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Complete List of Authors:	Herder, Christian; Deutsches Diabetes-Zentrum Leibniz-Zentrum fur Diabetes-Forschung, Institute for Clinical Diabetology Faerch, Kristine; Steno Diabetes Centre, Clinical Epidemiology Carstensen-Kirberg, Maren; Deutsches Diabetes-Zentrum Leibniz-Zentrum fur Diabetes-Forschung, Institute for Clinical Diabetology Lowe, Gordon; University of Glasgow, Institute of Cardiovascular and Medical Sciences Haapakoski, Rita; University College London, Department of Epidemiology and Public Health Witte, Daniel; Aarhus Universitet, Department of Public Health Brunner, Eric; University College London, Department of Epidemiology and Public Health Roden, Michael; Deutsches Diabetes-Zentrum Leibniz-Zentrum fur Diabetes-Forschung, Institute for Clinical Diabetology Tabák, Ádám; University College London, Department of Epidemiology and Public Health Kivimaki, Mika; University College London, Epidemiology and Public Health Vistisen, Dorte; Steno Diabetes Center AS, Clinical Epidemiology
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Biomarkers of subclinical inflammation and increases in glycaemia, insulin resistance and beta-cell function in non-diabetic individuals: the Whitehall II study

Short title: Inflammation and glucose metabolism

Christian Herder[1,2], Kristine Færch[3], Maren Carstensen-Kirberg[1,2], Gordon D. Lowe[4], Rita Haapakoski[5], Daniel R. Witte[5,6], Eric J. Brunner[5], Michael Roden[1,2,8], Adam G. Tabák[5,9], Mika Kivimäki[5], Dorte Vistisen[3]

[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany; [2]German Center for Diabetes Research, München-Neuherberg, Germany; [3]Steno Diabetes Center A/S, Gentofte, Denmark; [4]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; [5]Department of Epidemiology and Public Health, University College London, London, UK; [6]Department of Public Health, Aarhus University, Aarhus, Denmark; [7]Danish Diabetes Academy, Odense, Denmark; [8]Department of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany; [9]First Department of Medicine, Semmelweis University, Faculty of Medicine, Budapest, Hungary.

Keywords: subclinical inflammation, cytokines, adiponectin, insulin resistance, prediabetes, cohort

Corresponding author:

Dr. Christian Herder MSc, Institute for Clinical Diabetology, German Diabetes Center, Auf'm Hennekamp 65, 40225 Düsseldorf, Germany. Phone: +49 211 3382 647. Fax: +49 211 3382 603.

Email: christian.herder@ddz.uni-duesseldorf.de

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Abstract

Objective: Higher systemic levels of proinflammatory biomarkers and low adiponectin are associated with increased risk for type 2 diabetes, but their associations with changes in glycaemic deterioration before onset of diabetes are poorly understood. We aimed to study whether inflammation-related biomarkers associated with 5-year changes in glucose and insulin, HbA1c, insulin sensitivity and beta-cell function before the diagnosis of type 2 diabetes and whether these associations may be bidirectional.

Design and Methods: We used multiple repeat measures (17,891 person-examinations from 7,683 non-diabetic participants) from the Whitehall II study to assess whether circulating high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), IL-1 receptor antagonist (IL-1Ra) and adiponectin associated with subsequent changes in glycaemia, insulin, insulin resistance and beta-cell function (based on oral glucose tolerance tests). We examined bidirectionality by testing if parameters of glucose metabolism at baseline associated with changes in inflammation-related biomarkers.

Results: Higher hsCRP and IL-6 were associated with increases in fasting insulin, insulin resistance and, for IL-6, with beta-cell function after adjustment for confounders. Higher adiponectin associated with decreases in fasting glucose, HbA1c, fasting insulin, insulin resistance and beta-cell function. The reverse approach showed that 2-hour glucose and insulin sensitivity associated in opposite directions with changes in IL-1Ra. Fasting insulin and insulin resistance showed inverse associations with changes in adiponectin.

Conclusions: Subclinical inflammation associated with development of increased glycaemia, insulin resistance and beta-cell function in non-diabetic individuals. These findings are consistent with the hypothesis that inflammation-related processes may increase insulin resistance and lead to a compensatory upregulation of beta-cell function.

Introduction

Biomarkers of subclinical inflammation are associated with incident type 2 diabetes (1,2), but prospective data on glycaemic deterioration before the onset of diabetes are scarce. Cross-sectional studies suggest differential time-courses for changes in biomarkers of subclinical inflammation before type 2 diabetes. Regarding circulating C-reactive protein (CRP), for example, higher levels were observed in prediabetes (i.e. impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)) compared to normal glucose tolerance (NGT), whereas only minor differences in CRP levels were observed between people with prediabetes and type 2 diabetes (3). In contrast, systemic levels of interleukin (IL)-6 or IL-18 seemed to be similar in individuals with NGT and prediabetes, but higher in those with type 2 diabetes compared to those with prediabetes (3,4). Thus, different biomarkers of subclinical inflammation are related to early versus late stages of glycaemic deterioration, but little is known about the underlying pathophysiology (5).

If subclinical inflammation influences early deterioration of glycaemic control, biomarkers of subclinical inflammation should be associated with development of prediabetes, when individuals with NGT are followed-up longitudinally. To date two small studies have failed to provide evidence for an association of proinflammatory cytokines or adiponectin with incident IFG or IGT (6,7). An alternative approach with higher statistical power is to investigate whether baseline levels of biomarkers of subclinical inflammation are associated with subsequent changes in measures of glucose metabolism (8,9).

In this study, we adopted that latter approach to examine whether biomarkers of subclinical inflammation are associated with 5-year changes in glucose and insulin levels, HbA_{1c}, insulin sensitivity and beta-cell function before the diagnosis of type 2 diabetes in a large population-based cohort. The study was based on three 5-year observation cycles, which were combined by means of a mixed model (10). Since there is evidence for an impact of hyperglycaemia and

hyperinsulinaemia on subclinical inflammation and hypoadiponectinaemia (11,12), we also considered a potentially bidirectional relationship by investigating to what extent markers of glucose metabolism may also be associated with changes in biomarkers of subclinical inflammation.

Materials and Methods

Study participants, procedures and measurements

Participants are from the Whitehall II Study, an occupational cohort of 10,308 British civil servants (6,896 men and 3,412 women aged 35–55 years) of mainly white ethnicity recruited between 1985 and 1988 (phase 1) (13). The UK NHS Health Research Authority London-Harrow ethics committee reviewed and approved the study. Written informed consent was obtained from each participant at each examination phase. The study was conducted according to the principles of the Helsinki Declaration. The cohort has been followed at eight subsequent phases, 2.5 years apart. All study phases included a questionnaire, and every second phase (5 years apart) also included a clinical health examination (phases 1, 3, 5, 7, and 9). Phase 3 (1991–1993) was the first phase with an oral glucose tolerance test (OGTT), therefore phase 1 was not used. In the Whitehall II cohort 8,815 participated at phase 3 (1991–1993); 7,870 at phase 5 (1997–1999); 6,967 at phase 7 (2002–2004); and 6,761 at phase 9 (2007–2009) with the same individual participating in several phases. During follow-up, participants were censored if they died, were lost to follow-up or developed diabetes. Anthropometric, demographic, clinical and lifestyle characteristics are summarised in Table 1. At phases 3, 5, 7, and 9 a standard 2-hour 75 g OGTT was performed in the morning after an overnight fast (≥8 hours of fasting). For around one third of the examinations, the OGTT was administered in the afternoon after a light fat-free breakfast (≥5 hours of fasting). These examinations were not considered in this study. Diabetes was diagnosed by a doctor outside the study or at screening by OGTT. Screen-detected diabetes was ascertained throughout

follow-up by OGTTs administered every 5 years and defined according to the OGTT criteria defined by the World Health Organization (14).

Information on smoking habits (never/ex/current), alcohol consumption (units per week) and physical activity (hours per week of mild, moderate and vigorous physical activity) were collected using a self-administered questionnaire (15).

Plasma glucose, serum insulin, HbA_{1c} and serum lipids were measured as described previously (16,17). Insulin sensitivity and beta-cell function were estimated based on fasting plasma glucose and serum insulin using the homeostasis model assessment for insulin resistance (HOMA-IR) and beta-cell function (HOMA-β). In addition, whole-body insulin sensitivity was assessed using the insulin sensitivity index ($ISI_{0–120}$) based on fasting and 2-hour values of glucose and insulin (18).

High-sensitivity CRP (hsCRP) was measured using a high-sensitivity immunonephelometric assay, IL-6 was measured using a high-sensitivity ELISA assay, IL-1 receptor antagonist (IL-1Ra) and total adiponectin were measured with Quantikine ELISA kits (R&D Systems, Wiesbaden, Germany) in a diabetes case-cohort sample (19,20).

Statistical analysis

Statistical analyses were performed in R version 3.1.3 (The R Foundation for Statistical Computing) and SAS version 9.2 (SAS Institute, Cary, NC, USA).

In the main analysis the following outcomes were studied: fasting plasma glucose, 2-hour plasma glucose, HbA_{1c}, fasting and 2-hour serum insulin, HOMA-IR, HOMA-β and ISI_{0-120}. We excluded 10,529 (36.5%) person-examinations for which the participant had been fasting for <8 hours (OGTTs administered in the afternoon). Outcomes with a skewed distribution (fasting and 2-hour insulin, HOMA-IR, HOMA-β and ISI_{0-120}) were log-transformed prior to analysis.

The following biomarkers of subclinical inflammation were included as exposures: high-sensitivity (hs)CRP, IL-6, IL-1 receptor antagonist (IL-1Ra) and adiponectin (all Log2 transformed prior to analysis). As adiponectin and IL-1Ra were measured only in a case-cohort subsample nested within the Whitehall II study (19,20), analyses were restricted to the subcohort with these measurements. We excluded 412 (2.3%) person-examinations with hsCRP >10 mg/l as indicator of acute infections.

Up to a total of 17,891 person-examinations for 7,683 non-diabetic participants were analysed (8,303 person-examinations for 2,965 participants in the subcohort). We studied the associations of baseline levels of inflammation-related biomarkers and 5-year follow-up levels of the different outcomes, including the baseline level of the outcome as a covariate. The main analysis is based on all available data after the aforementioned exclusions and provides effect estimates per doubling in baseline levels of the respective biomarker. In addition, we used the subset of the population for whom all four biomarkers were available at the same time-points to calculate regression coefficients that were standardised per 1-SD difference in the Log of the biomarker to allow direct comparisons of effect sizes between the exposure variables.

All analyses were adjusted for age, sex, study phase and baseline value of the outcome studied (model 1). We further adjusted the analyses for other variables in a successive manner:

- model 2, further adjustment for baseline BMI;
- model 3, further adjustment for baseline lifestyle factors (smoking, physical activity, alcohol intake) and lipids (triacylglycerols, HDL-C, LDL-C);
- model 4, further adjustment for 5-year change in BMI after baseline.

To compare the estimated associations across models 1-4 for a given outcome and exposure, we used a complete-case approach, limiting the analyses to data with complete information on all covariates in model 4. Except for HbA_{1c}, which was only measured at phases 7 and 9, the

same individual may contribute with more than one observation to the analyses. To account for the likely correlation of repeated measurements within the same participant, we used mixed-effects models with a random intercept and a random slope for time. For HbA_{1c}, a standard linear model was used. In a sensitivity analysis, we further tested whether the associations were changed when using waist circumference instead of BMI.

In the reverse approach, we interchanged exposures and outcomes and studied the associations of the baseline levels of glycaemia, insulin, insulin sensitivity and beta-cell function with 5-year changes in inflammation-related biomarkers. These analyses were performed using the same methods and models as described above.

A two-sided 5% level of significance was adjusted for multiple testing with the method of Benjamini and Hochberg (21). This method controls the false discovery rate and is considered more powerful than the more simple Bonferroni adjustment of the error rate, because the risk of false negative results is lower with the Benjamini-Hochberg method.

Results

Associations between biomarkers of inflammation at baseline and 5-year changes in glycaemia, insulin, insulin sensitivity and beta-cell function

Higher systemic concentrations of hsCRP, IL-6 and IL-1Ra were associated with higher changes in fasting and 2-hour glucose and fasting and 2-hour insulin, but not HbA_{1c}, whereas adiponectin was inversely associated with all these five outcomes (Table 2, model 1). After adjustment for baseline BMI, lipids, lifestyle factors and change in BMI, the positive associations of hsCRP and IL-6 with fasting insulin and the inverse associations between adiponectin and fasting glucose, HbA_{1c} and fasting insulin remained significant (Table 2, models 2-4).

High baseline levels of hsCRP, IL-6 and IL-1Ra were also associated with increases in insulin resistance (i.e. increase in HOMA-IR and decrease in $ISI_{0,120}$) and beta-cell function, while

baseline adiponectin showed inverse associations (Table 2, model 1). Effect sizes were attenuated by adjustment for the aforementioned covariables, but the associations of hsCRP, IL-6 and adiponectin with changes in HOMA-IR and the associations of IL-6 and adiponectin with HOMA-β remained significant in the final model (model 4). Associations with $ISI_{0,120}$ lost statistical significance after adjustment.

To compare effect sizes between exposures, we standardised our estimates per 1 population SD of one Log unit of the concentrations of the four biomarkers of subclinical inflammation (Fig. 1; Supplementary Tables 1 and 2). Effect sizes were similar for hsCRP, IL-6 and IL-1Ra, but of larger magnitude (and in the opposite direction) for adiponectin.

We substituted BMI with waist circumference in a sensitivity analysis. In general this changed little (<10%) of the effect estimates in Table 2 (data not shown). Some effect estimates showed greater changes (≥10%), but these were only observed for non-significant associations.

Associations of glycaemia, insulin, insulin sensitivity and beta-cell function at baseline with 5-year changes in biomarkers of inflammation

When interchanging exposures and outcomes, we observed fewer significant associations (Fig. 2). None of the measures of glycaemia was associated with changes in hsCRP, IL-6, IL-1Ra or adiponectin when further adjusting for 5-year change in BMI after baseline (fully adjusted model), except an inverse association between 2-hr glucose and IL-1Ra (Supplementary Tables 3 and 4). Fasting insulin and HOMA-IR showed inverse associations with changes in adiponectin in the fully adjusted models, but neither insulin levels nor HOMA-IR were related to changes in hsCRP, IL-6 or IL-1Ra (Supplementary Tables 3 and 4). High baseline levels of $ISI_{0,120}$ were positively associated with increases in IL-1Ra (Supplementary Tables 3 and 4).

Discussion

This study examined the temporal relationship between biomarkers of subclinical inflammation and changes in glucose metabolism before the diagnosis of type 2 diabetes using repeat data. Baseline levels of hsCRP and IL-6 were positively associated with subsequent increases in fasting insulin, HOMA-IR and beta-cell function, while adiponectin was inversely associated with future changes in fasting glucose, HbA_{1c}, fasting insulin, HOMA-IR and beta-cell function. In the reverse analysis, baseline fasting insulin and HOMA-IR were associated with decreases in adiponectin, while 2-hour glucose and $ISI_{0,120}$ showed associations with changes in IL-1Ra.

Subclinical inflammation and glycaemia

Serum hsCRP, IL-6 and IL-1Ra were associated with 5-year increases in fasting and 2-hour glucose in age and sex-adjusted models, but further adjustment attenuated these associations to non-significance with BMI being the most important confounder. In contrast, adiponectin levels showed an independent inverse association with fasting glucose, but not with 2-hour glucose. These data are novel and may point towards a specific role of adiponectin in the early deterioration of glycaemia.

Fasting glucose levels are mainly determined by hepatic glucose production, whereas increased 2-hour glucose mainly reflects peripheral glucose uptake (22). Adiponectin receptors (ADIPOR)-1 and 2 are expressed on both hepatocytes and skeletal muscle cells with ADIPOR2 being the predominant receptor in the liver and ADIPOR1 the predominant receptor in skeletal muscle (23). Therefore, it can be speculated that ADIPOR2-mediated signaling and downstream effects on peroxisome proliferator-activated receptor-α and regulation of glucose uptake, fatty acid oxidation, oxidative stress and inflammation may mediate the observed association between adiponectin and deterioration of fasting glycaemia in our study. Importantly, chronically decreased adiponectin levels are indicators of adipose

tissue dysfunction and not only related to increased risk of type 2 diabetes, but also to diabetic complications (1,2,24,25).

With respect to HbA_{1c}, we observed an inverse association between adiponectin and increases in HbA_{1c}, but no associations of the other three biomarkers. Based on the findings for fasting glucose, associations may have been expected for all four biomarkers at least for the age and sex-adjusted model. However, this discrepancy may be due to the fact that glucose levels are only weak determinants of HbA_{1c} in non-diabetic individuals (26). Furthermore, the sample size for the HbA_{1c} analysis was smaller than that for other glycaemic traits. Our data are only partly in line with previous observations in the KORA study showing a positive association between hsCRP and 7-year changes in HbA_{1c}, but no association between adiponectin and HbA_{1c} (9). There are no obvious differences in baseline characteristics between the two studies, so the relevance of subclinical inflammation for HbA_{1c} levels in non-diabetic individuals merits further studies.

Subclinical inflammation and insulin resistance

Our study revealed consistent associations between all four biomarkers and fasting insulin and HOMA-IR, although the associations of IL-1Ra were not independent of 5-year changes in BMI. In contrast, for 2-hour insulin and $IS_{0\text{-}120}$, which were based on post-load measures, associations with hsCRP, IL-6 and adiponectin were only found in the initial regression models, but not after full adjustment.

So far, only one previous study employed a comparable design and found that high hsCRP levels were associated with increases in HOMA-IR in a young non-diabetic population (8). Thus, the use of a more comprehensive assessment of subclinical inflammation and dynamic measures of insulin resistance represents an extension of the current literature. Our observations for changes in fasting insulin and HOMA-IR complemented and corroborated our findings for fasting glucose and pointed towards an association between subclinical

inflammation and hepatic rather than peripheral insulin resistance in non-diabetic individuals. Associations were weaker for changes in IL-1Ra. IL-1Ra levels are considered as indicators of IL-1β-mediated processes. IL-1β has been demonstrated to induce insulin resistance in hepatocytes (27). Therefore, an association between IL-1Ra and hepatic insulin resistance is plausible.

Subclinical inflammation and beta-cell function

This is apparently the first study to show that higher hsCRP, IL-6 and IL-1Ra and lower adiponectin at baseline are associated with 5-year increases in beta-cell function assessed in the fasting state. After full adjustment, high IL-6 levels and low adiponectin levels remained associated with increases in fasting beta-cell function.

Although an increase in beta-cell function does not seem intuitively related to an increased risk of type 2 diabetes, our findings have to be seen in context of the aforementioned associations with worsening fasting glycaemia and increased insulin resistance. The associations of IL-6 and adiponectin with increases in beta-cell function were most likely a consequence of their associations with increased insulin resistance. In other words, increases in HOMA-IR in our non-diabetic study sample may reflect a compensatory upregulation of insulin secretion in response to decreases in insulin action, which was still sufficient to maintain glucose levels.

However, our data are also in line with the alternative hypothesis that biomarkers of subclinical inflammation have a direct impact on beta-cell function. At least IL-6 has been reported to stimulate insulin secretion through an incretin-mediated mechanism in experimental models of diabetes (28). The interpretation of our findings regarding beta-cell function would have been facilitated by the investigation of associations between subclinical inflammation and changes in the disposition index. Unfortunately, the assessment of dynamic beta-cell function is not possible with the available data in the Whitehall II cohort.

Bidirectionality in temporal associations between subclinical inflammation and markers of glucose metabolism

Our study is unique because our design allowed us to assess the potential bidirectionality in the associations of subclinical inflammation and glucose metabolism. Reversing our initial analysis led to two main results: First, fasting insulin and HOMA-IR were associated with decreases in adiponectin. Second, 2-hour glucose showed inverse and ISI_{0-120} showed direct associations with changes in IL-1Ra.

It has been proposed that hypoadiponectinaemia in obesity and type 2 diabetes may be a consequence rather than a cause of insulin resistance (12). The regulation of adiponectin is still poorly understood in humans, so we cannot draw firm conclusions. However, the results are consistent with our previous observations of continuous and faster decrease in adiponectin levels preceding the development of type 2 diabetes compared to healthy adults (20). Our study suggests that adiponectin and insulin resistance are linked in a bidirectional way with potential deleterious consequences for the regulation of glucose metabolism.

The associations between 2-hour glucose, ISI_{0-120} and changes in IL-1Ra point towards a potential link between peripheral insulin action and regulation of IL-1Ra. Such a link is plausible given the fact that the release of both IL-1β and IL-1Ra after exercise is part of normal skeletal muscle physiology (29). However, it is currently unclear how impairments in muscle insulin sensitivity could influence circulating levels of both proteins.

From a pathophysiological point-of-view, any bidirectionality in the relationship between subclinical inflammation and insulin resistance could reflect a positive feed-back loop, potentially fueling a vicious cycle resulting in progressive worsening of glycaemic control. Our finding of a limited degree of bidirectionality consequently argues in favour of a deleterious impact of hypoadiponectinaemia and subclinical inflammation in the development of dysglycaemia.

321

322 **Strengths and limitations**

323 Strengths of our study are its large sample size and the analysis of quantitative traits entailing
324 a larger statistical power than the analysis of a dichotomous outcome (e.g. prediabetes).
325 Further strengths are the use of multiple measures of glucose metabolism reflecting different
326 pathophysiological aspects and the availability of repeat data from up to four study phases,
327 which allowed us to assess potential bidirectional relationships. Moreover, we adjusted for
328 baseline BMI and its 5-year changes and thus demonstrated that associations were not solely
329 mediated by obesity.

330 One limitation is the observational design that provides evidence for temporal, but not for
331 causal relationships. Moreover, HOMA-IR and ISI_{0-120} correlate only moderately well with
332 the euglycaemic-hyperinsulinaemic clamp (30), but clamp measurements were not available.
333 Thus, our assessment of insulin resistance was less precise than the gold standard, and we had
334 to rely on indirect estimates to compare hepatic versus peripheral insulin resistance. HOMA-β
335 can only be used to estimate fasting beta-cell function, and our study did not include dynamic
336 assessments of beta-cell function. This limits the precision of the measurmenent, and we
337 could not examine beta-cell function relative to insulin sensitivity using the disposition index.
338 We used a complete case approach in our analyses. The fraction of missingness of hsCRP and
339 IL-6 in the cohort was around 5% and between 10-15% for IL-1Ra and adiponectin in the
340 subcohort. Therefore, and because we are studying associations, the effect of any potential
341 non-randomness of the missing data for biomarkers of subclinical inflammation is considered
342 negligible.

343 A final limitation of our study is the selection of four biomarkers, which left out others that
344 also merit further research. We focused on hsCRP, IL-6, IL-1Ra and adiponectin as pro- and
345 anti-inflammatory biomarkers because of their well established associations with incident
346 type 2 diabetes in prospective studies (1,2,31). Based on experimental data and other

epidemiological studies, cytokines such as IL-1β (32-34), tumour necrosis factor (TNF)-α (35,36) and transforming growth factor (TGF)-β (37,38) and chemokines such as monocyte chemoattractant protein-1/chemokine (C-C motif) ligand 2 (MCP-1/CCL2) (39,40) undoubtedly represent interesting candidates because of their impact on insulin sensitivity and/or beta-cell function. However, circulating levels of IL-1β are below the limit of detection for a large proportion of individuals in population-based studies with currently available assays, and experimental data on TNFα and insulin resistance do not appear to be translated into an association between circulating levels of this protein and risk of type 2 diabetes in cohort studies (41,42). Data on most other inflammation-related biomarkers and incident type 2 diabetes are based on only one or very few cohorts, so that further studies on their relevance both for early deterioration of glucose metabolism and for the manifestation of type 2 diabetes would be important.

Conclusion

Our study demonstrates multiple associations between baseline levels of biomarkers of subclinical inflammation and subsequent 5-year changes in glycaemia, insulin resistance and beta-cell function in a large population-based cohort of non-diabetic individuals. These findings are consistent with the hypothesis that subclinical inflammation may increase hepatic insulin resistance and upregulate beta-cell function. We observed less consistent evidence for a bidirectionality in these temporal relationships, suggesting that low-grade inflammation precedes insulin resistance rather than vice versa.

372 **Author contribution statement**

373 C Herder, K Færch, E J Brunner, A G Tabak, M Kivimäki and D Vistisen contributed to the
374 study concept and design. C Herder, M Carstensen-Kirberg, G D Lowe, R Haapakoski, D R
375 Witte, E J Brunner, M Roden, A G Tabak and M Kivimäki contributed data. C Herder, K
376 Færch and D Vistisen planned the statistical analysis. D Vistisen conducted the statistical
377 analysis. C Herder and D Vistisen drafted the paper. All authors contributed to, critically
378 revised and approved the final version of the manuscript.

379

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384 all members of the Whitehall II study team. The Whitehall II Study team comprises research
385 scientists, statisticians, study coordinators, nurses, data managers, administrative assistants
386 and data entry staff, who make the study possible. Whitehall II data, protocols, and other
387 metadata are available to bona fide researchers for research purposes. Please refer to the
388 Whitehall II data sharing policy at http://www.ucl.ac.uk/whitehallII/data-sharing.

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Disclosure

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543 **Figure 1** Effect of one population standard deviation difference in the Log of the immune
544 marker at baseline (hsCRP, IL-6, IL-1RA, adiponectin) on subsequent 5-year changes in
545 markers of glucose regulation. The associations are adjusted for baseline age, sex, study
546 phase, BMI, smoking, physical activity, alcohol intake, triacylglycerols, HDL-C and LDL-C
547 and baseline value of the outcome.

548

549 **Figure 2** Effect of a difference in baseline glycaemia, insulin sensitivity or beta-cell function
550 on subsequent 5-year changes in immune markers (hsCRP, IL-6, IL-1RA, adiponectin). The
551 associations are adjusted for baseline age, sex, study phase, BMI, smoking, physical activity,
552 alcohol intake, triacylglycerols, HDL-C and LDL-C and baseline value of the outcome.

553

Table 1 Characteristics of the study population at each study phase

Variable	Phase 3 (1991-1993)	Phase 5 (1997-1999)	Phase 7 (2002-2004)	Phase 9 (2007-2009)
n	5310	4310	4498	3773
Men (%)	69.1 (67.8;70.3)	71.6 (70.3;73.0)	73.3 (71.9;74.5)	72.9 (71.4;74.3)
White ethnicity (%)	90.5 (89.7;91.3)	91.9 (91.1;92.7)	92.8 (92.0;93.5)	93.1 (92.2;93.8)
Age (years)	49.4 (6.0)	55.1 (5.9)	60.6 (5.9)	65.4 (5.9)
BMI (kg/m^2)	25.3 (3.7)	26.1 (3.9)	26.6 (4.2)	26.6 (4.3)
Waist circumference (cm)	85.7 (11.6)	90.5 (11.6)	93.3 (11.9)	94.4 (11.9)
Total cholesterol (mmol/l)	6.5 (1.2)	5.9 (1.1)	5.8 (1.0)	5.3 (1.1)
HDL cholesterol (mmol/l)	1.4 (0.4)	1.5 (0.4)	1.6 (0.4)	1.6 (0.4)
LDL cholesterol (mmol/l)	4.4 (1.0)	3.9 (0.9)	3.6 (0.9)	3.2 (1.0)
Triacylglycerols (mmol/l)	1.5 (1.1)	1.3 (0.9)	1.3 (0.9)	1.2 (0.7)
Systolic blood pressure (mmHg)	120.4 (13.6)	122.2 (16.3)	127.7 (16.8)	125.3 (15.9)
Diastolic blood pressure (mmHg)	79.7 (9.5)	77.3 (10.4)	74.5 (10.5)	71.5 (10.0)
Fasting plasma glucose (mmol/l)	5.3 (0.7)	5.2 (0.7)	5.3 (0.8)	5.2 (0.6)
2-hour plasma glucose (mmol/l)	5.3 (1.9)	5.9 (1.8)	6.3 (1.9)	6.4 (1.9)
HbA_{1c} (%)	-	-	5.3 (0.5)	5.6 (0.4)
HbA_{1c} (mmol/mol)	-	-	39.1 (5.9)	43.8 (5.3)
Fasting serum insulin (pmol/l)	5.7 (3.7;8.9)	7.0 (4.9;10.2)	7.0 (4.7;10.7)	6.6 (4.3;10.2)
2-hour serum insulin (pmol/l)	33.0 (18.5;56.5)	32.6 (19.8;53.2)	37.8 (23.3;63.7)	41.3 (25.6;69.1)
HOMA-IR	1.3 (0.8;2.1)	1.6 (1.1;2.4)	1.6 (1.1;2.6)	1.5 (1.0;2.4)
HOMA-β	67.1 (44.4;100.8)	92.0 (65.1;131.3)	80.0 (55.2;118.9)	82.2 (55.7;120)
ISI_{0-120}	40.7 (31.5;53.3)	38.1 (29.8;48.9)	34.4 (26.4;44.5)	33.6 (25.5;43.6)
hsCRP (mg/dl)	0.9 (0.4;1.8)	1.0 (0.5;2.0)	1.2 (0.6;2.4)	-
IL-6 (pg/ml)	1.4 (1.0;2.0)	1.4 (1.0;2.0)	1.7 (1.2;2.4)	-
IL-1Ra (pg/ml)[a]	0.2 (0.2;0.3)	0.3 (0.3;0.4)	0.3 (0.3;0.4)	0.3 (0.3;0.4)
Adiponectin (µg/ml)[a]	8.7 (6.4;12.4)	8.6 (6.2;12.2)	8.3 (6.0;11.7)	8.5 (5.6;13.4)
Family history of diabetes (%)	11.2 (10.4;12.1)	10.3 (9.4;11.3)	10.0 (9.1;10.9)	9.6 (8.7;10.6)
Current smoker (%)	13.7 (12.8;14.7)	10.4 (9.5;11.3)	8.4 (7.6;9.3)	5.7 (5.0;6.5)
Moderate to vigorous exercise (hours/week)	2.0 (1.0;5.0)	11.5 (4.5;20.0)	12.0 (4.5;20.5)	-
Alcohol intake (units/week)	6.0 (2.0;14.0)	10.0 (3.0;20.0)	9.0 (3.0;18.0)	7.0 (2.0;16.0)
Antihypertensive treatment (%)	7.3 (6.6;8.0)	11.3 (10.3;12.2)	22.2 (21;23.4)	33.0 (31.5;34.5)
Lipid-lowering treatment (%)	0.8 (0.6;1.1)	2.8 (2.4;3.4)	10.0 (9.2;11.0)	29.1 (27.6;30.6)

Data are means (SD), medians (25^{th};75^{th} percentiles) or proportions (95% CI).

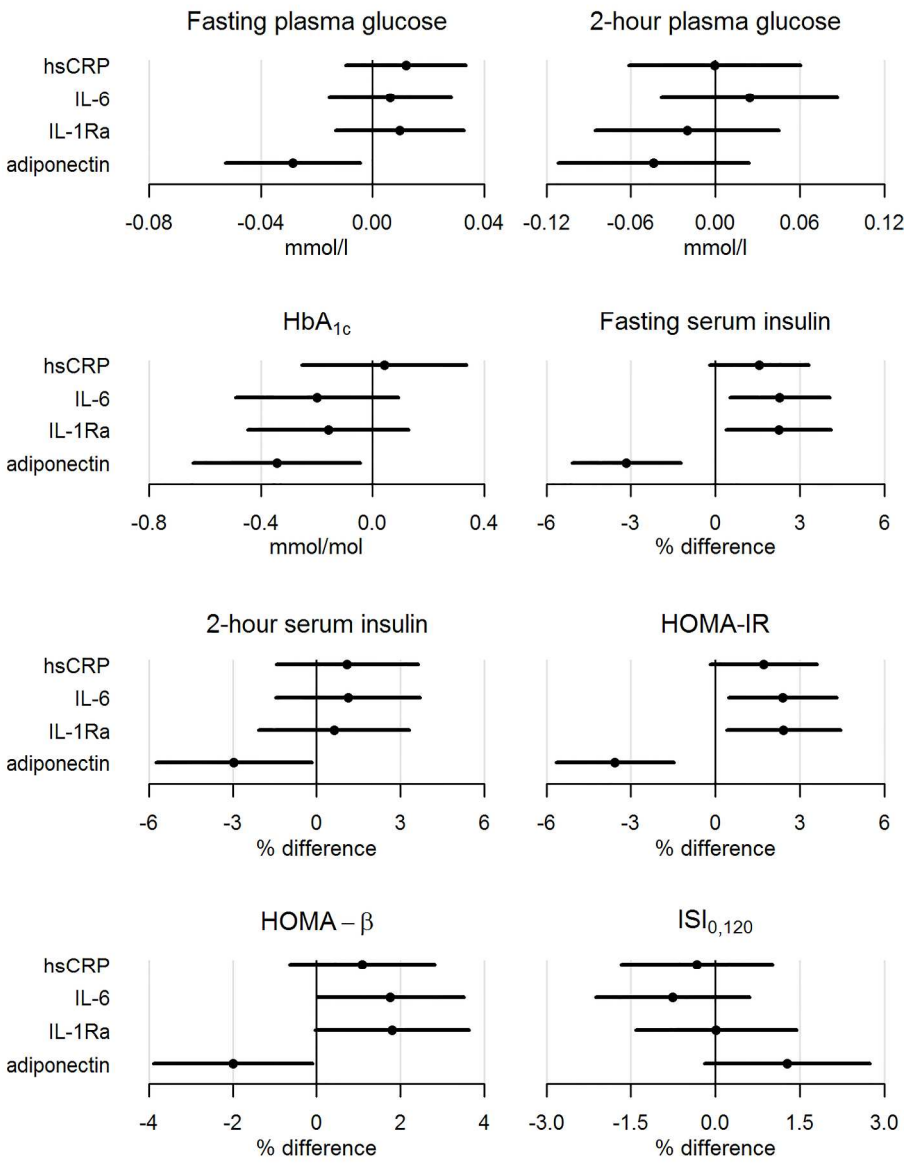
[a]Subsample (*n*=2636).

Table 2 Effects (with 95% CI) of a doubling in the inflammatory marker at baseline on 5-year changes in glycaemia, insulin, insulin sensitivity and beta-cell function

		hsCRP			IL-6			IL-1Ra			Adiponectin		
Outcome	Model	*n*	Estimate	*P*	*n*	Estimate	*P*	*n*	Estimate	*P*	*n*	Estimate	*P*
Fasting glucose (mmol/l)	1	6716	**0.02 (0.01;0.03)**	**<0.001**	6525	**0.02 (0.00;0.04)**	**0.027**	3651	**0.05 (0.02;0.08)**	**0.004**	3651	**-0.05 (-0.08;-0.02)**	**<0.001**
	2	6716	0.01 (0.00;0.02)	0.044	6525	0.01 (-0.01;0.03)	0.336	3651	0.03 (-0.01;0.06)	0.132	3651	**-0.04 (-0.07;-0.01)**	**0.003**
	3	6716	0.01 (0.00;0.02)	0.139	6525	0.00 (-0.02;0.02)	0.778	3651	0.02 (-0.02;0.05)	0.353	3651	**-0.04 (-0.07;-0.01)**	**0.020**
	4	6716	0.01 (0.00;0.02)	0.208	6525	0.00 (-0.02;0.02)	0.968	3651	0.01 (-0.03;0.05)	0.591	3651	**-0.04 (-0.07;-0.01)**	**0.011**
2-h glucose (mmol/l)	1	6033	**0.08 (0.05;0.10)**	**<0.001**	6029	**0.08 (0.03;0.13)**	**0.003**	3479	**0.11 (0.01;0.20)**	**0.027**	3479	**-0.13 (-0.21;-0.05)**	**0.001**
	2	6033	**0.04 (0.01;0.07)**	**0.004**	6029	0.03 (-0.02;0.08)	0.292	3479	0.00 (-0.10;0.10)	0.998	3479	-0.09 (-0.17;-0.01)	0.035
	3	6033	**0.03 (0.00;0.06)**	**0.028**	6029	0.01 (-0.04;0.07)	0.661	3479	-0.03 (-0.14;0.07)	0.509	3479	-0.07 (-0.16;0.02)	0.135
	4	6033	0.03 (0.00;0.06)	0.044	6029	0.01 (-0.05;0.06)	0.806	3479	-0.05 (-0.16;0.05)	0.309	3479	-0.07 (-0.16;0.02)	0.109
HbA_{1c} (mmol/mol)	1	2535	0.06 (-0.05;0.16)	0.285	2363	-0.11 (-0.31;0.08)	0.263	1190	-0.01 (-0.42;0.40)	0.961	1190	**-0.53 (-0.88;-0.18)**	**0.003**
	2	2535	0.02 (-0.09;0.14)	0.712	2363	-0.16 (-0.36;0.04)	0.120	1190	-0.10 (-0.55;0.34)	0.648	1190	**-0.52 (-0.88;-0.16)**	**0.005**
	3	2535	0.01 (-0.11;0.12)	0.904	2363	-0.17 (-0.38;0.04)	0.107	1190	-0.23 (-0.69;0.23)	0.325	1190	**-0.45 (-0.84;-0.06)**	**0.026**
	4	2535	-0.01 (-0.12;0.11)	0.874	2363	-0.22 (-0.42;-0.02)	0.033	1190	-0.28 (-0.73;0.18)	0.238	1190	**-0.44 (-0.83;-0.05)**	**0.028**
Fasting insulin (% diff.)	1	6186	**2.5 (1.7;3.3)**	**<0.001**	6177	**4.8 (3.2;6.4)**	**<0.001**	3617	**7.2 (4.3;10.2)**	**<0.001**	3617	**-5.6 (-7.8;-3.3)**	**<0.001**
	2	6186	**1.4 (0.6;2.3)**	**0.001**	6177	**3.4 (1.8;5.0)**	**<0.001**	3617	**4.9 (1.9;7.9)**	**0.001**	3617	**-4.8 (-7.0;-2.6)**	**<0.001**
	3	6186	**1.1 (0.3;2.0)**	**0.010**	6177	**2.7 (1.2;4.4)**	**<0.001**	3617	**4.0 (1.0;7.1)**	**0.009**	3617	**-4.1 (-6.5;-1.7)**	**0.001**
	4	6186	**0.9 (0.1;1.7)**	**0.024**	6177	**2.2 (0.7;3.7)**	**0.003**	3617	2.4 (-0.4;5.3)	0.094	3617	**-4.6 (-6.8;-2.3)**	**<0.001**

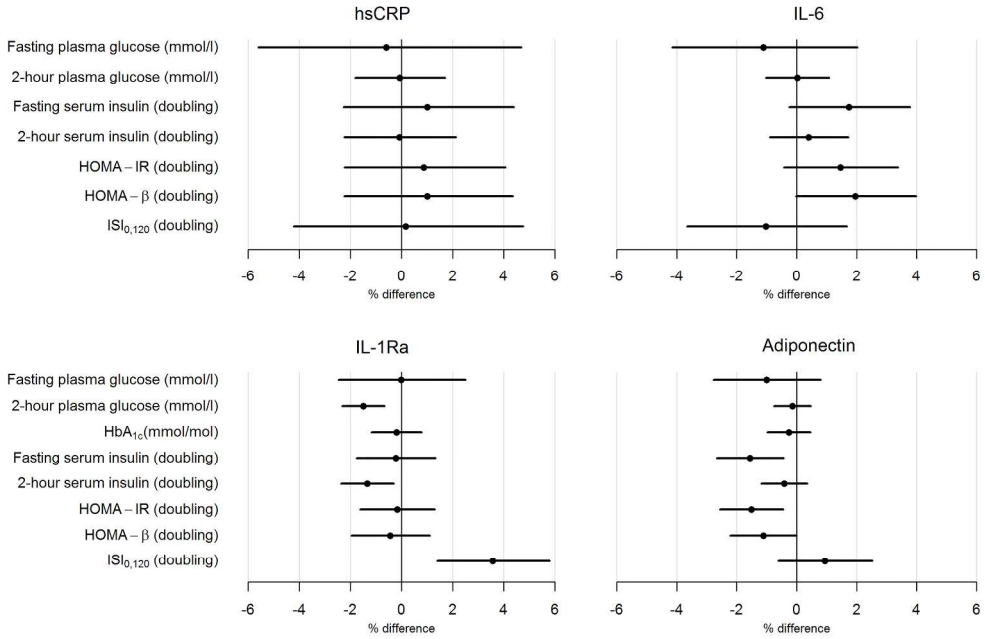
2-h insulin (% diff.)	1	5951	**2.4 (1.3;3.5)**	**<0.001**	5946	**2.5 (0.4;4.8)**	**0.021**	3428	4.0 (0.0;8.1)	0.050	3428	**-5.1 (-8.2;-1.8)**	**0.002**
	2	5951	**1.7 (0.5;3.0)**	**0.005**	5946	1.5 (-0.7;3.8)	0.188	3428	2.0 (-2.2;6.4)	0.349	3428	**-4.4 (-7.6;-1.1)**	**0.010**
	3	5951	**1.4 (0.2;2.6)**	**0.028**	5946	0.9 (-1.4;3.2)	0.457	3428	1.0 (-3.3;5.4)	0.667	3428	-3.8 (-7.2;-0.2)	0.038
	4	5951	1.2 (0.0;2.4)	0.057	5946	0.5 (-1.7;2.7)	0.681	3428	-0.6 (-4.7;3.7)	0.791	3428	-4.0 (-7.4;-0.6)	0.024
HOMA-IR (% diff.)	1	6168	**2.7 (1.8;3.6)**	**<0.001**	6159	**5.0 (3.3;6.7)**	**<0.001**	3612	**7.9 (4.7;11.1)**	**<0.001**	3612	**-6.2 (-8.6;-3.8)**	**<0.001**
	2	6168	**1.5 (0.6;2.5)**	**0.001**	6159	**3.5 (1.8;5.2)**	**<0.001**	3612	**5.3 (2.1;8.7)**	**0.001**	3612	**-5.4 (-7.8;-3.0)**	**<0.001**
	3	6168	**1.2 (0.3;2.2)**	**0.010**	6159	**2.7 (1.0;4.5)**	**0.002**	3612	**4.3 (1.0;7.7)**	**0.010**	3612	**-4.6 (-7.2;-2.0)**	**<0.001**
	4	6168	**1.0 (0.1;1.9)**	**0.025**	6159	**2.2 (0.6;3.8)**	**0.008**	3612	2.6 (-0.5;5.7)	0.096	3612	**-5.2 (-7.5;-2.7)**	**<0.001**
HOMA-β (% diff.)	1	6164	**2.1 (1.3;2.9)**	**<0.001**	6155	**4.3 (2.7;5.8)**	**<0.001**	3611	**5.3 (2.5;8.2)**	**<0.001**	3611	**-3.9 (-6.1;-1.7)**	**<0.001**
	2	6164	**1.1 (0.3;2.0)**	**0.009**	6155	**3.0 (1.5;4.6)**	**<0.001**	3611	**3.7 (0.8;6.7)**	**0.012**	3611	**-3.3 (-5.5;-1.0)**	**0.005**
	3	6164	1.0 (0.1;1.8)	0.031	6155	**2.6 (1.0;4.2)**	**0.001**	3611	3.2 (0.2;6.3)	0.036	3611	-2.7 (-5.0;-0.2)	0.032
	4	6164	0.8 (0.0;1.6)	0.064	6155	**2.2 (0.7;3.8)**	**0.005**	3611	2.0 (-0.8;5.0)	0.163	3611	**-3.0 (-5.3;-0.7)**	**0.012**
ISI0-120 (% diff.)	1	5800	**-1.6 (-2.2;-1.0)**	**<0.001**	5793	**-1.9 (-3.1;-0.8)**	**<0.001**	3419	**-2.5 (-4.5;-0.5)**	**0.015**	3419	**2.9 (1.1;4.7)**	**0.002**
	2	5800	**-1.0 (-1.6;-0.3)**	**0.003**	5793	-1.0 (-2.2;0.2)	0.090	3419	-0.6 (-2.8;1.6)	0.596	3419	**2.1 (0.3;4.0)**	**0.023**
	3	5800	**-0.8 (-1.4;-0.1)**	**0.022**	5793	-0.6 (-1.8;0.6)	0.320	3419	0.0 (-2.2;2.4)	0.970	3419	1.8 (-0.2;3.7)	0.075
	4	5800	-0.7 (-1.3;0.0)	0.046	5793	-0.4 (-1.6;0.8)	0.519	3419	0.9 (-1.4;3.1)	0.457	3419	1.9 (0.0;3.9)	0.049

n: number of person-examinations used in the particular analysis. *P*: *P* value for the test of the effect being equal to zero. Values in bold print indicate significant associations after adjustment for multiple testing (128 tests) with the method of Benjamini and Hochberg.
Model 1: Adjusted for baseline age, sex, study phase and baseline value of the outcome.
Model 2: Further adjustment for baseline BMI.
Model 3: Further adjustment for baseline smoking, physical activity, alcohol intake, triacylglycerols, HDL-C and LDL-C.
Model 4: Further adjustment for 5-year change in BMI after baseline.



Effect of one population standard deviation difference in the Log of the immune marker at baseline (hsCRP, IL-6, IL-1RA, adiponectin) on subsequent 5-year changes in markers of glucose regulation. The associations are adjusted for baseline age, sex, study phase, BMI, smoking, physical activity, alcohol intake, triacylglycerols, HDL-C and LDL-C and baseline value of the outcome.

159x213mm (600 x 600 DPI)



Effect of a difference in baseline glycaemia, insulin sensitivity or beta-cell function on subsequent 5-year changes in immune markers (hsCRP, IL-6, IL-1RA, adiponectin). The associations are adjusted for baseline age, sex, study phase, BMI, smoking, physical activity, alcohol intake, triacylglycerols, HDL-C and LDL-C and baseline value of the outcome.

126x84mm (600 x 600 DPI)

Supplementary Table 1 Effects (with 95% CI) of a standard deviation increase in Log of the inflammatory marker at baseline on 5-year changes in glycaemia and insulin.

		hsCRP			IL-6			IL-1Ra			Adiponectin		
Outcome	**Model**	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***
Fasting plasma glucose (mmol/l)	1	3589	**0.03 (0.01;0.05)**	**0.004**	3589	**0.02 (0.00;0.04)**	**0.036**	3589	**0.03 (0.01;0.05)**	**0.005**	3589	**-0.04 (-0.06;-0.02)**	**<0.001**
	2	3589	0.02 (-0.01;0.04)	0.143	3589	0.01 (-0.01;0.03)	0.264	3589	0.02 (-0.01;0.04)	0.160	3589	**-0.03 (-0.05;-0.01)**	**0.004**
	3	3589	0.01 (-0.01;0.03)	0.274	3589	0.01 (-0.02;0.03)	0.572	3589	0.01 (-0.01;0.03)	0.406	3589	**-0.03 (-0.05;0.00)**	**0.020**
	4	3589	0.01 (-0.01;0.03)	0.326	3589	0.00 (-0.02;0.03)	0.670	3589	0.01 (-0.02;0.03)	0.640	3589	**-0.03 (-0.05;-0.01)**	**0.011**
2-hour plasma glucose (mmol/l)	1	3421	**0.08 (0.02;0.13)**	**0.005**	3421	**0.08 (0.02;0.14)**	**0.006**	3421	**0.07 (0.01;0.12)**	**0.028**	3421	**-0.09 (-0.16;-0.03)**	**0.003**
	2	3421	0.02 (-0.04;0.08)	0.468	3421	0.04 (-0.02;0.10)	0.169	3421	0.00 (-0.06;0.06)	0.959	3421	-0.06 (-0.12;0.00)	0.058
	3	3421	0.00 (-0.06;0.06)	0.981	3421	0.02 (-0.04;0.09)	0.452	3421	-0.02 (-0.09;0.04)	0.541	3421	-0.04 (-0.11;0.02)	0.205
	4	3421	-0.01 (-0.07;0.05)	0.853	3421	0.02 (-0.04;0.08)	0.512	3421	-0.03 (-0.10;0.03)	0.343	3421	-0.05 (-0.11;0.02)	0.166
HbA_{1c} (mmol/mol)	1	1184	0.10 (-0.17;0.37)	0.463	1184	-0.13 (-0.4;0.14)	0.335	1184	-0.02 (-0.27;0.24)	0.889	1184	**-0.40 (-0.67;-0.14)**	**0.003**
	2	1184	0.06 (-0.23;0.35)	0.681	1184	-0.20 (-0.48;0.09)	0.178	1184	-0.08 (-0.36;0.20)	0.574	1184	**-0.40 (-0.67;-0.12)**	**0.005**
	3	1184	0.04 (-0.25;0.34)	0.780	1184	-0.20(-0.49;0.09)	0.183	1184	-0.16 (-0.45;0.13)	0.282	1184	**-0.34 (-0.64;-0.04)**	**0.025**
	4	1184	0.03 (-0.27;0.32)	0.860	1184	-0.24 (-0.53;0.05)	0.102	1184	-0.18 (-0.47;0.10)	0.213	1184	**-0.33 (-0.63;-0.04)**	**0.028**
Fasting insulin (% diff.)	1	3555	**3.2 (1.6;4.9)**	**<0.001**	3555	**3.8 (2.1;5.6)**	**<0.001**	3555	**4.2 (2.5;6.0)**	**<0.001**	3555	**-4.2 (-5.9;-2.5)**	**<0.001**
	2	3555	**1.8 (0.0;3.5)**	**0.046**	3555	**2.8 (1.0;4.6)**	**0.002**	3555	**2.8 (0.9;4.7)**	**0.003**	3555	**-3.6 (-5.4;-1.9)**	**<0.001**
	3	3555	1.6 (-0.2;3.4)	0.083	3555	**2.3 (0.5;4.1)**	**0.012**	3555	**2.3 (0.4;4.2)**	**0.018**	3555	**-3.1 (-5.0;-1.2)**	**0.001**
	4	3555	1.3 (-0.4;2.9)	0.136	3555	**2.0 (0.3;3.7)**	**0.021**	3555	1.3 (-0.4;3.1)	0.138	3555	**-3.5 (-5.2;-1.7)**	**<0.001**

2-hour insulin (% diff.)	1	3371	**2.8 (0.5;5.2)**	**0.018**	3371	**2.6 (0.1;5.2)**	**0.039**	3371	**2.5 (0.0;5.0)**	**0.048**	3371	**-3.9 (-6.3;-1.4)**	**0.003**
	2	3371	1.8 (-0.7;4.3)	0.170	3371	1.8 (-0.7;4.4)	0.167	3371	1.3 (-1.4;4.0)	0.344	3371	**-3.3 (-5.8;-0.8)**	**0.011**
	3	3371	1.1 (-1.4;3.7)	0.400	3371	1.1 (-1.5;3.8)	0.395	3371	0.6 (-2.1;3.4)	0.654	3371	**-2.9 (-5.6;-0.2)**	**0.037**
	4	3371	0.7 (-1.8;3.3)	0.579	3371	0.9 (-1.6;3.5)	0.501	3371	-0.3 (-2.9;2.4)	0.840	3371	**-3.1 (-5.7;-0.5)**	**0.022**

n: the number of person-examinations used in the particular analysis. *P*: *P* value for the test of the effect being equal to zero. Values in bold print indicate significant associations after adjustment for multiple testing (128 tests) with the method of Benjamini and Hochberg.

Model 1: adjusted for baseline age, sex, study phase and baseline value of the outcome

Model 2: further adjustment for baseline BMI

Model 3: further adjustment for baseline smoking, physical activity, alcohol intake, triacylglycerols, HDL-C and LDL-C

Model 4: further adjustment for 5-year change in BMI after baseline

Supplementary Table 2 Effects (with 95% CI) of a standard deviation increase in Log of the inflammatory marker at baseline on 5-year changes in insulin sensitivity and beta-cell function **.**

		hsCRP			IL-6			IL-1Ra			Adiponectin		
Outcome	**Model**	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***
HOMA-IR (% diff.)	1	3550	**3.6 (1.8;5.4)**	**<0.001**	3550	**4.2 (2.2;6.1)**	**<0.001**	3550	**4.6 (2.7;6.6)**	**<0.001**	3550	**-4.7 (-6.5;-2.8)**	**<0.001**
	2	3550	**2.0 (0.1;3.9)**	**0.040**	3550	**3.0 (1.1;5.0)**	**0.002**	3550	**3.0 (1.0;5.1)**	**0.003**	3550	**-4.1 (-5.9;-2.2)**	**<0.001**
	3	3550	1.7 (-0.2;3.7)	0.078	3550	**2.4 (0.5;4.4)**	**0.015**	3550	**2.4 (0.4;4.5)**	**0.019**	3550	**-3.5 (-5.5;-1.5)**	**<0.001**
	4	3550	1.4 (-0.4;3.2)	0.133	3550	**2.1 (0.2;3.9)**	**0.027**	3550	1.4 (-0.5;3.4)	0.141	3550	**-3.9 (-5.8;-2.0)**	**<0.001**
HOMA-β (% diff.)	1	3549	**2.2 (0.6;3.9)**	**0.008**	3549	**2.8 (1.1;4.5)**	**0.001**	3549	**3.1 (1.4;4.9)**	**<0.001**	3549	**-2.9 (-4.6;-1.2)**	**0.001**
	2	3549	1.2 (-0.6;2.9)	0.186	3549	**2.0 (0.3;3.8)**	**0.022**	3549	**2.1 (0.3;4.0)**	**0.021**	3549	**-2.4 (-4.2;-0.7)**	**0.006**
	3	3549	1.1 (-0.6;2.9)	0.218	3549	**1.8 (0.0;3.6)**	**0.049**	3549	1.8 (0.0;3.7)	0.055	3549	**-2.0 (-3.8;-0.1)**	**0.039**
	4	3549	0.9 (-0.8;2.6)	0.311	3549	1.5 (-0.2;3.3)	0.076	3549	1.2 (-0.6;3.0)	0.209	3549	**-2.3 (-4.0;-0.4)**	**0.015**
ISI_{0-120} (% diff.)	1	3363	**-1.8 (-3.0;-0.6)**	**0.003**	3363	**-1.9 (-3.2;-0.7)**	**0.003**	3363	**-1.6 (-2.8;-0.3)**	**0.016**	3363	**2.1 (0.7;3.5)**	**0.003**
	2	3363	-0.7 (-2.0;0.6)	0.264	3363	-1.2 (-2.5;0.1)	0.080	3363	-0.4 (-1.8;1.0)	0.592	3363	**1.5 (0.1;2.9)**	**0.030**
	3	3363	-0.3 (-1.7;1.0)	0.631	3363	-0.8 (-2.1;0.6)	0.273	3363	0.0 (-1.4;1.4)	0.987	3363	1.3 (-0.2;2.8)	0.090
	4	3363	-0.1 (-1.4;1.2)	0.853	3363	-0.6 (-1.9;0.7)	0.359	3363	0.5 (-0.9;1.9)	0.494	3363	1.4 (0.0;2.9)	0.058

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Model 1: adjusted for baseline age, sex, study phase and baseline value of the outcome

Model 2: further adjustment for baseline BMI

Model 3: further adjustment for baseline smoking, physical activity, alcohol intake, triacylglycerols, HDL-C and LDL-C

Model 4: further adjustment for 5-year change in BMI after baseline

Supplementary Table 3 Effects (with 95% CI) of a difference in glycaemia and insulin on 5-year changes in the inflammatory marker (% difference)

		Fasting plasma glucose (mmol/l)			2-hour plasma glucose (mmol/l)			HbA_{1c} (mmol/mol)			Fasting insulin (doubling)			2-hour insulin (doubling)		
Outcome	**Model**	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***
hsCRP (% diff.)	1	4043	2.0 (-3.1;7.3)	0.457	3954	0.3 (-1.4;2.1)	0.707		NA		3919	**4.6 (1.7;7.7)**	**0.002**	3911	1.2 (-0.9;3.3)	0.270
	2	4043	-1.2 (-6.1;4.1)	0.658	3954	-0.3 (-2.0;1.5)	0.760		NA		3919	0.9 (-2.3;4.1)	0.587	3911	-0.3 (-2.4;1.8)	0.767
	3	4043	-0.6 (-5.6;4.7)	0.823	3954	-0.1 (-1.8;1.7)	0.937		NA		3919	1.0 (-2.3;4.4)	0.550	3911	-0.1 (-2.2;2.1)	0.944
	4	4043	-0.2 (-5.1;4.9)	0.944	3954	0.4 (-1.3;2.1)	0.659		NA		3919	1.3 (-1.9;4.5)	0.443	3911	0.4 (-1.7;2.5)	0.738
IL-6 (% diff.)	1	3946	0.7 (-2.3;3.9)	0.637	3867	0.3 (-0.7;1.4)	0.559		NA		3821	**4.3 (2.5;6.1)**	**<0.001**	3828	1.3 (0.0;2.5)	0.045
	2	3946	-1.4 (-4.4;1.7)	0.371	3867	-0.2 (-1.2;0.9)	0.734		NA		3821	1.8 (-0.1;3.8)	0.063	3828	0.2 (-1.0;1.5)	0.745
	3	3946	-1.1 (-4.2;2.0)	0.482	3867	0.0 (-1.0;1.1)	0.965		NA		3821	1.7 (-0.2;3.8)	0.087	3828	0.4 (-0.9;1.7)	0.547
	4	3946	-1.0 (-4.0;2.1)	0.530	3867	0.1 (-0.9;1.2)	0.794		NA		3821	1.8 (-0.2;3.8)	0.072	3828	0.5 (-0.8;1.8)	0.433
IL-1Ra (% diff.)	1	2632	0.7 (-1.7;3.2)	0.563	2587	**-1.3 (-2.1;-0.5)**	**0.001**	242	0.0 (-1.0;0.9)	0.949	2632	1.0 (-0.4;2.4)	0.155	2563	-1.0 (-1.9;0.0)	0.054
	2	2632	-0.2 (-2.7;2.3)	0.867	2587	**-1.5 (-2.3;-0.7)**	**<0.001**	242	-0.1 (-1.1;0.9)	0.848	2632	-0.1 (-1.6;1.4)	0.859	2563	**-1.3 (-2.3;-0.3)**	**0.009**
	3	2632	0.0 (-2.5;2.5)	0.992	2587	**-1.5 (-2.3;-0.7)**	**<0.001**	242	-0.2 (-1.2;0.8)	0.694	2632	-0.2 (-1.8;1.3)	0.773	2563	**-1.3 (-2.4;-0.3)**	**0.012**
	4	2632	0.3 (-2.0;2.8)	0.780	2587	**-1.2 (-2.0;-0.4)**	**0.004**	242	-0.1 (-1.0;0.9)	0.898	2632	0.2 (-1.3;1.7)	0.791	2563	-0.9 (-1.9;0.1)	0.073
Adiponectin (% diff.)	1	2631	-0.9 (-2.7;0.9)	0.311	2586	-0.1 (-0.7;0.5)	0.635	242	-0.2 (-0.9;0.5)	0.519	2631	**-1.3 (-2.3;-0.3)**	**0.009**	2562	-0.5 (-1.2;0.3)	0.207
	2	2631	-0.9 (-2.7;0.9)	0.314	2586	-0.1 (-0.7;0.5)	0.650	242	-0.3 (-1.0;0.4)	0.438	2631	**-1.6 (-2.6;-0.5)**	**0.005**	2562	-0.5 (-1.2;0.3)	0.223
	3	2631	-1.0 (-2.8;0.8)	0.274	2586	-0.1 (-0.8;0.5)	0.644	242	-0.3 (-1.0;0.5)	0.482	2631	**-1.6 (-2.7;-0.4)**	**0.007**	2562	-0.4 (-1.2;0.4)	0.286
	4	2631	-1.3 (-3.0;0.4)	0.125	2586	-0.4 (-1.0;0.2)	0.177	242	-0.4 (-1.1;0.3)	0.284	2631	**-1.9 (-2.9;-0.8)**	**<0.001**	2562	-0.8 (-1.5;0.0)	0.039

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Model 2: further adjustment for baseline BMI

Model 3: further adjustment for baseline smoking, physical activity, alcohol intake, triacylglycerols, HDL-C and LDL-C

Model 4: further adjustment for 5-year change in BMI after baseline

Supplementary Table 4 Effects (with 95% CI) of a difference in insulin sensitivity or beta-cell function on 5-year changes in the inflammatory marker (% difference)

		HOMA-IR (doubling)			HOMA-β (doubling)			ISI_{0-120} (doubling)		
Outcome	**Model**	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***	***n***	**Estimate**	***P***
hsCRP (% diff.)	1	3909	**4.3 (1.5;7.2)**	**0.003**	3908	**4.2 (1.2;7.3)**	**0.006**	3777	-2.0(-6.1;2.2)	0.340
	2	3909	0.7 (-2.3;3.8)	0.646	3908	1.1 (-2.0;4.3)	0.505	3777	0.8(-3.5;5.2)	0.726
	3	3909	0.9 (-2.2;4.1)	0.586	3908	1.0 (-2.2;4.4)	0.548	3777	0.2(-4.2;4.8)	0.941
	4	3909	1.1 (-1.9;4.2)	0.462	3908	1.2 (-2;4.4)	0.466	3777	-0.9(-5.1;3.5)	0.671
IL-6 (% diff.)	1	3811	**3.9 (2.2;5.6)**	**<0.001**	3810	**4.3 (2.5;6.1)**	**<0.001**	3691	-2.6(-5;-0.1)	0.043
	2	3811	1.5 (-0.3;3.4)	0.105	3810	2.1 (0.2;4.1)	0.029	3691	-0.5(-3;2.1)	0.727
	3	3811	1.5 (-0.4;3.4)	0.129	3810	2.0 (0.0;4.0)	0.053	3691	-1.0(-3.7;1.7)	0.452
	4	3811	1.5 (-0.3;3.4)	0.108	3810	2.0 (0.0;4.0)	0.046	3691	-1.3(-3.9;1.3)	0.327
IL-1Ra (% diff.)	1	2629	1.0 (-0.3;2.3)	0.139	2629	0.7 (-0.7;2.1)	0.348	2559	**2.6(0.6;4.7)**	**0.012**
	2	2629	-0.1 (-1.5;1.3)	0.880	2629	-0.3 (-1.7;1.2)	0.725	2559	**3.5(1.4;5.6)**	**<0.001**
	3	2629	-0.2 (-1.6;1.3)	0.822	2629	-0.4 (-2.0;1.1)	0.569	2559	**3.6(1.4;5.8)**	**0.001**
	4	2629	0.3 (-1.2;1.7)	0.728	2629	-0.1 (-1.6;1.4)	0.887	2559	**2.6(0.5;4.8)**	**0.013**
Adiponectin (% diff.)	1	2628	**-1.3 (-2.2;-0.3)**	**0.008**	2628	-1.1 (-2.1;-0.1)	0.038	2558	1.0(-0.5;2.5)	0.192
	2	2628	**-1.5 (-2.5;-0.5)**	**0.004**	2628	-1.2 (-2.2;-0.1)	0.031	2558	1.0(-0.5;2.5)	0.207
	3	2628	**-1.5 (-2.6;-0.4)**	**0.006**	2628	-1.1 (-2.2;0.0)	0.050	2558	0.9(-0.6;2.5)	0.238
	4	2628	**-1.8 (-2.8;-0.8)**	**<0.001**	2628	-1.4 (-2.4;-0.3)	0.012	2558	1.7(0.2;3.2)	0.023

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