Relation of antioxidant capacity of diet and markers of oxidative status with C-reactive protein and adipocytokines: a prospective study.

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ABSTRACT

Background: The role of dietary antioxidants and plasma oxidant-antioxidant status in low-grade chronic inflammation and adipocytokine levels is not established yet.

Objectives: We aimed to evaluate whether total dietary antioxidant capacity (assessed by dietary ferric reducing antioxidant potential (FRAP)), serum uric acid (UA) and gamma glutamyltransferase (GGT) were associated with low-grade chronic inflammation and circulating adipocytokines.

Methods: Data of 4,506 participants aged ≥55 years from the Rotterdam Study were analyzed. Baseline (1990-1993) FRAP score was assessed by a food frequency questionnaire. Baseline UA and GGT levels were assessed in non-fasting serum samples. Serum high sensitivity C-reactive protein (hs-CRP) was measured at baseline and 10 years later. Plasma leptin, adiponectin, plasminogen activator inhibitor-1 (PAI-1) and resistin levels were assessed 10 years later.

Results: A high FRAP score was associated with lower levels of UA and GGT. Overall, no association was found between FRAP and hs-CRP levels. FRAP score was associated with lower levels of leptin and PAI-1, higher levels of adiponectin, and no difference in resistin levels. Increased levels of UA were associated with higher levels of hs-CRP, PAI-1 and leptin; lower levels of adiponectin and no difference in resistin levels. Similarly, GGT was associated with higher levels of hs-CRP whereas no association was observed between GGT and adipocytokines.

Conclusion: These findings suggest that overall antioxidant capacity of diet and low levels of UA are associated with circulating adipocytokines whereas no consistent association was found with hs-CRP.

Key words: total antioxidant capacity of diet, uric acid, gamma- glutamyltransferase, C- reactive protein, adipocytokines, low-grade inflammation.

1. INTRODUCTION

Low-grade chronic inflammation has been involved in the pathogenesis of atherosclerosis and development of coronary heart disease (CHD) [1, 2]. C-reactive protein (CRP), an acute phase reactant, is a general marker of low-grade chronic inflammation and has been associated with markers of atherosclerosis and CHD [2-4]. Plasma sensitive CRP (hs-CRP) correlates with obesity and obesity-related disorders, including insulin resistance and type 2 diabetes [5]. Adipose tissue synthesizes and releases many inflammatory mediators into the systemic circulation termed adipocytokines, and include leptin, adiponectin, plasminogen activator inhibitor-1 (PAI-1) and resistin, all of which can initiate the development of chronic inflammation and may directly contribute to metabolic and vascular diseases [6-15].

An imbalance between plasma oxidants-antioxidants (oxidative stress) as well as dietary antioxidants have been suggested to play a role in systemic low-grade chronic inflammation [16]. Oxidative stress, defined as an increased load of free radicals, induces the activation of NF-κB, a transcription factor involved in cell survival, differentiation, and inflammation [17]. Antioxidant molecules neutralize such free radicals and therefore diminish low-grade inflammation. Dietary antioxidants, including vitamin A, E and C, can counteract oxidative stress and therefore its adverse effect on inflammation [18]. However, studies evaluating the role of individual antioxidants on inflammation have shown contradictory results, which can be due to not taking into account the interactive effect among nutrients [19]. Hence, assessing the overall effects of antioxidants in the diet instead of the individual effects can provide further information regarding the association between diet and inflammation [19]. The ferric reducing antioxidant potential (FRAP) measures the overall antioxidant capacity of diet by measuring the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+})[20] and, has been used as a marker of the overall effects of

antioxidants in many studies. FRAP has been associated with inflammatory related diseases, including cardiovascular disease and cancer [21, 22]. However, only a few studies have assessed its role on inflammation and adipocytokine levels [22-24]. Furthermore, serum levels of uric acid (UA) and gamma-glutamyl transferase (GGT) are considered endogenous markers of oxidative stress [25]. Both levels of UA and GGT positively correlate with markers of low-grade inflammation including hs-CRP, but how UA and GGT levels relate longitudinally with hs-CRP and adipocytokine levels remains unclear [26-29].

Therefore, we aimed to assess whether FRAP and endogenous markers of oxidative stress, UA and GGT, were associated with low-grade chronic inflammation and circulating adipocytokine concentrations in a prospective cohort of middle aged and elderly men and women.

2. MATERIAL AND METHODS

The study was performed within the Rotterdam Study (RS), a population-based cohort among individuals 55 years and over in the Ommoord district of Rotterdam, the Netherlands. The rationale and design of the RS is described elsewhere [30]. The baseline examination (RS-I) took place in 1990-1993. Trained research assistants collected data on medical history, current health status, use of medication, lifestyle and risk indicators for chronic diseases during an extensive home interview. Subsequently the participants visited the study center for detailed clinical examinations and assessment of diet. Follow up visits were held every 3-4 years.

2.1 MEASUREMENTS

2.1.1 Assessment of ferric reducing antioxidant potential (FRAP)

Dietary antioxidant capacity was assessed from the FFQ (Online Supplemental Material) the participants filled in during the interview. We used the Antioxidant Food Table published by the Institute of Nutrition Research, University of Oslo, which includes measurements of >3,000 foods [31], to calculate each food's contribution to ferric reducing antioxidant potential. The FRAP assay assesses the antioxidant capacity of individual food items to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) [20]. Since the food table consisted of foods from several manufacturers, we consulted nutritional experts at Wageningen University (the Netherlands) to determine the linkage of foods from several manufacturers that were closest to the Dutch food products. For each participant, we multiplied the consumption frequency of each food by the corresponding FRAP value (in mmol/100g), and summed these values across all dietary sources. Vitamin supplementation was not included in the FRAP assessment because there were no detailed data available. Most variation in dietary FRAP score was explained by intakes of coffee (65%) and tea (21%) as described previously[21].

2.1.2 Assessment of Uric Acid and Gamma–glutamyltransferase (GGT)

Values of serum UA and GGT were obtained from baseline (1990-1993) non-fasting blood samples, which were centrifuged and the serum was subsequently frozen (−20°C) for 1 week. UA was determined with a Kone Diagnostica reagent kit and a Kone autoanalyzer. In order to check the calibration, 3 control samples were included every 10 samples. If the average values of the control samples of each run (100 samples) were not within 2.5% of the true value, the run was repeated. Day-by-day variation had to be within 5% [32]. Serum GGT levels were determined within two weeks using a Merck Diagnostica kit (Merck, Whitehouse Station, NJ, USA) on an Elan Autoanalyzer (Merck).

2.1.3 Assessment of hs-CRP and adipocytokines

hs-CRP was measured in non-fasting frozen serum of study participants at baseline (1990-1993) and at the third center visit (1997-1999). A rate near-infrared particle immunoassay (Immage Immunochemistry System, Beckman Coulter, Fullerton, CA, USA) was used. This system measures concentrations from 0.2 to 1440mg/l, with a within-run precision of 0.5%, a total precision <7.5% and a reliability coefficient of 0.995. Undetectable CRP was scored as 0.2 (n=72).

For assessment of adipocytokines, fasting blood samples were collected at the research center, in the third center visit (1997-1999). Plasma was isolated and immediately put on ice and stored at -80°C. Citrate plasma (200Ul) was sent in July 2008 to Rules-Based Medicine, Austin, Texas (www.myriadrbm.com). Fifty inflammatory biomarkers were quantified using multiplex immunoassay on a custom designed human multianalyte profile. The intra-assay variability was less than 4% and the inter assay variability was less than 13%. Biomarkers with more than 60% completeness of measurements were selected for imputation and further analysis. Data on leptin, adiponectin, plasminogen activator inhibitor 1 (PAI-1) and resistin, major inflammatory markers released by adipose tissue [7], were available. The inflammatory markers investigated in the current study have no standard international calibration reference therefore, interpretation of the absolute values should be with caution. Since the current study is conducted within one set of individuals, the use of relative measures should not affect the effect estimates.

2.2 POPULATION FOR ANALYSIS

2.2.1 FRAP and inflammation

In the baseline examination (1990-1993) of the first cohort of the Rotterdam Study, 7,983 participants were included. Of out 7,983 participants, 6,521 participants were invited for dietary intake interview, out of which only 5,435 (83%) participants completed food frequency questionnaire and therefore had complete information on dietary intake. Moreover, out of 7,983 participants, randomly we invited 7,129 participants to assess cardiovascular risk factors, including CRP. However, only 6658 (93.3%) had C-reactive protein assessed. Participants with available information on both dietary and C-reactive protein levels were 5104. Further, we excluded 598 participants who reported use of anti-inflammatory drugs at baseline and/or during the follow-up (n=598), leaving 4,506 participants for the analysis of FRAP with CRP (Figure 1). In addition, leptin, adiponectin, PAI-1 and resistin were measured in a random subsample of 971 participants, hence only 798 participants were included in the analysis of FRAP with adipocytokines (Figure 1).

2.2.2 Uric acid, gamma-glutamyltransferase and inflammation

In the baseline examination (1990-1993) of the first cohort of the Rotterdam Study, 7,983 participants were included. Uric acid and GGT data were available for 5,047 subjects (Figure 2). Out of these, 893 participants were excluded either because they did not have CRP measured at the first visit or because they reported use of anti-inflammatory drugs at baseline and/or during the follow-up, leaving 4,154 participants for the analysis of uric acid with CRP and GGT with CRP. 3,447 participants were further excluded because they did not have measures of other inflammatory markers, hence 707 participants were included in the analysis of uric acid and GGT with leptin, adiponectin, PAI-1 and resistin (Figure 2).

2.3 STATISTICAL ANALYSES

Data are presented as mean (± standard deviation) for normally distributed continuous variables, median (range) for continuous variables that are not normally distributed, and percentages for categorical variables. We used natural log-transformed values of serum CRP concentrations, GGT, non-fasting serum glucose, leptin, adiponectin, PAI-1 and resistin to better approximate a normal distribution. Pearson correlations were used to assess the correlations between inflammatory markers. To account for systematic measurement error in FRAP, FRAP was adjusted for total energy intake by using the residual method in the analysis[33]. FRAP was analyzed continuously. For analyses evaluating CRP as outcome, we fitted linear regression models using generalized estimating equations with exchangeable correlation structure adjusting for the within-subject correlations due to the repeated measurements of CRP in the same individual (inter-class correlation coefficient = 0.682 for natural log-transformed CRP) [34]. Multivariable linear regression was used to examine whether FRAP, GGT and UA were independently associated with blood levels of adiponectin, leptin, resistin and PAI-1. Regression coefficients (βs) and 95% confidence intervals were obtained on the basis of robust standard errors (95% CI). First, we calculated age and gender adjusted coefficients (Model 1) for the following exposure: FRAP, GGT and UA. Subsequently in Model 2, we adjusted for potential confounders when the covariates changed the effect estimate by more than 10% in univariate models of each exposure with any of the outcomes assessed. The following potential confounding factors, were evaluated: body mass index (BMI) (continuous), energy intake (continuous), physical activity(continuous), smoking status (never or former, current), lipid lowering medication use (Yes, No), systolic blood pressure(continuous), total cholesterol(continuous), vitamin supplementation (Yes, No), hormone replacement therapy (HRT) (Yes, No), prevalent chronic diseases (CVD or T2D) (yes, no), non-fasting blood

glucose(continuous), education (low, intermediate, high), income (low, intermediate, high), alcohol, energy-adjusted processed meat intake (continuous), energy-adjusted unprocessed meat intake (continuous), Dutch Healthy Diet index (DHDI)(continuous). For the analysis on leptin, adiponectin, PAI-1 and resistin as outcomes, we also adjusted for CRP in the first visit (1990-1993) as a proxy of chronic inflammation at baseline as adipocytokines were measured only at the third round visit (1997-1999). To check for non-linear relation, a quadratic term was tested in multivariable model 2. Since there is evidence that the association between diet antioxidants and inflammatory biomarkers differs by sex [35], we tested for statistical interaction by adding a product term in model 2. Furthermore, stratified analysis was performed and the results were presented for model 2. We further checked the association of FRAP with uric acid, and FRAP with GGT using multivariable linear regression models. We also performed sensitivity analyses (i) restricting all main analyses to participants with available information on all exposures and outcomes investigated (N=633), (ii) excluding participants with chronic diseases (CVD or T2D) and (iii) further adjusted for BMI change from first to the third visit. A P-value lower than 0.05 was considered as statistically significant, but to account for multiple testing, we adjusted the p-value from 0.05 to 0.0166 by applying the Bonferroni correction for the number of exposures studied (N=3).

To adjust for potential bias associated with missing data we used multiple imputation procedure (N= 5 imputations). All analyses were done using SPSS statistical software (SPSS, version 21.0; SPSS Inc., Chicago, Illinois).

3. RESULTS

The main characteristics of the study population are shown by gender in Table 1. FRAP score and GGT levels were lower in women compare to men (FRAP: 20.02±5.07 mmol/day vs. 20.83±5.95 mmol/day; GGT: median 21 U/l, range 351U/l vs median 27U/l, range 576 U/l) whereas UA levels were higher in women (296.62±71.44 µmol/l vs. 352.88±74.40 µmol/l) (**Table 1**). CRP levels at baseline were slightly lower in women whereas no significant difference was observed in the CRP levels at the third visit (**Table 1**). Also, women had slightly higher BMI (26.55 vs. 25.68 kg/m^2) and leptin levels (median: 14.0 vs 4.02 ng/mL) than men. Although the energy intake was lower in women (1796 vs. 2246.2 kcal/day), they had higher physical activity (89.45 vs 69.15 MET) as well as a healthier diet (DHDI: 31.95 vs. 27.95) than men. Among the adipocytokines, PAI-1 and leptin (r=0.466, p=0.01), PAI-1 and CRP (r=0.325, p=0.01), PAI-1 and adiponectin (r=-0.270, p=0.01), leptin and CRP (r=0.254, p=0.01) showed the highest correlation (**Supplementary Table S1**). Compared to subjects who did not have information on leptin, adiponectin, PAI-1 and resistin, subjects who had information on these inflammatory markers did not differ with respect to FRAP, but had higher levels of CRP, BMI, systolic blood pressure and higher prevalence of chronic disease (**Supplementary Table S2**).

3.1 The association between FRAP score and inflammatory markers

There was no association between FRAP and hs-CRP levels in the age and gender-adjusted model or multivariable model **(Table 2).** In the multivariable models, FRAP score was associated with lower levels of leptin (β=-0.01, 95%CI=-0.02; -0.001), PAI-1 (β=-0.02, 95%CI=-0.03; -0.01) and higher levels of adiponectin (β=0.01, 95%CI=0.002; 0.015). No association was observed between FRAP and resistin. **(Table 2).**

3.2 The association between UA, GGT and inflammatory markers

After multivariable adjustment, increased levels of UA were associated with higher levels of hs-CRP (β=0.12, 95%CI=0.09; 0.16), leptin (β=0.10, 95%CI=0.05; 0.15) PAI-1 (β=0.15, 95%CI=0.09; 0.20), and lower levels of adiponectin (β=-0.07, 95%CI=-0.10; -0.03) **(Table 3).** No association was observed between UA and resistin (**Table 3**). Similarly, after correcting for confounding factors, GGT was associated with higher levels of hs-CRP (β=0.06, 95%CI=0.13; 0.19) whereas no association was observed between GGT and adipocytokines (**Table 3**).

3.3 Effect modification by gender

A significant effect modification by sex was found for the association between FRAP score and hs-CRP (*P*-interaction= 0.009). After stratification, a high dietary FRAP score was associated with lower levels of hs-CRP in women (β=-0.01, 95%CI=-0.02; -0.003), whereas no association was observed in men (**Supplementary Table S3**). No effect modification by sex was observed for the association between FRAP score with the adipocytokine levels (All *P*-interaction > 0.05). Similarly, the analyses were not different between strata of sex (**Supplementary Table S3**). Also, no sex differences were observed for the association of UA and GGT with CRP and adipocytokines (All *P*-intercation > 0.05) (**Supplementary Table S4**).

3.4 Sensitivity analyses

Higher levels of FRAP score were associated with lower levels of both UA (β=-0.003, 95%CI=-0.005; -0.002) and GGT (β=-0.006, 95%CI=-0.009; -0.003), after correcting for confounders **(Supplementary Figure S1 and Supplementary Table S5)**. There was no evidence against a linear relation in all the main analyses (all *P*-values for quadratic term >0.05, data not shown). Also, all associations that were statistically significant in the main analyses remained unchanged in terms of statistical significance when the analyses were restricted to (i) participants with

available measures of FRAP, UA, GGT, CRP, leptin, adiponectin, PAI-1 and resistin (n=633) (data not shown), (ii) to subjects without chronic diseases (**Supplementary Table S6 and S7**) or (iii) when we further adjusted for changes in BMI between the first and third visit (data not shown). The associations of FRAP with adiponectin and PAI-1, of UA with hs-CRP, leptin, adiponectin, and PAI-1, and the association of GGT with hs-CRP, remained significant after we applied the Bonferroni correction (all $p<0.0166$).

4. DISCUSSION

Overall a higher FRAP score was associated with leptin, adiponectin, and PAI-1 but not with CRP levels. Furthermore, increased levels of both GGT and UA levels were associated with higher levels of pro-inflammatory markers and lower levels of anti-inflammatory markers.

In the current investigation, no association was found between FRAP and CRP levels in the overall population, however, in women, a higher FRAP score was associated with diminished chronic inflammation. Similar to our findings, Detopoulou et al in a cross-sectional study of 532 men and women found no association between FRAP and CRP levels in the total population [36]. In contrast, a cross-sectional study from Brighenti et al [23] , which used the TAC assay to measure antioxidant capacity, showed an association with lower levels of CRP in an adult Italian population including both men and women. We did find an interaction with gender, suggesting that the association between FRAP and CRP levels is present only in women, which is in line with the results of previous studies conducted in women. For example, the study from Kobayashi at al.[24] showed that dietary total antioxidant capacity was associated with lower serum CRP concentrations in young Japanese women (474 women, aged 18-22 years) regardless of assay used to measure it. Also, in a 9-month observational study among postmenopausal women,

Wang and his colleagues showed that consumption of diets rich in total antioxidants was associated with lower plasma CRP levels [37].

Several studies show a stronger defense against oxidative damage in the female liver tissue, which is the major determinant of CRP levels [38]. Animal studies have shown that, compared to males, antioxidant capacity of diet assessed by FRAP and other methods is higher in liver tissue [38]. Also, females have greater mean hepatic alpha-tocopherol levels, total capacity of the cellular systems that detoxify reactive oxygen species or free radical-drug metabolites seems to be higher in the female rat liver[39]. These evidence may account for the sex differences observed in the association between FRAP and CRP levels in our study, which merits further investigation.

Similar to our findings, previous studies [27, 40] have shown that increased UA levels are significantly associated with increased hs-CRP levels. Also in a study of Park et al [41] in postmenopausal women uric acid was associated with lower adiponectin levels. Another study from Ali et al [42] found that high GGT levels are associated with high hs-CRP levels implicating that elevated GGT levels are associated with burden of subclinical vascular inflammation.

To our knowledge, this is the first study to show that the FRAP score was a determinant of leptin and PAI-I concentrations. In line with our findings, a previous study has shown an association between FRAP score and higher adiponectin levels [36]. Previous studies [43] have indicated that total antioxidant capacity of diet is associated with less central adiposity, as well as to metabolic (e.g. insulin resistance index) and oxidative stress markers in healthy young adults (e.g. oxidized-LDL, malondialdehyde). Central adiposity, mainly abdominal adiposity is the

main producer of anti-inflammatory (adiponectin) and pro-inflammatory markers (leptin, resistin and PAI-1)[12, 44, 45]. Leptin is an adipocyte-derived hormone that reduces food intake and increases energy expenditure by acting in the hypothalamus [46, 47] and has also pro-inflammatory effects [7, 8]. Leptin levels correlate with higher indices of adiposity, however, individuals with similar degrees of adiposity have variations in serum leptin levels [46, 48]. Adiponectin is one of the most abundant adipocyte-derived hormones and appears to improve insulin sensitivity and vascular inflammation through its actions in liver and muscle [7]. Several studies have demonstrated that adiponectin is a marker and a mediator of metabolic risk, including the risk for conversion to diabetes and risk of myocardial infarction [49]. PAI-1, is another hormone secreted from fat cells, and is suggested to be a possible contributor to obesity-induced diabetes and atherosclerosis [50]. Resistin, on the other hand, is almost an exclusively white adipose tissue-expressed polypeptide, and has also been linked to energy homeostasis and diet-induced obesity, insulin resistance and diabetes[51]. Other factors, including hormonal and nutritional factors have been suggested to influence concentrations of these inflammatory markers[52]. Our study also indicates that the antioxidant diet, GGT and UA may affect the levels of leptin, adiponectin, and PAI-I but not resistin independent of obesity. It was reported that uric acid induces CRP expression by implication on cell proliferation and nitric oxide production of human vascular cells [53]. Elevation of serum GGT is involved in the inflammatory response. It is plausible that elevation in GGT might occur before elevation in CRP, if oxidative stress leads to an inflammatory response [54]. These data imply that inflammation may be one of the underlying mechanism linking an antioxidant diet, GGT and UA with cardiometabolic outcomes, which needs to be elucidated by future studies. However future studies are needed to clarify specific inflammatory markers that may be involved in the pathway.

Probably oxidative stress is the pathway that links antioxidants with a low inflammatory profile. The human body has a number of defense mechanisms against oxidative stress including antioxidants, preventive and repair mechanism and physical defense [17]. Antioxidants themselves can be divided into enzymatic antioxidants (glutathione peroxidase, peroxide dismutase and catalase) and non-enzymatic antioxidants like ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), carotenoids, flavonoids. Coffee and tea are the main contributors of FRAP in Rotterdam Study and in other studies as well [21, 55]. The anti-inflammatory effects of both coffee and tea have been previously reported [56]. On the other hand, the anti-inflammatory effect of fruits and vegetables is supposed to come from vitamins and flavonoids they contain [19]. Antioxidants act scavenging ROS and inhibit NF-κβ, even though not all at the same level. This may lead to decreased oxidative stress, and therefore in diminished low-grade chronic inflammation.

Our study is unique among previous investigations because of its prospective design, large population-based study group and adjustment for a broad range of confounders. Also, to our knowledge, this is one of the first prospective studies to use measures of CRP in two time points. Also, in our study, we could assess the association between FRAP and markers of oxidative stress, such as GGT and UA, showing a strong association, and therefore supporting internal validity. Nevertheless, it has some limitations. First, assessment of diet was done at baseline and there may have been changes in antioxidant consumption over time. However, it has been shown that dietary habits change very little over time in middle-aged adults [57]. Second, the FFQ can be limited by errors in reporting and recall and by incomplete assessment of all sources of antioxidant intake, which may introduce misclassification in dietary intake and would bias results toward the null. Third, we did not have repeated measures for leptin, adiponectin, PAI-1

and resistin. Also, these markers were assessed 10 years later from FRAP, UA and GGT measurements. Moreover, we had no measurements of other adipocyte-derived inflammatory markers like interleukin-6 or tumor necrosis factor-α or more accurate measures of oxidative stress such as ROS, that could have strengthened the results. Furthermore, we used a subpopulation for the analysis regarding adiponectin, resistin, leptin and PAI-1 as outcome, which may have introduced selection bias since this population was different with respect to some health characteristics. However, it has been shown that using a restricted source population for a cohort study usually leads to bias towards the null which may have led to an underestimation of the observed associations in our study of the exposure[58]. Moreover, it has been shown that using a selected source population for a cohort study usually leads to bias towards the null. Furthermore, the restriction of the main analysis in the participants with available information on all exposures and outcomes investigated in this study provided similar results, and therefore, selection bias is less likely to have happened. Finally, physical activity was measured at the third round of the Rotterdam Study. Therefore, we cannot fully exclude residual confounding by physical activity levels.

5. CONCLUSIONS

In conclusion, we found no consistent association between FRAP and CRP levels, while both UA and GGT were associated with low CRP. Furthermore, high overall dietary antioxidant capacity of diet and lower levels of UA were associated with lower levels of pro-inflammatory adipocytokines and higher levels of anti-inflammatory adipocytokines.

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DISCLOSURE

The authors declare no conflict of interest.

CONTRIBUTORS/AUTHORSHIP

TM and OHF conceived and designed the study. NS, TM and OHF participated in the statistical analyses, data interpretation, manuscript writing and revising and had primary responsibility for the final content of the manuscript. JCK participated in data synthesis/analysis and interpretation

of the data. NS, AD, TM, JCK and OHF drafted the final manuscript. AH designed the Rotterdam Study and participated in data interpretation, manuscript writing and revising. All authors contributed to the critical revision of the manuscript and approved the final version.

REFERENCES

[1] Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. Am J Med. 2004;116 Suppl 6A:9S-16S.

[2] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105:1135-43.

[3] Rifai N, Ridker PM. Inflammatory markers and coronary heart disease. Curr Opin Lipidol. 2002;13:383-9.

[4] Tremblay J, Hamet P. Biomarkers of vascular complications in type 2 diabetes. Metabolism: Clinical & Experimental. 2015;64:S28-32.

[5] Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Jama. 2001;286:327-34.

[6] Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. 2006;444:875-80.

[7] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11:85-97.

[8] Sainz N, Barrenetxe J, Moreno-Aliaga MJ, Martinez JA. Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. Metabolism: Clinical & Experimental. 2015;64:35-46.

[9] Nakanishi S, Yamane K, Kamei N, Nojima H, Okubo M, Kohno N. A protective effect of adiponectin against oxidative stress in Japanese Americans: the association between adiponectin or leptin and urinary isoprostane. Metabolism: Clinical & Experimental. 2005;54:194-9.

[10] Park HK, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. Metabolism: Clinical & Experimental. 2015;64:24-34.

[11] Gasbarrino K, Gorgui J, Nauche B, Cote R, Daskalopoulou SS. Corrigendum to "circulating adiponectin and carotid intima-media thickness: a systematic review and meta-analysis" [Metabolism 65 (2016) 968-986]. Metabolism: Clinical & Experimental. 2016;65:1434-42.

[12] Kizer JR. Adiponectin, cardiovascular disease, and mortality: parsing the dual prognostic implications of a complex adipokine. Metabolism: Clinical & Experimental. 2014;63:1079-83.

[13] Potter van Loon BJ, Kluft C, Radder JK, Blankenstein MA, Meinders AE. The cardiovascular risk factor plasminogen activator inhibitor type 1 is related to insulin resistance. Metabolism: Clinical & Experimental. 1993;42:945-9.

[14] Nakamura T, Adachi H, Hirai Y, Satoh A, Ohuchida M, Imaizumi T. Association of plasminogen activator inhibitor-1 with insulin resistance in Japan where obesity is rare. Metabolism: Clinical & Experimental. 2003;52:226-9.

[15] Putz DM, Goldner WS, Bar RS, Haynes WG, Sivitz WI. Adiponectin and C-reactive protein in obesity, type 2 diabetes, and monodrug therapy. Metabolism: Clinical & Experimental. 2004;53:1454-61.

[16] Savini I, Catani MV, Evangelista D, Gasperi V, Avigliano L. Obesity-Associated Oxidative Stress: Strategies Finalized to Improve Redox State. International Journal of Molecular Sciences. 2013;14:10497-538.

[17] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44-84.

[18] Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr. 2004;44:275-95.

[19] Alissa EM, Ferns GA. Dietary Fruits and Vegetables and Cardiovascular Diseases Risk. Crit Rev Food Sci Nutr. 2015:0.

[20] Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. Analytical Biochemistry. 1996;239:70-6.

[21] Pantavos A, Ruiter R, Feskens EF, de Keyser CE, Hofman A, Stricker BH, et al. Total dietary antioxidant capacity, individual antioxidant intake and breast cancer risk: the Rotterdam Study. Int J Cancer. 2015;136:2178-86.
[22] Korkmaz GG, Altinoglu E, Civelek S, Sozer V, Erdenen F, Tabak O, et al. The association of oxidative stress markers with conventional risk factors in the metabolic syndrome. Metabolism: Clinical & Experimental. 2013;62:828-35.
[23] Brighenti F, Valtuena S, Pellegrini N, Ardigo D, Del Rio D, Salvatore S, et al. Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects. Br J Nutr. 2005;93:619-25.
[24] Kobayashi S, Murakami K, Sasaki S, Uenishi K, Yamasaki M, Hayabuchi H, et al. Dietary total antioxidant capacity from different assays in relation to serum C-reactive protein among young Japanese women. Nutr J. 2012;11:91.
[25] Lee DH, Blomhoff R, Jacobs DR, Jr. Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic Res. 2004;38:535-9.
[26] Wijnands JMA, Boonen A, Dagnelie PC, van Greevenbroek MMJ, van der Kallen CJH, Ferreira I, et al. The cross-sectional association between uric acid and atherosclerosis and the role of low-grade inflammation: the CODAM study. Rheumatology. 2014;53:2053-62.
[27] Ruggiero C, Cherubini A, Ble A, Bos AJ, Maggio M, Dixit VD, et al. Uric acid and inflammatory markers. Eur Heart J. 2006;27:1174-81.
[28] Liu X, Hamnvik OP, Chamberland JP, Petrou M, Gong H, Christophi CA, et al. Circulating alanine transaminase (ALT) and gamma-glutamyl transferase (GGT), but not fetuin-A, are associated with metabolic risk factors, at baseline and at two-year follow-up: the prospective Cyprus Metabolism Study. Metabolism. 2014;63:773-82.
[29] Fukui M, Tanaka M, Toda H, Asano M, Yamazaki M, Hasegawa G, et al. The serum concentration of allograft inflammatory factor-1 is correlated with metabolic parameters in healthy subjects. Metabolism: Clinical & Experimental. 2012;61:1021-5.
[30] Hofman A, Brusselle GG, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A, et al. The Rotterdam Study: 2016 objectives and design update. Eur J Epidemiol. 2015;30:661-708.
[31] Carlsen MH, Halvorsen BL, Holte K, Bøhn SK, Dragland S, Sampson L, et al. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutrition Journal. 2010;9:3-.
[32] Bos MJ, Koudstaal PJ, Hofman A, Witteman JCM, Breteler MMB. Uric Acid Is a Risk Factor for Myocardial Infarction and Stroke: The Rotterdam Study. Stroke. 2006;37:1503-7.
[33] Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. The American Journal of Clinical Nutrition. 1997;65:1220S-8S.
[34] Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics. 1988;44:1049-60.
[35] Oliveira A, Rodriguez-Artalejo F, Lopes C. The association of fruits, vegetables, antioxidant vitamins and fibre intake with high-sensitivity C-reactive protein: sex and body mass index interactions. Eur J Clin Nutr. 2009;63:1345-52.
[36] Detopoulou P, Panagiotakos DB, Chrysohoou C, Fragopoulou E, Nomikos T, Antonopoulou S, et al. Dietary antioxidant capacity and concentration of adiponectin in apparently healthy adults: the ATTICA study. Eur J Clin Nutr. 2010;64:161-8.
[37] Wang Y, Yang M, Lee SG, Davis CG, Koo SI, Fernandez ML, et al. Diets high in total antioxidant capacity improve risk biomarkers of cardiovascular disease: a 9-month observational study among overweight/obese postmenopausal women. Eur J Nutr. 2014;53:1363-9.

[38] Katalinic V, Modun D, Music I, Boban M. Gender differences in antioxidant capacity of rat tissues determined by 2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and ferric reducing antioxidant power (FRAP) assays. Comp Biochem Physiol C Toxicol Pharmacol. 2005;140:47-52.
[39] Julicher RH, Sterrenberg L, Haenen GR, Bast A, Noordhoek J. Sex differences in the cellular defence system against free radicals from oxygen or drug metabolites in rat. Arch Toxicol. 1984;56:83-6.
[40] Keenan T, Blaha MJ, Nasir K, Silverman MG, Tota-Maharaj R, Carvalho JAM, et al. Relation of Uric Acid to Serum Levels of High-Sensitivity C-Reactive Protein, Triglycerides, and High-Density Lipoprotein Cholesterol and to Hepatic Steatosis. The American Journal of Cardiology. 2012;110:1787-92.
[41] Park JS, Kang S, Ahn CW, Cha BS, Kim KR, Lee HC. Relationships between serum uric acid, adiponectin and arterial stiffness in postmenopausal women. Maturitas. 2012;73:344-8.
[42] Ali SS, Shaharyar S, Blaha MJ, Jamal O, Agatston AS, Blumenthal RS, et al. ELEVATED GAMMA-GLUTAMYL TRANSFERASE IS INDEPENDENTLY ASSOCIATED WITH BURDEN OF SUBCLINICAL VASCULAR INFLAMMATION. Journal of the American College of Cardiology. 2013;61.
[43] Hermsdorff HH, Puchau B, Volp AC, Barbosa KB, Bressan J, Zulet MA, et al. Dietary total antioxidant capacity is inversely related to central adiposity as well as to metabolic and oxidative stress markers in healthy young adults. Nutr Metab (Lond). 2011;8:59.
[44] Nakatsuji H, Kishida K, Sekimoto R, Komura N, Kihara S, Funahashi T, et al. Accumulation of adiponectin in inflamed adipose tissues of obese mice. Metabolism. 2014;63:542-53.
[45] Bluher M, Mantzoros CS. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. Metabolism. 2015;64:131-45.
[46] Brydon L. Adiposity, leptin and stress reactivity in humans. Biol Psychol. 2011;86:114-20.
[47] Friedman JM, Mantzoros CS. 20 years of leptin: from the discovery of the leptin gene to leptin in our therapeutic armamentarium. Metabolism. 2015;64:1-4.
[48] Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334:292-5.
[49] Snijder MB, Heine RJ, Seidell JC, Bouter LM, Stehouwer CD, Nijpels G, et al. Associations of adiponectin levels with incident impaired glucose metabolism and type 2 diabetes in older men and women: the hoorn study. Diabetes Care. 2006;29:2498-503.
[50] Lyon CJ, Hsueh WA. Effect of plasminogen activator inhibitor-1 in diabetes mellitus and cardiovascular disease. Am J Med. 2003;115 Suppl 8A:62S-8S.
[51] Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature. 2001;409:307-12.
[52] Alexe DM, Syridou G, Petridou ET. Determinants of early life leptin levels and later life degenerative outcomes. Clin Med Res. 2006;4:326-35.
[53] Kang DH, Park SK, Lee IK, Johnson RJ. Uric acid-induced C-reactive protein expression: implication on cell proliferation and nitric oxide production of human vascular cells. J Am Soc Nephrol. 2005;16:3553-62.
[54] Lee DH, Jacobs DR, Jr. Association between serum gamma-glutamyltransferase and C-reactive protein. Atherosclerosis. 2005;178:327-30.
[55] Qureshi SA, Lund AC, Veierod MB, Carlsen MH, Blomhoff R, Andersen LF, et al. Food items contributing most to variation in antioxidant intake; a cross-sectional study among Norwegian women. BMC Public Health. 2014;14:45.
[56] Tangney CC, Rasmussen HE. Polyphenols, inflammation, and cardiovascular disease. Curr Atheroscler Rep. 2013;15:324.
[57] Goldbohm RA, Vantveer P, Vandenbrandt PA, Vanthof MA, Brants HAM, Sturmans F, et al. Reproducibility of a Food Frequency Questionnaire and Stability of Dietary Habits Determined from 5 Annually Repeated Measurements. European Journal of Clinical Nutrition. 1995;49:420-9.

524 [58] Nilsen RM, Suren P, Gunnes N, Alsaker ER, Bresnahan M, Hirtz D, et al. Analysis of self-selection bias
525 in a population-based cohort study of autism spectrum disorders. Paediatr Perinat Epidemiol.
526 2013;27:553-63.

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Figure 1: Flow chart of participants included in the analysis of overall antioxidant capacity of diet and inflammation : the Rotterdam Study.

FRAP, ferric reducing antioxidant potential; PAI-1, Plasminogen activator inhibitor-1;

Figure 2: Flow Chart of participants included in the analysis of uric acid and gamma-glutamyltransferse (GGT) with inflammatory markers: the Rotterdam Study.

PAI-1, Plasminogen activator inhibitor-1;

573 **Table 1** Baseline characteristics of study participants (N=4506): the Rotterdam Study.

	Total (N=4506)	Women (N=2571)	Men (N=1935)	P - value[b]
FRAP (mmol/day)	20.37±5.48	20.02±5.07	20.83±5.95	<0.001
Age (years)	67.64±7.74	67.93±8.01	67.26±7.36	0.004
Energy intake (kcal/day)	1989.51±504.48	1796.30±405.97	2246.17±508.24	<0.001
Physical activity (MET hours/week)	78.30±44.28	89.45±43.90	69.15±41.56	<0.001
BMI (kg/m^2)	26.18±3.57	26.55±3.99	25.68±2.85	<0.001
CRP first round[a] (mg/ml)	1.78 (0.86-3.39)	1.74 (0.85-3.13)	1.85 (0.87-3.79)	<0.001
CRP third round[a] (mg/ml)	2.34 (1.16-4.34)	2.36 (1.15-4.26)	2.30 (1.16-4.46)	0.583
Non-fasting serum glucose[a] (mml/l)	6.20 (5.45-7.40)	6.10 (5.40-7.10)	6.40 (5.60-7.70)	<0.001
SBP (mmHg)	183.84±22.05	139.10±22.22	138.50±21.83	0.363
DBP (mmHg)	78.80±11.26	73.29±11.14	74.47±11.39	<0.001
Total Cholesterol (mmol/l)	6.68±1.19	6.92±1.18	6.35±1.12	<0.001
Hormone replacement therapy, n (%)	65 (1.4%)	63 (2.5%)	2 (0.1%)	<0.001

Uric Acid (µmol/l)	320.16±77.76	296.62±71.44	352.88±74.40	<0.001
Vitamin supplement use, n (%)	329 (7.3%)	245 (9.5%)	84 (4.3%)	<0.001
GGT[a] (U/l)	23.00 (18.00-32.00)	21.00 (16.00-28.00)	27.00 (21.00-38.00)	<0.001
Lipid reducing agents, n (%)	119 (2.6%)	66 (2.6%)	53 (2.7%)	0.395
DHDI	30.23±9.20	31.95±9.11	27.95±8.83	<0.001
Prevalent diseases*, n (%)	1490 (33.1%)	712 (27.7%)	778 (40.2%)	<0.001
Smoking: Never or former, n (%)	3440 (76.3%)	2079 (80.9%)	1361 (70.3%)	<0.001
Current, n (%)	1066 (23.7%)	492 (19.1%)	574 (29.7%)	
Income: Low, n (%)	1014 (22.5%)	829 (32.2%)	185 (9.6%)	<0.001
Middle, n (%)	2002 (44.4%)	1062 (41.3%)	940 (48.6%)	
High, n (%)	1490 (33.1%)	680 (26.4%)	810 (41.9%)	
Education: Low, n (%)	2321 (51.5%)	1597 (62.1%)	724 (37.4%)	<0.001
Middle, n (%)	1781 (39.5%)	862 (33.5%)	919(47.5%)	
High, n (%)	404 (9.0%)	112 (4.4%)	292 (15.1%)	
Processed meat intake (servings/day)	1.47±1.24	1.19±1.05	1.84±1.37	<0.001

Unprocessed meat intake (servings/day)	0.74±0.47	0.69±0.42	0.82±0.53	0.048
Alcohol[#]:				<0.001
Quartile I (<0.1886g), n (%)	1126 (25.0%)	847 (32.9%)	279 (14.4%)	
Quartile II (0.1886-3.6813g), n (%)	1127 (25.0%)	798 (31.0%)	329 (17.0%)	
Quartile III (3.6813-15.1401g), n (%)	1127 (25.0%)	572 (22.2%)	555 (28.7%)	
Quartile IV (>15.1401g), n (%)	1126 (25.0%)	354 (13.8%)	772 (39.9%)	
Leptin[c] (ng/mL)	7.63 (3.82-16.20)	14.00 (7.85-22.00)	4.02 (2.44-6.64)	<0.001
Adiponectin[c] (µg/mL)	3.42 (2.25-5.00)	4.34 (3.17-5.89)	2.7 (1.94-3.63)	<0.001
PAI-1[c] (ng/mL)	17.15 (9.98-28.63)	17.90 (10.30-33.20)	16.10 (9.66-26.15)	0.009
Resistin[c] (ng/mL)	0.42 (0.31-0.58)	0.42 (0.31-0.58)	0.43 (0.31-0.59)	0.951

574 FRAP, ferric reducing antioxidant potential; BMI, Body mass index; CRP, C-reactive protein;
575 DHDI, Dutch healthy diet index (excluding fruits and vegetables); DBP, diastolic blood pressure;
576 GGT, Gamma glutamyltransferase; PAI-1, Plasminogen activator inhibitor - 1; SBP, systolic
577 blood pressure
578 [a] Median (Range between 25th percentile and 75th percentile)
579 [b] Comparison between men and women. For continuous variables = Independent sample T-Test;

For categorical variables = Chi^2 (χ^2)

[c] N=798 included in the analyses of FRAP and adipocytokines.

*Prevalent disease include cardiovascular disease and type 2 diabetes.

[#] Quartile I refers to values < 25^{th} percentile; Quartile II refers to values between 25^{th} and 50^{th} percentile; Quartile III refers to values between 50^{th} and 75^{th} percentile; Quartile IV refers to values >75^{th} percentile.

Table 2 Association of ferric reducing antioxidant potential with C-reactive protein and adipocytokines: the Rotterdam Study.

	Model 1 β (95% CI)	Model 2 β (95%CI)
CRP§ (N=4507)	0.001 (-0.004,0.007)	-0.002(-0.007,0.003)a
Leptin§ (N=798)	-0.012 (-0.023, -0.001)	-0.009(-0.017, -0.00005)b
Adiponectin§ (N=798)	0.009 (0.002,0.015)*	0.009(0.003,0.016)*b
PAI-1§ (N=798)	-0.018(-0.028, -0.008)*	-0.018(-0.027, -0.008)*b
Resistin§ (N= 798)	0.002 (-0.006,0.009)	0.001(-0.006,0.009)b

CI, confidence interval; FRAP, ferric reducing antioxidant potential; CRP, C-reactive protein; PAI-I, Plasminogen Activator Inhibitor-1.

§ Variables were log transformed to better approximate normal distribution.

*remains significant after Bonferroni correction (p=0.0166)

βs and 95% confidence intervals were estimated using generalized estimated equations (for C-reactive protein as outcome) and linear regression models (for leptin, adiponectin, PAI-1 and resistin as outcomes) adjusted for age and gender (Model 1), and additionally adjusted for body mass index, smoking status, prevalent diseases, systolic blood pressure, non-fasting glucose, total cholesterol, index1(time), energy intake, income, alcohol, statin use (Model 2a). For adipocytokines, model 2 was further adjusted for C-reactive protein (Model 2b). Additional adjustment for other covariates did not change the effect estimate with >10%.

Table 3 Association of uric acid and gamma glutamyltransferase with C-reactive protein and adipocytokines: the Rotterdam study.

	Uric acid (per SD)		GGT (per SD)[§]	
	Model 1	Model 2	Model 1	Model 2
	β (95% CI)	β (95%CI)	β (95% CI)	β (95% CI)
CRP[§](N=4154)	0.198	0.123	0.213	0.160
	(0.167,0.228)*	(0.091,0.155)*[a]	(0.181,0.245)*	(0.128,0.191)* [a]
Leptin[§](N=707)	0.257	0.100	0.101	-0.020
	(0.197,0.316)*	(0.048,0.152)*[b]	(0.040,0.161)*	(-0.070,0.030)[b]
Adiponectin[§](N=707)	-0.099	-0.066	-0.041	-0.005
	(-0.135,-0.064)*	(-0.103,-0.028)*[b]	(-0.075,-0.006)*	(-0.041,0.032)[b]
PAI-1[§](N=707)	0.246	0.147	0.148	0.047
	(0.193,0.300)*	(0.091,0.203)*[b]	(0.094,0.202)	(-0.007,0.100)[b]
Resistin[§](N=707)	0.014	0.026	0.006	0.012
	(-0.028,0.056)	(-0.020,0.072)[b]	(-0.035,0.046)	(-0.032,0.055)[b]

CI, confidence interval; CRP, C-reactive protein; GGT, gamma glutamyltransferase; PAI-1, Plasminogen Activator Inhibitor-1; SD, standard deviation.

[§] Variables were log transformed to better approximate normal distribution.

* remains significant after Bonferroni correction (p=0.0166)

βs and 95% confidence intervals were estimated using generalized estimated equations (for C-reactive protein as outcome) and linear regression models (for leptin, adiponectin, PAI-1 and resistin as outcomes) adjusted for age and sex (Model 1) and additionally adjusted for baseline body mass time, time of measurement, non-fasting glucose, energy intake, total cholesterol,

611 hormone replacement therapy, systolic blood pressure, diastolic blood pressure, statin use,
612 income, alcohol+ GGT/uric acid (adjustment for GGT when uric acid was the exposure and vice
613 versa) (Model 2a). For adipocytokines as outcomes, model 2 was further adjusted for baseline C-
614 reactive protein (Model 2b). Results are presented per standard deviation uric acid (for CRP as
615 outcome: 1SD= 80.5611 µmol/L; for adipocytokines as outcome: 1SD=73,1832 µmol/L) and
616 GGT levels (for CRP as outcome: 1SD=29,9731 U/L ; for adipocytokines as outcome:
617 1SD=22,4034 U/L).

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