



Biomarkers of subclinical inflammation and increases in glycaemia, insulin resistance and beta-cell function in non-diabetic individuals: the Whitehall II study

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3 **Biomarkers of subclinical inflammation and increases in glycaemia, insulin resistance**
4 **and beta-cell function in non-diabetic individuals: the Whitehall II study**

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6 Short title: Inflammation and glucose metabolism

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For Review Only

36 **Abstract**

37

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

44 *Design and Methods:* We used multiple repeat measures (17,891 person-examinations from
45 7,683 non-diabetic participants) from the Whitehall II study to assess whether circulating
46 high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), IL-1 receptor antagonist
47 (IL-1Ra) and adiponectin associated with subsequent changes in glycaemia, insulin, insulin
48 resistance and beta-cell function (based on oral glucose tolerance tests). We examined
49 bidirectionality by testing if parameters of glucose metabolism at baseline associated with
50 changes in inflammation-related biomarkers.

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

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

61

62 **Introduction**

63

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

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

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

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

92

93 **Materials and Methods**

94 **Study participants, procedures and measurements**

95 Participants are from the Whitehall II Study, an occupational cohort of 10,308 British civil
96 servants (6,896 men and 3,412 women aged 35–55 years) of mainly white ethnicity recruited
97 between 1985 and 1988 (phase 1) (13). The UK NHS Health Research Authority London-
98 Harrow ethics committee reviewed and approved the study. Written informed consent was
99 obtained from each participant at each examination phase. The study was conducted
100 according to the principles of the Helsinki Declaration. The cohort has been followed at eight
101 subsequent phases, 2.5 years apart. All study phases included a questionnaire, and every
102 second phase (5 years apart) also included a clinical health examination (phases 1, 3, 5, 7, and
103 9). Phase 3 (1991–1993) was the first phase with an oral glucose tolerance test (OGTT),
104 therefore phase 1 was not used. In the Whitehall II cohort 8,815 participated at phase 3
105 (1991–1993); 7,870 at phase 5 (1997–1999); 6,967 at phase 7 (2002–2004); and 6,761 at
106 phase 9 (2007–2009) with the same individual participating in several phases. During follow-
107 up, participants were censored if they died, were lost to follow-up or developed diabetes.
108 Anthropometric, demographic, clinical and lifestyle characteristics are summarised in Table 1.
109 At phases 3, 5, 7, and 9 a standard 2-hour 75 g OGTT was performed in the morning after an
110 overnight fast (≥ 8 hours of fasting). For around one third of the examinations, the OGTT was
111 administered in the afternoon after a light fat-free breakfast (≥ 5 hours of fasting). These
112 examinations were not considered in this study. Diabetes was diagnosed by a doctor outside
113 the study or at screening by OGTT. Screen-detected diabetes was ascertained throughout

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

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

119 Plasma glucose, serum insulin, HbA_{1c} and serum lipids were measured as described
120 previously (16,17). Insulin sensitivity and beta-cell function were estimated based on fasting
121 plasma glucose and serum insulin using the homeostasis model assessment for insulin
122 resistance (HOMA-IR) and beta-cell function (HOMA-β). In addition, whole-body insulin
123 sensitivity was assessed using the insulin sensitivity index (ISI₀₋₁₂₀) based on fasting and 2-
124 hour values of glucose and insulin (18).

125 High-sensitivity CRP (hsCRP) was measured using a high-sensitivity immunonephelometric
126 assay, IL-6 was measured using a high-sensitivity ELISA assay, IL-1 receptor antagonist (IL-
127 1Ra) and total adiponectin were measured with Quantikine ELISA kits (R&D Systems,
128 Wiesbaden, Germany) in a diabetes case-cohort sample (19,20).

129

130 **Statistical analysis**

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

133 In the main analysis the following outcomes were studied: fasting plasma glucose, 2-hour
134 plasma glucose, HbA_{1c}, fasting and 2-hour serum insulin, HOMA-IR, HOMA-β and ISI₀₋₁₂₀.

135 We excluded 10,529 (36.5%) person-examinations for which the participant had been fasting
136 for <8 hours (OGTTs administered in the afternoon). Outcomes with a skewed distribution
137 (fasting and 2-hour insulin, HOMA-IR, HOMA-β and ISI₀₋₁₂₀) were log-transformed prior to
138 analysis.

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

145 Up to a total of 17,891 person-examinations for 7,683 non-diabetic participants were analysed
146 (8,303 person-examinations for 2,965 participants in the subcohort). We studied the
147 associations of baseline levels of inflammation-related biomarkers and 5-year follow-up
148 levels of the different outcomes, including the baseline level of the outcome as a covariate.
149 The main analysis is based on all available data after the aforementioned exclusions and
150 provides effect estimates per doubling in baseline levels of the respective biomarker. In
151 addition, we used the subset of the population for whom all four biomarkers were available at
152 the same time-points to calculate regression coefficients that were standardised per 1-SD
153 difference in the Log of the biomarker to allow direct comparisons of effect sizes between the
154 exposure variables.

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

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

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

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

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

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

178

179 **Results**

180 **Associations between biomarkers of inflammation at baseline and 5-year changes in** 181 **glycaemia, insulin, insulin sensitivity and beta-cell function**

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

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

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

196 To compare effect sizes between exposures, we standardised our estimates per 1 population
197 SD of one Log unit of the concentrations of the four biomarkers of subclinical inflammation
198 (Fig. 1; Supplementary Tables 1 and 2). Effect sizes were similar for hsCRP, IL-6 and IL-
199 1Ra, but of larger magnitude (and in the opposite direction) for adiponectin.

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

204

205 **Associations of glycaemia, insulin, insulin sensitivity and beta-cell function at baseline** 206 **with 5-year changes in biomarkers of inflammation**

207 When interchanging exposures and outcomes, we observed fewer significant associations
208 (Fig. 2). None of the measures of glycaemia was associated with changes in hsCRP, IL-6, IL-
209 1Ra or adiponectin when further adjusting for 5-year change in BMI after baseline (fully
210 adjusted model), except an inverse association between 2-hr glucose and IL-1Ra
211 (Supplementary Tables 3 and 4). Fasting insulin and HOMA-IR showed inverse associations
212 with changes in adiponectin in the fully adjusted models, but neither insulin levels nor
213 HOMA-IR were related to changes in hsCRP, IL-6 or IL-1Ra (Supplementary Tables 3 and
214 4). High baseline levels of $ISI_{0,120}$ were positively associated with increases in IL-1Ra
215 (Supplementary Tables 3 and 4).

216

217 Discussion

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

226

227 Subclinical inflammation and glycaemia

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

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

10

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

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

256

257 **Subclinical inflammation and insulin resistance**

258 Our study revealed consistent associations between all four biomarkers and fasting insulin and
259 HOMA-IR, although the associations of IL-1Ra were not independent of 5-year changes in
260 BMI. In contrast, for 2-hour insulin and IS₀₋₁₂₀, which were based on post-load measures,
261 associations with hsCRP, IL-6 and adiponectin were only found in the initial regression
262 models, but not after full adjustment.

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

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

274

275 **Subclinical inflammation and beta-cell function**

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

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

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

295

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

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

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

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

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

347 epidemiological studies, cytokines such as IL-1 β (32-34), tumour necrosis factor (TNF)- α
348 (35,36) and transforming growth factor (TGF)- β (37,38) and chemokines such as monocyte
349 chemoattractant protein-1/chemokine (C-C motif) ligand 2 (MCP-1/CCL2) (39,40)
350 undoubtedly represent interesting candidates because of their impact on insulin sensitivity
351 and/or beta-cell function. However, circulating levels of IL-1 β are below the limit of detection
352 for a large proportion of individuals in population-based studies with currently available
353 assays, and experimental data on TNF α and insulin resistance do not appear to be translated
354 into an association between circulating levels of this protein and risk of type 2 diabetes in
355 cohort studies (41,42). Data on most other inflammation-related biomarkers and incident type
356 2 diabetes are based on only one or very few cohorts, so that further studies on their relevance
357 both for early deterioration of glucose metabolism and for the manifestation of type 2 diabetes
358 would be important.

359

360 **Conclusion**

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

368

369

370

371

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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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>.

389

390 **Disclosure**

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407

408

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542

For Review Only

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

Review Only

1 **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)
<i>n</i>	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)

2

3 Data are means (SD), medians (25th;75th percentiles) or proportions (95% CI).4 ^aSubsample (*n*=2636).

- 1 **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
 2 beta-cell function

Outcome	Model	hsCRP			IL-6			IL-1Ra			Adiponectin		
		<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>
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

3

4 *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

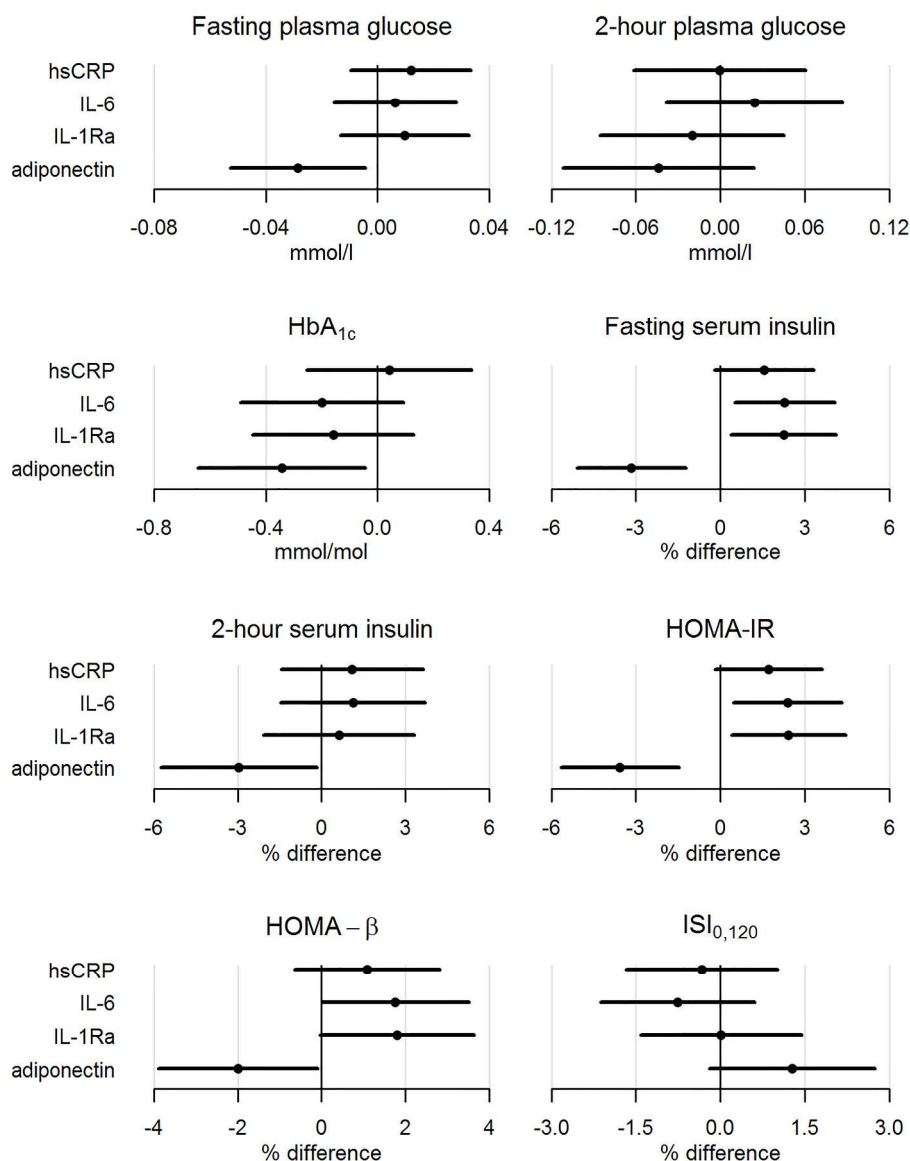
5 indicate significant associations after adjustment for multiple testing (128 tests) with the method of Benjamini and Hochberg.

6 Model 1: Adjusted for baseline age, sex, study phase and baseline value of the outcome.

7 Model 2: Further adjustment for baseline BMI.

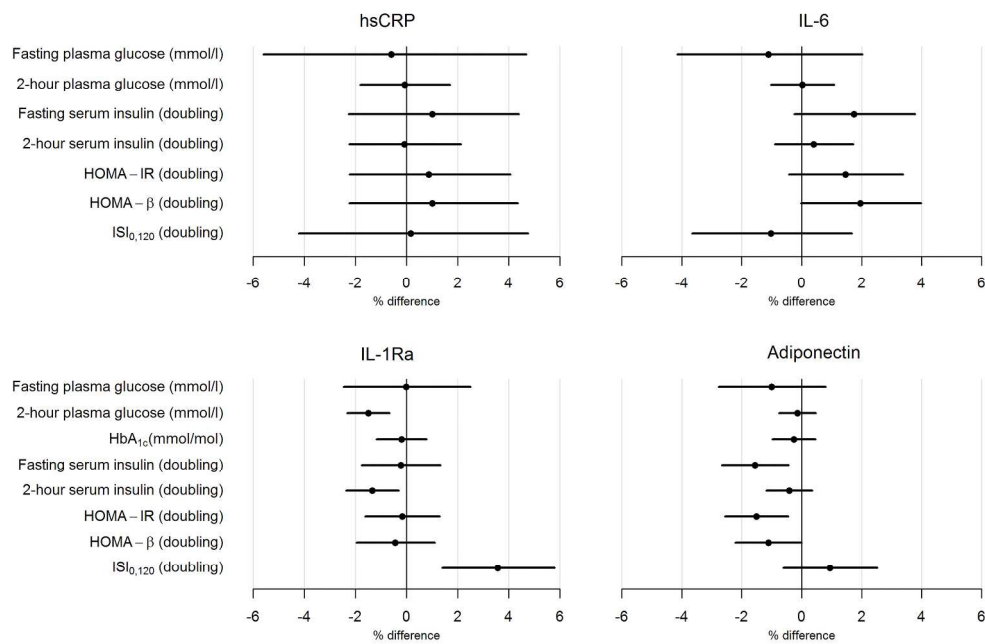
8 Model 3: Further adjustment for baseline smoking, physical activity, alcohol intake, triacylglycerols, HDL-C and LDL-C.

9 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.

Outcome	Model	hsCRP			IL-6			IL-1Ra			Adiponectin		
		<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>
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 .

Outcome	Model	hsCRP			IL-6			IL-1Ra			Adiponectin		
		<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>
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

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

Outcome	Model	Fasting plasma glucose (mmol/l)			2-hour plasma glucose (mmol/l)			HbA _{1c} (mmol/mol)			Fasting insulin (doubling)			2-hour insulin (doubling)		
		<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>
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

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

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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)

Outcome	Model	HOMA-IR (doubling)			HOMA- β (doubling)			ISI ₀₋₁₂₀ (doubling)		
		<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>	<i>n</i>	Estimate	<i>P</i>
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

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

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