Early Markers of Atherosclerotic Disease in Individuals with Excess Weight and Dyslipidemia
Eduardo Menti, Denise Zaffari, Thais Galarraga, João Regis da Conceição e Lessa, Bruna Pontin, Lucia Campos Pellanda, Vera Lúcia Portal
Instituto de Cardiologia – Fundação Universitária de Cardiologia, Porto Alegre, RS – Brazil

Abstract

Background: Excessive weight is a cardiovascular risk factor since it generates a chronic inflammatory process that aggravates the endothelial function.

Objective: To evaluate the endothelial function in individuals with excess weight and mild dyslipidemia using brachial artery flow-mediated dilation (BAFMD), and the association of endothelial function with anthropometric and biochemical variables.

Methods: Cross-sectional study that included 74 individuals and evaluated anthropometric variables (body mass index [BMI], waist-hip ratio [WHR], waist circumference [AC], and percentage of body fat [PBF]), biochemical (blood glucose, insulinemia, ultrasensitive C-reactive protein, fibrinogen, total cholesterol, HDL-cholesterol, triglycerides, and LDL-cholesterol) and endothelial function (BAFMD, evaluated by ultrasound). The statistical analysis was performed with SPSS, version 16.0. To study the association between the variables, we used chi-square, Student’s t and Mann-Whitney tests, and Pearson’s correlation. Logistic regression analyzed the independent influence of the factors. Values of p < 0.05 were considered significant.

Results: The participants had a mean age of 50.8 years, and 57% were female. BMI, WC, WHR, and PBF showed no significant association with BAFMD. The male gender (p = 0.02) and higher serum levels of fibrinogen (p = 0.02) were significantly and independently associated with a BAFMD below 8%.

Conclusions: In individuals with excess weight and mild untreated dyslipidemia, male gender and higher levels of fibrinogen were independently associated with worse BAFMD. (Arq Bras Cardiol. 2016; 106(6):457-463)

Keywords: Atherosclerosis; Biomarkers; Endothelium; Obesity; Dyslipidemias.

Introduction

When endothelial cells are exposed to risk factors such as hypertension, smoking, insulin resistance, and obesity, they are stimulated to express adhesion molecules on their surface, recruiting several classes of leukocytes and promoting the initial signaling mechanisms for cellular changes and atheroma formation. Endothelial dysfunction may be detected even before the occurrence of obstructive atherosclerotic plaques. The amount of nitric oxide released by endothelial cells depends on the integrity of the endothelium and determines the degree of vasodilation. The most used method to estimate endothelial dysfunction is the evaluation of the brachial artery diameter before and after distal tissue ischemia (hyperemic reaction). This measurement has applications in population studies, but its individual application has not been established yet. Dilatation values between 8 and 10% seem to be the best discriminators between normal and abnormal endothelial functions.

Obesity and excessive weight are able to change the vascular endothelium function. There is growing recognition that obesity is characterized by a low degree of chronic and subclinical inflammation. The exact mechanisms that stimulate this sustained inflammation have not been elucidated yet but are highly relevant to the atherothrombotic process.

It is, thus, crucial to identify variables that could predict the progression of the disease and the occurrence of clinically significant events in obese individuals. This study evaluated the occurrence of associations of anthropometric measures and metabolic and inflammatory markers with endothelial function assessed by brachial artery dilation in individuals with excess weight and mild untreated dyslipidemia. The objective was to identify the variable with a better ability to predict the occurrence of subclinical atherosclerosis and, consequently, more useful in the clinical follow-up of individuals with excess weight.

Methods

This study is part of a research conducted at Instituto de Cardiologia involving individuals with excess weight and...
dyslipidemia. The sample was obtained by convenience, and the study of the endothelial function was performed in one in every four participants undergoing nutritional and anthropometric follow-up, in a total of 74 individuals.

Inclusion criteria
The study included men and women aged 35–60 years, with dyslipidemia and excess weight, and without a history of clinically manifested cardiovascular disease. Dyslipidemia was considered present when the levels of at least one of the following biochemical parameters was abnormal: total cholesterol (TC) > 200 mg/dL, and/or triglycerides (TG) > 150 mg/dL, and/or HDL-cholesterol < 40 mg/dL in men and < 50 mg/dL in women. Excess weight was assessed with the body mass index (BMI), and the participants had BMI values between 25 and 35 kg/m².

Exclusion criteria
Exclusion criteria were the occurrence of neoplasms, infections, and liver, kidney and gastrointestinal disorders; levels of LDL-cholesterol > 160 mg/dL and TG > 400 mg/dL; pregnancy and lactation; alcohol consumption above four doses a day; use of estrogen, nonsteroidal anti-inflammatory, antiobesity agents, and vitamin supplementation; use of statins, fibrates, and other lipid-lowering medications; unexplained weight loss (greater than 2 kg) in the last 30 days.

Ethical aspects
The study was approved by the Ethics Committee in Research (Comitê de Ética em Pesquisa, COEP) at Fundação Universitária de Cardiologia. All patients were informed about the study by reading and analyzing the free and informed consent form and agreed to participate. The research protocol did not interfere with any medical recommendation or prescription.

Study protocol
The selected individuals answered a standardized questionnaire and their anthropometric measurements (BMI, waist circumference [WC], waist-hip ratio [WHR], and body fat percentage), metabolic profile (blood glucose, insulin, TC, HDL-cholesterol, and TG), and inflammatory profile (C-reactive protein [CRP] and fibrinogen) were analyzed. The endothelial function was assessed with brachial artery flow-mediated dilatation (BAFMD). The technique used in this study was that recommended by the American Society of Echocardiography and Society of Vascular Medicine and Biology, based on the percentage modification of the brachial artery diameter by reactive hyperemia.

Statistical analysis
The results are presented as mean ± standard deviation for continuous variables. WC, WHR, and BMI were treated as qualitative variables using cutoff points described in the literature for values considered abnormal. Values of WC and WHR were considered abnormal in men when above 102 cm and 0.9, respectively, and in women when above 88 cm and 0.85, respectively. Values of BMI between 25 and 30 kg/m² were considered as overweight and those equal to or above 30 kg/m² as obesity. The association of the variables was analyzed with the chi-square test for dichotomous variables, Student's t test for parametric continuous variables, and Mann-Whitney test for nonparametric continuous variables. Results of ultrasensitive CRP (usCRP) are presented as median since this is a variable with a non-Gaussian distribution. Differences were considered statistically significant for p values < 0.05. Additionally, logistic regression was conducted to assess the independent influence of factors significantly associated with the endothelial vasodilation response and Pearson's correlation test to estimate the degree of linear relationship between the serum level of fibrinogen and the percentage of dilation of the brachial artery. We used the statistical program SPSS, version 16.0 (SPSS Inc., Chicago, USA).

Results
The participants had a mean age of 50.88 ± 6.14 years, and 57% were female. All individuals had excess weight with a mean BMI value of 28.82 ± 2.60 kg/m² and some degree of dyslipidemia, with mean values of TC of 222.67 ± 34.24 mg/dL, HDL-cholesterol of 45.68 ± 14.83 mg/dL, LDL-cholesterol of 146.05 ± 32.02 mg/dL, and TG of 154.66 ± 79.37 mg/dL (Table 1). The WC was increased in 46.9% of the men and 75.0% of the women while the WHR was abnormal in 90.5% of the men and 38.1% of the women. The percentage of body fat varied between 14.81% and 36.14%, with a mean value of 22.64% ± 6.87%.

Table 1 – Characteristics of the cohort

| Characteristic | n  | Statistics |
|---------------|----|------------|
| Age (years)   | 74 | 50.88 ± 6.14 |
| Female gender (%) | 74 | 42 (57%) |
| Smokers (%)   | 74 | 11 (14.8%) |
| Body mass index (kg/m²) | 74 | 28.82 ± 2.60 |
| Waist circumference (cm) | 74 | M: 101.48 ± 7.25 |
| Waist/hip ratio | 74 | M: 0.93 ± 0.05 |
| Percentage of body fat (%) | 74 | M: 21.53 ± 3.28 |
| Insulin       | 74 | 10.57 ± 6.09 |
| Blood glucose (mg/dL) | 74 | 101.45 ± 29.45 |
| Total cholesterol (mg/dL) | 74 | 222.67 ± 34.24 |
| HDL-cholesterol (mg/dL) | 74 | M: 39.52 ± 8.44 |
| LDL-cholesterol (mg/dL) | 74 | F: 50.24 ± 16.73 |
| Triglycerides (mg/dL) | 74 | 146.05 ± 32.02 |
| Fibrinogen (mg/dL) | 74 | 154.66 ± 29.45 |
| Ultrasensitive C-reactive protein (mg/L) | 74 | 266.00 ± 63.06 |

Data are presented as mean ± standard deviation and median or value (percentage). HDL-cholesterol: high-density cholesterol; LDL-cholesterol: low-density lipoprotein cholesterol; M: male; F: females.
23.19 ± 4.12%. Only eight individuals had body fat percentage values above those compatible with obesity (25% in men and 32% in women). The individuals were then subdivided into groups of overweight and obesity. According to this criterion, 29.7% of the sample was composed of obese individuals.

The diameter of the brachial artery varied 7.80 ± 6.41% during the BAFMD when compared with its baseline value (Table 1). The median BAFMD value was 8%, which served as a cutoff point for a qualitative analysis between individuals with vasodilation responses above and below this value.

WC, WHR, and BMI, treated as qualitative variables, showed no association with the degree of vasodilation response treated as a continuous variable (verified by Student's t test) or qualitative variable (verified with the chi-square test, with a cutoff point of 8% for the BAFMD result) (Table 2). The male gender showed a significant association with a worse vasodilation response, i.e., men had more frequently BAFMD values below 8% (p = 0.03) (Figure 1).

The biochemical results of the metabolic parameters and inflammatory markers were treated as quantitative variables and their associations with the endothelial function were verified with Student's t test (Table 2). Fibrinogen was the only biochemical parameter significantly associated with the endothelial function (p = 0.02) (Figure 2). When this association was evaluated by quartiles of dilation, we observed that for dilation values below 3.7%, the mean serum fibrinogen was of 295.50 ± 50.41 mg/dL, whereas for dilation values greater than 13.03%, the mean was 229.41 ± 48.95 mg/dL (Figure 3).

After we had observed the association of the male gender and serum fibrinogen level with worse brachial artery vasodilation response, we performed a logistic regression analysis to verify whether this would be an independent association. The results demonstrated that the associations between endothelial function with male gender and serum levels of fibrinogen remained significant. The male gender increased the chances of a worse vasodilation response by approximately three times (odds ratio [OR] 3.33; 95% confidence interval [CI] 1.19 – 9.28, p = 0.02), while an increase in 1 mg/dL in serum fibrinogen level increased this risk in 1% (OR 1.01, 95% CI 1.00 – 1.01, p = 0.02). Therefore, it would be expected that an increase of 100 mg/dL in serum fibrinogen level would increase in approximately two times the risk of a worse vasodilation brachial artery response.

The variables were additionally evaluated with Pearson's correlation test, and the correlation factor with the dilation of the brachial artery for fibrinogen was -0.31 (p = 0.008).

**Table 2** – Association between anthropometric, metabolic and inflammatory variables with brachial artery flow-mediated dilatation

| Variable                   | BAFMD < 8% | BAFMD ≥ 8% | p     |
|----------------------------|------------|------------|-------|
| Male gender                | 21         | 11         | p = 0.03 |
| BMI > 30 kg/m² †           | 10         | 12         | p = 0.09 |
| Abnormal WC † Men: > 102 cm; Women: > 88 cm | 24         | 29         | p = 0.03 |
| Abnormal WHR † Men: > 0.85; Women: > 0.90 | 21         | 19         | p = 0.51 |
| Percentage of body fat ‡   | 23.04      | 23.34      | p = 0.22 |
| Insulin ‡                  | 9.60       | 11.63      | p = 0.15 |
| Blood glucose              | 99.60      | 103.00     | p = 0.59 |
| LDL-cholesterol ‡          | 146.50     | 145.57     | p = 0.90 |
| HDL-cholesterol ‡          | 42.63      | 49.00      | p = 0.06 |
| Triglycerides ‡            | 167.11     | 141.14     | p = 0.16 |
| Fibrinogen ‡               | 281.55     | 248.62     | p = 0.02 |
| UsCRP *                    | 0.17       | 0.36       | p = 0.14 |

*nonparametric variable, association verified with the Mann-Whitney test; † association verified with the chi-square test; ‡ parametric variables, association verified with Student's t test. BAFMD: brachial artery flow-mediated dilatation; BMI: body mass index; WC: waist circumference; WHR: waist/hip ratio; LDL-cholesterol: low-density lipoprotein cholesterol; HDL-cholesterol: high-density cholesterol; UsCRP: ultrasensitive C-reactive protein.

**Discussion**

In a cohort of individuals with excess weight, mild dyslipidemia, and without clinically significant atherosclerotic disease, we found that the male gender and high levels of serum fibrinogen were associated with worse endothelial function determined by BAFMD. Our study suggests the relevance of measuring circulating fibrinogen as a marker of subclinical atherosclerosis in individuals with excess weight without manifested atherosclerotic disease.

The association of the male gender with worse endothelial function is aligned with clinical and epidemiological observations that the male gender is an important risk factor for atherosclerotic disease. By studying the influence of risk factors on endothelial function in asymptomatic individuals, different researchers have demonstrated an independent and significant association of the male gender with worse BAFMD.18-20
The inclusion of individuals with a mean age of 50 years in our study confirms this association, since at this age men have a higher cardiovascular risk than women.

Elevated fibrinogen levels are strongly associated with atherosclerotic disease. The ARIC (Atherosclerosis Risk in Communities) study has shown an increased risk of coronary disease with higher levels of fibrinogen, with a relative risk of 1.76. In the PROCAM (Prospective Cardiovascular Münster) study, the occurrence of death due to coronary disease and nonfatal infarction was greater among individuals with higher levels of fibrinogen. In that study, fibrinogen levels were better risk predictors than BMI and levels of LDL-cholesterol. In a meta-analysis that included 22 studies evaluating the association between serum concentration of fibrinogen and cardiovascular disease, the estimated risk of events in individuals with levels of fibrinogen in the highest tertile was two times greater than that in individuals with levels in the lowest tertile (OR 1.99, 95%CI 1.85 – 2.12). In children or adolescents with overweight or obesity, fibrinogen has also been associated with hsCRP elevation and with the
occurrence of four or more cardiovascular risk factors. In contrast, the association between fibrinogen and markers of early atherosclerosis has already been demonstrated in studies evaluating the carotid myointimal thickening and BAFMD. In a series of asymptomatic individuals, elevated fibrinogen levels were significantly related to increased myointimal thickening, independent of other potentially confounding variables. The same has been observed in another study that evaluated fibrinogen and usCRP as markers of subclinical carotid atherosclerosis.

Similarly, greater myointimal carotid thickening, worse BAFMD, and higher concentrations of E-selectin and thrombomodulin have shown association with serum fibrinogen levels in obese children. Fibrinogen has also been described as more frequently increased in individuals with type 2 diabetes mellitus with metabolic syndrome than in those without metabolic syndrome. In addition, fibrinogen increases the risk of microvascular diseases, including diabetic retinopathy. A small study that has only evaluated the influence of fibrinogen in endothelium-dependent vasodilatation has observed an inverse relationship between plasma levels of fibrinogen and degree of BAFMD. When individuals with manifested heart disease are considered, fibrinogen also appears as a marker of worse brachial artery vasodilation response.

High serum levels of fibrinogen may promote vascular disease by increasing blood viscosity, stimulating fibrin formation, or increasing platelet-platelet interaction. Fibrinogen may also be simply a marker of vascular disease without contributing for its progression. The hepatic production of fibrinogen is regulated by cytokines whose concentrations increase in response to different inflammatory processes. In this context, excess weight has been associated with a higher production of inflammatory cytokines by the adipose tissue. This inflammatory status is due to a dysfunction in the interaction between adipocytes and tissue macrophages. CRP is also an acute phase inflammatory protein and its baseline levels are independent risk predictors of myocardial infarction and stroke, showing correlation with fibrinogen levels. Our study did not confirm an association between CRP and fibrinogen, which can be explained in part by the non-normal distribution of the CRP levels and the low levels detected in the serum. Similarly, the study lacked power to test the association between fibrinogen levels and degree of excess weight. This relationship has already been demonstrated in previous studies focusing on WC, body fat, BMI, and WHR. The narrow range of variation of the anthropometric parameters in our cohort seems to have influenced the lack of association of the adiposity measurements with endothelium-dependent vasodilation.

Obese individuals have a low-degree chronic inflammatory condition that manifests with worse flow-mediated vasodilation response. A relationship has already been demonstrated between markers of prothrombotic status, like fibrinogen and prothrombin activity, with the degree of visceral adiposity and other cardiovascular risk factors.

Weight reduction is able to revert the deleterious effect of excessive weight on endothelial function through mechanisms not yet fully known. These observations about fibrinogen levels in obese individuals bring an additional element to the final consideration that fibrinogen is intimately related to subclinical atherosclerotic disease in individuals with excess weight.
Study limitations

The results of this study suggest an association between male gender and fibrinogen levels with endothelial function in individuals with excess weight and dyslipidemia. However, since this was a cross-sectional study, it is unable to determine a cause-effect relationship between these variables.

The verification of the association between inflammatory markers and degrees of excess weight, as well as between the degrees of excess weight and endothelial dysfunction may have been compromised by the uniformity of the degrees of adiposity and the sample size.

Conclusion

The results of this study suggest that fibrinogen is associated with subclinical atherosclerosis in individuals with excess weight. New studies should clarify this association and establish the benefit of including fibrinogen as a marker in clinical practice to evaluate this group of patients.

Author contributions

Conception and design of the research: Menti E, Zaffari D, Galarraga T, Pontin B, Portal VL. Acquisition of data: Menti E, Zaffari D, Galarraga T, Portal VL. Analysis and interpretation of the data: Menti E, Zaffari D, Portal VL. Statistical analysis: Menti E, Zaffari D, Portal VL. Obtaining financing: Menti E, Zaffari D, Portal VL. Writing of the manuscript: Menti E, Portal VL. Critical revision of the manuscript for intellectual content: Menti E, Portal VL.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of master submitted by Eduardo Menti, from Fundação Universitária de Cardiologia do Rio Grande do Sul.

References

1. Ross R. Atherosclerosis – an inflammatory disease. N Engl J Med. 1999;340(2):115-26.
2. Toth PP. Subclinical atherosclerosis: what it is, what it means and what we can do about it. Int J Clin Pract. 2008;62(8):1246-54.
3. Ministério da Saúde. Datasus. Morbidade hospitalar do SUS. Por local de internação. Brasil. [Acesso em 2011 jul 7]. Disponível em: http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sih/cnv/nuf.def
4. Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clin Chem. 2008;54(1):124-38.
5. Kharbanda RK, Deanfield JE. Functions of the healthy endothelium. Coron Artery Dis. 2001;12(6):485-1.
6. Kuvin JT, Karas RH. Clinical utility of endothelial function testing: ready for prime time? Circulation. 2003;107(25):3243-7.
7. Coretti C, Anderson TJ, Benjamin EJ, Celemajer D, Charbonneau F, Creager MA, et al; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilatation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65. Erratum in: J Am Coll Cardiol 2002;39(6):1082.
8. Roman MJ, Narvi TZ, Gardin JM, Gerhard-Herman M, Jaffe M, Mohler E; American Society of Echocardiography; Society of Vascular Medicine and Biology. Clinical application of noninvasive vascular ultrasound in cardiovascular risk stratification: a report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology. J Am Soc Echocardiogr. 2006;19(8):943-54.
9. Al-Qaisi M, Kharbanda RK, Mittal TK, Donald AE. Measurement of endothelial function and its clinical utility for cardiovascular risk. Vasc Health Risk Manag. 2008;4(3):647-52.
10. Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzolani JO, et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. J Am Coll Cardiol. 2003;41(10):1769-75.
11. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. J Am Coll Cardiol. 2002;40(3):505-10.
12. Williams IL, Chowienczyk PJ, Wheatcroft SB, Patel AG, Sherwood RA, Momin A, et al. Endothelial function and weight loss in obese humans. Obes Surg. 2005;15(7):1055-60.
13. Benjamin EJ, Larson MG, Keyes MJ, Mitchell CF, Vasan RS, Keaney JF, et al. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. Circulation. 2004;109(5):613-9. Erratum in: Circulation. 2004;109(25):3256.
14. Hajer GR, van Haaften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes and vascular diseases. Eur Heart J. 2008;29(24):2959-71.
15. Avogaro A, de Kreutzgenburg SV. Mechanisms of endothelial dysfunction in obesity. Clin Chim Acta. 2005;360(1-2):1-10.
16. Grover-Pazé F, Zavaloa-Gómez AB. Endothelial dysfunction and cardiovascular risk factors. Diabetes Res Clin Pract. 2009;84(1):1-9.
17. Brook RD, Bard RI, Rubenfire M, Ridker PM, Rajagopalan S. Usefulness of visceral obesity (waist/hip ratio) in predicting vascular endothelial function in healthy overweight adults. Am J Cardiol. 2001;88(11):1264-9.
18. Celemajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol. 1994;24(6):1468-74.
19. Juonala M, Kähönen M, Laitinen T, Huhtti-Kähönen N, Jokinen E, Trautmann L, et al. Effect of age and sex on carotid intima-media thickness, elasticity and brachial endothelial function in healthy adults: the cardio-risk vascular study in Young Finns Study. Eur Heart J. 2008;29(9):1198-206.
20. Perreux D, Chauhduri A, Mohanty P, Bukhari L, Wilson MF, Sung BH, et al. Effect of gender differences and estrogen replacement therapy on vascular reactivity. Metabolism. 1999;48(2):227-32.
21. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 1997;96(4):1102-8.

22. Heinrich J, Balleisen L, Schulte H, Asmann G, Loo J. Fibrinogen and factor VII in the prediction of coronary risk: results from the PROCAM study in healthy men. Arterioscler Thromb. 1994;14(1):54-9.

23. Maresca G, Di Blasio A, Marchioli R, Di Minno G. Measuring plasma fibrinogen to predict stroke and myocardial infarction: an update. Arterioscler Thromb Vasc Biol. 1999;19(6):1368-77.

24. Azevedo WF, Cantalice AS, Gonzaga NC, Simões MO, Guimarães AL, Carvalho DF, et al. Fibrinogen: cardiometabolic risk marker in obese or overweight children and adolescents. J Pediatr (Rio J). 2015;91(5):464-70.

25. Páramo JA, Orbe J, Beloqui O, Benito A, Colina I, Martínez-Vila E, et al. Prothrombin fragment 1+2 is associated with carotid intima-media thickness in subjects free of clinical cardiovascular disease. Stroke. 2004;35(5):1085-9.

26. Kawase Ishihara K, Kokubo Y, Yokota C, Hida E, Miyata T, Toyoda K, et al. Associations of body mass index, waist circumference and plasma fibrinogen levels in overweight children and adolescents. J Pediatr (Rio J). 2011;88(19-20):839-45.

27. Meyers MR, Gokce N. Endothelial dysfunction in obesity: etiological role in atherosclerosis. Curr Opin Endocrinol Diabetes Obes. 2007;14(5):365-9.

28. Mahendra JV, Satish KD, Anuradha TS, Prashanth T, Nagaraj RS, Vishali V. Effect of plasma fibrinogen, high-sensitive C-reactive protein, and cigarette smoking on carotid atherosclerosis: the Suita study. J Stroke Cerebrovasc Dis. 2015;24(10):2385-9.

29. Allen JD, Wilson JB, Tulley RT, Lefevre M, Welsch MA. Influence of age and FDP on vascular smooth muscle cells by IL-6, TNF-α and iNOS. Life Sci. 2011;88(19-20):839-45.

30. Bosevski M, Borozanov V, Peovska I, Georgievska-Ismail L. Endothelial dysfunction correlates with plasma fibrinogen and HDL-cholesterol in type 2 diabetic patients with coronary artery disease. Bratisl Lek Listy. 2007;108(7):297-300.

31. Lu P, Liu J, Liu N, Guo F, Ji Y, Pang X. Pro-inflammatory effect of fibrinogen and FDP on vascular smooth muscle cells by IL-6, TNF-α and iNOS. Life Sci. 2011;88(19-20):839-45.

32. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. Circ Res. 2005;96(9):939-49.

33. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA. 1998;279(18):1477-82.

34. Dhangana R, Murphy TP, Pencina MJ, Zafar AM. Prevalence of low ankle-brachial index, elevated plasma fibrinogen and CRP across Framingham risk categories: Data from the National Health and Nutrition Examination Survey (NHANES) 1999–2004. Atherosclerosis. 2011;216(1):174-9.

35. Rana S, Arsenault BJ, Després JP, Côté M, Talmud PJ, Ninio E, et al. Inflammatory biomarkers, physical activity, waist circumference, and risk of future coronary heart disease in healthy men and women. Eur Heart J. 2011;32(3):336-44.

36. Saito I, Yonematsu K, Inami F. Association of body mass index, body fat, and weight gain with inflammation markers among rural residents in Japan. Circ J. 2003;67(4):323-9.

37. Maple-Brown LJ, Cunningham J, Nandi N, Hodge A, O’Dea K. Fibrinogen and associated risk factors in a high-risk population: urban indigenous Australians, the DRUID study. Cardiovasc Diabetol. 2010;9:69-75.

38. Gustafson B. Adipose tissue, inflammation and atherosclerosis. J Atheroscler Thromb. 2010;17(4):332-41.

39. Fain J. Release of inflammatory mediators by human adipose tissue is enhanced in obesity and primarily by the nonfat cells: a review. Mediators Inflamm. 2010;2010:513948.

40. Montilla M, Santi MJ, Carrozas M, Ruiz FA. Biomarkers of the prothrombotic state in abdominal obesity. Nutr Hosp. 2014;31(3):1059-66.

41. Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M, et al. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. Circulation. 2002;105(7):804-9.

42. Pierce GL, Beske SD, Lawson BR, Southall KL, Benay FJ, Donato AJ, et al. Weight loss alone improves conduit and resistance artery endothelial function in young and older overweight/obese adults. Hypertension 2008;52(1):72-9.

43. Raffidi LS, Lekakis J, Kolomvatsou A, Zampelas A, Yamvakou G, Efstathiou S, et al. Close adherence to a Mediterranean diet improves endothelial function in subjects with abdominal obesity. Am J Clin Nutr. 2009;90(2):263-8.

44. Dutschmann HH, Flechtner-Mors M, Adler G. Fibrinogen in obesity before and after weight reduction. Obes Res. 1995;3(1):43-8.