HHS Public Access

Author manuscript

Diabetologia. Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

Diabetologia. 2019 January ; 62(1): 58–69. doi:10.1007/s00125-018-4748-2.

Non-traditional biomarkers and incident diabetes in the Diabetes Prevention Program: comparative effects of lifestyle and metformin interventions

Ronald B. Goldberg[1], **George A. Bray**[2], **Santica M. Marcovina**[3], **Kieren J. Mather**[4], **Trevor J. Orchard**[5], **Leigh Perreault**[6], **Marinella Temprosa**[7], and **the Diabetes Prevention Program Research Group**

[1.]Division of Endocrinology, Diabetes and Metabolism, Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL, USAClinical Obesity, Pennington Biomedical Research Center, Louisiana State University Medical Center, Baton Rouge, LA, USA

[2.]Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington, Northwest Lipid Research Labs, Seattle, WA, USA

[3.]Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

[4.]Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA

[5.]Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

[6.]Department of Epidemiology and Biostatistics, Biostatistics Center and Milken Institute School of Public Health, George Washington University, Rockville, MD, USA

[7.]c/o Diabetes Prevention Program Coordinating Center, The Biostatistics Center, Milken Institute School of Public Health George Washington University, 6110 Executive Blvd, Suite 750 Rockville, MD 20852, USA

Abstract

Aims/hypothesis—We compared the associations of circulating biomarkers of inflammation, endothelial and adipocyte dysfunction and coagulation with incident diabetes in the placebo, lifestyle and metformin intervention arms of the Diabetes Prevention Program, a randomised clinical trial, to determine whether reported associations in general populations are reproduced in individuals with impaired glucose tolerance, and whether these associations are independent of

Corresponding author: DPP Research Group, c/o Diabetes Prevention Program Coordinating Center, The Biostatistics Center, Milken Institute School of Public Health George Washington University, 6110 Executive Blvd, Suite 750 Rockville, MD 20852, USA.

Contribution statement: MT and RG wrote the manuscript, SM performed the assays, MT did the major analyses and RG, MT, GB, SM, TO, LP and KM, who comprised the Writing Group, all made substantial contributions to study design, analysis and interpretation of data, and revised the manuscript critically for intellectual content and gave final approval of the version to be published. MT is the guarantor of the work.

A complete list of DPP and Diabetes Prevention Program Outcomes Study (DPPOS) centres, investigators and staff can be found in the electronic supplementary material (ESM).

Data availability In compliance with our sponsor's data-sharing policies, the data used for this paper are available in the National Institute of Diabetes and Digestive and Kidney (NIDDK) data repositories for DPP (https://repository.niddk.nih.gov/studies/dpp/) and DPPOS (https://repository.niddk.nih.gov/studies/dppos/).

traditional diabetes risk factors. We further investigated whether biomarker–incident diabetes associations are influenced by interventions that alter pathophysiology, biomarker concentrations and rates of incident diabetes.

Methods—The Diabetes Prevention Program randomised 3234 individuals with impaired glucose tolerance into placebo, metformin (850 mg twice daily) and intensive lifestyle groups and showed that metformin and lifestyle reduced incident diabetes by 31% and 58%, respectively compared with placebo over an average follow-up period of 3.2 years. For this study, we measured adiponectin, leptin, tissue plasminogen activator (as a surrogate for plasminogen activator inhibitor 1), high-sensitivity C-reactive protein, IL-6, monocyte chemotactic protein 1, fibrinogen, E-selectin and intercellular adhesion molecule 1 at baseline and at 1 year by specific immunoassays. Traditional diabetes risk factors were defined as family history, HDL-cholesterol, triacylglycerol, BMI, fasting and 2 h glucose, HbA_{1c}, systolic blood pressure, inverse of fasting insulin and insulinogenic index. Cox proportional hazard models were used to assess the effects of each biomarker on the development of diabetes assessed semi-annually and the effects of covariates on these.

Results—E-selectin, (HR 1.19 [95% CI 1.06, 1.34]), adiponectin (0.84 [0.71, 0.99]) and tissue plasminogen activator (1.13 [1.03, 1.24]) were associated with incident diabetes in the placebo group, independent of diabetes risk factors. Only the association between adiponectin and diabetes was maintained in the lifestyle (0.69 [0.52, 0.92]) and metformin groups (0.79 [0.66, 0.94]). E-selectin was not related to diabetes development in either lifestyle or metformin groups. A novel association appeared for change in IL-6 in the metformin group (1.09 [1.021, 1.173]) and for baseline leptin in the lifestyle groups (1.31 [1.06, 1.63]).

Conclusions/interpretation—These findings clarify associations between an extensive group of biomarkers and incident diabetes in a multi-ethnic cohort with impaired glucose tolerance, the effects of diabetes risk factors on these, and demonstrate differential modification of associations by interventions. They strengthen evidence linking adiponectin to diabetes development, and argue against a central role for endothelial dysfunction. The findings have implications for the pathophysiology of diabetes development and its prevention.

Keywords

Adiponectin; Biomarkers; C-reactive protein; Diabetes prevention; E-selectin; Interleukin 6; Leptin; Lifestyle change; Metformin; Tissue plasminogen activator

Introduction

Although type 2 diabetes is known to result from deficits of insulin secretion and action, the metabolic disturbances underlying these abnormalities remain incompletely understood. Evidence implicates activation of inflammatory pathways in adipose and other tissues, with accompanying vascular endothelial, adipocyte and coagulant dysfunction, in the pathophysiology of diabetes (1). Population studies show that circulating biomarkers of these processes associate with incident diabetes, (2) but few studies have been carried out in cohorts with impaired glucose tolerance. It remains unclear whether such associations are distinct from the traditional diabetes risk factors (DRFs), as weight gain, insulin resistance, dyslipidaemia and hyperglycaemia have each been shown to activate pathways leading to

inflammation, endothelial dysfunction and imbalanced coagulation (1). Furthermore, there is limited information on the effects of interventions that slow progression to diabetes on biomarker–incident diabetes associations. An intervention-induced alteration of an association between a given biomarker and incident diabetes may provide a deeper perspective on the relationship of the biomarker to the development of diabetes and on the mechanism(s) underlying the prevention effect.

The Diabetes Prevention Program (DPP; ClinicalTrials.gov registration no. NCT00004992) was a randomised multicentre clinical trial that demonstrated that both intensive lifestyle modification (ILS) and metformin therapy can reduce incident diabetes in high-risk individuals compared with standard care (3). Well-recognised risk factors for diabetes development, namely increased BMI, dysglycaemia and impairments of insulin secretion and action were shown to be determinants of incident diabetes in all groups (4). We report here on the associations between incident diabetes and a composite group of biomarkers of inflammation, coagulant imbalance and endothelial and adipocyte dysfunction in the DPP cohort with prediabetes, exploring treatment-specific effects and relationships with established DRFs.

Methods

Study participants and procedures

Eligibility criteria, design, methods and primary results of the DPP have been reported in detail elsewhere (3). The selection criteria included: age ≥ 25 years, BMI ≥ 24 kg/m² (≥22 kg/m² in Asian Americans), fasting plasma glucose levels between 5.3 and 6.9 mmol/l (<6.9 mmol/l in American Indians) and impaired glucose tolerance (2 h post-load glucose of 7.8–11.0 mmol/l). Written informed consent was obtained from all participants before screening, consistent with the Declaration of Helsinki and the guidelines of each centre's institutional review board. In total, 3234 participants were randomly assigned to one of three intervention groups: metformin 850 mg twice daily; placebo twice daily; or an intensive programme of lifestyle modification (ILS). Allocation to metformin and placebo groups was double blinded. The goals of ILS were to achieve and maintain weight reduction of ≥ 7% through consumption of a low-calorie, low-fat diet and to engage in moderate physical activity for ≥ 150 min/week. The placebo group was managed according to standard healthcare recommendations only. Diabetes was diagnosed by an annual OGTT or a semi-annual fasting glucose test according to American Diabetes Association criteria (5) throughout the follow-up period of 3.2 years. The diagnosis required confirmation by a second test, usually within 6 weeks. Semi-annual measurements of weight, BP and fasting glucose and annual measurements of HDL-cholesterol, triacylglycerol and HbA_{1c}, and glucose tolerance by OGTT, were performed.

Clinical and metabolic variables

Standardised interviewer-administered questionnaires were used to obtain demographic information, and BP and anthropometrics (BMI and waist circumference) were measured by standard techniques. Glucose, insulin, HbA_{1c} and biomarker measurements were performed at the Central Biochemistry Laboratory (Northwest Lipid Research Laboratories, University

of Washington, Seattle, WA, USA) as previously reported (3, 4). The biomarker measurements other than fibrinogen, tissue plasminogen activator (tPA) and C-reactive protein (CRP) were specifically performed for the current study, whereas all other assessments were part of the original DPP study (3). Indirect measures of insulin resistance defined as inverse of fasting insulin (IFI) and insulin secretory capacity defined as insulinogenic index (IGI; 30–0 min change in plasma insulin over 30–0 min change in plasma glucose) were obtained from the annual OGTT (4). A total of 3195 samples were available for biomarker assay at baseline and 3009 samples had paired measurements at both baseline and 1 year.

We evaluated at least two biomarkers representing each of the distinct biological processes of inflammation, procoagulant state and endothelial and adipocyte dysfunction for this study. Markers of inflammation included high-sensitivity CRP measured immunochemically using Dade-Behring reagent on the Behring Nephelometer autoanalyser (Deerfield, IL, USA), and IL-6 and Monocyte chemoattractive protein 1 (MCP-1) measured by ELISAs from R&D Systems (Minneapolis, MN, USA). Procoagulant state was assessed by fibrinogen levels in plasma (using the same method as CRP). Total tPA level measured in citrated plasma using an ELISA (Asserachrom tPA; Diagnostica Stago, Parsippany, NJ, USA) was used as a surrogate for plasminogen activator inhibitor 1 (PAI-1), as previously documented (6). Endothelial dysfunction was assessed using soluble E-selectin (sE-selectin) and soluble intercellular adhesion molecule 1 (sICAM-1), measured by an R&D Systems ELISA. Adipocyte dysfunction was assessed by total adiponectin levels, measured using a latex particle-enhanced turbidimetric assay (Otsuka Pharmaceutical, Tokyo, Japan), and leptin levels, measured by radioimmunoassay (Millipore, Darmstadt, Germany). The within-run and between-run CVs for all biomarker assays ranged between 2.10% and 7.40%, and 2.60% and 9.25%, respectively.

Statistical analysis

We applied the intent-to-treat approach to analysis. Differences in baseline and 1 year change in biomarkers among treatment groups were assessed by the median test using quantile regression (7) and ANOVA, respectively, with the significance level set at 0.01 and using unadjusted p values for the three pairwise treatment group comparison. This is slightly more conservative than the Bonferroni significance level of 0.0167 for three tests (i.e. 0.05/3). Spearman correlations were used to describe the bivariate relationships among the anthropometric and metabolic variables, with adjustment for demographic factors. Traditional DRFs were defined as family history of diabetes, BMI, waist circumference, IFI and IGI, fasting and 2 h glucose, HbA_{1c}, HDL-cholesterol, triacylglycerol and systolic BP (SBP). BMI was chosen over waist circumference for Cox models as, in our population, the two measurements predict incident diabetes equally well (8).

We assessed the distributional characteristics of each biomarker (electronic supplementary material [ESM] Fig 1) and used natural log transformations for CRP, IL-6 and IFI. Correlations and multicollinearity among biomarkers within the same pathway (inflammation, coagulation, endothelial dysfunction and adipose dysregulation) were assessed to ensure it was appropriate to include them in the same model. None of the

biomarkers was found to be collinear. Missing data were assumed missing at random. Cox proportional hazards models were used to assess the effects of each biomarker on the development of diabetes. The added effects of biomarker change at 1 year were also assessed in separate models, though HbA_{1c} and fasting glucose were excluded in this analysis as the outcome was diabetes. Graphical procedures were used to examine the proportionality assumption. HRs are expressed per 1 standard deviation. For the association of biomarkers and incident diabetes, we set a nominal significance level of 0.05 and for tests of heterogeneity between groups, $p<0.1$. We deem these analyses as both validation of previously reported associations in general populations and exploratory to assess possible moderating effects of treatment, although the study is underpowered.

Results

Baseline and 1 year change in traditional diabetes risk factors and biomarker values

The results are presented in Table 1. The DPP cohort included 3234 participants with mean age of 51 years, 67% were women and 45% were ethnic minorities and a positive family history was present in 70% of the cohort. Table 1 depicts BMI, waist circumference, SBP, HDL-cholesterol, triacylglycerol, fasting and 2 h glucose, HbA_{1c}, IFI, IGI and biomarker values at baseline for the entire cohort that had baseline samples available for biomarker measurements (n=3195), and 1 year changes in these variables by intervention group and grouped according to the biological pathways represented. The baseline biomarker values are displayed by intervention group in ESM Table 1. As there were no differences among randomised treatment groups at baseline, median baseline values with interquartile ranges (IQRs) for the entire cohort are shown. As previously reported (3), ILS and metformin improved BMI, HDL-cholesterol, fasting glucose, HbA_{1c} and fasting insulin compared with placebo at 1 year, with ILS having a more robust effect, while ILS only reduced 2 h glucose, SBP and triacylglycerol. The 1 year change in all of the evaluated biomarkers differed among treatment groups when the biomarkers were considered individually. For markers of adipocyte dysfunction, adiponectin and leptin were improved by ILS vs placebo and metformin, whereas metformin only reduced leptin (and not adiponectin) compared with placebo. Among inflammatory markers, CRP was reduced only by ILS compared with placebo and metformin, while IL-6 was decreased by both active intervention groups. For the procoagulant markers, tPA was reduced by both ILS and metformin vs placebo and more in the ILS group than in the metformin group, while fibrinogen was lowered only by ILS compared with placebo and metformin. The markers of endothelial dysfunction sE-selectin and sICAM-1 were reduced by ILS compared with the other two interventions, whereas metformin only reduced sICAM-1 (and not sE-selectin) vs placebo.

Correlations between traditional diabetes risk factors and biomarkers

Figure 1 depicts Spearman correlation coefficients between biomarkers and DRFs (BMI, waist circumference, fasting glucose, HbA_{1c}, 2 h plasma glucose, IGI, IFI, HDL-cholesterol, tracylglycerol and SBP) at baseline, ordered by r values for BMI. All of the evaluated biomarkers correlated significantly with BMI, waist circumference and IFI. Leptin, CRP, and IL-6 and fibrinogen had the strongest correlations with BMI and waist circumference, while adiponectin (directly), and tPA and leptin (inversely) were most strongly correlated

with IFI. Associations between biomarkers and IGI were weaker, with leptin (directly) and adiponectin (inversely) more strongly associated than others. Associations with fasting and 2 h glucose were weak or non-existent except for moderate associations for adiponectin and tPA with fasting glucose. HbA_{1c} was more strongly correlated with the inflammatory markers CRP, IL-6, MCP-1 and fibrinogen than were the glucose measurements. sICAM-1 and sE-selectin showed similar moderate associations with BMI and waist circumference and inversely with IFI. Overall, these observations indicate that the biomarkers evaluated were more strongly but differentially related to adiposity and insulin resistance than to measures of glycaemia or insulin secretion.

Correlations among biomarkers at baseline

Figure 2 illustrates correlations between baseline biomarkers. Strong associations were noted between CRP, IL-6, leptin and fibrinogen. The endothelial dysfunction markers sICAM-1 and sE-selectin correlated with each other and had similar associations with IL-6 and tPA; sICAM-1 was moderately correlated with CRP and sE-selectin more weakly so. Adiponectin correlated inversely with tPA and sE-selectin but had weak or no associations with inflammatory markers (CRP, IL-6 and MCP-1). These observations suggest that the inflammatory markers and fibrinogen tended to form one group of interrelated factors, whereas adiponectin, tPA and endothelial function markers tended to form a separate group, with sICAM-1 overlapping the two groups.

Biomarkers as predictors of incident diabetes and effects of interventions

Table 2 presents Cox proportional hazards models testing the effects of baseline biomarker HRs for incident diabetes by intervention group. There were 1344 participants who developed diabetes (506 in the placebo group, 446 in the metformin grop and 392 in the ILS group). Each baseline biomarker was tested in three successive stepwise models. Model 1 describes the HR for each baseline biomarker adjusted for demographic factors only (age at randomisation, sex, race/ethnicity). Model 2 adds the effect of baseline DRFs, which include family history of diabetes, HbA_{1c}, fasting glucose, BMI, IGI, IFI, SBP, triacylglycerol and HDL-cholesterol. When waist circumference was substituted for BMI in these models, there were no significant differences in the HRs and so the data are shown with only BMI in the model. Fasting glucose was chosen over 2 h glucose as it associated better with the biomarkers, and is more relevant clinically. Model 3 adds the 1 year changes in non-glycaemic DRFs (BMI, IGI, IFI, SBP, triacylglycerol, HDL-cholesterol). A final model, which included baseline and year 1 changes in all biomarkers and DRFs to evaluate biomarker interactions, is shown in ESM Table 2. A separate analysis tested whether 1 year biomarker changes adjusted for demographics and DRFs associated with incident diabetes (Fig. 3). We observed no significant associations for fibrinogen or MCP-1 with incident diabetes in any group; therefore, these data are not shown. Models were evaluated separately for each treatment group to explore treatment-specific mediators of treatment effects.

Placebo group—Adiponectin was inversely and CRP, sE-selectin, sICAM-1 and tPA values were directly associated with incident diabetes after adjustment for demographic factors (model 1); although attenuated, this association remained significant for adiponectin (HR 0.84 [95% CI 0.71, 0.99]), tPA (1.10 [1.01, 1.21]) and sE-selectin (1.16 [1.04, 1.28])

after adjustment for baseline DRFs (model 2), and both sE-selectin (1.19 [1.06, 1.34]) and tPA (1.13 [1.03, 1.24]) remained significantly associated after adjustment for the modest DRF changes in this group (model 3) and also after all other biomarkers were included in the model (ESM Table 2). Neither leptin nor IL-6 demonstrated any relationship with incident diabetes. In the change analysis (Fig. 3a, b), adiponectin change was associated with diabetes even after adjustment for risk factor changes at 1 year.

ILS group—Baseline adiponectin (inversely) and leptin (directly) were robustly associated with incident diabetes independent of DRFs and in the case of adiponectin even after changes in DRFs at 1 year (model 3) as well as after adjustment for all other biomarkers (ESM Table 2). As in the placebo group, baseline CRP was associated with diabetes development as was tPA (model 1), but both lost significance when DRFs were included in the model (model 2). A major difference between the ILS and placebo groups was the absence of an association between sE-selectin and incident diabetes. Although sICAM-1 was not associated with incident diabetes in models 1 and 2, this became significant after adjustment for changes in DRFs (model 3). The changes in biomarkers at 1 year, with the exception of IL-6, all associated with incident diabetes (Fig. 3a) but all lost significance, including adiponectin, after risk factors changes were included in the analysis (Fig. 3b).

Metformin group—In parallel with findings in the placebo and ILS, baseline adiponectin was inversely associated with incident diabetes in models 1 and 2 and, like ILS, this association persisted in model 3 and after adjustment for other biomarkers (ESM Table 2). CRP was directly associated only in model 1. Like ILS, metformin differed from the placebo in that sE-selectin did not associate with incident diabetes but, unlike ILS, neither baseline leptin, tPA nor sICAM-1 was associated. In the change analysis, as for the ILS group, CRP, sE-selectin and tPA associated with incident diabetes (Fig. 3a) but this association was lost after adjustment for changes in DRFs (Fig 3b). Only in the metformin goup was change in IL-6 associated with incident diabetes even after adjustment for change in DRFs (1.09 [1.021, 1.173]) (HR [95% CI]).

In all instances in which biomarker associations with incident diabetes differed between groups, heterogeneity by group was present.

Discussion

In this study of biomarker associations with incident diabetes, we first sought to determine whether previously well-documented findings in population studies applied to a high-risk population with impaired glucose tolerance, such as that recruited in the DPP. Using the placebo group that received standard care only, we confirmed that baseline sE-selectin, adiponectin, tPA, sICAM-1 and CRP were all significantly associated with incident diabetes, as has been reported in previous population surveys (2, 8–16) but, unlike another study (17), leptin and IL-6 were not. The similarities of our findings to previously published reports may not be surprising as incident diabetes presumably develops from undiagnosed prediabetes in population studies. Although we used tPA as a surrogate for PAI-1, it performs well as a marker of incident diabetes (16). These observations confirm the

generalisability of the biomarker associations we found and, by inference, support the generalisability of the treatment-specific associations that we observed.

Adiponectin, tPA and sE-selectin had the strongest associations with incident diabetes in the placebo group. Importantly, we were able to show that these associations, unlike those for CRP and sICAM-1, were at least partly independent of traditional DRFs. This suggests that the pathways through which adiponectin, tPA and sE-selectin are linked to incident diabetes operate, in part, independently of factors such as obesity, insulin resistance, dyslipidaemia and dysglycaemia at baseline, whereas those marked by CRP and sICAM-1 do not. Although adiponectin is strongly related to insulin resistance (18), the fact that its association with diabetes development was largely independent of IFI, an admittedly imperfect measure of insulin resistance, raises the possibility that its association with incident diabetes extends to other pathophysiological pathways (19). PAI-1 is thought to play a major role in fibrinolysis (20), but has also been implicated in vascular remodelling, adipocyte differentiation and macrophage function (21), all of which could underlie the relationship between tPA and diabetes independent of traditional DRFs and their changes. The association of sE-selectin with incident diabetes may reflect a linkage between endothelial dysfunction and development of diabetes (22) that is not accounted for by DRFs and their changes, and appeared also to be independent of the other biomarkers. However, this association need not imply a direct role for endothelial dysfunction in diabetes development. Instead, it could reflect the specificity of sE-selectin for vascular damage (22) that has been proposed to be part of the common soil in which vascular disease and type 2 diabetes emerge (23).

Given these findings, it was of interest to determine whether these well-established biomarker associations with incident diabetes were modified or remained intact after ILS and metformin interventions. At issue is whether the favourable changes in biomarker levels, DRFs and incident diabetes induced by these two different interventions affect the pathways reflected by these biomarkers in a manner that alters the biomarker–incident diabetes associations seen in the non-intervened state and whether this was similar or different in ILS vs metformin groups. Persistence or strengthening of these associations with ILS or metformin intervention would bolster evidence in support of a central robust pathophysiological role for the underlying pathway or pathways marked by the biomarker in diabetes development. On the other hand, loss of one or more of these associations in the ILS or metformin groups could constitute evidence lessening the relevance of that biomarker and the biochemical pathway it reflects, or alternatively that the biomarker reflects only one of multiple processes related to diabetes development that are differentially affected by these interventions.

With these considerations in mind, we found first that baseline adiponectin was even more strongly associated with incident diabetes in both ILS and metformin groups than in the placebo group, even after full adjustment for DRFs as well as their changes at 1 year and without evidence of heterogeneity by intervention group. These results, which we have partly reported previously (24) and which were independent of the other biomarkers, argue for a direct strong relationship between adiponectin and incident diabetes (25) that remains intact with or without interventions that change pathophysiology. It was notable, however,

that although the ILS-induced adiponectin change was quite sizeable and was inversely related to diabetes development, this was no longer true after adjustment for risk factor changes. No associations with diabetes for the smaller adiponectin change in the metformin group were found. It is possible that baseline adiponectin levels reflect a metabolic setting that has a consistent and persisting 'conditioning' effect on diabetes development in all three different intervention scenarios, with the induced change in level resulting from ILS being more closely tied to changes in the metabolic state, such as an improvement in insulin sensitivity, than to the more distal effect on diabetes incidence per se.

Second, while baseline tPA had a similar association with incident diabetes in the ILS group to that in the placebo group, adjustment for DRFs attenuated this association and there was no association between tPA and diabetes development in the metformin group. Thus, in contrast to what was observed with adiponectin, it appears that ILS modifies diabetes development by weakening the link between tPA and incident diabetes noted in the placebo group, and metformin intervention disconnects it altogether. Furthermore, although the ILS-induced change in tPA was strongly associated with incident diabetes in line with a previous report (26), as for the adiponectin change, this was accounted for by the effects of change in ILS-related risk factors on diabetes development. Interestingly, there was no relationship between the tPA change and incident diabetes in the metformin group, even though metformin led to a similar significant reduction in tPA as did ILS. Thus, it appears that the pathways marked by tPA (as a surrogate for PAI-1) are tied to diabetes development in the ILS group through its link to metabolic risk factors and are not independent of them, whereas in the metformin group, tPA was not even indirectly linked to incident diabetes through metabolic risk factors. This was initially unexpected as metformin, like ILS, did produce favourable changes in body weight, insulin resistance, dysglycaemia and dyslipidaemia, all of which are correlated with tPA. However, the principal mechanism by which metformin is thought to lower glucose levels involves direct inhibition of hepatic glucose production, an effect not known to be influenced by tPA/PAI-1. A final point is that although the effect of metformin to reduce tPA may not be related to its effect on diabetes incidence, the tPA effect may still mark a benefit of metformin in the prevention of vascular disease, with which tPA/PAI is thought to be associated (20).

A third observation is that while baseline sE-selectin was the most robust of the biomarker–incident diabetes associations in the placebo, consistent with findings from the Framingham Study (12), and sICAM-1 was also associated although not after DRF adjustment, surprisingly there were no such associations in the ILS or metformin groups. This suggests that both active interventions reduce diabetes development in a manner that disconnects its association with the biomarkers of endothelial function that are present in the placebo group. Both interventions produced favourable changes in sICAM-1 and ILS lowered sE-selectin levels at 1 year, and the changes in these markers were associated with incident diabetes but not after adjustment for changes in DRFs. Thus, as for tPA, endothelial dysfunction was not directly related to development of diabetes in participants receiving ILS or metformin. While both ILS and metformin have been shown to favourably modify endothelial dysfunction using direct testing (27, 28), it is possible that this effect of these interventions may be more relevant for preventing progressive vascular damage than preventing incident diabetes.

Last, we found that the active interventions exposed biomarker associations with incident diabetes not evident in the placebo group and in a differential manner. In the metformin group only, change in IL-6 independently associated with incident diabetes. Although baseline CRP, which is closely correlated with IL-6, and CRP changes in the active intervention groups were related to incident diabetes, this was not significant after adjustment for baseline DRFs and change in DRFs, respectively, and probably reflects the strong association of this biomarker with obesity (29). Also, in the ILS group only, baseline and change in leptin associated with incident diabetes in a manner independent of baseline DRFs, such as BMI, though these associations became non-significant after adjustment for changes in risk factors at 1 year. Although the meaning of these findings is unclear, they appear to reflect unique effects of ILS and metformin. In the case of metformin, one could speculate that the effect of metformin, but not ILS, on IL-6, an adipokine marker of inflammation, is uniquely linked to its effect on diabetes development independent of effects on body weight and insulin resistance; this would allow for the emergence of a selective association with incident diabetes in the metformin group only. Along the same lines, the novel leptin–incident diabetes association in the ILS group might reflect the possibility that participants with higher leptin levels were more likely to develop diabetes in the ILS group only, suggesting that lifestyle intervention exposed a hyperleptinaemic subgroup that was relatively resistant to the benefits of the intervention for development of diabetes, a hypothesis that deserves more direct testing.

Although the study design provided the opportunity to examine the associations between biomarkers and incident diabetes in a novel manner, there are several limitations to our findings. First, the study was not powered to test for differences in biomarker–incident diabetes associations between treatment groups. Second, we used surrogate measurements for insulin sensitivity and secretion because of the large number of participants in the study. Third, as our cohort was selected, the results are not generalisable to all people with prediabetes.

In summary, we show that among an extensive group of biomarkers, adiponectin, tPA and sE-selectin associate with incident diabetes independent of traditional DRFs in the placebo group, but that ILS and metformin interventions modify these associations significantly and differently, revealing new treatment-emergent biomarker associations. These observations attest to the complexity of the relationships between the processes reflected by these biomarkers and incident diabetes, offering clues to the further elucidation of the pathophysiology of diabetes development. These differences point to possible investigative opportunities for more precise approaches to targeting interventions for diabetes prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This manuscript is dedicated to the late Abbas Kitabchi MD, in recognition of his many contributions to the field of diabetes.

The Research Group gratefully acknowledges the commitment and dedication of the participants of the DPP and Diabetes Prevention Program Outcomes Study (DPPOS). A complete list of centres, investigators and staff can be found in the ESM.

Funding During the DPP and DPPOS, the NIDDK of the National Institutes of Health provided funding to the clinical centres and the coordinating centre for the design and conduct of the study and collection, management, analysis and interpretation of the data (U01 DK048489). Funding for measurement of biomarkers was provided by an NIDDK grant (1R01DK078907–01A1). The Southwestern American Indian centres were supported directly by the NIDDK, including its intramural research programme, and the Indian Health Service. The General Clinical Research Center Program, National Center for Research Resources and the Department of Veterans Affairs supported data collection at many of the clinical centres. Funding was also provided by the National Institute of Child Health and Human Development, the National Institute on Aging, the National Eye Institute, the National Heart Lung and Blood Institute, the National Cancer Institute, the Office of Research on Women's Health, the National Institute on Minority Health and Health Disparities, the Centers for Disease Control and Prevention and the American Diabetes Association. Bristol-Myers Squibb and Parke-Davis provided additional funding and material support during the DPP, Lipha (Merck-Sante) provided medication and LifeScan donated materials during the DPP and DPPOS. This research was also supported, in part, by the intramural research programme of the NIDDK. LifeScan, Health O Meter, Hoechst Marion Roussel, Merck-Medco Managed Care, Merck, Nike Sports Marketing, SlimFast Foods and Quaker Oats donated materials, equipment or medicines for concomitant conditions. McKesson BioServices, Matthews Media Group and the Henry M. Jackson Foundation provided support services under subcontract with the coordinating centre. The sponsor of this study was represented on the Steering Committee and played a part in study design, how the study was done and publication. The funding agency was not represented on the writing group, although all members of the Steering Committee had input into the report's contents. All authors in the writing group had access to all data. The opinions expressed are those of the investigators and do not necessarily reflect the views of the funding agencies. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Duality of interest KM received an investigator-initiated project grant from Novo and has also received donations of medications or supplies with aggregate value >$10,000 for other research studies from Novo Nordisk, Sanofi, Merck and Abbott. MT received honoraria for speaking at symposium from Merck. All other authors declare that there is no duality of interest associated with their contribution to this manuscript.

Abbreviations

DRF	Diabetes risk factor
DPP	Diabetes Prevention Program
ILS	Intensive lifestyle modification
CRP	C-reactive protein
MCP-1	Monocyte chemoattractive protein 1
tPA	Tissue plasminogen activator
PAI-1	Plasminogen activator inhibitor 1
IFI	Inverse of fasting insulin
IGI	Insulinogenic index
SBP	Systolic BP
sE-selectin	Soluble E-selectin
sICAM-1	Soluble intercellular adhesion molecule 1

References

1. Goldberg RB. Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. J Clin Endocrinol Metab 2009; 94:3171–182. [PubMed: 19509100]
2. Sattar N, Wannamethee SG, Forouhi NG. Novel biochemical risk factors for type 2 diabetes: pathogenic insights or prediction possibilities? Diabetologia 2008; 51: 926–940. [PubMed: 18392804]
3. Diabetes Prevention Program Research Group; Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002; 346:393–340. [PubMed: 11832527]
4. Kitabchi AE, Temprosa M, Knowler WC, et al. Diabetes Prevention Program Research Group. Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the Diabetes Prevention Program: effects of lifestyle intervention and metformin. Diabetes 2005; 54:2404–2414. [PubMed: 16046308]
5. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care1997; 20: 1183–1197. [PubMed: 9203460]
6. Lowe GD, Danesh J, Lewington S, et al. Tissue plasminogen activator antigen and coronary heart disease: prospective study and meta-analysis. Eur Heart J 2004; 25:252–259. [PubMed: 14972427]
7. Koenker R Machado AF Goodness of Fit and Related Inference Processes for Quantile Regression. Journal of the American Statistical Association, 1999: 94, 1296–1310.
8. Bray GA, Jablonski KA, Fujimoto WY, et al. Diabetes Prevention Program Research Group. Relation of central adiposity and body mass index to the development of diabetes in the Diabetes Prevention Program. Am J Clin Nutr 2008; 87:1212–1218. [PubMed: 18469241]
9. Schmidt MI, Duncan BB, Sharrett AR, et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet 1999; 353:1649–1652. [PubMed: 10335783]
10. Lindsay RS, Funahashi T, Hanson RL, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet 2002; 360:57–58. [PubMed: 12114044]
11. Festa A, D'Agostino R Jr, Tracy RP, et al. Insulin Resistance Atherosclerosis Study. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes 2002; 51:1131–1137. [PubMed: 11916936]
12. Duncan BB, Schmidt MI, Pankow JS, et al.; Atherosclerosis Risk in Communities Study. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes 2003: 52:1799–1805. [PubMed: 12829649]
13. Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA 2004; 291:1978–1986. [PubMed: 15113816]
14. Herder C, Baumert J, Thorand B, et al. Chemokines as risk factors for type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984–2002. Diabetologia 2006; 49:921–929. [PubMed: 16532324]
15. Hernestål-Boman J, Norberg M, Jansson JH, et al. Signs of dysregulated fibrinolysis precede the development of type 2 diabetes mellitus in a population-based study. Cardiovasc Diabetol 2012; 11:15224.
16. Julia C, Czernichow S, Charnaux N, et al. Relationships between adipokines, biomarkers of endothelial function and inflammation and risk of type 2 diabetes. Diabetes Res Clin Pract 2014; 105:231–238. [PubMed: 24931702]
17. Wannamethee SG, Lowe GD, Rumley A, et al. Adipokines and risk of type 2 diabetes in older men. Diabetes Care 2007; 30:1200–1205. [PubMed: 17322479]
18. Kadowaki T, Yamauchi T, Kubota N, et al. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006; 116: 1784–1792. [PubMed: 16823476]

19. Tao C, Sifuentes A, Holland WL. Regulation of glucose and lipid homeostasis by adiponectin: effects on hepatocytes, pancreatic β cells and adipocytes. Best Pract Res Clin Endocrinol Metab 2014; 28:43–58. [PubMed: 24417945]

20. Cesari M, Pahor M, Incalzi RA. Plasminogen activator inhibitor-1 (PAI-1): a key factor linking fibrinolysis and age-related subclinical and clinical conditions. Cardiovasc Ther 2010; 28:e72–91. [PubMed: 20626406]

21. Juhan-Vague I, Thompson SG, Jespersen J. Involvement of the hemostatic system in the insulin resistance syndrome: a study of 1500 patients with angina pectoris. Arterioscler Thromb 1993; 13:1865–1873. [PubMed: 8241109]

22. Roldán V, Marín F, Lip GY, Blann AD. Soluble E-selectin in cardiovascular disease and its risk factors. A review of the literature. Thromb Haemost 2003: 90; 1007–1020. [PubMed: 14652631]

23. Stern MP. Diabetes and cardiovascular disease. The “common soil” hypothesis. Diabetes 1995: 44:369–374. [PubMed: 7698502]

24. Mather KJ, Funahashi T, Matsuzawa Y, et al. Diabetes Prevention Program. Adiponectin, change in adiponectin, and progression to diabetes in the Diabetes Prevention Program. Diabetes 2008; 57: 980–986. [PubMed: 18192541]

25. 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; 302:179–188. [PubMed: 19584347]

26. Festa A, Williams K, Tracy RP, Wagenknecht LE, Haffner SM. Progression of plasminogen activator inhibitor-1 and fibrinogen levels in relation to incident type 2 diabetes. Circulation 2006; 113:1753–1759. [PubMed: 16585388]

27. Hamdy O, Ledbury S, Mullooly C, et al. Lifestyle modification improves endothelial function in obese subjects with the insulin resistance syndrome. Diabetes Care 2003;26: 2119–2125. [PubMed: 12832323]

28. Mather KJ, Verma S, Anderson TJ. Improved endothelial function with metformin in type 2 diabetes mellitus. J Am Coll Cardiol 2001; 37:1344–1350. [PubMed: 11300445]

29. Florez H, Castillo-Florez S, Mendez A, et al. C-reactive protein is elevated in obese patients with the metabolic syndrome. Diabetes Res Clin Pract 2006;71:92–100. [PubMed: 16002176]

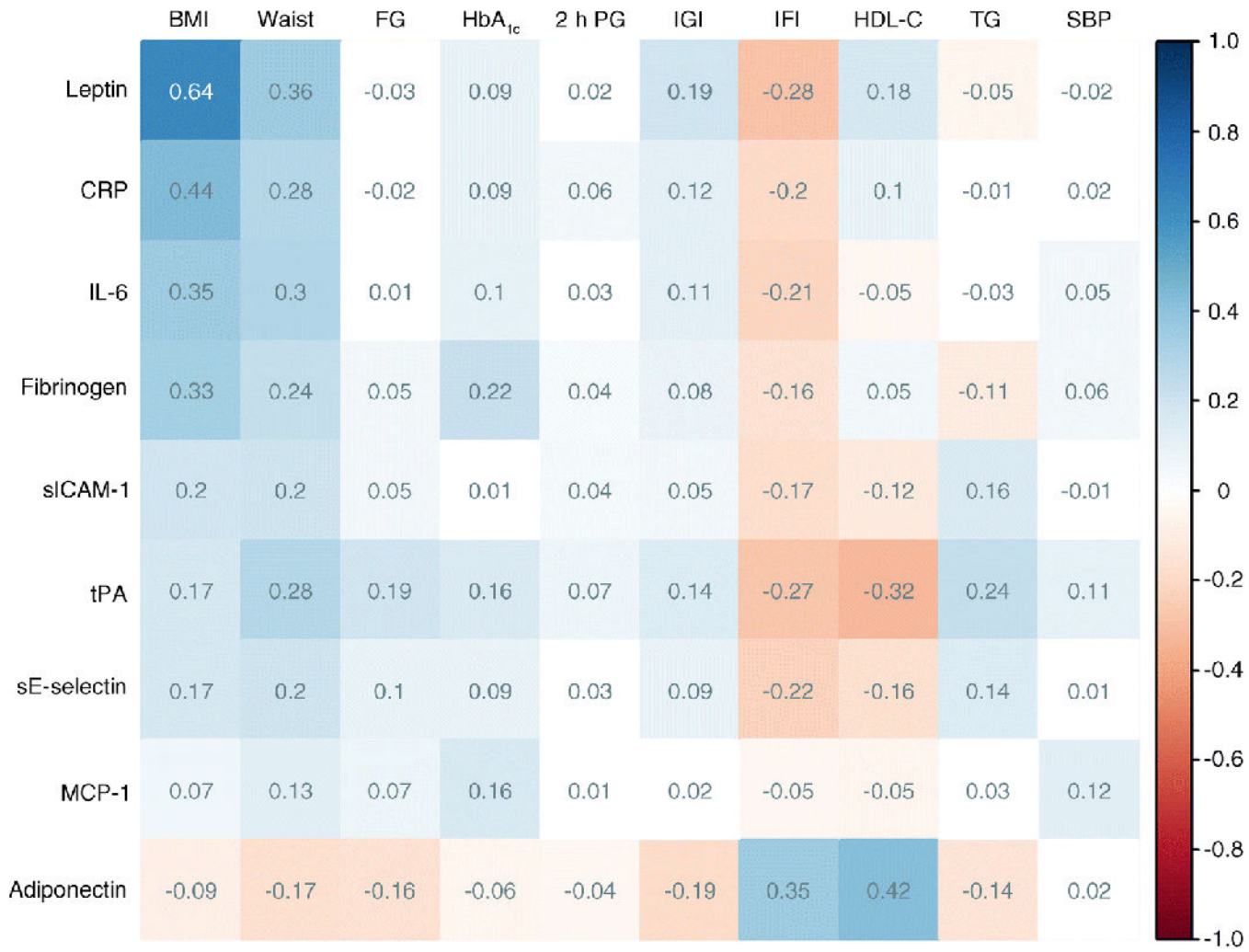

Fig. 1.

Heat map showing Spearman correlations of biomarkers and DRFs at baseline. Correlations are characterised according to direction (positive in blue, negative in orange) and strength (intensity of colour). Spearman correlations with $p>0.05$ are indicated by a white background colour. FG, fasting glucose; HDL-C, HDL-cholesterol; PG, plasma glucose; TG, triacylglycerol; Waist, waist circumference

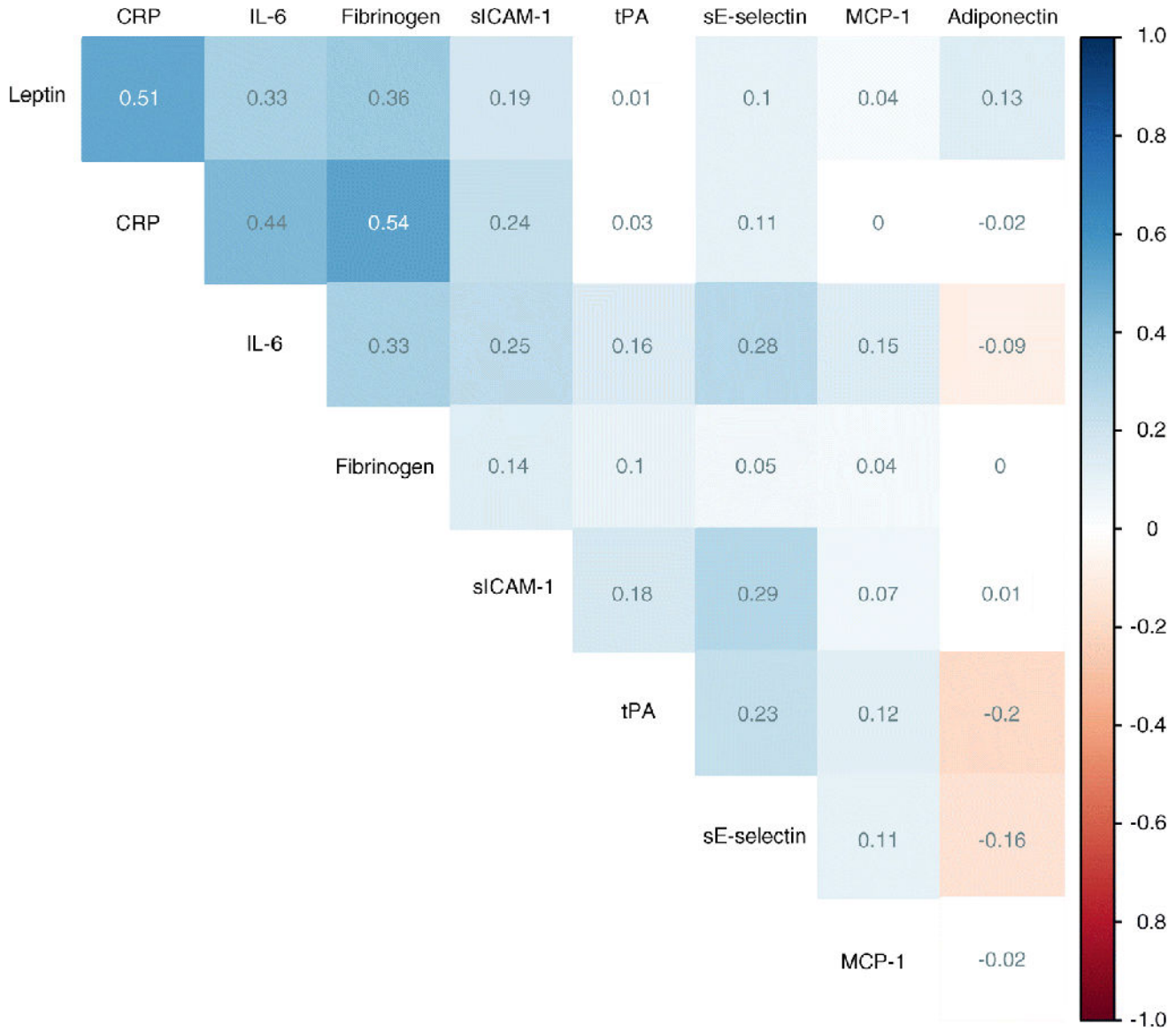

Fig. 2.

Heat map showing Spearman correlations between baseline biomarkers. Correlations are characterised according to direction (positive in blue, negative in orange) and strength (intensity of colour). Spearman correlations with $p>0.05$ are indciated by a white background colour

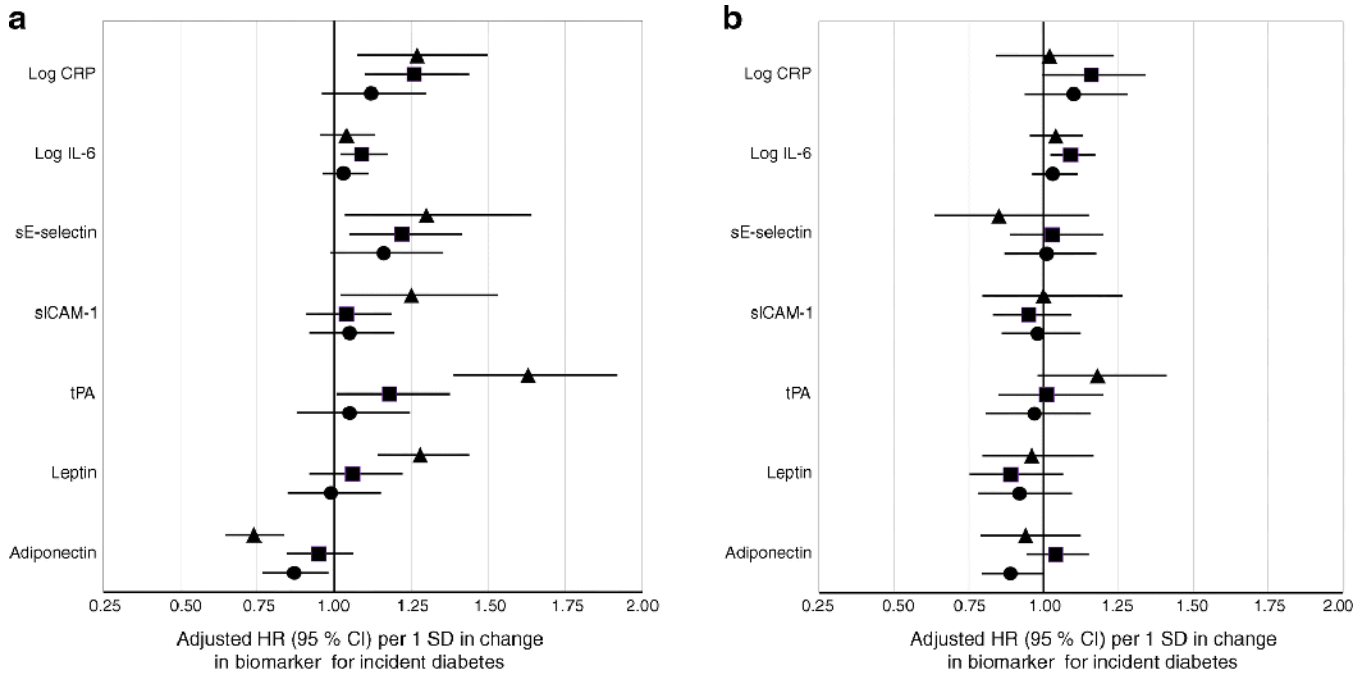

Fig. 3.

Adjusted HRs of change in biomarker for incident diabetes by treatment group. Treatment group-specific HR associations per 1 SD change in each biomarker for developing diabetes. (**a**) Adjustments include demographics (age at randomisation, sex, race/ethnicity), baseline DRFs (family history of diabetes, HbA_{1c}, fasting glucose, BMI, IGI, IFI, SBP, triacylglycerol, HDL-cholesterol) and baseline biomarker. (**b**) Adjustments as in (**a**), with further adjustment for 1 year change in non-glycaemic DRFs (waist circumference, IGI, IFI, SBP, triacylglycerol, HDL-cholesterol). Circles, placebo; squares, metformin; and triangles, ILS

Table 1

Baseline and year 1 changes in biomarkers and DRFs by treatment assignment

Characteristic	Overall Median at baseline		Placebo 1 year change		Metformin 1 year change		ILS 1 year change		Group *p* value for 1 year changes
	n	Median (IQR)	n	Mean change (95% CI)	n	Mean change (95% CI)	n	Mean change (95% CI)	
n	3195		1068		1060		1068		
Traditional DRFs									
BMI (kg/m^2)	3195	32.8 (29.0–37.5)	1011	−0.2 (−0.3, −0.1) *	1002	−1.0 (−1.1, −0.9) *	1012	−2.4 (−2.6, −2.3) *	<.001 †‡§
Waist circumference (cm)	3192	103.8 (94.7– 113.6)	1008	−0.66 (−1.01, −0.31) *	1002	−2.24 (−2.63, −1.85) *	1010	−6.46 (−6.91, −6.01) *	<.001 †‡§
Fasting glucose (mmol/l)	3195	5.8 (5.6– 6.2)	1010	0.0 (−0.0, 0.1)	1003	−0.2 (−0.3, −0.2) *	1014	−0.3 (−0.3, −0.2) *	<.001 †‡
2 h glucose (mmol/l)	3195	8.99 (8.32–9.88)	977	−0.34 (−0.48, −0.20) *	994	−0.46 (−0.59, −0.33) *	1009	−1.32 (−1.44, −1.20) *	<.001 ‡§
HbA_{1c} (%)	3188	5.9 (5.6–6.2)	1005	0.09 (0.06, 0.11) *	1001	0.0 (−0.01, 0.02)	1010	−0.09 (−0.11, −0.07) *	<.001 †‡§
HbA_{1c} (mmol/mol)	3188	41 (38–44)	1005	0.94 (0.70, 1.2) *	1001	0.05 (−0.15, 0.25)	1010	−1.01 (−1.22, −0.82) *	<.001 †‡§
Fasting insulin (pmol/l)	3192	167 (111–229)	988	6.4 (−0.8, 13.7)	982	−24.5 (−30.3, −18.8) *	996	−36.4 (−43.3, −29.6) *	<.001 †‡§
IGI (pmol/mmol)	3135	13014 (8347–19721)	947	37.5 (−901, 963)	960	−501 (−1264, 250)	976	−526 (−1313, 275)	0.69
Systolic BP (mmHg)	3195	122 (113–132)	997	−0.74 (−1.54, 0.06)	994	−1.15 (−2.01, −0.30) *	1000	−3.23 (−4.06, −2.40) *	<.001 ‡§
HDL-C (mmol/l)	3190	1.14 (0.96–1.35)	1009	−0.002 (−0.01,0.008)	1004	0.02 (0.01, 0.03) *	1012	0.03 (0.02, 0.04) *	<.001 †‡
Triacylglycerol (mmol/l)	3190	1.60 (1.13–2.27)	1012	−0.28 (−0.33, −0.23) *	1004	−0.08 (−0.12, −0.03) *	1009	−0.11 (−0.17, −0.06) *	<.001 ‡§
Markers of adipocyte dysfunction									
Adiponectin (μg/ml)	3102	7.3 (5.5–9.6)	948	0.1 (0.0, 0.2)	941	0.2 (0.1, 0.3) *	938	0.8 (0.7, 0.9) *	<.001 ‡§
Leptin (ng/ml)	3195	22 (12.7–32.6)	1002	0.4 (−0.1, 1.0)	994	−1.7 (−2.3, −1.2) *	1002	−3.6 (−4.2, −3.0) *	<.001 †‡§
Markers of inflammation									
CRP (nmol/l)	3191	35.2 (16.2–72.4)	1010	−19 (−59, 20)	1003	−52 (−90, −16) *	1014	−130 (−167, −93) *	<.001 ‡§
IL-6 (pg/ml)	3192	1.9 (1.3–3.0)	1007	0.0 (−0.1, 0.1)	998	−0.2 (−0.3, −0.1) *	1008	−0.3 (−0.4, −0.2) *	<.001 †‡
MCP-1 (pg/ml)	3194	138.0 (115.7–167.3)	1004	−9.5 (−12.4, −6.5) *	997	−8.5 (−11.7, −5.4) *	1004	−12.5 (−15.4, −9.7) *	0.012
Markers of endothelial dysfunction									

Characteristic	Overall Median at baseline		Placebo 1 year change		Metformin 1 year change		ILS 1 year change		Group *p* value for 1 year changes
	n	Median (IQR)	n	Mean change (95% CI)	n	Mean change (95% CI)	n	Mean change (95% CI)	
sE-selectin (ng/ml)	3194	44.1 (32.9–56.8)	1007	−0.2 (−1.0, 0.6)	998	−0.2 (−0.9, 0.4)	1008	−4.6 (−5.2, −4.0) *	<.001 ‡§
sICAM-1 (ng/ml)	3194	246 (210–288)	1006	−4.7 (−7.4, −1.9) *	998	−14.8 (−17.9, −11.8) *	1007	−19.9 (−22.7, −17.0) *	<.001 †‡§
Markers of procoagulant state									
tPA (ng/ml)	3181	10.9 (8.7–13.4)	1004	−0.7 (−0.9, −0.5) *	990	−2.1 (−2.3, −1.9) *	1008	−2.5 (−2.7, −2.3) *	<.001 †‡§
Fibrinogen (g/l)	3187	3.74 (3.28–4.33)	1009	0.24 (−0.02, 0.70)	1002	0.01 (−0.04, 0.05)	1014	−0.11 (−0.15, −0.06) *	<.001 ‡§

* $p<0.05$ for change from baseline value in that group

Group *p* value results are from the overall test of treatment group comparisons of the change from baseline to year 1. Significant pairwise differences between groups are assessed when group difference meets nominal significance of $p<0.01$ and are noted for:

† placebo vs metformin

‡ placebo vs ILS

§ metformin vs ILS

HDL-C, HDL-cholesterol

Table 2

Adjusted HR (95% CI) associated with each baseline biomarker for the development of diabetes stratified by treatment group

Baseline biomarker (SD unit)/model	n	Placebo	Metformin	ILS	Homogeneity in groups p
Adiponectin (3 μg/ml)					
1	2843	0.77 (0.66, 0.91) *	0.75 (0.63, 0.89) *	0.61 (0.48, 0.78) *	0.27
2	2780	0.84 (0.71, 0.99) *	0.77 (0.64, 0.93) *	0.66 (0.51, 0.86) *	0.33
3	2699	0.88 (0.73, 1.05)	0.79 (0.66, 0.94) *	0.69 (0.52, 0.92) *	0.37
Leptin (14 ng/ml)					
1	2933	1.13 (0.98, 1.30)	1.12 (0.96, 1.29)	1.40 (1.18, 1.65) *	0.09 †
2	2870	0.96 (0.81, 1.15)	1.06 (0.85, 1.31)	1.31 (1.06, 1.63) *	0.09 †
3	2786	0.98 (0.81, 1.19)	1.05 (0.85, 1.30)	1.22 (0.93, 1.59)	0.43
Log CRP (30%)					
1	2929	1.05 (1.02, 1.08) *	1.03 (1.00, 1.06) *	1.06 (1.01, 1.11) *	0.62
2	2869	1.02 (0.98, 1.05)	1.03 (0.99, 1.07)	1.01 (0.96, 1.07)	0.85
3	2785	1.02 (0.99, 1.06)	1.03 (0.99, 1.07)	0.99 (0.93, 1.04)	0.44
Log IL-6 (30%)					
1	2931	1.05 (0.997, 1.10)	1.06 (0.998, 1.12)	1.05 (0.98, 1.12)	0.98
2	2868	1.00 (0.95, 1.06)	1.05 (0.98, 1.12)	1.00 (0.92, 1.09)	0.50
3	2784	1.00 (0.95, 1.06)	1.06 (0.99, 1.13)	0.97 (0.88, 1.06)	0.22
sE-selectin (18 ng/ml)					
1	2933	1.23 (1.13, 1.33) *	1.15 (0.99, 1.33)	1.05 (0.89, 1.24)	0.23
2	2870	1.16 (1.04, 1.28) *	1.00 (0.86, 1.16)	0.90 (0.76, 1.08)	0.039 †
3	2776	1.19 (1.06, 1.34) *	1.01 (0.87, 1.17)	0.93 (0.77, 1.13)	0.05 †
sICAM-1 (77 ng/ml)					
1	2933	1.19 (1.06, 1.33) *	1.09 (0.95, 1.24)	1.00 (0.85, 1.19)	0.25
2	2870	1.09 (0.97, 1.23)	1.02 (0.89, 1.17)	0.85 (0.70, 1.04)	0.10
3	2786	1.08 (0.96, 1.22)	1.04 (0.89, 1.21)	0.81 (0.66, 0.98) *	0.043 †
tPA (4 ng/ml)					

Baseline biomarker (SD unit)/model	n	Placebo	Metformin	ILS	Homogeneity in groups p
1	2920	1.19 (1.09, 1.30)*	1.03 (0.92, 1.15)	1.20 (1.07, 1.35)*	0.08†
2	2857	1.10 (1.01, 1.21)*	0.90 (0.79, 1.04)	1.08 (0.92, 1.26)	0.06†
3	2775	1.13 (1.03, 1.24)*	0.88 (0.75, 1.03)	1.05 (0.89, 1.24)	0.028†

HRs and 95% CIs are presented per 1 SD in baseline biomarker except for log-transformed biomarkers (IL-6 and CRP), which are presented per 30% difference

Cox models were adjusted for the following covariates: model 1, demographics (age at randomisation, sex, race/ethnicity); model 2, demographics and baseline DRFs (family history of diabetes, HbA_{1c}, fasting glucose, BMI, IGI, IFI, SBP, triacylglycerol, HDL-cholesterol); and model 3, demographics, baseline DRFs and 1 year change in non-glycaemic DRFs (BMI, IGI, IFI, SBP, triacylglycerol, HDL-cholesterol)

*$p<0.05$ for the HR

†$p<0.1$ for non-homogeneity among treatment group