Original Article

Systemic Immune Mediators and Lifestyle Changes in the Prevention of Type 2 Diabetes

Results From the Finnish Diabetes Prevention Study

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The Finnish DPS (Diabetes Prevention Study) demonstrated that lifestyle intervention, aimed at increasing physical activity, improving diet, and decreasing body weight, reduced the incidence of type 2 diabetes in individuals with overweight and impaired glucose tolerance by 58%. Here, we studied which immunological markers at baseline predicted subsequent type 2 diabetes and whether there are immunologically defined subsets of subjects who are more or less responsive to the protective effects of lifestyle intervention. We randomly assigned 522 participants to a control group (n = 257) or a lifestyle intervention group (n = 265). Immunological parameters at baseline included high-sensitivity C-reactive protein (CRP), serum amyloid A, interleukin-6, regulated on activation normal T-cell expressed and secreted (RANTES), macrophage migration inhibitory factor (MIF), and soluble intercellular adhesion molecule. In the control group, CRP was the best immunological predictor for progression to overt type 2 diabetes. In the intervention group, progression to type 2 diabetes was significantly higher in subjects with the highest RANTES concentrations and was lower in subjects with the highest MIF levels. Ratios of RANTES to MIF in the upper tertile were highly predictive of incident type 2 diabetes in the intervention group (P = 0.006), whereas the association was less pronounced in the control group (P = 0.088). Thus, systemic concentrations of immune mediators appear to be associated with the progression to type 2 diabetes and the prevention of type 2 diabetes by lifestyle changes. *Diabetes* 55:2340–2346, 2006

Type 2 diabetes is one of the most prevalent chronic diseases, and its complications, including cardiovascular disease, nephropathy, neuropathy, and retinopathy, cause substantial human and economic burdens (1,2). Thus, the prevention of type 2 diabetes is a major challenge for clinicians and public health policy makers worldwide. Lifestyle intervention including physical activity and dietary modification has been shown to be effective in the prevention of type 2 diabetes in individuals with overweight and impaired glucose tolerance (IGT) (3,4). The risk-reducing effect of lifestyle intervention has been partly attributable to weight reduction (3–5), improved insulin sensitivity (6), and increased physical activity (7).

There is increasing evidence that plasma markers of low-grade inflammation and immunological activation, such as C-reactive protein (CRP), interleukin (IL)-6, and E-selectin, predict the risk of developing type 2 diabetes (8–16). Other markers of inflammation and immunological activation suggested to play a role in the development of type 2 diabetes include serum amyloid A (SAA), soluble intercellular adhesion molecule (sICAM), macrophage migration inhibitory factor (MIF), and regulated on activation, normal T-cell expressed and secreted (RANTES), although existing evidence is limited. Whether inflammatory or immunological markers modify the effect of lifestyle intervention on the risk of developing type 2 diabetes and on metabolic risk factors for type 2 diabetes is unknown.

We therefore investigated in the Finnish Diabetes Prevention Study (DPS) *1*) whether systemic levels of immunological markers at baseline predict the risk of developing type 2 diabetes and changes in metabolic risk factors for type 2 diabetes in individuals with overweight and IGT and *2*) whether there are immunologically defined subsets of individuals who are more or less responsive to the protective effects of lifestyle intervention.

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CRP, C-reactive protein; DPS, Diabetes Prevention Study; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; IL, interleukin; KORA, Cooperative Health Research in the Region of Augsburg (Kooperative Gesundheitsforschung in der Region Augsburg); MIF, macrophage migration inhibitory factor; OGTT, oral glucose tolerance test; RANTES, regulated on activation, normal T-cell expressed and secreted; SAA, serum amyloid A; sICAM, soluble intercellular adhesion molecule.

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RESEARCH DESIGN AND METHODS

The Finnish DPS is a multicenter randomized controlled trial designed to investigate whether lifestyle intervention aimed at increasing physical activity, improving diet, and decreasing body weight reduces the risk of developing type 2 diabetes in high-risk individuals. The DPS study design has been described in detail elsewhere (17). Briefly, the study population consisted of 522 men and women 40–65 years of age who were overweight or obese (BMI $\geq$25 kg/m^2) and had IGT, defined as 2-h plasma glucose 140–200 mg/dl (7.8–11.0 mmol/l) in an oral glucose tolerance test (OGTT) and fasting plasma glucose <140 mg/dl (<7.8 mmol/l) at baseline. The study protocol was approved by the ethics committee of the National Public Health Institute in Helsinki, Finland, and all study participants gave written informed consent.

Intervention. The study participants were randomly assigned to the intervention group (n = 265) or the control group (n = 257) (3). The subjects in the intervention group were given detailed advice on how to achieve the goals of the intervention, which were *1*) moderate to vigorous exercise for $\geq$30 min per day, *2*) a reduction in intake of fat to <30% of total energy intake, *3*) a reduction of intake of saturated fat to <10% of total energy intake, *4*) an increase in fiber intake to $\geq$15 g per 1,000 kcal, and *5*) a reduction in body weight of $\geq$5%. The subjects in the intervention group were individually guided to increase their overall level of physical activity. This was done by the nutritionist during the dietary counseling sessions and highlighted by the study physicians at the annual visits. Endurance exercise was recommended to increase aerobic capacity and cardiorespiratory fitness. Supervised, progressive, individually tailored circuit-type moderate-intensity resistance training sessions to improve the functional capacity and strength of the large muscle groups of the upper and lower body were also offered free of charge beginning 4–6 months after the randomization. At baseline, the control group was given general information about lifestyle and diabetes risk. This was done either individually or in one group session, and some printed material was delivered.

Clinical, lifestyle, and anthropometric assessments. At baseline and at each annual visit, all subjects underwent a 2-h OGTT and completed the validated KIHD (Kuopio Ischemic Heart Disease Risk Factor Study) 12-month Leisure Time Physical Activity Questionnaire (7,18) and a 3-day food diary (5). Measurements of height, weight, waist circumference, and blood pressure have been described in detail previously (17).

Blood sampling and laboratory measurements. Blood was taken from the antecubital vein with the participant in a sitting position and allowed to clot at room temperature for 30–60 min. After centrifugation at 8,000–11,000*g*, sera were stored at −70°C for future analyses, although for logistic reasons, storage at −20°C was allowed for a maximum of 3 months. Concomitant analyses of similarly handled samples of recent versus past population-based surveys (MONICA [Monitoring of Trends and Determinants in Cardiovascular Disease]/Cooperative Health Research in the Region of Augsburg [KORA; Kooperative Gesundheitsforschung in der Region Augsburg]) did not reveal loss of signals over time (14,19). Serum concentrations of CRP and SAA were assessed by a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany) (20) and by immunonephelometry (21), respectively. Interleukin (IL)-6 concentrations in serum were determined by enzyme-linked immunosorbent assay, using recombinant IL-6 and an antibody pair from Sanquin (Amsterdam) (19). Circulating concentrations of sICAM-1, MIF, and RANTES were assessed in serum samples, using commercially available enzyme-linked immunosorbent assays from Diaclone (Besancon, France; for sICAM-1) or R&D Systems (Wiesbaden, Germany; for MIF and RANTES). Plasma glucose was measured by means of standard methods, as described in detail previously (3). The serum insulin concentration was measured by radioimmunoassay (Pharmacia, Uppsala, Sweden), and serum levels of total, LDL, and HDL cholesterol were determined by enzymatic assay in the central laboratory in Helsinki.

Determination of diabetes. Diabetes was defined according to the 1985 criteria of the World Health Organization (22) as either a fasting plasma glucose concentration $\geq$7.8 mmol/l or a plasma glucose concentration $\geq$11.1 mmol/l at 2 h after a 75-g oral glucose challenge. Participants were asked to fast and to refrain from strenuous exercise for 12 h before the OGTT. If the diagnosis of diabetes was not confirmed by a second 2-h OGTT, the subject continued in the study (3). The current analyses are based on a mean follow-up period of 3.9 years.

Statistical analysis. Baseline characteristics of study participants are given as frequency (for sex), means ± SD (for age, BMI, waist circumference, fasting glucose, 2-h glucose, fasting insulin, homeostasis model assessment [HOMA] of insulin resistance, LDL and HDL cholesterol, and systolic and diastolic blood pressure), or median and interquartile range (IQR; for RANTES, MIF, IL-6, CRP, SAA, and sICAM-1) and were compared using the χ^2 test, unpaired Student's t test (two-tailed), or Mann-Whitney test, respectively. Insulin resistance measured by HOMA was calculated as follows: HOMA of insulin resistance = fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5. Baseline correlations between immunological markers and other variables were assessed by using Pearson's coefficients for correlation. Because immunological markers were not normally distributed, log-transformed values for these variables were used. Relative risks for developing type 2 diabetes in the middle and highest tertiles of immunological markers, the lowest tertile as the reference group, were assessed with Cox proportional hazards models adjusted for age, sex, BMI, and 2-h glucose at baseline. Changes in BMI, waist circumference, LDL and HDL cholesterol, insulin, as well as systolic and diastolic blood pressure during the 1st study year in the tertiles of immunological markers were assessed with ANCOVA adjusted for age, sex, and BMI at baseline. All of these analyses were performed separately in the control and intervention groups. Associations with P values <0.05 were considered statistically significant. In addition, to take into account the multiple tests performed, we have provided adjusted P values according to Holm's step-down Bonferroni procedure (23). Statistical analyses were performed, using the Stata statistics package (release 8.0; StataCorp, College Station, TX).

RESULTS

Baseline characteristics of study participants. The subjects in the intervention group had significantly higher systolic blood pressure (P = 0.026) and higher circulating SAA concentrations (P = 0.014) than those in the control group, but they did not differ significantly regarding any other baseline variable tested (Table 1). In all subjects, among the immunological markers, the strongest correlations were found between CRP, SAA, IL-6, and sICAM-1 (Table 2). RANTES and MIF were also correlated with CRP and SAA but not with IL-6, sICAM-1, or each other. CRP, SAA, IL-6, sICAM-1, and RANTES were correlated with BMI, whereas their correlations with waist circumference were weaker.

Incidence of type 2 diabetes according to levels of immunological markers. In the control group, incidence rates for type 2 diabetes were higher in the middle tertile ($P < 0.05$) and in the highest tertile ($P < 0.1$) of CRP than in the lowest tertile after adjustment for age, sex, and BMI at baseline and after taking into account two tests within this group (Table 3). In the intervention group, the incidence rates between CRP tertiles did not differ significantly, but they increased significantly with increasing RANTES levels and decreased significantly with increasing MIF levels (Table 3). The association between RANTES and diabetes incidence remained significant when adjusted for multiple testing, whereas the significance for the association between MIF and diabetes incidence was lost.

As shown in Table 4, in the intervention group, the subjects in the highest tertile of RANTES had a 2.6 times higher risk of developing type 2 diabetes and the subjects in the highest tertile of MIF a 65% lower risk of type 2 diabetes than those in the lowest tertiles after adjusting for age, sex, BMI, 2-h glucose, and other immunological markers. CRP was not associated with the risk of type 2 diabetes in the intervention group. In the control group, the subjects in the middle tertile of CRP had a higher risk of type 2 diabetes and the subjects in the highest tertile also tended to have a higher risk than those in the lowest CRP tertile. RANTES or MIF were not associated with the risk of type 2 diabetes in the control group (Table 4). The associations between incidence and immune markers (intervention group: RANTES and MIF; control group: CRP) were significant by descriptive statistics and lost significance when adjusted for multiple testing.

The independent associations of RANTES and MIF with the risk of diabetes led us to assess whether the ratio of RANTES to MIF would be of predictive relevance. In the intervention group, the subjects in the highest tertile of the RANTES-to-MIF ratio had an approximately threefold in-

TABLE 1
Baseline characteristics of the study participants

	Intervention group		Control group	
	n	Mean or median	*n*	Mean or median
Sex (M/F)	91/174	—	81/176	—
Age (years)	265	55.4 ± 7.3	257	54.9 ± 7.0
BMI (kg/m^2)	265	31.4 ± 4.5	257	31.1 ± 4.5
Waist (cm)	264	102.0 ± 11.0	256	100.5 ± 10.9
Glucose at 0 h (mmol/l)	265	6.1 ± 0.8	257	6.2 ± 0.7
Glucose at 2 h (mmol/l)	265	8.9 ± 1.5	257	8.9 ± 1.5
Insulin at 0 h (mU/l)	240	14.7 ± 7.3	235	14.9 ± 7.7
HOMA for insulin resistance	240	4.1 ± 2.5	235	4.2 ± 2.4
LDL cholesterol (mmol/l)	262	3.6 ± 0.9	257	3.6 ± 0.8
HDL cholesterol (mmol/l)	264	1.20 ± 0.31	257	1.22 ± 0.28
Systolic blood pressure (mmHg)	264	139.7 ± 17.7*	253	136.2 ± 17.5
Diastolic blood pressure (mmHg)	264	85.7 ± 9.3	253	85.7 ± 10.1
RANTES (ng/ml)	262	52.2 (38.7–81.3)	252	54.2 (35.4–81.3)
MIF (pg/ml)	262	6,202 (4,234–9,135)	252	5,828 (3,772–8,931)
IL-6 (pg/ml)	262	1.88 (1.09–2.83)	252	1.64 (1.08–2.51)
CRP (mg/l)	253	2.28 (1.12–5.02)	245	1.89 (0.95–4.27)
SAA (mg/l)	255	4.6 (3.0–7.4)*	245	4.1 (2.9–5.8)
sICAM-1 (ng/ml)	256	928 (768–1,126)	248	933 (798–1,108)

Data are means ± SD or median (interquartile range). *$P < 0.05$ compared with control subjects.

creased risk of progression to type 2 diabetes compared with those in the middle or lowest tertiles (78.2, 27.2, and 22.4 cases per 1,000 person-years, respectively; $P = 0.006$ for trend) after adjusting for age, sex, and BMI at baseline (Fig. 1). In the control group, the association between RANTES-to-MIF ratio and the risk of type 2 diabetes was weaker and nonsignificant ($P = 0.088$) because the incidence rates were much higher in the lowest and middle tertiles of the RANTES-to-MIF ratio in the control group than in the intervention group. Further adjustment for CRP, 2-h glucose, LDL and HDL cholesterol, and systolic blood pressure had no effect on the association in the intervention group. RANTES-to-MIF ratio was not correlated with age, waist circumference, 2-h glucose, fasting insulin, LDL or HDL cholesterol, systolic or diastolic blood pressure, CRP, SAA, IL-6, or sICAM-1 in the intervention group or the control group ($|r| \leq 0.1$, $P > 0.05$) (data not shown). There was only a weak correlation between RANTES-to-MIF ratio and BMI in the intervention group ($r = 0.139$).

Inflammatory and immunological markers and 1-year changes in components of the metabolic syndrome. BMI and insulin decreased less and systolic and diastolic blood pressure tended to decrease less during the 1st year of intervention in the subjects with the highest RANTES-to-MIF ratio. On the other hand, HDL cholesterol increased most in those with the highest RANTES-to-MIF ratio (data not shown). Whereas insulin decreased less among subjects in the lowest tertile of MIF than in the other groups (−0.65, −2.90, and −2.33 mU/l for lowest, middle, and highest tertiles, respectively; $P = 0.025$ for difference), CRP or RANTES levels did not modify the

TABLE 2
Correlation of systemic immune mediator concentrations with clinical, biochemical, and immunological markers for all study participants (intervention and control groups combined)

Variable	RANTES	MIF	IL-6	CRP	SAA	sICAM-1
Age	0.007	0.011	0.053	−0.036	0.042	0.019
BMI	0.112*	0.023	0.211*	0.402*	0.237*	0.168*
Waist	0.016	0.026	0.160*	0.266*	0.069	0.111*
Glucose at 0 h	0.020	−0.056	−0.015	0.069	0.040	0.005
Glucose at 2 h	0.108*	−0.040	0.010	0.144*	0.088*	−0.014
Insulin at 0 h	0.026	−0.038	0.137*	0.086	0.062	0.176*
HOMA for insulin resistance	0.025	−0.041	0.124*	0.088	0.052	0.162*
LDL cholesterol	0.078	0.035	−0.011	−0.057	0.022	−0.001
HDL cholesterol	0.065	0.032	0.015	0.078	0.223*	−0.020
Systolic blood pressure	0.022	0.049	0.072	0.082	0.146*	0.102*
Diastolic blood pressure	0.018	0.001	0.008	0.084	0.099*	0.068
RANTES	1	−0.014	0.039	0.205*	0.152*	0.037
MIF	—	1	0.002	0.101*	0.091*	0.073
IL-6	—	—	1	0.276*	0.271*	0.151*
CRP	—	—	—	1	0.636*	0.192*
SAA	—	—	—	—	1	0.207*
sICAM-1	—	—	—	—	—	1

Correlation is assessed by Pearson's correlation coefficients. Immune marker concentrations were log transformed due to non-Gaussian distribution. *$P < 0.05$.

TABLE 3
Incidence rates of type 2 diabetes per 1,000 person-years by tertiles of systemic immune mediator concentrations

	Tertile 1 (low)	Tertile 2 (intermediate)	Tertile 3 (high)	*P* (trend)	$P_{adj.}$ (trend)
RANTES					
Intervention	21.9	39.3	64.0*	0.008	0.048
Control	74.2	47.3	98.9	0.254	1.000
MIF					
Intervention	53.4	43.7	22.2†	0.039	0.195
Control	80.3	74.3	62.5	0.341	1.000
IL-6					
Intervention	36.8	40.3	41.2	0.795	1.000
Control	75.5	71.9	68.9	0.269	1.000
CRP					
Intervention	29.8	42.8	42.9	0.779	1.000
Control	45.5	92.8*	82.2†	0.114	0.684
SAA					
Intervention	38.8	46.8	30.4	0.244	0.976
Control	61.9	75.9	80.3	0.437	1.000
sICAM-1					
Intervention	43.0	42.0	31.1	0.396	1.000
Control	83.3	66.9	65.4	0.316	1.000

Tertiles of intervention group or control group contain ~85 subjects. *P* values for the linear trend were adjusted for sex, age, and BMI at baseline. *$P < 0.05$; †$P < 0.10$ in comparison to tertile 1 taking into account two tests within a treatment group. $P_{adj.}$, *P* value for the linear trend adjusted for 6 multiple tests within a treatment group, using Holm's step-down Bonferroni procedure.

effect of lifestyle intervention on the components of the metabolic syndrome (data not shown).

DISCUSSION

In recent years, prospective population-based studies have found associations of mildly elevated plasma levels of acute-phase proteins, cytokines, and soluble adhesion molecules with an increased incidence of type 2 diabetes (rev. in 24,25). Whether these inflammatory and immunological makers predict the risk of incident type 2 diabetes and responses to lifestyle interventions in individuals with the metabolic syndrome remains unknown. This question is an important public health issue because targeting individuals with the highest risk for type 2 diabetes and who are most responsive to lifestyle changes would be the most cost-effective strategy for prevention of type 2 diabetes.

TABLE 4
Association of immunological variables with type 2 diabetes incidence

Variable	Tertile parameters	Hazard ratio	95% CI	*P*	$P_{adj.}$
Intervention group					
RANTES (ng/ml)					
1st tertile	8.9–42.9	1.00	—	—	—
2nd tertile	43.4–69.0	1.46	0.54–3.99	0.455	1.000
3rd tertile	70.1–893.5	2.59	1.06–6.37	0.038	0.190
MIF (pg/ml)					
1st tertile	158–4,910	1.00	—	—	—
2nd tertile	4,923–8,381	0.81	0.37–1.74	0.585	1.000
3rd tertile	8,394–44,231	0.35	0.14–0.90	0.029	0.174
CRP (mg/l)					
1st tertile	0.19–1.44	1.00	—	—	—
2nd tertile	1.45–3.97	0.96	0.39–2.36	0.925	1.000
3rd tertile	4.01–42.8	0.98	0.37–2.59	0.963	1.000
Control group					
RANTES (ng/ml)					
1st tertile	6.4–39.7	1.00	—	—	—
2nd tertile	41.0–69.8	0.82	0.41–1.61	0.562	1.000
3rd tertile	70.7–719.0	1.48	0.83–2.64	0.183	0.732
MIF (pg/ml)					
1st tertile	220–4,604	1.00	—	—	—
2nd tertile	4,608–7,270	0.85	0.48–1.53	0.595	1.000
3rd tertile	7,292–82,480	0.87	0.49–1.55	0.635	1.000
CRP (mg/l)					
1st tertile	0.17–1.17	1.00	—	—	—
2nd tertile	1.18–3.51	2.48	1.22–5.00	0.012	0.072
3rd tertile	3.56–39.6	1.94	0.86–4.37	0.109	0.545

Cox regression model using tertiles of RANTES, MIF, and CRP (the first tertile is the reference group). The model also included age, sex, BMI, and 2-h glucose. $P_{adj.}$, *P* value adjusted for 6 multiple tests within a treatment group, using Holm's step-down Bonferroni procedure.

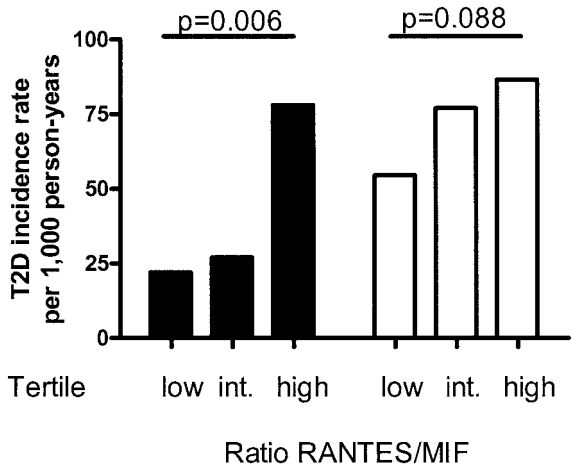

FIG. 1. Incidence rates of type 2 diabetes (T2D) per 1,000 person-years according to tertiles of RANTES-to-MIF ratio. Tertiles of the intervention group (■) and control group (□) contain ~85 subjects. Data for linear trend were adjusted for age, sex, and BMI at baseline. *P* values were adjusted taking into account two multiple tests, using Holm's step-down Bonferroni procedure. int., intermediate.

We therefore investigated the relevance of immune markers as risk factors for the progression from the metabolic syndrome to overt type 2 diabetes in individuals participating in the Finnish DPS. Because of the randomization of participants in this prospective trial into two groups differing in lifestyle, it was also possible to compare immune parameters at baseline and the impact of a healthier lifestyle on the progression to diabetes. First, we found that in the group with little changes in lifestyle (control group), elevated levels of CRP were associated with increased type 2 diabetes incidence. Elevated CRP is by far the best characterized immunological risk factor for incident type 2 diabetes because international standards for the assessment of CRP levels exist (26,27) and because this association could be confirmed in a range of independent studies (9–12,14,15). Previous studies have shown that elevated CRP levels indicate increased risk of type 2 diabetes in population-based cohorts (8–10). In another population-based study, the KORA Survey 2000, we demonstrated that concentrations of CRP and IL-6 are positively associated with obesity and already significantly elevated in IGT compared with normoglycemic subjects, whereas the difference between IGT and type 2 diabetic patients was relatively small (19). Keeping in mind that the KORA Survey 2000 was a cross-sectional survey, these data are in line with the hypothesis that elevated CRP levels may be more informative regarding increased type 2 diabetes risk in a relatively healthy (i.e., at least in general not obese and with normal glucose tolerance) cohort compared with a high-risk study sample as selected for the Finnish DPS. Because CRP levels are associated with insulin resistance and increased BMI also in nondiabetic individuals, there does not seem to be a direct association between CRP and diabetes per se. In the current study, serum concentrations of CRP still provide information about the risk of progression to diabetes, although systemic CRP and IL-6 levels are already elevated in individuals with IGT. No such information could be gained from IL-6 levels here, which may in part be attributable to the limited statistical power in the current study.

A surprising finding was that baseline CRP levels were not associated with diabetes outcome in the lifestyle intervention group. Although it cannot be excluded that this finding was due to chance, it is tempting to consider that lifestyle intervention is effective in individuals with high baseline CRP levels, i.e., that individuals with higher CRP might be more responsive to the protective effects of lifestyle changes. This would fit with the observation that increased physical activity and loss of body weight indeed decreases systemic CRP levels (rev. in 25, and 28,29).

Instead of CRP, we identified two novel immune markers that predicted progression from IGT to type 2 diabetes in the lifestyle intervention group, RANTES and MIF. This finding persisted after adjusting for the potential confounding variables age, sex, BMI, postchallenge glucose levels, and CRP, but it was attenuated when correcting for multiple testing. In the intervention group, subjects with low RANTES and high MIF levels had a lower diabetes risk than subjects with high RANTES-to-MIF ratio and thus appeared to benefit more from lifestyle intervention. The observation that predictive immune parameters differed between "natural" progression and intensive lifestyle intervention suggests an interaction between the immune system and lifestyle intervention. Support for this notion comes from the observation that subjects with high RANTES-to-MIF ratio exhibited in general a less favorable development of metabolic parameters in the 1st year of the study compared with subjects in the low and intermediate tertiles, who were also characterized by lower type 2 diabetes incidence. RANTES and MIF may therefore be useful markers for the immunological identification of a subset of high-risk individuals who respond better or in a less pronounced way to lifestyle changes.

The interaction of MIF levels with lifestyle changes may occur at the level of β-cell function (30). MIF is strongly expressed in β-cells and has been reported to be secreted together with insulin from β-cells. Immunohistochemical studies confirmed the presence of MIF in insulin granula (31). Moreover, MIF acts as an autocrine factor promoting insulin production and release. MIF production is stimulated by high glucose concentrations, whereas neutralization of MIF or inhibition of MIF gene expression impaired glucose-induced insulin secretion (31–33). Taken together, high MIF levels may promote β-cell function. By contrast, high RANTES levels have been observed to be associated with severe inflammation. RANTES plays important roles in allergic inflammation (34) and leukocyte recruitment to atherosclerotic lesions (35). Moreover, RANTES appears to be involved in inflammation of the central nervous system, and certain RANTES gene polymorphisms increase the risk for multiple sclerosis (36–37). Support for the apparent opposing association of MIF and RANTES with diabetes risk comes from the finding that the ratio of concentrations of RANTES divided by MIF was also associated with diabetes risk. These findings are suggestive, but confirmation from independent cohorts should be awaited.

Regarding limitations and strengths of our study, it needs to be mentioned that it is based on relatively low numbers of study participants and incident cases. Because of the limited power, we might have missed associations of the investigated markers with incident type 2 diabetes. However, it appears unlikely that the small sample size was the reason for the significant associations of RANTES and MIF with diabetes risk. Our analyses were explorative and hence required multiple analyses. When formally correcting for multiple testing, *P* values were attenuated, and the associations between immune markers and diabe-

tes incidence lost significance in some cases without affecting the overall message. The current study has the advantage that diabetes diagnosis relied on OGTTs according to World Health Organization standards, so that there were probably no undetected cases at baseline, which would have led to incorrect estimations of the impact of baseline immune marker levels on diabetes risk.

In summary, the data suggest that increased levels of RANTES and decreased levels of MIF are associated with increased risk of developing type 2 diabetes in the intervention group of the DPS, and they identify high-risk patients who tend to be resistant to lifestyle intervention. The assessment of inflammatory and immunological markers may provide additional information about the risk of developing type 2 diabetes beyond traditional risk factors.

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