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Serum high sensitive C reactive protein is associated with dietary intake in diabetic patients with and without hypertension: a cross-sectional study

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Declarations

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Abstract

Background: Serum C reactive protein (CRP) concentrations independently predict the development of diabetes, metabolic syndrome and cardiovascular disease. However, the impact of dietary factors on serum CRP concentrations in diabetic patients has received limited attention. We aimed to investigate the association between dietary factors and serum CRP, measured using a high sensitivity (hs-)assay, among diabetic patients with and without hypertension and healthy subjects.

Methods: In this cross-sectional study, diabetics with (n=325) and without hypertension (n=599) and healthy individuals (n=1220) were recruited in Mashhad, Iran. Dietary intake was assessed by 24-hour recall. Biochemical parameters including serum hs-CRP were measured using standard protocols. Stepwise multiple regression analysis was used to predict whether serum hs-CRP concentration was associated with dietary constituents.

Results: hs-CRP was significantly higher among hypertensive and non-hypertensive diabetic patients compared to healthy subjects (p<0.001). The dietary intake of zinc +6.4% and calcium - 3.4%, and BMI +3.9% explained approximately 13.7% of the variation in serum hs-CRP among diabetic hypertensive patients. Approximately, 9.7% of the variation in serum hs-CRP in diabetic non-hypertensive patients could be explained by BMI, and intake of sodium, iron and cholesterol. In the healthy subjects approximately 4.4% of the total variation in serum hs-CRP concentration could be explained by cholesterol consumption and waist circumference.

Conclusion: Serum hs-CRP concentrations were found to be a significant predictor for hypertensive and non-hypertensive diabetic subjects. There was a significant association between dietary factors include zinc, iron, sodium and cholesterol and serum hs-CRP whilst there was an inverse association between dietary calcium and serum hs-CRP in diabetic hypertensive individuals.

Keywords: hs-CRP; inflammation; diabetes; hypertension; dietary intake.

Introduction

The prevalence of diabetes mellitus is increasing globally and has become a challenging issue for public health in both developed and developing nations.^{1, 2} In 2015, the number of people with diabetes mellitus was estimated to be approximately 415 million by the International Diabetes Federation (IDF).³ Approximately 7.7% of Iranian adults (2 million adults) have diabetes mellitus.⁴

Raised serum inflammatory markers, that include high sensitive C reactive protein (hs-CRP), are reported to be associated with several non-communicable conditions including obesity⁵ hypertension,⁶ metabolic syndrome,⁷ diabetes mellitus and cardiovascular disease.⁸ hs-CRP is a marker of systemic inflammation, and is produced by the liver in response to pro-inflammatory cytokines such as interleukin 6 (IL-6).⁹

Inflammatory factors are thought to be involved in the pathogenesis of insulin resistance,¹⁰ diabetes mellitus¹¹ and metabolic syndrome,¹² and it has been suggested that an elevated hs-CRP should be added to the definition of metabolic syndrome.⁸ Although there is a positive association between several factors such as smoking,¹³ physical inactivity,¹⁴ waist circumferences,¹⁵ BMI¹⁵ and serum hs-CRP, the relationship between specific dietary components and serum hs-CRP is unclear. A significant inverse association has been shown between some dietary items include fruits and vegetables,¹⁶ fish and poultry,¹⁷ dietary fiber,¹⁸ oleic acid,¹⁹ and serum hs-CRP, whereas, a significant positive association has been reported between the consumption of red meat,²⁰ trans-fat,²¹ and saturated fat,²² and serum hs-CRP. Other studies have reported no significant association between consumption of fiber,²³ carbohydrate, protein, total fat,²² and trans fatty acid,²⁴ and serum hs-CRP. In this current study, we aimed to investigate the association between the intake of energy and macronutrients including carbohydrate, fat and protein, antioxidants including vitamin E and C, and the other specific dietary components such as potassium, calcium, magnesium, phosphorus, iron, copper, zinc, selenium, iodine, folate and sodium and the level of serum hs-CRP among diabetics with and without hypertension and within healthy subjects, derived from a large sample of Iranian adults.

Methods

Study design and subject selection

This cross-sectional study is a part of ongoing large cohort study entitled Mashhad stroke and heart atherosclerotic disorder (MASHAD) study.²⁵ As previously described in detail,²⁵ in the first phase of the MASHAD study, participants were drawn from three regions in Mashhad using stratified cluster randomised sampling. Each region was divided into nine sites centered upon Mashhad Healthcare Center divisions. Households with individuals of eligible age between 35 and 65 years were identified and contacted by telephone to arrange an appointment for the formal physical examination.²⁵ Finally, from a total of 2427117 residents of Mashhad city, 11800 individuals were chosen to participate in the study, of whom a total of 9761 participants, mean age 48.1 years old agreed to participate. The demographic, anthropometric and lifestyle data were obtained by two expert health care professionals and a nurse. Exclusion criteria were pregnancy and lactation, established cardiovascular disease, cancer or chronic kidney and consumption of dietary supplements. All diabetic subjects, who were either hypertensive (n=325)or non-hypertensive (n=559) were selected for this sub-study. Healthy individuals (subjects without any metabolic abnormalities or clinical disorders categorizes as healthy individuals), drawn from the same region were matched with the case participants as a healthy group (n=1220) (control group).

Anthropometric and Biochemical Measurements

Anthropometric measurements, including: weight, height, and waist and hip circumference were measured by trained nurses. All of the anthropometric measurements were made in duplicate and the average value recorded. Body mass index (BMI) was computed as weight in kilograms divided by the square of height in meters. Resting blood pressure was measured three times using a standardized protocol and the mean of these records was reported as subject's blood pressure. Fasting blood samples were obtained early in morning after a 12 h fast and stored at -80° C. Enzymatic methods (Pars Azmun, Karaj, Iran), were used to measure serum triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose and hs-CRP. The levels of biochemical parameters (serum triglycerides, total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], fasting blood glucose [FBG] and hs-CRP were determined

using an autoanalyser (BT3000 biotechnical instruments company, Spain) and commercial kits purchased from Pars Azmon. The intra- and inter-assay coefficients of variation of FBG were $\leq 1.72\%$ and $\leq 1.16\%$, respectively. The intra- and inter-assay coefficients of variation of lipids profile were less than 5%. The intra- and inter-assay coefficients of variation of hs-CRP were $\leq 2.27\%$ and $\leq 8.52\%$, respectively. The standard protocols for the anthropometric and biochemical assessment have been reported previously.²⁵

Assessments of dietary intakes

Data on the dietary intake of the study participants were collected using 24 hour recall, by a trained interviewer. For the analysis of the dietary intake, Dietplan6 software was used (Forest field Software Ltd., UK).

Assessment of metabolic disorders

Blood pressure equal or greater than 140/90 mmHg was defined as hypertension.²⁶ Individuals with a fasting blood glucose \geq 7 mmol/L) or higher on two separate tests, or those under treatment with oral hypoglycemic agents or insulin were considered to be diabetic.²⁷

Assessment of other variables

Demographic and lifestyle information including age, smoking status, medical history and drug use was obtained by expert interviewers using a structure questionnaire. Physical activity was measured using the James and Schofield human energy requirements equations,²⁸ and was calculated as the total energy expenditure (TEE):BMR (Basal Metabolic Rate) ratio over a twenty-four hour period. Questions on physical activity were based on the James and Schofield equations, and were selected from those used in the Scottish Heart Health Study (SHHS)/ MONICA questionnaire. Questions were included to assess the time spent on activities during work (including housework), outside work, and in bed (resting and sleeping).²⁹

Statistical methods

Statistical analysis was undertaken with SPSS software version 16.0 (SPSS® Inc., Chicago, IL). Normality of data was assessed by using the Kolomogorov-Smirnov test. We used parametric tests for normally distributed data and non-parametric test for non-normally distributed data. Between groups comparisons of continuous variables were assessed by analysis of covariance (ANCOVA). Chi-square test was used for assessment of categorical data between groups. The dietary intakes were found to be non-normally distributed and were therefore compared using Mann-Whitney tests. Nutrient intake adjusted for total energy intake through the residual method and expressed in grams, milligrams and micrograms. To predict the associations of serum hs-CRP concentrations and dietary intake, we used linear regression analysis. To enable adjustment for potential confounding factors, we entered into the equation the factors age, sex, smoking, physical activity, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, LDL, HDL, glucose, total cholesterol and triglyceride. A P value of <0.05 was considered significant.

Results

General characteristics and anthropometric measurements in diabetic hypertensive, diabetic non-hypertensive patients and healthy subjects

The general characteristics and anthropometric measurements in diabetic hypertensive and nonhypertensive patients and healthy subjects are shown in table 1. There were no significant differences between smoking status and the percentage of males and females between all three groups. However, participants in both diabetic groups were older than the healthy subjects (p<0.001). In addition, diabetic hypertensive patients were older than diabetic non-hypertensive patients. Body mass index, waist circumference, systolic blood pressure and diastolic blood pressure were significantly higher sub-groups of diabetic patients in comparison to the healthy subjects. Furthermore, hypertensive diabetic patients had a significant higher body mass index and waist circumference, compared with diabetic non-hypertensive subjects. Moreover, patients in the diabetic hypertensive group were less active compared to diabetic non-hypertensive patients and healthy individuals, Table 1.

Clinical and biochemical parameters

Table 2. Shows the clinical and biochemical measurement between patient groups and healthy group. Serum hs-CRP, LDL, , total cholesterol, and triglyceride were significantly higher among diabetic hypertensive and non-hypertensive patients, in comparison to the healthy subjects. Furthermore, total cholesterol, and triglyceride were significantly higher among diabetic

hypertensive patients compared with non-hypertensive subjects. However, there were no significant differences between serum HDL in all three groups (Table 2).

Dietary intake of macro- and micronutrients in diabetic hypertensive, diabetic nonhypertensive patients and healthy subjects

There were no significant differences between the dietary energy, intake of fat and several dietary micronutrients including vitamin E, calcium, iron, copper, zinc, selenium, iodine and folate in diabetic hypertensive patients and healthy subjects (Table 3). In addition, there were no significant differences between dietary energy and dietary micronutrients including vitamins C and E, calcium, copper, selenium, iodine and folate in diabetic non-hypertensive patients and healthy individuals (Table 3). However, diabetic patients in both sub-groups consumed significantly less carbohydrate and more protein, potassium, magnesium, phosphorus and sodium than healthy individuals. Moreover, a lower intake of vitamin C was found in the diabetic hypertensive patients compared to healthy subjects. Dietary intake of fat, iron and zinc were significantly higher among diabetic non-hypertensive patients (Table 3). The differences in dietary intake (include energy, macro and micronutrients) of diabetic hypertensive and non-hypertensive patients were not significant.

Multivariate analysis

The dietary intake of zinc (p=0.001, +6.4%) and calcium (p=0.02, -3.4%), and BMI values (p=0.01, +3.9%) explained approximately 13.7% of the variation in serum hs-CRP among diabetic hypertensive patients using the best fitting models derived from stepwise multiple linear regression analysis (Table 4). Approximately, 9.7% of the variation in serum hs-CRP in diabetic non-hypertensive patients could be explained by BMI, and intake of sodium, iron and cholesterol (Table 5). In the healthy subjects about 4.4% of total variation of hs-CRP concentration could be explained by cholesterol consumption and waist circumferences using a similar analysis (Table 6).

Discussion

Results of the current study, showed that serum hs-CRP concentrations in diabetics with and without concomitant hypertension, were significantly higher than healthy individuals. In addition, some dietary items as well as some anthropometric parameters were significantly

related to serum hs-CRP concentrations. To the best of our knowledge, this is the first study investigating the association between serum high sensitive C reactive protein with dietary intake in healthy and diabetic hypertensive and diabetic non-hypertensive patients. It appears that hs-CRP concentrations and increased in association with both diabetes mellitus⁸ and hypertension,⁶ and hypertension is highly prevalent among diabetic patients,³⁰ we therefore divided the diabetic patients into two sub-groups.

Whilst it has been shown previously that zinc supplementation reduce serum inflammatory markers such as hs-CRP and IL-6 among obese young women³¹in our study zinc was positively associated with hs-CRP in the diabetic hypertensive patients suggesting more studies particularly longitudinal studies might be needed to clarify this relationship.

We found, a significant association between the intake of iron and hs-CRP among diabetic nonhypertensive patients. It has been shown previously that a high consumption of iron is associated with increased risk of coronary heart disease and type 2 diabetes.^{32, 33} Indeed, has been previously suggested that iron could cause an increased production of reactive oxygen species, oxidative stress, and inflammation.³⁴ Results of a recent meta-analysis has shown that the intake of heme iron is positively associated with coronary heart diseases (CHD),³⁵ possibly related to the potential tissue-damaging inflammation which may be caused by heme iron in the oxidation of LDL.³⁵⁻³⁷

We found that, serum hs-CRP concentrations in both diabetic sub-groups patients, was approximately two-fold higher than for healthy individuals. In addition, BMI was significantly associated with elevated serum hs-CRP in both diabetic groups. Earlier reports have indicated that obesity, BMI, insulin resistance and metabolic syndrome are positively associated with low-grade inflammation particularly elevated blood hs-CRP.^{12, 15, 38-41} It has been suggested that an increased production of inflammatory markers such as IL-6 and tumor necrosis factor-alpha (TNF- α) associated with obesity, result in an increased synthesis and secretion of hs-CRP by the liver, and this may also lead to insulin resistance and diabetes mellitus among obese individuals.^{40, 42} Following a healthy lifestyle, including a healthy diet and increased physical activity, both of which can normalize weight, might be helpful to protect against elevated hs-CRP and several chronic diseases related to it.

We found an inverse association between dietary calcium and serum hs-CRP among diabetic hypertensive. There are limited data on the relationship between dietary calcium and serum hs-CRP concentrations. However, in a previous study examining the association between dairy products (highly source of calcium) intake and inflammatory markers such as hs-CRP and IL-6 and TNF- α , a significant reverse association was found between consumption of servings of dairy products per week and inflammatory markers.⁴³ In addition, in a study of Iranian women, a significant inverse association was found between the intake of low-fat dairy and hs-CRP though the relationship between intake of total dairy products and hs-CRP was not significant.⁴⁴ With regard to the inverse association between calcium intake and insulin resistance, obesity and CHD risk,⁴⁵⁻⁴⁸ and the positive association between BMI and serum hs-CRP level,^{15, 38} it is possible that our results on calcium intake and hs-CRP might be explained through an impact of dietary calcium on body weight.

Dietary intake of sodium was also associated with hs-CRP among diabetic non-hypertensive patients. A direct role of sodium in stimulation of inflammatory response and hypothesis that proposed sodium may increase gene expression of inflammatory factors might be the possible mechanisms to explain the role of sodium in increasing inflammatory response.^{49, 50} An association between sodium intake and serum CRP, which may influenced by BMI was found in a previous observational study,⁵¹ which is consistent with our results. In their study, Zhu et.al found a significant direct association between higher sodium intake and adipocyte dysfunction and inflammation among adolescents.⁵² However, in a recent randomized controlled clinical trial, investigating the effect of Dietary Approaches to Stop Hypertension (DASH diet), which contained restricted amount of sodium, on inflammation in gestational diabetes, there was no significant difference between serum hs-CRP among intervened and control groups.⁵³

We also found a significant positive association between cholesterol intake and hs-CRP concentration in the diabetic, non-hypertensive and healthy individuals. Hypercholesterolemia and atherosclerosis are related to the consumption of high-fat, high-cholesterol diets, causing an accumulation of cholesterol in immune cells such as macrophages, which increases inflammatory response.⁵⁴ Furthermore, results of a previous rodent study indicated that hepatic inflammation occurs by dietary cholesterol, rather than liver steatosis suggesting dietary cholesterol should be considered as a salient factor of hepatic inflammation.⁵⁵

With respect to the significant positive association between waist circumferences and plasma hs-CRP in healthy participants in this study, previous studies have investigated the relationship between component of metabolic syndrome and serum hs-CRP, and it has been shown that central obesity is a major determinant of increased hs-CRP in metabolic syndrome.⁵⁶ Similarly, other reports have indicated that waist circumference is an important determinent of serum hs-CRP.^{15, 57} In a study conducted among healthy men, there was a significant association between abdominal adipose tissue and elevated hs-CRP, suggesting that although the best predictor of CRP levels is the amount of total body fat, abdominal fat deposition should be considered as a major determinant of an inflammatory metabolic state.⁵⁸

This present study has some limitations. The cross-sectional study design cannot be used to impute causality. Furthermore we obtained dietary intake of the study participants using self-report questionnaire, which indicated measurement bias. Finally, we used 24-hours food recall to collect dietary intake. Although this method has been widely applied, it depends on memory and under- or over-reporting might lead to imprecision.

In conclusion, our findings highlight the significant association between serum hs-CRP concentration in diabetics with and without hypertension, and demonstrate that BMI and some dietary constituents including zinc, iron, sodium and cholesterol have a significant association with serum hs-CRP. Dietary calcium might have a protective effect against elevated hs-CRP in diabetic patients. More longitudinal study, particularly randomized clinical trial using more population will be required to clarify the exact association between serum high sensitive C reactive protein and dietary intake in diabetic patients.

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References

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus present and future perspectives. Nature Reviews Endocrinology. 2012;8:228-36.

2. Zimmet P, Alberti KG, Magliano DJ, Bennett PH. Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. Nature Reviews Endocrinology. 2016;12:616-22.

3. International Diabetes Federation. IDF Diabetes Atlas

7th edn 2016.

4. Esteghamati A, Gouya MM, Abbasi M, Delavari A, Alikhani S, Alaedini F, et al. prevalence of diabetes and impaired fasting glucose in the adult population of Iran national survey of risk factors for non-communicable diseases of Iran. Diabetes care. 2008;31:96-8.

5. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF- α and IL-6. Diabetes research and clinical practice. 2005;69:29-35.

6. Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. Hypertension. 2005;46:1118-22.

7. Ridker PM, Wilson PW, Grundy SM. Should C-reactive protein be added to metabolic syndrome and to assessment of global cardiovascular risk? Circulation. 2004;109:2818-25.

8. Haffner SM. The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease. The American journal of cardiology. 2006;97:3-11.

9. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. The Journal of clinical investigation. 2003;111:1805-12.

10. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. The Journal of clinical investigation. 2006;116:1793-801.

11. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes care. 2004;27:813-23.

12. Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation. 2005;111:1448-54.

13. Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM. Relation between markers of systemic vascular inflammation and smoking in women. American Journal of Cardiology. 2002;89:1117-9.

14. Yu Z, Ye X, Wang J, Qi Q, Franco OH, Rennie KL, et al. Associations of physical activity with inflammatory factors, adipocytokines, and metabolic syndrome in middle-aged and older chinese people. Circulation. 2009;119:2969-77.

15. Huffman FG, Whisner S, Zarini GG, Nath S. Waist circumference and BMI in relation to serum high sensitivity C-reactive protein (hs-CRP) in Cuban Americans with and without type 2 diabetes. International journal of environmental research and public health. 2010;7:842-52.

16. Ko A, Kim H, Han C-J, Kim J-M, Chung H-W, Chang N. Association between high sensitivity Creactive protein and dietary intake in Vietnamese young women. Nutrition research and practice. 2014;8:445-52.

17. Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. The American journal of clinical nutrition. 2004;80:1029-35.

18. Ma Y, Griffith JA, Chasan-Taber L, Olendzki BC, Jackson E, Stanek EJ, et al. Association between dietary fiber and serum C-reactive protein. The American journal of clinical nutrition. 2006;83:760-6.

19. Yoneyama S, Miura K, Sasaki S, Yoshita K, Morikawa Y, Ishizaki M, et al. Dietary intake of fatty acids and serum C-reactive protein in Japanese. Journal of epidemiology. 2007;17:86-92.

20. Montonen J, Boeing H, Fritsche A, Schleicher E, Joost H-G, Schulze MB, et al. Consumption of red meat and whole-grain bread in relation to biomarkers of obesity, inflammation, glucose metabolism and oxidative stress. European journal of nutrition. 2013;52:337-45.

21. Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, et al. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. The Journal of nutrition. 2005;135:562-6.

22. Arya S, Isharwal S, Misra A, Pandey RM, Rastogi K, Vikram NK, et al. C-reactive protein and dietary nutrients in urban Asian Indian adolescents and young adults. Nutrition. 2006;22:865-71.

23. Ma Y, Hébert JR, Li W, Bertone-Johnson ER, Olendzki B, Pagoto SL, et al. Association between dietary fiber and markers of systemic inflammation in the Women's Health Initiative Observational Study. Nutrition. 2008;24:941-9.

24. Mozaffarian D, Pischon T, Hankinson SE, Rifai N, Joshipura K, Willett WC, et al. Dietary intake of trans fatty acids and systemic inflammation in women. The American journal of clinical nutrition. 2004;79:606-12.

25. Ghayour-Mobarhan M, Moohebati M, Esmaily H, Ebrahimi M, Parizadeh SMR, Heidari-Bakavoli AR, et al. Mashhad stroke and heart atherosclerotic disorder (MASHAD) study: design, baseline characteristics and 10-year cardiovascular risk estimation. International journal of public health. 2015:1-12.

26. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. The lancet. 2005;365:217-23.

27. Alberti KGMM, Zimmet Pf. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabetic medicine. 1998;15:539-53.

28. James WPT S. Human energy requirement. Oxford University Press. 1990.

29. James WPT, Schofield EC. Human energy requirements. A manual for planners and nutritionists: Oxford University Press; 1990.

30. Epstein M, Sowers JR. Diabetes mellitus and hypertension. Hypertension. 1992;19:403-18.

31. Kim J, Ahn J. Effect of zinc supplementation on inflammatory markers and adipokines in young obese women. Biological trace element research. 2014;157:101-6.

32. Bao W, Rong Y, Rong S, Liu L. Dietary iron intake, body iron stores, and the risk of type 2 diabetes: a systematic review and meta-analysis. BMC medicine. 2012;10:119.

33. Ascherio A, Willett WC, Rimm EB, Giovannucci EL, Stampfer MJ. Dietary iron intake and risk of coronary disease among men. Circulation. 1994;89:969-74.

34. Wagener FA, Volk H-D, Willis D, Abraham NG, Soares MP, Adema GJ, et al. Different faces of the heme-heme oxygenase system in inflammation. Pharmacological reviews. 2003;55:551-71.

35. Hunnicutt J, He K, Xun P. Dietary iron intake and body iron stores are associated with risk of coronary heart disease in a meta-analysis of prospective cohort studies. The Journal of nutrition. 2014;144:359-66.

36. Meyers D. The iron hypothesis: does iron play a role in atherosclerosis? Transfusion. 2000;40:1023-9.

37. McCord JM, editor Iron, free radicals, and oxidative injury. Seminars in hematology; 1998.

38. Rawson ES, Freedson PS, Osganian SK, Matthews CE, Reed G, Ockene IS. Body mass index, but not physical activity, is associated with C-reactive protein. Medicine and science in sports and exercise. 2003;35:1160-6.

39. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends in immunology. 2004;25:4-7.

40. Bastard J-P, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. European cytokine network. 2006;17:4-12.

41. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. The Journal of clinical investigation. 2003;112:1796-808.

42. Barzilay JI, Abraham L, Heckbert SR, Cushman M, Kuller LH, Resnick HE, et al. The relation of markers of inflammation to the development of glucose disorders in the elderly the Cardiovascular Health Study. Diabetes. 2001;50:2384-9.

43. Panagiotakos DB, Pitsavos CH, Zampelas AD, Chrysohoou CA, Stefanadis CI. Dairy products consumption is associated with decreased levels of inflammatory markers related to cardiovascular disease in apparently healthy adults: the ATTICA study. Journal of the American College of Nutrition. 2010;29:357-64.

44. Esmaillzadeh A, Azadbakht L. Dairy consumption and circulating levels of inflammatory markers among Iranian women. Public health nutrition. 2010;13:1395-402.

45. Davies KM, Heaney RP, Recker RR, Lappe JM, Barger-Lux MJ, Rafferty K, et al. Calcium intake and body weight 1. The Journal of Clinical Endocrinology & Metabolism. 2000;85:4635-8.

46. Jacqmain M, Doucet E, Després J-P, Bouchard C, Tremblay A. Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. The American journal of clinical nutrition. 2003;77:1448-52.

47. Varenna M, Binelli L, Casari S, Zucchi F, Sinigaglia L. Effects of dietary calcium intake on body weight and prevalence of osteoporosis in early postmenopausal women. The American journal of clinical nutrition. 2007;86:639-44.

48. Teegarden D. Calcium intake and reduction in weight or fat mass. The Journal of nutrition. 2003;133:249S-51S.

49. Telini LSR, de Carvalho Beduschi G, Caramori JCT, Castro JH, Martin LC, Barretti P. Effect of dietary sodium restriction on body water, blood pressure, and inflammation in hemodialysis patients: a prospective randomized controlled study. International urology and nephrology. 2014;46:91-7.

50. Dinarello CA, editor Hyperosmolar sodium chloride, p38 mitogen activated protein and cytokinemediated inflammation. Seminars in dialysis; 2008.

51. Fogarty AW, Lewis SA, McKeever TM, Britton JR. Is higher sodium intake associated with elevated systemic inflammation? A population-based study. The American journal of clinical nutrition. 2009;89:1901-4.

52. Zhu H, Pollock NK, Kotak I, Gutin B, Wang X, Bhagatwala J, et al. Dietary sodium, adiposity, and inflammation in healthy adolescents. Pediatrics. 2014;133:e635-e42.

53. Asemi Z, Samimi M, Tabassi Z, Sabihi S-s, Esmaillzadeh A. A randomized controlled clinical trial investigating the effect of DASH diet on insulin resistance, inflammation, and oxidative stress in gestational diabetes. Nutrition. 2013;29:619-24.

54. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. Nature Reviews Immunology. 2015;15:104-16.

55. Wouters K, van Gorp PJ, Bieghs V, Gijbels MJ, Duimel H, Lütjohann D, et al. Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis. Hepatology. 2008;48:474-86.

56. Santos A, Lopes C, Guimaraes J, Barros H. Central obesity as a major determinant of increased high-sensitivity C-reactive protein in metabolic syndrome. International journal of obesity. 2005;29:1452-6.

57. Shemesh T, Rowley K, Jenkins A, Brimblecombe J, Best J, O'dea K. Differential association of Creactive protein with adiposity in men and women in an Aboriginal community in northeast Arnhem Land of Australia. International journal of obesity. 2007;31:103-8.

58. Lemieux I, Pascot A, Prud'homme D, Alméras N, Bogaty P, Nadeau A, et al. Elevated C-reactive protein another component of the atherothrombotic profile of abdominal obesity. Arteriosclerosis, thrombosis, and vascular biology. 2001;21:961-7.

Table 1							
General characteristics and anthropometric measurements in patient and control subjects [*]							
Group	Diabetic hypertensive (n=325)	Diabetic non-hypertensive (n=559)	Control (n=1220)	P^{I}	P^2	P^3	
Age (y)	54±6.9 [#]	50.8±7.4	46.5±7.9	< 0.001	< 0.001	< 0.001	
Males/females	131/194	210/349	495/725	0.94	0.23	0.33	
Current smoking [n (%)]	15.7	22	19.5	0.12	0.22	0.05	
Physical activity level (PAL)	1.52±0.3	1.58 ± 0.27	1.58±0.26	0.001	0.97	0.002	
BMI (Kg/m2)	29.8±4.6	28.3±4.6	27.2±4.5	< 0.001	< 0.001	< 0.001	
Waist circumference	101.6±11.1	97.8±11.7	93.5±11.5	< 0.001	< 0.001	< 0.001	
Systolic blood pressure	148±16	118.4±11.3	114.4±11.7	< 0.001	< 0.001	< 0.001	
Diastolic blood pressure	91.6±8.4	75.9±7.5	74.7±8.2	< 0.001	0.005	< 0.001	

* Continuous variables were compared by chi-square test and categorical variables were compared by ANCOVA * Mean \pm standard deviation ¹ Diabetic hypertensive in compared to the control ² Diabetic non-hypertensive in compared to the control

³ Diabetic hypertensive in compared to the Diabetic non-hypertensive

Table 2 Serum biochemical measurements in patient and control subjects *						
Diabetic hypertensive	Diabetic non-hypertensive	Control	P^2	P^3	P^4	
2.9 (4.3) #	2.5 (4.06)	1.3 (1.75)	< 0.001	< 0.001	0.16	
$125.5 \pm 43.2^{\dagger}$	121±39.7	116.3±32	< 0.001	0.01	0.3	
42.3±9.6	41.7±10.1	42.3±9.6	0.93	0.23	0.62	
195.1±59.6	196±61.1	78.6±9.9	< 0.001	< 0.001	0.77	
213.3±49	202.4±46.1	186.8±35.2	< 0.001	< 0.001	0.005	
172 (120)	153 (123)	112 (80)	< 0.001	< 0.001	0.002	
	$\frac{1 \text{ measurements in patien}}{\text{Diabetic hypertensive}}$ $\frac{2.9 (4.3)^{\#}}{125.5 \pm 43.2^{\ddagger}}$ $\frac{42.3 \pm 9.6}{195.1 \pm 59.6}$ $\frac{213.3 \pm 49}{172 (120)}$	1 measurements in patient and control subjects *Diabetic hypertensiveDiabetic non-hypertensive $2.9 (4.3)^{\#}$ $2.5 (4.06)$ $125.5\pm43.2^{\ddagger}$ 121 ± 39.7 42.3 ± 9.6 41.7 ± 10.1 195.1 ± 59.6 196 ± 61.1 213.3 ± 49 202.4 ± 46.1 $172 (120)$ $153 (123)$	I measurements in patient and control subjects *Diabetic hypertensiveDiabetic non-hypertensiveControl $2.9 (4.3)^{\#}$ $2.5 (4.06)$ $1.3 (1.75)$ $125.5 \pm 43.2^{\ddagger}$ 121 ± 39.7 116.3 ± 32 42.3 ± 9.6 41.7 ± 10.1 42.3 ± 9.6 195.1 ± 59.6 196 ± 61.1 78.6 ± 9.9 213.3 ± 49 202.4 ± 46.1 186.8 ± 35.2 $172 (120)$ $153 (123)$ $112 (80)$	I measurements in patient and control subjects *Diabetic hypertensiveDiabetic non-hypertensiveControl P^2 $2.9 (4.3)^{\#}$ $2.5 (4.06)$ $1.3 (1.75)$ <0.001 $125.5\pm43.2^{\ddagger}$ 121 ± 39.7 116.3 ± 32 <0.001 42.3 ± 9.6 41.7 ± 10.1 42.3 ± 9.6 0.93 195.1 ± 59.6 196 ± 61.1 78.6 ± 9.9 <0.001 213.3 ± 49 202.4 ± 46.1 186.8 ± 35.2 <0.001 $172 (120)$ $153 (123)$ $112 (80)$ <0.001	I measurements in patient and control subjects *Diabetic hypertensiveDiabetic non-hypertensiveControl P^2 P^3 $2.9 (4.3)^{\#}$ $2.5 (4.06)$ $1.3 (1.75)$ <0.001 <0.001 $125.5\pm43.2^{\dagger}$ 121 ± 39.7 116.3 ± 32 <0.001 0.01 42.3 ± 9.6 41.7 ± 10.1 42.3 ± 9.6 0.93 0.23 195.1 ± 59.6 196 ± 61.1 78.6 ± 9.9 <0.001 <0.001 213.3 ± 49 202.4 ± 46.1 186.8 ± 35.2 <0.001 <0.001 $172 (120)$ $153 (123)$ $112 (80)$ <0.001 <0.001	

* Continuous variables were compared by chi-square test and categorical variables were compared by ANCOVA or Mann-Whitney # Median (Interquartile range) * Mean ± standard deviation

²Diabetic hypertensive in compared to the control

³ Diabetic non-hypertensive in compared to the control

⁴ Diabetic hypertensive in compared to the Diabetic non-hypertensive

Table 3							
Comparison of macro- and micronutrient dietary intakes between patients and control subjects *							
	Groups	Diabetic hypertensive	Diabetic non-hypertensive	Healthy subjects	P^{I}	P^2	P^3
		subjects	subjects				
Γ	Dietary intakes						
N	lacronutrients						
	Energy (Kcal)	1966 (1555-2447)#	1867 (1422-2310)	1820 (1443-2197)	0.14	0.54	0.42
	Carbohydrate (g)	240.7 (201.7-279.7)	229.1 (196.9-261.2)	243.2 (212.6-273.8)	0.01	< 0.001	0.33
	Fat (g)	69 (55-83)	72.4 (58.7-86)	69.3 (257.3-281.3)	0.12	0.01	0.74
	Protein (g)	69.3 (58.1-80.4)	72.4 (61.6-83.2)	68.3 (59-77.6)	0.04	< 0.001	0.9
A	Antioxidants						
	Vitamin E (mg)	16.3 (9.2-23.3)	17.1 (9.9-24.3)	16.5 (10.8-22.2)	0.8	0.57	0.8
	Vitamin C (mg)	61.6 (14.3-108.9)	78.6 (25.7-131.4)	77.5 (28.2-126.7)	0.03	0.56	0.4
C	Others						
	Potassium (mg)	2915 (2303-3527)	2898 (2238-3558)	2766 (2282-3250)	0.01	0.005	0.7
	Calcium (mg)	859 (564-1154)	839 (576-1102)	841 (632-1049)	0.2	0.92	0.23
	Magnesium (mg)	252.5 (190.1-314.7)	253.7 (210.5-296.8)	230.6 (150.6-310.6)	0.003	< 0.001	0.78
	Phosphorus (mg)	1377 (1120-1634)	1343 (1105-1581)	1286 (1101.3-1470.7)	0.001	0.005	0.24
	Iron (mg)	9.5 (6-13)	10.8 (7.5-14)	9.5 (6.7-12.3)	0.2	< 0.001	0.76
	Cooper (mg)	1.8 (1.47-2.13)	1.8 (1.53-2.07)	1.7 (1.47-1.93)	0.34	0.3	0.41
	Zinc (mg)	9.1 (7.1-11.1)	9.5 (7.5-11.5)	9 (7.4-10.6)	0.22	0.004	0.7
	Selenium (mg)	29.4 (15.9-42.9)	31.3 (17.6-44.9)	34.2 (22.7-45.7)	0.07	0.4	0.65
	Iodine (mcg)	91.2 (32.4-149.9)	97.4 (35.5-159.2)	108.3 (50.3-166.3)	0.35	0.14	0.21
	Folate (mcg)	220.9 (144-297.7)	229.5 (166.3-292.6)	217.9 (159.8-276)	0.5	0.4	0.24
	Sodium (mg)	2435 (1453-3416)	2425 (1510-3339)	1908 (1105-2710)	< 0.001	< 0.001	0.41
*Obtained using Mann-Whitney test							
[#] Median (Interquartile range)							
1	Diabatic hypertongia	e in compared to the con	trol				
1	Diabetic hypertensive in compared to the control						

² Diabetic non-hypertensive in compared to the control
 ³ Diabetic hypertensive in compared to the Diabetic non-hypertensive

Table 4					
Multifactorial analysis of serum C-Reactive Protein * (Diabetic hypertensive)					
Group and confounders	β	<i>R</i> 2	P		
		%			
Zinc	0.914	6.4	0.001		
BMI	0.526	3.9	0.01		
Calcium	-0.005	3.4	0.02		
Sodium	0.001	0.02	0.33		
Iron	0.07	0.006	0.29		
Cholesterol	0.006	0.01	0.13		
Waist circumference	0.05	0.003	0.38		
*Obtained from linear regression.					

Table 5						
Multifactorial analysis of C-Reactive Protein [*] (Diabetic non-hypertensive)						
Group and confounders	β	R2	Р			
BMI	0.324	3.1	0.009			
Sodium	0.001	2.9	0.005			
Iron	0.226	2.1	0.025			
Cholesterol	0.025	1.6	0.047			
Waist circumference	0.06	0.006	0.19			
Zinc	0.004	0.12	0.28			
Calcium	-0.001	0.001	0.57			
*Obtained from linear regression.						

Table 6						
Multifactorial analysis of C-Reactive Protein [*] (control group)						
Group and confounders	β	R2	P			
	%					
Cholesterol	0.007	2.2	0.002			
Waist circumference	0.104	2.2	0.002			
BMI	-0.05	0.000	0.49			
Sodium	0.001	0.000	0.88			
Iron	0.04	0.001	0.36			
Zinc	-0.01	0.000	0.81			
Calcium	0.000	0.000	0.61			
*Obtained from linear regression.						