Association of Systemic Chemokine Concentrations With Impaired Glucose Tolerance and Type 2 Diabetes

Results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4)

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Chemokines are crucial immune mediators in many physiological and pathophysiological conditions. Chemokines have been hypothesized to be involved in macrophage infiltration into adipose tissue in obesity and might therefore play an important role in the development of obesity-related disorders like type 2 diabetes. Out of 1,653 individuals aged 55–74 years participating in a population-based survey in southern Germany (the Kooperative Gesundheitsforschung in der Region Augsburg [KORA] [Cooperative Health Research in the Region of Augsburg] Survey S4, 1999–2001), 236 individuals with type 2 diabetes, 242 subjects with impaired glucose tolerance (IGT), and 244 normoglycemic control subjects were studied for circulating concentrations of interleukin (IL)-8; RANTES (regulated on activation, normal T-cell expressed, and secreted); interferon-γ–inducible protein-10 (IP-10), and eotaxin. Systemic concentrations of RANTES were higher in individuals with IGT or type 2 diabetes than in control subjects, whereas IL-8 levels were elevated in type 2 diabetic patients only ($P < 0.001$ for all comparisons). IP-10 and eotaxin were not significantly associated with IGT or type 2 diabetes. Adjustment for age, sex, BMI, hypertension, LDL cholesterol, HDL cholesterol, uric acid, C-reactive protein, and IL-6 did not alter these findings. Our findings indicate that RANTES and IL-8 may be involved in the development of type 2 diabetes independent of metabolic syndrome–related risk factors and of each other. There is no general upregulation of chemokine production in type 2 diabetes, but rather an association of the disease with specific members of the chemokine family.
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CRP, C-reactive protein; IGT, impaired glucose tolerance; IL, interleukin; IP-10, interferon-γ–inducible protein-10; KORA, Kooperative Gesundheitsforschung in der Region Augsburg [Cooperative Health Research in the Region of Augsburg]; RANTES, regulated on activation, normal T-cell expressed, and secreted; Th, T-helper.

Numerous reports demonstrated that chronic low-grade inflammation and activation of the innate immunity are involved in the pathogenesis of type 2 diabetes. Cross-sectional and prospective studies showed not only that systemic concentrations of acute-phase proteins and cytokines are elevated in most type 2 diabetic patients, but that these immune abnormalities precede type 2 diabetes by several years (1–5). It is notable that the data indicate neither a general upregulation of inflammatory immune mediators nor a random upregulation, but rather demonstrate that impaired glucose tolerance (IGT) and type 2 diabetes are associated with a differential, specific, and coordinated immune activation.

Although many studies addressed the association of type 2 diabetes and acute-phase proteins or proinflammatory cytokines, much less is known about the potential role of chemokines in the development of type 2 diabetes. Chemokines are a superfamily of secreted low–molecular weight proteins with crucial roles in physiological and pathophysiological processes such as hematopoiesis, angiogenesis, inflammation, atherosclerosis, and allergic, infectious, or autoimmune diseases (6,7). Their primary function is the regulation of leukocyte migration along concentration gradients, but chemokines also activate various cell types to produce and secrete inflammatory mediators ranging from histamine to cytokines. Chemokine research is complicated by the facts that chemokines usually bind to several G-protein–coupled seven-transmembrane domain receptors and that the various chemokine receptors show overlapping ligand specificities; therefore, in most experimental systems, chemokines exhibit overlapping and redundant functions.

Recently, chemokines came into the focus of diabetes research, since three studies found that in mouse models and in humans, obesity was associated with infiltration of macrophages into adipose tissue (8–10). Adipose tissue from obese mice exhibited a significant upregulation of immune genes including chemokines, which would appear to be interesting candidates to control this cell migration (8,9). Several small studies investigated the association of interleukin (IL)-8, the first chemokine to be discovered, or interferon-γ–inducible protein-10 (IP-10), with diabetes but came to conflicting results (11–13). The absence of data from population-based studies on the role of chemokines in type 2 diabetes led to the present investigation of samples from 722 participants of the Kooperative Gesund-

heitsforschung in der Region Augsburg (KORA) [Cooperative Health Research in the Region of Augsburg] Survey S4 (14). We hypothesized that if chemokines are relevant for the development of type 2 diabetes, the analysis of systemic concentrations of IL-8 and other chemokines should reveal an increase or decrease of chemokine levels in individuals with IGT or type 2 diabetes. We decided to concentrate on IL-8, IP-10, eotaxin, and RANTES (regulated on activation, normal T-cell expressed, and secreted), which represent considerably different chemotactic properties due to the differential expression of their respective receptors on leukocyte subsets and that can be associated with both T-helper (Th)-1 (IL-8, IP-10) and Th2 (eotaxin, RANTES) reactivities (15,16).

This study was performed to address the following questions: *1*) Are systemic levels of IL-8, IP-10, eotaxin, and RANTES associated with type 2 diabetes? *2*) If yes, is the respective chemokine also associated with IGT to support a potential role in diabetes development? *3*) To what extent are chemokines correlated with each other and/or the metabolic risk factors of diabetes?

RESEARCH DESIGN AND METHODS

The KORA Survey S4 study population, laboratory, and statistical methods have been described extensively (14,17,18). Briefly, the KORA Survey S4 studied a sample of the adult general population of German nationality in the region of Augsburg; these subjects were recruited from October 1999 to April 2001. The sampling design followed the guidelines of three previous surveys conducted in this region within the frame of the multinational World Health Organization–MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) Augsburg project (19). The investigations were carried out in accordance with the Declaration of Helsinki as revised in 1996, including written informed consent of all participants. All study methods were approved by the ethics committee of the "Bayerische Landesärztekammer" Munich. In the age range of 55–74 years, 1,653 individuals participated in a standardized interview followed by biochemical and clinical analyses. An oral glucose tolerance test and biochemical and immunological analyses were performed as described (14). Diabetes was diagnosed according to the 1999 World Health Organization criteria (14). Blood samples were obtained and were fasting in nondiabetic subjects and in newly detected diabetic patients and nonfasting in subjects with a known history of diabetes. A total of 236 individuals with type 2 diabetes and 242 with IGT were available for analysis. A total of 244 normoglycemic control subjects were randomly selected after frequency matching for age and sex. The diabetic patients comprised 116 individuals with type 2 diabetes previously diagnosed by their treating physicians (mean duration ± SD from diagnosis 9.1 ± 6.7 years) and 120 subjects with type 2 diabetes diagnosed during the survey, who did not yet receive antidiabetic treatment. Subjects who were positive for GAD autoantibodies were excluded. Autoantibodies to GAD were measured as described (20). The study group (n = 722) is slightly larger than the previously described group used for immunogenetic analyses (n = 704) (17). This difference is mainly due to missing DNA samples for some individuals, who were included however in the serological analysis.

Bioelectrical impedance analysis and laboratory measurements. Bioelectrical impedance analysis was conducted to estimate total fat-free mass and body fat using the B.I.A.-2000-S Analyzer (Data Input, Frankfurt, Germany). Relevant parameters were estimated using the equation developed by Segal et al. (21).

Blood samples were drawn and prepared according to the recommendations of the International Committee for Standardization in Hematology (22). Clinical and metabolic markers, plasma C-reactive protein (CRP), and serum IL-6 were measured as described (3). Serum concentrations of IL-8, RANTES, IP-10, and eotaxin were measured by sandwich enzyme-linked immunosorbent assay using antibody pairs and recombinant proteins from Sanquin (Amsterdam, the Netherlands; IL-8), R&D Systems (Wiesbaden, Germany; RANTES), or PharMingen (San Diego, CA; IP-10 and eotaxin). The detection limits for IL-8, RANTES, IP-10, and eotaxin were 1.68, 0.14, 14.4, and 12.7 pg/ml, respectively. Patients with chemokine concentrations lower than the detection limit were assigned a value of 0.5 of the detection limit (IL-8, n = 5; RANTES, n = 1; IP-10, n = 2; eotaxin, n = 71).

Statistical analysis. Data with Gaussian distribution were described by means ± SD, whereas data with non-Gaussian distribution were reported as median and interquartile range (all other continuous variables). Differences between subjects with IGT or type 2 diabetes versus control subjects were analyzed by the unpaired t test or Wilcoxon's test, respectively. For dichotomous variables, absolute numbers were given and the corresponding probabilities were compared with Fisher's exact test (all tests two-sided). Hypertension was defined as use of antihypertensive treatment or systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Spearman's rank correlation coefficients were used to analyze associations between chemokine concentrations and clinical, metabolic, or immunological variables. The analysis of the associations of increasing chemokine serum concentrations and diabetes risk was assessed after subdivision of the sample into quartiles based on the distribution of normoglycemic control subjects. Multiple logistic regression models were fitted using diabetes or IGT as dependent variables and the chemokines as independent variables (trend: quartiles as ordinal variables; quartile analysis: quartiles as indicator variables). These models were estimated on the subpopulations of diabetic subjects and control subjects or IGT subjects and control subjects, respectively. Adjustment for clinical and biochemical confounders was performed by including them as independent variables in the logistic regression model. Odds ratios (ORs) and 95% CIs were estimated from the logistic regression model. The level of significance was 5%. Calculations were carried out using the SAS statistical package, version 8.2 TS2M0.

RESULTS

Basic characteristics of study participants. The study population is described in Table 1. Individuals with type 2 diabetes or IGT showed significantly elevated mean or median levels of BMI, waist-to-hip ratio, body fat content, insulin resistance measured by homeostasis model assessment, HbA_{1c} (A1C), fasting triglycerides, systolic and diastolic blood pressure, leukocyte count, CRP, and IL-6, whereas HDL cholesterol concentrations were lower than in normoglycemic control subjects (Table 1). In addition, patients with type 2 diabetes exhibited significantly elevated fat-free mass and decreased total and LDL cholesterol compared with control subjects (Table 1). The three groups did not differ significantly regarding smoking behavior, alcohol consumption, or frequency of respiratory infections or other inflammatory conditions during the week before the examination (data not shown).

Systemic levels of IL-8 and RANTES, but not IP-10 or eotaxin, are elevated in patients with impaired glucose metabolism. Patients with type 2 diabetes exhibited significantly higher serum concentrations of IL-8 and RANTES than normoglycemic control subjects (IL-8: median [interquartile range], 16.59 [13.07–22.25] vs. 13.48 [10.03–16.88] pg/ml, $P < 0.001$; RANTES: 28.29 [17.87–44.77] vs. 19.93 [14.13–29.32] ng/ml, $P < 0.001$) (Table 2). Furthermore, RANTES levels were already significantly elevated in individuals with IGT (25.96 [18.05–38.33] ng/ml; $P < 0.001$). No significant differences between the IGT and type 2 diabetic groups compared with control subjects were detected for IP-10 and eotaxin (Table 2). Because the type 2 diabetic group consisted of almost equal numbers of newly and previously diagnosed cases, these two subgroups were also compared. Chemokine levels between the two groups did not differ significantly (data not shown).

Logistic regression analyses were performed to estimate quartile-specific ORs for the associations of circulating chemokine concentrations with IGT and type 2 diabetes. Quartiles were defined by the chemokine distribution in normoglycemic subjects. Tables 3, 4, 5, and 6 summarize the results for IL-8, RANTES, IP-10, and eotaxin, respectively.

As shown in Table 3, increasing levels of IL-8 were associated with a higher risk of type 2 diabetes compared with control subjects, since the ORs for increasing quar-

TABLE 1
Characteristics of the subjects with IGT and type 2 diabetes and of the control subjects from the KORA Survey S4 population (age 55–74 years, 1999–2001)

	Control subjects	IGT	Type 2 diabetes
Sex (M/F)	137/107	130/112	137/99
Age (years)	65.3 ± 5.3 (244)	65.3 ± 5.2 (242)	65.1 ± 5.1 (236)
BMI (kg/m^2)	27.4 ± 3.7 (242)	29.7 ± 4.0* (241)	30.9 ± 4.5* (232)
Waist-to-hip ratio	0.90 ± 0.08 (243)	0.92 ± 0.07† (242)	0.94 ± 0.08* (235)
Body fat (kg)	27.3 ± 6.6 (238)	30.7 ± 6.9* (240)	32.8 ± 8.4* (224)
Body fat (%)	35.8 ± 5.5 (238)	38.1 ± 5.5* (240)	38.6 ± 5.9* (224)
Fat-free mass (kg)	48.7 ± 8.7 (238)	49.8 ± 8.1 (240)	51.7 ± 8.6* (224)
HOMA-IR	1.95 (1.37–2.99) (243)	3.06* (2.20–4.60) (240)	4.54* (2.65–7.61) (118)‡
A1C (%)	5.6 (5.3–5.8) (244)	5.6† (5.4–5.9) (242)	6.4* (5.9–7.2) (233)
Total cholesterol (mg/dl)	243 ± 45 (243)	243 ± 41 (242)	235 ± 46§ (233)
LDL cholesterol (mg/dl)	155 ± 43 (242)	156 ± 38 (242)	146 ± 41§ (232)
HDL cholesterol (mg/dl)	59 ± 17 (242)	55 ± 15§ (242)	50 ± 14* (233)
Fasting triglycerides (mg/dl)	106 (80–144) (237)	125* (96–174) (241)	141* (107–196) (135)‡
Systolic blood pressure (mmHg)	131 ± 18 (169)‖	139 ± 20* (118)‖	146 ± 21* (106)‖
Diastolic blood pressure (mmHg)	78 ± 10 (169)‖	81 ± 9§ (118)‖	84 ± 11* (106)‖
Uric acid (mg/dl)	5.5 ± 1.3 (244)	6.1 ± 1.4* (242)	6.1 ± 1.5* (233)
Serum albumin (g/l)	38.2 ± 4.1 (243)	38.5 ± 4.0 (241)	38.5 ± 4.1 (228)
Leukocyte count ($\times 10^{-3}/\mu$l)	5.88 ± 1.28 (244)	6.30 ± 1.61† (242)	6.99 ± 1.99* (233)
CRP (mg/l)	1.27 (0.68–2.98) (243)	2.39* (1.25–4.44) (241)	2.52* (1.13–5.63) (228)
IL-6 (pg/ml)	1.69 (0.68–2.90) (241)	2.32* (1.32–3.54) (240)	2.48* (1.28–4.80) (230)

Data with Gaussian distribution are means ± SD (n), whereas data with non-Gaussian distribution are medians (interquartile range) (n). For dichotomous variables, absolute numbers are given. Dichotomous variables, variables with Gaussian distribution, and variables with non-Gaussian distribution were compared with controls using the Fisher's exact test, t test, and Wilcoxon's test, respectively. HOMA-IR, insulin resistance measured by homeostasis model assessment. *$P < 0.001$, †$P < 0.01$ compared with normoglycemic control subjects. ‡Patients with a history of diabetes who did not provide fasting blood samples were excluded. §$P < 0.05$ compared with normoglycemic control subjects. ‖Patients on antihypertensive medication were excluded.

tiles in a crude analysis were 1.0, 2.30, 3.26, and 5.98, respectively (model 1; $P < 0.0001$ for trend). Adjustment for BMI did not change the results (model 2; $P < 0.0001$ for trend). Further adjustment for sex, age, hypertension, LDL cholesterol, HDL cholesterol, and uric acid (model 3) and additional inclusion of CRP and IL-6 (fully adjusted model 4) slightly attenuated the association of type 2 diabetes and IL-8 values, but the trend remained highly significant in both models ($P < 0.0001$). The fully adjusted model 4 demonstrated that IL-8 levels within quartiles 3 and 4 were strongly associated with type 2 diabetes, with ORs of 2.74 (95% CI 1.33–5.67; $P = 0.0065$) and 4.69 (2.32–9.50; $P < 0.0001$), respectively. A similar analysis for IGT did not show a significant association with IL-8.

Results for RANTES differed from the IL-8 data (Table 4). In the nonadjusted model 1, high RANTES concentrations were associated not only with type 2 diabetes, but also with IGT. The inclusion of BMI (model 2) and the aforementioned metabolic (model 3) and immunological (model 4) parameters weakened the association, but in the fully adjusted model 4, the association of RANTES with type 2 diabetes and IGT remained significant. In the type 2 diabetic group, the ORs for increasing quartiles were 1.0, 1.07, 1.07, and 1.96 ($P = 0.035$), respectively ($P = 0.024$ for trend). In the IGT group, the ORs for increasing quartiles were 1.0, 1.09, 2.07 ($P = 0.018$), and 2.47 ($P = 0.0026$), respectively ($P = 0.0004$ for trend).

The same analyses were performed for IP-10 and eotaxin, but in contrast to IL-8 and RANTES, these two chemokines were not significantly associated with type 2 diabetes or IGT (Tables 5 and 6).

Association between chemokine concentrations and clinical, metabolic, and inflammatory markers. By Spearman's analysis, we tested whether chemokine levels were correlated with age and key metabolic markers as well as with CRP, IL-6, and with each other (Table 7). The strongest correlations were observed in the analysis of the diabetes-associated chemokines IL-8 (waist-to-hip ratio, $r = 0.18$; A1C, $r = 0.23$) and RANTES (BMI, $r = 0.15$; A1C, $r = 0.16$; uric acid, $r = 0.16$; HDL, $r = -0.15$), whereas the Spearman rank coefficients for the association of IP-10 and eotaxin with the investigated variables were close to zero. Notably, IL-8, RANTES, IP-10, and eotaxin differed from CRP and IL-6 in their degree of correlation with immunological variables. CRP and IL-6 appeared co-regulated in the study population ($r = 0.42$), and both mediators were highly correlated with leukocyte count ($r = 0.33$ and $r = 0.25$, respectively), whereas the highest Spearman rank

TABLE 2
Serum chemokine levels in control subjects and in individuals with IGT or type 2 diabetes

	Control subjects	IGT	Type 2 diabetes
IL-8 (pg/ml)	13.48 (10.03–16.96) (244)	13.52 (9.63–16.96) (239)	16.59* (13.12–22.08) (230)
RANTES (ng/ml)	19.93 (14.22–29.28) (242)	25.96* (18.05–38.33) (239)	28.29* (17.95–44.50) (230)
IP-10 (pg/ml)	281.0 (193.8–438.4) (243)	310.2 (214.7–445.8) (238)	291.8 (186.7–426.5) (227)
Eotaxin (pg/ml)	74.4 (40.6–106.8) (241)	74.3 (41.5–109.6) (239)	76.6 (48.2–113.3) (229)

Data are medians (interquartile range) (n) and are compared with control subjects using the Wilcoxon's test. *$P < 0.001$ compared with normoglycemic control subjects.

TABLE 3
Association of IL-8 with type 2 diabetes and IGT (multiple logistic regression models)

IL-8 (pg/ml)	Quartile 1 (≤10.03)	Quartile 2 (>10.03/≤13.48)	Quartile 3 (>13.48/≤16.88)	Quartile 4 (>16.88)	*P* for trend
Type 2 diabetes vs. control subjects					
Model 1	1.0	2.30 (1.20–4.41)*	3.26 (1.75–6.09)†	5.98 (3.28–10.90)†	<0.0001
Model 2	1.0	2.55 (1.26–5.18)‡	2.92 (1.48–5.75)‡	5.76 (3.00–11.07)†	<0.0001
Model 3	1.0	1.99 (0.95–4.18)	2.89 (1.43–5.86)‡	4.98 (2.50–9.89)†	<0.0001
Model 4	1.0	1.93 (0.90–4.15)	2.74 (1.33–5.67)‡	4.69 (2.32–9.50)†	<0.0001
IGT vs. control subjects					
Model 1	1.0	0.84 (0.51–1.40)	0.92 (0.56–1.52)	0.97 (0.59–1.59)	NS
Model 2	1.0	0.86 (0.51–1.46)	0.86 (0.51–1.45)	0.97 (0.58–1.62)	NS
Model 3	1.0	0.82 (0.47–1.43)	0.84 (0.49–1.45)	0.92 (0.54–1.58)	NS
Model 4	1.0	0.79 (0.45–1.40)	0.85 (0.49–1.48)	0.87 (0.50–1.52)	NS

Data are ORs (95% CI) for association of type 2 diabetes or IGT with IL-8. Quartiles are based on data from normoglycemic control subjects. *$P < 0.05$, †$P < 0.001$, ‡$P < 0.01$, compared with quartile 1. Model 1: crude; model 2: adjusted for BMI; model 3: adjusted for BMI, sex, age, hypertension, LDL cholesterol, HDL cholesterol, and uric acid; model 4: model 3 with additional adjustment for log-CRP and log–IL-6.

coefficient for association of any of the four chemokines with any other of the immunological variables was $r = 0.13$. Overall, the correlation of IL-8, RANTES, IP-10, and eotaxin with metabolic syndrome–associated risk factors and with each other therefore appeared moderate.

DISCUSSION

The present study investigates the association of circulating chemokines with IGT and type 2 diabetes in the well-characterized KORA Survey S4 population and provides three major findings.

First, our data identify RANTES and IL-8 as type 2 diabetes–associated chemokines. The lack of association of IP-10 and eotaxin shows that there is no general or coordinated upregulation of chemokines in type 2 diabetes, which is in good accordance with previous findings regarding the differential and specific upregulation of cytokines in type 2 diabetes (3). Chemokines have previously been shown to exert crucial functions in common disorders such as allergies, atherosclerosis, and type 1 diabetes (6,7), but their role in type 2 diabetes had not been investigated.

Second, the analysis of subjects with IGT revealed that RANTES, but not IL-8, is strongly associated with IGT. Thus, the upregulation of these chemokines appears to occur during different stages of the disease; it can be hypothesized therefore that both chemokines have different roles in diabetes development. The lack of association between IP-10 and eotaxin with IGT or type 2 diabetes in this cross-sectional study does not preclude a transient involvement of these mediators in the different stages of diabetes onset and the manifestation of diabetic complications. Prospective studies will be needed to further evaluate the relevance of RANTES, IL-8, IP-10, and eotaxin in the disease process and to clarify whether RANTES can be considered as an early risk factor for type 2 diabetes.

Third, although RANTES and IL-8 display a moderate correlation with several metabolic syndrome–related parameters, our analysis indicates for the first time that both chemokines are associated with type 2 diabetes (and in the case of RANTES, also with IGT) independently of "classic" metabolic syndrome–related and inflammatory variables. Moreover, the correlation between systemic concentrations of RANTES, IL-8, IP-10, and eotaxin is rather moderate, which suggests that their basal expression levels are not coordinated.

Given the lack of data on chemokines in type 2 diabetes, the pathophysiological roles of RANTES and IL-8 are difficult to assess. It is striking that type 2 diabetes is associated with both Th1 (IL-8) and Th2 (RANTES)-associated chemokines. Th1 and Th2 reactivities can be considered polarized forms of the immune response, and characterization of type 2 diabetes as a disorder associated with either extreme seems too simplistic (5). A

TABLE 4
Association of RANTES with type 2 diabetes and IGT (multiple logistic regression models)

RANTES (ng/ml)	Quartile 1 (≤14.22)	Quartile 2 (>14.22/≤19.93)	Quartile 3 (>19.93/≤29.28)	Quartile 4 (>29.28)	*P* for trend
Type 2 diabetes vs. control subjects					
Model 1	1.0	1.11 (0.62–1.99)	1.47 (0.84–2.58)	3.26 (1.93–5.51)*	<0.0001
Model 2	1.0	0.98 (0.53–1.84)	1.13 (0.62–2.08)	2.42 (1.38–4.23)†	0.0006
Model 3	1.0	1.05 (0.54–2.03)	1.12 (0.59–2.13)	2.09 (1.15–3.81)‡	0.0093
Model 4	1.0	1.07 (0.54–2.11)	1.07 (0.55–2.09)	1.96 (1.05–3.66)‡	0.024
IGT vs. control subjects					
Model 1	1.0	1.18 (0.65–2.13)	2.37 (1.38–4.09)†	2.99 (1.75–5.11)*	<0.0001
Model 2	1.0	1.13 (0.61–2.08)	2.32 (1.31–4.08)†	2.76 (1.58–4.80)*	<0.0001
Model 3	1.0	1.17 (0.62–2.21)	2.30 (1.28–4.13)†	2.71 (1.52–4.83)*	0.0001
Model 4	1.0	1.09 (0.57–2.09)	2.07 (1.14–3.78)‡	2.47 (1.37–4.45)†	0.0004

Data are ORs (95% CI) for association of type 2 diabetes or IGT with RANTES. Quartiles are based on data from normoglycemic control subjects. *$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$ compared with quartile 1. Model 1: crude; model 2: adjusted for BMI; model 3: adjusted for BMI, sex, age, hypertension, LDL cholesterol, HDL cholesterol, and uric acid; model 4: model 3 with additional adjustment for log-CRP and log–IL-6.

TABLE 5
Association of IP-10 with type 2 diabetes and IGT (multiple logistic regression models)

IP-10 (pg/ml)	Quartile 1 (≤193.8)	Quartile 2 (>193.8/≤281.0)	Quartile 3 (>281.0/≤438.4)	Quartile 4 (>438.4)	*P* for trend
Type 2 diabetes vs. control subjects					
Model 1	1.0	0.89 (0.53–1.50)	1.19 (0.72–1.96)	0.91 (0.54–1.52)	NS
Model 2	1.0	0.91 (0.52–1.60)	1.06 (0.61–1.82)	0.74 (0.42–1.30)	NS
Model 3	1.0	0.89 (0.49–1.62)	0.98 (0.55–1.74)	0.58 (0.32–1.07)	NS
Model 4	1.0	0.88 (0.47–1.64)	0.97 (0.53–1.77)	0.48 (0.26–0.92)*	NS
IGT vs. control subjects					
Model 1	1.0	1.03 (0.61–1.76)	1.62 (0.98–2.67)	1.31 (0.78–2.19)	NS
Model 2	1.0	1.13 (0.65–1.97)	1.86 (1.10–3.15)*	1.33 (0.77–2.28)	NS
Model 3	1.0	1.07 (0.60–1.93)	1.73 (0.99–3.03)	1.18 (0.66–2.10)	NS
Model 4	1.0	1.02 (0.56–1.87)	1.69 (0.95–3.00)	0.97 (0.53–1.77)	NS

Data are ORs (95% CI) for association of type 2 diabetes or IGT with IP-10. Quartiles are based on data from normoglycemic control subjects. *$P < 0.05$ compared with quartile 1. Model 1: crude; model 2: adjusted for BMI; model 3: adjusted for BMI, sex, age, hypertension, LDL cholesterol, HDL cholesterol, and uric acid; model 4: model 3 with additional adjustment for log-CRP and log–IL-6.

substantial number of studies exists on the role of chemokines in metabolic syndrome–related disorders such as atherosclerosis and obesity, with important implications for the assessment of our data.

First, RANTES appears to have a causal role in atherosclerosis, since inactivation of RANTES receptors in a hypercholesterolemic mouse model prevented the progression of disease (23). The role of IL-8 is less clear, but it has been reported that it has angiogenic properties (24), mediates glucose-stimulated monocyte/endothelial adhesion (25), and may thereby contribute to plaque formation in coronary atherosclerosis. It is therefore conceivable that the expected higher prevalence of coronary heart disease in the IGT and type 2 diabetic groups could lead to false-positive association with RANTES or IL-8 (26). A similar effect was evident for monocyte chemotactic protein-1 in a population-based sample in the Dallas Heart Study where a positive association of type 2 diabetes and monocyte chemotactic protein-1 could be seen only in the whole study group, but not after exclusion of subjects with coronary heart disease (27). Because the association of RANTES and IL-8 with IGT and type 2 diabetes was not significantly attenuated by adjustment for well-established cardiovascular risk factors and because we cannot detect elevated monocyte chemotactic protein-1 concentrations in the IGT or type 2 diabetic groups of the KORA Survey S4 (C. Herder, H. Kolb, unpublished data), a confounding effect of atherosclerosis in our study is unlikely.

Second, obesity is associated with increased chemokine expression in adipose tissue (8,9) and might therefore increase circulating chemokine concentrations, as shown for IL-8 (28). Because IL-8 is expressed in adipose tissue (29), we included BMI into the logistic regression analysis, which however did not alter the results. Moreover, Spearman's correlation analysis of chemokines and indexes of obesity showed that systemic chemokine levels did not correlate strongly with obesity parameters and therefore confirmed the lack of confounding effects of obesity. It is important to note that IL-8 expression in adipose tissue and in endothelial cells was found positively regulated by glucose (30,31) and that systemic IL-8 levels increased in obese subjects with IGT during an oral glucose tolerance test (31). These findings support the association of IL-8 and type 2 diabetes, as identified in the present work.

Taken together, the present study demonstrates in the well-characterized population-based KORA Survey S4 participants that systemic concentrations of RANTES and IL-8 are significantly elevated in type 2 diabetes. The finding that levels of RANTES, but not IL-8, are already significantly increased in subjects with IGT argues for a different role of these chemokines in the development of type 2 diabetes. These associations are independent of metabolic syndrome–related anthropomorphic, biochemical, and inflammatory variables, but prospective studies are required to evaluate the novel hypothesis that RANTES and/or IL-8 are risk factors for type 2 diabetes.

TABLE 6
Association of eotaxin with type 2 diabetes and IGT (multiple logistic regression models)

Eotaxin (pg/ml)	Quartile 1 (≤40.64)	Quartile 2 (>40.64/≤74.35)	Quartile 3 (>74.35/≤106.80)	Quartile 4 (>106.80)	*P* for trend
Type 2 diabetes vs. control subjects					
Model 1	1.0	1.27 (0.75–2.13)	1.14 (0.67–1.92)	1.41 (0.85–2.36)	NS
Model 2	1.0	1.12 (0.63–1.98)	1.15 (0.65–2.04)	1.27 (0.72–2.23)	NS
Model 3	1.0	0.98 (0.53–1.79)	1.06 (0.57–1.96)	1.19 (0.66–2.17)	NS
Model 4	1.0	0.91 (0.49–1.70)	1.02 (0.55–1.92)	1.15 (0.62–2.14)	NS
IGT vs. control subjects					
Model 1	1.0	1.12 (0.68–1.86)	0.98 (0.59–1.63)	1.14 (0.69–1.88)	NS
Model 2	1.0	0.99 (0.58–1.68)	0.99 (0.58–1.68)	1.08 (0.64–1.82)	NS
Model 3	1.0	0.92 (0.53–1.61)	1.00 (0.57–1.75)	1.05 (0.61–1.83)	NS
Model 4	1.0	0.87 (0.49–1.54)	0.95 (0.54–1.68)	0.98 (0.56–1.73)	NS

Data are ORs (95% CI) for association of type 2 diabetes or IGT with eotaxin. Quartiles are based on data from normoglycemic control subjects. Model 1: crude; model 2: adjusted for BMI; model 3: adjusted for BMI, sex, age, hypertension, LDL cholesterol, HDL cholesterol, and uric acid; model 4: model 3 with additional adjustment for log-CRP and log-IL-6.

TABLE 7
Correlation of chemokine levels (Spearman's correlation coefficient r) with clinical, metabolic, and inflammatory parameters

	IL-8	RANTES	IP-10	Eotaxin
Age	0.03	−0.00	0.11*	−0.01
BMI	0.12*	0.15†	0.03	−0.00
Waist-to-hip ratio	0.18†	0.05	0.04	−0.07
Body fat (kg)	0.11*	0.13†	0.05	0.00
Body fat (%)	0.03	0.11*	0.09‡	0.06
Fat-free mass (kg)	0.09‡	0.01	−0.06	−0.10*
HOMA-IR	0.13*	0.10‡	0.09‡	−0.01
A1C	0.23†	0.16†	0.02	−0.03
Total cholesterol	−0.12*	0.04	−0.10*	0.03
LDL cholesterol	−0.12*	0.05	−0.10‡	0.01
HDL cholesterol	−0.06	−0.15†	−0.03	0.00
Fasting triglycerides§	0.03	0.09‡	−0.02	0.04
Systolic blood pressure‖	0.10‡	0.05	0.07	0.02
Diastolic blood pressure‖	0.06	0.04	0.03	0.02
Uric acid	0.07	0.16†	0.03	−0.05
Serum albumin	−0.03	0.03	0.00	−0.00
Leukocyte count	−0.08‡	0.13†	−0.02	0.07‡
CRP	0.03	0.11*	0.06	−0.03
IL-6	0.12†	0.10*	0.07	0.05
IL-8	—	0.07	0.13†	0.10‡
RANTES	0.07	—	0.01	0.05
IP-10	0.13†	0.01	—	0.07
Eotaxin	0.10‡	0.05	0.07	—

HOMA-IR, insulin resistance measured by homeostasis model assessment. *$P < 0.01$; †$P < 0.001$; ‡$P < 0.05$. §Patients with a history of diabetes who did not provide fasting blood samples were excluded. ‖Patients on antihypertensive medication were excluded.

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