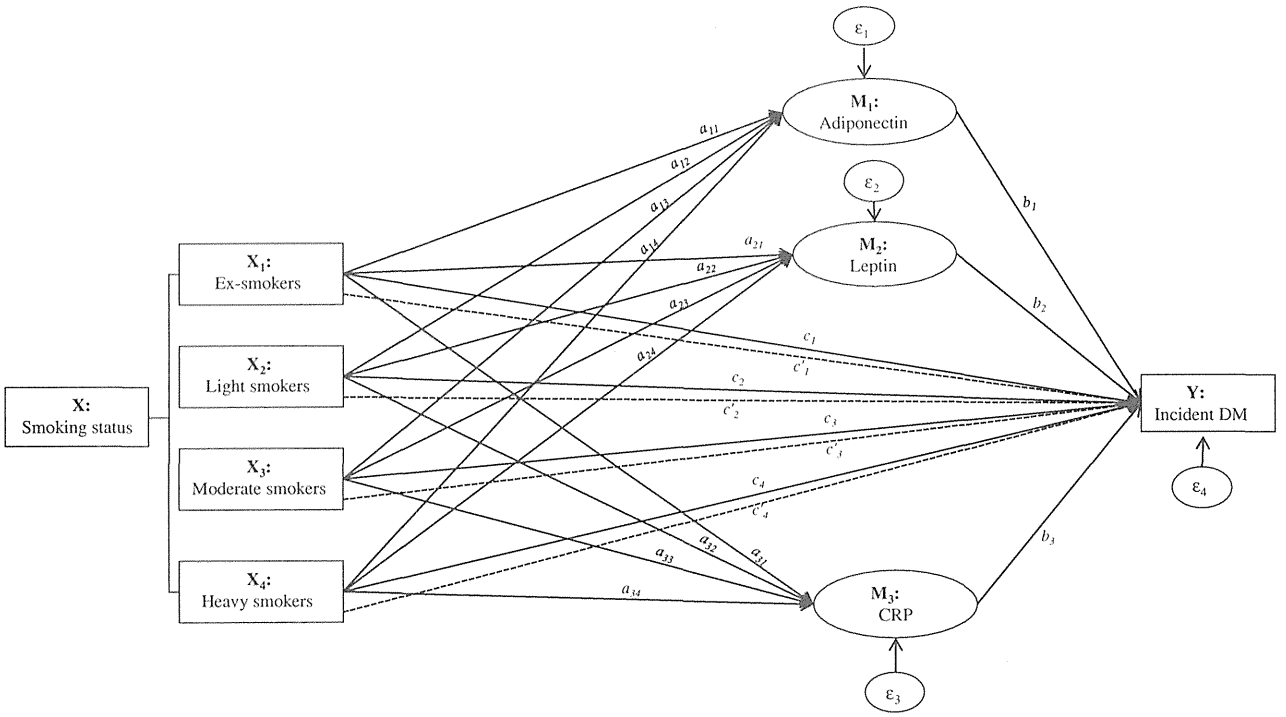

Figure 1. A multiple mediation model illustrating potential mediatory roles of adiponectin, leptin, and CRP concentrations in the smoking-diabetes association. This figure demonstrates how we estimated the indirect effects through the proposed mediators of being in one smoking category (X_1, X_2, X_3, or X_4) on DM incidence (Y) relative to the never smokers category. The 'c' paths represent the overall association—relative total effect—of being in a smoking category with DM incidence, and are equivalent to the unstandardized regression coefficients (β). Mediation is said to occur when (part of) this overall association is explained by a hypothesized mediator variable: adiponectin (M_1), leptin (M_2), and CRP (M_3). Associations of the potential mediators with each smoking category (*a*-paths) and their associations with DM incidence independent *of* one another and of the smoking category (paths b_1, b_2, and b_3) are assessed simultaneously. The product of regression coefficients *a*'s and *b*'s (*ab*'s) quantifies the relative indirect effect of being in one smoking category, relative to the reference, on DM incidence through the mediators. For instance, the relative indirect effect of being an ex-smoker (X_1) relative to never smokers on DM incidence via adiponectin (M_1), leptin (M_2), and CRP (M_3) is given by products $a_{11}b_1$, $a_{21}b_2$, and $a_{31}b_3$, respectively. The part of the relative total effect (c_1) not explained by these mediating paths is the relative direct effect (c'_1) of being an ex-smoker on DM incidence. Similarly, the relative total direct and indirect effects of being in the other smoking categories on DM incidence relative to the never smokers can be represented by the respective paths linking the independent, dependent, and potential mediator variables. DM, diabetes mellitus; CRP, C-reactive protein.

ex-smokers and 1.82 (95% CI 1.29–2.56) among current smokers. After further adjustment for physical activity, family history of DM, mean arterial pressure, and BMI, as well as log-transformed levels of HOMA2-IR, triglycerides, and adiponectin, the hazard ratios for DM were slightly attenuated but remained significantly elevated among both ex-smokers (HR 1.54, 95% CI 1.07–2.22) and current smokers (HR 1.75, 95% CI 1.25–2.46).

Analysis for current smokers stratified by the number of cigarettes smoked per day revealed a dose-dependently increased risk of developing the disease: compared with never smokers, the hazard ratios of DM were 1.35 (95% CI 0.79–2.32) among light smokers, 1.68 (95% CI 1.10–2.58) for moderate smokers, and 2.30 (95% CI 1.47–3.60) for heavy smokers in the maximally adjusted model (Table 3). Similar results of association between smoking status and DM incidence were produced when the analysis was done in a logistic model as part of the mediation analysis (*c* paths in Table 4).

Association between smoking and adiponectin, leptin, and CRP

Table 4 shows the association of each smoking category, relative to never smokers, with concentrations of adiponectin, leptin, and CRP. Significant inverse associations between current smoking and adiponectin levels were observed. The magnitudes of association with being a light, moderate, or heavy smoker were (β [SE] = −0.03 [0.01], $P = 0.017$), (β [SE] = −0.04 [0.01], $P < 0.001$) and (β [SE] = −0.05 [0.01], $P < 0.001$), respectively. Adiponectin concentrations in ex-smokers, however, were not significantly different from those in never smokers (β [SE] = −0.01 [0.01], $P = 0.194$). Leptin concentrations were negatively associated with all

Table 1. Demographic and lifestyle characteristics of study subjects at baseline by smoking status, Aichi, 2002–2011

Characteristics	Smoking status at recruitment					*P*-value[b]
	Never smokers	Ex-smokers	Current smokers, by cigarettes per day			
			Light smokers (<20)	Moderate smokers (20–<30)	Heavy smokers (30+)	
n	1608	776	264	421	269	
Demographic factors						
Sex, % men	58.0	94.5	84.1	96.9	99.3	<0.001
Age, years	46.2 (7.1)	49.7 (6.5)	47.6 (7.2)	48.7 (6.7)	49.8 (6.3)	<0.001
Smoking metrics						
Age at smoking debut, years		20.0 (2.3)	20.8 (2.7)	20.5 (2.9)	19.9 (2.1)	0.253
Pack-years of smoking[c]		21.5 (20.1–23.0)	13.0 (12.0–14.0)	27.8 (27.0–28.5)	50.0 (48.3–51.8)	<0.001
Median years since quitting		11				
Physically active[a], %	52.4	64.2	55.3	55.1	54.6	0.314
Alcohol consumption, g/day[c]	4.2 (3.8–4.6)	10.0 (9.0–11.2)	8.8 (7.1–11.1)	13.1 (11.3–15.4)	16.1 (13.4–19.5)	<0.001
Body mass index, kg/m^2	22.5 (2.7)	23.2 (2.5)	23.1 (2.8)	23.0 (2.7)	23.1 (2.6)	<0.001
Sleep duration, minutes/day	387.0 (51.5)	402.4 (49.2)	398.3 (49.9)	400.1 (55.5)	395.9 (53.8)	<0.001
Dietary factors						
Fat consumption, g/day	56.9 (20.0)	57.4 (20.2)	55.7 (22.5)	54.2 (20.1)	52.4 (21.3)	<0.001
Energy, kcal	1891.9 (579.6)	2015.5 (583.6)	1966.5 (617.8)	2001.6 (587.7)	1953.8 (610.7)	0.001
Carbohydrate consumption, g/day	254.9 (87.1)	264.3 (91.0)	259.1 (85.8)	261.5 (85.2)	256.0 (94.2)	0.344
Sugar consumption, mg/day[c]	11.6 (11.2–12.0)	10.2 (9.6–10.8)	9.4 (8.5–10.4)	10.2 (9.5–11.0)	9.6 (8.6–10.7)	<0.001
Glycemic load	154.5 (59.1)	158.6 (62.1)	156.6 (58.2)	156.9 (59.3)	153.9 (65.3)	0.704

Each continuous variable is being reported as mean (SD) unless mentioned otherwise.
[a]Physical activity: self-reported exercise for a total of 60 minutes or more for more than a day per month.
[b]Using ANOVA and Chi-square test for continuous and categorical variables, respectively.
[c]Values are expressed as geometric mean (95% confidence interval).

Table 2. Subjects' baseline levels of metabolic risk factors, serum adiponectin, leptin, and CRP by smoking status, Aichi, 2002–2011

Characteristics	Smoking status at recruitment					*P*-value[a]
	Never smokers	Ex-smokers	Current smokers, by cigarettes per day			
			Light smokers (<20)	Moderate smokers (20–<30)	Heavy smokers (30+)	
n	1608	776	264	421	269	
Positive family history of DM[b], %	16.5	13.3	12.9	14.5	14.9	0.192
Total cholesterol, mg/dL	211.5 (33.7)	212.9 (33.5)	210.1 (37.3)	208.5 (34.3)	205.7 (33.4)	0.006
HDL cholesterol, mg/dL	64.6 (15.3)	61.1 (15.4)	59.2 (14.7)	56.5 (15.3)	53.4 (13.7)	<0.001
Triglycerides, mg/dL[e]	88.7 (86.4–91.1)	107.2 (103.2–111.5)	110.7 (103.0–118.9)	121.5 (115.3–128.0)	131.6 (122.8–141.0)	<0.001
Total-to-HDL cholesterol ratio	3.4 (0.98)	3.7 (1.00)	3.8 (1.1)	3.9 (1.1)	4.1 (1.2)	<0.001
Fasting blood sugar, mg/dL	89.2 (10.5)	92.1 (10.6)	90.5 (10.7)	91.3 (11.2)	90.7 (11.6)	0.001
Fasting plasma insulin, μU/mL[e]	6.1 (6.0–6.3)	6.4 (6.1–6.7)	6.3 (5.8–6.9)	6.3 (5.9–6.7)	6.1 (6.0–6.7)	0.632
Insulin resistance, HOMA2-IR[e]	0.68 (0.66–0.70)	0.72 (0.68–0.75)	0.70 (0.64–0.77)	0.70 (0.65–0.75)	0.68 (0.62–0.75)	0.565
Hypertensive[c], %	15.5	26.5	14.0	18.5	24.5	0.006
Systolic blood pressure, mm Hg	123.8 (14.6)	129.1 (15.1)	124.5 (14.6)	126.3 (15.2)	128.3 (16.4)	<0.001
Diastolic blood pressure, mm Hg	75.6 (11.3)	79.9 (11.7)	76.4 (10.5)	78.3 (10.8)	79.2 (12.2)	<0.001
Mean arterial pressure[d], mm Hg	91.7 (11.7)	96.3 (12.1)	92.4 (11.0)	94.3 (11.5)	95.6 (13.0)	<0.001
Adiponectin, μg/mL[e]	7.7 (7.5–7.8)	6.3 (6.1–6.5)	6.3 (5.9–6.7)	5.9 (5.6–6.1)	5.7 (5.4–6.0)	<0.001
CRP, mg/dL[e]	0.031 (0.029–0.033)	0.036 (0.034–0.039)	0.034 (0.030–0.040)	0.043 (0.039–0.048)	0.064 (0.056–0.073)	<0.001
Leptin, ng/mL[e]	5.3 (5.1–5.4)	4.3 (4.2–4.5)	4.5 (4.2–4.8)	3.8 (3.6–4.0)	3.8 (3.5–4.0)	<0.001
Incident diabetes by 2011, %	4.7	8.4	6.4	8.6	11.9	<0.001

Each continuous variable is being reported as mean (standard deviation) unless mentioned otherwise.
[a]Using ANOVA and Chi-square test for continuous and categorical variables, respectively.
DM, diabetes mellitus; HDL, high-density lipoprotein; HOMA2-IR, homeostatic model assessment 2–insulin resistance; CRP, C-reactive protein.
[b]History of diabetes or raised blood glucose in one or more of first-degree relatives.
[c]Hypertension is defined as systolic/diastolic blood pressure of ≥140/90 mm Hg and/or use of antihypertensive medication.
[d]Diastolic blood pressure + 1/3 (systolic blood pressure-diastolic blood pressure).
[e]Values are expressed as geometric mean (95% confidence intervals).

smoking categories, although the associations were significant only among moderate (β [SE] = −0.035 [0.01], $P < 0.001$) and heavy smokers (β [SE] = −0.034 [0.012], $P = 0.003$). Concentrations of CRP were negatively but non-significantly associated with ex- and light smokers. In contrast, CRP concentration was positively and significantly associated with being a moderate (β [SE] = 0.076 [0.027], $P = 0.005$) or heavy smoker (β [SE] = 0.235 [0.032], $P < 0.001$).

Table 3. Hazards of diabetes mellitus by baseline smoking status, Aichi, 2002–2011

	Smoking status at recruitment					
			Current smokers, by cigarettes per day			
	Never smokers	Ex-smokers	Light smokers	Moderate smokers	Heavy smokers	All current smokers
n	1608	776	264	421	269	954
Number of cases	75	65	17	36	32	85
Person-years	12264	5855	1977	3134	1910	7021
Crude incidence rate/1000 person-years	6.1	11.1	8.6	11.5	16.8	12.1
Model 1[a]	1	1.62 (1.13–2.34)	1.36 (0.80–2.33)	1.76 (1.15–2.69)	2.45 (1.56–3.82)	1.82 (1.29–2.56)
Model 2[b]	1	1.64 (1.14–2.36)	1.35 (0.79–2.32)	1.74 (1.13–2.66)	2.41 (1.54–3.77)	1.80 (1.28–2.53)
Model 3[c]	1	1.54 (1.07–2.23)	1.39 (0.82–2.38)	1.74 (1.14–2.67)	2.44 (1.57–3.82)	1.83 (1.30–2.57)
Model 4[d]	1	1.54 (1.07–2.22)	1.35 (0.79–2.32)	1.68 (1.10–2.58)	2.30 (1.47–3.60)	1.75 (1.25–2.46)

[a]Adjusted for age (continuous) and sex at baseline.
[b]Model 1 + adjustments for physical activity, consumptions of alcohol, sugar and energy (all continuous), and sleep duration/day (continuous).
[c]Model 2 + adjustments for family history of diabetes mellitus, mean arterial pressure, body mass index (continuous), total cholesterol to high-density lipoprotein cholesterol ratio, and log-transformed values of homeostatic model assessment 2–insulin resistance (HOMA2-IR) and triglyceride.
[d]Model 3 + adjustment for log-transformed serum adiponectin, C-reactive protain, and leptin levels (continuous).

Table 4. Associations among smoking status, diabetes, and three potential mediators in a multiple mediation model, Aichi, 2002–2011

Antecedent	Consequent											
	Adiponectin (M_1)			Leptin (M_2)			CRP (M_3)			DM incidence (Y)		
Smoking status (X)	Path	β (SE)	*P*-value	Path	β (SE)	*P*-value	Path	β (SE)	*P*-value	Path	β (SE)	*P*-value
Never smokers	Referent											
Ex-smokers	a_{11}	−0.011 (0.009)	0.194	a_{21}	−0.002 (0.008)	0.793	a_{31}	−0.019 (0.022)	0.392	c_1	0.485 (0.196)	0.014
Light smokers	a_{12}	−0.033 (0.014)	0.017	a_{22}	−0.006 (0.011)	0.564	a_{32}	−0.013 (0.032)	0.68	c_2	0.330 (0.286)	0.249
Moderate smokers	a_{13}	−0.044 (0.011)	<0.001	a_{23}	−0.035 (0.01)	<0.001	a_{33}	0.076 (0.027)	0.005	c_3	0.605 (0.229)	0.008
Heavy smokers	a_{14}	−0.054 (0.013)	<0.001	a_{24}	−0.034 (0.012)	0.003	a_{34}	0.235 (0.032)	<0.001	c_4	0.927 (0.243)	<0.001
Adiponectin (M_1)	b_1										−0.992 (0.381)	0.009
Leptin (M_2)	b_2										0.134 (0.445)	0.763
CRP (M_3)	b_3										−0.062 (0.158)	0.693

CRP, C-reactive protein; DM, diabetes mellitus; SE, standard error.

Association between DM incidence and adiponectin, leptin, and CRP

Adiponectin concentration was inversely and significantly associated with DM incidence (β [SE] = −0.992 [0.381], $P = 0.009$). However, concentrations of leptin (β [SE] = 0.134 [0.445], $P = 0.763$) and CRP (β [SE] = −0.062 [0.158], $P = 0.693$) were not significantly associated (Table 4). These associations with adiponectin, leptin, and CRP concentrations were independent of one another and all other covariates described above.

Mediating role of adiponectin, leptin, and/or CRP

Table 5 shows the direct effects, and the indirect effects through the three potential mediators, of being in each smoking category on DM incidence relative to never smokers. When adjustments for the three potential mediators were made simultaneously, the direct effects of being an ex-, moderate, and heavy smoker on DM incidence remained significant with β (SE) = 0.480 (0.196), $P = 0.015$; β (SE) = 0.574 (0.230), $P = 0.013$; and β (SE) = 0.880 (0.247), $P < 0.001$, respectively.

Adiponectin levels appeared to partially mediate the association between the three categories of current smoking and DM incidence: the indirect effects of being a light (point estimate 0.033; BC 95% CI 0.005–0.082), moderate (point estimate 0.044; BC 95% CI 0.010–0.094) or heavy smoker (point estimate 0.054; BC 95% CI 0.013–0.113) on DM, relative to never smokers, were statistically significant. In terms of the ratio of indirect effect to total effect, the contribution of adiponectin concentration as a mediator in the smoking-DM association was 10%, 7.2%, and 5.8% among light, moderate, and heavy smokers, respectively. In contrast, neither levels of leptin nor CRP seemed to mediate the smoking-DM association, as the corresponding BC 95% CIs included zero (Table 5).

Additional analyses

We also conducted mediation analyses using other cardiovascular risk factors, including lipids, glucose, and blood pressure, which were significantly associated with smoking in our study. However, the indirect effects of

Table 5. Direct and indirect effects though three potential mediators of smoking status on diabetes incidence, Aichi, 2002–2011

Smoking status	Direct effect of Smoking on DM incidence (c′ paths)			Indirect effect of smoking on DM incidence (path *ab*'s)					
				Through Adiponectin (M_1)		Through Leptin (M_2)		Through CRP (M_3)	
	Path	β (SE)	95% CI	Point estimate (SE)	BC 95% CI[a]	Point estimate (SE)	BC 95% CI[a]	Point estimate (SE)	BC 95% CI[a]
Never smoker	Referent								
Ex-smoker	c'_1	0.480 (0.196)	0.095 to 0.864	0.011 (0.010)	−0.003 to 0.040	−0.0003 (0.004)	−0.012 to 0.006	0.001 (0.004)	−0.004 to 0.017
Light smokers	c'_2	0.299 (0.286)	−0.263 to 0.860	0.033 (0.019)	0.005 to 0.082	−0.0009 (0.006)	−0.020 to 0.007	0.0008 (0.005)	−0.006 to 0.020
Moderate smokers	c'_3	0.574 (0.230)	0.122 to 1.025	0.044 (0.021)	0.010 to 0.094	−0.0047 (0.017)	−0.041 to 0.027	−0.0047 (0.012)	−0.033 to 0.015
Heavy smokers	c'_4	0.880 (0.247)	0.396 to 1.365	0.054 (0.025)	0.013 to 0.113	−0.005 (0.017)	−0.042 to 0.027	−0.015 (0.034)	−0.086 to 0.049

BC, bias-corrected; CI, confidence interval; CRP, C-reactive protein; SE, standard error.
[a]Bias-corrected confidence intervals after running 10 000 bootstrap samples.

smoking on DM incidence were statistically insignificant when each of those variables was used as a potential mediator in the model (data not shown).

DISCUSSION

The main findings of our study are threefold. First, we confirmed that risk of developing DM was significantly elevated among ex- and current smokers. Second, we found that adiponectin levels were inversely associated with both smoking and DM incidence, and that leptin and CRP levels were associated with smoking but not with DM. Finally, of the three potential mediators assessed, we found that only adiponectin mediated the association between current smoking and DM.

A positive association between active smoking and DM incidence has been noted in a number of studies conducted among various populations,[44–52] as well as in a recent meta-analysis of 25 prospective cohort studies.[1] The substantial increase in the risk of DM among current smokers that we observed in our study is consistent with these previous reports. Our results affirm the putative influence of active smoking on the incidence of DM. Being an ex-smoker was also significantly associated with increased risk of DM compared to never smokers in the present study, although the risk is far less than the one exhibited among current heavy smokers. An increased risk of DM among ex-smokers' has been shown in some[44,53,54] but not all[47,50,52,55] previous studies. The discrepancy might be related to differences in ex-smokers' intensity of smoking and intervals since quitting among subjects in the respective studies.

Serum adiponectin levels were inversely associated with both current smoking and DM incidence in our study. This corroborates the findings of previous studies that reported the inverse associations of adiponectin levels independently with active smoking[6–10] and DM incidence.[11–14] The present study revealed, for the first time, that adiponectin may play a significant mediating role in the smoking-DM association; the effect of being a light, moderate, or heavy smoker on DM incidence may be due in part to its indirect effect through adiponectin. However, the indirect effect of being an ex-smoker on DM through adiponectin was not significant. These results may indicate that the effect of smoking on adiponectin is short-lived after smoking cessation, as shown in previous studies that found that ex-smokers' serum adiponectin were able to increase[56,57] and be restored to normal levels in as little as 2 months after quitting smoking.[57]

There are several biologically plausible mechanisms that may explain how the speculated smoking-adiponectin-DM causal pathway might work. Smoking-induced oxidative stress decreases the secretion and expression of plasma adiponectin via inhibition of the activation of phosphatidylinositol 3-kinase,[9] a key molecule in the secretion of adiponectin in adipocytes.[58] Nicotine in tobacco smoke can also directly inhibit the secretion and expression of adiponectin in adipocytes.[9,59] Moreover, persistent production of tumor necrosis factor α induced by chronic exposure to cigarette smoke may promote the development of hypoadiponectinemia.[60,61] Hypoadiponectinemia may, in turn, cause insulin resistance and DM; adiponectin is believed to stimulate the phosphorylation and activation of 5′-adenosine monophosphate-activated protein kinase in the liver and skeletal muscles, thereby directly regulating glucose metabolism and insulin sensitivity.[4,62] Adiponectin may also increase fatty-acid combustion and energy consumption, in part via peroxisome proliferator-activated receptor α activation, leading to decreased triglyceride content and a

corresponding coordinated increase in insulin sensitivity in the liver and skeletal muscles.[4,63]

Contrary to our initial hypothesis, neither leptin nor CRP appeared to mediate the smoking-DM association. This finding ruled out the smoking-leptin-DM or smoking-CRP-DM pathways as alternative causal pathways in the smoking-DM association. There are hardly any previous studies on this issue with which to compare our findings. However, the present finding that only adiponectin partially mediated the smoking-DM association may indicate that smoking is related to DM development through adipocyte dysfunction[64] secondary to smoking-induced adipocyte inflammation, and leptin and CRP are likely markers of such a state.

Moderate and heavy smokers had significantly lower leptin but higher CRP levels relative to never smokers in our study. Although previous studies on the influence of smoking on leptin are few, findings in available reports were inconsistent: increasing,[20,21] reducing,[15–18] or no effects[19] of smoking on leptin were documented. One of the reasons for such a discrepancy might be the fact that smoking may interact with other factors such as diet, exercise, and other lifestyle factors, as well as hormones and host inflammatory responses, and these interactions may further impair leptin regulations.[15,16] Further studies are warranted to elucidate how these interactions may mediate the effect of smoking on leptin levels. With regard to the smoking-CRP association, the significantly elevated levels of CRP observed among moderate and heavy smokers in our study was consistent with previous findings,[29–33] reinforcing the acknowledged pro-inflammatory impact of smoking.[65]

Neither leptin nor CRP levels were significantly associated with DM incidence in the present study. Findings of previous studies investigating the association of both biomarkers individually with DM have been equivocal: independent positive associations with the disease of either leptin or CRP were reported in some studies[22–24,34–36,66] but not all.[25–27,37,38] These inconsistencies may be due in part to variations in the potential confounders considered among studies, in addition to differences in population characteristics. For instance, the lack of a significant association with DM incidence of both leptin and CRP observed in our study was independent of adiponectin; this was not the case in previous studies reporting a positive association.[22–24,34,36,66] Conversely, the results of studies that reported no significant association between DM and leptin or CRP levels were obtained after controlling for the possible effect of adiponectin.[27,38] Since adiponectin is associated with both leptin and CRP,[67,68] we speculate that adiponectin might negatively confound the association of leptin with DM and qualitatively confound the association of CRP with DM. Further studies specifically designed to test this hypothesis are warranted.

Our study has several limitations. First, there may have been misclassification bias, as smoking status was self-reported. However, we believe that any such bias would be non-differential and not expected to affect the results significantly. Second, some of the participants in the cohort were censored at the time of retirement. However, we believe censoring due to retirement was non-differential to the outcome and would not introduce significant bias. Third, the analysis of the association of smoking status with the three biomarkers was cross-sectional, and claims of causality should be made with caution. But the possibility of reverse causality is remote, as the levels of the biomarkers are not likely to influence smoking behavior. Fourth, total serum adiponectin was used in our analysis. The high-molecular-weight complex of adiponectin is believed to correlate with glucose tolerance similarly to or better than total serum adiponectin.[69,70] Finally, our study was conducted in middle-aged Japanese workers; hence, further research is needed before the findings are extrapolated to other groups of subjects and races.

However, despite those limitations, our findings have the following clinical and public health implications. First, they contribute to the understanding of the pathogenesis of smoking-related DM and could aid clinicians and public health workers in advising smoking clients. Second, they indicate that an adiponectin-focused intervention in the future may help avert at least 6% of smoking-related cases of DM among current smokers. The public health implications of such an intervention could be even bigger in populations with high prevalence of smoking. In fact, the potential therapeutic role of adiponectin in the treatment of DM, insulin resistance, metabolic syndrome, and cardiovascular diseases has long been documented.[71,72] It might also be useful to explore other lifestyle behaviors and traits of individuals that decrease or increase adiponectin concentrations and investigate them in relation to DM incidence. Such studies may identify other targets of DM prevention.

In conclusion, in addition to confirming the positive association between smoking and DM incidence, our study has revealed that serum levels of adiponectin may mediate the association, at least in part. However, levels of leptin and CRP did not appear to have a mediating role in the smoking-DM association nor were they associated individually with DM incidence. Further research is needed to elucidate the role of these biomarkers and their interaction in the pathogenesis of smoking-related DM.

ONLINE ONLY MATERIAL

Abstract in Japanese.

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ELSEVIER

ORIGINAL ARTICLE

Independent association of liver fat accumulation with insulin resistance

Hiroshi Yatsuya[a,b,*], Takashi Nihashi[c], Yuanying Li[b], Yo Hotta[e], Kunihiro Matsushita[f], Takashi Muramatsu[g], Rei Otsuka[h], Masaaki Matsunaga[i], Kentaro Yamashita[j], Chaochen Wang[b], Mayu Uemura[b], Akiko Harada[k], Hiroshi Fukatsu[l], Hideaki Toyoshima[m], Atsuko Aoyama[b], Koji Tamakoshi[n]

[a] *Department of Public Health, Fujita Health University, Toyoake, Japan*
[b] *Department of Public Health and Health Systems, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[c] *Department of Radiology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[e] *Geriatric Health Services Facility Mizusato, Nagoya, Japan*
[f] *Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA*
[g] *Department of Interventional Cardiology, Erasmus Medical Center, Thoraxcenter, Rotterdam, The Netherlands*
[h] *Department for Development of Preventive Medicine, National Center for Geriatrics and Gerontology, Obu, Japan*
[i] *Inazawa City Hospital, Inazawa, Japan*
[j] *Department of Cardiology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[k] *Institute of Stress Science, Public Health Research Foundation, Tokyo, Japan*
[l] *Division of Medical Infomatics, Aichi Medical University Hospital, Nagakute, Japan*
[m] *Health Care Center, Anjo Kosei Hospital, Anjo, Japan*
[n] *Department of Nursing, Nagoya University School of Health Sciences, Nagoya, Japan*

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KEYWORDS
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Summary
Background: To examine the association of intrahepatic fat with homeostasis model assessment-insulin resistance (HOMA-IR), a marker of insulin resistance, in Japanese

* Corresponding author at: Department of Public Health, Fujita Health University, 1-98 Dengakugakubo Kutsukake-cho, Toyoake 470-1192, Japan. Tel.: +81 562 93 2453; fax: +81 562 93 3079.
E-mail address: yatsuya@gmail.com (H. Yatsuya).

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adults, and whether intrahepatic fat is associated with insulin resistance independent of waist circumference and other measures of obesity.
Methods: Fifty-three individuals aged 37–69 were studied. Spectrum obtained using a 3-T magnetic resonance imager was analysed with LCModel to quantify intrahepatic fat. Blood levels of insulin, glucose and other biochemical markers were obtained after 8 h or more fasting. Percent body fat was estimated by a bioelectrical impedance analyzer. HOMA-IR and intrahepatic fat content were log-transformed in the analysis.
Results: We found a positive correlation between intrahepatic fat and HOMA-IR, which was independent of the anthropometric measures of obesity. In contrast, significant and positive correlations of body mass index, percent body fat, and waist circumference with HOMA-IR were largely explained by their associations with intrahepatic fat. Intrahepatic fat was positively associated with alanine transaminase and triglycerides even after adjustment for HOMA-IR.
Conclusion: Intrahepatic fat was associated with insulin resistance independent of age, sex, and measures of obesity in Japanese adults. Hypertriglyceridemia and liver injury may directly occur subsequent to intrahepatic fat accumulation.

Introduction

Ectopic fat observed in the obese has been suggested to be involved in the development of the metabolic abnormalities by inducing lipotoxicity [1] or through the potential upstream abnormality such as dysfunctional adipose tissue [2]. Indeed, intrahepatic fat, evaluated by magnetic resonance spectroscopy (MRS) [3], is associated with insulin resistance in healthy [4] as well as in diabetic subjects [5]. However, it has not been confirmed whether the correlation between intrahepatic fat and insulin resistance, a central pathophysiology of metabolic syndrome [6], is independent of measures of adiposity such as waist circumference, body fat, or body weight. Furthermore, few reports exist in Eastern Asians, an ethnic group with greater predisposition to develop diabetes at a given level of adiposity as compared to Caucasian [7].

Therefore, the aim of the present paper is to examine the association of MRS-quantified intrahepatic fat with a marker of insulin resistance, homeostasis model assessment-insulin resistance (HOMA-IR) in Japanese adults. HOMA-IR has been shown to correlate strongly with clamp-measured total glucose disposal [8,9]. Subsequently, whether liver fat *per se* is associated with insulin resistance independent of waist circumference and other measures of obesity is examined.

Participants and methods

Subjects

Prior to the recruitment, pretests were conducted for six individuals using the same procedure, which yielded the mean intrahepatic fat value of 0.105/water signal and the standard deviation of 0.068. Using these values, the sample size necessary to detect intrahepatic fat difference of 0.07 was calculated as 43 with two-sided $\alpha = 0.05$ and $\beta = 0.1$. Considering potential loss of participants at the time of analysis due to possible technical or logistical reasons, we planned to recruit 50 subjects or more. Civil servant retirees were contacted as potential participants by mail ($n = 671$). They had been followed by us for the occurrence of cardiovascular diseases since 2002. We enrolled all the individuals who responded to participate except for those with liver disease ($n = 55$). We did not exclude subjects with alcohol drinking habit or those with medical treatment for diabetes in the present analysis. Instead, we performed additional analyses by excluding these subjects. One man who could not finish liver MR scanning and one man with ungradable liver MRS both due to technical reasons were excluded, leaving 53 subjects aged 37–69 without overt liver disease for the present analysis.

Written informed consent was obtained after explanation of study aims and procedures. The study protocol was approved by Ethics Review Committee of Nagoya University School of Medicine.

Magnetic resonance spectroscopy

A 3-T MR imager (Trio, Siemens Medical Solutions, Erlangen, Germany) was used. All patients were examined in supine position. A dedicated body surface coil was used for both MR imaging and spectroscopy. T1-weighted axial and coronal MR images were used for localisation of the volume of interest (VOI) within the right lobe of the liver. And then, single voxel spectroscopy (SVS) was performed.

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研究代表者　　岡村 智教
〒160-8582
東京都新宿区信濃町 35
慶應義塾大学医学部衛生学公衆衛生学
TEL:03-3353-1211　FAX:03-3359-3686

印　　刷　　有限会社 MOA
〒105-0004
東京都港区新橋 2-10-3 ブラザービル 8F
TEL:03-3500-3248　FAX:03-3591-3370