Original Article

Elevated Levels of Interleukin-18 Predict the Development of Type 2 Diabetes

Results From the MONICA/KORA Augsburg Study, 1984–2002

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We investigated prospectively the association between serum levels of interleukin (IL)-18 and the risk of type 2 diabetes in a case-cohort study conducted in middle-aged men and women who represented 7,936 participants of the three MONItoring of trends and determinants in CArdiovascular disease (MONICA)/Cooperative Research in the Region of Augsburg (KORA) surveys. Levels of IL-18 were measured in stored samples of 527 case subjects with incident type 2 diabetes and 1,698 noncase subjects. Elevated levels of IL-18 were associated with a significantly increased risk of type 2 diabetes after adjustment for age, sex, survey, BMI, systolic blood pressure, ratio of total cholesterol to HDL cholesterol, physical activity, alcohol intake, smoking status, and parental history of diabetes. Hazard ratios and 95% confidence intervals comparing quartile extremes were 1.73 (1.25–2.40). Further adjustment for C-reactive protein and IL-6 had no impact on the observed associations. However, the risk of developing type 2 diabetes was highest among subjects with elevated levels of both IL-18 and CRP or IL-18 and IL-6, respectively. In conclusion, elevated levels of IL-18 are associated with a considerably increased risk of type 2 diabetes. This association is independent of a generalized proinflammatory state, but subjects with elevated levels of several inflammatory markers seem to be particularly prone to develop type 2 diabetes. *Diabetes* 54:2932–2938, 2005

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Received for publication 18 March 2005 and accepted in revised form 23 June 2005.

CRP, C-reactive protein; IL, interleukin; MI, myocardial infarction; WHR, waist-to-hip ratio.

It has been postulated that type 2 diabetes represents the manifestation of a chronic subclinical inflammatory state (1). This hypothesis has recently been supported by several prospective studies that have shown that subjects who developed diabetes during the follow-up period had elevated levels of markers of inflammation such as C-reactive protein (CRP), serum amyloid A, or interleukin (IL)-6 at the baseline examination compared with subjects who did not develop the disease (2–13).

IL-18, which was originally identified as an interferon-γ–inducing factor (14), is another potent proinflammatory cytokine that plays a central role in the inflammatory cascade (15). It is a member of the proinflammatory IL-1 family and can induce either T helper 1 or T helper 2 immune response depending on the immunologic context (16). IL-18 seems to be involved in atherosclerotic plaque destabilization (17), and elevated levels have been shown to predict cardiovascular death in patients with coronary heart disease (18) and acute coronary events in healthy middle-aged men (19). Furthermore, IL-18 could be involved in the pathogenesis of type 2 diabetes. Elevated levels of IL-18 have been found in subjects with type 2 diabetes (20–22), and levels of IL-18 were associated with fasting plasma glucose and HbA_{1c} (A1C) (20,22). However, to our knowledge, there are no published prospective data evaluating the association between IL-18 and the development of type 2 diabetes. Therefore, the purpose of this study is to examine the role of IL-18 in the prediction of type 2 diabetes in middle-aged men and women, independent of known risk factors for diabetes. Of particular importance is to evaluate whether IL-18 adds to the prediction of type 2 diabetes independently of other markers of inflammation that have previously been linked to the disease.

RESEARCH DESIGN AND METHODS

We designed a prospective case-cohort study (23) within the population-based MONItoring of trends and determinants in CArdiovascular disease (MONICA) /Cooperative Research in the Region of Augsburg (KORA) Augsburg studies conducted between 1984 and 2002. The MONICA Augsburg project was part of the multinational World Health Organization MONICA project (24). Three independent cross-sectional population-based surveys covering the city area of Augsburg, Germany, and two adjacent counties were conducted in 1984/85 (S1), 1989/90 (S2), and 1994/95 (S3) to estimate the prevalence and distribu-

tion of cardiovascular risk factors among men and women aged 25–64 (S1) or 25–74 years (S2, S3). The study was approved by the local authorities, and all participants provided written informed consent. The total number of participants was 13,427 (6,725 men and 6,702 women). All subjects were prospectively followed within the frame of the KORA. The present study was restricted to subjects aged 35–74 years at baseline, since the incidence of type 2 diabetes is low in younger subjects. Altogether, 10,718 persons (5,382 men and 5,336 women) of this age range participated in at least one of the three baseline surveys. After exclusion of 1,187 subjects with missing blood samples, 509 participants with self-reported prevalent diabetes, 14 subjects with incident diabetes other than type 2 diabetes (e.g., type 1 or secondary diabetes), 30 subjects with self-reported incident diabetes where the diagnosis could not be validated, 988 subjects without follow-up information, and 54 subjects with a follow-up time of <1 year, the source population for the present study comprised 7,936 subjects (3,894 men and 4,042 women). The sample sizes of the source population by survey were 1,156 (S1), 1,463 (S2), and 1,275 (S3) for men and 1,339 (S1), 1,438 (S2), and 1,265 (S3) for women.

For the case-cohort study, a stratified random sample of the source population, called the subcohort here and containing 1,885 subjects (1,018 men, 867 women), was selected for each group of women and men stratifying by survey. From these, we excluded 55 men and 12 women with missing values of IL-18 or any of the covariables used in the present analysis, leading to a subcohort of 1,818 subjects (963 men, 855 women). The sample sizes for the subcohort by survey were 388 (S1), 372 (S2), and 203 (S3) for men and 345 (S1), 300 (S2), and 210 (S3) for women. These final stratum-specific sample sizes were used together with the stratum-specific sizes of the cohort of interest to compute sampling fractions, and the inverse of the sampling fractions yields the survey- and sex-specific sampling weights: 2.98, 3.93, and 6.28 for men and 3.88, 4.79, and 6.02 for women.

A total of 555 incident cases of type 2 diabetes (329 men, 226 women) were observed between participants' study start dates and 31 December 2002. Of these, 24 men and 4 women were excluded because of incomplete information on any of the inflammatory markers or other covariables, leaving 305 men and 222 women with incident type 2 diabetes for the final analyses. Since 74 male and 46 female cases were also part of the randomly drawn subcohort, the present analysis comprised a total of 2,225 participants (305 men with incident diabetes, 222 women with incident diabetes, 889 male noncases, and 809 female noncases).

To determine the incidence of diabetes, a written follow-up questionnaire was sent to all participants of the three baseline surveys in 1997–1998 and in 2002–2003. Furthermore, all subjects who participated in the first survey were invited to participate in a follow-up examination conducted in 1987–1988. At follow-up, all subjects were asked whether they had diabetes and whether the disease had been diagnosed by a physician. In addition, the year of diagnosis was assessed. Participants with self-reported incident diabetes were validated by a questionnaire mailed to the treating physician or medical chart review. The mean follow-up time for the study population was 10.8 years with a standard deviation of 5.1 and a range from 1.0 to 18.2 years.

Standardized interviews were conducted by trained medical staff (mainly nurses) to assess information concerning sociodemographic variables, smoking habits, leisure-time physical activity level, alcohol consumption, and parental history of diabetes. In addition, participants underwent standardized medical examinations including collection of a nonfasting venous blood sample. All assessment procedures have been described elsewhere in detail (25–27). Total serum cholesterol and HDL cholesterol were measured by enzymatic methods (CHOD-PAP, Boehringer Mannheim, Mannheim, Germany). HDL cholesterol was precipitated with phosphotungstic acid and magnesium ions. Serum and plasma samples stored at −80°C were used to analyze IL-18, IL-6, and CRP. Serum levels of IL-18 were measured by Luminex technology using an antibody pair and recombinant IL-18 protein from Medical and Biological Laboratories (Nagoya, Japan). The assay was based on a protocol of de Jager et al. (28). Serum levels of IL-6 were determined using a previously described sandwich ELISA (29). CRP concentrations were measured using a high-sensitivity immunoradiometric assay (IRMA) (range 0.05–10 mg/l) (S1: men aged 45–64; S3) (30) or a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer (S1: men aged 35–44 and all women; S2) (Dade Behring, Marburg, Germany). Both methods gave similar results when the same samples were analyzed (31). The intra- and interassay coefficients of variation (CV) of quality control test sera for CRP and cytokines were as follows: CRP-IRMA, 4.0 and 12.0%; CRP nephelometric assay, 2.5 and 5.1%; IL-6, <10.0 and <10.0%; and IL-18, <10.0 and <25.0%.

Means or proportions for baseline demographic and clinical characteristics were computed by linear or logistic regression using the SAS macros SURVEYREG (32) or LOGITSE (33), which estimated standard errors (SEs) appropriate to the sampling scheme. Tests of differences between subjects with and without incident diabetes were based on these procedures. In case of nonnormality, tests were carried out with log-transformed variables, and results were presented as geometric means with antilogs of SEs of the adjusted log means.

Weighted Pearson correlations were used to describe univariate associations between markers of inflammation and continuous risk factors for diabetes, and *P* values were obtained from weighted regression models using the SAS macro SURVEYREG (32).

Cox proportional hazards analysis was used to assess the association between IL-18 and incident type 2 diabetes. Due to the case-cohort design, SEs were corrected using a SAS macro with a "sampling weight" approach developed by Barlow et al. (23). The weighted quartiles of IL-18 in the subcohort were used to classify subjects in different risk groups. In multivariable analyses, we adjusted for sex, age (continuous), survey, BMI (continuous), smoking status (never smoker, former smoker, or current smoker), alcohol consumption (0, 0.1–39.9 vs. ≥ 40 g/day for men; 0, 0.1–19.9 vs. ≥ 20 g/day for women), physical activity (inactive versus active, i.e., regular physical activity in summer and in winter with a duration of at least 1 hour per week in either season), systolic blood pressure (continuous), ratio of total cholesterol to HDL cholesterol (continuous), and parental history of diabetes (negative, positive, or unknown). Moreover, to assess whether the impact of IL-18 on incident type 2 diabetes was independent of a generalized proinflammatory state, additional Cox models that also included CRP and IL-6 as covariables were calculated. Results are presented for each quartile of IL-18 (coded as dummy variables with the first quartile as the reference category) as hazard ratios (HRs) together with 95% CIs. To assess the joint effects of either IL-18 and CRP or IL-18 and IL-6 in the prediction of type 2 diabetes, subjects were divided into four groups based on weighted median cut points in the subcohort for each biomarker. *P* values are based on robust variance estimates using the Barlow macro. To test for trends, quartiles were coded with their median values. Interactions between quartiles of IL-18 (coded as dummy variables) and other risk factors were examined using likelihood ratio tests that compared the −2 log (likelihood) between the model that contained only the main effects and the model that contained both the main effects and interaction terms. For all statistical analyses, a *P* value less than 0.05 was considered to be statistically significant. All evaluations were performed with the statistical software package SAS (version 8.02 for Unix, SAS Institute, Cary, NC).

RESULTS

Selected baseline demographics, lifestyle, and clinical characteristics of subjects who developed incident type 2 diabetes during the follow-up period (cases) and those who remained free of diabetes (noncases) are shown in Table 1. As expected, cases were older, less educated, less physically active, and more obese. Furthermore, cases suffered more often from hypertension and previous myocardial infarction (MI); they had a higher ratio of total cholesterol to HDL cholesterol and were more likely to have a positive or unknown parental history of diabetes. Also, levels of inflammatory markers including IL-18, IL-6, and CRP were elevated in subjects who developed incident type 2 diabetes.

IL-18 was not associated with many of the established risk factors for diabetes or cardiovascular disease, including physical activity, alcohol consumption, parental history of diabetes, BMI, and total cholesterol (Tables 2 and 3). Significant associations were observed for smoking, actual hypertension, systolic and diastolic blood pressure, HDL cholesterol, waist-to-hip ratio (WHR), IL-6, and CRP, but the strength of these associations was low with correlation coefficients below 0.10 for all continuous variables. For IL-6 and CRP, in contrast, correlations with other risk factors were much stronger.

Elevated concentrations of IL-18 were strongly associated with an increased risk of type 2 diabetes (Table 4). Crude HRs were 1.96 (95% CI 1.49–2.58) for the fourth and 1.57 (1.18–2.09) for the third quartile compared with the first quartile of IL-18 (model 1). Adjustment for age, sex, and survey (model 2) hardly influenced the HRs. Additional adjustment for BMI, systolic blood pressure, ratio of total cholesterol to HDL cholesterol, level of leisure-time

TABLE 1
Baseline demographic, lifestyle, and clinical characteristics of the study participants by diabetes status at follow-up

Characteristic	Subjects with incident type 2 diabetes during follow-up	Subjects without incident type 2 diabetes during follow-up	*P* value*
Demographic			
n (women/men)	222/305	809/889	
Age (years)	56.1 ± 0.4	51.7 ± 0.3	<0.001
Education <12 years (%)	82.4	75.1	0.039
Lifestyle			
Smoking status (%)			0.285
Current smoker	26.9	24.1	
Former smoker	31.7	27.8	
Never smoker	41.4	48.1	
Frequency of exercise (%)			0.011
Active	30.2	40.6	
Inactive	69.8	59.4	
Alcohol consumption†			0.473
0 g/day	33.4	29.0	
>0–39.9/19.9 g/day	39.7	44.1	
≥ 40/20 g/day	26.9	26.9	
Current HRT‡	8.2	11.7	0.461
Current use of oral contraceptives§	7.7	15.3	0.420
Clinical			
BMI (kg/m^2)	30.2 ± 0.2	26.7 ± 0.1	<0.001
WHR‖	0.924 ± 0.004	0.865 ± 0.003	<0.001
History of actual hypertension	67.6	39.7	<0.001
History of MI	5.1	2.0	0.033
Total-to-HDL cholesterol ratio	5.6 ± 0.1	4.5 ± 0.04	<0.001
Parental history of diabetes			0.002
Positive	28.3	19.8	
Unknown	26.8	20.5	
Negative	45.0	59.7	
CRP (mg/l)	2.6 (1.0)	1.4 (1.0)	<0.001
IL-6 (pg/ml)	3.0 (1.0)	2.0 (1.0)	<0.001
IL-18 (pg/ml)	188.7 (1.0)	159.4 (1.0)	<0.001
Survey			0.039
S1	36.2	30.9	
S2	40.8	36.4	
S3	23.0	32.8	

Data are weighted percentages for categorical variables, weighted means ± SE for normally distributed continuous variables, and weighted geometric means (antilog of SEs of log means) for skewed continuous variables. *t test for continuous variables and χ^2 test for categorical variables. †Men, 0, >0–39.9 g/day, ≥ 40 g/day; women, 0, >0–19.9 g/day, ≥ 20 g/day. ‡Only for women aged ≥50 years (n = 606) with no current use of oral contraceptives. §Only for women aged <50 years (n = 413) with no current HRT. ‖Only measured in participants of surveys 2 and 3 (cases: n = 333; noncases: n = 1,028). Weights: cases = 1; noncases = 1/sampling fraction with sampling fraction = subcohort/full cohort without cases for each sex and survey. HRT, hormone replacement therapy.

physical activity, alcohol intake, smoking status, and parental history of diabetes (model 3) lead to slightly attenuated HRs for the fourth quartile, but the association remained highly significant. In multivariable-adjusted models, subjects with levels of IL-18 in the upper quartile had a >70% increased risk of developing type 2 diabetes (1.73 [1.25–2.40]). To determine whether the observed association was independent of a generalized proinflammatory state, models were also adjusted for concentrations of CRP and IL-6, which have previously been shown to predict the development of type 2 diabetes. Adjustment for these markers of inflammation had no impact on the observed HRs (model 4) (HR [95% CI] for the fourth quartile versus the first quartile 1.73 [1.25-2.40]). In women, adjustment for hormone replacement therapy and use of oral contraceptives had virtually no impact on the observed HRs (data not shown).

As previously described, IL-18 was more strongly correlated with WHR than with BMI. Therefore, we examined the effect of additional adjustment for WHR, which is a measure of abdominal adiposity, in a subgroup where these measurements were available (n = 1,361, 333 cases, 1,028 noncases). HRs were generally somewhat lower in this subgroup than in the whole study sample, particularly for the fourth quartile (crude HR [95% CI] 1.64 [1.17–2.31]), but the impact of additional adjustment for WHR was relatively small. HRs comparing quartile extremes were 1.33 (0.88–2.03) after adjustment for all variables in model 3 and 1.25 (0.83–1.88) after additional adjustment for WHR.

Table 5 describes the association between IL-18 and type 2 diabetes stratified by sex, BMI, smoking status, level of physical activity, and parental history of diabetes. Significant associations were seen in most subgroups except for men, subjects who were overweight (BMI 25 to <30 kg/m^2), former smokers, and subjects with an unknown parental history of diabetes. Associations were slightly stronger in women than in men, in active compared with inactive persons, and in subjects with a positive parental history of diabetes compared with those with

TABLE 2
Age-, sex-, and survey-adjusted geometric mean concentrations of serum IL-18 and antilog of SEs of the adjusted log means by selected categorical covariates in the randomly sampled subcohort

		IL-18 (pg/ml)		
	n	Geometric mean	Antilog of SE of log mean	P value*
	1,818			
Sex				0.742
Men	963	160.9	1.03	
Women	855	159.0	1.02	
Education				0.392
<12 years	1,364	161.4	1.02	
≥12 years	454	155.4	1.04	
Smoking status				0.007
Never smoker	835	152.8	1.03	
Former smoker	525	158.3	1.03	
Current smoker	458	176.8	1.04	
Frequency of exercise				0.808
Inactive	1,107	160.5	1.02	
Active	711	159.0	1.03	
Alcohol consumption				0.761
0 g/day	513	163.1	1.04	
>0–39.9 g/day	791	157.6	1.03	
≥ 40 g/day	514	160.2	1.03	
Parental history of diabetes				0.263
Negative	1,060	156.5	1.02	
Positive	376	161.5	1.04	
Unknown	382	168.1	1.04	
Actual hypertension				0.001
No	1,078	151.6	1.03	
Yes	740	172.6	1.03	
History of MI				0.690
No	1,776	159.7	1.02	
Yes	42	166.9	1.11	
Current HRT†				0.098
No	417	154.8	1.05	
Yes	50	183.5	1.10	
Current use of oral contraceptives‡				0.112
No	322	155.3	1.08	
Yes	54	180.1	1.13	
Survey				0.911
S1	733	160.6	1.03	
S2	672	161.0	1.03	
S3	413	157.9	1.04	

*Test for equality of means; †only for women aged ≥50 years (n = 467) with no current use of oral contraceptives; ‡only for women aged <50 years (n = 376) with no current HRT.

a negative parental history, but P values from all tests for interaction were nonsignificant.

The joint effect of IL-18 combined with either CRP or IL-6 is shown in Fig. 1. In both cases, the risk was highest in subjects with elevated levels of both markers of inflammation, but there was no significant interaction on a multiplicative scale (P values for interaction were 0.221 [Figure 1*A*] and 0.200 [*B*], respectively).

We conducted several sensitivity analyses. To ensure that the observed associations were not caused by undiagnosed prevalent cases of diabetes at baseline, we excluded all subjects with ≤3 years of follow-up (n = 186, 96 cases, 90 noncases). Multivariable-adjusted HRs estimated from these models were very similar to the HRs estimated for the whole group (HR [95% CI] comparing extreme quartiles was 1.81 [1.27–2.58], P for trend = 0.001, after multivariable adjustment including CRP and IL-6). To exclude any potential confounding effect by the presence of cardiovascular disease at baseline, we performed fur-

TABLE 3
Correlation of inflammatory markers with age and continuous risk factors for diabetes in the randomly sampled subcohort

	Pearson correlation coefficient*		
	Log IL-18	Log IL-6	Log CRP
Age	0.002	0.188†	0.228†
BMI	0.022	0.249†	0.402†
WHR‖	0.073‡	0.188†	0.243†
Systolic blood pressure	0.064¶	0.156†	0.224†
Diastolic blood pressure	0.056‡	0.092†	0.111†
Total cholesterol	−0.025	0.082†	0.120†
HDL cholesterol	−0.053‡	−0.162†	−0.159†
Log IL-18	—	0.075¶	0.085†
Log IL-6	0.075¶	—	0.367†
Log CRP	0.085†	0.367†	—

*Weighted to reflect the sampling strategy. P values were obtained from weighted regression models; †$P < 0.001$; ‡$P < 0.05$; ¶$P < 0.01$; ‖only for n = 1,082.

TABLE 4
HRs (95% CI) of developing diabetes comparing quartiles of IL-18 levels

	Quartile of IL-18				
	1	2	3	4	
Median (lower, upper limit) (pg/ml)	80.09 (4.9, 116.7)	145.18 (116.8, 173.5)	205.04 (173.6, 244.6)	322.08 (244.7, 8,792.4)	*P* value for trend
n (nondiabetic/diabetic)	424/95	429/108	417/146	428/178	
Model 1	1.0	1.13 (0.84–1.52)	1.57 (1.18–2.09)	1.96 (1.49–2.58)	<0.001
Model 2	1.0	1.18 (0.87–1.60)	1.64 (1.22–2.20)	1.93 (1.45–2.56)	<0.001
Model 3	1.0	1.10 (0.78–1.57)	1.62 (1.16–2.25)	1.73 (1.25–2.40)	<0.001
Model 4	1.0	1.10 (0.78–1.57)	1.61 (1.16–2.24)	1.73 (1.25–2.40)	<0.001

Model 1, crude; model 2, adjusted for age, sex, and survey; model 3, adjusted for factors in model 2 + BMI, systolic blood pressure, ratio of total cholesterol to HDL cholesterol, physical activity, alcohol intake, smoking status, and parental history of diabetes; and model 4, adjusted for factors in model 3 + CRP and IL-6.

ther analyses after exclusion of subjects with prevalent MI, prevalent stroke, or incident MI (n = 192, 75 cases, 117 noncases). Results were virtually unchanged after these exclusions (the HR [95% CI] comparing quartile extremes was 1.73 [1.20–2.48] in the fully adjusted model).

Moreover, in a subgroup of 512 subjects (118 cases, 394 noncases) with available A1C measurements, we assessed the potential confounding effect of chronic hyperglycemia. The addition of A1C to the multivariable-adjusted model had virtually no impact on the observed HRs, but multivariable-adjusted HRs were generally not significant in this relatively small subgroup (HR [95% CI] for the fourth quartile versus the first quartile in the fully adjusted model, including A1C 1.75 [0.75–4.09]).

DISCUSSION

The results of the present study demonstrate for the first time a strong positive association between serum levels of IL-18 and risk of type 2 diabetes in a population-based cohort of middle-aged men and women. The association between IL-18 and type 2 diabetes was independent of other known risk factors for type 2 diabetes and other inflammatory markers. In fully adjusted analyses, subjects with IL-18 concentrations in the highest quartile (≥244.7 pg/ml) had a >70% increased risk of developing type 2 diabetes compared with those in the lowest quartile (≤116.7 pg/ml). This relationship was slightly stronger in women than in men, in physically active than in inactive subjects, and in subjects with a positive parental history of diabetes compared with those with a negative parental history, but differences did not reach statistical significance.

The present observations extend the results from previous studies, which have shown that subjects with prevalent type 2 diabetes have elevated concentrations of IL-18 (20–22) by demonstrating that increased IL-18 concentrations are already present before onset of overt clinical

TABLE 5
Multivariable-adjusted* HRs of developing diabetes comparing quartiles of IL-18 levels stratified by sex, BMI, smoking status, physical activity, and parental history of diabetes

		Quartile of IL-18					
		1	2	3	4		
Median (lower, upper limit) (pg/ml)	*n*	80.09 (4.9, 116.7)	145.18 (116.8, 173.5)	205.04 (173.6, 244.6)	322.08 (244.7, 8,792.4)	*P* value for trend	*P* value for interaction
Sex							
Men	1,194	1.0	1.07 (0.67–1.69)	1.48 (0.97–2.24)	1.45 (0.95–2.21)	0.061	0.707
Women	1,031	1.0	1.17 (0.67–2.04)	1.54 (0.88–2.71)	2.06 (1.21–3.52)	0.003	
BMI (kg/m^2)†							
<25	659	1.0	1.02 (0.36–2.90)	0.94 (0.30–2.94)	2.33 (0.93–5.85)	0.031	0.986
25 to <30	1,006	1.0	1.22 (0.75–1.97)	1.60 (1.02–2.52)	1.49 (0.94–2.36)	0.083	
≥30	560	1.0	1.05 (0.60–1.83)	1.82 (1.07–3.09)	1.84 (1.07–3.17)	0.008	
Smoking status							
Never smoker	1,011	1.0	1.24 (0.72–2.13)	1.65 (0.95–2.88)	1.88 (1.10–3.22)	0.013	0.886
Former smoker	651	1.0	1.13 (0.61–2.08)	1.31 (0.76–2.26)	1.36 (0.77–2.39)	0.277	
Current smoker	563	1.0	0.92 (0.42–2.02)	1.86 (0.92–3.76)	1.90 (0.93–3.91)	0.028	
Frequency of exercise							
Inactive	1,391	1.0	1.00 (0.65–1.52)	1.60 (1.07–2.39)	1.53 (1.03–2.27)	0.013	0.645
Active	834	1.0	1.64 (0.88–3.08)	1.95 (1.05–3.64)	2.40 (1.30–4.43)	0.006	
Parental history of diabetes							
Negative	1,248	1.0	1.02 (0.61–1.72)	1.60 (1.00–2.57)	1.96 (1.26–3.06)	0.001	0.597
Positive	485	1.0	1.40 (0.68–2.91)	2.00 (0.97–4.13)	2.61 (1.25–5.44)	0.006	
Unknown	492	1.0	0.91 (0.46–1.82)	1.35 (0.68–2.67)	0.97 (0.50–1.90)	0.976	

Data are median ranges (lower, upper limits) or HRs (95% CIs). *Models were adjusted for age, sex, survey, BMI, systolic blood pressure, ratio of total cholesterol to HDL cholesterol, physical activity, alcohol intake, smoking status, and parental history of diabetes. †BMI was included as a continuous variable in the main effects model and in the model with the interaction term.

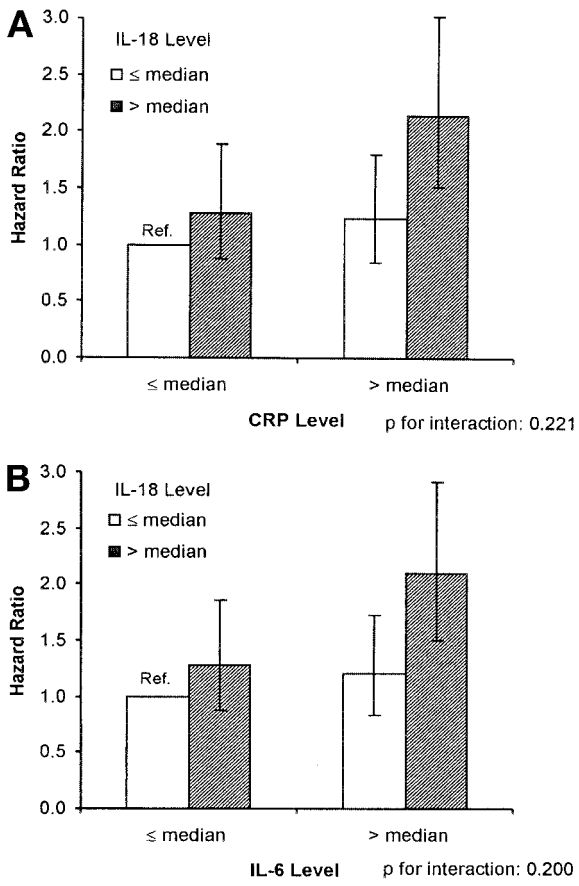

FIG. 1. The joint effect of IL-18 combined with either CRP (*A*) or IL-6 (*B*). Variables were stratified based on weighted median cut points for each biomarker using the distribution in the randomly drawn subcohort; median values were 173.5 pg/ml for IL-18, 1.4 mg/l for CRP, and 2.3 pg/ml for IL-6. Error bars indicate 95% CIs.

diabetes. Thus, as previously suggested for other proinflammatory cytokines such as IL-6 (3,9,12,13), IL-18 could be involved in the pathogenesis of type 2 diabetes. Of note in this context is the observation that the association between elevated concentrations of IL-18 and type 2 diabetes was independent of IL-6 and CRP. Thus, IL-18 seems to induce specific inflammatory responses that may promote the development of insulin resistance and type 2 diabetes.

IL-18 is a very potent proinflammatory cytokine that plays a central role in the proinflammatory cascade (15). IL-18 originates from a wide variety of cells, including Kupffer cells, macrophages, T-cells, B-cells, osteoblasts, keratinocytes, dendritic cells, astrocytes, and microglias (16). Recently, it has been shown that adipocytes are also a source of IL-18 (34). Furthermore, weight loss leads to reduced concentrations of IL-18 (35). Thus, IL-18 could be one link through which obesity influences the risk for type 2 diabetes and insulin resistance. This hypothesis is supported by the observation that adjustment for BMI and WHR attenuated HRs for IL-18. However, adjustment for measures of body fat had a much smaller impact on the associations between IL-18 and incident diabetes than on associations between CRP or IL-6 and incident diabetes (data not shown), and thus, other still unknown pathophysiologic mechanisms seem to be responsible for the detrimental effects of IL-18 concerning the development of type 2 diabetes. Alternatively, the association between IL-18 and type 2 diabetes could be explained by unmeasured correlates of IL-18.

This study has several limitations that need to be considered. First, we cannot rule out that selection bias due to missing blood samples and incomplete follow-up information could have influenced the results. However, there is little evidence that supports the assumption that the association between IL-18 and type 2 diabetes is different between the two groups. Also, baseline levels of IL-18 were similar in subjects with available follow-up information and those who were excluded due to missing follow-up data. Second, incident cases of diabetes were initially identified by self-report only, and we did not perform an oral glucose tolerance test at baseline or follow-up. Thus, we most likely missed subjects with undiagnosed diabetes on both occasions. Misclassification of incident cases at follow-up would, however, have biased our results toward the null and thus cannot explain the positive associations that were observed. Misclassification of subjects at baseline could in theory have caused spurious positive associations; however, most of these subjects probably would have been diagnosed during the 1st year of follow-up, and exclusion of cases diagnosed within the 1st 3 years of follow-up hardly affected the results. Also, interassay CVs for IL-18 were relatively high, which might have attenuated the observed associations. Finally, some misclassification of exposure could have occurred since levels of IL-18 were only measured at a single point in time at baseline.

The strengths of the study are its population-based prospective design, the large number of incident cases, a long follow-up period, and detailed assessment of potential confounding factors.

In conclusion, we report a strong positive association between levels of IL-18 and risk of type 2 diabetes, which was independent of known risk factors for diabetes. Furthermore, the association was independent of a generalized proinflammatory state as reflected by elevated levels of CRP and IL-6; however, subjects with elevated levels of both IL-18 and CRP or IL-18 and IL-6 had the highest risk of developing type 2 diabetes. Our observations suggest that upregulation of specific proinflammatory cytokines favors the development of type 2 diabetes, but the exact pathophysiologic mechanisms remain to be elucidated.

ACKNOWLEDGMENTS

This study was supported by research grants from the German Research Foundation (TH-784/2-1); by the European Foundation for the Study of Diabetes; and by additional funds provided by the University of Ulm, Germany; the German Diabetes Center; the GSF National Research Center for Environment and Health, Neuherberg, Germany; and the Federal Ministry of Education and Research, Berlin, Germany (NGFN). The MONICA/KORA Augsburg cohort study was financed by the GSF National Research Center for Environment and Health and supported by grants from the Federal Ministry of Education and Research, Berlin, Germany.

We thank all members of the GSF Institute of Epidemiology and the field staff in Augsburg who were involved in the planning and conduct of the MONICA/KORA Augsburg studies. Specifically, we would like to thank Professor Ulrich Keil (University of Münster, Germany), who was the principal investigator of the MONICA Augsburg study, and Andrea Schneider and Mainsi Marowsky-Köppl, who were

responsible for the data management. Furthermore, we thank Gerlinde Trischler (University of Ulm), Gabi Gornitzka, Ulrike Poschen, and Karin Röhrig (all from the German Diabetes Center) for their excellent technical assistance, and Wilco de Jager (University Medical Center, Utrecht) for his support with establishing the Luminex assay for IL-18 measurements. Finally, we express our appreciation to all study participants.

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