Treatment with Anakinra Improves Disposition Index But Not Insulin Sensitivity in Nondiabetic Subjects with the Metabolic Syndrome: A Randomized, Double-Blind, Placebo-Controlled Study

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Context: Obesity induces low-grade inflammation that may promote the development of insulin resistance. IL-1 is one of the key inflammatory factors.

Objective: The objective of the study was to demonstrate improvement of insulin sensitivity by blocking IL-1.

Design: This was a randomized, double-blind, crossover study.

Setting: The study was based on ambulatory care.

Participants: Participants included nondiabetic, obese subjects with the metabolic syndrome.

Intervention: Intervention included 150 mg anakinra sc once daily or matching placebo for 4 wk.

Main Outcome Measure: Insulin sensitivity as measured by euglycemic hyperinsulinemic clamp.

Results: A total of 13 of 19 subjects completed the study. Although anakinra treatment resulted in a significantly lower level of inflammation illustrated by a reduction in circulating C-reactive protein concentrations and leukocyte numbers, insulin sensitivity was not significantly different after anakinra treatment (2.8 \times 10^{-2} \pm 0.5 \times 10^{-2}) compared with placebo treatment (2.4 \times 10^{-2} \pm 0.3 \times 10^{-2} \mu mol/kg^{-1} \cdot min^{-1} \cdot pmol^{-1}, P = 0.15). Adipose tissue examination, performed to analyze local effects of IL-1 receptor antagonist, showed an increased influx of macrophages after treatment with anakinra most likely due to an injection site reaction caused by the vehicle (0.28 \pm 0.05 vs. 0.11 \pm 0.01 macrophages per adipocyte, P = 0.005). The differences in individual subject insulin sensitivity after anakinra as compared with placebo between subjects were negatively correlated with macrophage infiltration into the adipose tissue (r^{2} = 0.46, P = 0.01). The disposition index increased significantly after anakinra treatment (P = 0.04), reflecting an improvement in \beta-cell function.

Conclusions: Our results suggest that anakinra does not improve insulin sensitivity in obese, insulin-resistant, nondiabetic subjects. (J Clin Endocrinol Metab 96: 2119–2126, 2011)

The global prevalence of obesity is rapidly rising and paralleled by an increasing incidence of insulin resistance and type 2 diabetes mellitus. Although the pathophysiological basis underlying obesity-associated insulin resistance has not been fully unraveled, many reports suggest that chronic low-grade inflammation originating from adipose tissue represents an important link (1).

Abbreviations: CD68, Cluster of differentiation 68; CI, confidence interval; FABP4, fatty acid binding protein 4; HbA1c, glycosylated hemoglobin; hsCRP, high-sensitivity C-reactive protein; IL-1R, IL-1 receptor; IL-1Ra, IL-1 receptor antagonist; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator-activated receptor.
Storage of excess energy results in expansion of adipose tissue mass and an increase in adipocyte size. Concurrent with this expansion, oxygen delivery to the adipocyte is decreased and may eventually lead to microhypoxia and adipocyte death. Together with the enhanced proinflammatory profile of enlarged adipocytes (2), this results in the recruitment of macrophages that further amplify the inflammatory status of the adipose tissue (3). Proinflammatory cytokines such as TNFα and IL-6 that are released in increased amounts by adipocytes and activated macrophages of obese individuals can induce insulin resistance (4, 5).

The proinflammatory cytokine IL-1 is also involved in the development of diabetes mellitus. By inducing β-cell destruction, IL-1 contributes to the pathogenesis of type 1 diabetes mellitus (6). Furthermore, a positive association between IL-1 levels and the presence of obesity has been found (7). IL-1β induces insulin resistance in cultured human adipocytes (8) and primary rat hepatocytes (9), and IL-1β knockout mice are more insulin sensitive as their wild-type littermates (10, 11). Based on these observations, one might expect that blockade of IL-1β would improve insulin resistance in humans.

IL-1 exerts its effects by binding to the type 1 IL-1 receptor (IL-1R) (12). This results in recruitment of IL-1 receptor accessory protein, necessary for signal transduction (13). Activation of IL-1R leads to fever, synthesis of acute phase proteins, leukocytosis, thrombocytosis, and anemia (14). IL-1 receptor antagonist (IL-1Ra) inhibits IL-1 action by competing with IL-1 for binding to the IL-1R. Binding of IL-1Ra to the IL-1R does not result in recruitment of IL-1 receptor accessory protein and thereby blocks intracellular signaling (13).

Blocking the IL-1 effects in patients with type 2 diabetes mellitus by anakinra, a recombinant human IL-1Ra, has been shown to improve glycemic control (13). Whether IL-1 blockade has a beneficial effect on insulin sensitivity in nondiabetic subjects characterized by insulin resistance remains to be determined.

In the present study, we investigated whether blocking IL-1 by recombinant IL-1Ra in humans for 4 wk can improve insulin sensitivity in nondiabetic, obese subjects with the metabolic syndrome.

Subjects and Methods

Study design

Overweight subjects with the metabolic syndrome, yet no type 2 diabetes mellitus, were included in this randomized, double-blind, placebo-controlled, two-period, crossover study. Subjects were recruited by means of advertisements in a local newspaper and from the outpatient clinic of the Radboud University Nijmegen Medical Centre. We compared anakinra with matching placebo. Study medication was self-administered sc once daily for 4 wk. Between both treatment periods, there was a 4-wk washout. Because prior studies suggest that the conventional dose of 100 mg anakinra once daily is not sufficient in patients with high body weight (15), the dose was increased to 150 mg daily. This dose has been applied in patients with rheumatoid arthritis (16).

Anakinra was purchased from the regular manufacturer (Biovitrum, Stockholm, Sweden). The Pharmacy Department of Radboud University Nijmegen Medical Centre prepared all study medication and was responsible for the blinding and randomization procedure. Subjects were randomized in blocks of four in a 1:1 allocation ratio.

Participants

The study was approved by the institutional ethics review board and assessed by an independent monitor. Written informed consent was obtained from all subjects before randomization.

Inclusion criteria were age 18 yr or older, body mass index greater than 30 kg/m², and three or more characteristics of the metabolic syndrome according to the definition of the third report of the National Cholesterol Education Program’s Adult Treatment Panel (17). Exclusion criteria were the known presence of diabetes mellitus, a fasting plasma glucose 7.0 mmol/liter or higher, glycosylated hemoglobin (HbA1c) 6.2% or higher, immunodeficiency or immunosuppressive treatment, current use of antiinflammatory medication (≤100 mg aspirin per day was allowed), signs of current infection or treatment with antibiotics, a history of recurrent infections or tuberculosis, pregnancy or breast-feeding, a serum alanine aminotransferase or aspartate aminotransferase level of more than 3 times the upper limit of the normal range, a serum creatinine level higher than 130 μmol/liter, neutropenia (a leukocyte count of less than 2 × 10⁹/liter), or the presence of any other medical condition that might interfere with the current study protocol and inability to give informed consent.

Study procedures

At the end of both treatment periods, we performed a euglycemic hyperinsulinemic clamp (insulin infusion rate 360 pmol/m² min) (18) and on a separate day a 75-g oral glucose tolerance test.

A sc fat biopsy was obtained. All study procedures were performed after an overnight fast.

Subcutaneous adipose tissue biopsy

A sc adipose tissue biopsy was taken just before the clamp, about 30 min after a sc injection with placebo or anakinra to analyze local effects of the active substance (recombinant IL-1Ra) of anakinra. Biopsies were taken under local anesthesia (2% Lidocaine HCl) from an area that was not affected by a local injection site reaction caused by the vehicle of anakinra, about 10 cm lateral of the umbilicus using a Hepafix Luer lock syringe (Braun, Melsungen, Germany) and a 2.10- × 80-mm Braun medical Sterican needle (Braun). The adipose tissue was washed using a 0.9% normal saline solution. The adipose tissue was snap fro-
zen and stored at −80°C until further analysis or fixated in 4% paraformaldehyde for embedding in paraffin.

**Immunohistochemistry**

An antibody against the cluster of differentiation 68 (CD68; AbD Serotec, Kidlington, UK) was used to stain macrophages. Immunohistochemistry was performed as described earlier (19). Macrophage influx was quantified by counting the number of adipocytes and macrophages in four representative fields of adipose tissue. A mean number of 609 ± 24 adipocytes were counted per subject for each treatment period.

**RNA isolation and real-time PCR analysis**

Total RNA was extracted from sc adipose tissue using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA concentration was determined using the NanoDrop (NanoDrop Technologies, Wilmington, MA). cDNA synthesis was performed using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Real-time PCR was performed using Power-SYBR Green master mix and the 7300 real-time PCR system (Applied Biosystem, Warrington, UK). Expression of genes was normalized to β2M gene expression levels. Used primer sequences were: β2M, ATGAGATGCGCTGGGTGGC (sense), CCAAATGCGCATCTTCAAC (antisense); peroxisome proliferator-activated receptor (PPARγ), ATTGACCGAAGGCGATTCC (sense), TCTTCCATTACGGAGAG, ATCCAC (antisense); fatty acid binding protein 4 (FABP4), GAAAGCGATTCC (sense), TCTTCCATTACGGAGAG, ATCCAC (antisense); adiponectin, ATCGGTGAAACCGGAGTACC (sense), GCATGTGGGGATAGTAACGTAA (antisense).

**Biochemical analysis**

Glucose concentrations were measured using the oxidation method (Glucose Analyzer 2; Beckman Instruments Inc., Fullerton, CA). Plasma concentrations of total adiponectin, IL-6, IL-1Ra, high-sensitivity C-reactive protein (hsCRP), and proinsulin were determined using ELISA [R&D Systems, Minneapolis, MN; proinsulin: Millipore, Billerica, MA; hsCRP in-house developed ELISA by University Hospital Maastricht (20)]. Insulin levels were determined by a RIA. For C-peptide determination, chemiluminescence was used. Inter- and intraassay coefficients of variation were, respectively: adiponectin, 2.7 and 1.3%; IL-6, 3.3 and 5.0%; IL-1Ra, 5.0 and 8.0%; hsCRP, 4.7 and 3.8%; insulin, 9.7 and 4.7%; and proinsulin 4.7 and 5.6%.

Measurements of other parameters were performed in the clinical laboratory unit of the Radboud University Nijmegen Medical Centre.

**Statistical analysis**

We considered a 20% improvement of the primary end point, insulin sensitivity, as determined by euglycemic hyperinsulineemic clamp, as clinically relevant. Assuming a test-to-test correlation coefficient of 0.5 (paired tests) and a mean glucose infusion rate of 30 ± 7 μmol/kg/min would require a total of 11 subjects to detect a 20% change in insulin sensitivity with a power of 80% at a significance level of 0.05. Dropout rates were calculated using the Student’s t test. Correlation analysis was performed by regression analysis. Two-tailed P ≤ 0.05 was considered to denote significance. Data are presented as mean ± SEM.

**Results**

Nineteen of the 32 initially screened subjects underwent randomization and were enrolled in the study between June and October of 2009. A total of 13 subjects, nine females and four males, completed the study. Nine of the 13 subjects who completed the study used anakinra in the second treatment period. Two of the six dropouts used anakinra in the second treatment period. Two subjects were discontinued from the study due to an infection, one had an H1N1 infection (during anakinra treatment) and one had a wound infection (during placebo treatment in the first treatment period). Two subjects withdrew due to the physical load of the tests and two subjects withdrew due to injection site reactions (Fig. 1). Dropouts were significantly younger (P = 0.008), had a higher body mass index (P = 0.02), and had a larger waist circumference (P = 0.05) in comparison with study subjects that completed the study. All other parameters were similar between study subjects that withdrew or completed the study.

The trial was terminated after the last subject completed all studies in February 2010. Baseline characteristics of all subjects that completed the study are shown in Table 1.

Twelve of 13 subjects experienced local injection site reactions (Fig. 2B) of varying severity. No other adverse
events were observed during the study. Sufficiency of the intervention was assessed by measuring serum and adipose tissue levels of IL-1Ra. The serum levels of IL-1Ra 30 min after the last injection of anakinra or placebo were 73.5 ± 86 after anakinra and 0.7 ± 0.1 µg/liter after placebo, P < 0.001. Levels of IL-1Ra in adipose tissue lysates were 29.2 ± 7.5 after anakinra and 1.8 ± 0.4 ng/liter after placebo, corrected for protein levels, P < 0.05. There were no carry-over effects detected.

**Systemic inflammation**

Inasmuch IL-1Ra has been attributed with antiinflammatory effects (21), we measured several markers indicative of systemic inflammation. hsCRP levels (anakinra 3.57 ± 1.40 vs. placebo 5.37 ± 1.78 mg/liter, P = 0.05) (Fig. 3A) and leukocyte counts (anakinra 5.32 ± 0.41 vs. placebo 6.39 ± 0.57 × 10⁹/liter, P = 0.002) (Fig. 3B) were significantly reduced in study subjects after anakinra treatment.

**Insulin sensitivity and β-cell function**

Although participants were clearly insulin resistant as illustrated by an insulin sensitivity index of 2.4 × 10⁻² ± 0.3 × 10⁻² µmol/kg⁻¹·min⁻¹·pmol⁻¹ insulin after placebo treatment (normal range in lean subjects 4.0–7.0 × 10⁻² µmol/kg⁻¹·min⁻¹·pmol⁻¹ insulin) (22), treatment with anakinra for 4 wk did not result in an improvement in insulin sensitivity (anakinra 2.8 × 10⁻² ± 0.5 × 10⁻² vs. placebo 2.4 × 10⁻² ± 0.3 × 10⁻² µmol/kg⁻¹·min⁻¹·pmol⁻¹ insulin, P = 0.15) (Fig. 2A). Average HbA1c (anakinra 5.57 ± 0.08 vs. placebo 5.58 ± 0.09, P = 0.05) (Fig. 3C) and fasting proinsulin to insulin ratio (Fig. 3E) were also not significantly different between anakinra and placebo groups (P = 0.41) (Fig. 3D).
0.11 vs. placebo 5.62 ± 0.08%, P = 0.35) and fasting glucose (anakinra 5.34 ± 0.13 vs. placebo 5.46 ± 0.15 mmol/liter, P = 0.17) levels were unchanged after anakinra treatment. Inasmuch no stable isotopes were used during the clamp, we were unable to determine the effects of anakinra treatment on liver insulin sensitivity levels. Alternatively, liver insulin sensitivity was estimated by using data obtained during the oral glucose tolerance test (OGTT) (23). Liver insulin sensitivity was not different between placebo vs. anakinra treatment (P = 0.66).

The disposition index during the OGTT [change in insulin (0–30 min)/change in glucose (0–30 min)] improved after anakinra treatment, P = 0.04 (Fig. 3C), suggestive for a somewhat enhanced β-cell function. However, other parameters indicative for β-cell function including stimulated C-peptide levels (area under the curve during the OGTT) (Fig. 3D) and fasting proinsulin to insulin ratio (Fig. 3E) did not differ significantly between placebo and anakinra treatment. Raw OGTT data are represented in Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org.

Based on OGTT criteria, five of the study participants had impaired glucose tolerance. In this group no statistically significant differences in insulin sensitivity or β-cell function were found, perhaps based on the small sample size.

No significant differences in triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total cholesterol were present between placebo and anakinra treatment.

Adipose tissue
The systemic low-grade inflammatory reaction characteristic for obesity is mainly inflicted by inflammation in the adipose tissue (3). The inhibition of IL-1 by IL-1Ra (active substance of anakinra) is expected to reduce this inflammatory reaction. However, the vehicle of anakinra is known to induce an injection site reaction during the first weeks of treatment in a majority of patients (16). We performed fat biopsies to study the local effects of anakinra treatment in abdominal sc adipose tissue.

Immunohistochemical visualization of macrophages using CD68 staining revealed an increased number of macrophages in the adipose tissue after anakinra treatment (0.28 ± 0.05 vs. 0.11 ± 0.01 macrophages per adipocyte, P = 0.005) (Fig. 2C). In addition, IL-6 concentrations in adipose tissue tended to be higher after anakinra treatment (anakinra 26.9 ± 12.4 μg/g adipose tissue vs. placebo 5.7 ± 1.2 μg/g adipose tissue, P = 0.07), suggestive of an enhancement in inflammatory properties of the adipose tissue. Moreover, expression levels of adipogenic genes determined by quantitative PCR analysis in sc adipose tissue revealed significantly decreased levels of PPARγ (P = 0.03) and FABP4 (P = 0.04), whereas adiponectin tended to decrease (P = 0.07) after anakinra treatment.

The severity of the inflammatory reaction in the abdominal sc adipose tissue, reflected by the number of infiltrating macrophages after anakinra treatment, was negatively correlated with the difference in individual subject insulin sensitivity level after anakinra treatment (R² = 0.46, P = 0.01) (Fig. 2D).

Subgroup analysis, using an increase in macrophage influx of greater than 300% after anakinra treatment as a cutoff point, unveiled that in study subjects with low levels of infiltrating macrophages, insulin sensitivity after anakinra treatment improved significantly by 39 ± 9% [95% confidence interval (CI) 18–61%, P = 0.003] compared with placebo, whereas in subjects with more than a 3-fold increase in infiltrating macrophages, insulin sensitivity decreased by −14 ± 12% (95% CI −47 to 19%, P = 0.31). The average insulin sensitivity of subjects with low macrophage influx was 53% (95% CI 20−86%, P = 0.006) better as compared with subjects with high macrophage influx. Levels of circulating hsCRP were comparable in both groups. Adipose tissue IL-6 appeared lower in subjects with no or mild infiltration of macrophages compared with the subjects with extensive infiltration, although the difference did not attain statistical significance (13.7 ± 5.9 vs. 53.2 ± 34.4 μg/g adipose tissue, P = 0.14).

Discussion
The present study did not find an improvement in insulin sensitivity after 4 wk of treatment with the IL-1Ra anakinra in an insulin-resistant population. The selected study population enabled us to analyze the effects of an antiinflammatory intervention on insulin sensitivity without the potential interference of antidiabetic medication and the confounding effects of changing glycemic control.

In accordance with earlier findings in a population of type 2 diabetic patients (15), we were unable to detect an improvement in insulin sensitivity after anakinra treatment our study population. However, an animal study using XOMA 052 (anti-IL-1β antibody) has reported a positive effect on both insulin sensitivity and β-cell function (11). Another animal study using 1400.24.17 (anti-IL-1β antibody) showed reduced HbA1c after 18 wk of treatment without consistent improvements in insulin sensitivity and glucose tolerance (24). An important difference between the animal studies vs. the human trial of Larsen et al. (15) and our trial is the use of specific anti-
IL-1β antibodies in the animal trials. The human trials do use IL-1Ra, which has effects on both IL-1α and IL-1β.

There are a number of explanations for the lack of improvement in insulin sensitivity in our study.

First, circulating levels of IL-1Ra in study participants may vary considerably. It has been suggested that IL-1Ra levels at baseline determine the effects of anakinra treatment, with those with the lowest level showing the highest response (25). Nevertheless, in our study we were unable to find any association between IL-1Ra levels at baseline and changes in insulin sensitivity after anakinra treatment, which largely excludes the possibility that insufficient production of IL-1Ra contributes to the development of insulin resistance in our study subjects. Noticeably, also in our population, the baseline IL-1Ra levels were quite variable.

Second, the dose of IL-1Ra used in our study or infiltration into target tissues may have been insufficient. However, the dosage used in our study was 1.5 times higher than normally used and concentrations of IL-1Ra in the circulation were 1000-fold higher compared with placebo treatment, accompanied by a 16-fold increase in IL-1Ra levels in adipose tissue after anakinra treatment. Moreover, the reduction in inflammatory markers including hsCRP and leukocytes suggest that treatment with anakinra did provoke a systemic anti-inflammatory effect.

Third, because adipose tissue is a causative factor for systemic low-grade inflammation during the development of obesity (3) and is partly responsible for determining systemic insulin sensitivity, we evaluated local effects of anakinra in adipose tissue biopsies. Based on earlier results of in vitro studies that demonstrated lower insulin sensitivity and reduced levels of the adipogenic markers PPARγ and FABP4 after IL-1β treatment of adipocytes, we hypothesized that inhibiting IL-1 signaling by anakinra treatment would enhance adipogenic gene expression levels (8, 26). In contrast, anakinra treatment led to reduced gene expression levels of PPARγ and FABP4 and increased levels of macrophage infiltration. This finding is surprising because treatment with anakinra reduced systemic levels of inflammation. The influx of macrophages, probably accompanied by other immune cells, is most likely not the direct effect of IL-1Ra but might be the effect of one of the additives present in the commercial preparations of anakinra. Injection site reaction is a well-known side effect of anakinra during the first weeks of treatment and is known to result in the infiltration of macrophages (27). Earlier studies have demonstrated the importance of infiltrating macrophages in the sc adipose tissue in provoking systemic insulin resistance (28). The lack of positive findings of our study on insulin sensitivity may thus (partly) be explained by negative effects of local adipose tissue inflammatory injection site reactions on insulin sensitivity. In line with this explanation, the severity of the inflammatory reaction caused by the injection site reaction in sc adipose tissue turned out to be inversely correlated with the improvement in systemic insulin sensitivity after anakinra treatment.

The relation between IL-1, inflammation in adipose tissue, and its effect on systemic insulin sensitivity by affecting systemic inflammation is well known. However, in this study local inflammation in the abdominal sc adipose tissue is correlated with systemic insulin sensitivity, without influencing systemic inflammation. This can partly be explained by a direct negative effect of local inflammation on the local adipose tissue. Alternatively, the inflamed adipose tissue might have led to an altered adipokine secretion profile that negatively interfered with insulin sensitivity in peripheral organs including liver and muscle.

It should be stressed that the adipose tissue biopsy was not taken out of an area with macroscopic inflammation. However, because injection sites were varied over time (as advised) over different abdominal areas, the diffuse inflammation observed may reflect tissue responses from earlier injections.

In respect to β-cell function, we found a hint toward improved insulin secretion capacity as reflected by the disposition index, although the other evaluated parameters for β-cell function did not improve significantly. However, the disposition index is known to be an independent predictor for the development of type 2 diabetes mellitus (29). It should also be stressed that the participants in this study were selected for insulin resistance (obesity/metabolic syndrome) but not insulin secretion deficits. All subjects had glucose levels in the nondiabetic range.

This is the first study that has specifically studied the effects of IL-1 blockade on insulin sensitivity in human nondiabetic subjects. A similar study has been performed in subjects with type 2 diabetes (15). This latter study measured both insulin sensitivity and insulin secretion before and after 13 wk of treatment. Glycemic control improved after 4 and 13 wk of treatment, and this was attributed to an improvement in β-cell function, whereas insulin sensitivity appeared to be unchanged. As such, our findings are in line with the results of the study performed by Larsen et al. (15). The fact that we did not find a clear improvement in insulin secretion may be explained by the differences in study population. The subjects included in our study were nondiabetic and had normal levels of HbA1c and fasting glucose at baseline and had no indication of defective β-cell function, whereas Larsen et al. included patients with type 2 diabetes mellitus.

The dose and duration of treatment were different in our study compared with Larsen et al. (15) (100 mg sc once
daily for 13 wk by Larsen et al. vs. 150 mg sc once daily for 4 wk in our study). The larger dose and shorter treatment period used in our study may have aggravated the injection site reaction because adverse effects are dose dependent and vanish after prolonged treatment (16). Theoretically a prolonged treatment of subjects in our study could have resulted in waning of side effects because injection site reactions of anakinra do disappear after 4–6 wk of treatment and may have revealed an improvement in insulin sensitivity. However, a prolonged treatment period would substantially extend the duration of the (cross-over) trial, increasing the risk for carry-over effects, whereas biological effects of IL-1 blockade were expected to be present within weeks.

In summary, the results of this study do not support the concept that blockade of IL-1 by anakinra treatment improves insulin sensitivity. It cannot be fully excluded, though, that potentially positive effects on insulin sensitivity are (partly) offset by local inflammatory reactions at the injection site of anakinra. Whether treatment with other modalities that block IL-1 but do not produce local injection site effects potentially has a beneficial effect on insulin sensitivity remains to be determined. The present study results support a beneficial effect of blocking IL-1 on β-cell function.

Acknowledgments

Microscopy was performed at the Microscopic Imaging Centre of the Radboud University Nijmegen Medical Centre. The assistance of research nurses, laboratory analysts, and clinical pharmacy employees of the Radboud University Nijmegen Medical Centre was highly appreciated. The clinical trial registration number is NCT00928876 (www.Clinicaltrials.gov).

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This work was supported by a grant from the European Association for the Study of Diabetes and the Dutch Diabetes Research Foundation. M.G.N. was supported by a Vici grant from The Netherlands Organization for Scientific Research.

Disclosure Summary: All authors declare no conflict of interest.

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