Diabetes, Obesity and Metabolism 16: 1269–1273, 2014.

The interleukin-1 receptor antagonist anakinra improves first-phase insulin secretion and insulinogenic index in subjects with impaired glucose tolerance

Inflammation at the level of the β cell appears to be involved in progressive β-cell dysfunction in type 2 diabetes. We assessed the effect of blocking interleukin-1 (IL-1) by anakinra [recombinant human interleukin-1 receptor antagonist (IL-1Ra)] on β-cell function. Sixteen participants with impaired glucose tolerance were treated with 150 mg anakinra daily for 4 weeks in a double blind, randomized, placebo-controlled cross-over study with a wash-out period of 4 weeks. At the end of each treatment period, oral glucose tolerance tests (OGTTs) and hyperglycaemic clamps were performed. First-phase insulin secretion improved after anakinra treatment compared with placebo, 148 ± 20 versus 123 ± 14 mU/l, respectively ($p = 0.03$), and the insulinogenic index was higher after anakinra treatment. These results support the concept of involvement of IL-1β in the (progressive) decrease of insulin secretion capacity associated with type 2 diabetes.

Keywords: β cell, diabetes mellitus, drug mechanism, experimental pharmacology, type 2 diabetes

Date submitted 30 April 2014; date of first decision 26 May 2014; date of final acceptance 17 June 2014

Introduction

Type 2 diabetes mellitus occurs when β cells fail to appropriately increase insulin secretion in response to insulin resistance [1]. β-Cell function progressively deteriorates over time [2,3] and inflammation at the level of the β cell appears to be involved [4].

The proinflammatory cytokine interleukin-1 (IL-1) appears to be an important mediator in the inflammatory process in the β cell. *In vitro*, high glucose levels increase β-cell production of IL-1β followed by functional impairment and apoptosis of β cells. β Cells can be rescued from hyperglycaemia-induced apoptosis by addition of the interleukin-1 receptor antagonist (IL-1Ra), a naturally occurring inhibitor of IL-1β [5].

Blocking the effects of IL-1 in patients with type 2 diabetes mellitus by anakinra, a recombinant human IL-1Ra, has been shown to improve glycaemic control through enhanced β cell function [6]. However, in patients with diabetes, improvement of β-cell function can be either direct or indirect due to improvement in glucose levels, as hyperglycaemia itself impairs both insulin secretion and insulin sensitivity (concept of glucotoxicity) [7].

We hypothesized that treatment with IL-1Ra anakinra can improve β-cell function in subjects with impaired glucose tolerance (IGT).

Correspondence to : Cees J. Tack, Department of Internal Medicine 463, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands.
E-mail: cees.tack@radboudumc.nl

Materials and Methods

Study Population

Included were participants with IGT defined as either impaired fasting glucose (IFG) (fasting glucose 5.6–6.9 mmol/l) and/or IGT (2 h plasma glucose 7.8–11.0 mmol/l) by 75 g oral glucose tolerance test (OGTT) and/or haemoglobin A1c (HbA1c) levels of 5.7–6.4% [8]. Other inclusion criteria were body mass index >25 kg/m^2 and age 40–70 years.

Protocol

This was a randomized, double-blind, placebo-controlled cross-over study (clinical trials.gov number NCT01285232). Participants were randomly assigned to treatment with anakinra 150 mg or placebo subcutaneous once daily for four consecutive weeks. After a wash-out period of 4 weeks, participants crossed over to the other treatment arm. At the end of each treatment, a hyperglycaemic clamp and a 75 g OGTT were performed. The Pharmacy Department provided anakinra and matching placebo injections and was responsible for blinding and randomization.

Hyperglycaemic Clamp

The hyperglycaemic clamp procedure was performed after an overnight fast. After a 30-min equilibration period, the clamp was started ($t = 0$) by an intravenous bolus of 0.8 ml glucose, 20% solution per kg body weight followed by a variable glucose 20% infusion in order to maintain a blood glucose level of 10 mmol/l for a total duration of 120 min. At 120 min, an

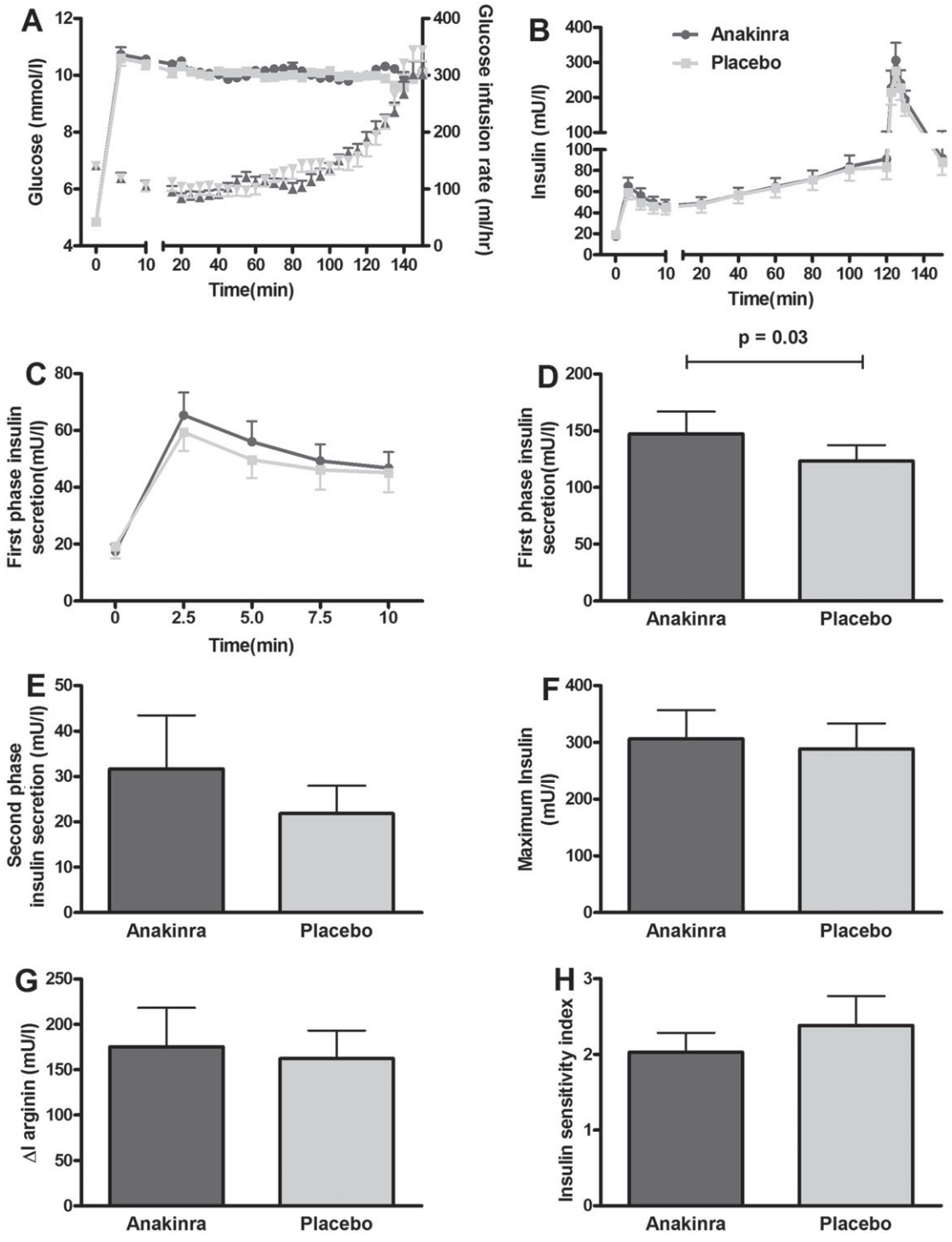

Figure 1. Hyperglycaemic clamp. Glucose (A) levels and glucose infusion rate (GIR) during the hyperglycaemic clamp after treatment with anakinra (dark grey) and placebo treatment (light grey). The glucose levels are depicted as symbols with connecting line and the GIR as symbols only. During the hyperglycaemic clamp, glucose levels were nearly identical in both treatment periods (anakinra 9.98 ± 0.04 mmol/l vs. placebo 10.02 ± 0.04 mmol/l, respectively, p = 0.43). The mean CV of the hyperglycaemic clamp was below 4% in both treatment periods. Insulin (B) levels during the hyperglycaemic clamp after treatment with anakinra and placebo. First-phase insulin secretion was calculated as the sum of increments of plasma insulin levels from 2.5 to 10 min of the clamp. First-phase insulin secretion after treatment with anakinra (dark grey) and placebo treatment (light grey) are shown as insulin levels (C) and as the mean sum of increments (D). Second-phase insulin secretion (E) was taken as the average increment in plasma insulin levels from 80 to 120 min of the clamp. The maximal insulin concentration (F) was the single highest post arginine insulin level. The acute insulin response to arginine (ΔI arginine) (G) was calculated by subtracting as the insulin level at 120 min from the mean insulin level of 122.5 and 125 min. Insulin sensitivity was assessed as an insulin sensitivity index (ISI) (H), defined as GIR to maintain hyperglycaemia from 80 to 120 min divided by the mean plasma insulin level during the same interval. Data are expressed as mean ± s.e.m.

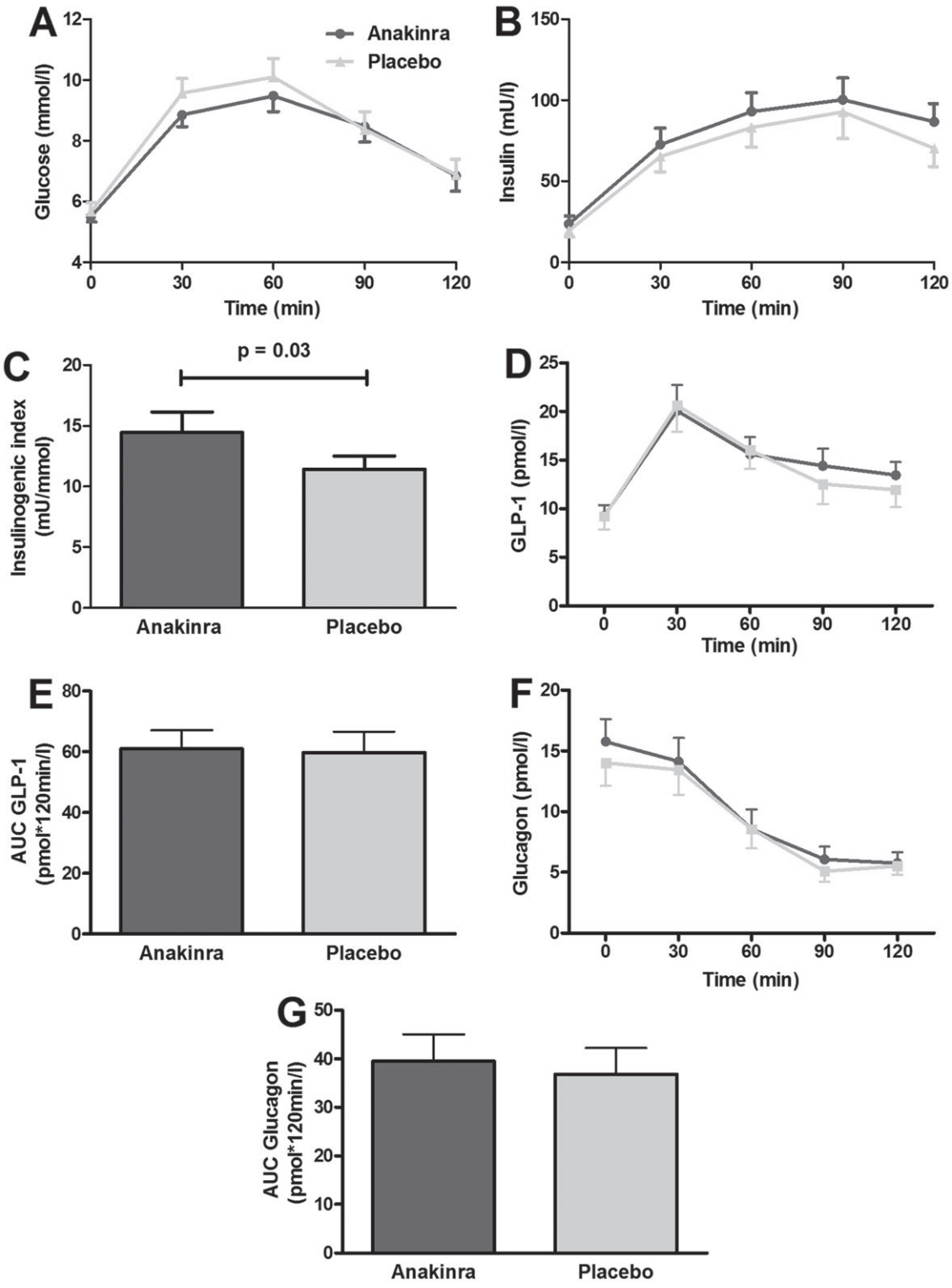

Figure 2. Oral glucose tolerance test (OGTT). Glucose (A) and insulin (B) levels during OGTT for anakinra (dark grey) and placebo (light grey) treatment. The area under the curve (AUC) for glucose during the OGTT did not differ between anakinra and placebo treatment, while the AUC for insulin tended to be higher after anakinra treatment (321 ± 40 vs. 286 ± 41 mU·120 min/l, p = 0.09). The insulinogenic index (C) was calculated as the increase in insulin level from 0 to 30 min divided by the increase in plasma glucose level from 0 to 30 min. The insulinogenic index was higher after treatment with anakinra compared with placebo treatment. The glucagon-like peptide (GLP) levels during OGTT (D) and AUC for GLP-1 (E) are shown for both anakinra (dark grey) and placebo treatment (light grey). Glucagon levels during OGTT (F) and AUC for glucagon (G) are also depicted. Data are expressed as mean ± s.e.m.

arginine bolus of 5 g was administered to measure maximum insulin secretory capacity.

Blood glucose levels were measured in whole blood using the oxidation method (Biosen C-line, EKF diagnostics, GmbH, Cardiff, UK) every 5 min to allow precise adjustment of the glucose infusion rate. Blood samples were taken at $t = 0$, 2.5, 5, 7.5, 10, 20, 40, 60, 80, 100, 120, 122.5, 125, 127.5, 130 and 150 min and for assessment of insulin concentration. After 150 min of hyperglycaemia, glucose infusion was discontinued.

The OGTT was performed in the morning after an overnight fast, at least 24 h before or after the hyperglycaemic clamp. After collection of the fasting blood samples, the subject drank 75 g of anhydrous glucose in 250–300 ml water over the course of 5 min. Blood samples were collected every 30 min for 2 h after the test load. Insulin measurements were performed (Magpix, Luminex, Austin, TX, USA), and samples were assayed for total glucagon-like peptide-1 (GLP-1) and glucagon [9].

Statistical Analysis

Statistical analyses were performed using GRAPHPAD 5.0. Differences in means were tested by paired student's *t*-test for normally distributed and Wilcoxon signed rank test for non-normally distributed data. Results were expressed as mean ± standard error of mean (s.e.m.) unless otherwise indicated. Significance was set at a p-value of less than 0.05.

Results

A total of 16 participants (7 females and 9 males) completed the trial (Figure S1, Supporting information). Baseline characteristics of the participants were (mean ± s.d.): age 55 ± 9 years, body mass index (BMI) 32 ± 5 kg/m^2, HbA1c 5.8 ± 0.5% and fasting glucose level 5.3 ± 0.71 mmol/l (Table S1).

Leucocyte (anakinra $5.4 \pm 0.3 \times 10^9$ /l vs. placebo $6.2 \pm 0.4 \times 10^9$ /l, $p = 0.017$) and neutrophil counts (anakinra $2.9 \pm 0.3 \times 10^9$ /l vs. placebo $3.7 \pm 0.3 \times 10^9$ /l, $p = 0.001$) were significantly reduced after anakinra treatment, as were CRP levels (anakinra 0.9 ± 0.2 µg/ml vs. placebo 2.7 ± 0.6 µg/ml, $p < 0.001$).

First-phase insulin secretion improved after anakinra treatment compared with placebo (148 ± 20 vs. 123 ± 14 mU/l, respectively, $p = 0.03$). Second-phase insulin secretion, insulin response after arginine stimulus and maximal insulin secretion did not differ between the two treatment arms (Figure 1).

Anakinra had no effect on the insulin sensitivity index (2.0 ± 0.3 mmol/mU anakinra and 2.4 ± 0.43 mmol/mU placebo treatment, $p = 0.29$).

In line with the first-phase clamp results, the insulinogenic index derived from the OGTT improved after anakinra compared with placebo treatment (14.5 ± 1.7 vs. 11.4 ± 1.1 mU/mmol, respectively, $p = 0.036$) (Figure 2).

Anakinra treatment had no effect on GLP-1 and glucagon levels during OGTT.

Anakinra treatment did not change fasting glucose levels (5.5 ± 0.2 vs. 5.5 ± 0.3 mmol/l) or HbA1c levels compared with placebo (5.6 ± 0.1% vs. 5.7 ± 0.1%, $p = 0.068$).

Fourteen participants experienced injection site reactions during anakinra treatment. One subject withdrew because of the local reaction. All reactions disappeared after the cessation of anakinra treatment. One participant developed erysipelas and one participant developed a thrombophlebitis of the antecubital vein, which was used for infusion of glucose 20% during the clamp, both after anakinra treatment. There were no other infections or serious adverse events.

Conclusion

The main finding of this study is that 4 weeks of anakinra treatment improves the insulinogenic index and augments the first-phase insulin secretion. These observations suggest that anakinra can improve β-cell function. However, second-phase insulin secretion, insulin response after arginine and the maximal insulin concentration were not influenced by anakinra. The improved first-phase insulin response was not mediated by an enhanced incretin response.

These findings are in line with Larsen et al. [6] and with our earlier study in non-diabetic subjects with the metabolic syndrome, where anakinra improved the disposition index [10]. A recent study using canakinumab, a human monoclonal anti-IL-1β antibody, found a trend towards improving insulin secretion rates in patients with type 2 diabetes mellitus and an improved insulin secretion in subjects with IGT [11].

A number of mechanisms may underlie these results. First, anakinra might inhibit intra-islet inflammation and thereby preserve β-cell function. *In vitro* studies show that high glucose levels induce IL-1β production by β cells followed by β-cell dysfunction and apoptosis, and that these deleterious effects can be prevented by IL-1Ra [5]. Second, anakinra may improve β-cell function by decreasing systemic inflammation. Elevated levels of IL-1β, CRP and IL-6 are predictive of type 2 diabetes mellitus [12]. The reduction in inflammatory markers in this study suggests that anakinra treatment induces a systemic anti-inflammatory effect. Third, an improvement in β-cell function could be explained by a compensatory response to a decline in insulin sensitivity. However, we did not find significant changes in the insulin sensitivity index.

In summary, this study supports the concept of involvement of IL-1β in the decrease of insulin secretion capacity associated with type 2 diabetes mellitus. These findings are relevant as improvement in β-cell function can delay and/or prevent progression to frank diabetes.

P. C. M. van Poppel[1], E. J. P. van Asseldonk[1], J. J. Holst[2], T. Vilsbøll[3], M. G. Netea[1,4] & C. J. Tack[5]

[1]Department of Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
[2]Department of Biomedical Sciences, Panum Institute University of Copenhagen, Copenhagen, Denmark
[3]Department of Medicine, Copenhagen University Hospital, Copenhagen, Denmark
[4]Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Nijmegen, the Netherlands
[5]Department of Internal Medicine (463), Radboud University Medical Center, Nijmegen, the Netherlands

Acknowledgements

This study was supported by a grant of the European Federation for the Study of Diabetes. The assistance of research nurses, laboratory analysts and clinical pharmacy employees of the Radboud University Nijmegen Medical Centre was highly appreciated.

Conflict of Interest

All authors declare no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Design, enrollment, withdrawal and completion of the trial.

Table S1. Baseline characteristics (mean±SD).

References

1. Gerich JE. Contributions of insulin-resistance and insulin-secretory defects to the pathogenesis of type 2 diabetes mellitus. Mayo Clin Proc 2003; **78**: 447–456.
2. Cnop M, Vidal J, Hull RL et al. Progressive loss of beta-cell function leads to worsening glucose tolerance in first-degree relatives of subjects with type 2 diabetes. Diabetes Care 2007; **30**: 677–682.
3. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. Diabetes 1995; **44**: 1249–1258.
4. Donath MY, Storling J, Berchtold LA, Billestrup N, Mandrup-Poulsen T. Cytokines and beta-cell biology: from concept to clinical translation. Endocr Rev 2008; **29**: 334–350.
5. Maedler K, Sergeev P, Ris F et al. Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. J Clin Invest 2002; **110**: 851–860.
6. Larsen CM, Faulenbach M, Vaag A et al. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 2007; **356**: 1517–1526.
7. Yki-Jarvinen H. Glucose toxicity. Endocr Rev 1992; **13**: 415–431.
8. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; **33**(Suppl. 1): S62–S69.
9. Knop FK, Aaboe K, Vilsboll T et al. Impaired incretin effect and fasting hyperglucagonaemia characterizing type 2 diabetic subjects are early signs of dysmetabolism in obesity. Diabetes Obes Metab 2012; **14**: 500–510.
10. van Asseldonk EJ, Stienstra R, Koenen TB, Joosten LA, Netea MG, Tack CJ. Treatment with Anakinra improves disposition index but not insulin sensitivity in nondiabetic subjects with the metabolic syndrome: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab 2011; **96**: 2119–2126.
11. Rissanen A, Howard CP, Botha J, Thuren T, for the Global Investigators. Effect of anti-IL-1beta antibody (canakinumab) on insulin secretion rates in impaired glucose tolerance or type 2 diabetes: results of a randomized, placebo-controlled trial. Diabetes Obes Metab 2012; **14**: 1088–1096.
12. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001; **286**: 327–334.