greater increases in arterial stiffness and thickness.

Are specific clusters of MetS component having differing, cytokine levels differentially associated with arterial function and structure?

Supplement Tables 5– 6 – 7 summarize the metabolic, arterial properties, and cytokine profile of subjects according to their clusters of MetS components.

Finally, we estimated the role of cytokines in the arterial burden associated with specific clusters of MetS components. The 2–2.5 fold higher odds of presenting an extremely high PWV that was associated with clusters of T-B-W or G-B-W or G-T-B (-W) components, as previously described (4), was **not** affected by cytokine levels. Similarly, the 2.5–3 fold higher odds of extremely thick CCA IMT associations with clusters of altered H-B-W or G-T-B (-W) components, as previously described (4), was also **not** affected by cytokine levels.

DISCUSSION

A major strength of the present study is the large study population, its broad age range, and inclusion of key arterial parameters and of several cytokines in addition to MetS components. Indeed, most published studies investigating the relationship of cytokines with arterial properties have focused on fewer cytokines, introduced individually (i.e. not simultaneously) in the predictive models, often without including MetS components amongst the covariates.

The main findings of our study are that: (1) cytokine levels – except for MCP1 - differ between control subjects and MetS; (2) MetS remained significantly associated with arterial aging, independent of age, sex, and levels of individual MetS components; (3) the association between MetS and arterial stiffness or thickness, however, is independent of cytokine levels; (4) nonetheless, specific cytokines are independently associated with arterial stiffness (higher leptin and higher hsCRP) or thickness (lower adiponectin and higher IL6) – independent of age, sex, and other traditional CV risk factors; and (5) the presence of both

MetS and elevated cytokine levels is associated with a much greater stiffening and thickening of central arteries.

Cytokine levels have been reported to be increased (decreased for adiponectin) in subjects with altered components of MetS or diabetes mellitus (10), and there is evidence to support their possible role as causative factors or mediators of vascular damage observed in MetS (5). Cytokines are involved in complex signaling pathways and some of them regulate the elaboration of others (e.g., IL6 stimulates hepatic production of hsCRP). The present study confirms that significantly lower level of adiponectin and higher levels of leptin, hsCRP, and IL6 occur in subjects with the MetS, as well as in different clusters of MetS components.

These findings further support the hypothesis that structural and functional changes in adipocytes may represent one of the primary alterations underlying the metabolic “derangement” leading to the metabolic syndrome and linking MetS with CV disease. Indeed, increased number, size, and turnover of adipocytes, infiltration of adipose by mononuclear cells, and rarefaction of blood vessels in the adipose tissue have been described as an early alteration in obesity (13). Adipose tissue bioactive secreted products, for instance lower levels of adiponectin, have, in turn, been associated with reduced endothelium-dependent vasodilation (14) and higher risk of CV events (15–16).

With specific regards to arterial properties, prior studies had reported that CRP levels are associated with increased arterial stiffness in various study populations (normal subjects, hypercholesterolemia, hypertension, population study) (17–20). Of note, in all of these studies – including the present one - CRP levels were within the normal range, reflecting an association of “subclinical inflammation” and arterial stiffness. In healthy adults, circulating levels of leptin are also associated with reduced arterial distensibility (21). In some studies, adiponectin levels were inversely associated with CCA IMT (22–24). The Malmo Study, for example, reported a gender-specific inverse relationship between circulating adiponectin levels and carotid IMT (significant in men, but not in women), but this became insignificant after accounting for MetS components (25). In contrast, a lack of significant association between adiponectin and CCA IMT has been reported by the European Group on Insulin Resistance (26). These conflicting results may, at least in part, be explained by differences in selection of covariates or in the population-based study design.

The study design does not allow speculation about pathophysiological pathways linking selectively specific cytokines to arterial aging nor allow to hypothesize whether the increased levels of circulating inflammatory markers that we observed to be associated with arterial aging (adiponectin, leptin, hsCRP, IL6) may reflect inflammation generated by cells within the arterial wall (27), and not only cytokines derived from adipose tissue (28) – a key factor in the natural history of MetS (29).

Potential clinical impact

The present study shows that MetS and circulating cytokine levels are independently associated with accelerated arterial thickening and stiffening. Nonetheless, when MetS and elevated cytokine levels occur together, their impact on central arterial aging is additive resulting in much greater arterial stiffness and thickness. Thus, prevention or treatment

strategies to reduce the risk of CV events associated with increased arterial stiffness and thickness might need to be more intensive in subjects presenting both subclinical inflammation, reflected in cytokine levels, and metabolic alterations that underlie MetS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. 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). Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. JAMA. 2001; 285:2486–2497. [PubMed: 11368702]
2. Scuteri A, Najjar SS, Morrell CH, Lakatta EG. The metabolic syndrome in older individuals: prevalence and prediction of cardiovascular events: the Cardiovascular Health Study. Diabetes Care. 2005; 28:882–887. [PubMed: 15793190]
3. Scuteri A, Najjar SS, Muller DC, Andres R, Hougaku H, Metter EJ, Lakatta EG. Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. JACC. 2004; 43:1388–1395. [PubMed: 15093872]
4. Scuteri A, Najjar SS, Orru' M, Usala G, Piras MG, Ferrucci L, Cao A, Schlessinger D, Uda M, Lakatta EG. The central arterial burden of the metabolic syndrome is similar in men and women: the SardiNIA Study. Eur Heart J. 2010; 31:602–613. [PubMed: 19942601]
5. Grundy SM. Inflammation, hypertension, and the metabolic syndrome. JAMA. 2003; 290:3000–3002. [PubMed: 14665663]
6. Scuteri A, Tesauro M, Rizza S, Iantorno M, Federici M, Lauro D, Campia U, Turriziani M, Fusco A, Cocciolillo G, Lauro R. Endothelial function and arterial stiffness in normotensive normoglycemic first-degree relatives of diabetic patients are independent of the metabolic syndrome. Nutr Metab Cardiovasc Dis. 2008; 18:349–356. [PubMed: 17935958]
7. Beltowski J. Leptin and atherosclerosis. Atherosclerosis. 2006; 189:47–60. [PubMed: 16580676]
8. Shimada K, Miyazaki T, Daida H. Adiponectin and atherosclerotic disease. Clin Chim Acta. 2004; 344:1–12. [PubMed: 15149866]
9. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation. 1998; 98:731–733. [PubMed: 9727541]
10. Barzilay JI, Abraham L, Heckbert SR, Cushman M, Kuller LH, Resnick HE, Tracy RP. The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. Diabetes. 2001; 50:2384–2389. [PubMed: 11574423]
11. Pilia G, Chen WM, Scuteri A, Orru M, Albai G, Dei M, Lai S, Usala G, Lai M, Loi P, Mameli C, Vacca L, Deiana M, Olla N, Masala M, Cao A, Najjar SS, Terracciano A, Nedorezov T, Sharov A, Zonderman AB, Abecasis GR, Costa P, Lakatta E, Schlessinger D. Heritability of cardiovascular and personality traits in 6,148 Sardinians. PLoS Genet. 2006; 2:e132. [PubMed: 16934002]

12. Scuteri A, Najjar SS, Orru' M, Albai G, Strait J, Tarasov KV, Piras MG, Cao A, Schlessinger D, Uda M, Lakatta EG. Age- and gender-specific awareness, treatment, and control of cardiovascular risk factors and subclinical vascular lesions in a founder population: The SardiNIA Study. *Nutr Metab Cardiovasc Dis.* 2009; 19:532–534. [PubMed: 19321325]
13. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue *J Clin Invest.* 2003; 112:1785–1788.
14. Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, Kumada M, Ohashi K, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Nagasawa A, Kobayashi H, Hiraoka H, Komai N, Kaibe M, Rakugi H, Ogiwara T, Matsuzawa Y. Hypertension. 2003; 42:231–234.
15. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules. *Circulation.* 1999; 100:2473–2476. [PubMed: 10604883]
16. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA.* 2004; 291:1730–1737. [PubMed: 15082700]
17. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-Reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arterioscler Thromb Vasc Biol.* 2004; 24:969–974. [PubMed: 15001456]
18. Mahmud A, Freely J. Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension.* 2005; 46:1118–1122. [PubMed: 16216991]
19. Mattace-Raso FU, van der Cammen TJM, van der Meer IM, Schalekamp MA, Asmar R, Hofman A, Witteman JC. C-reactive protein and arterial stiffness in older adults: the Rotterdam Study. *Atherosclerosis.* 2004; 176:111–116. [PubMed: 15306182]
20. Tsioufis C, Dimitriadis K, Selima M, Thomopoulos C, Mihos C, Skiadas I, Tousoulis D, Stefanadis C, Kalikazaros I. Low-grade inflammation and hypoadiponectinaemia have an additive detrimental effect on aortic stiffness in essential hypertensive patients. *European Heart Journal.* 2007; 28:1162–1169. [PubMed: 17446227]
21. Singhal A, Farooqi IS, Cole TJ, O'Rahilly S, Fewtrell M, Kattenhorn M, Lucas A, Deanfield J. Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? *Circulation.* 2002; 106:1919–1924. [PubMed: 12370213]
22. Pilz S, Horejsi R, Moller R, Almer G, Scharnagl H, Stojakovic T, Dimitrova R, Weihrauch G, Borkenstein M, Maerz W, Schauenstein K, Mangge H. Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. *J Clin Endocrinol Metab.* 2005; 90:4792–4796. [PubMed: 15928248]
23. Iglseder B, Mackevics V, Stadlmayer A, Tasch G, Ladurner G, Paulweber B. Plasma adiponectin levels and sonographic phenotypes of subclinical carotid artery: data from the SAPHIR Study. *Stroke.* 2005; 36:2577–2582. [PubMed: 16282542]
24. Lo J, Dolan SE, Kanter JR, Hemphill LC, Connelly JM, Lees RS, Grinspoon SK. Effects of obesity, body composition, and adiponectin on carotid intima-media thickness in healthy women. *J Clin Endocrinol Metab.* 2006; 91:1677–1682. [PubMed: 16522690]
25. Nilsson PM, Engstrom G, Hedblad B, Frystyk J, Persson M, Berglund G, Flyvbjerg A. Plasma adiponectin levels are inversely associated with carotid intima media thickness and markers of insulin resistance in middle-aged men. *Arterioscler Thromb Vasc Biol.* 2006; 26:2758–2762. [PubMed: 17038635]
26. Patel S, Flyvbjerg A, Kozakova M, Frystyk J, Ibrahim IM, Petrie JR, Avery PJ, Ferrannini E, Walker M. the RISC Investigators. Variation in the ADIPOQ gene promoter is associated with carotid intima media thickness independent of plasma adiponectin levels in healthy subjects. *Eur Heart J.* 2008; 29:386–393. [PubMed: 18084018]
27. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res.* 2001; 89:763–771. [PubMed: 11679405]
28. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab.* 1998; 83:847–850. [PubMed: 9506738]
29. Scuteri A, Morrell CH, Najjar SS, Muller D, Andres R, Ferrucci L, Lakatta EG. Longitudinal paths to the metabolic syndrome: can the incidence of the metabolic syndrome be predicted? The

Baltimore Longitudinal Study of Aging. J Gerontol A Biol Sci Med Sci. 2009; 64:590–598.
[PubMed: 19270183]

Figure 1.

Least square means (after adjustment for age, sex, levels in each factor contributing to the MetS, and LDL cholesterol) for PWV in subjects with or without MetS according to the presence of extreme values of each cytokine that emerged as a significant determinant of PWV in multiple regression analysis (TOP PANELS) and for CCA IMT in subjects with or without MetS according to the presence of extreme values of each cytokine that emerged as a significant determinant of CCA IMT in multiple regression analysis (BOTTOM PANELS).

C= controls, i.e. subjects without MetS

Table 1

Comparison of cytokine levels in control subjects and in those with MetS

	C	MetS	P&
N=	5724	399	
Age	43 ± 17	60 ± 13	0.0001
Male Sex	42.3	46.4	
Adiponectin (mg/ml)	2.8 ± 1.9	2.4 ± 1.6	0.0001
Leptin (pg/ml)	7465 ± 7554	11587 ± 10520	0.0001
HsCRP (mg/ml)	2.5 ± 3.6	4.4 ± 5.2	0.0001
MCP1 (pg/ml)	277 ± 168	303 ± 178	0.84
IL6 (pg/ml)	2.9 ± 2.5	4.4 ± 3.1	0.0001
Adiponectin 25 th percentile (<1.54 mg/dl)(%)	25.9	35.3	0.0001
Leptin 75 th percentile (>11000 pg/ml) (%)	22.6	38.6	0.0001
hsCRP 75 th percentile (> 3.07 mg/ml) (%)	22.0	44.1	0.0001
MCP1 75 th percentile (>331 pg/ml) (%)	23.9	30.6	0.80
IL6 75 th percentile (>3.64 pg/ml) (%)	22.5	45.1	0.0001
N of cytokine in the extreme quartile (%)			0.0001
0	30.3	9.8	
1	36.7	29.8	
2	21.8	29.6	
3	8.5	20.3	
4-5	2.7	10.5	

& After adjustment for age and sex

Table 2

Multiple regression analyses to identify the role of MetS or proinflammatory cytokines as independent determinants of arterial function (PWV) and structure (CCA IMT)

	PWV				CCA IMT			
	Model 1		Model 2		Model 1		Model 2	
	Beta ± SE	P	Beta ± SE	P	Beta ± SE [°]	P	Beta ± SE [°]	P
Age	6.34 ± 0.16	\$	6.20 ± 0.18	\$	40.5 ± 0.9	\$	39.5 ± 1.0	\$
Female Sex	-23.8 ± 4.8	\$	-13.5 ± 6	*	185.6 ± 25.9	\$	208.9 ± 32.2	\$
Waist	2.12 ± 0.22	\$	1.70 ± 0.25	\$	2.2 ± 1.2	.06	1.9 ± 1.4	
SBP	2.95 ± 0.18	\$	2.97 ± 0.18	\$	8.5 ± 0.9	\$	8.2 ± 1.0	\$
DBP	-1.03 ± 0.29	&	-0.99 ± 0.31	**	-9.6 ± 1.6	\$	-8.7 ± 1.6	\$
LDL Chol	0.05 ± 0.06		0.07 ± 0.07		-1.2 ± 0.3	&	-0.8 ± 0.4	*
HDL Chol	0.10 ± 0.15		0.24 ± 0.16		-2.8 ± 0.8	&	-2.7 ± 0.9	**
Triglycerides	0.05 ± 0.04		0.04 ± 0.05		-0.5 ± 0.2	*	-0.6 ± 0.2	*
F. Glucose	0.49 ± 0.10	\$	0.51 ± 0.11	\$	0.3 ± 0.5		1.0 ± 0.6	
MetS	51.1 ± 10.0	\$	46.9 ± 10.5	\$	224.6 ± 53.6	\$	194.0 ± 56.2	&
Adiponectin	---		-1.31 ± 1.30		---		14.9 ± 6.6	*
Leptin [°]	---		11.6 ± 3.3	&	---		30.7 ± 17.7	
HsCRP	---		2.20 ± 0.63	&	---		-0.9 ± 3.4	
MCP1	---		-0.02 ± 0.01		---		0.1 ± 0.1	
IL-6	---		1.41 ± 0.96		---		18.3 ± 5.1	&

[°] multiplied 10⁻⁴

* p<0.05

** p<0.01

& p<0.001

\$ p<0.0001