

Leptin and incident type 2 diabetes: risk or protection?

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Abstract

Aims/hypothesis The aim of this study was to investigate the association of leptin levels with incident diabetes in middle-aged adults, taking into account factors purportedly related to leptin resistance.

Subjects and methods We conducted a case-cohort study (570 incident diabetes cases and 530 non-cases) representing the 9-year experience of 10,275 participants of the Athero-

sclerosis Risk in Communities Study. Plasma leptin was measured by direct sandwich ELISA.

Results In proportional hazards models adjusting for age, study centre, ethnicity and sex, high leptin levels (defined by sex-specific cut-off points) predicted an increased risk of diabetes, with a hazard ratio (HR) comparing the upper with the lower quartile of 3.9 (95% CI 2.6–5.6). However, after further adjusting additionally for obesity indices, fasting insulin, inflammation score, hypertension, triglycerides and adiponectin, high leptin predicted a lower diabetes risk (HR=0.40, 95% CI 0.23–0.67). Additional inclusion of fasting glucose attenuated this protective association (HR=0.59, 95% CI 0.32–1.08, $p<0.03$ for linear trend across quartiles). In similar models, protective associations were generally seen across subgroups of sex, race, nutritional status and smoking, though not among those with lower inflammation scores or impaired fasting glucose (interaction $p=0.03$ for both).

Conclusions/interpretation High leptin levels, probably reflecting leptin resistance, predict an increased risk of diabetes. Adjusting for factors purportedly related to leptin resistance unveils a protective association, independent of adiponectin and consistent with some of leptin's described protective effects against diabetes.

Keywords Inflammatory markers · Leptin · Leptin resistance · Risk factors · Type 2 diabetes

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Abbreviations

ARIC Atherosclerosis Risk in Communities
CRP C-reactive protein
HR hazard ratio
OR odds ratio

Introduction

Diabetes has become a major health priority worldwide. The epidemic rise in obesity and type 2 diabetes has stimulated new avenues of investigation, such as the role of inflammation in the progression from obesity to diabetes. Along these lines, we found that a mild chronic state of inflammation precedes and predicts incident type 2 diabetes [1, 2]. These associations have been confirmed in different ethnic groups, and analyses have been expanded to include various circulating molecules with inflammatory and metabolic properties, as recently reviewed [3].

One important adipocyte-derived signalling molecule operating within this context, leptin [4–7], has been little investigated in epidemiological studies of incident type 2 diabetes. In Japanese Americans, greater baseline leptin levels were associated with an increased risk of developing diabetes in men but not in women [8]. In Mauritius, risk of diabetes increased with increasing levels of leptin, with a tendency to plateau at high levels [9]. More recently, elevated leptin levels have been found to predict worsening of glucose intolerance in middle-aged white adults [10].

These risk associations could result from direct noxious effects of leptin on glucose metabolism, though such effects have not been consistently found across different experimental models [6, 11, 12]. In fact, many animal studies suggest a protective role for leptin in the development of diabetes [13–17]. Thus, as leptin levels are elevated in obese individuals, suggesting resistance to its action in the control of food intake and energy expenditure [18], it is possible that the strong associations observed between leptin and diabetes in the above epidemiological studies result from resistance to central and/or peripheral leptin actions in obese subjects progressing to diabetes.

Leptin's protective actions may not have been noted in epidemiological studies of type 2 diabetes because high leptin levels are usually accompanied by leptin resistance. One way of overcoming this problem is to adjust leptin–diabetes associations not only for obesity, which reflects directly the pool of leptin production, but also for central fat distribution, hyperinsulinemia/insulin resistance and subclinical inflammation, all known to be linked to a metabolic dysregulation purportedly related to leptin resistance [19–21].

Thus, the purpose of this study was to examine the relationship of leptin levels with incident diabetes, both when minimally adjusted and when taking into account the effects of obesity, WHR, fasting insulin, inflammation markers, and other components of the metabolic syndrome purportedly related to leptin resistance. Analyses were based on a large case–cohort study designed to investigate chronic inflammation and incident diabetes in white and

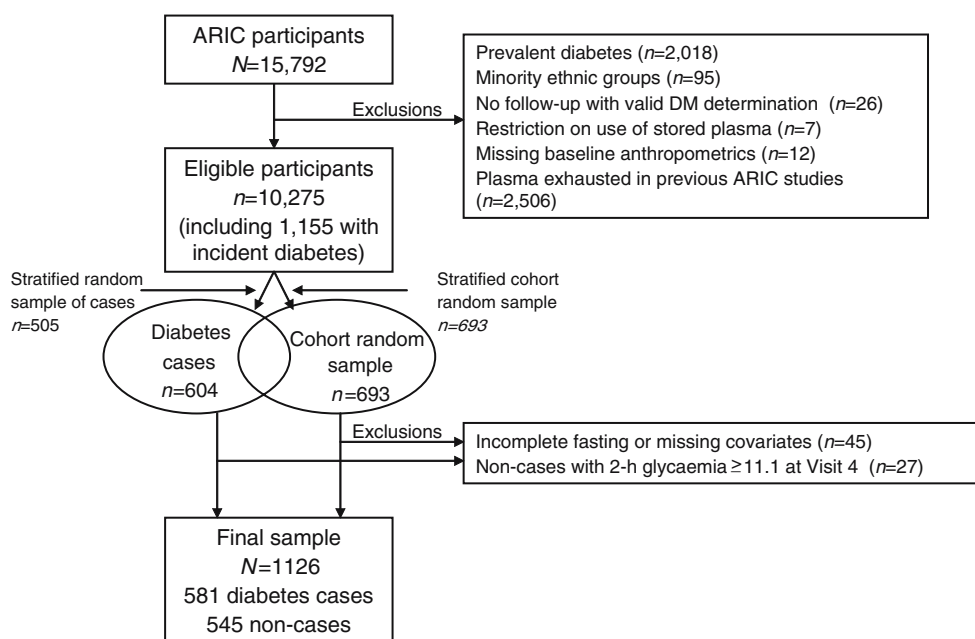
African-American adults participating in the Atherosclerosis Risk in Communities (ARIC) study.

Subjects and methods

Subjects

Between 1987 and 1989 the ARIC study recruited a population-based cohort of 15,792 men and women aged 45–64 years from four US communities [22]. All subjects were invited to return to three clinic visits, at approximately 3-year intervals, at which incident diabetes was ascertained. Human subjects' research review committees at the involved institutions approved the study, and all participants gave written consent.

We chose the same case-cohort design previously used to investigate the role of inflammation in the development of diabetes [2], which permits evaluation of the association between leptin and incident diabetes within the context of a sample well characterised with respect to inflammation markers. As an OGTT was not performed at baseline, we defined both prevalent and incident diabetes on the basis of the following: a reported physician diagnosis, use of glucose-lowering medications, a fasting (≥ 8 h) glucose of ≥ 7.0 mmol/l, or a non-fasting glucose of ≥ 11.1 mmol/l. The date of diabetes incidence was estimated by linear interpolation using glucose values at the ascertaining visit and the previous one, as previously described [2]. Prior to sampling, we excluded 2,018 participants with prevalent diabetes, 95 members of minority ethnic groups with small numbers, 853 individuals who did not return to any follow-up visit, 26 with no valid diabetes determination at follow-ups, seven with restrictions on stored plasma use, 12 with missing baseline anthropometric measurements, and 2,506 participants in previous ARIC case–control and case–cohort studies involving cardiovascular disease for whom stored plasma was either previously exhausted or held in reserve (Fig. 1). This resulted in a final sample of 10,275 individuals (75% of those in the full cohort without diabetes at baseline), of whom 1,155 (11.2%) were ascertained as developing diabetes during follow-up. From these 10,275 eligible members of this baseline cohort, we selected and measured several analytes on ethnicity-stratified (50% white, 50% African-American) random samples of both cases of incident diabetes and of eligible members of the full cohort, a total of 1,198 individuals. A few of the incident cases of diabetes overlapped with the cohort random sample, and a few were selected only via the cohort sample. Of those sampled, we excluded 45 for incomplete fasting (< 8 h) or for not having values for all covariates, and 27 identified as non-cases but having 2-h glycaemia ≥ 11.1 mmol/l during the OGTT performed at the last follow-up visit, leaving a total of

Fig. 1 Selection of case-cohort participants

1,126 subjects, including 581 diabetes cases and 545 non-cases, for analysis. The cohort random sample contained 4.2% of eligible white participants and 13.6% of eligible African-Americans. Among those with incident diabetes, 40% of eligible white and 70% of eligible African-American cohort participants were included. For the purpose of maintaining the same number of participants across models of the association between leptin and incident diabetes, we excluded an additional 26 subjects from the proportional hazards analyses owing to missing information on covariates.

Laboratory measurements

We measured glucose at baseline and at follow-up visits by a hexokinase method, fasting serum insulin by non-specific RIA. We measured waist girth at the umbilical level and hip circumference at the maximum hip girth in order to obtain the WHR. We defined parental history of diabetes as a report of diabetes in either parent, and BMI as weight divided by height squared. The definitions and methods for other baseline measurements (height, weight, smoking status, systolic blood pressure, hypertension, physical activity, triglycerides, HDL cholesterol, insulin, white blood cell count, fibrinogen, IL-6, sialic acid, C-reactive protein [CRP], orosomucoid and adiponectin) have been previously reported [1, 2].

Leptin was measured in duplicate by direct sandwich ELISA (Linco Research, St Charles, MI, USA) at a central laboratory on plasma specimens frozen at baseline. These samples, stored for approximately 15 years at -70°C , were thawed and maintained at 4°C until measured, no longer than 24 h later. A reliability coefficient of 0.94 for leptin, expressing

between-person: total variance, was obtained analysing replicate pairs of samples drawn at baseline from a subset of 35 subjects.

As in a previous report [2], we created a score, ranging from 0–6, to indicate low-grade systemic inflammation, attributing 1 point for a value greater than the median of the cohort sample for each of the six inflammation markers: IL-6, CRP, orosomucoid, sialic acid, white cell count and fibrinogen.

Statistical analysis

Statistical analysis was based on our case-cohort sampling design, weights being defined as the inverse of the ethnicity-specific sampling fractions, thus permitting statistical estimation and inference relevant to the entire cohort. In the cohort random sample, we performed weighted analysis of covariance to compute adjusted means and proportions of socio-demographic variables and risk factors, and weighted Spearman's correlations to describe unadjusted associations between study variables.

The adjusted relative risk of developing diabetes at different quantiles of leptin was estimated in proportional hazards models fitted using SUDAAN (Research Triangle Institute, Research Triangle Park, NC, USA) to account for the case-cohort design, with stratified sampling both from the whole cohort and from the incident diabetes cases [23]. The proportional hazards assumption was examined through plots of martingale and Schoenfeld residuals [24]. To adjust for obesity, WHR, fasting insulin, inflammation markers and other related metabolic variables, we included continuous variables with their quadratic terms, retaining the quadratic terms in the models when statistically significant at the level

of 0.20. Collinearity across independent variables was investigated with linear regression models, suspicion being raised if a particular variable presented a variance inflation factor ≥ 5 . To test heterogeneity across categories of covariates in the associations between leptin and incident diabetes, we characterised leptin tertiles and covariate categories using indicator variables and employed a chunk test of interaction terms. Analyses were performed using SAS (SAS Institute, Cary, NC, USA) and SUDAAN. A p value of less than 0.05 was considered statistically significant.

Results

Among incident diabetes cases there were 153 (26%) white men, 151 (26%) white women, 76 (13%) African-American men and 201 (35%) African-American women. Among non-cases, the corresponding numbers were 117 (21%), 189 (35%), 75 (14%), and 164 (30%), respectively. Additional characteristics of cases and non-cases have previously been reported [2, 25].

The distribution of leptin values was considerably right-skewed, and normality was not achieved despite various transformations. As shown in Table 1, median and interquartile range values varied according to subgroups, particularly across sex and BMI categories.

Also shown in Table 1 are means (95% CI) adjusted for age, sex, race and study centre. Similar to the non-parametric comparisons, leptin means were lower in the non-obese,

male, white, current smoking and low inflammation subgroups. These differences were generally attenuated after further adjustment for BMI, WHR, fasting insulin, the inflammation score and other metabolic syndrome elements, those for race and smoking groups no longer being statistically significant. Even with this full adjustment, women continued to have four-fold higher leptin levels than men.

Spearman correlations in the cohort random sample for leptin levels with inflammatory and metabolic factors are shown in Table 2. Although leptin correlations were generally of modest magnitude, they were markedly confounded by sex, as can be seen examining these correlations separately in men and women. For example, the correlation between WHR and leptin was -0.05 overall, but 0.40 and 0.44 in men and women, respectively. Furthermore, among women, correlations were frequently stronger, especially those with inflammatory markers. For example, the correlation with CRP was 0.48 in women vs 0.20 in men; that with fibrinogen, 0.35 in women and 0.04 in men.

The median (interquartile range) follow-up was 3.0 (1.7–5.9) years for those who developed diabetes and 8.9 (8.7–9.0) years for those who did not. Among the incident cases, 499 (86%) were ascertained by a fasting glucose of ≥ 7.0 mmol/l, and five (1%) by a non-fasting glucose of ≥ 11.1 mmol/l. Independently of their glucose values, 125 (22%) were additionally ascertained by a reported physician diagnosis or hypoglycaemic medication use. Those who developed diabetes had higher levels of leptin after adjustment for age, sex, race and study site (24.1 vs 16.8 ng/ml; $p < 0.001$). After

Table 1 Leptin levels at baseline in the cohort random sample of 693 individuals

Variable	Strata	Median (ng/ml)	Interquartile range	Minimal adjustment			Full adjustment		
				Mean (ng/ml)	95% CI	p value	Mean (ng/ml)	95% CI	p value
Overall		10.6	4.9–21.7	16.9	15.4–18.4		17.1	16.0–18.2	
BMI	Normal	8.4	3.6–13.5	8.0	6.8–9.2		11.0	9.7–12.2	
	Overweight	8.2	4.5–18.2	15.9	14.4–17.4		15.5	14.1–16.9	
	Obese	35.2	17.0–51.7	36.4	31.5–41.3	<0.001	32.1	27.4–36.8	<0.001
Sex	Female	17.0	10.4–32.9	23.4	21.1–25.7		23.6	21.8–25.3	
	Male	4.4	2.4–7.0	5.9	4.9–6.8	<0.001	6.0	4.5–7.5	<0.001
Race	Black	20.3	6.9–37.1	24.4	21.8–27.0		18.6	16.3–20.8	
	White	9.8	4.7–17.4	15.0	13.2–16.8	<0.001	16.7	15.2–18.2	0.26
Smoking	Current	8.3	3.9–16.2	13.3	11.1–15.6		15.2	13.2–17.2	
	Ex	7.4	4.4–17.7	19.1	15.6–22.6		17.7	15.1–20.3	
	Never	13.8	7.4–29.1	17.3	15.2–19.3	0.003	17.6	16.2–19.0	0.14
Inflammation score	Low	9.2	4.5–16.6	13.0	11.8–14.3		15.9	14.8–17.0	
	High	16.0	6.2–36.8	23.9	20.6–27.2	<0.001	19.1	17.1–21.2	0.005

The ARIC study, 1987–1999

Results are presented as medians (interquartile range) or adjusted means (95% CI)

Minimal adjustment: age, sex, centre, race

Full adjustment: age, sex, centre, race, BMI, BMI², WHR, insulin, insulin², inflammation score, hypertension, triglycerides, triglycerides², glucose

Note that the variable defining the strata in question was not included as a covariable in analyses estimating its strata-specific leptin parameters

Table 2 Spearman correlations for leptin levels with inflammation and metabolic variables at baseline in the cohort random sample of 693 individuals

Variable	Overall		Women		Men	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
CRP	0.36	<0.001	0.48	<0.001	0.20	0.003
Orosomuroid	0.27	<0.001	0.43	<0.001	0.14	0.04
Fibrinogen	0.26	<0.001	0.35	<0.001	0.04	0.56
IL-6	0.19	<0.001	0.31	<0.001	0.12	0.07
Total sialic acid	0.15	<0.001	0.25	<0.001	0.04	0.52
Fasting insulin	0.46	<0.001	0.65	<0.001	0.52	<0.001
BMI	0.49	<0.001	0.75	<0.001	0.67	<0.001
Waist circumference	0.34	<0.001	0.71	<0.001	0.65	<0.001
WHR	−0.05	0.22	0.44	<0.001	0.40	<0.001
Systolic blood pressure	0.10	0.02	0.33	<0.001	0.14	0.03
Fasting glucose	0.09	0.02	0.27	<0.001	0.20	0.003
Triglycerides	0.06	0.11	0.26	<0.001	0.19	0.005
HDL cholesterol	0.13	<0.001	−0.23	<0.001	−0.15	0.03

The ARIC study, 1987–1999

additional adjustment for obesity indices and other elements of the metabolic syndrome, identical to that performed for analyses shown in Table 1, the direction of the association changed, with those who developed diabetes presenting lower leptin levels (14.1 vs 18.3 ng/ml; *p*<0.001).

Given the marked sex differences observed for leptin levels and for leptin correlations with metabolic syndrome variables, we examined associations of leptin with incident diabetes using sex-specific cut-off values for leptin quantiles. Table 3 summarises the hazard ratios and their 95% CIs for these associations in models with increasing numbers of covariates. The first leptin quartile always serves as the reference category.

A strong, monotonic, positive association was noted between leptin levels and incident diabetes in the model adjusted only for age, sex, race and study centre, the hazard ratio for diabetes being 3.9 (95% CI 2.6–5.6) comparing extreme leptin quartiles. Addition of BMI to the model (Model 2) abolished this association, suggesting that confounding by obesity explains the apparent risk seen for leptin in the initial model. Further addition of WHR, fasting insulin and the inflammation score (Models 3–5) successively decreased the magnitude of the hazard ratios, higher leptin levels being associated with decreased risk (for the model including these three variables, comparing extreme quartiles, hazard ratio [HR]=0.39, CI 0.23–0.65). Addition

Table 3 HRs and 95% CIs of incident diabetes for each sex-specific quartile of leptin

Model	2nd Quartile		3rd Quartile		4th Quartile		Linear trend <i>p</i> value
	HR	95% CI	HR	95% CI	HR	95% CI	
Model 1	1.60	1.05–2.43	2.25	1.52–3.35	3.86	2.63–5.65	<0.001
Model 2	1.09	0.69–1.72	1.10	0.70–1.73	1.01	0.60–1.69	0.92
Model 3	1.07	0.68–1.70	0.93	0.59–1.48	0.79	0.47–1.33	0.23
Model 4	0.94	0.59–1.52	0.62	0.38–1.02	0.41	0.23–0.71	<0.001
Model 5	0.94	0.59–1.48	0.59	0.36–0.94	0.39	0.23–0.65	<0.001
Model 6	0.87	0.55–1.38	0.57	0.35–0.92	0.37	0.22–0.63	<0.001
Model 7	0.89	0.56–1.41	0.59	0.37–0.96	0.40	0.23–0.67	<0.001
Model 8	1.06	0.63–1.77	0.71	0.40–1.25	0.59	0.32–1.08	0.03

Case-cohort sample of 1,100 individuals, the ARIC study

Model 1: Adjusted for age, sex and race/centre indicators

Model 2: Model 1 plus BMI, BMI²

Model 3: Model 2 plus WHR

Model 4: Model 3 plus ln-insulin and ln-insulin²

Model 5: Model 4 plus inflammation score

Model 6: Model 5 plus hypertension and ln-triglycerides, ln-triglycerides²

Model 7: Model 6 plus adiponectin

Model 8: Model 7 plus glucose

of two elements of the metabolic syndrome—hypertension and triglycerides (Model 6)—produced minimal change. The inclusion of adiponectin (Model 7), a cytokine with antidiabetic properties, slightly increased hazard ratios (comparing extreme quartiles, HR=0.40, CI 0.23–0.67). Adding fasting glucose (Model 8) further increased hazard ratios (comparing extreme quartiles, HR=0.59; CI 0.32–1.08; test for linear trend across quartiles $p=0.03$).

Repeat analyses, in which all continuous covariables were modelled as quartiles, revealed a similar pattern of associations for all models (data not shown).

To examine the consistency of these associations across subgroups, leptin was categorised in tertiles, so as to provide an adequate number of incident cases across leptin tertiles in each subgroup. Table 4 shows that men and women presented the same pattern of associations between leptin and incident diabetes as seen overall, although leptin presented somewhat greater risk in minimally adjusted models for men and somewhat greater protection in more adjusted models for women. Although the differences were not statistically significant (interaction $p=0.41$ in the final model), the protective association seen in the final model appeared stronger in women (third vs first tertile leptin, HR=0.34, 95% CI 0.18–0.65) than in men (HR=0.67, 95% CI 0.30–1.53). Figures 2 and 3 show that the pattern of the associations described for the whole sample was generally seen across various other subgroups.

In the minimally adjusted models, a notable exception was that higher levels of leptin were protective in obese participants (interaction $p=0.01$). To investigate whether the

greater risk associated with higher leptin levels among the non-obese individuals could be explained by residual confounding by BMI, we additionally adjusted for BMI within this stratum. With this adjustment, increased risk was no longer seen for the non-obese (third vs first tertile leptin, HR=0.94; 95% CI 0.56–1.57), suggesting that the heterogeneity seen across BMI strata in minimal models was largely due to residual confounding by BMI.

In fully adjusted models, additional exceptions to the general pattern were that a protective association was present only in those with higher inflammation scores (interaction $p=0.03$) and was notably smaller in those with impaired fasting glucose (interaction $p=0.04$).

Further analyses considering cases ascertained in each of the three follow-up visits separately showed similar protective associations, supporting the proportional hazards assumption. Additionally, multiple plots of martingale and Schoenfeld residuals were consistent with this assumption. Collinearity among covariates was examined in all models and judged not to be present to an important degree, as indicated by the fact that the variance inflation factors were never greater than 5 (data not shown).

Discussion

Our results, which indicate that high levels of leptin are associated with an increased risk of developing diabetes in minimally adjusted models, are consistent with previous epidemiological findings [8–10]. However, that further

Table 4 HRs and 95% CIs of incident diabetes for each sex-specific tertile of leptin, by sex

Model	Women					Men				
	2nd Tertile		3rd Tertile		Linear trend <i>p</i> value	2nd Tertile		3rd Tertile		Linear trend <i>p</i> value
	HR	95 % CI	HR	95 % CI		HR	95% CI	HR	95% CI	
Model 1	1.33	0.86–2.07	2.53	1.65–3.87	<0.001	1.59	0.93–2.73	3.07	1.84–5.12	<0.001
Model 2	0.77	0.47–1.25	0.60	0.32–1.12	0.12	0.93	0.50–1.73	0.91	0.45–1.86	0.83
Model 3	0.72	0.45–1.18	0.50	0.28–0.93	0.03	0.85	0.45–1.60	0.74	0.35–1.54	0.43
Model 4	0.54	0.34–0.88	0.28	0.15–0.50	<0.001	0.84	0.43–1.64	0.50	0.23–1.10	0.06
Model 5	0.53	0.33–0.85	0.26	0.15–0.45	<0.001	0.86	0.44–1.69	0.46	0.20–1.03	0.04
Model 6	0.50	0.32–0.80	0.25	0.15–0.44	<0.001	0.79	0.41–1.53	0.44	0.20–0.95	0.02
Model 7	0.51	0.32–0.82	0.27	0.15–0.48	<0.001	0.75	0.39–1.44	0.47	0.22–1.01	0.04
Model 8	0.61	0.36–1.05	0.34	0.18–0.65	<0.001	0.82	0.43–1.59	0.67	0.30–1.53	0.35

Case-cohort sample of 1,100 individuals, the ARIC study

Model 1: Adjusted for age, sex and race/centre indicators

Model 2: Model 1 plus BMI, BMI²

Model 3: Model 2 plus WHR

Model 4: Model 3 plus ln-insulin and ln-insulin²

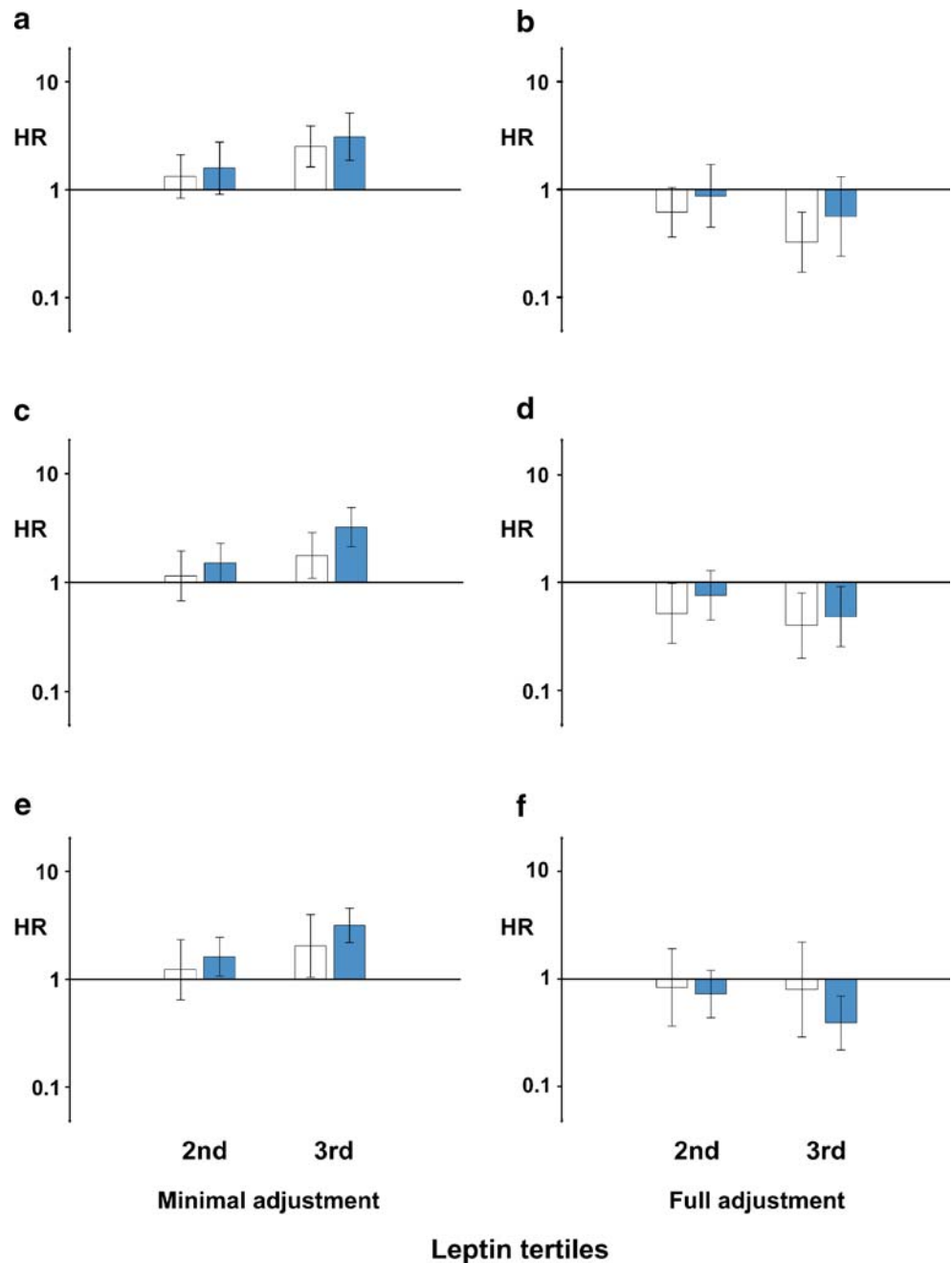
Model 5: Model 4 plus inflammation score

Model 6: Model 5 plus hypertension and ln-triglycerides, ln-triglycerides²

Model 7: Model 6 plus adiponectin

Model 8: Model 7 plus glucose

Fig. 2 Heterogeneity in the association of leptin with incident diabetes by sex (**a, b**; open columns: women, filled columns: men), race (**c, d**; open columns: African-Americans, filled columns: whites) and smoking (**e, f**; open columns: smokers, filled columns: non-smokers). Left panel, minimally adjusted model (adjusted for age, centre, ethnicity and sex); right panel, fully adjusted model (adjusted additionally for BMI, BMI², WHR, inflammation score, hypertension, triglycerides, triglycerides², fasting glucose, insulin, insulin²)

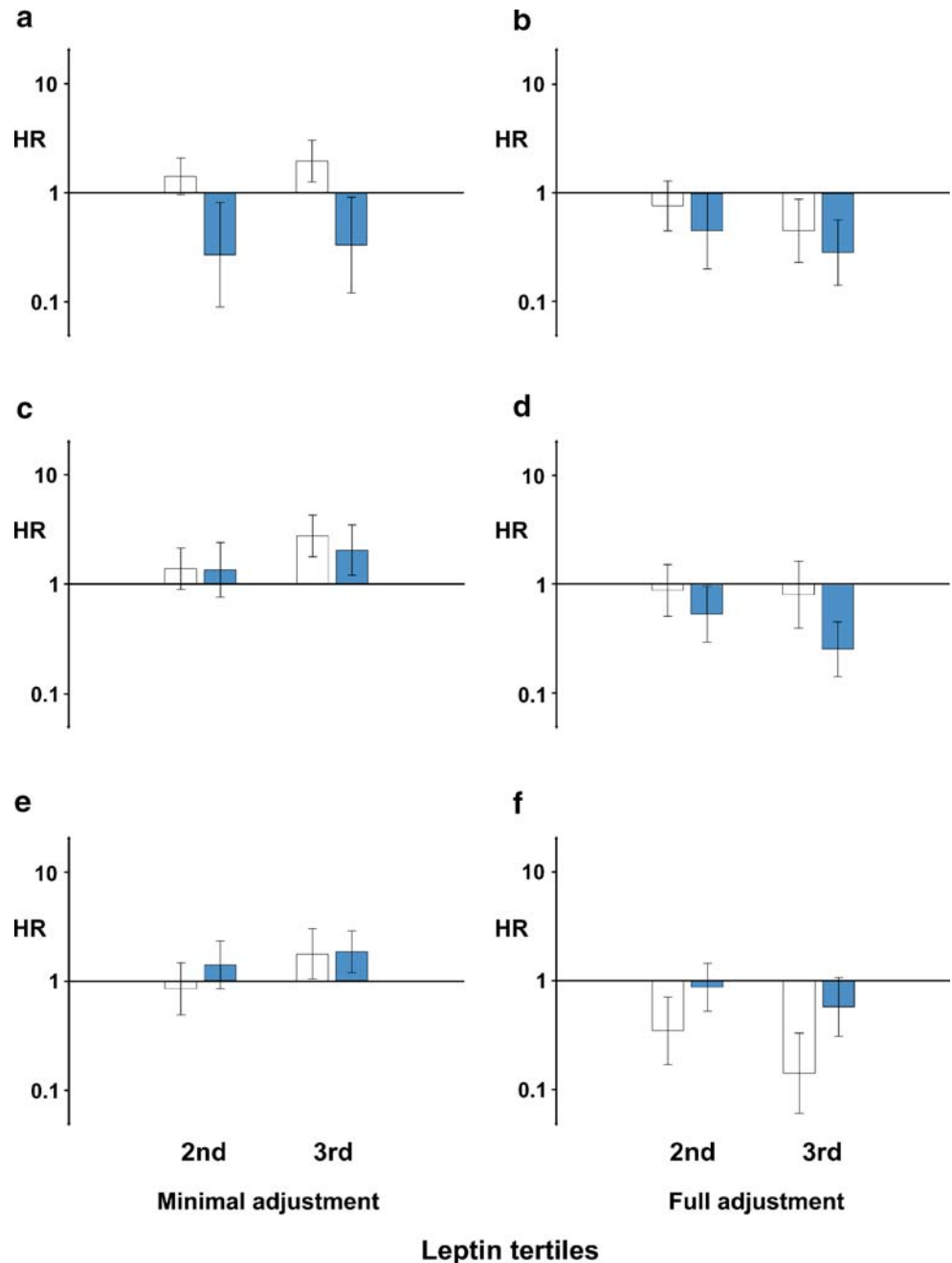


statistical adjustment for BMI, WHR, fasting insulin, inflammation score, hypertension and triglycerides revealed protective associations—those with highest adjusted leptin levels having only 40% the incidence of diabetes of those with lowest levels, independently of adiponectin—is novel, and, if confirmed, potentially relevant.

Interestingly, in the Japanese American study (with a relatively small number of incident cases, $n=40$), while men with higher leptin levels had an increased risk of diabetes (odds ratio [OR]=1.8, 95% CI 1.02–3.17 for a 1-SD change in leptin) in analyses adjusting for total fat, the point estimate found for women, though not statistically signif-

icant, was similar in magnitude to that found in the ARIC women (OR=0.66, 95% CI 0.30–1.48) [8]. In the Mauritian study, after adjustment for obesity indices and metabolic syndrome elements, a complex association was found, with increasing risk at the lower end of the leptin spectrum and a plateau of risk at the higher end of the spectrum [9]. Although differences in study populations may in part explain the heterogeneous findings, for example, ARIC participants were more frequently obese and of African-American ancestry, other factors may have been involved. First, our analysis of men and women together used sex-specific cut-off points for leptin, given the four-fold sex

Fig. 3 Heterogeneity in the association of leptin with incident diabetes by adiposity (a, b; *open columns: BMI ≥ 30 kg/m², filled columns BMI < 30 kg/m²*), inflammation (c, d; *open columns: low score, filled columns: high score*) and baseline impaired fasting glucose (e, f; *open columns: absent, filled columns: present*). *Left panel*, minimally adjusted model (adjusted for age, centre, ethnicity and sex); *Right panel*, fully adjusted model (adjusted additionally for BMI, BMI², WHR, inflammation score, hypertension, triglycerides, triglycerides², fasting glucose, insulin, insulin²)



difference in mean leptin levels. Second, we adjusted more fully for factors purportedly related to leptin resistance, including central fat deposition [19], hyperinsulinaemia/insulin resistance [7, 20, 26] and inflammation markers [21, 27]. Third, as most of our cases were ascertained by fasting glucose, it is possible that their pathogenesis is somewhat different from cases in the other three studies, mostly ascertained by an OGTT. In this regard, dysregulation of hepatic gluconeogenesis in leptin-resistant states may be of particular importance [28, 29].

Leptin levels were markedly increased in obese subjects, and the increased risk of diabetes in those with

high leptin levels seen in minimally adjusted models was neutralised after adjusting for obesity indices. Thus, confounding by obesity—a measure of body fat, the source of leptin—appears to explain most of the crude hyperleptinaemia–diabetes association. As additional adjustments unveiled a large protective association, this suggests that the variables included in the models were also confounders of the initial increased risk association. It is possible that such adjustments controlled more fully for factors related to obesity and leptin resistance. The inclusion of baseline glucose, while presented here also as confounding, may well represent over-adjustment, as

adjusted leptin action at baseline would be expected to lower concurrently measured glucose, and this glucose lowering could mediate part of the protective leptin effect seen in the final model.

Heterogeneity of leptin associations merits discussion. While the pattern of associations was consistent across subgroup categories of sex, ethnicity, smoking and obesity, the decrease in diabetes incidence was minimal in those with impaired fasting glucose (interaction $p=0.04$) and was present only in those with higher inflammation scores (interaction $p=0.03$). We find no clear explanation in the literature for this heterogeneity. Nonetheless, these findings suggest, first, that leptin's protective actions may be less effective or less important once impaired fasting glucose is established; and second, that leptin, perhaps because one of its functions is to facilitate the inflammatory response [4], may best exert its metabolic effects in a proinflammatory milieu. Although adjusted higher leptin levels predicted less diabetes in both men and women, the association appeared to be stronger, though not statistically significantly so, among women, perhaps reflecting the important role of leptin in female physiology [30]. A physiological basis for our epidemiological findings exists, as leptin, at a molecular level, elicits several actions potentially protective against diabetes. It decreases energy intake, increases energy expenditure, and partitions caloric surplus. With variable effects on glucose homeostasis, overall, leptin appears to improve glucose utilisation [6]. Like adiponectin, leptin activates AMP-activated protein kinase (AMPK), which in turn stimulates glucose uptake through pathways that are independent of insulin signalling [16, 31]. By promoting fatty acid oxidation, leptin reduces ectopic fat accumulation in non-adipose tissues, thereby increasing insulin sensitivity and decreasing insulin secretion [13, 14, 32, 33]. Leptin stimulates expression of the gene for peroxisome proliferator-activator receptor- γ co-activator-1 α (PGC-1 α), and upregulation of uncoupling proteins 1 and 2 and mitochondrial biogenesis might offer additional mechanisms for its role in the regulation of caloric surplus [34].

In the liver, leptin increases fatty acid oxidation, probably by repressing stearoyl-CoA desaturase-1 (SCD-1) [35, 36]. In vivo evidence suggests a role for leptin in the acute regulation of hepatic triglyceride metabolism and triglyceride homeostasis [20]. Resistance to leptin's hepatic actions leads not only to hepatic steatosis [37], but also to increased hepatic gluconeogenesis [28].

Leptin effects on beta cell function are less clear [12]. although leptin has been shown to decrease insulin secretion [32, 33], this effect was seen in parallel with others that led to a marked improvement in metabolic parameters in one animal model of diabetes [17]. Its ability to partition caloric surplus to avoid deposition of fat in non-adipose tissues could minimise beta cell lipotoxicity and apoptosis [38].

On balance, we believe that these basic science findings, coupled with our epidemiological data, make it unlikely that leptin itself directly causes diabetes. The more likely alternative is that being overweight, or factors intimately related to the overweight state, such as chronic overeating (and or specific patterns of eating) [39–41] and a sedentary lifestyle [42, 43], lead to leptin resistance with resulting hyperleptinaemia.

Leptin resistance/hyperleptinaemia may result from defects in the transport of leptin through the blood–brain barrier or from increased expression of genes encoding inhibitors of leptin signalling pathways, such as suppressor of cytokine signalling 3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B) [18]. Additionally, leptin action/resistance may vary according to different fractions of free and bound leptin and different levels of plasma soluble leptin receptors [41, 44].

Our findings, coupled with the literature, support the hypothesis that leptin resistance, with resultant dysregulation of leptin action, not leptin levels per se, is related to processes, such as altered hepatic metabolism, ectopic fat deposition, lipotoxicity, insulin resistance, impaired insulin secretion and decreased whole-body glucose utilisation, that produce diabetes and other related diseases in humans [45]. Additionally, our data showing higher adjusted levels of leptin in the obese and those with higher inflammation scores (Table 1) support bench research demonstrating that adipocytes and proinflammatory mediators have important roles in stimulating hyperleptinaemia and/or leptin resistance. In fact, the leptin resistance so produced may be an important pathway by which obesity causes diabetes. Additionally, the much higher adjusted levels found in women, as previously reported, attests its special importance in female physiology [30].

Potential limitations to our study merit comment. First, selection bias, either due to participants not returning for follow-up or not having a sample available for measurement, could conceivably have influenced our results. However, we have little a priori reason to believe that the association between leptin and incident diabetes should be stronger or weaker among those without an available sample or who were lost to follow-up. Furthermore, exclusion of some participants with cardiovascular disease has not prevented the demonstration of associations of incident diabetes with factors such as inflammation markers and adiponectin in this sample. Second, epidemiological studies such as ours are restricted in their ability to assess independent effects of inter-related variables, such as leptin, obesity, inflammation, glucose and insulin resistance. Finally, as we ascertained diabetes without use of an OGTT, some misclassification in diabetes ascertainment was likely to have occurred. However, because our follow-up was relatively long, and as, if anything, leptin's protective association was stronger among those with normal fasting glucose than those in whom it was

impaired, we believe this misclassification is unlikely to have produced associations as a result of reverse causality.

In conclusion, high leptin levels, probably reflecting leptin resistance, predict an increased risk of type 2 diabetes. Yet, once the effects of overall and central obesity, hyperinsulinaemia, inflammation and other metabolic syndrome components are taken into account, high leptin levels predict a decreased risk of diabetes, independently of adiponectin. If this result is confirmed, it validates, at a human population level, bench research suggesting that leptin's basic physiological functions protect against diabetes.

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