# Low Production Capacity of Interleukin-10 Associates With the Metabolic Syndrome and Type 2 Diabetes

## The Leiden 85-Plus Study

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It has been suggested that the metabolic syndrome and type 2 diabetes are manifestations of the inflammatory host response. This host response is orchestrated by the production of pro- and anti-inflammatory cytokines that are under genetic control. We therefore hypothesized that a low production capacity of interleukin-10 (IL-10), a centrally operating cytokine with strong anti-inflammatory properties, associates with the metabolic syndrome and type 2 diabetes in old age. In the current study, 599 inhabitants of the city of Leiden, age 85 years, were visited at their place of residence. The production capacity of the anti-inflammatory cytokine IL-10 was assessed in a whole-blood assay in which lipopolysaccharide was used as a stimulus. Serum concentrations of lipids, lipoproteins, glucose, and HbA$_{1c}$ were determined, and a history of type 2 diabetes was obtained. Serum concentrations of total cholesterol, LDL cholesterol, triglycerides, glucose, and HbA$_{1c}$ gradually decreased over strata representing higher IL-10 production capacity, whereas the concentration of HDL cholesterol gradually increased (all $P < 0.01$). The odds ratio for type 2 diabetes was 2.7 (95% confidence interval 1.5–4.9) when subjects with the lowest IL-10 production capacity were compared with those with the highest IL-10 production capacity. These findings showed that low IL-10 production capacity (i.e., a pro-inflammatory response) is associated with the metabolic syndrome and type 2 diabetes. *Diabetes* 51: 1088–1092, 2002

**T**he metabolic syndrome is a convergence of dyslipidemia, impaired glucose tolerance, and hypertension (1). It has been found that ~70% of all obese adults have at least one of the major characteristics of the syndrome (2). It has long been recognized that the clustering of these risk factors carries an increased risk of type 2 diabetes and cardiovascular disease (3,4).

Insulin resistance has been proposed as the underlying cause for this metabolic and cardiovascular syndrome, although its molecular basis has not yet been identified (3). One of the biological mechanisms that may be involved is the innate immune system (5,6). Several studies have shown that markers of inflammation, such as C-reactive protein (7), fibrinogen (8), and pro-inflammatory cytokines such as interleukin-6 (9−11) and tumor necrosis factor-α (TNF-α) (5,9,11), associate with the metabolic syndrome, type 2 diabetes, and dyslipidemia. In particular, it has been suggested that TNF-α is associated with insulin resistance and type 2 diabetes, given that TNF-α down-regulates the tyrosine kinase activity of the insulin receptor (5,12,13). The infusion of anti−TNF-α antibodies in patients with type 2 diabetes, however, has been shown to have no effect on those patients' insulin sensitivity (14); this observation raises doubts about the contribution of TNF-α to type 2 diabetes and the metabolic syndrome.

Interleukin (IL)-10 is a centrally operating anti-inflammatory cytokine that plays a crucial role in the regulation of the innate immune system. It has strong deactivating properties on the inflammatory host response mediated by macrophages and lymphocytes, and potently inhibits the production of pro-inflammatory cytokines such as IL-6 and TNF-α (15−17). IL-10 is produced by T-cells, B-cells, monocytes, and macrophages, and is under tight genetic control, with heritability estimates as high as 75% (18). We therefore propose that low IL-10 production capacity is associated with the metabolic syndrome and type 2 diabetes. To this end, in the Leiden 85-Plus Study, we analyzed the relation among IL-10 production capacity (using a standardized whole-blood assay), dyslipidemia, and parameters of glucose metabolism.

## RESEARCH DESIGN AND METHODS

**Subjects.** The Leiden 85-Plus Study is a population-based study of inhabitants of Leiden, The Netherlands. From 1997 to 1999, all members of the 1912 to 1914 birth cohort ($n = 705$) were enrolled in the month of their 85th birthday. There were no selection criteria based on health or demographic characteristics. Those who were eligible for the study were informed by mail. They were then contacted by telephone or were visited at home and asked for informed consent. When subjects were cognitively impaired, informed consent was obtained from a guardian. The Medical Ethical Committee of the Leiden University Medical Center approved the study. Subjects were visited three times at their place of residence. At these visits, face-to-face interviews were conducted, an electrocardiogram was performed, and the subject's BMI was obtained. All blood samples were collected early in the morning under

TABLE 1
Clinical characteristics of the study sample

| | |
|---|---|
| $n$ | 553 |
| Female/male | 370/183 |
| Age (years) | 85 |
| Type 2 diabetes | |
|    All | 89 (16) |
|    Use of sulphonylureas or biguanides | 49 (9) |
|    Use of insulin | 8 (1) |
| BMI (kg/m$^2$) | 27.2 (26.8–27.7) |
| Use of nonsterodial anti-inflammatory drugs | 152 (28) |

Data are $n$ or $n$ (%), unless otherwise noted. BMI shown as mean (95% CI).

nonfasting conditions. In addition, information on the use of medication was obtained from the subject's pharmacist.

**Production capacity of IL-10 and TNF-α.** The production capacity of IL-10 and TNF-α was assessed with a standardized whole-blood assay (19). The methods by which whole-blood samples were stimulated with 10 ng/ml of lipopolysaccharide (LPS) have been described elsewhere, including data on reproducibility (19). In short, heparinized whole blood was diluted twofold with RPMI-1640. LPS (endotoxin, 10 ng/ml) was used as the primary stimulus. After the addition of LPS, samples were incubated for 4 or 24 h at 37°C and 5% $CO_2$. After centrifugation, the supernatants were stored at $-80$°C until being assayed for the pro-inflammatory cytokine TNF-α in the 4-h samples, and the anti-inflammatory cytokine IL-10 in the 24-h samples. The production capacity of IL-10 and TNF-α was assayed using standard enzyme-linked immunosorbent assay techniques. Unstimulated baseline samples were obtained to serve as a control for contamination. Subjects with detectable TNF-α under unstimulated conditions (TNF-α >100 pg/ml) were excluded from further analysis (19,20). The coefficients of variation for the day-to-day variation in the whole blood stimulation ranged from 8 to 12%. The intra-individual variation was 15% for TNF-α production capacity and 19% for IL-10 production capacity (19).

All subjects were grouped into three equal strata representing decreasing IL-10 production capacity or increasing TNF-α production capacity. This was done separately for women and men, as women have lower IL-10 and TNF-α production capacity than men. The advantage of this stratification is that it intrinsically adjusts for gender-based differences.

**Lipids and lipoproteins.** Total cholesterol and triglycerides levels were analyzed on a fully automated Hitachi 747. HDL cholesterol was measured using a Hitachi 911. LDL cholesterol was estimated using the Friedenwald equation (21). We excluded five subjects with a triglyceride concentration >5 mmol/l.

**Glucose metabolism and type 2 diabetes.** HbA$_{1c}$ and glucose concentrations were determined in serum. Subjects were classified as having type 2 diabetes when they met at least one of the following criteria: *1*) history of type 2 diabetes obtained from the general practitioner or the subject's treating physician; *2*) use of sulfonylureas, biguanides, or insulin, based on information obtained from the subject's pharmacist; or *3*) nonfasting glucose of ≥11.1 mmol/l. In all, 8 subjects had a previous diagnosis of type 2 diabetes and used insulin (median age of diagnosis 72 years, range 61–82 years), and 10 subjects were newly diagnosed as having type 2 diabetes, based on nonfasting glucose levels of ≥11.1 mmol/l. However, these newly diagnosed subjects did not fulfill all of American Diabetes Association criteria for type 2 diabetes, as it was unknown whether these subjects had symptoms of diabetes (22).

**Data analysis.** The primary outcome measures were the serum concentrations of glucose, HbA$_{1c}$, lipids, and lipoproteins, expressed as means with a corresponding 95% confidence interval (CI). We used the one-way ANOVA procedure to determine the $P$ value for trend over strata of IL-10 production capacity. Univariate odds ratios for type 2 diabetes over strata of IL-10 production capacity and the corresponding 95% CI were obtained by cross-tabulation. Multivariate odds ratios were obtained by logistic regression analysis. We tested for trend using the log-likelihood statistic, with one degree of freedom.

## RESULTS

Between 1 September 1997 and 1 September 1999, 705 inhabitants of Leiden reached age 85 years and were eligible to participate in the study. Of those 705, 14 died before they could be enrolled in the study. The total response rate was 87% (599 of 691 subjects; 397 women and 202 men). There were no statistical significant differences between the 599 participating subjects and the source population with respect to the following characteristics: gender, marital status, socioeconomic status, and mortality rate.

IL-10 production capacity could not be determined in 7 subjects who died before a blood sample could be drawn, and 30 subjects refused to give a blood sample. Under unstimulated conditions, nine subjects had detectable TNF-α concentrations (>100 pg/ml) that were suspect for contamination of the whole-blood system and were therefore excluded from the analyses (19,20). Table 1 shows the demographic and clinical characteristics of the 553 subjects included in the present analysis. After stimulation with LPS in whole blood samples, the mean IL-10 concentration was 945 pg/ml (CI 861–1,030 pg/ml) in men and 799 pg/ml (CI 750–884 pg/ml) in women (Student's $t$ test, $P = 0.002$).

**IL-10, lipids, lipoproteins, glucose, and HbA$_{1c}$** We divided subjects into three equal strata depending on their IL-10 production capacity, keeping men and women separate, to study the relation between IL-10 production and lipids, lipoproteins, glucose, and HbA$_{1c}$ (Table 2). The serum concentrations of total cholesterol, LDL cholesterol, triglycerides, glucose, and HbA$_{1c}$ gradually decreased over strata representing increasing IL-10 production capacity, whereas the concentration of HDL cholesterol gradually increased (all $P < 0.01$). There was no association between IL-10 production capacity and BMI. In an additional analysis, we ex-

TABLE 2
Effect of IL-10 production on lipids, lipoproteins, and glucose levels

| | IL-10 production | | | $P$ |
|---|---|---|---|---|
| | Low ($n = 184$) | Intermediate ($n = 185$) | High ($n = 184$) | |
| IL-10 pg/ml | 377 (358–396) | 763 (746–780) | 1402 (1331–1474) | — |
| BMI (kg/m$^2$) | 27.2 (26.4–27.9) | 27.6 (26.8–28.4) | 27.0 (26.3–27.6) | 0.7 |
| Total cholesterol (mmol/l) | 5.95 (5.77–6.12) | 5.67 (5.53–5.84) | 5.55 (5.41–5.71) | 0.001 |
| LDL-cholesterol (mmol/l) | 3.86 (3.71–4.02) | 3.67 (3.53–3.80) | 3.55 (3.42–3.69) | 0.002 |
| Triglycerides (mmol/l) | 1.77 (1.63–1.91) | 1.61 (1.48–1.74) | 1.36 (1.28–1.44) | <0.001 |
| HDL-cholesterol (mmol/l) | 1.28 (1.21–1.34) | 1.29 (1.23–1.34) | 1.39 (1.33–1.44) | 0.009 |
| Glucose (mmol/l) | 7.50 (7.09–7.91) | 7.08 (6.68–7.48) | 6.31 (6.00–6.61) | <0.001 |
| HbA1c (%) | 6.01 (5.83–6.19) | 5.82 (5.65–6.00) | 5.55 (5.46–5.65) | <0.001 |

Data are presented as means (95% CI). Production capacity of IL-10 was assessed in a whole blood stimulation and were grouped into three equal strata. This was done separately for women and men, as women have lower IL-10 production than men.
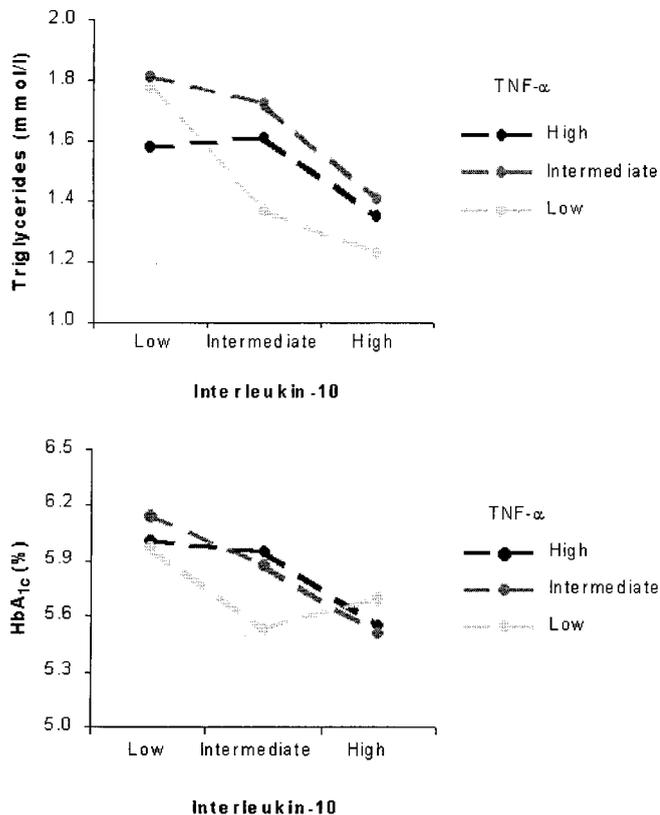
**FIG. 1. Mean concentrations of triglycerides and HbA$_{1c}$ in strata of IL-10 and TNF-$\alpha$. Levels of triglycerides and HbA$_{1c}$ over strata of IL-10 production, $P < 0.05$; levels of triglycerides and HbA$_{1c}$ over strata of TNF-$\alpha$ production, $P > 0.1$.**

cluded subjects with type 2 diabetes to ascertain that our findings were not only attributable to the effect of type 2 diabetes on IL-10 production capacity. The trends among IL-10 production capacity, lipids, lipoproteins, glucose, and HbA$_{1c}$ remained similar (all $P < 0.05$). The trend between IL-10 production capacity and parameters of the metabolic syndrome could also be distorted by the use of nonsteroidal anti-inflammatory drugs that are often prescribed at old age. In a restricted sample of 401 subjects who did not use nonsteroidal anti-inflammatory drugs, however, we could still obtain the statistical significant trends presented in Table 2. Those trends also remained statistically significant when we adjusted for TNF-$\alpha$ using linear regression.

In Fig. 1 we present the mean concentrations of triglycerides and HbA$_{1c}$ over strata of IL-10 and TNF-$\alpha$ production capacity to determine whether low, intermediate, or high TNF-$\alpha$ production capacity had an additional effect on these metabolic outcomes. In each stratum of TNF-$\alpha$ production capacity, there was a significant decrease of triglycerides and HbA$_{1c}$ when IL-10 production capacity increased ($P < 0.05$ for trend in each stratum). There was no significant increase of triglycerides and HbA$_{1c}$ when TNF-$\alpha$ production increased in any strata of IL-10 production capacity ($P > 0.1$ for trend in each stratum). Trends similar to those shown in Fig. 1 were obtained for total cholesterol, HDL cholesterol, LDL cholesterol, and glucose (data not shown).

**IL-10 and type 2 diabetes.** The proportion of subjects with type 2 diabetes gradually decreased over the strata representing a higher IL-10 production capacity ($P = 0.001$ for trend) (Table 3). The odds ratio for type 2 diabetes increased to 2.7 (CI 1.5–4.9) when subjects with the lowest IL-10 production capacity were compared to those with the highest IL-10 production capacity. The odds ratio for type 2 diabetes was slightly higher after adjustment for TNF-$\alpha$. In an additional analysis, we excluded the newly diagnosed diabetic subjects, as it was unknown whether these subjects had symptoms of diabetes and therefore fulfilled all American Diabetes Association criteria for a diagnosis of diabetes (22). The results shown in Table 3 were unaffected.

## DISCUSSION

This analysis of the Leiden 85-Plus Study showed that low IL-10 production capacity (i.e., a pro-inflammatory cytokine response) is associated with high plasma glucose, high HbA$_{1c}$, type 2 diabetes, and dyslipidemia. When the production capacity of IL-10 was taken into account, the production capacity of TNF-$\alpha$ added only little to these metabolic parameters.

**Possible mechanisms.** Pro-inflammatory cytokines have earlier been associated with the development of the metabolic syndrome and type 2 diabetes. Experimental studies in humans and animals have shown that treatment with pro-inflammatory cytokines induces hypertriglyceridemia and insulin resistance (9,12). TNF-$\alpha$ downregulates the tyrosine kinase activity of the insulin receptor, thereby increasing insulin resistance (5,12,13). Serum IL-6 and C-reactive protein concentrations are higher in subjects with the metabolic syndrome or type 2 diabetes compared with control subjects (10). Pro-inflammatory cytokines also contribute to dyslipidemia by increasing lipolysis (7,9). We hypothesize that IL-10 at least partly represents the effect of an anti-inflammatory response on the metabolic syndrome and type 2 diabetes, as studies on the innate immune system

TABLE 3
Odds ratios for type 2 diabetes in relation to IL-10 production

| | IL-10 production | | | |
| --- | --- | --- | --- | --- |
| | Low | Intermediate | High | $P$ |
| Type 2 diabetes | | | | |
| Present | 42 (23) | 29 (16) | 18 (10) | 0.001 |
| Absent | 142 (77) | 156 (84) | 166 (90) | — |
| Odds ratio | | | | |
| Crude | 2.7 (1.5–4.9) | 1.7 (0.9–3.2) | 1* | 0.001 |
| Adjusted | 3.4 (1.6–7.1) | 1.9 (1.0–3.6) | 1* | 0.001 |

Data are $n$ (%) or $n$ (95% CI). * Reference category. Adjustments for TNF-$\alpha$ production were made using logistic regression.

suggest that IL-10 is a key regulator and a powerful suppressor of the immune response (15). We hypothesize that high IL-10 levels prevent the development of the metabolic syndrome and type 2 diabetes by limiting the effects of the inflammatory response—that is, by counterregulating the effects of pro-inflammatory cytokines such as TNF-$\alpha$ and IL-6. This hypothesis is partly derived from our findings, which suggest that when the production capacity of IL-10 is taken into account, the production capacity of TNF-$\alpha$ adds little to markers of the metabolic syndrome, such as triglycerides and $HbA_{1c}$ High levels of IL-10 should theoretically cause an upregulation of tyrosine kinase activity of the insulin receptor and decrease lipolysis, by counterregulating the effects of TNF-$\alpha$ and IL-6 (5,7,9,12,13). Therefore, a high IL-10 production capacity could confer protection against the metabolic syndrome and type 2 diabetes, whereas a low IL-10 production capacity would predispose one to the metabolic syndrome and type 2 diabetes.

It has been questioned why the characteristics of the metabolic syndrome are so prevalent in humans, as these have such deleterious effects later in life. Evolutionary theories on aging can explain this paradox. To explain this phenomenon, it is critical to understand that the force of selection decreases with age (23). Therefore, pleiotropic genes that have beneficial effects early in life are favored by selection even if these genes have deleterious effects later in life (24). Selection for genes encoding for the metabolic syndrome fit within these theories. Subjects with a pro-inflammatory cytokine response (i.e., a low IL-10 production capacity) (18) and hypercholesterolemia (25,26) are relatively protected against infection early in life. In times when infant mortality from infectious disease was high, survivors were likely to have had an innate pro-inflammatory host response. The trade-off for this survival benefit is the pro-inflammatory host response that predisposes one for the metabolic syndrome, type 2 diabetes, and the development of atherosclerosis in later life.
**Limitations.** The cross-sectional nature of the relation between low IL-10 production capacity and the lipid and glucose metabolism is one limitation of our study. It is tempting to speculate that the association between IL-10 production capacity, the metabolic syndrome, and type 2 diabetes is causal, as we have previously shown in family studies of first-degree relatives and twins that as much as 75% of the variance in IL-10 production capacity in humans derives from genetic factors (18). Causality among IL-10 production capacity, the metabolic syndrome, and type 2 diabetes, however, can only be determined when these associations can be confirmed in healthy, first-degree relatives of subjects with the metabolic syndrome or type 2 diabetes, or by using a prospective design in younger subjects, with a long-term follow-up.

A second limitation could be that the blood samples were collected under nonfasting conditions. It is likely that we have thus underestimated the effect of IL-10 on glucose and triglyceride levels, as it could be argued that the relation among IL-10 production capacity, glucose, and triglycerides was diluted by nondifferential misclassification (i.e., misclassification of glucose and triglycerides independent of IL-10 production capacity). We found, however, that low IL-10 production capacity was associated with high glucose and high $HbA_{1c}$, suggesting that the association between IL-10 production capacity and glucose metabolism is real because postprandial changes in $HbA_{1c}$ are absent. Finally, we argue that it is necessary to determine fasting triglycerides when absolute levels of triglycerides are assessed for clinical purposes (i.e., to diagnose hypertriglyceridemia in individuals). However, this premise can be somewhat relaxed when we tried to elucidate the relation among inflammation, the metabolic syndrome, and type 2 diabetes in the population at large.

A final question that could arise is whether age 85 years is a rather late age to study the association among IL-10 production capacity, the metabolic syndrome, and type 2 diabetes, given that subjects age 85 years are long-term survivors from a far larger birth cohort. This does not alter the validity of the association among IL-10 production capacity, the metabolic syndrome, and type 2 diabetes, as found in the most elderly individuals. However, as with all studies, it would be valuable to demonstrate the same association in different populations, using different study designs and younger subjects.

**Conclusions.** We found an association between low IL-10 production capacity (i.e., a pro-inflammatory host response) and high serum glucose, high $HbA_{1c}$, type 2 diabetes, and dyslipidemia. We are not aware of other studies reporting on the effects of IL-10, a strong anti-inflammatory cytokine, on these metabolic parameters. Further studies need to be conducted to confirm the hypothesis that low IL-10 production capacity (i.e., a pro-inflammatory response) predisposes one to the metabolic syndrome and type 2 diabetes.

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