

Adipokines and risk of type 2 diabetes in older men

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ABSTRACT

Aim The aim was to assess the relationship between adipokines including interleukin 6 (IL-6), leptin and adiponectin with development of type 2 diabetes and assess the role of obesity and insulin resistance in these relationships.

Methods A prospective study of 3599 non-diabetic men aged 60-79 followed up for a mean period of 5 years during which there were 108 incident cases of type 2 diabetes.

Results Elevated IL-6 and leptin and low adiponectin were associated with increased risk of type 2 diabetes even after adjustment for BMI, lifestyle factors, pre-existing cardiovascular disease and systolic blood pressure. The relative risks (95% CI) (3rd versus 1st third) were 2.02 (1.14,3.58) for IL6, 1.91 (0.97,3.76) for leptin, and 0.40 (0.23,0.70) for adiponectin. Further adjustment for insulin resistance made minor differences to the IL-6 diabetes relationship [adjusted RR 2.1 (1.18,3.81), weakened the associations with adiponectin (adjusted RR 0.59 (0.33,1.04) and abolished the association between leptin and diabetes [adjusted RR 1.12 (0.55,2.26)]. The inverse relation between low adiponectin and diabetes was significantly stronger in men who were obese (waist circumference >102 or BMI > 30kg/m) [adjusted RR 0.30 (0.11,0.7)] relative to leaner men [adjusted RR 0.93 (0.44,1.55)] (test for interaction p=0.04).

Conclusion The association between leptin and incident diabetes is mediated by insulin resistance. By contrast the positive association between IL-6 and diabetes appeared to be independent of obesity and insulin resistance. Finally, the association between low adiponectin and increased risk of diabetes appears to be significantly stronger in obese men than leaner counterparts.

INTRODUCTION

Adipose tissue, in addition to being a fat store, secretes a number of hormones and proteins collectively termed adipokines [1,2]. Several adipokines, including adiponectin, leptin and interleukin 6 (IL-6) have been linked to the development of diabetes [3-17]. In most studies, low adiponectin [3-10] and elevated IL-6 [13-17] have been associated with the development of subsequent diabetes independent of obesity and some have shown the associations to be independent of measures of insulin [3,7,8,15]. However, the extent to which leptin is independently related to risk of diabetes has been variable. While one study has indicated a positive effect [11], another suggested a protective effect against diabetes [12] and one indicated no independent relationship between leptin and diabetes [10]. Finally, data on the whether the effects of these adipokines are independent of each other in the prediction of diabetes are relatively sparse. Inflammatory markers are predictive of diabetes [13-17] and, as with adiponectin and leptin, are synthesised by adipocytes. Hence, it remains possible that the associations of adiponectin and leptin with subsequent diabetes are partly mediated via cytokines such as IL-6, or vice versa.

We have therefore examined the independent prospective relationships between adipokines (leptin, adiponectin and IL-6) and risk of type 2 diabetes. We also assessed the role of insulin resistance in these relationships and the possible interaction with obesity, an important point given recent intriguing evidence that agents (e.g. glitazones) that increase adiponectin and lower inflammatory marker levels [18] may work best to attenuate diabetes risk in at risk individuals who have greater baseline levels of obesity [19].

SUBJECTS AND METHODS

The British Regional Heart Study is a prospective study of cardiovascular disease involving 7735 men aged 40-59 years selected from the age-sex registers of one general practice in each of 24 British towns, who were screened between 1978 and 1980 [20]. In 1998-2000, all surviving men, now aged 60-79 years, were invited for a 20th year follow-up

examination. Ethics approval was provided by all relevant local research ethics committees. All men provided informed written consent to the investigation, which was carried out in accordance with the Declaration of Helsinki and completed a questionnaire (Q20) which included questions on their medical history and lifestyle behaviour. The men were asked to fast for a minimum of 6 hours, during which they were instructed to drink only water and to attend for measurement at a pre-specified time between 0800 and 1800h. They then provided a blood sample, collected using the Sarstedt Monovette system. The samples were stored at -20°C on the day of collection and transferred in batches for storage at -70°C until analysis, carried out after no more than one freeze-thaw cycle. 4252 men (77% of survivors) attended for examination. 4086 men had at least one measurement of the adipocyte variables (leptin, adiponectin or interleukin 6). We excluded 487 men with a doctor diagnosis of diabetes or those with a fasting glucose of > 7 mmol per litre (WHO criteria) who were considered to have prevalent diabetes. A total of 3599 men then were available for analysis

Adipokine measurements

Plasma leptin was measured by an 'in house' radioimmunoassay validated thoroughly against the commercially available Linco assay, as previously described [21]. The intra- and inter-assay coefficients of variation (CV) were $<7\%$ and $<10\%$, respectively, over the sample concentration range. The detection limit of the assay was 0.5ng/ml. Plasma adiponectin concentrations were determined using ELISA (R&D systems, UK) and the intra-assay and inter-assay CVs were each $<7.5\%$. Interleukin-6 was assayed using a high-sensitivity ELISA (R & D Systems, Oxford, UK). The intra-assay and inter-assay CVs were 7.5% and 8.9% respectively. There is no evidence that the adipokines measured in the present study are influenced by prolonged storage or repeat free-thawing of samples. Of the 3599 men with at least one measure of adipokines, 32 men had missing data on adiponectin, 33 men on IL-6, and 192 men on leptin. More men had missing leptin levels since this assay required a larger sample volume.

Cardiovascular risk factors

Weight, height and waist circumference (WC) were measured. Body mass index (BMI; weight/height² in kg/m²) was calculated for each man at re-examination. Details of questionnaire assessment and classification of smoking status, physical activity, social class, alcohol intake [20,22], and the measurement of blood pressure and blood lipids in this cohort have been described elsewhere [20,23-24]. Men were asked to recall a doctor diagnosis of coronary heart disease (myocardial infarction or angina), stroke and diabetes. Plasma glucose was measured by a glucose oxidase method using a Falcor 600 automated analyser. Serum insulin was measured using an ELISA assay which does not cross-react with proinsulin [25]. Triglycerides, blood glucose and insulin concentrations were adjusted for the effects of fasting duration and time of day [24]. Insulin resistance was estimated according to the homeostasis model assessment (HOMA-IR - the product of fasting glucose (mmol/L) and insulin (units/mL) divided by the constant 22.5) [26]. C-reactive protein (CRP) was assayed by ultra sensitive nephelometry (Dade Behring, Milton Keynes, UK).

Follow-up

All men have been followed up for all cause mortality, cardiovascular morbidity and development of a diagnosis of type 2 diabetes from initial examination to June 2004 [27] and follow-up has been achieved for 99% of the cohort. This analysis is based on follow-up from re-screening in 1998-2000, a mean follow-up period of 5 years (4-6 years). Information on death was collected through the established "tagging" procedures provided by the National Health Service central registers. Information on new cases of diabetes was obtained by regular two-yearly reviews of the patients' notes (including hospital and clinic correspondence) through to the end of the study period and from repeated personal questionnaires to surviving subjects after initial examination. Cases are based on self-reported diagnoses confirmed by primary care records; an approach which has been validated in the present study [28]

Statistical Methods

The distributions of adiponectin, leptin and IL-6 were skewed and log transformation was

used and geometric means and interquartile ranges were presented. The men were divided into three equal thirds on the basis of adiponectin, leptin and IL-6 distributions. Cox's proportional hazards model was used to assess the multivariate-adjusted relative risk for each third compared with the reference group (lowest third). Person time years was used to calculate the incidence of diabetes with men censored at time of death. In the adjustment, smoking (never, long term ex-smokers (>15 years), recent ex-smokers (<15 years) and current smokers), social class (manual workers, non-manual workers, Armed Forces), physical activity (4 groups), alcohol intake (5 groups), pre-existing CHD (yes/no), stroke (yes/no), use of statins (yes/no), treatment for hypertension (yes/no) were fitted as categorical variables. Inactive men included men who reported no physical activity or who were only occasionally active [22]. BMI, HOMA-IR, systolic blood pressure, HDL-C and CRP were fitted as continuous variables. Tests for interaction were carried out by adding an interaction term (obesity X adipokine variable) to the regression model with the adipokine groups fitted continuously (1-3). All analyses were carried out using SAS (version 8.2) SAS Institute Inc, Cary NC.

RESULTS

Baseline characteristics of incident cases and controls

During the mean follow-up period of 5 years there were 108 incident diabetes cases (rate 6.0/1000 person-years). Table 1 shows the baseline characteristics in the men who developed diabetes and in men who remained free of diabetes. Men who developed diabetes had higher BMI and waist circumference and were more likely to be physically inactive and to have a higher prevalence of CHD. They had significantly higher mean levels of metabolic risk factors, CRP, leptin, IL-6 and lower levels of adiponectin.

Association of adipokines with incident diabetes

Table 2 shows the correlations between the adipokine measures and age, BMI, WC, metabolic risk factors and CRP. The incidence rates and adjusted relative risks of type 2 diabetes by tertiles of the adipocyte markers, using those in the lowest third as the reference

group, are shown in Table 3. Adiponectin (inversely), and leptin and IL-6 (positively) were significantly predictive of type 2 diabetes even after adjustment for age, social class, physical activity, smoking status, alcohol intake, pre-existing CHD, stroke, use of statins, treatment of hypertension, systolic blood pressure and BMI. We repeated the analyses in Table 3 adjusting for waist circumference instead of BMI; similar results were obtained.

Further adjustment for HOMA-IR attenuated the relationship between adiponectin and diabetes, abolished the association between leptin and diabetes, but made little difference to the association between IL-6 and diabetes. The relationship between IL-6 and incident diabetes remained significant even after further adjustment for HDL-cholesterol and CRP (Table 3). Since adiponectin and IL-6 were not correlated, further adjustment for each other made little difference to the associations seen (adjusted RR=2.01 95% CI (1.06-3.81) for IL6, and 0.63 95% CI (0.35-1.11) for adiponectin (top vs bottom third). The IL6:adiponectin ratio showed similar magnitude of association as seen for IL6 (adjusted RR 2.20 95% CI 1.22-3.97) after adjustment for HOMA (IR).

Adipokines, obesity and diabetes

We examined the relationships between the adipokine variables and risk of diabetes separately in obese men (WC > 102cm or BMI > 30kg/m²) and non obese men (Table 4). High adiponectin was associated with significantly decreased risk of diabetes in obese men but the benefit was less apparent in non obese men (test for interaction p=0.04). This decreased risk in obese men was seen even after adjustment for HOMA-IR; no association was seen in non-obese men. By contrast, no interaction was seen between obesity and IL6 and risk of diabetes. Elevated IL-6 was associated with increased risk of diabetes in both groups of men even after adjustment for HOMA-IR (Table 4). Further adjustment for CRP and HDL-C made little difference to the associations seen in Table 4. The associations seen for adiponectin in obese men persisted even after further adjustment for IL6. Leptin showed no association after adjustment for insulin in either group

DISCUSSION

In this large prospective study of men aged 60-79 at baseline, we have shown that low adiponectin and high leptin levels, associate with a higher risk of incident type 2 diabetes independent of age and obesity and a comprehensive range of other potential confounders or explanatory factors. However, further adjustment for HOMA-IR attenuated these relationships, especially for leptin. By contrast, elevated IL-6 levels remain significantly and independently associated with incident diabetes following additional adjustment for HOMA-IR. Perhaps more importantly, our results suggest that the relationship between low adiponectin and incident diabetes is potentially dependent upon baseline adiposity levels.

These results have several potential implications. Firstly, the novel observation of a significant impact of obesity on the relationship between adiponectin and subsequent diabetes may have clinical relevance. Recent data from the DREAM study [19] indicates that rosiglitazone reduces the risk of progression to diabetes most in those at risk subjects who had higher baseline BMI. The mechanism of action of glitazones is not fully elucidated but may include enhancing both beta-cell function and adiponectin synthesis. Adiponectin promotes hepatic fatty acid oxidation and reduces hepatic fat. In DREAM, a significant reduction in ALT concentrations implies a reduction in liver fat [19]. One may speculate therefore that a stronger link of low adiponectin to subsequent diabetes in obese subjects could partly explain a greater relative risk reduction in diabetes seen with glitazones in obese subjects [19]. Interestingly, rimonabant, a selective cannabinoid-1 receptor (CB1) blocker, also increases adiponectin [29] and improves glycaemic control [30]

As regards leptin's link with diabetes, recent nested case-control work from the ARIC study (including both men and women and different ethnicities) suggested that following adjustment for age, gender, ethnicity, obesity indices, fasting insulin, inflammation score, hypertension, triglycerides and adiponectin, higher leptin was significantly associated with a 40% lower risk of subsequent diabetes [12].

The authors suggested that their findings were commensurate with a possible protective effect of leptin against diabetes. By contrast, higher leptin levels predicted higher risk of diabetes in Japanese men but not women [11], although the number of cases of diabetes in that study were small (n=23 men, 17 women), and other studies showed no independent association [10]. Our results generated in a more homogenous population of predominantly white men, but adjusted for similar range of confounders, did not confirm a protective effect of leptin on diabetes risk. Rather, much of the association between leptin and subsequent diabetes could be accounted for by obesity and insulin resistance [31]. Further prospective studies are needed to disentangle the relationship between leptin and subsequent diabetes.

Interestingly, high IL-6 was independently associated with subsequent diabetes, even with further adjustment for insulin resistance or CRP. Although, high IL-6 has been previously associated with subsequent diabetes [13-17], few studies have assessed the role of insulin resistance as a potential confounder, and in the one other study that has, IL-6 has also been shown to be related to diabetes independent of insulin [15]. This is important since low grade systemic inflammation could mediate higher diabetes risk via insulin resistance [32] but our observation suggests IL-6 may associate with diabetes via alternative mechanisms; at present there is no evidence for an independent role of IL-6 in impaired beta-cell function/apoptosis. As such our work adds to the literature on the potential links between IL-6 and diabetes. A recent comprehensive review which took account of a range of in-vitro, in vivo and genetic studies, together with studies on diverse tissues such as liver, muscle, adipose tissue and pancreas, concluded that chronically elevated IL-6 may indeed contribute to development of type 2 diabetes via mechanisms including altered insulin signaling in hepatocytes / adipocytes and effects on CNS to impair energy regulation [33]. High IL-6 may also drive hepatic fatty acid synthesis and cause endothelial dysfunction [34]. Indeed, high IL-6 is consistently linked to a range of metabolic abnormalities typical of an insulin resistant state [34]. Finally, that IL-6 was linked to incident diabetes independently of CRP is

noteworthy, particularly since CRP may not be causally linked to insulin resistance and related features [35]. Such observations direct attention towards upstream cytokines, such as IL-6. In terms of causality, an IL-6 receptor antagonist (Tocilizumab) [36] may offer the potential to directly block this pathway and determine metabolic effects in obese individuals.

Our study is not without some limitations. Our study was carried out in an older predominantly white Caucasian male population and we cannot generalise our findings to women, younger men or other ethnic groups. Moreover, the numbers developing diabetes were modest and greater follow-up time would enhance power and narrow confidence intervals. We also acknowledge that adiponectin association with other risk factors appeared weaker than in other studies but we feel this may be related to the more advanced age of our cohort since adiponectin rises with age. It is also relevant that we measured total adiponectin; the high molecular weight adiponectin fraction, which is as yet not easily measured in such large numbers, may be more strongly associated with incident diabetes, but this requires further study. Finally, we acknowledge that we did not measure other cytokines, such as TNF- α , which may have relevance to diabetes pathogenesis and thus prediction.

Conclusion

On the basis of our prospective cohort study, the findings indicate that 1) the association between leptin and diabetes is largely explained by obesity and insulin resistance, 2) the association between IL-6 and diabetes remains independent of obesity and insulin resistance, and 3) the adiponectin-diabetes association is mediated to some extent by insulin resistance and appears to be stronger in obese individuals.

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Table 1 Distribution of risk factors and inflammatory/haemostatic markers in 3599 non-diabetic subjects aged 60-79 years at re-examination according to diabetes status at follow-up. The British Regional Heart Study.

	Developed diabetes		p-value difference
	No N=3491	Yes N=108	
Age	68.6 (5.5)	68.6 (5.4)	0.87
BMI	26.6 (3.5)	29.7(3.7)	<0.0001
% current smokers	13.2	10.2	0.84
% inactive	32.6	44.2	<0.0001
% manual workers	64.8	53.2	0.02
% heavy drinkers (> 35 drinks/week)	3.7	2.9	0.32
% CHD	17.6	38.9	<0.0001
% use of statins	6.6	17.6	<0.0001
% stroke	5.0	6.5	0.67
SBP	148.2 (23.9)	154.1 (23.4)	0.01
Triglyceride [#] (mmol/l)	1.57 (1.12-2.13)	2.14 (1.55-2.81)	<0.0001
HDL-C (mmol/l)	1.34 (0.34)	1.16 (0.30)	<0.0001
Glucose [#] (mmol/l)	5.52 (5.21-5.89)	5.99 (5.63-6.58)	<0.0001
Log HOMA-IR	0.66 (0.59)	1.30 (0.54)	<0.0001
CRP [#] mg/L	1.66 (0.80-3.33)	2.56 (1.27-4.28)	<0.0001
IL-6 (pg/ml)	2.41 (1.55,3.42)	3.00 (2.30,4.48)	<0.0001
Leptin (ng/ml)	8.93 (5.6,14.1)	14.30 (10.2,21.9)	<0.0001
Adiponectin (microgram/mL)	7.04(4.49-11.41)	4.96 (3.33-7.36)	<0.0001

Data are means (SD), geometric means (interquartile range) for skewed variables

geometric mean

Table 2 Spearman correlation coefficients between adipokines and risk factors in 3599 non-diabetic subjects aged 60-79 years at re-examination. The British Regional Heart Study.

	Adiponectin	Leptin	IL-6
Leptin	-0.08**		
IL-6	0.008	0.14***	
Age	0.17***	0.02	0.22***
BMI	-0.15***	0.57***	0.11***
WC	-0.13***	0.57***	0.14***
SBP	-0.01	0.08**	0.13***
DBP	-0.04	0.16***	0.11***
triglyceride	-0.25***	0.26***	0.05*
HDL-C	0.26***	-0.21***	-0.15***
glucose	-0.006	0.06*	0.01
HOMA-IR	-0.19***	0.54***	0.10***
CRP	-0.05*	0.21***	0.57***

SBP systolic blood pressure;DBP diastolic blood pressure;
*** p<0.0001;

**

p<0.001;p<0.01

Table 3. Adjusted relative risk of incident type 2 diabetes by tertiles of adiponectin, leptin and IL-6 in 3599 non-diabetic men aged 60-79 years. The British Regional Heart Study.

Tertiles	Rates/100 0 per- years (No of cases)	Adjusted relative risk (95% CI)			
		A	B	C	D
Adiponectin (microgram/mL)					
<5.44 (1189)	9.3 (55)	1.00	1.00	1.00	1.00
5.44- (1189)	5.5 (32)	0.59 (0.38,0.91)	0.66 (0.42,1.04)	0.85 (0.54,1.36)	0.93 (0.58,1.49)
9.68- (1189)	3.2 (18)	0.33 (0.19,0.56)	0.40 (0.23,0.70)	0.59 (0.33,1.04)	0.67 (0.38,1.20)
Trend across groups		P<0.00001	P=0.0007	P=0.07	P=0.20
Leptin (ng/ml)					
<1.91 (1135)	2.4 (13)	1.00	1.00	1.00	1.00
1.91- (1135)	4.0 (22)	1.68 (0.85,3.35)	1.17 (0.58,2.35)	0.97 (0.48,1.95)	0.84 (0.41,1.70)
2.49- (1137)	11.8 (65)	4.98 (2.75,9.04)	1.91 (0.97,3.76)	1.12 (0.55,2.26)	1.01 (0.50,2.06)
Trend across groups		P<0.0001	P=0.03	P=0.68	P=0.82
IL-6 (pg/ml)					
<1.77 (1197)	2.9 (18)	1.00	1.00	1.00	1.00
1.77- (1175)	5.57 (32)	1.92 (1.07,3.44)	1.57 (0.87,2.83)	1.64 (0.90,3.00)	1.64 (0.88,3.07)
2.92- (1193)	10.5 (58)	3.71 (2.17,6.34)	2.02 (1.14,3.58)	2.12 (1.18,3.81)	2.10 (1.10,4.02)
Trend across groups		P<0.0001	P=0.01	P=0.01	P=0.03

A=age-adjusted

B= adjusted for age, social class, physical activity, smoking status, alcohol intake, pre-existing CHD or stroke, use of statins, systolic blood pressure, treatment for hypertension and BMI.

C= adjusted for above and HOMA-IR.

D=adjusted for above and HDL-cholesterol and CRP.

Table 4: Obesity, adipokines and adjusted relative risk of type 2 diabetes in 3599 non-diabetic men aged 60-79 years. The British Regional Heart Study.

Tertiles	Non-obese (No of cases/no of men=40/2586;3.2/1000 per- yrs)			Obese (No of cases/No of men=68/1012;14.1/1000 per-yrs)			
	Rate/100 0 per-yrs	Relative Risk+	Relative Risk ++	Rate/1000 per-yrs	Relative Risk +	Relative Risk ++	
Adiponectin							
1	3.5	1.00	1.00	17.5	1.00	1.00	
2	2.4	0.53 (0.23,1.25)	0.58 (0.24,1.36)	11.2	0.73 (0.44,1.27)	0.98 (0.56,1.70)	
3	3.0	0.73 (0.35,1.55)	0.93 (0.44,1.96)	3.5	0.20 (0.06,0.51)	0.30 (0.11,0.79)	
Trend across groups		P=0.93	P=0.58		P<0.0001	P=0.01	
Test for interaction between obesity and adiponectin +		P=0.04					
Leptin							
1	1.6	1.00	1.00	8.4	1.00	1.00	
2	2.7	1.03 (0.41,2.59)	0.80 (0.31,2.01)	6.4	0.67 (0.21,2.14)	0.62 (0.19,1.99)	
3	5.9	1.77 (0.69,4.58)	0.97 (0.36,2.63)	15.2	0.94 (0.33,2.74)	0.61 (0.21,1.81)	
Trend accros groups		P=0.24	P=0.33		P=0.79	P=0.77	
Test for interaction between		P=0.52					

obesity and
leptin+
IL-6

1	1.8	1.00	1.00	5.9	1.00	1.00
2	2.7	1.79 (0.73,4.39)	1.88 (0.74,4.80)	10.8	1.31 (0.60,2.85)	1.39 (0.62,3.61)
3	5.2	2.33 (0.96,5.67)	2.43 (0.95,6.19)	17.1	1.72 (0.83,3.57)	1.83 (0.86,3.90)
Trend across groups		P=0.12	P=0.13		P=0.05	P=0.04
Test for interaction between obesity and IL-6+		P=0.98				

+adjusted for age, social class, physical activity, smoking status, alcohol intake, pre-existing CHD, stroke, use of statins, systolic blood pressure, treatment for hypertension and BMI

++ adjusted for above and HOMA-IR.