Inflammatory Cytokines and the Risk to Develop Type 2 Diabetes

Results of the Prospective Population-Based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study

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A subclinical inflammatory reaction has been shown to precede the onset of type 2 (non–insulin-dependent) diabetes. We therefore examined prospectively the effects of the central inflammatory cytokines interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) on the development of type 2 diabetes. We designed a nested case-control study within the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study including 27,548 individuals. Case subjects were defined to be those who were free of type 2 diabetes at baseline and subsequently developed type 2 diabetes during a 2.3-year follow-up period. A total of 192 cases of incident type 2 diabetes were identified and matched with 384 non–disease-developing control subjects. IL-6 and TNF-α levels were found to be elevated in participants with incident type 2 diabetes, whereas IL-1β plasma levels did not differ between the groups. Analysis of single cytokines revealed IL-6 as an independent predictor of type 2 diabetes after adjustment for age, sex, BMI, waist-to-hip ratio (WHR), sports, smoking status, educational attainment, alcohol consumption, and HbA_{1c} (4th vs. the 1st quartile: odds ratio [OR] 2.6, 95% CI 1.2–5.5). The association between TNF-α and future type 2 diabetes was no longer significant after adjustment for BMI or WHR. Interestingly, combined analysis of the cytokines revealed a significant interaction between IL-1β and IL-6. In the fully adjusted model, participants with detectable levels of IL-1β and elevated levels of IL-6 had an independently increased risk to develop type 2 diabetes (3.3, 1.7–6.8), whereas individuals with increased concentrations of IL-6 but undetectable levels of IL-1β had no significantly increased risk, both compared with the low-level reference group. These results were confirmed in an analysis including only individuals with HbA_{1c} <5.8% at baseline. Our data suggest that the pattern of circulating inflammatory cytokines modifies the risk for type 2 diabetes. In particular, a combined elevation of IL-1β and IL-6, rather than the isolated elevation of IL-6 alone, independently increases the risk of type 2 diabetes. These data strongly support the hypothesis that a subclinical inflammatory reaction has a role in the pathogenesis of type 2 diabetes. *Diabetes* 52:812–817, 2003

Low physical activity and hyperalimentation are lifestyle factors associated with an increased risk of type 2 diabetes (1). Despite more than 100 million patients affected worldwide and a dramatic socioeconomic burden due to vascular complications, the etiology of type 2 diabetes is not yet completely understood.

It has been hypothesized that type 2 diabetes is a manifestation of an ongoing acute-phase response that is primarily characterized by alterations of the so-called acute-phase proteins, such as C-reactive protein (CRP) (2,3). Cross-sectional and prospective studies demonstrated increased concentrations of markers of the acute-phase response (including CRP, serum amyloid-A, and sialic acid) in patients with type 2 diabetes (2–9). In one of these studies, elevated levels of IL-6, which is known to be a main stimulator of the production of most acute-phase proteins (10,11), were shown to increase the risk of diabetes (9). However, in addition to IL-6, other cytokines, such as interleukin (IL)-1β or tumor necrosis factor-α (TNF-α), are central mediators of inflammatory reactions. It is well known that cytokines operate as a network in stimulating the production of acute-phase proteins. For example, the effects of IL-6 on CRP synthesis largely depend on an interaction with IL-1β (11). The acute-phase response in various artificial inflammatory models requires

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CRP, C-reactive protein; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; EPIC, European Prospective Investigation into Cancer and Nutrition; IL, interleukin; LOQ, limit of quantification; OR, odds ratio; TNF-α, tumor necrosis factor-α; WHR, waist-to-hip ratio.

both IL-6 and IL-1β, as demonstrated in the respective knockout mouse models (12,13). These data strongly suggest that inflammatory reactions do not depend on single mediators, but rather that the pattern of various cytokines is crucially important for the perpetuation of an acute-phase response.

Until now, there has been neither prospective evidence concerning the individual regulation of the inflammatory cytokines IL-1β or TNF-α nor prospective evidence about the combined role of IL-1β, IL-6, and TNF-α preceding type 2 diabetes, although the combined effects of these cytokines are likely to be more important than the circulating levels of the single cytokines. We therefore designed a nested case-control study within the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort of 27,548 individuals to further evaluate the role of CRP, IL-1β, IL-6, and TNF-α in the development of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study population. The EPIC-Potsdam study, as part of the multicenter population-based cohort study EPIC, aims to explore the relation between dietary and lifestyle factors and the development of complex diseases. Details of the recruitment procedure of EPIC-Potsdam have been published (14). In brief, 27,548 subjects (women aged 35–65 years and men aged 40–65 years) were recruited from the general population. Baseline examinations, including anthropometric measurements, blood sampling, a self-administered food frequency questionnaire, and a personal interview on lifestyle habits and medical history were conducted between 1994 and 1998. Follow-up questionnaires are sent to the study participants every 2–3 years to obtain information on, among other things, current medication and newly developed diseases, including diabetes.

Anthropometry and lifestyle characteristics. Anthropometric measurements (body height and weight, waist and hip circumference) were performed by trained personnel, with the participants wearing only light underwear and without shoes (15). BMI was calculated as body weight (in kilograms) divided by body height (in meters) squared. Waist-to-hip ratio (WHR) was calculated as waist divided by hip circumference.

Information on lifestyle characteristics were obtained from self-administered questionnaires and a personal, computer-guided interview by trained and quality-monitored personnel (16). Sporting activities (hours per week) were calculated as the average of hours of sports per week during the summer and the winter season.

End points and disease status. We studied 27,548 participants of the population-based EPIC-Potsdam study. The follow-up procedure was successful in receiving completely filled-in questionnaires from 96% of all cohort participants attending the baseline examination (17).

Case subjects were those who were free of type 2 diabetes at baseline and developed type 2 diabetes during the first 2- to 3-year follow-up, depending on the time of recruitment. Potential cases of incident diabetes were identified from self-reports on incident disease, current medications, and/or current dietary treatment for diabetes ($n = 399$). For each potentially incident subject, a special questionnaire was sent to the primary care physician. The study subject was considered as a case subject only if the diagnosis of newly developed diabetes was confirmed by this physician. A total of 201 cases of incident diabetes were identified by this verification process until 1 November 2001. At that time, another 10 potential incident cases were pending confirmation by the primary care physician. These subjects were not further considered. The biochemical analysis regarding diabetes-associated antibodies GAD65 and IA-2 revealed that nine of the case subjects should be considered as case subjects with type 1 diabetes, leaving 192 medically confirmed cases of type 2 diabetes. Each of the 192 individuals with confirmed type 2 diabetes was matched with two control subjects by age (±1 year) and sex ($n = 384$). For statistical analysis, those individuals with missing values in one of the variables being used in the statistical models were not considered ($n = 4$ for case subjects, $n = 7$ for control subjects), thus leaving 188 case subjects and 377 control subjects for the final analysis.

Laboratory procedures. Peripheral venous citrate-blood samples were taken at enrollment into the study. The blood samples were centrifuged at 1,000*g* for 10 min at 4°C. Plasma was then removed and stored in aliquots in freezers at −80°C until assays of the markers of interest were performed. IL-1β, IL-6, and TNF-α were measured by enzyme-linked immunosorbent assays (ELISAs; R&D Systems, Minneapolis, MN). CRP was determined by high-sensitivity ELISA (Immun Diagnostik, Bensheim, Germany). All assay procedures were performed as described by the manufacturer. Blood samples were analyzed in random order to exclude systemic bias due to interassay variation. Control specimens were analyzed simultaneously on each plate for every marker. The intra-assay coefficient of variation (CV) ranged between 6.4 and 10.2% for IL-1β, 3.8 and 11.1% for IL-6, 8.7 and 14.8% for TNF-α, and 4.9 and 6.2% for CRP. The interassay CV was 10.3% for IL-1β, 9.9% for IL-6, 16.1% for TNF-α, and 13.2% for CRP. The limits of detection were 0.1 pg/ml for IL-1β, 0.094 pg/ml for IL-6, 0.12 pg/ml for TNF-α, and 0.124 ng/ml for CRP. HbA_{1c} was determined by enzyme immunoassay (DAKO Diagnostika, Hamburg, Germany). The intra-assay CV ranged from 2 to 3%, and the interassay CV was 1.9%. Values of subjects without diabetes have been shown to range from 4.8 to 6.9% in this assay, according to the information of the manufacturer. Diabetes-associated antibodies GAD65 and IA-2 were analyzed by radioimmunoassay (Medipan Diagnostica, Selchow, Germany), which was performed as described by the manufacturer.

Statistical analyses. For all analyses, we used SAS software release 8.0 (SAS Institute, Cary, NC). In a first step, measurement values of the laboratory parameters (IL-1β, TNF-α, and CRP) below the limit of quantification (LOQ) were set at 0.7 × the respective LOQ (18). Means, standard deviations, and proportions of baseline characteristics of case and control subjects were calculated. The means ± SD are reported. Significance was considered at two-tailed $\alpha < 0.05$.

The nonparametric Wilcoxon's rank-sum test was used to test for differences in continuous variables between case and control subjects, and a χ^2 test with 1 degree of freedom (Mantel-Haenszel test) was used to describe differences in proportions between case and control subjects. Spearman correlation coefficients were used to test the association between anthropometric, lifestyle variables, and cytokines.

Associations were initially investigated separately for IL-1β, IL-6, and TNF-α. IL-6 and TNF-α were divided into quartiles, and CRP was dichotomized. Because of the high numbers of undetectable values, IL-1β was dichotomized. Because of case and control exclusions due to missing values, primarily unconditional logistic regression analysis was used to estimate odds ratios (ORs) and corresponding 95% CIs. As previously demonstrated, the estimation of the OR approximates the relative risk given an infrequent disease occurrence (19). We therefore calculated the OR to estimate the relative risk to develop type 2 diabetes that is associated with increasing categories of the investigated cytokine. Because the design of the study also allows conditional logistic analysis, study results were recalculated using conditional logistic regression analysis. In the article, the results of the unconditional regression and major results of the conditional regression analysis are shown. Estimates of relative risk were first obtained from age-adjusted (continuous) and sex-adjusted (categorical: female and male) analyses; this analysis was followed by further adjustment for BMI (continuous). The subsequent model also considers sex-normalized WHR (continuous), sporting activities in hours/week (continuous), smoking status (categorical: current smoker and nonsmoker), alcohol consumption in grams/day (continuous), and educational attainment (categorical: basic training, technical school, and university). In the last model, we additionally added HbA_{1c} (continuous) to the fully adjusted model to reduce potential bias caused by undetected cases of prevalent diabetes.

To investigate the role of cytokine patterns, formal interaction terms including the cytokines in question were analyzed. Product terms were built from dichotomized subgroups. IL-1β was therefore dichotomized as previously described (setting individuals with undetectable levels as 0 and those with detectable levels as 1), whereas IL-6 and TNF-α were dichotomized by using the 75th percentile as the cutoff point (setting individuals who were <75th percentile for both IL-6 and TNF-α as 0 and those who were >75th quartile as 1). Quartile cut points were estimated from the combined group of control and case subjects. In addition, ORs and 95% CIs were estimated for each combination, including two of these cytokines with the low-level category (<75th percentile for IL-6 and TNF-α and nondetectable values of IL-1β, respectively) as the reference category.

To reduce bias by individuals with undetected prevalent type 2 diabetes at baseline, we repeated the analysis, including only those case and control subjects with HbA_{1c} <5.8%.

RESULTS

Clinical parameters. Baseline characteristics of the participants are shown in Table 1. As expected, case subjects had higher BMI and WHR, and they exercised less (Table 1). Elevated glucose levels, such as impaired fasting glucose or impaired glucose tolerance, are well known to

TABLE 1
Baseline characteristics of the participants

Characteristic	Case subjects	Control subjects	*P*
n	188	377	
Age (years)	56 ± 7	56 ± 7	Matching variable
BMI (kg/m^2)	30.7 ± 4.8	26.7 ± 3.5	<0.0001
WHR	0.95 ± 0.09	0.89 ± 0.09	<0.0001
Sports (h/week)	0.5 ± 1.1	0.9 ± 1.6	0.0100
Alcohol consumption (g/day)	18.5 ± 28.2	16.1 ± 16.4	0.4678
IL-1β (pg/ml)	0.57 ± 0.93	0.47 ± 0.79	0.1959
IL-6 (pg/ml)	2.45 ± 1.80	1.67 ± 1.59	<0.0001
TNF-α (pg/ml)	2.04 ± 1.51	1.79 ± 1.28	0.0094
CRP (μg/ml)	4.14 ± 5.1	2.45 ± 4.38	<0.0001
Men	111 (59)	222 (59)	Matching variable
Current smokers	36 (19)	80 (21)	0.5661
Less than high school education	83 (44)	142 (38)	0.1383
Prevalence of hypertension	148 (79)	195 (52)	<0.0001
Prevalence of hyperlipoproteinemia	81 (43)	120 (32)	0.0085

Data are means ± SD or *n* (%).

be a risk factor for future type 2 diabetes (20), which is reflected in our study group by significantly elevated HbA_{1c} levels in case subjects (6.39 ± 2.16%) compared with control subjects (4.73 ± 0.74%). Of the participants, 2.8% had undetectable levels of CRP (5% for TNF-α and 62% for IL-1β, respectively). IL-6 levels were detectable in all participants. As demonstrated in previous studies (8,9), elevated levels of CRP were found to be associated with an increased risk of type 2 diabetes in the fully adjusted model (OR 1.9, 95% CI 1.2–3.2). With respect to clinical parameters and CRP, subgroup analysis (HbA_{1c} <5.8%) yielded results comparable to the analysis that included all participants. For example, the risk of individuals with elevated CRP levels to develop type 2 diabetes was 2.1 (95% CI 1.2–3.7) in the fully adjusted model in this subgroup. The mean HbA_{1c} was 4.5 ± 0.5% for control and 4.9 ± 0.5% for case subjects ($P < 0.001$) within the restricted subcohort. Correlations of cytokines, CRP, BMI, WHR, and HbA_{1c} are demonstrated in Table 2.

Effects of single cytokines on diabetes risk

IL-6 independently predicts the risk of type 2 diabetes. Mean baseline levels of IL-6 were higher among case subjects compared with control subjects ($P < 0.0001$). Elevated levels of IL-6 were associated with an increased risk of type 2 diabetes (risk estimates of individuals according to IL-6 quartiles are demonstrated in Table 3). After adjustment for all covariables (BMI, WHR, sports, age, sex, smoking status, educational attainment, alcohol consumption, and HbA_{1c}), IL-6 was found to be an independent predictor of type 2 diabetes (OR 2.57, 95% CI 1.24–5.47) This result was confirmed in conditional regression analysis (2.6, 1.2–5.9). Comparable and significant results were observed in analyses restricted to case subjects with HbA_{1c} <5.8% only. Within this subgroup analysis, the risk of individuals within the 4th quartile of IL-6 was also substantially increased (3.1, 1.3–7.4 [unconditional]; 3.7, 1.16–11.9 [conditional]) in the fully adjusted model.

Elevated levels of TNF-α and IL-1β alone are not independently associated with future type 2 diabetes. Mean concentrations of TNF-α were higher in case subjects (2.04 ± 1.51 pg/ml) compared with control subjects (1.79 ± 1.28 pg/ml, $P < 0.01$). The risk of type 2 diabetes increased with increasing quartiles of TNF-α (Table 3). However, after adjustment for BMI or WHR, this association was no longer significant. Correlation analysis showed a mild correlation between TNF-α and BMI ($r = 0.17$, $P < 0.001$) or WHR ($r = 0.15$, $P < 0.001$). Again, analysis of participants with HbA_{1c} <5.8% yielded a similar picture as analysis of all participants. For example, the ORs in the fully adjusted model including HbA_{1c} were 1.0, 1.8 (95% CI 0.8–3.8), 0.9 (0.4–1.9), and 1.3 (0.6–3.1) for the increasing quartiles. Using conditional regression analysis also yielded no significant results regarding TNF-α–dependent relative risks after adjustment for BMI and/or WHR.

We found that 41.0% of case subjects and 36.6% of control subjects had detectable levels of IL-1β, which was not a statistically significant difference. According to these data, the relative risk of developing type 2 diabetes was not associated with circulating levels of IL-1β, independent of the model or type of analysis applied (Table 3).

Combined effects of cytokines modify the risk of future type 2 diabetes. Participants with a combined elevation of IL-6 levels and detectable levels of IL-1β were

TABLE 2
Correlations of cytokines, CRP, BMI, WHR, and HbA_{1c}

	HbA_{1c}	BMI	WHR	IL-6	TNF-α
HbA_{1c}	—	—	—	—	—
BMI	0.224 ($P < 0.001$)	—	—	—	—
WHR	0.239 ($P < 0.001$)	0.425 ($P < 0.001$)	—	—	—
IL-6	0.099 ($P = 0.019$)	0.302 ($P < 0.001$)	0.264 ($P < 0.001$)	—	—
TNF-α	0.031 (NS)	0.17 ($P < 0.001$)	0.15 ($P < 0.001$)	0.177 ($P < 0.001$)	—
CRP	0.1 ($P = 0.017$)	0.242 ($P < 0.001$)	0.11 ($P = 0.01$)	0.51 ($P < 0.001$)	0.135 ($P = 0.001$)

TABLE 3
Risk estimates of individuals according to circulating cytokine levels

	Median	Age- and sex-adjusted analysis	+BMI-adjusted analysis	Adjusted for all risk factors*	Adjusted for all risk factors and HbA_{1c}
IL-1β					
Category 1	0.09 (0.09–0.09)†	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Category 2	0.84 (0.13–5.52)†	1.20 (0.84–1.72)	1.18 (0.79–1.75)	1.19 (0.78–1.79)	1.20 (0.74–1.96)
CRP					
Category 1	0.62 (0.09–1.49)†	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Category 2	3.57 (1.50–39.7)†	3.5 (2.4–5.1)	2.2 (1.5–3.3)	1.9 (1.2–2.9)	1.9 (1.2–3.2)
IL-6					
Category 1	0.66 (0.11–0.90)‡	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Category 2	1.19 (0.90–1.45)‡	1.77 (0.97–3.29)	1.36 (0.72–2.6)	1.22 (0.64–2.38)	1.14 (0.54–2.45)
Category 3	1.80 (1.45–2.24)‡	3.88 (2.21–7.03)	2.17 (1.18–4.07)	1.92 (1.02–3.67)	1.72 (0.83–3.60)
Category 4	3.30 (2.24–10.8)‡	7.34 (4.18–13.32)	3.26 (1.76–6.19)	2.75 (1.44–5.34)	2.57 (1.24–5.47)
TNF-α					
Category 1	0.79 (0.35–1.11)‡	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Category 2	1.34 (1.11–1.56)‡	1.85 (1.11–3.11)	1.66 (0.94–2.95)	1.62 (0.90–2.94)	1.39 (0.95–3.85)
Category 3	1.82 (1.56–2.26)‡	1.40 (0.83–2.39)	1.05 (0.58–1.89)	1.04 (0.57–1.92)	1.06 (0.51–2.21)
Category 4	2.94 (2.26–15.15)‡	2.30 (1.37–3.90)	1.70 (0.96–3.04)	1.52 (0.84–2.78)	1.57 (0.78–3.18)

Data are OR (95% CI), unless otherwise indicated. †Median (category limits), ‡Median (interquartile range). Individuals were divided into quartiles according to their baseline plasma concentrations of IL-6 or TNF-α. They were dichotomized according to their levels of IL-1β and CRP; *adjusted for age, sex, BMI, WHR, sporting activities, smoking status, alcohol consumption, and educational attainment.

found to have an increased risk of future type 2 diabetes (OR 3.3, 95% CI 1.7–6.8 [unconditional]; 3.479, 1.078–11.222 [conditional]) compared with the low-level reference group in the fully adjusted model. In contrast, individuals with elevated levels of IL-6 but nondetectable levels of IL-1β had no significantly increased risk to develop type 2 diabetes (1.2, 0.6–2.5 [unconditional]; 1.5, 0.5–4.4 [conditional]) compared with the low-level reference group in the fully adjusted model. These results were confirmed by the inclusion of a formal interaction term between detectable IL-1β and elevated IL-6; this term was 3.3 (95% CI 1.1–9.7) and was significant (Table 4). Similar results were obtained including only individuals with HbA_{1c} <5.8%; here, the interaction term was 2.3 (1.1–5.1).

In crude analysis, individuals with a combined elevation of IL-6 and TNF-α or with a combined elevation of TNF-α and IL-1β had a substantially increased risk compared with individuals with elevated levels of IL-6 alone or compared with the low-level reference group. These effects of a combined elevation of IL-6 and TNF-α (OR 3.2, 95% CI 1.6–6.4) or IL-1β and TNF-α (2.3, 1.1–4.9) was still significant in the analysis restricted to participants with HbA_{1c} <5.8%. However, as described for TNF-α alone, this effect did not remain significant in the fully adjusted model. Results were again confirmed using conditional regression analysis and by calculation of formal interaction terms (Table 4).

TABLE 4
Interaction between IL-1β, IL-6, and TNF-α on diabetes risk

	OR (95% CI)
Interaction TNF-α/IL-1β	
Reference (TNF-α low/IL-1β undetectable)	1
TNF-α (high)	0.89 (0.45–1.73)
IL-1β (undetectable)	0.95 (0.54–1.67)
Interaction term TNF*IL-1β (high/detectable)	2.51 (0.84–7.57)
Interaction TNF-α/IL-6	
Reference (TNF-α low/IL-6 low)	1
TNF-α (high)	1.32 (0.68–2.52)
IL-6 (high)	2.15 (1.12–4.11)
Interaction term TNF*IL-6 (both high)	0.7 (0.23–2.16)
Interaction IL-1β/IL-6	
Reference (IL-6 low/IL-1β undetectable)	1
IL-1β (undetectable)	0.8 (0.43–1.44)
IL-6 (high)	1.14 (0.55–2.32)
Interaction term IL-1β*IL-6 (detectable/high)	3.31 (1.14–9.87)

Data shown were calculated after adjustment for age, sex, BMI, WHR, sporting activities, HbA_{1c} smoking status, alcohol consumption, and educational attainment.

DISCUSSION

We evaluated the effects of various inflammatory cytokines on the risk of type 2 diabetes. Participants with a combined elevation of IL-6 and IL-1β had a roughly threefold increased risk of developing type 2 diabetes compared with the low-level reference group. In contrast, participants with elevated levels of IL-6 alone (and undetectable levels of IL-1β) had no substantial increase of their diabetes risk. In this regard, IL-1β appears to have a permissive role in the IL-6–mediated acute-phase response preceding the onset of type 2 diabetes. Elevated levels of TNF-α were associated with an increased diabetes risk in the crude analysis. However, this TNF-α–dependent effect was no longer significant after adjustment for BMI or WHR. Furthermore, we found no significant effects of TNF-α on IL-1β– or IL-6–dependent risk estimates.

To the best of our knowledge, this is the first study to describe the combined effects of the three inflammatory cytokines IL-1β, IL-6, and TNF-α on the risk to develop type 2 diabetes. However, some limitations of this study need to be considered. It is well known that there is a relatively high proportion of individuals with undiagnosed type 2 diabetes among the general population (21,22). We therefore adjusted for blood glucose control by including HbA_{1c} into the fully adjusted model. To reduce the remaining potential bias of prevalent diabetes in case or control subjects at baseline, we also performed separate analyses among participants (case and control subjects) with HbA_{1c} <5.8%. A total of 94 individuals in our cohort provided

fasting blood specimens. Within this subgroup, all case individuals with HbA_{1c} <5.8% had a baseline fasting glucose <7.0 mmol/l, which is sufficient to exclude diabetes in epidemiological studies, according to American Diabetes Association criteria. Thus, the number of individuals with prevalent diabetes at baseline (in case and control subjects) appears to be small in the analyses restricted to individuals with HbA_{1c} <5.8%. However, physician-diagnosed cases (as in this study) are likely to represent more progressed stages of diabetes compared with those diagnosed, for example, by an oral glucose tolerance test. Given that cytokine changes at baseline may be associated with the stage of diabetes, one might expect weaker associations if earlier stages of diabetes are investigated. In addition, some of the control subjects may have developed diabetes by the end of follow-up but were not diagnosed by a physician.

The robustness of results was additionally confirmed by inclusion of further covariates (preexisting hypertension and hyperlipidemia) into the model, although there is no clear functional evidence that these factors influence the development of type 2 diabetes. It is important to note that all of these additional analyses confirmed the findings described. Although BMI and WHR are more common clinical measures of obesity and central obesity, respectively, they may not fully account for the metabolic consequences of obesity and residual confounding even though adjustment for these parameters may exist. We analyzed various components of the metabolic syndrome, and cytokine effects were found to be independent of hypertension or hyperlipidemia. Although previous studies demonstrated that inflammatory markers are associated with future type 2 diabetes, even after adjustment for fasting insulin (6,8), it remains to be elucidated whether this holds true for the cytokine changes described here. Another potential bias may result from different precision of cytokine measurement. When two or more cytokines are entered into the statistical models, the relative strength of association between cytokines and disease can be expected to be highest for the cytokine with the least measurement error. In our study, inter- and intra-assay coefficients of variation were comparable in measurements of the three cytokines. However, additional sources of measurement variability (related to venipuncture, blood processing, or effects of long-term storage) may have differed for the three cytokines. Thus, we cannot entirely exclude that some of the above-mentioned limitations may have influenced our results.

Our data suggest that the pattern of inflammatory cytokines is important in the pathogenesis of type 2 diabetes. These findings are in line with the fact that inflammatory reactions depend on a cluster of cytokines rather than on single cytokines only. Patterns of cytokine production differ with different inflammatory conditions, and cytokines are components of a large complex signaling network (11–13). Several mechanisms, such as considerable influence of cytokines on lipid metabolism, may be important for the effects of combined elevations of different cytokines. For example, both IL-6 and IL-1β act on the liver to produce the characteristic dyslipidemia of the metabolic syndrome, with increased VLDL and decreased HDL (3). Combined elevation of IL-6 and IL-1β dramatically increased the expression of the acute-phase proteins, compared with the effect of each cytokine alone (11). Another potential molecular mechanism how inflammation may be involved in the pathogenesis of type 2 diabetes has been elucidated in recent elegant studies showing that sensitizing of insulin signaling by salicylates is induced via inhibition of the activity of IκB kinase β (23–25). IL-1β is well known to activate the IκB kinase β and might thereby induce insulin resistance.

In conclusion, our data support the concept that subclinical activation of the immune system is involved in the pathogenesis of type 2 diabetes. We demonstrated that a specific pattern of cytokines was associated with an increased risk of type 2 diabetes, rather than isolated elevation of the respective cytokines.

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REFERENCES

1. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC: Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* 345:790–797, 2001
2. Pickup JC, Mattock MB, Chusney GD, Burt D: NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* 40:1286–1292, 1997
3. Pickup JC, Crook MA: Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia* 41:1241–1248, 1998
4. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G: Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet* 353:1649–1652, 1999
5. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA: High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51:455–461, 2002
6. Festa A, D'Agostino R Jr, Tracy RP, Haffner SM: Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 51:1131–1137, 2002
7. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD, O'Reilly DS, Packard CJ, Sattar N: C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes* 51:1596–600, 2002
8. Barzilay JI, Abraham L, Heckbert SR, Cushman M, Kuller LH, Resnick HE, Tracy RP: The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. *Diabetes* 50:2384–2389, 2001
9. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327–334, 2001
10. Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H: Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived

hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci U S A* 84:7251–7255, 1987
11. Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340:448–454, 1999
12. Fattori E, Cappelletti M, Costa P, Sellitto C, Cantoni L, Carelli M, Faggioni R, Fantuzzi G, Ghezzi P, Poli V: Defective inflammatory response in interleukin 6-deficient mice. *J Exp Med* 180:1243–1250, 1994
13. Zheng H, Fletcher D, Kozak W, Jiang M, Hofmann KJ, Conn CA, Soszynski D, Grabiec C, Trumbauer ME, Shaw A, et al.: Resistance to fever induction and impaired acute-phase response in interleukin-1 beta-deficient mice. *Immunity* 3:9–19, 1995
14. Boeing H, Korfmann A, Bergmann MM: Recruitment procedures of EPIC-Germany: European Investigation into Cancer and Nutrition. *Ann Nutr Metab* 43:205–215, 1999
15. Klipstein-Grobusch K, Georg T, Boeing H: Interviewer variability in anthropometric measurements and estimates of body composition. *Int J Epidemiol* 26:S174–S180, 1997
16. Kroke A, Bergmann MM, Lotze G, Jeckel A, Klipstein-Grobusch K, Boeing H: Measures of quality control in the German component of the EPIC study: European Prospective Investigation into Cancer and Nutrition. *Ann Nutr Metab* 43:216–224, 1999
17. Bergmann MM, Bussas U, Boeing H: Follow-up procedures in EPIC-Germany—data quality aspects: European Prospective Investigation Into Cancer and Nutrition. *Ann Nutr Metab* 43:225–234, 1999
18. Hallez S, Derouane A: Novelle methode de traitement de séries de données tronquées dans l'étude de la pollutions atmospherique. *Sci Total Environ* 22:115–123, 1982
19. Cornfield J: A method of estimating comparative rates from clinical data: applications to cancer of the lung, breast and cervix. *J Natl Cancer Inst* 11:1269–1275, 1951
20. de Vegt F, Dekker JM, Jager A, Hienkens E, Kostense PJ, Stehouwer CD, Nijpels G, Bouter LM, Heine RJ: Relation of impaired fasting and postload glucose with incident type 2 diabetes in a Dutch population: the Hoorn Study. *JAMA* 285:2109–2113, 2001
21. Warram JH, Kopczynski J, Janka HU, Krolewski AS: Epidemiology of non-insulin-dependent diabetes mellitus and its macrovascular complications: a basis for the development of cost-effective programs. *Endocrinol Metab Clin North Am* 26:165–188, 1997
22. Harris MI, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, Wiedmeyer HM, Byrd-Holt DD: Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults: the Third National Health and Nutrition Examination Survey 1988–1994. *Diabetes Care* 21:518–524, 1998
23. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, Shulman GI: Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest* 108:437–446, 2001
24. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE: Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293:1673–1677, 2001
25. Hundal RS, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, Shulman GI: Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *J Clin Invest* 109:1321–1326, 2002