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ORIGINAL ARTICLE

Adipocytokines and the risk of coronary heart disease in healthy middle aged men: the PRIME Study

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Background: Adipokines play an important role in glucose, lipid and lipoprotein metabolisms, as well as in coagulation and inflammatory processes. So far, studies have evaluated the association of individual adipokines with future coronary heart disease (CHD) event and provided mixed results.
Objectives: We sought to investigate the association of a set of adipocytokines, including total adiponectin, adipsin, resistin, leptin and plasminogen activator inihibitor-1 (PAI-1), with future CHD events in apparently healthy men.
Methods: We built a nested case–control study within the PRIME Study, a multicenter prospective cohort of 9779 healthy European middle-aged men. Total adiponectin, adipsin, resistin, leptin and PAI-1 were measured in the baseline plasma sample of 617 men who developed a first CHD event (coronary death, myocardial infarction, stable or unstable angina) during 10 years of follow-up and in 1215 study-matched controls, by multiplex assays using commercial kits. HRs for CHD were estimated by conditional logistic regression analysis.
Results: Median concentrations of total adiponectin, adipsin and resistin were similar in cases and in controls, whereas those of leptin and PAI-1 were higher in cases than in controls, 6.30 vs 5.40 ng ml^{-1}, and 10.09 vs 8.48 IU ml^{-1}, respectively. The risk of future CHD event increased with increasing quintiles of baseline leptin and PAI-1 concentrations only in unadjusted analysis (*P*-value for trend <0.003 and <0.0001, respectively). However, these associations were no longer significant after adjustment for usual CHD risk factors including hypertension, diabetes, smoking, total cholesterol, triglycerides and HDL cholesterol. Conversely, baseline CRP and IL-6 levels remained associated with CHD risk in multivariate analysis.
Conclusions: In apparently healthy men, circulating total adiponectin, adipsin, resistin, leptin and PAI-1 were not independent predictors of future CHD event.
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Introduction

It has long been recognized that obese adults have reduced life expectancy,[1] and the risks appear to be attendant on excess intra-abdominal fat.[2] Among potential causes of mortality associated with obesity, cardiovascular disorders such as myocardial infarction and stroke are well documented.[3,4] Indeed, greater abdominal obesity is strongly associated with insulin resistance, dyslipidemia and systemic inflammation, which play essential roles in the pathogenesis of cardiovascular disease.[5]

Adipose tissue is composed of adipocytes but also of cells of many other types as fibroblasts, lymphocytes, macrophages and capillary cells. Over the two last decades it has become clear that adipose tissue is an active tissue which expressed a number of biological active molecules named adipokines.[6] It is now well acknowledged that the sequelae of obesity, particularly insulin resistance, type 2 diabetes, and high blood pressure, are influenced to a great extent by

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the action of these adipokines. Adipokines are numerous and can be categorized according to their cellular expression. Adiponectin, adipsin and leptin are secreted by adipocytes, whereas other proteins such as resistin, plasminogen activator inihibitor-1 (PAI-1) and interleukin (IL)-6 have their origin both in adipocytes and adipose macrophages.[7–9]

Close relationships between lipoprotein and glucose metabolism, coagulation process and artery inflammation and reactivity, and the onset of coronary heart disease (CHD) have been extensively documented. Furthermore, it is becoming increasingly apparent from *in vitro* and *in vivo* studies of humans and animals that the biological actions of adipokines are affected both locally and systemically, and that changes of their plasma level in overweight subjects might have prothrombotic and proatherogenic effects, which might result in increased CHD risk.

However, only a few prospective cohort studies have evaluated these associations and the results have been conflicting for most adipokines. On the other hand, C-reactive protein (CRP) level, a ubiquitous indicator of systemic inflammation positively correlated with obesity, has been consistently found associated with CHD risk in cohort studies.

Therefore, the aim of the present study was to assess the association of total adiponectin, resistin, adipsin, leptin, PAI-1 and IL-6 together with CRP, with the risk of a first ischemic coronary event in an initially healthy population. Adipokine levels, measured by a novel, high-throughput multiplex assay that allows simultaneous quantification of multiple adipokines in a single-plasma sample,[10,11] were investigated within the prospective epidemiological study of myocardial infarction (PRIME) prospective cohort study using a nested case-control design.

Materials and methods

Study population

The PRIME Study is a population-based, prospective cohort designed to identify risk factors for CHD. Details on recruitment, baseline examination and follow-up of the PRIME Study have been described previously.[12] During 1991–1994, 10 600 European-Caucasian men aged 50–59 years living in the areas of Lille, Strasbourg and Toulouse in France, and Belfast in Northern Ireland, were recruited in various employment groups, health screening centers and general practices. Each sub-sample of approximately 2500 men was built to broadly match the social class structure of the underlying population. Approval from the appropriate local ethics committee was obtained and all subjects gave written informed consent.

Baseline examination in the entire cohort

The entry examination included standardized questionnaires relating to medical history, drug intake, presence of CHD and various habits including tobacco and alcohol consumptions, and a clinical examination. Waist circumference, which was measured at the mid distance between the last rib margin and the top of the iliac crest, and weight were standardized between centers. A participant was considered diabetic if he took hypoglycemic drugs. Blood pressure was measured twice in the sitting position with the same automatic device (Spengler SP9). Of those 10 600 recruited men, 9779 were free of CHD at baseline examination.

Biological measurements

Blood was drawn after a 12-h fast into EDTA, citrate and dry tubes. Lipid measurements were carried out for the whole cohort in fresh plasma in a central laboratory (Pasteur Institute of Lille, Lille, France) using commercial kits as previously described.[12] Aliquots of serum and plasma were thereafter frozen in liquid nitrogen until analysis for the analysis of biomarkers.

Follow-up and ascertainment of cases

During the 10-year follow-up, subjects were contacted annually by letter and, if necessary, by telephone, and a clinical event questionnaire was completed. For all possible events, clinical information was sought directly from hospital or general practitioners' notes. All details of ECG, hospital admissions, enzymes, surgery, angioplasty, treatment, and so on, were collected and classified according to the MONICA criteria.[13] Whenever possible, circumstances of death were obtained from the practitioner or the family. In the few cases where the circumstances surrounding the death were not available from the practitioner or the family, death certificates were checked for supporting clinical and post-mortem information on cause of death. A Medical Committee comprising one member from each PRIME Centre and the Coordinating Centre, and three cardiologists was established, in order to provide independent validation of coronary events. A description of the coronary endpoint definitions has been published elsewhere.[14] Angina pectoris was defined not only by the occurrence of chest pain at rest and/or on exertion, but also by one of the following criteria: (1) angiographic stenosis over 50%; (2) a positive scintigraphy (if no angiographic data); (3) positive exercise stress (if no angiographic or scintigraphy data) and (4) electrocardiogram changes at rest (if no data from angiographic, scintigraphy or exercise stress test were available) but without myocardial infarction and no evidence of non-coronary cause in the clinical history. Unstable angina was defined as a crescendo pain or chest pain at rest, with either enzyme changes or electrical changes. In the absence of enzyme or electrical data, the diagnosis was not upheld. Myocardial infarction was defined by one of the following set of conditions: (1) new diagnostic Q-wave or other fresh typical electrocardiographic signs of necrosis; (2) typical or atypical pain symptoms and new (or increased) ischemia and myocardial enzyme levels higher than twice the upper limit

and (3) post-mortem evidence of fresh myocardial infarction or thrombosis. Coronary death was defined as death with a documented coronary event.

After 10 years of follow-up, the CHD event status was available for more than 98% of the cohort.

The case–control study

At the end of the 10 years of follow-up, 661 men developed first coronary event, but plasma samples were available for 617 men. A nested case–control study within the PRIME prospective cohort study was conducted using the baseline plasma samples from those 617 men (cases) and from 1215 matched controls (two controls per case). The matched controls were study participants recruited in the same center on the same day (± 3 days) and of the same age (± 3 years) as the corresponding case, and were free of CHD at the date of the ischemic event of the case. Circulating levels of total adiponectin, resistin, adipsin, leptin and CRP were determined in baseline samples with a multiplex bioassay using commercially available kits. Multiplex beads were purchased from the following two manufacturers: R&D Systems (Billerica, MA, USA) for measurements of CRP (LOB1707), leptin (LUB398), resistin (LOB1359) and adipsin (LOB1824), and Linco Research Inc. (Billerica, MA, USA) for total adiponectin (Human cardiovascular disease panel 1 multiplex immunoassay). Each multiplex assay was performed according to the manufacturer's specifications. The plates were read on a Luminex 200 instrument system. IL-6 was determined by high-sensitivity ELISA (BMS213HS; Bender MedSystems, Vienna, Austria). PAI-1 activity was measured by a two-stage amidolytic method using a commercially available kit (SpectrolyseTM/Fibrin; Biopool, Umea, Sweden). The coefficients of variation were 12.4, 6.6, 9.9, 7.2, 9.5, 8.0 and 9.7% for adiponectin, adipsin, resistin, leptin, PAI-1, IL-6 and CRP, respectively.

Statistical analysis

Variables with a skewed distribution including triglycerides, total adiponectin, adipsin, resistin, leptin, PAI-1, IL-6, and CRP were log-transformed. For these last variables, reported means are geometric means ± s.d. Baseline characteristics between cases and controls were compared by conditional logistic regression analysis suited to the matched design. We determined interrelations among biological parameters in the control group by using Spearman correlations. Associations of adipokine and CRP concentrations with smoking, hypertension and diabetes in the control group were examined using unadjusted analysis of variance. To assess the shape of the relationships between adipokines and CHD risk, subjects were grouped according to quintiles of their distribution in the control group. Hazard ratios (HRs) of CHD for each quintile relative to the lowest one was estimated by separate conditional logistic regression analysis, successively without and with adjustment for usual CHD risk factors, namely diabetes, hypertension, smoking status, total and high-density lipoprotein (HDL) cholesterol and triglycerides. HRs were similarly calculated for 1-s.d. increase (estimated in the control group) in the explanatory variable. Analyses were two-sided and $P<0.05$ was considered to be significant. All computations were carried out with SAS software, version 8.1 (SAS Institute, Cary, IL, USA).

Results

Baseline characteristics

The baseline characteristics of subjects who developed a first CHD event during the follow-up period (cases) and of the controls are compared in Table 1. As expected, cases had higher body mass index, waist circumference, total cholesterol, triglycerides, lower HDL cholesterol and were more likely current smoker, hypertensive or to have diabetes. Moreover, they had significantly higher leptin, PAI-1, IL-6 and CRP concentrations than controls, as shown by a respective relative increase of 17, 19, 86 and 28% of their concentrations. In contrast, plasma total adiponectin, resistin and adipsin concentrations were not significantly different between the two groups.

Adipokines and risk factors in controls

As outlined in Table 2, smoking status affected the concentrations of leptin, IL-6 and CRP. Current smokers had higher IL-6 and CRP, and lower leptin levels than those who never smoked. Hypertension was associated with elevated levels of adipsin, leptin, PAI-1, IL-6 and CRP, and diabetes with higher levels of adiponectin, leptin, PAI-1 and CRP. In contrast, resistin levels were independent of any of these factors.

With the exception of resistin, the level studied adipokines and CRP significantly correlated with anthropometry (Table 3), plasma leptin and PAI-1 level being strongly correlated with body mass index ($r=0.67$; $P<0.0001$ and $r=0.43$; $P<0.0001$, respectively) and with waist circumference ($r=0.65$, $P<0.0001$ and $r=0.40$, $P<0.0001$, respectively). Adipsin, resistin, leptin, PAI-1, IL-6 and CRP levels also correlated positively with the level of plasma triglycerides and negatively with that of HDL cholesterol. On the other hand, adiponectin level correlated negatively with that of triglycerides and positively with that of HDL cholesterol. Adipokines and CRP were mutually associated at moderate levels.

Adipokines and future CHD events

The univariate HRs for CHD increased significantly from the bottom to the highest quintile of PAI-1 (P for trend <0.0001), leptin ($P<0.003$), IL-6 ($P<0.0001$) and CRP ($P<0.0001$) (Figure 1a). Comparing the highest with the lowest quintile, CHD risk increased 1.6-fold for leptin and 2–2.5-fold for CRP, IL-6 and PAI-1. No increase in risk was

Table 1 Baseline characteristics of cases and matched controls. The PRIME Study

	Cases (n = 617)	*Controls (n = 1215)*	P-*value*
Body mass index (kg m^{-2})	27.4 ± 3.6	26.6 ± 3.3	<0.0001
Waist circumference (cm)	95.8 ± 10.0	94.2 ± 9.6	0.006
Cholesterol (mg dl^{-1})	232 ± 40	222 ± 36	<0.0001
HDL cholesterol (mg dl^{-1})	45 ± 12	49 ± 13	<0.0001
Triglycerides[a] (mg dl^{-1})	152 (91–252)	131 (78–220)	<0.0001
Current smokers (%)	36	27	<0.0001
Hypertension (%)	40	26	<0.0001
Diabetes mellitus (%)	6	3	<0.0001
Total adiponectin[a] (mg dl^{-1})	10.6 (5.4–20.6)	11.0 (5.5–22.0)	0.26
Adipsin[a] (ng ml^{-1})	2.81 (1.79–4.41)	2.78 (1.73–4.47)	0.38
Resistin[a] (ng ml^{-1})	2.97 (1.18–7.45)	3.00 (1.21–7.47)	0.79
Leptin[a] (ng ml^{-1})	6.30 (2.86–13.86)	5.40 (2.27–12.87)	0.0002
PAI-1[a] (IU ml^{-1})	10.09 (1.99–51.09)	8.48 (1.70–42.35)	0.03
IL-6[a] (pg ml^{-1})	0.13 (0.01–2.02)	0.07 (0.01–1.37)	<0.0001
CRP[a] (mg l^{-1})	2.85 (1.12–7.27)	2.23 (0.84–5.94)	<0.0001

Abbreviations: CRP, C-reactive protein; HDL, high-density lipoprotein; IL, interleukin; PAI, plasminogen activator inhibitor. Continuous variables are expressed as arythmetic means ± s.d. and as geometric means ± s.d. for log-transformed variables (triglycerides, total adiponectin, adipsin, resistin, leptin, PAI-1, IL-6 and CRP), and as % for smoking, hypertension and diabetes, respectively. [a]Log-transformed variable.

Table 2 Mean levels of adipocytokines according to categorical risk factors in controls. The PRIME Study

	Total adiponectin (mg dl^{-1})	*Adipsin (ng ml^{-1})*	*Resistin (ng ml^{-1})*	*Leptin (ng ml^{-1})*	*PAI-1 (IU ml^{-1})*	*IL-6 (pg ml^{-1})*	*CRP (mg l^{-1})*
Smoking							
Never	11.6	2.84	3.06	5.50	7.61	0.05	1.91
Former	10.7	2.76	2.93	6.15	8.95	0.07	2.21
Current	10.9	2.74	3.05	4.33	8.86	0.13	2.69
P-value[a]	0.18	0.57	0.73	<0.0001	0.28	<0.0001	<0.0001
Hypertension							
No	11.1	2.69	2.95	4.80	7.68	0.07	2.04
Yes	10.9	3.06	3.18	7.57	11.22	0.10	2.86
P-value[a]	0.67	<0.0001	0.21	<0.0001	<0.0003	0.07	<0.0001
Diabetes							
No	11.1	2.78	3.01	5.35	8.34	0.07	2.20
Yes	8.73	2.96	2.85	7.41	15.75	0.09	3.63
P-value[a]	0.05	0.77	0.93	0.04	0.02	0.69	0.004

Abbreviations: CRP, C-reactive protein; IL, interleukin; PAI, plasminogen activator inhibitor. [a]Unadjusted general linear regression analysis.

Table 3 Spearman correlation coefficients between baseline adipokines and CRP with BMI, waist circumference, total cholesterol, triglycerides, HDL-cholesterol in the controls

	Adiponectin	*Adipsin*	*Resistin*	*Leptin*	*PAI-1*	*IL-6*	*CRP*
BMI	−0.13†	0.17†	0.01	0.67†	0.43†	0.06*	0.22†
Waist circumference	−0.14†	0.17†	−0.02	0.65†	0.40†	0.05	0.19†
Cholesterol	−0.05	0.04	0.03	0.11†	0.16†	0.00	0.06*
Triglycerides	−0.10§	0.16†	0.08§	0.35†	0.40†	0.16†	0.18†
HDL cholesterol	0.17†	−0.14†	−0.12†	−0.25†	−0.19†	−0.10†	−0.19†
Adiponectin	—	0.11†	0.09§	−0.05	−0.15†	−0.07*-	0.00
Adipsin	—	—	0.29†	0.30†	0.14†	0.04	0.30†
Resistin	—	—	—	0.08†	0.09§	−0.03	0.07*
Leptin	—	—	—	—	0.48†	0.06*	0.29†
PAI-1	—	—	—	—	—	0.11†	0.20†
IL-6	—	—	—	—	—	—	0.20†

Abbreviations: BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; IL, interleukin; PAI, plasminogen activator inhibitor. The PRIME Study. *$P<0.05$; $^{\S}P<0.01$; $^{\dagger}P<0.001$.

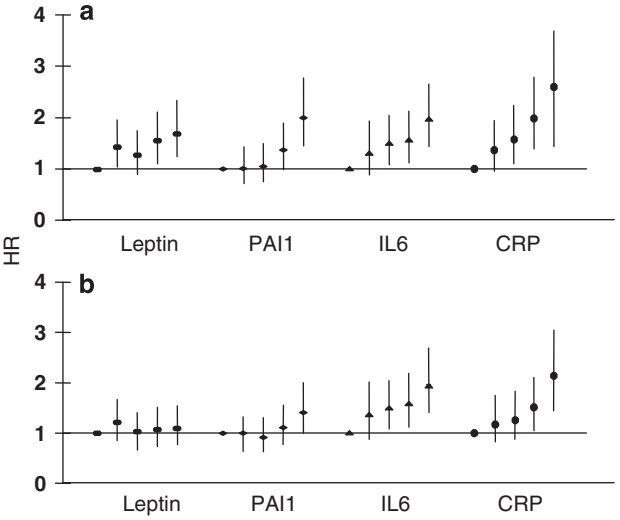

Figure 1 HRs for combined coronary events according to quintiles of CRP, IL-6, PAI-1 and leptin in univariate analysis (**a**) and after adjustment for total cholesterol, HDL cholesterol, triglycerides, hypertension, diabetes and smoking status (**b**). The PRIME Study. The dots indicate HR and vertical bars denote 95% CIs. CI, confidence interval; CRP, C-reactive protein; HDL, high-density cholesterol; HR, hazards ratio; IL, interleukin.

found among quintiles for adiponectin, adipsin and resistin (data not shown).

After adjustment for the usual risk factors, only the HR of the top quintile of PAI-1, but not leptin, remained borderline significant, with a relative increased risk of 42% for PAI-1 (95% confidence intervals (CI) = 1.00–2.03; $P<0.05$; Figure 1b). A more detailed analysis showed that the correlations of these two adipokines with triglycerides and HDL cholesterol (and hypertension for leptin) were responsible for these confounding effects. Conversely, HRs associated with quintiles 4 and 5 of IL-6 were not modified after adjustment and remained significant as shown by HRs of 1.58 (95% CI = 1.14–2.19; $P<0.006$) and 1.93 (95% CI = 1.39–2.66; $P<0.0001$), respectively. CHD risk associated with quintiles 4 and 5 of CRP decreased moderately to 1.48 (95% CI = 1.03–2.14; $P<0.03$) and 2.12 (95% CI = 1.47–3.06; $P<0.0001$) (Figure 1b), respectively.

The adjusted HRs for 1-s.d. increment of each adipokine for first CHD event are summarized in Table 4. Total adiponectin, adipsin, resistin, leptin and PAI-1 were not significantly associated to CHD risk. Only, IL-6 and CRP were significant predictors, with a similar standardized HR (1.22 and 1.24, respectively). The inclusion of both CRP and IL-6 in the same model showed that the two parameters remained independently associated with CHD risk (data not shown).

Table 4 Separate adjusted HR for combined coronary events according to 1-s.d. increase of log-transformed variables total adiponectin, adipsin, resistin, leptin, PAI-1, IL-6 and CRP

	HR	*95% CI*	P-*value*
Adiponectin	0.99	0.88–1.12	0.87
Adipsin	0.97	0.83–1.05	0.59
Resistin	0.92	0.73–1.13	0.53
Leptin	1.04	0.92–1.17	0.75
PAI-1	1.00	0.89–1.12	0.89
IL-6	1.22	1.09–1.38	0.0008
CRP	1.24	1.10–1.40	0.0003

Abbreviations: CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; IL, interleukin; PAI, plasminogen activator inhibitor. The PRIME Study. HRs were estimated by separate conditional logistic regression adjusted for total cholesterol, HDL cholesterol, triglycerides, diabetes, hypertension and smoking status.

Discussion

Using a new multiplex technology, we sought to investigate the association between a set of adipokines, including adiponectin, resistin, adipsin, leptin, PAI-1, measured initially in middle-aged men and subsequent occurrence of CHD during 10 years of follow-up in the PRIME Study. Total adiponectin, resistin, adipsin were not predictors of CHD even in unadjusted analysis. The significant graded increased risk of CHD associated with increasing level of leptin and PAI-1 was no longer observed after adjustment for established coronary risk factors such as smoking, hypertension, diabetes, total cholesterol, triglycerides and HDL cholesterol. Finally, CRP and IL-6 remained the only independent predictors of CHD.

Previous results relating adipokines with future CHD events have been inconsistent. However, most of these studies investigated a single adipokine and included few CHD events. To date, simultaneous assessment of several adipokines within the same cohort and their association with the risk of future CHD events has not been reported. Briefly, in the following section, our results are discussed and related to what is already known about each adipokine.

Risks associated with low adiponectin level was evaluated in a meta-analysis,[15] including results of seven cohort studies.[15–21] It suggested no association but much heterogeneity was present between studies. Since then, further reports have confirmed this result, a few showing an inverse association between adiponectin levels and CHD,[22–24] and several others showing no association.[25–28] Recently a high-molecular-weight adiponectin form was claimed to be more important for vascular protection than the total amount of adiponectin.[29,30] However, cohort studies have not shown a clearer relationship between levels of high-molecular-weight adiponectin and CHD risk, two studies reporting no association,[25,31] and one showing a protective effect.[32] On the other hand, all studies, including the present one, have reported associations of levels of total or high-molecular-weight adiponectin with the main characteristics of the insulin-resistance syndrome such as weight and visceral adipose mass, triglycerides and HDL cholesterol.[33–36] Clinical and cell cultures studies also describe other mechanisms for a high level of adiponectin such as a favorable effect on lipoprotein size[37] and a reduced lipid accumulation in

macrophages,[38] both effects which would be responsible for decreased CHD risk.

If low plasma level of adiponectin is now recognized as a predictor for the onset of type-2 diabetes,[39] we may conclude that it is not strongly predictive of a future coronary event in the general population.

Adipsin is mainly expressed in adipocytes[40] and is involved in the activation of the alternative pathway of complement with the acyl-stimulating protein as the final component. To our knowledge, this is the first population-based study evaluating the association between adipsin and future CHD events. A previous retrospective case–control study has suggested a significant higher level of acyl-stimulating protein in CHD patients as compared with that in controls.[41] The positive correlation between adipsin levels and body mass index observed in the present study as well as in others[42] suggests a role of adipsin in the increase of fat mass through acyl-stimulating protein synthesis. Indeed, by enhancing differentiation of pre-adipocytes in adipocytes, and synthesis of triglycerides, acyl-stimulating protein could stimulate an increase in body weight. However, the specific biological role of adipsin is unclear and no interpretation of the possibly lower CHD risk we observed in subjects with high adipsin but low CRP could be proposed.

An association between CHD and resistin was first reported in retrospective case–control studies[43,44] and resistin level has also been related to the presence of coronary artery calcification.[45] More recently, one prospective cohort study concluded that resistin was an independent risk factor of CHD after adjustment for usual risk factors.[46] Divergence with the present results is anomalous, since correlation coefficients between resistin and body mass index, HDL cholesterol and CRP level were similar. We observed no association between resistin and waist girth or diabetes in accordance with the previously described comparability of resistin levels between normal, insulin-resistant and type-2 diabetes subjects.[47] This finding might be explained by circulating monocytes as the main source of resistin in humans[48] even though adipocytes also secrete the protein.[49] The role of resistin is not yet well known, and whereas some studies support a role as an inflammatory adipokine, others suggest antioxidant properties for this protein,[50] which would, therefore, have opposite effects on the artery wall.

Reports of the association between leptin and CHD risk yielded divergent results, with three studies describing no association,[51–53] one a positive[54] and one reporting significant negative association.[55] An unadjusted high leptin level was in fact a predictor of a first coronary event in our study, but this association disappeared when other risk factors, particularly triglycerides, HDL cholesterol and presence of hypertension, were included into the model, similar to the results of the Quebec Cardiovascular Study. In contrast to the west of Scotland coronary prevention Study (WOSCOPS), which disclosed a positive association, the Quebec Cardiovascular Study and the PRIME Study included angina events and not only 'hard CHD' events. However, the exclusion of angina cases in our study did not modify the results (data not shown). It should be noted that the populations were also different as WOSCOPS recruited exclusively hypercholesterolemic subjects. Leptin is an extremely robust circulating marker of excess body weight, so its correlation with CRP level is biologically plausible because both leptin and cytokines such as IL-6, which promotes hepatic CRP secretion, are produced by adipocytes. This correlation can be also explained by hyperleptinemia as part of the acute-phase reaction.[56] As it is unlikely that leptin has direct atherogenic properties, our results strengthen the hypothesis that this adipokine might be a candidate link between overweight and cardiovascular risk through its direct or indirect effects on lipid metabolism and inflammatory process.

While PAI-1 was associated with CHD risk in the Caerphilly Study,[57] this was not the case in several other cohort studies[33,35,36]. Paralleling our results, Thogersen *et al*.[34] and Juhan-Vague *et al*.[58] noted a significant association between PAI-1 and the development of a first myocardial infarction, but this association disappeared after adjustment for established risk factors and particularly lipid factors. It should be noted, however, that association between the top quintile of PAI-1 with CHD in the multivariate analysis was of borderline significance, and we may have lacked of power to detect a true, but small, association between PAI-1 and CHD risk.

Finally, the results of the present study confirm the predictive values of CRP and IL-6 for a future CHD event, which has been observed in previously published studies and synthesized in two meta-analyzes.[59,60] Most studies yielded standardized HRs for CRP similar to that estimated in the present study.[59] IL-6 was also described as a risk marker for CHD, the association being as strong as that of major established risk factors.[60] The consistency of these associations introduces a clear distinction between these molecules and the adipokines measured in the present study at least regarding their potential role in CHD etiology.

Limitations of the study

The present study has some limitations. We acknowledged that the definition of diabetes lacked of accuracy as it was defined by the self report of diabetes and/or the use of anti diabetic medication at baseline examination. Reliable fasting glycemia concentrations were available only in a subgroup of 825 subjects in whom 4.7% had fasting glycemia greater than 7 mmol l^{-1}. Of note, half of these diabetic men self-reported having diabetes or using antidiabetic medication at baseline examination. It is, therefore, rather unlikely that our current definition of diabetes was responsible for the lack of independent association between circulating adipokines and CHD risk. Plasma sample were kept frozen more than 10 years and protein degradation cannot be excluded. Serial examination was not performed in the PRIME Study so that changes in risk factor levels, including that of adipokines, and therapy during follow-up were not controlled for.

Our sample included middle-aged European-Caucasian men so that current results should be confirmed for older, for women and for other ethnic groups. The procedures of selection of subjects free of CHD at entry and the procedures of identification of cases and controls during follow up did not permit taking into account some silent forms of CHD episodes and consequently the possibility of some bias towards the null hypotheses could not be ruled out.

In conclusion, this study does not suggest independent, clinically relevant associations between total adiponectin, adipsin, resistin, leptin and PAI-1, and risk for future CHD in apparently healthy subjects. However, our results do not exclude that adipokines could have an impact on the atherosclerotic process through different metabolic pathways.

Conflict of interest

The authors declare no conflict of interest.

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Appendix

The PRIME Study Group

The PRIME Study is organized under an agreement between INSERM and the Merck, Sharpe and Dohme-Chibret Laboratory, with the following participating Laboratories:

The Strasbourg MONICA Project, Laboratoire d'Epidemiologie et de Sante Publique, EA1801, Strasbourg, F-67085, France, and Universite Louis Pasteur, Faculte de Medecine, Strasbourg, F-67085, France (D Arveiler, B Haas); The Toulouse MONICA Project, INSERM U558, and Département d'Epidemiologie, Universite Paul Sabatier—Toulouse Purpan, Toulouse, France (J Ferrières, JB. Ruidavets); The Lille MONICA Project, INSERM, U744, Lille, France, and Institut Pasteur de Lille, Lille, France; Université de Lille 2, Lille, France (P. Amouyel, M. Montaye); The Department of Epidemiology and Public Health, Queen's University, Belfast, Northern Ireland (A Evans, J Yarnell, F Kee), The Department of Atherosclerosis, INSERM, U545, Lille, Institut Pasteur de Lille, Lille, Université de Lille 2, Lille, France (G Luc, JM Bard); The Laboratory of Haematology, INSERM, U626, Marseille, Hôpital La Timone, Marseille, France (I Juhan-Vague, P Morange), The Laboratory of Endocrinology, INSERM U563, Toulouse, France (B Perret); The Vitamin Research Unit, The University of Bern, Bern, Switzerland (F Gey); The Nutrition and Metabolism Group, Centre for Clinical and Population Sciences, Queen's University Belfast, Northern Ireland (Woodside, I Young); The DNA Bank, INSERM U525, Paris, France (F Cambien); The Coordinating Center, INSERM, Unit 909, Villejuif, F-94807, France, and Université Paris V, Paris Cardiovascular Research Centre (PAARC), Paris, F-75015, France (P Ducimetiere, A Bingham)