

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

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28 **ABSTRACT**

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

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

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

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

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

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

51

52

53 1. INTRODUCTION

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

63 An imbalance between plasma oxidants-antioxidants (oxidative stress) as well as dietary
64 antioxidants have been suggested to play a role in systemic low-grade chronic inflammation [16].
65 Oxidative stress, defined as an increased load of free radicals, induces the activation of NF- κ B, a
66 transcription factor involved in cell survival, differentiation, and inflammation [17]. Antioxidant
67 molecules neutralize such free radicals and therefore diminish low-grade inflammation. Dietary
68 antioxidants, including vitamin A, E and C, can counteract oxidative stress and therefore its
69 adverse effect on inflammation [18]. However, studies evaluating the role of individual
70 antioxidants on inflammation have shown contradictory results, which can be due to not taking
71 into account the interactive effect among nutrients [19]. Hence, assessing the overall effects of
72 antioxidants in the diet instead of the individual effects can provide further information regarding
73 the association between diet and inflammation [19]. The ferric reducing antioxidant potential
74 (FRAP) measures the overall antioxidant capacity of diet by measuring the reduction of ferric
75 iron (Fe³⁺) to ferrous iron (Fe²⁺)[20] and, has been used as a marker of the overall effects of

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

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

86 **2. MATERIAL AND METHODS**

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

94 **2.1 MEASUREMENTS**

95 **2.1.1 Assessment of ferric reducing antioxidant potential (FRAP)**

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

109 **2.1.2 Assessment of Uric Acid and Gamma-glutamyltransferase (GGT)**

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

118 **2.1.3 Assessment of hs-CRP and adipocytokines**

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

125 For assessment of adipocytokines, fasting blood samples were collected at the research center, in
126 the third center visit (1997-1999). Plasma was isolated and immediately put on ice and stored at
127 -80°C. Citrate plasma (200UI) was sent in July 2008 to Rules-Based Medicine, Austin, Texas
128 (www.myriadrbm.com). Fifty inflammatory biomarkers were quantified using multiplex
129 immunoassay on a custom designed human multianalyte profile. The intra-assay variability was
130 less than 4% and the inter assay variability was less than 13%. Biomarkers with more than 60%
131 completeness of measurements were selected for imputation and further analysis. Data on leptin,
132 adiponectin, plasminogen activator inhibitor 1 (PAI-1) and resistin, major inflammatory markers
133 released by adipose tissue [7], were available. The inflammatory markers investigated in the
134 current study have no standard international calibration reference therefore, interpretation of the
135 absolute values should be with caution. Since the current study is conducted within one set of
136 individuals, the use of relative measures should not affect the effect estimates.

137 **2.2 POPULATION FOR ANALYSIS**

138 **2.2.1 FRAP and inflammation**

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

151 **2.2.2 Uric acid, gamma-glutamyltransferase and inflammation**

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

160 **2.3 STATISTICAL ANALYSES**

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

184 glucose(continuous), education (low, intermediate, high), income (low, intermediate, high),
185 alcohol, energy-adjusted processed meat intake (continuous), energy-adjusted unprocessed meat
186 intake (continuous), Dutch Healthy Diet index (DHDI)(continuous). For the analysis on leptin,
187 adiponectin, PAI-1 and resistin as outcomes, we also adjusted for CRP in the first visit (1990-
188 1993) as a proxy of chronic inflammation at baseline as adipocytokines were measured only at
189 the third round visit (1997-1999). To check for non-linear relation, a quadratic term was tested
190 in multivariable model 2. Since there is evidence that the association between diet antioxidants
191 and inflammatory biomarkers differs by sex [35], we tested for statistical interaction by adding a
192 product term in model 2. Furthermore, stratified analysis was performed and the results were
193 presented for model 2. We further checked the association of FRAP with uric acid, and FRAP
194 with GGT using multivariable linear regression models. We also performed sensitivity analyses
195 (i) restricting all main analyses to participants with available information on all exposures and
196 outcomes investigated (N=633), (ii) excluding participants with chronic diseases (CVD or T2D)
197 and (iii) further adjusted for BMI change from first to the third visit. A P-value lower than 0.05
198 was considered as statistically significant, but to account for multiple testing, we adjusted the p-
199 value from 0.05 to 0.0166 by applying the Bonferroni correction for the number of exposures
200 studied (N=3).

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

204 **3. RESULTS**

205 The main characteristics of the study population are shown by gender in Table 1. FRAP score
206 and GGT levels were lower in women compare to men (FRAP: 20.02 ± 5.07 mmol/day vs.
207 20.83 ± 5.95 mmol/day; GGT: median 21 U/l, range 351U/l vs median 27U/l, range 576 U/l)
208 whereas UA levels were higher in women (296.62 ± 71.44 μ mol/l vs. 352.88 ± 74.40 μ mol/l)
209 (**Table 1**). CRP levels at baseline were slightly lower in women whereas no significant
210 difference was observed in the CRP levels at the third visit (**Table 1**). Also, women had slightly
211 higher BMI (26.55 vs. 25.68 kg/m²) and leptin levels (median: 14.0 vs 4.02 ng/mL) than men.
212 Although the energy intake was lower in women (1796 vs. 2246.2 kcal/day), they had higher
213 physical activity (89.45 vs 69.15 MET) as well as a healthier diet (DHDI: 31.95 vs. 27.95) than
214 men. Among the adipocytokines, PAI-1 and leptin ($r=0.466$, $p=0.01$), PAI-1 and CRP ($r=0.325$,
215 $p=0.01$), PAI-1 and adiponectin ($r=-0.270$, $p=0.01$), leptin and CRP ($r=0.254$, $p=0.01$) showed
216 the highest correlation (**Supplementary Table S1**). Compared to subjects who did not have
217 information on leptin, adiponectin, PAI-1 and resistin, subjects who had information on these
218 inflammatory markers did not differ with respect to FRAP, but had higher levels of CRP, BMI,
219 systolic blood pressure and higher prevalence of chronic disease (**Supplementary Table S2**).

220 **3.1 The association between FRAP score and inflammatory markers**

221 There was no association between FRAP and hs-CRP levels in the age and gender-adjusted
222 model or multivariable model (**Table 2**). In the multivariable models, FRAP score was
223 associated with lower levels of leptin ($\beta=-0.01$, 95%CI= -0.02 ; -0.001), PAI-1 ($\beta=-0.02$, 95%CI= $-$
224 0.03 ; -0.01) and higher levels of adiponectin ($\beta=0.01$, 95%CI= 0.002 ; 0.015). No association was
225 observed between FRAP and resistin. (**Table 2**).

226 **3.2 The association between UA, GGT and inflammatory markers**

227 After multivariable adjustment, increased levels of UA were associated with higher levels of hs-
228 CRP ($\beta=0.12$, 95%CI=0.09; 0.16), leptin ($\beta=0.10$, 95%CI=0.05; 0.15) PAI-1 ($\beta=0.15$,
229 95%CI=0.09; 0.20), and lower levels of adiponectin ($\beta=-0.07$, 95%CI=-0.10; -0.03) (**Table 3**).
230 No association was observed between UA and resistin (**Table 3**). Similarly, after correcting for
231 confounding factors, GGT was associated with higher levels of hs-CRP ($\beta=0.06$, 95%CI=0.13;
232 0.19) whereas no association was observed between GGT and adipocytokines (**Table 3**).

233 **3.3 Effect modification by gender**

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

242 **3.4 Sensitivity analyses**

243 Higher levels of FRAP score were associated with lower levels of both UA ($\beta=-0.003$, 95%CI=-
244 0.005; -0.002) and GGT ($\beta=-0.006$, 95%CI=-0.009; -0.003), after correcting for confounders
245 (**Supplementary Figure S1 and Supplementary Table S5**). There was no evidence against a
246 linear relation in all the main analyses (all P -values for quadratic term >0.05, data not shown).
247 Also, all associations that were statistically significant in the main analyses remained unchanged
248 in terms of statistical significance when the analyses were restricted to (i) participants with

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

255 **4. DISCUSSION**

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

259 In the current investigation, no association was found between FRAP and CRP levels in the
260 overall population, however, in women, a higher FRAP score was associated with diminished
261 chronic inflammation. Similar to our findings, Detopoulou et al in a cross-sectional study of 532
262 men and women found no association between FRAP and CRP levels in the total population
263 [36]. In contrast, a cross-sectional study from Brighenti et al [23], which used the TAC assay to
264 measure antioxidant capacity, showed an association with lower levels of CRP in an adult Italian
265 population including both men and women. We did find an interaction with gender, suggesting
266 that the association between FRAP and CRP levels is present only in women, which is in line
267 with the results of previous studies conducted in women. For example, the study from Kobayashi
268 et al.[24] showed that dietary total antioxidant capacity was associated with lower serum CRP
269 concentrations in young Japanese women (474 women, aged 18-22 years) regardless of assay
270 used to measure it. Also, in a 9-month observational study among postmenopausal women,

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

273 Several studies show a stronger defense against oxidative damage in the female liver tissue,
274 which is the major determinant of CRP levels [38]. Animal studies have shown that, compared to
275 males, antioxidant capacity of diet assessed by FRAP and other methods is higher in liver tissue
276 [38]. Also, females have greater mean hepatic alpha-tocopherol levels, total capacity of the
277 cellular systems that detoxify reactive oxygen species or free radical-drug metabolites seems to
278 be higher in the female rat liver[39]. These evidence may account for the sex differences
279 observed in the association between FRAP and CRP levels in our study, which merits further
280 investigation.

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

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

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

316 Probably oxidative stress is the pathway that links antioxidants with a low inflammatory profile.
317 The human body has a number of defense mechanisms against oxidative stress including
318 antioxidants, preventive and repair mechanism and physical defense [17]. Antioxidants
319 themselves can be divided into enzymatic antioxidants (glutathione peroxidase, peroxide
320 dismutase and catalase) and non-enzymatic antioxidants like ascorbic acid (vitamin C), alpha-
321 tocopherol (vitamin E), carotenoids, flavonoids. Coffee and tea are the main contributors of
322 FRAP in Rotterdam Study and in other studies as well [21, 55]. The anti-inflammatory effects of
323 both coffee and tea have been previously reported [56]. On the other hand, the anti-inflammatory
324 effect of fruits and vegetables is supposed to come from vitamins and flavonoids they contain
325 [19]. Antioxidants act scavenging ROS and inhibit NF- κ B, even though not all at the same level.
326 This may lead to decreased oxidative stress, and therefore in diminished low-grade chronic
327 inflammation.

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

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

354 **5. CONCLUSIONS**

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

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375 **DISCLOSURE**

376 The authors declare no conflict of interest.

377 **CONTRIBUTORS/AUTHORSHIP**

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

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

384

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

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

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

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571 PAI-1, Plasminogen activator inhibitor-1;

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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 (mmol/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 ($\mu\text{mol/l}$)	320.16 \pm 77.76	296.62 \pm 71.44	352.88 \pm 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 \pm 9.20	31.95 \pm 9.11	27.95 \pm 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 \pm 1.24	1.19 \pm 1.05	1.84 \pm 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;

580 For categorical variables = Chi² (χ^2)

581 ^c N=798 included in the analyses of FRAP and adipocytokines.

582 *Prevalent disease include cardiovascular disease and type 2 diabetes.

583 # Quartile I refers to values < 25th percentile; Quartile II refers to values between 25th and 50th
584 percentile; Quartile III refers to values between 50th and 75th percentile; Quartile IV refers to
585 values >75th percentile.

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

	Model 1	Model 2
	β (95% CI)	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

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

591 § Variables were log transformed to better approximate normal distribution.

592 *remains significant after Bonferroni correction (p=0.0166)

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

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601 **Table 3** Association of uric acid and gamma glutamyltransferase with C-reactive protein and
 602 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 (0.167,0.228)*	0.123 (0.091,0.155)* ^a	0.213 (0.181,0.245)*	0.160 (0.128,0.191)* ^a
Leptin [§] (N=707)	0.257 (0.197,0.316)*	0.100 (0.048,0.152)* ^b	0.101 (0.040,0.161)*	-0.020 (-0.070,0.030) ^b
Adiponectin [§] (N=707)	-0.099 (-0.135,-0.064)*	-0.066 (-0.103,-0.028)* ^b	-0.041 (-0.075,-0.006)*	-0.005 (-0.041,0.032) ^b
PAI-1 [§] (N=707)	0.246 (0.193,0.300)*	0.147 (0.091,0.203)* ^b	0.148 (0.094,0.202)	0.047 (-0.007,0.100) ^b
Resistin [§] (N=707)	0.014 (-0.028,0.056)	0.026 (-0.020,0.072) ^b	0.006 (-0.035,0.046)	0.012 (-0.032,0.055) ^b

603 CI, confidence interval; CRP, C-reactive protein; GGT, gamma glutamyltransferase; PAI-1,

604 Plasminogen Activator Inhibitor-1; SD, standard deviation.

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

606 * remains significant after Bonferroni correction (p=0.0166)

607 β s and 95% confidence intervals were estimated using generalized estimated equations (for C-

608 reactive protein as outcome) and linear regression models (for leptin, adiponectin, PAI-1 and

609 resistin as outcomes) adjusted for age and sex (Model 1) and additionally adjusted for baseline

610 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 $\mu\text{mol/L}$; for adipocytokines as outcome: 1SD=73,1832 $\mu\text{mol/L}$) 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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