**Association of Changes in Inflammation with Changes in Glycemia, Insulin Resistance and Secretion: a DIRECT study based on KORA**

Tonia de las Heras Gala1,2, Christian Herder2,3, Femke Rutters4, Maren Carstensen-Kirberg2,3, Cornelia Huth1,2, Coen DA Stehouwer5, Giel Nijpels4, Casper Schalkwijk5, Allan Flyvbjerg6, Paul W. Franks7, Jacqueline Dekker4,Christa Meisinger1,2,8, Wolfgang Koenig9,10,11, Michael Roden2,3,12, Wolfgang Rathmann2,13, Annette Peters1,2,10, Barbara Thorand1,2

1 Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

2 German Center for Diabetes Research (DZD), München-Neuherberg, Germany

3 Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany

4 Department of Epidemiology and Biostatistics and EMGO+ Institute for Health and Care Research, VUMC, Amsterdam, the Netherlands

5 Department of Internal Medicine and Cardiovascular Research Institute Maastricht (CARIM),

Maastricht University Medical Centre, Maastricht, the Netherlands.

6 Steno Diabetes Center Copenhagen, The Capital Region of Denmark, Copenhagen, Denmark

7 Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University Diabetes Centre, Lund University, Malmö, Sweden

8 Chair of Epidemiology, Ludwig-Maximilians-Universität München,UNIKA-T Augsburg, Germany

9 Deutsches Herzzentrum München, Technische Universität München, Munich, Germany

10 Department of Internal Medicine II- Cardiology, University of Ulm, Medical Center, Ulm, Germany

11 DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany

12 Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

13 Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany

***Short running title:*** Inflammation and Glycemic Changes

***Keywords:*** Glycemic deterioration, HbA1c, Insulin resistance, ß-cell function, Inflammation

***Word count abstract:*** 239

***Word count main body of text:*** 3499 ***(***excluding references, tables and figures) (maximum 3500)

***Number of references:*** 40

***Number of tables:***2

***Number of figures:*** 2

***Number of supplementary tables:*** 3

***Corresponding author and person to whom reprint requests should be addressed:***

Prof. Dr. Barbara Thorand

Helmholtz Zentrum München GmbH

Institute of Epidemiology

Ingolstädter Landstraße 1, D-85764 Neuherberg, Germany

Phone: +49-(0)89-3187-4480

Fax: +49-(0)89-3187-3667

E-mail: thorand@helmholtz-muenchen.de

**Abstract**  239 words (max. 250)

*Aims:* Subclinical systemic inflammation may contribute to the development of type 2 diabetes, but its association with early progression of glycemic deterioration in persons without diabetes has not been fully investigated. Our primary aim was to assess longitudinal associations of changes in pro- (leukocytes, high-sensitivity C-reactive protein (hsCRP)) and anti-inflammatory (adiponectin) markers with changes in markers that assessed glycemia, insulin resistance and secretion (HbA1c, HOMA-IR, HOMA-ß). Furthermore, we aimed to directly compare longitudinal with cross-sectional associations.

*Materials and methods:* This study includes 819 initially non-diabetic individuals with repeated measurements from the KORA S4/F4 cohort study (median follow-up: 7.1 years). Longitudinal and cross-sectional associations were simultaneously examined using linear mixed growth models. Changes in markers of inflammation were used as independent and changes in markers of glycemia/insulin resistance/insulin secretion as dependent variables. Models were adjusted for age, sex and major lifestyle and metabolic risk factors for diabetes using time-varying variables in the final model.

*Results:* Changes of leukocyte count were positively associated with changes in HbA1c and HOMA-ß while changes in adiponectin were inversely associated with changes in HbA1c. All examined cross-sectional associations were statistically significant; they were generally stronger and mostly directionally consistent to the longitudinal association estimates.

*Conclusions:* Adverse changes in low-grade systemic inflammation go along with glycemic deterioration and increased insulin secretion independently of changes in other risk factors, suggesting that low-grade inflammation may contribute to the development of hyperglycemia and a compensatory increase in insulin secretion.

**Introduction**

Elevated concentrations of markers of low-grade systemic inflammation and decreased concentrations of the anti-inflammatory adipokine, adiponectin, have consistently been associated with the development of type 2 diabetes[1-3](#_ENREF_1). However, whether the observed associations are causal remains to be elucidated, as Mendelian randomization studies yielded mixed results[4-8](#_ENREF_4). Most prospective studies on inflammatory biomarkers focused on incident type 2 diabetes, whereas only few studies addressed associations between inflammatory biomarkers and changes in markers of glycemia such as HbA1c, and insulin resistance or secretion such as homeostasis model assessment-insulin resistance (HOMA-IR) and HOMA-ß in initially non-diabetic persons[9-12](#_ENREF_9). Particularly studies examining the association between changes in low-grade systemic inflammation over time and concomitant changes in glycemia, insulin resistance or insulin secretion are scarce. We are aware of only one study which assessed the longitudinal association between changes in hsCRP and changes in HbA1c[13](#_ENREF_13), while similar studies regarding markers of insulin resistance or secretion as the outcome or addressing associations for other markers than hsCRP are lacking. These data are important to better understand the temporal sequence of metabolic changes before the onset of type 2 diabetes. Furthermore, as higher HbA1c and HOMA-IR have been shown to be predictors of microvascular and macrovascular outcomes in people with prediabetes[14-17](#_ENREF_14) and have even been linked to higher risk of cardiovascular disease and all-cause mortality in non-diabetic individuals[18-20](#_ENREF_18), it is important to identify the determinants of early adverse changes in glycemia, insulin resistance and secretion as this may open new avenues for early prevention.

Therefore, the primary aim of the present study was to investigate longitudinal associations between changes in the proinflammatory markers leukocyte count and hsCRP as well as the anti-inflammatory adipokine adiponectin with glycemic deterioration and changes in insulin resistance and secretion, measured by levels of HbA1c, HOMA-IR and HOMA-β, respectively, in participants without diabetes from a prospective, population-based cohort study. In order to directly compare longitudinal and cross-sectional effect estimates we aimed to simultaneously assess corresponding cross-sectional associations using linear mixed growth models.

**Materials and Methods**

*Study population and methods*

The Cooperative Health Research in the Region of Augsburg (KORA) Study S4 is a prospective population-based study with baseline examination in 1999-2001 (n=4,261), including individuals aged 25 to 74 years from the city of Augsburg and the two adjacent counties located in the state of Bavaria in Southern Germany. Individuals were re-investigated in the follow-up study KORA F4 in 2006-2008 (n=3,080; response: 80% of eligible persons). The median follow-up time was 7.1 years (25th percentile: 7.0; 75th percentile: 7.1).

At baseline and at follow-up glucose tolerance status was determined by performing 75 g oral glucose tolerance tests (OGTT) in all individuals without known type 2 diabetes after an overnight fasting period of ≥8 h. Normal glucose metabolism (i.e. fasting glucose <6.1 mmol/l and 2 h glucose <7.8 mmol/l), prediabetes (fasting glucose ≥6.1 mmol/l but <7.0mmol/l, and 2 h glucose <7.8 mmol/l [isolated impaired fasting glucose (IFG)] or fasting glucose <6.1 mmol/l and 2 h glucose ≥7.8 mmol/l but <11.1 mmol/l [isolated impaired glucose tolerance (IGT)], or both [IFG and IGT]), and newly diagnosed diabetes (fasting glucose ≥7.0 mmol/l or 2 h glucose ≥11.1 mmol/l) were defined using the 1999 WHO diagnostic criteria for fasting and 2 h glucose levels [21](#_ENREF_21), [22](#_ENREF_22). HbA1c was measured using a turbidimetric immunological method (Tinaquant; Roche Diagnostics, Mannheim, Germany) with a Hitachi 717 analyzer in KORA S4 and with a reverse-phase cation-exchange HPLC method using the Menarini–Arkray analyzer HA-8160 (Menarini Diagnostics, Florence, Italy) in KORA F4. Fasting glucose was determined using a hexokinase method (KORA S4: Gluco-quant, Roche Diagnostics, Mannheim, Germany; KORA F4: GLU Flex, Dade Behring, Marburg, Germany). In KORA S4, fasting insulin was measured using a microparticle enzyme immunoassay (Abbott Laboratories, Ludwigshafen, Germany) and in KORA F4 using an electrochemiluminescence immunoassay (ECLIA) on a COBAS system (Roche Diagnostics, Mannheim, Germany). Using the HOMA model[23](#_ENREF_23), [24](#_ENREF_24), insulin resistance and insulin secretion were calculated as follows: HOMA-IR = fasting glucose (mmol⁄ l) \* fasting insulin (µU⁄ l) ⁄ 22.5; HOMA-β = (20 \* fasting insulin (µU⁄ l)) / (fasting glucose (mmol⁄ l) - 3.5).

Inflammatory variables were measured as follows: Leukocyte count was determined using a Coulter STKS Hematology analyzer in KORA S4 and a Coulter LH750 Hematology analyzer in KORA F4, respectively (both analyzers were from Block Scientific, New York, NY, USA). Plasma concentrations of hsCRP were assessed using a high-sensitivity latex-enhanced nephelometric assay on a BN II System analyzer (Siemens, Erlangen, Germany)[25](#_ENREF_25) in KORA S4 and F4. Serum levels of adiponectin were assessed using the human adiponectin RIA from Linco Research (St. Charles, MO, USA)[26](#_ENREF_26) in KORA S4 and the Human Total Adiponectin/Acrp30 Quantikine ELISA from R&D Systems (Wiesbaden, Germany) in KORA F4[27](#_ENREF_27).

Detailed information on the assessment of anthropometric and lifestyle variables has been published elsewhere[10](#_ENREF_10), [22](#_ENREF_22), [28](#_ENREF_28). Briefly, alcohol intake was categorized as no (0 g/day), moderate (men 0.1–39.9 g/day; women 0.1–19.9 g/day) and high (men ≥40 g/day; women ≥20 g/day) alcohol consumption. Individuals were considered to be physically active if they participated in leisure time physical activity in summer and in winter and if they were active for at least one hour per week in either season. Smoking status was categorized as never, former or current smoking, where smoking was defined as smoking at least one cigarette per day. Parental history was defined as positive (at least one parent with diabetes), negative (both parents without diabetes) or unknown diabetes status of mother and/or father.

Study protocols have been approved by the local Ethics Committee of the Bavarian Medical Association. All investigations were performed in accordance with the Declaration of Helsinki, including obtaining written informed consent from all participants.

*Exclusions*

Since fasting measurements of glucose and insulin were only available for KORA S4 individuals aged 55-74 years, all individuals outside this age range were excluded (n=2,608). After further exclusions as described in Figure 1, 819 individuals without diabetes at baseline were included in the present analysis. As missing values varied by parameter, the number of participants used in the final models varied slightly. In total, n=492 participants within the age range 55-74 years were lost-to-follow-up. After applying the same exclusion criteria as for the main analysis, n=371 of these were used for an attrition analysis.

*Statistical analysis*

Characteristics of the study participants are presented as percentages (number) for categorical variables and as median (25th, 75th percentiles) for continuous variables.

To model change in markers of glycemic deterioration, we used a two-level growth model, where observation times nested within individuals represent level 1 and individuals represent level 2[29](#_ENREF_29). We applied a specific approach to discriminate between cross-sectional (between-subject) and longitudinal (within-subject) effects of the inflammatory markers[29](#_ENREF_29), [30](#_ENREF_30). Markers of inflammation were the independent and markers of glycemia/insulin resistance/insulin secretion the dependent variables. Thus, the between-subject effect estimate refers to the cross-sectional association between an inflammatory marker and HbA1c, HOMA-IR or HOMA-β, whereas the within-subject effect estimate (longitudinal effect) represents the association between changes in inflammatory markers and changes in markers of glycemia/insulin resistance/insulin secretion over time. For the growth models, all inflammatory markers, HOMA-IR, HOMA-β, and triglycerides were loge-transformed to approximate normality. We estimated effects for a one unit change in markers of inflammation on the loge-scale which corresponds to a one percent change on the original scale. Thus, for instance, longitudinal effect estimates can be interpreted as follows: A one percent increase in markers of inflammation from baseline to follow-up is associated with a (ß/100) unit increase in HbA1c and a ß percent increase in HOMA-IR or HOMA-ß, respectively. Since follow-up time was almost identical for all subjects, we created a binary dummy variable (baseline=0, follow-up=1) to represent observation period. All regression models were adjusted for sex and age at baseline (model 1). Model 2 also included baseline information about smoking status, alcohol intake, physical inactivity and parental history of diabetes. Model 3 extended model 2 by additionally adjusting for baseline levels of waist circumference, ratio of total and HDL cholesterol, loge-transformed triglycerides and systolic blood pressure. In model 4, we included all covariates of model 3 as time-varying variables, except for parental history of diabetes, where we only used the follow-up information in this model.

In sensitivity analyses, we replaced waist circumference in models 3 and 4 with body mass index (BMI). Furthermore, we included interaction terms between categorized baseline levels of inflammation markers (<76%/≥76% of the data (hsCRP <3.1 (reference) / ≥3.1 mg/l; adiponectin <6.2 / ≥6.2 (reference) µg/ml; leukocyte count <6.7 (reference) / ≥6.7 /nl) and longitudinal effect estimates. For hsCRP and leukocyte count, persons with low values and for adiponectin persons with high values were set as reference group. In further sensitivity analyses, we adjusted the results in model 3 for use of drugs potentially affecting inflammation and/or glucose metabolism and insulin secretion/action: i.e. regular use of steroids, regular use of acetylsalicylic acid, use of thiazide diuretics, use of beta-blockers and use of statins as well as use of antineoplastic/anti-inflammatory drugs. A two-tailed p-value of < 0.05 was considered statistically significant without correction for multiple testing as outcome markers are highly correlated. Statistical analysis was conducted with R version 3.4.1 (2017-06-30). For the two-level regression models, we used the R-package *nlme*[31](#_ENREF_31), [32](#_ENREF_32).

**Results**

*Characteristics of the study populations*

**Table 1** shows the characteristics of the study participants at baseline and follow-up. At baseline, median age was 63 years (IQR: 9), most participants were overweight or obese and 26% were prediabetic. A comparison of baseline characteristics (where available) of those with and without follow-up information is shown in **Supplementary Table S1**. Participants without follow-up information were somewhat older, had higher levels of markers of inflammation and tended to have a more adverse metabolic risk factor profile.

*Two-level growth models*

Results of the two-level growth models for the association of inflammatory markers with HbA1c, HOMA-IR and HOMA-β are depicted in **Table 2. Figure 2** illustrates longitudinal and cross-sectional effect estimates from model 4, adjusted for changes in metabolic or lifestyle factors.

Regarding longitudinal changes, we showed that increases in leukocyte count between baseline and follow-up were significantly associated with increased levels of all outcomes, i.e. HbA1c, HOMA-IR and HOMA-ß after adjustment for baseline covariates (model 3). Further adjustment for time-varying covariates (model 4) attenuated the effect estimates somewhat and associations remained significant for HbA1c and HOMA-ß only. For instance, a doubling of leukocyte count from baseline to follow-up (i.e. an increase by 100%) was associated with a 0.168 unit increase in HbA1c , i.e. an increase of 0.168 % HbA1c and a 23.4% increase in insulin secretion (HOMA-ß) between baseline and follow-up. Increases in hsCRP were associated with increased HOMA-IR in model 3, but associations became non-significant after adjustment for time-varying covariates (model 4). Increases in adiponectin were significantly associated with decreased HbA1c levels and decreased HOMA-IR after adjustment for baseline covariates (model 3). After further adjustment for time-varying covariates (model 4) associations remained significant for HbA1c only.

Cross-sectional associations were directionally consistent and generally stronger compared to longitudinal associations. For instance, a doubling of leukocyte count was associated with a 0.187 unit higher HbA1c level and a 25.3% higher insulin secretion (HOMA-ß). The only exception was the association between adiponectin and HbA1c, where the longitudinal ß-estimate was higher (i.e. in this case more negative: -0.165) than the cross-sectional ß-estimate (-0.103).

*Sensitivity analyses*

The sensitivity analysis, in which we replaced waist circumference adjustment with BMI adjustment, showed very similar results to the main analyses with respect to effect sizes and p-values (**Supplementary Table S2**). Further sensitivity analyses regarding interactions between categories of baseline markers of inflammation and longitudinal effect estimates (**Supplementary Table S3**) demonstrated that longitudinal effect estimates were mainly independent of baseline inflammatory status. Only for the association of hsCRP with HbA1c, we observed a significant interaction demonstrating that change in hsCRP was less strongly associated with change in HbA1c in persons who had higher hsCRP concentrations at baseline.

Adjustment for use of drugs potentially affecting inflammation and/or glucose metabolism and insulin secretion/action had virtually no effect on the observed results (data not shown).

**Discussion**

This study primarily aimed to investigate the relationship between changes in systemic inflammation and concomitant changes in glycemia and insulin resistance and insulin secretion in order to better understand the temporal sequence of metabolic derangements in the development towards type 2 diabetes. We identified significant longitudinal associations of changes in markers of systemic inflammation with glycemic deterioration and changes in HOMA-IR and HOMA-ß in initially non-diabetic participants followed up for a time period of 7 years. Associations were generally stronger for leukocyte count and adiponectin than for hsCRP. After adjustment for changes in other type 2 diabetes risk factors, changes in leukocyte count and adiponectin remained significantly associated with glycemic deterioration. Furthermore, changes in leukocyte count additionally remained significantly associated with changes in HOMA-ß. Overall, longitudinal associations were weaker than cross-sectional associations, except for adiponectin where we observed larger longitudinal effect estimates regarding changes in HbA1c.

As described above, few other studies have investigated associations between inflammatory markers and changes in glycemia and insulin resistance and secretion[9-13](#_ENREF_9), and only one of them addressed longitudinal associations between changes in markers of inflammation and changes in glycemic traits[13](#_ENREF_13). The latter study specifically analyzed associations between changes in hsCRP and changes in HbA1c levels. In contrast to our results, an increase in hsCRP over 13 years of follow-up was associated with a significant increase in HbA1c, even after adjusting for changes in other covariates[13](#_ENREF_13). The reasons for the discrepant findings are not entirely clear, but the shorter follow-up time and smaller sample size of our study could have contributed to the diverging results. Previous studies which analyzed associations of baseline hsCRP with changes in HbA1c over time also yielded conflicting results: While in our own analyses of data from the KORA S4/F4 study[10](#_ENREF_10) baseline hsCRP significantly predicted change in HbA1c over 7 years after multivariable adjustment, there was no evidence for association in the Whitehall II study [12](#_ENREF_12), the European Prospective Investigation into Cancer (EPIC) Norfolk study[13](#_ENREF_13) and the Anglo-Danish-Dutch study of Intensive Treatment In PeOple with ScreeN-detected Diabetes in Primary Care (ADDITION-PRO) cohort[11](#_ENREF_11) after multivariable adjustment.

We are not aware of any other study which analysed associations between changes in markers of low-grade inflammation and concomitant changes in insulin resistance or insulin secretion. Thus, our finding that changes in low-grade inflammation, particularly in leukocyte count, not only go along with glycemic deterioration but are also associated with increased HOMA-ß over time is novel. At first sight, this observation may seem counterintuitive, since one expects increased ß-cell function and insulin secretion to go along with lower glucose and HbA1c concentrations. However, our findings have to be interpreted in the light of the *positive* longitudinal association observed between leucocyte count and HOMA-IR. Thus, our results support the notion that in a non-diabetic population insulin secretion is increased to compensate an increased peripheral insulin resistance. If this compensatory increase in ß-cell function is not sufficient to overcome the increasing insulin resistance, glycemic deterioration will occur as seen in the present study. To better understand the present findings, it would have been interesting to also look at the disposition index as an integrated measure of ß-cell compensation[33](#_ENREF_33) or similar measures. Unfortunately, the data that were available in the present study preclude the assessment of dynamic ß-cell function.

Other prospective studies which assessed associations between baseline measures of low-grade inflammation and changes in insulin secretion observed significant positive associations for interleukin (IL)-6[12](#_ENREF_12), soluble CD163, a specific monocyte/macrophage-derived biomarker relecting macrophage activation during inflammation[11](#_ENREF_11), hsCRP[11](#_ENREF_11) and significant inverse associations for adiponectin[11](#_ENREF_11), [12](#_ENREF_12) further supporting a link of systemic low-grade inflammation and adipocyte metabolism with ß-cell function.

In line with our results, Ahmadi-Abhari et al.[13](#_ENREF_13) also observed stronger cross-sectional than longitudinal associations. This difference can presumamby be explained by the fact, that cross-sectional analyses are more prone to reverse causality than longitudinal analyses and points towards the importance of longitudinal studies to estalish the temporal sequence of metablic changes. Nonetheless, due to the observational nature of our study, it remains to be elucidated whether the observed associations are causal. While experimental and animal studies clearly point towards insulin-sensitizing properties of adiponectin[34](#_ENREF_34), Mendelian randomization (MR) studies yielded mixed evidence regarding a causal role of adiponectin in the development of insulin resistance and type 2 diabetes[5-7](#_ENREF_5). In line with the weak associations seen for hsCRP in the present analysis, a large previous MR study concluded that associations between CRP and insulin resistance, glycemia and diabetes are most likely non-causal[4](#_ENREF_4). With regard to leukocyte count, results of another MR study also suggested that the association with diabetes incidence is most likely non-causal[8](#_ENREF_8). However, the actions of various pro-inflammatory cytokines which are released by leukocytes may explain the observed associations: Besides total leukocyte count, particularly neutrophils and lymphocytes are predictors of type 2 diabetes[35](#_ENREF_35). Human neutrophils secrete tumor necrosis factor α (TNFα) which may contribute to diabetes development through its interaction with insulin signaling pathways and beta-cell function[36](#_ENREF_36), [37](#_ENREF_37). Higher total leukocyte as well as neutrophil and lymphocyte counts have also been found to be associated with a polymorphism in the *IL6* gene which results in elevated IL-6 concentrations[38](#_ENREF_38). Hence, IL-6 could be another cytokine explaining the mechanistic link between leukocyte count and glycemic deterioration.

Thus, further research is necessary to confirm or refute a direct causal link between the examined pro- or anti-inflammatory markers and changes in glycemic deterioration and insulin secretion/resistance. Therefore, at this point, it cannot be recommended to target specific inflammatory markers with medical treatment in order to prevent glycemic deterioration in healthy persons. However, as the results from the present study and the wealth of available data generally support the idea that subclinical inflammation is related to the development of type 2 diabetes, lifestyle habits that are associated with an overall lower inflammatory state should be promoted. These include the maintenance of normal body weight, healthy dietary habits (e.g. high fiber intake, low alcohol consumption, avoidance of excess caloric intake, a “prudent” dietary pattern), regular physical activity, non-smoking and avoidance of sleep deprivation[39](#_ENREF_39), [40](#_ENREF_40).

**Strengths and Limitations**

The strengths of this study are the large sample size and the population-based longitudinal design with repeated measurements which enabled us to take into account changes in risk factors for type 2 diabetes over time as potential confounders. Nevertheless, inclusion of more than two time points would have enriched our analysis, e.g. by enabling us to look at trajectories in more detail. Another limitation is the observational design of our study due to which we were only able to establish temporal but not causal relationships. Furthermore, we were only able to adjust for BMI and waist circumference as markers of obesity and body fat distribution and used a relatively crude measure of physical activity. Therefore, residual confounding by body fat distribution or physical activity cannot be excluded. Additionally, we restricted our analysis to individuals who participated in both the baseline and follow-up examination which resulted in a study sample which was slightly younger and healthier than the original KORA S4 study population. Furthermore, HOMA-IR and HOMA-ß are rather crude estimates of insulin resistance and ß-cell function. Moreover, these estimates do not differentiate between hepatic and peripheral insulin resistance. Furthermore, the available data precluded assessment of ß-cell function in relation to insulin resistance such as the disposition index. Finally, we cannot rule out a potential bias by using different measurement methods at baseline and follow-up, particularly regarding the measurement of HbA1c, insulin and adiponectin.

**Conclusion**

Our results support the hypothesis that low-grade inflammation is associated with glycemic deterioration. Furthermore, they provide evidence that increased compensatory insulin secretion to counterbalance increased insulin resistance is an early step linking inflammation with glycemic deterioration. If the observed associations are confirmed to be causal, low-grade inflammation may provide a potential target to prevent early glycemic deterioration and ultimately the onset of type 2 diabetes mellitus and diabetic complications.

**Acknowledgements**

We thank Andrea Schneider for excellent data handling and Michael Laxy for explanations on multilevel growth models.

**Declaration of conflict of interest:** The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Funding:** The work leading to this publication has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement no115317 (DIRECT), resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies’ in kind contribution (<http://www.direct-diabetes.org/>). This study was also supported in part by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.). The KORA study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of the LMUinnovativ and the German Diabetes Center (DDZ) which is funded by the German Federal Ministry of Health (BMG) and the Ministry of Culture and Science of the State North Rhine-Westphalia.

**References**

1. Li S, Shin HJ, Ding EL, van Dam RM**.** Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2009;302:179-88.

2. Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y, Yao P, Liu LG**.** Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care 2013;36:166-75.

3. Gkrania-Klotsas E, Ye Z, Cooper AJ, Sharp SJ, Luben R, Biggs ML, Chen LK, Gokulakrishnan K, Hanefeld M, Ingelsson E, Lai WA, Lin SY, Lind L, Lohsoonthorn V, Mohan V, Muscari A, Nilsson G, Ohrvik J, Chao Qiang J, Jenny NS, Tamakoshi K, Temelkova-Kurktschiev T, Wang YY, Yajnik CS, Zoli M, Khaw KT, Forouhi NG, Wareham NJ, Langenberg C**.** Differential white blood cell count and type 2 diabetes: systematic review and meta-analysis of cross-sectional and prospective studies. PLoS One 2010;5:e13405.

4. Brunner EJ, Kivimaki M, Witte DR, Lawlor DA, Davey Smith G, Cooper JA, Miller M, Lowe GD, Rumley A, Casas JP, Shah T, Humphries SE, Hingorani AD, Marmot MG, Timpson NJ, Kumari M**.** Inflammation, insulin resistance, and diabetes--Mendelian randomization using CRP haplotypes points upstream. PLoS Med 2008;5:e155.

5. Mente A, Meyre D, Lanktree MB, Heydarpour M, Davis AD, Miller R, Gerstein H, Hegele RA, Yusuf S, Anand SS**.** Causal relationship between adiponectin and metabolic traits: a Mendelian randomization study in a multiethnic population. PLoS One 2013;8:e66808.

6. Yaghootkar H, Lamina C, Scott RA, Dastani Z, Hivert MF, Warren LL, Stancakova A, Buxbaum SG, Lyytikainen LP, Henneman P, Wu Y, Cheung CY, Pankow JS, Jackson AU, Gustafsson S, Zhao JH, Ballantyne CM, Xie W, Bergman RN, Boehnke M, el Bouazzaoui F, Collins FS, Dunn SH, Dupuis J, Forouhi NG, Gillson C, Hattersley AT, Hong J, Kahonen M, Kuusisto J, Kedenko L, Kronenberg F, Doria A, Assimes TL, Ferrannini E, Hansen T, Hao K, Haring H, Knowles JW, Lindgren CM, Nolan JJ, Paananen J, Pedersen O, Quertermous T, Smith U, Lehtimaki T, Liu CT, Loos RJ, McCarthy MI, Morris AD, Vasan RS, Spector TD, Teslovich TM, Tuomilehto J, van Dijk KW, Viikari JS, Zhu N, Langenberg C, Ingelsson E, Semple RK, Sinaiko AR, Palmer CN, Walker M, Lam KS, Paulweber B, Mohlke KL, van Duijn C, Raitakari OT, Bidulescu A, Wareham NJ, Laakso M, Waterworth DM, Lawlor DA, Meigs JB, Richards JB, Frayling TM**.** Mendelian randomization studies do not support a causal role for reduced circulating adiponectin levels in insulin resistance and type 2 diabetes. Diabetes 2013;62:3589-98.

7. Gao H, Fall T, van Dam RM, Flyvbjerg A, Zethelius B, Ingelsson E, Hagg S**.** Evidence of a causal relationship between adiponectin levels and insulin sensitivity: a Mendelian randomization study. Diabetes 2013;62:1338-44.

8. Borné Y, Smith JG, Nilsson PM, Melander O, Hedblad B, Engstrom G**.** Total and Differential Leukocyte Counts in Relation to Incidence of Diabetes Mellitus: A Prospective Population-Based Cohort Study. PLoS One 2016;11:e0148963.

9. Park K, Steffes M, Lee DH, Himes JH, Jacobs DR, Jr.Association of inflammation with worsening HOMA-insulin resistance. Diabetologia 2009;52:2337-44.

10. Klüppelholz B, Thorand B, Koenig W, de las Heras Gala T, Meisinger C, Huth C, Giani G, Franks PW, Roden M, Rathmann W, Peters A, Herder C**.** Association of subclinical inflammation with deterioration of glycaemia before the diagnosis of type 2 diabetes: the KORA S4/F4 study. Diabetologia 2015;58:2269-77.

11. Deichgræber P, Witte DR, Moller HJ, Skriver MV, Richelsen B, Jorgensen ME, Johansen NB, Sandbaek A**.** Soluble CD163, adiponectin, C-reactive protein and progression of dysglycaemia in individuals at high risk of type 2 diabetes mellitus: the ADDITION-PRO cohort. Diabetologia 2016;59:2467-76.

12. Herder C, Faerch K, Carstensen-Kirberg M, Lowe G, Haapakoski R, Witte DR, Brunner EJ, Roden M, Tabak AG, Kivimaki M, Vistisen D**.** Biomarkers of subclinical inflammation and increases in glycaemia, insulin resistance and beta-cell function in non-diabetic individuals: the Whitehall II study. Eur J Endocrinol 2016;175:367-77.

13. Ahmadi-Abhari S, Kaptoge S, Luben RN, Wareham NJ, Khaw KT**.** Longitudinal association of C-reactive protein and Haemoglobin A1c over 13 years: the European Prospective Investigation into Cancer--Norfolk study. Cardiovasc Diabetol 2015;14:61.

14. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR**.** Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405-12.

15. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimaki M**.** Prediabetes: a high-risk state for diabetes development. Lancet 2012;379:2279-90.

16. Bonora E, Formentini G, Calcaterra F, Lombardi S, Marini F, Zenari L, Saggiani F, Poli M, Perbellini S, Raffaelli A, Cacciatori V, Santi L, Targher G, Bonadonna R, Muggeo M**.** HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study. Diabetes Care 2002;25:1135-41.

17. Laakso M**.** Is Insulin Resistance a Feature of or a Primary Risk Factor for Cardiovascular Disease? Curr Diab Rep 2015;15:105.

18. Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N**.** Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. Ann Intern Med 2004;141:413-20.

19. Mossmann M, Wainstein MV, Goncalves SC, Wainstein RV, Gravina GL, Sangalli M, Veadrigo F, Matte R, Reich R, Costa FG, Bertoluci MC**.** HOMA-IR is associated with significant angiographic coronary artery disease in non-diabetic, non-obese individuals: a cross-sectional study. Diabetology & Metabolic Syndrome 2015;7.

20. Gast KB, Tjeerdema N, Stijnen T, Smit JW, Dekkers OM**.** Insulin resistance and risk of incident cardiovascular events in adults without diabetes: meta-analysis. PLoS One 2012;7:e52036.

21. WHO**.** Report of a WHO consultation: definition, diagnosis and classification of diabetes mellitus and its complications. World Health Organization, Geneva. 1999.

22. Rathmann W, Haastert B, Icks A, Lowel H, Meisinger C, Holle R, Giani G**.** High prevalence of undiagnosed diabetes mellitus in Southern Germany: target populations for efficient screening. The KORA survey 2000. Diabetologia 2003;46:182-9.

23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC**.** Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

24. Muniyappa R, Lee S, Chen H, Quon MJ**.** Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 2008;294:E15-26.

25. Müller S, Martin S, Koenig W, Hanifi-Moghaddam P, Rathmann W, Haastert B, Giani G, Illig T, Thorand B, Kolb H**.** Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. Diabetologia 2002;45:805-12.

26. Herder C, Hauner H, Haastert B, Rohrig K, Koenig W, Kolb H, Muller-Scholze S, Thorand B, Holle R, Rathmann W**.** Hypoadiponectinemia and proinflammatory state: two sides of the same coin?: results from the Cooperative Health Research in the Region of Augsburg Survey 4 (KORA S4). Diabetes Care 2006;29:1626-31.

27. Herder C, Bongaerts BW, Rathmann W, Heier M, Kowall B, Koenig W, Thorand B, Roden M, Meisinger C, Ziegler D**.** Association of subclinical inflammation with polyneuropathy in the older population: KORA F4 study. Diabetes Care 2013;36:3663-70.

28. Rathmann W, Strassburger K, Heier M, Holle R, Thorand B, Giani G, Meisinger C**.** Incidence of Type 2 diabetes in the elderly German population and the effect of clinical and lifestyle risk factors: KORA S4/F4 cohort study. Diabet Med 2009;26:1212-9.

29. Hedeker D**.** An introduction to growth modeling. Thousand Oaks CA: Sage Publications; 2004.

30. Laxy M, Holle R, Doring A, Peters A, Hunger M**.** The longitudinal association between weight change and health-related quality of life: the KORA S4/F4 cohort study. Int J Public Health 2014;59:279-88.

31. R Core Team**.** R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; 2015.

32. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team**.** nlme: Linear and Nonlinear Mixed Effects Models. 2016.

33. Retnakaran R, Shen S, Hanley AJ, Vuksan V, Hamilton JK, Zinman B**.** Hyperbolic relationship between insulin secretion and sensitivity on oral glucose tolerance test. Obesity (Silver Spring) 2008;16:1901-7.

34. Lai H, Lin N, Xing Z, Weng H, Zhang H**.** Association between the level of circulating adiponectin and prediabetes: A meta-analysis. J Diabetes Investig 2015;6:416-29.

35. Zhang H, Yang Z, Zhang W, Niu Y, Li X, Qin L, Su Q**.** White blood cell subtypes and risk of type 2 diabetes. J Diabetes Complications 2017;31:31-7.

36. Senn JJ, Klover PJ, Nowak IA, Mooney RA**.** Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes 2002;51:3391-9.

37. Smedman C, Gardlund B, Nihlmark K, Gille-Johnson P, Andersson J, Paulie S**.** ELISpot analysis of LPS-stimulated leukocytes: human granulocytes selectively secrete IL-8, MIP-1beta and TNF-alpha. J Immunol Methods 2009;346:1-8.

38. Fernandez-Real JM, Broch M, Vendrell J, Gutierrez C, Casamitjana R, Pugeat M, Richart C, Ricart W**.** Interleukin-6 gene polymorphism and insulin sensitivity. Diabetes 2000;49:517-20.

39. Kolb H, Mandrup-Poulsen T**.** The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. Diabetologia 2010;53:10-20.

40. O'Connor MF, Irwin MR**.** Links Between Behavioral Factors and Inflammation. Clinical Pharmacology & Therapeutics 2010;87:479-82.

**Figure Legends**

**Figure 1:** Flowchart showing the final study population after applying exclusion criteria

Figure 2: Beta coefficients with 95% CI for longitudinal (solid line) and cross-sectional (dashed line) association of inflammatory markers with HbA1c (unit=%) (A), log*e*-transformed HOMA-IR (B) and log*e*-transformed HOMA-β (C). Adjustments for age, sex, smoking, alcohol intake, physical inactivity, parental history of diabetes (follow-up only), waist circumference, ratio of total and HDL cholesterol, log*e*-transformed triglycerides and systolic blood pressure (model 4).

**Table 1:** Description of characteristics of study population

|  |  |
| --- | --- |
|  | **KORA F4N = 819** |
|  | **Baseline** | **Follow-Up** |
| Sex, male | 49.9 (409) | 49.9 (409) |
| Age (years) | 63 (58, 67) | 70 (65, 74) |
| Waist circumference (cm) | 94.4 (86.7, 101.4) | 97.1 (89.6, 104.9) |
| BMI (kg/m2) | 27.7 (25.5, 30.1) | 27.9 (25.5, 30.8) |
| Systolic blood pressure (mm Hg) | 131.0 (119.5, 145.0) | 128.0 (115.2, 138.5) |
| Ratio total/HDL-cholesterol | 4.3 (3.4, 5.3) | 4.1 (3.3, 4.9) |
| Triglycerides (mg/dl) | 110.0 (80.0, 154.0) | 111.0 (81.0, 154.0) |
| Smoking Status: |  |  |
| *Never* | 51.8 (424) | 51.5 (422) |
| *Former* | 36.8 (301) | 41.8 (342) |
| *Current* | 11.5 (94) | 6.7 (55) |
| Parental history of diabetes: |  |  |
| *No* | 57.8 (473) | 55.6 (455) |
| *Positive* | 24.1 (197) | 25.3 (207) |
| *Unknown* | 18.2 (149) | 19.2 (157) |
| Alcohol consumption: |  |  |
| *No consumption* | 24.1 (197) | 30.8 (252) |
| *Moderate consumption* | 55.6 (455) | 51.9 (425) |
| *High consumption* | 20.4 (167) | 17.3 (142) |
| Physical inactivity | 52.0 (426) | 47.4 (388) |
| hsCRP (mg/l) | 1.5 (0.8, 3.0) | 1.5 (0.8, 3.1) |
| Adiponectin (µg/ml) | 9.1 (6.3, 12.3) | 10.4 (7.0, 15.7) |
| Leukocyte count (/nl) | 5.7 (5.0, 6.6) | 5.7 (4.8, 6.6) |
| Glucose tolerance status: |  |  |
| *Normoglycemic* | 74.0 (606) | 61.1 (500) |
| *Prediabetic* | 26.0 (213) | 29.7 (243) |
| *Diabetic* | 0 (0) | 9.3 (76) |
| Fasting glucose (mmol/l) | 5.4 (5.1, 5.8) | 5.4 (5.1, 5.8) |
| Fasting insulin (µU/ml) | 9.8 (6.9, 13.9) | 9.8 (7.1, 14.0) |
| HbA1c (%) | 5.6 (5.4, 5.8) | 5.6 (5.4, 5.8) |
| HbA1c (mmol/mol) | 37.7 (35.5, 39.9) | 37.7 (35.5, 39.9) |
| HOMA-IR  | 2.3 (1.6, 3.5) | 2.3 (1.7, 3.5) |
| HOMA-β  | 101.9 (75.2, 140.0) | 105.3 (75.2, 142.1) |

BMI, body mass index; HDL, high density lipoprotein; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-ß, beta-cell function

Characteristics of the study participants are presented as percentages (number) for categorical variables and as median (25th, 75th percentiles) for continuous variables.

**Table 2:** Shown are regression coefficients (95% CI) per one percent change of inflammation markers for cross-sectional and longitudinal associations with changes in HbA1c (unit=%), log*e*-transformed HOMA-IR and log*e*-transformed HOMA-β, respectively.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Model 1** | **Model 2** | **Model 3** | **Model 4** |
| **HbA1c** |  |  |  |  |  |
| Leukocyte count |  |  |  |  |  |
| N = 819 | cross-sectional | 0.235 (0.144;0.325)\*\*\* | 0.232 (0.137;0.327)\*\*\* | 0.202 (0.107;0.297)\*\*\* | 0.187 (0.093;0.280)\*\*\* |
|  | longitudinal | 0.191 (0.056;0.325)\*\* | 0.191 (0.056;0.325)\*\* | 0.191 (0.056;0.325)\*\* | 0.168 (0.033;0.303)\* |
| hsCRP |  |  |  |  |  |
| N = 808 | cross-sectional | 0.055 (0.033;0.077)\*\*\* | 0.056 (0.034;0.078)\*\*\* | 0.047 (0.024;0.070)\*\*\* | 0.038 (0.015;0.061)\*\* |
|  | longitudinal | 0.020 (-0.011;0.050) | 0.020 (-0.011;0.050) | 0.020 (-0.011;0.050) | 0.017 (-0.013;0.047) |
| Adiponectin |  |  |  |  |  |
| N = 809 | cross-sectional | -0.140 (-0.185;-0.095)\*\*\* | -0.136 (-0.181;-0.092)\*\*\* | -0.111 (-0.159;-0.063)\*\*\* | -0.103 (-0.150;-0.056)\*\*\* |
|  | longitudinal | -0.177 (-0.254;-0.099)\*\*\* | -0.177 (-0.254;-0.099)\*\*\* | -0.177 (-0.254;-0.099)\*\*\* | -0.165 (-0.243;-0.087)\*\*\* |
| **HOMA-IR** |  |  |  |  |  |
| Leukocyte count |  |  |  |  |  |
| N = 809 | cross-sectional | 0.453 (0.285;0.621)\*\*\* | 0.478 (0.304;0.653)\*\*\* | 0.289 (0.145;0.432)\*\*\* | 0.280 (0.144;0.416)\*\*\* |
|  | longitudinal | 0.233 (0.055;0.411)\* | 0.233 (0.055;0.411)\* | 0.233 (0.055;0.411)\* | 0.167 (-0.005;0.339) |
| hsCRP |  |  |  |  |  |
| N = 799 | cross-sectional | 0.170 (0.130;0.210)\*\*\* | 0.166 (0.125;0.206)\*\*\* | 0.063 (0.027;0.099)\*\*\* | 0.058 (0.024;0.092)\*\*\* |
|  | longitudinal | 0.039 (0.000;0.079)\* | 0.039 (0.000;0.079)\* | 0.039 (0.000;0.079)\* | 0.024 (-0.014;0.062) |
| Adiponectin |  |  |  |  |  |
| N = 802 | cross-sectional | -0.423 (-0.502;-0.343)\*\*\* | -0.425 (-0.503;-0.346)\*\*\* | -0.250 (-0.321;-0.180)\*\*\* | -0.231 (-0.298;-0.164)\*\*\* |
|  | longitudinal | -0.179 (-0.282;-0.076)\*\*\* | -0.179 (-0.282;-0.076)\*\*\* | -0.179 (-0.282;-0.076)\*\*\* | -0.075 (-0.174;0.024) |
| **HOMA-β** |  |  |  |  |  |
| Leukocyte count |  |  |  |  |  |
| N = 809 | cross-sectional | 0.369 (0.223;0.515)\*\*\* | 0.349 (0.198;0.499)\*\*\* | 0.233 (0.096;0.370)\*\*\* | 0.253 (0.119;0.387)\*\*\* |
|  | longitudinal | 0.289 (0.122;0.456)\*\*\* | 0.289 (0.122;0.456)\*\*\* | 0.289 (0.122;0.456)\*\*\* | 0.234 (0.067;0.402)\*\* |
| hsCRP |  |  |  |  |  |
| N = 799 | cross-sectional | 0.115 (0.080;0.150)\*\*\* | 0.107 (0.071;0.143)\*\*\* | 0.042 (0.007;0.076)\* | 0.048 (0.015;0.082)\*\* |
|  | longitudinal | 0.005 (-0.032;0.042) | 0.005 (-0.032;0.042) | 0.005 (-0.032;0.042) | -0.003 (-0.041;0.034) |
| Adiponectin |  |  |  |  |  |
| N = 802 | cross-sectional | -0.247 (-0.318;-0.176)\*\*\* | -0.248 (-0.318;-0.178)\*\*\* | -0.136 (-0.205;-0.068)\*\*\* | -0.134 (-0.201;-0.067)\*\*\* |
|  | longitudinal | -0.049 (-0.146;0.048) | -0.049 (-0.146;0.048) | -0.049 (-0.146;0.048) | 0.022 (-0.075;0.119) |

hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-ß, beta-cell function

Adjustments: Model 1: age + sex; Model 2: M1 + smoking + alcohol intake + physical inactivity + parental history of diabetes; Model 3: M2 + waist circumference + ratio of total and HDL cholesterol + loge(triglycerides) + systolic blood pressure; Model 4: M3 + all adjusted covariates time-dependent (parental history of diabetes only from follow-up); \* p<0.05, \*\* p<0.01; \*\*\* p<0.001