

## ORIGINAL ARTICLE

# Association of coffee consumption with serum adiponectin, leptin, inflammation and metabolic markers in Japanese workers: a cross-sectional study

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**BACKGROUND:** Mechanisms underlying coffee's beneficial actions against cardiovascular disease and glucose metabolism are not well understood. Little information is available regarding association between coffee consumption and adipocytokines.

**OBJECTIVE:** We investigated potential associations between coffee consumption and adiponectin, leptin, markers for subclinical inflammation, glucose metabolism, lipids and liver enzymes. We then investigated whether adipocytokines played a role in the association between coffee consumption and these markers.

**DESIGN AND SUBJECTS:** This is a cross-sectional study comprising 2554 male and 763 female Japanese workers. Potential relations between coffee consumption and adipocytokines or other markers were evaluated using a multiple linear regression model adjusted for confounding factors. We evaluated whether adiponectin and leptin partly explain the associations between coffee consumption and each marker by multiple mediation analysis.

**RESULTS:** Coffee consumption showed significant positive associations with adiponectin and total and low-density lipoprotein cholesterol, and inverse associations with leptin, high sensitivity C-reactive protein (hs-CRP), triglycerides and liver enzymes (all  $P < 0.05$ ). An adjustment for adiponectin and leptin significantly attenuated the associations between coffee consumption and hs-CRP or triglycerides, but not for liver enzymes. No associations were observed between coffee consumption and glucose metabolism-related markers.

**CONCLUSION:** Coffee consumption was associated with high adiponectin and low leptin levels. We speculated that adipocytokines mainly explain the associations of coffee consumption with lipids and hs-CRP. Factors other than adipocytokines may explain the association between coffee consumption and liver function.

*Nutrition and Diabetes* (2012) 2, e33; doi:10.1038/nutd.2012.6; published online 2 April 2012

**Keywords:** coffee; adiponectin; leptin; lipids; inflammatory markers; cross-sectional study

## INTRODUCTION

Coffee is one of the world's most commonly consumed beverages, and its effects on health have been attracting considerable attention.<sup>1–3</sup> An inverse association of coffee consumption with triglycerides<sup>4,5</sup> and a positive association with high-density lipoprotein cholesterol levels were previously reported.<sup>6</sup> Consumption of unfiltered coffee showed greater positive associations with total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) than filtered coffee.<sup>6</sup> A meta-analysis of 15 large prospective studies has shown that four or more cups of coffee consumption per day was associated with a significantly lower risk of type 2 diabetes mellitus (DM).<sup>3</sup> Although it has been speculated that weight loss,<sup>7</sup> attenuation of subclinical inflammation,<sup>8</sup> reduction of oxidative stress<sup>5</sup> and ameliorating effects on liver function<sup>9,10</sup> might be potential mechanisms underlying the association, the precise mechanisms remain unknown. A few studies that assessed the association of coffee consumption with subclinical inflammation showed inconsistent results: positive<sup>11</sup> and negative<sup>8</sup> associations. A short-term clinical trial showed that

C-reactive protein (CRP) levels did not change after coffee consumption.<sup>6</sup>

Adiponectin and leptin are well known to have important roles in the lipid and glucose metabolisms as well as inflammatory regulation.<sup>12–17</sup> The association between coffee consumption and adiponectin is still debated. Two cross-sectional studies showed positive association of coffee consumption with adiponectin,<sup>18,19</sup> whereas one study showed that coffee consumption was not significantly associated with adiponectin levels.<sup>20</sup> In a clinical trial, adiponectin levels increased after eight cups of coffee per day compared with 0 cups per day.<sup>5</sup> Few studies have examined the association of coffee consumption with leptin levels and it remains to be elucidated.<sup>21</sup> There is a possibility that coffee consumption is associated with adipocytokines, markers of subclinical inflammation, lipids, glucose metabolism and liver function. Additionally, we hypothesized that these associations might be explained partly by adipocytokines.

In this study, we explored the potential association of coffee consumption with a number of biomarkers,

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Received 26 September 2011; revised 28 February 2012; accepted 4 March 2012

and examined whether adipocytokines might explain these associations.

## MATERIALS AND METHODS

### Study sample

The Aichi Workers' cohort included 6651 civil servants in Aichi Prefecture, an urban and suburban area located in central Japan between 35 and 69 years of age at recruitment in 2002 (participation rate: 62%). Most of the cohorts were engaged in clerical work. Police officers, firefighters and public school teachers were not included. Baseline information was obtained through self-administered questionnaires and mandatory annual health check-ups provided by the worksite. Participants not consenting to the use of health check-up data or who did not provide blood sample were excluded from the present analysis ( $n = 2570$ ). We further excluded those without coffee consumption data ( $n = 52$ ), those with a history of cardiovascular diseases as well as those who had been on medication for hypertension, DM or dyslipidemia ( $n = 732$ ), because the treatment and associated behavioral change could confound the association between coffee consumption and blood markers, leaving 2554 men and 763 women eligible for the present analysis. The study protocol was approved by the Ethics Review Committee of the Nagoya University School of Medicine.

### Main exposure variables

Coffee consumption frequency was assessed by a question item in the brief dietary history questionnaire that required recall of dietary habits over 1 month. The possible responses included eight frequency categories: never, less than one cup per week, one cup per week, two to three cups per week, four to six cups per week, one cup per day, two to three cups per day and four or more cups per day. For the descriptive analysis, the lower five categories were incorporated into a separate category of less than one cup per day, because of the small number of the subjects in these categories. In the multiple linear regression analysis, coffee consumption frequency was treated as a continuous variable. For each category of coffee consumption frequency, the following numbers were assigned: 0, 0.05, 0.1, 0.4, 0.7, 1, 2.5 and 4 cups per day, respectively. We also assessed whether they used sugar in their coffee, categorized as used, not used and no answer.

### Analysis of blood samples

Blood serum samples were drawn from the subjects overnight or after fasting for at least 8 h, and were stored at  $-80^{\circ}\text{C}$  until biochemical assay. All the assays were carried out at a commercial laboratory using the standard procedures. Adiponectin was determined using an enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan). The detection limit of the adiponectin assay was  $0.40\ \mu\text{g ml}^{-1}$ . Leptin was measured by a radioimmunoassay kit (HUMAN LEPTIN RIA KIT, Linco Research, Inc., St Charles, IL, USA). The detection limit of the leptin assay was  $0.5\ \text{ng ml}^{-1}$ , specificity of the human leptin was 100%.

High sensitivity C-reactive protein (hs-CRP) was measured by latex nephelometry (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA), which was sensitive enough to detect  $0.02\ \text{mg l}^{-1}$ . Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) was measured by enzyme immunoassay with a minimum detectable level of  $6\ \text{pg ml}^{-1}$  (R&D Systems Inc., Minneapolis, MN, USA). High-sensitivity interleukin 6 (IL-6) was also measured by enzyme immunoassay (R&D Systems Inc.).

Fasting blood glucose was enzymatically determined by the hexokinase method (GLU-HK; Shino-test Corp., Tokyo, Japan). Fasting immunoreactive insulin was measured by a solid-phase radioimmunoassay (RIABEAD2; Dinabot Co., Ltd, Chiba, Japan), while a homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as  $\text{FBS (mg dl}^{-1}) \times \text{fasting immunoreactive insulin (}\mu\text{U ml}^{-1})/405$ .

TC and triglycerides were determined enzymatically. High-density lipoprotein cholesterol was measured by the phosphotungstate method. LDL-C was measured by an enzymatic method (Cholestest; Sekisui Medical Co., Ltd, Tokyo, Japan).

Aspartate aminotransaminase, alanine aminotransaminase and gamma-glutamyl transpeptidase were measured by the consensus method of the Japan Society of Clinical Chemistry. Intra-assay coefficients of variation of each variable were as follows: adiponectin,  $< 8.6\%$ ; leptin,  $< 1.79\%$ ; hs-CRP,  $< 4.0\%$ ; TNF- $\alpha$ ,  $< 9.6\%$ ; IL-6,  $< 7.5\%$ ; Aspartate aminotransaminase,  $< 4.9\%$ ; alanine aminotransaminase,  $< 7.5\%$ ; and gamma-glutamyl transpeptidase,  $< 2.0\%$ . TNF- $\alpha$  and IL-6 were only available for a portion of the studied sample ( $n = 691$ ).

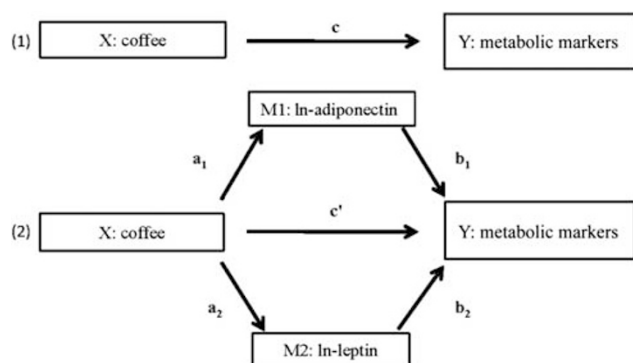
### Other variables

Height and weight were measured in a standing position at the annual health check-up, with body mass index (BMI) being calculated as weight in kilograms divided by the square of the height in meters. Smoking status was self-reported, and classified into current, ex- or never smokers. The number of cigarettes smoked per day was also obtained for current and ex-smokers. Physically active subjects were defined as those who engaged in moderate or vigorous exercise for more than 60 min per week.<sup>22</sup> Brief dietary history questionnaire was also used to assess other dietary habits. Total energy intake, and the intakes of magnesium and alcohol were calculated using the *ad hoc* computer algorithm based on the Standard Table of Food Composition in Japan, fifth revised edition.<sup>23</sup> Consumption frequency of green tea was assessed using the same categories as coffee consumption. In the present analysis, they were incorporated into two categories: one cup or less per day and two or more cups per day. The estimated glomerular filtration rate (eGFR) was calculated as  $194 \times \text{serum creatinine}^{-1.094} \times \text{Age}^{-0.287} \times 0.739$  (if female) ( $\text{ml min}^{-1}$  per  $1.73\ \text{m}^2$ ).<sup>24</sup>

### Statistical analysis

Adipocytokines, inflammatory and glucose metabolism-related markers, lipids, liver enzymes and eGFR were natural logarithmically transformed to approximately normalize the distribution and used in the analysis, then transformed back for data presentation, and shown as geometric means and 95% confidence intervals. A comparison of continuous variables among coffee consumption groups was conducted by one-way analysis of variance or covariance adjusted for covariates described below. The linear trend across coffee consumption categories was evaluated by using the polynomial procedure of SPSS. Multiple linear regression analysis, taking adipocytokines, inflammatory markers, lipids, glucose metabolism-related markers and liver enzymes as dependent variables, was also performed to evaluate the direction and magnitude of associations with coffee consumption. Independent variables included in the model were age, BMI, smoking status, cigarettes smoked per day for current smokers, physical activity, total energy intake, intakes of alcohol, magnesium and green tea, sugar use in coffee, and eGFR.

Then, we performed the multiple mediation analyses based on the structure equation modeling using SPSS macros provided by Preacher and Hayes<sup>25</sup> to examine how much of the association between coffee consumption and inflammatory or glucose metabolism-related markers, lipids or liver enzymes was attributable to the adipocytokines. Figure 1 shows the multiple mediation model, with path  $a(s)$  representing the relations of coffee consumption ( $X$ ) to adipocytokines ( $M(s)$ ), path  $b(s)$  representing the relations of  $M(s)$  to each marker ( $Y(s)$ ) adjusted for  $X$ , path  $c(s)$  representing the relations of  $X$  to  $Y(s)$  and path  $c(s)$  representing the relations of  $X$  to  $Y(s)$  adjusted for  $M(s)$ . All of these paths would typically be quantified with regression coefficients calculated by multiple linear regression analyses. The mediation analyses were carried out for variables in which only when all the paths  $a(s)$ ,  $b(s)$  and  $c(s)$  to be statistically significant<sup>25</sup> that is, Model 1  $P$ -values, and Model 2  $P$ -values for ln-leptin and ln-adiponectin in Table 2  $< 0.05$ . In the mediation analysis, the indirect effect of  $X$  on  $Y$  through  $M(s)$  is defined as the product of path  $a$  and path  $b$ , that is,  $a_1b_1$  and  $a_2b_2$ . And, the total effect of  $X$  on  $Y$  is the sum of the direct effect and all of indirect effects:  $c = c' + a_1b_1 + a_2b_2$ . We computed bootstrapped bias-corrected 95% confidence intervals (5000 samples) for the size of the specific mediation effects. All multiple mediation analyses were performed with adjustments for the covariates used in the multiple regression analysis. We presented standardized regression coefficients ( $\beta$ ) and respective 95% confidence intervals so that comparison of the



**Figure 1.** Multiple mediation model with two mediators ( $M_1$ , In-adiponectin;  $M_2$ , In-leptin) in the context of this study. (1) Total effect of coffee consumption ( $X$ ) on each marker ( $Y(s)$ ) – path  $c$ . (2) Coffee consumption is hypothesized to exert indirect effects on each marker through In-adiponectin ( $M_1$ ) and In-leptin ( $M_2$ ), and to exert a direct effect on each marker (path  $c'(s)$ ). The specific indirect effect of  $X$  on  $Y$  through  $M_1$  is defined by the product of the two paths linking  $X$  on  $Y$  through that mediator, that is,  $a_1b_1$ ; similarly, the specific indirect effect of  $X$  on  $Y$  through  $M_2$  is defined by  $a_2b_2$ . The total indirect effect of  $X$  on  $Y$  is the sum of the two specific indirect effects, that is,  $a_1b_1 + a_2b_2$ . The total effect of  $X$  on  $Y$  is thus the sum of the direct effect and all of the indirect effects:  $c = c' + a_1b_1 + a_2b_2$

strength of the associations investigated would be possible. All statistical analyses were conducted using the SPSS statistical package for Windows version 19.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

The mean  $\pm$  s.d. age was  $47.6 \pm 7.1$  years, and the mean BMI was  $22.8 \pm 2.7 \text{ kg m}^{-2}$  (range 15.0–35.8). Current and ex-smokers accounted for 29.6% and 21.1% of the sample, respectively. The geometric means of insulin, adiponectin and leptin were  $4.59 \mu\text{U ml}^{-1}$ ,  $6.78 \mu\text{g ml}^{-1}$  and  $6.58 \text{ ng ml}^{-1}$ , respectively. Greater coffee consumption was associated with younger age, a higher proportion of current smoking and higher total energy and magnesium intakes (all  $P < 0.001$ ) (Table 1). Coffee consumption was also associated positively with eGFR ( $P = 0.033$ ), but was not related to BMI or physical activity. Coffee consumption was inversely associated with the intakes of green tea ( $P = 0.003$ ).

Coffee consumption was positively and significantly associated with adiponectin levels (Tables 1 and 2) independent of potential confounding variables ( $P < 0.001$ ). In contrast, it was negatively associated with leptin ( $P < 0.001$ ), hs-CRP ( $P = 0.026$ ), triglycerides ( $P = 0.026$ ) and liver enzymes (all  $P < 0.001$ ). The inverse association of coffee consumption with hs-CRP or triglycerides was attenuated by additional adjustment for adipocytokines and became statistically insignificant, that is, completely explained. Although the associations of coffee consumption with liver enzymes were also attenuated by adipocytokines, they were nevertheless significant. Coffee consumption was positively associated with TC and LDL-C; interestingly, these associations were strengthened by the inclusion of adipocytokines in the model. Statistically significant associations were not observed between coffee consumption and high-density lipoprotein cholesterol or glucose metabolism-related markers.

The mediation effects by adiponectin and/or leptin on the association of coffee consumption were evaluated for hs-CRP, triglycerides or liver enzymes (Table 3). Adipocytokines explained 43.1% and 61.2% of the associations of coffee consumption with hs-CRP and triglycerides, respectively. Total mediation effects by adipocytokines on the associations of coffee consumption with

liver enzymes ranged from 19% for aspartate aminotransaminase to 33.3% for alanine aminotransaminase.

## DISCUSSION

In the present study, we found that the amount of coffee consumption was associated positively with adiponectin and inversely with leptin levels. Although coffee consumption was not related to BMI, our finding was independent of BMI and potential confounding variables. These associations of coffee with adipocytokines explained most of its association with triglycerides and some of those with hs-CRP and liver enzymes. A positive association of coffee consumption with adiponectin levels is consistent with previous reports from smaller studies.<sup>18,19</sup> As adiponectin is secreted from adipocytes,<sup>12</sup> coffee may have effects on adipocytes. Indeed, one of the major substances that coffee contains among several hundred other substances, caffeine, in an experimental study led to the upregulation of peroxisome proliferator-activated receptor  $\gamma$  expression,<sup>26</sup> which is an essential regulator of adipocyte differentiation and maintenance.<sup>27</sup> As no association between decaffeinated coffee consumption and adiponectin was reported,<sup>19</sup> caffeine contained in the coffee may have acted to increase adiponectin levels.

We observed a statistically significant inverse association between coffee consumption and leptin concentration in the present study. This was consistent with a previous study that found a statistically insignificant inverse association.<sup>21</sup> Although the pathophysiological significance of blood leptin levels is not fully understood, it is positively correlated with obesity and inflammatory markers.<sup>28</sup> In addition, increased circulating leptin has been implicated as a marker of leptin resistance.<sup>29,30</sup> As increased leptin levels may have effects on tissues and organs that remain sensitive to high leptin concentrations,<sup>31</sup> the decrease of leptin due to coffee consumption may attenuate such effects. However, it remains to be elucidated how coffee consumption might do so.

The level of hs-CRP is a marker of systemic low-grade inflammation. An inverse association of coffee consumption with hs-CRP was mainly explained by adiponectin and leptin, a finding consistent with previous studies reporting the role of leptin and adiponectin-determining serum CRP levels.<sup>32,33</sup> Our data also showed that mediation effects of adiponectin and leptin on hs-CRP were almost equal.

Coffee consumption was inversely associated with triglycerides, also consistent with previous reports.<sup>4–6</sup> However, in this study we were the first to reveal that the association was explained mainly by adiponectin. Coffee may have a potential to improve hypertriglyceridemia through its effects on adiponectin. In contrast, LDL-C and TC were positively associated with coffee consumption. That finding is also consistent with others even though consumption of unfiltered coffee would be very little in the present sample.<sup>6,34</sup> Our data showed that adipocytokines negatively mediated the associations of coffee consumption with TC and LDL-C. In other words, adipocytokines were suggested to have suppressed<sup>35</sup> coffee's cholesterol-increasing effect.<sup>36</sup>

In line with previous studies,<sup>9,10</sup> coffee consumption was inversely associated with blood concentration of the liver enzymes. The associations were partly explained by adipocytokines (from 18.9% in aspartate aminotransaminase to 33.5% in alanine aminotransaminase). Thus, other factors would likely explain the associations of coffee consumption with liver enzymes.

We found no associations between coffee consumption and the markers of glucose metabolism. The present finding seemingly contradicts coffee's reported inverse association with DM incidence.<sup>3</sup> A recent report also showed that coffee consumption was inversely associated with 2-h postload glucose of oral glucose tolerance test.<sup>37</sup> We also did not find associations of coffee consumption with DM prevalence or with markers of glucose

**Table 1.** Baseline characteristics and multivariate-adjusted values of adipocytokines, inflammatory markers, lipids, glucose metabolism-related markers and liver enzymes according to daily coffee consumption, Aichi, 2002

Characteristics and markers	Daily coffee consumption				P-value <sup>b</sup>
	< 1 Cup (n = 949)	1 Cup (n = 803)	2–3 Cups (n = 1336)	≥ 4 Cups (n = 229)	
Male (%)	80	76	75	82	0.010
Age (year)	48.2 ± 7.7	48.2 ± 7.0	46.9 ± 6.8	47.0 ± 6.9	< 0.001
Body mass index (kg m <sup>-2</sup> )	22.8 ± 2.7	22.9 ± 2.7	22.8 ± 2.7	22.6 ± 2.6	0.51
<i>Smoking status</i>					< 0.001
Current smoking (%)	20	26	34	50	
Past smoking (%)	22	22	21	18	
Never (%)	58	52	45	32	
Alcohol intake (g per day)	14.9 ± 22.3	15.0 ± 21.0	13.4 ± 19.4	13.4 ± 19.6	0.10
Physical activity <sup>a</sup> (%)	19	20	20	22	0.84
Total energy intake (kcal per day)	1918 ± 600	1911 ± 578	1975 ± 577	2117 ± 722	< 0.001
Magnesium intake (mg per day)	251 ± 90	255 ± 90	272 ± 86	302 ± 108	< 0.001
Sugar use in coffee (%)	22	16	15	10	< 0.001
Green tea intake (≥ 2 cups) (%)	56	48	51	48	0.003
eGFR (ml min <sup>-1</sup> per 1.73 m <sup>2</sup> )	76.2 (75.4–77.1)	77.2 (76.2–78.2)	77.4 (76.8–78.0)	78.9 (77.5–80.3)	0.033
<i>Adipocytokines</i>					
adiponectin (μg ml <sup>-1</sup> ) <sup>c</sup>	6.58 (6.40–6.76)	6.68 (6.48–6.88)	6.92 (6.76–7.08)	7.23 (6.84–7.65)	0.005 <sup>e</sup>
leptin (ng ml <sup>-1</sup> ) <sup>c</sup>	4.69 (4.58–4.81)	4.65 (4.53–4.78)	4.50 (4.41–4.59)	4.42 (4.20–4.64)	0.27
<i>Inflammatory markers</i>					
C-reactive protein (nmol l <sup>-1</sup> ) <sup>c</sup>	4.10 (3.90–4.48)	3.81 (3.52–4.10)	3.52 (3.33–3.81)	4.00 (3.43–4.57)	0.014
TNF-α (pg ml <sup>-1</sup> ) <sup>c,d</sup>	12.25 (11.64–12.90)	11.86 (11.25–12.50)	12.05 (11.54–12.59)	12.03 (10.89–13.29)	0.85
IL-6 (pg ml <sup>-1</sup> ) <sup>c,d</sup>	1.75 (1.60–1.92)	1.48 (1.34–1.63)	1.56 (1.44–1.69)	1.47 (1.22–1.76)	0.070
<i>Lipids</i>					
Total cholesterol (mmol l <sup>-1</sup> ) <sup>c</sup>	5.33 (5.28–5.38)	5.42 (5.36–5.48)	5.42 (5.37–5.46)	5.49 (5.38–5.60)	0.025 <sup>e</sup>
LDL cholesterol (mmol l <sup>-1</sup> ) <sup>c</sup>	3.05 (3.01–3.10)	3.14 (3.08–3.19)	3.15 (3.12–3.20)	3.21 (3.11–3.31)	0.005 <sup>e</sup>
HDL cholesterol (mmol l <sup>-1</sup> ) <sup>c</sup>	1.52 (1.50–1.54)	1.56 (1.54–1.59)	1.56 (1.54–1.58)	1.54 (1.49–1.58)	0.012
Triglycerides (mmol l <sup>-1</sup> ) <sup>c</sup>	1.21 (1.17–1.25)	1.15 (1.11–1.19)	1.12 (1.09–1.15)	1.17 (1.09–1.25)	0.006
<i>Glucose metabolism related markers</i>					
Glucose (mmol l <sup>-1</sup> ) <sup>c</sup>	5.05 (5.00–5.11)	5.13 (5.07–5.18)	5.05 (5.01–5.09)	5.08 (4.97–5.18)	0.16
Insulin (pmol l <sup>-1</sup> ) <sup>c</sup>	44.7 (42.8–46.8)	46.2 (44.0–48.5)	46.0 (44.3–47.7)	46.0 (42.0–50.3)	0.77
HOMA-IR <sup>c</sup>	1.45 (1.38–1.52)	1.52 (1.44–1.60)	1.49 (1.43–1.55)	1.49 (1.35–1.65)	0.65
<i>Liver enzymes</i>					
AST (μkat l <sup>-1</sup> ) <sup>c</sup>	0.375 (0.367–0.382)	0.365 (0.358–0.373)	0.357 (0.352–0.363)	0.355 (0.340–0.368)	0.004 <sup>e</sup>
ALT (μkat l <sup>-1</sup> ) <sup>c</sup>	0.326 (0.317–0.336)	0.319 (0.309–0.329)	0.309 (0.302–0.317)	0.296 (0.277–0.312)	0.007 <sup>e</sup>
γ-GTP (μkat l <sup>-1</sup> ) <sup>c</sup>	0.655 (0.630–0.680)	0.632 (0.607–0.658)	0.583 (0.565–0.603)	0.582 (0.538–0.630)	< 0.001 <sup>e</sup>

Abbreviations: ALT, alanine transaminase; AST, aspartate transferase; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; γ-GTP, γ-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistant; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α denotes tissue necrosis factor-α. Values are percentage, mean ± s.d. or geometric mean (95% CI). <sup>a</sup>Physical activity was defined as moderate and vigorous leisure-time physical activity 3 days or more and 60 min or more in total per week. <sup>b</sup>Differences in mean values were tested by one-way analysis of (co)variance, and differences in the proportions were tested by χ<sup>2</sup>-test. <sup>c</sup>Values were adjusted for age, BMI, smoking status, current smoking number, alcohol, physical activity, green tea intake, total energy intake, magnesium intake, green tea intake and eGFR. <sup>d</sup>n = 199, 175, 265 and 52 from low to high intake in the analysis of TNF-α and IL-6. <sup>e</sup>linear trend tested using polynomial procedure was statistically significant (P < 0.05).

metabolism even in a sample without excluding subjects on medication for DM (data not shown). Nevertheless, it would be possible that the association of coffee consumption with glucose or insulin could not be observed in apparently healthy young population with low inter-individual variability. Indeed, it has been shown that fasting glucose concentrations and insulin sensitivity show changes starting 3 to 5 years before a diagnosis of DM,<sup>38</sup> and that the onset of DM is often rapid rather than gradual.<sup>39</sup> It has also been reported that the prevalence of DM rapidly rises in people 60 years or older in Japan.<sup>40</sup> Thus, it may be possible that examination of older subjects or a sample with greater inter-individual variability of glucose level might reveal associations of coffee consumption with markers of the glucose metabolism.

This study has some potential limitations to be considered. First, because it is a cross-sectional study, causality can only be inferred. However, as it is less likely that adipocytokines or other markers influence coffee consumption, we consider our inference plausible that coffee consumption may have primary effects on adipocytokines and other markers investigated in the present study. In addition, we have excluded subjects who had been medically treated for several conditions. Although there may be individuals with other conditions (e.g., these chronic conditions without medication, gastrointestinal disorders and so on), which could confound the association, we considered this would have minor effects. Second, there may be some misclassification of daily coffee consumption doses based on the self-administered questionnaire. However, such a misclassification would have been

**Table 2.** Multivaria-adjusted associations of coffee consumption (continuous) with adipocytokines, inflammatory and glucose metabolism-related markers, lipids, and liver enzymes with or without further adjustment for adipocytokines, Aichi, 2002 (n = 3317)

Dependent variables	Model 1 <sup>a</sup>		Model 2 <sup>b</sup>					
	$\beta$ for coffee consumption	P-value	$\beta$ for coffee consumption <sup>g</sup>	P-value	$\beta$ for In-adiponectin <sup>e</sup>	P-value	$\beta$ for In-leptin <sup>e</sup>	P-value
<b>Adipocytokines</b>								
In-adiponectin	0.058 <sup>d</sup>	< 0.001	—	—	—	—	—	—
In-leptin	-0.038 <sup>d</sup>	< 0.001	—	—	—	—	—	—
<b>Inflammatory markers</b>								
In-hs-CRP	-0.039 <sup>f</sup>	0.026	-0.022	0.20	-0.132	< 0.001	0.237	< 0.001
In-TNF- $\alpha^c$	-0.017 <sup>f</sup>	0.68	-0.013	0.76	-0.015	< 0.001	-0.013	< 0.001
In-IL-6 <sup>c</sup>	-0.057 <sup>f</sup>	0.16	-0.049	0.23	-0.052	< 0.001	-0.052	< 0.001
<b>Lipids</b>								
In-TC	0.042 <sup>f</sup>	0.019	0.051	0.004	-0.016	0.42	0.207	< 0.001
In-LDL-C	0.055 <sup>f</sup>	0.002	0.066	< 0.001	-0.073	< 0.001	0.172	< 0.001
In-HDL-C	0.025 <sup>f</sup>	0.11	0.006	0.69	0.318	< 0.001	-0.025	0.26
In-triglycerides	-0.039 <sup>f</sup>	0.018	-0.015	0.34	-0.259	< 0.001	0.232	< 0.001
<b>Glucose metabolism-related markers</b>								
In-Glucose	-0.009 <sup>f</sup>	0.59	-0.004	0.81	-0.066	< 0.001	0.037	0.15
In-Insulin	0.010 <sup>f</sup>	0.57	0.031	0.065	-0.121	< 0.001	0.357	< 0.001
In-HOMA-IR	0.007 <sup>f</sup>	0.69	0.027	0.11	-0.124	< 0.001	0.331	< 0.001
<b>Liver enzymes</b>								
In-AST	-0.064 <sup>f</sup>	< 0.001	-0.052	0.002	-0.066	< 0.001	0.219	< 0.001
In-ALT	-0.059 <sup>f</sup>	< 0.001	-0.039	0.012	-0.143	< 0.001	0.302	< 0.001
In- $\gamma$ -GTP	-0.068 <sup>f</sup>	< 0.001	-0.051	< 0.001	-0.128	< 0.001	0.249	< 0.001

Abbreviations: ALT, alanine transaminase; AST, aspartate transferase;  $\gamma$ -GTP,  $\gamma$ -glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistant; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; In-, natural logarithmically transformed; TC, total cholesterol; TNF- $\alpha$  denotes tissue necrosis factor- $\alpha$ .  $\beta$  denotes standardized regression coefficients. <sup>a</sup>Adjusted for sex, age, body mass index, smoking status, cigarettes smoked per day for current smokers, physical activity, total energy intake, intakes of alcohol, green tea and magnesium, sugar use in coffee, and log-transformed estimated glomerular filtration rate. <sup>b</sup>Model 2 adjusted additionally for In-adiponectin and In-leptin. <sup>c</sup>n = 691 for the analyses of TNF- $\alpha$  and IL-6. <sup>d</sup>Path a(s), <sup>e</sup>Path b(s), <sup>f</sup>Path c(s), <sup>g</sup>Path c'(s) in multiple mediation analyses (see Figure 1).

**Table 3.** Mediation role of adiponectin and leptin on the associations of coffee consumption with hs-CRP, triglycerides and liver enzymes, Aichi, 2002

Dependent variables	Total effect of coffee consumption	Multiple mediation analyses					
		By In-adiponectin and In-leptin		By In-adiponectin		By In-leptin	
		$\beta$ (95% CI)	$\beta$ (95% CI)	Total mediation (%) <sup>a</sup>	$\beta$ (95% CI)	Specific mediation (%) <sup>a</sup>	$\beta$ (95% CI)
In-hs-CRP	-0.039 (-0.073, -0.005)	-0.017 (-0.025, -0.009)	43.1	-0.008 (-0.013, -0.004)	19.7	-0.009 (-0.016, -0.003)	23.4
In-TC	0.042 (0.002, 0.017)	-0.009 (-0.015, -0.004)	-21.4	-0.001 (-0.005, 0.002)	-2.4	-0.008 (-0.014, -0.004)	-19.0
In-LDL-C	0.055 (0.008, 0.032)	-0.011 (-0.019, -0.006)	-20.0	-0.004 (-0.008, -0.002)	-7.3	-0.007 (-0.012, -0.002)	-12.7
In-triglycerides	-0.039 (-0.071, -0.006)	-0.024 (-0.035, -0.014)	61.5	-0.015 (-0.023, -0.007)	38.7	-0.009 (-0.015, -0.004)	22.8
In-AST	-0.065 (-0.098, -0.032)	-0.012 (-0.019, -0.006)	18.9	-0.004 (-0.008, -0.001)	5.9	-0.008 (-0.014, -0.003)	13.0
In-ALT	-0.059 (-0.092, -0.027)	-0.020 (-0.030, -0.011)	33.5	-0.008 (-0.013, -0.004)	14.0	-0.012 (-0.020, -0.005)	19.5
In- $\gamma$ -GTP	-0.068 (-0.098, -0.038)	-0.017 (-0.025, -0.009)	25.2	-0.007 (-0.012, -0.003)	11.0	-0.010 (-0.016, -0.004)	14.1

Abbreviations: ALT, alanine transaminase; AST, aspartate transferase; CI, confidence interval;  $\gamma$ -GTP,  $\gamma$ -glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistant; hs-CRP, high sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; In-, natural logarithmically transformed; TC, total cholesterol.  $\beta$  denotes standardized regression coefficients <sup>a</sup>Calculated as proportion of the total indirect effects to the total effects of coffee consumption: for example,  $-0.017/(-0.039) \times 100$  for In-hs-CRP. Negative values indicate that  $\beta$  for coffee consumption was strengthened by adjustment for In-adiponectin and In-leptin.

nondifferential. Third, we did not obtain anthropometric measurements other than BMI. The possibility that a residual confounding of body fat distribution exists cannot be excluded. Finally, as all subjects in the present study were Japanese, the present findings may not apply directly to other races.

In conclusion, we found that coffee consumption was positively associated with adiponectin, and inversely with

leptin levels in middle-aged Japanese workers. Such associations explained most of the associations of coffee consumption with triglycerides, and some of those on hs-CRP and liver enzymes. Further studies are necessary to determine whether the mediation effects of adipocytokines on the associations of coffee consumption with metabolic markers are causal.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ACKNOWLEDGEMENTS**

The authors wish to express their sincere appreciation to the participants, and to the healthcare personnel of the local government office.

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