Lifestyle and Metformin Interventions Have a Durable Effect to Lower CRP and tPA Levels in the Diabetes Prevention Program Except in Those Who Develop Diabetes

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OBJECTIVE

We evaluate whether lifestyle and metformin interventions used to prevent diabetes have durable effects on markers of inflammation and coagulation and whether the effects are influenced by the development of diabetes.

RESEARCH DESIGN AND METHODS

The Diabetes Prevention Program was a controlled clinical trial of 3,234 subjects at high risk for diabetes who were randomized to lifestyle, metformin, or placebo interventions for 3.4 years. Diabetes was diagnosed semiannually by fasting glucose and annually by oral glucose tolerance testing. In addition to baseline testing, anthropometry was performed every 6 months; fasting insulin yearly; and hs-CRP, tissue plasminogen activator (tPA), and fibrinogen at 1 year and end of study (EOS).

RESULTS

CRP and tPA levels were unchanged in the placebo group but fell in the lifestyle and metformin groups at 1 year and remained lower at EOS. These reductions were not seen in those who developed diabetes over the course of the study despite intervention. Fibrinogen was lower at 1 year in the lifestyle group. Differences in weight and weight change explained most of the influence of diabetes on the CRP response in the lifestyle group, but only partly in the placebo and metformin groups. Weight, insulin sensitivity, and hyperglycemia differences each accounted for the influence of diabetes on the tPA response.

CONCLUSIONS

Lifestyle and metformin interventions have durable effects to lower hs-CRP and tPA. Incident diabetes prevented these improvements, and this was accounted for by differences in weight, insulin resistance, and glucose levels.

It is well established that lifestyle and pharmacologic interventions can slow progression to type 2 diabetes in adults with impaired glucose tolerance (IGT) by altering pathophysiological processes important in the development of diabetes and its complications (1,2). Among these processes, activation of inflammation

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**A complete list of centers, investigators, and staff can be found in the Supplementary Data online.*

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and coagulation leading to vascular dysfunction are recently recognized abnormalities that have been observed to occur in prediabetic individuals (3,4). There have been many short-term trials of these interventions and a few reports of 1-year interventions on markers of inflammation and procoagulant balance (5,6). However, little is known about the longer-term durability of these interventions on biomarkers of these processes and whether changes are linked to the ability of these interventions to slow progression to diabetes over time.

The Diabetes Prevention Program (DPP) compared the effect of intensive lifestyle (ILS) or metformin with placebo on the development of diabetes in a multicenter cohort with IGT (1). Subsequently, interventions were modified and participants entered an extended follow-up, open-label, long-term outcome study. During the trial, hs-CRP (as a marker of inflammation) and tissue plasminogen activator (tPA) and fibrinogen (as markers of a procoagulant state) were measured. Previous reports from the DPP have described the baseline levels of these biomarkers and their associations with other diabetes and cardiovascular risk factors (7), as well as the initial effects of ILS and metformin compared with the placebo group on CRP and fibrinogen levels after 1 year of intervention (6).

We report here the longer-term effect of metformin and ILS in the DPP on CRP, tPA, and fibrinogen before modification of interventions occurred. In addition, we compared these effects in participants who did or did not develop diabetes.

RESEARCH DESIGN AND METHODS

Participants, Interventions, and Data Collection

This report includes 3,234 participants in the ILS, metformin, and placebo treatment arms. Individuals were recruited based on an increased risk for development of diabetes (1). Eligibility was based on results of a 75-g oral glucose tolerance test. Inclusion criteria included a fasting plasma glucose value of 5.3–6.9 mmol/L ($\leq$6.9 mmol/L for American Indians), a 2-h plasma glucose of 7.8–11.1 mmol/L following the glucose load, age $\geq$25 years, and BMI $\geq$24 kg/m^2 ($\geq$22 kg/m^2 for Asian Americans). Major exclusions included a recent myocardial infarction, symptoms of coronary heart disease, major illness, prior diagnosis of diabetes, or use of medications known to impair glucose tolerance. Written informed consent was obtained from all participants prior to screening consistent with the Helsinki Declaration and the guidelines of each center's institutional review board.

Eligible participants were randomly assigned to one of three interventions: metformin 850 mg twice daily, placebo twice daily, or an intensive program of ILS. Random treatment assignments were stratified by clinical center and double-blind for the metformin and placebo groups. The goals of the ILS were to achieve and maintain a weight reduction of at least 7% of initial body weight through consumption of a low-calorie, low-fat diet and to engage in moderate physical activity for at least 150 min/week. Adherence to metformin was defined as taking $\geq$80% of assigned metformin and adherence to lifestyle as achieving the assigned weight loss of $\leq$7% of randomization weight. Diabetes was diagnosed on the basis of an annual oral glucose tolerance test or a semiannual fasting plasma glucose test according to American Diabetes Association criteria (8). The diagnosis required confirmation by a second test, usually within 6 weeks. Weight and glucose was assessed semiannually, while fasting insulin was assessed annually. The DPP study was stopped prematurely in May 2001 based on evidence of diabetes prevention in both active treatment groups, and an end of study (EOS) sample collection was conducted during a 6-month period from June through December 2001. At this time, participants entered a bridge period where interventions were unmasked and the lifestyle curriculum was offered in group sessions to the other treatment groups. CRP, tPA, and fibrinogen were measured at DPP baseline, at the first annual visit, and at EOS (median [25th–75th percentile] follow-up was 3.4 [2.8–4.0] years). CRP was also measured after 6 months. We excluded CRP, tPA, and fibrinogen measurements done after the initiation of the bridge (see below) to limit the analyses to the period of the DPP interventions. Since weight, glucose, and insulin measurements were obtained at protocol-specified annual visits that did not, for the most part, coincide with the EOS visits, these data were obtained from annual visits conducted within a 7-month period prior to or after the participant's EOS visit. Using this approach, 91.4% of participants had weight, fasting glucose, and fasting insulin results available for analysis. These data together with the biomarker values obtained from samples collected at the end of the study visit are collectively referred to as the EOS results. During 1 week in August 2001, metformin was discontinued temporarily to investigate the effect of short-term metformin washout on diabetes incidence (9).

Biochemical Measurements

Plasma glucose was measured on a chemistry AutoAnalyzer by the glucokinase method. Insulin measurements were performed by a polyethylene glycol–accelerated double antibody radioimmunoassay method. hs-CRP and fibrinogen levels in plasma were measured immunochemically using Dade Behring reagent on the Behring Nephelometer II analyzer (BN II). tPA levels were measured in citrated plasma using an ELISA assay (Asserachrom tPA; Diagnostica Stago).

Statistical Analysis

Analysis was conducted according to the intention-to-treat principle. Comparisons among groups at baseline were made using ANOVA for quantitative variables and χ^2 test for categorical variables. Nominal P values are shown without adjustment for multiple comparisons. Mixed models were used to assess how the interventions and diabetes status affected biomarker levels. Similar mixed-effects models were also used to evaluate whether changes in weight, fasting insulin, or fasting glucose could explain the effects of diabetes status on biomarkers. We excluded data for participants with a CRP level exceeding 32 mg/L (99th percentile for the population), as this reflects acute illness not related to intervention.

RESULTS

Effects of Interventions on Biomarker Levels

Table 1 shows characteristics at baseline and follow-up according to treatment assignment. For the metformin group, adherence (percentage of participants taking $\geq$80% assigned study metformin)

Table 1—Characteristics by treatment group

	Placebo	Metformin	Lifestyle
n			
Baseline	1,082	1,073	1,079
Year 0.5	1,019	1,024	1,044
Year 1	1,021	1,013	1,019
EOS	858	860	899
Weight (kg)			
Baseline	94.3 (93.1, 95.5)	94.3 (93.1, 95.5)	94.1 (92.8, 95.3)
Year 0.5	93.7 (93.4, 94.0)	91.8 (91.5, 92.1)*	87.4 (87.1, 87.7)†,‡
Year 1	93.6 (93.3, 94.0)	91.4 (91.0, 91.7)*	87.3 (87.0, 87.7)†,‡
EOS	94.3 (93.9, 94.8)	92.6 (92.1, 93.0)*	90.3 (89.9, 90.8)†,‡
Fasting glucose (mmol/L)			
Baseline	5.92 (5.90, 5.95)	5.91 (5.88, 5.94)	5.90 (5.87, 5.93)
Year 0.5	5.92 (5.89, 5.95)	5.69 (5.66, 5.72)*	5.65 (5.62, 5.68)†
Year 1	5.95 (5.91, 5.98)	5.68 (5.64, 5.72)*	5.64 (5.60, 5.68)†
EOS	6.23 (6.17, 6.29)	5.94 (5.88, 6.00)*	5.97 (5.91, 6.03)†
Fasting insulin (pmol/L)			
Baseline	161 (156, 167)	163 (158, 168)	158 (153, 163)
Year 1	164 (160, 169)	138 (135, 142)*	124 (121, 128)†,‡
EOS	164 (159, 170)	147 (142, 152)*	140 (135, 144)†
CRP (mg/L)			
Baseline	3.52 (3.30, 3.75)	3.34 (3.13, 3.57)	3.53 (3.31, 3.76)
Year 0.5	3.50 (3.35, 3.66)	3.09 (2.96, 3.23) *	2.78 (2.66, 2.90)†,‡
Year 1	3.40 (3.25, 3.55)	2.99 (2.86, 3.12)*	2.36 (2.26, 2.46)†,‡
EOS	3.44 (3.27, 3.62)	2.98 (2.83, 3.14)*	2.82 (2.68, 2.96)†
tPA (ng/mL)			
Baseline	11.40 (11.16, 11.64)	11.25 (10.99, 11.50)	11.34 (11.09, 11.60)
Year 1	10.60 (10.43, 10.78)	9.23 (9.06, 9.41)*	8.82 (8.64, 8.99)†,‡
EOS	11.75 (11.51, 11.99)	10.70 (10.46, 10.94)*	10.71 (10.47, 10.94)†
Fibrinogen (μmol/L)			
Baseline	386 (381, 391)	380 (375, 385)	385 (380, 390)
Year 1	387 (384, 391)	383 (379, 387)	374 (370, 378)†,‡
EOS	376 (371, 381)	372 (368, 377)	372 (367, 377)

Data are expressed as mean and 95% CI except for fasting insulin and CRP, which are expressed as the geometric mean. *$P < 0.05$ for placebo vs. metformin. †$P < 0.05$ for placebo vs. lifestyle. ‡$P < 0.05$ for metformin vs. lifestyle.

was 71.6 and 49.1% at year 1 and EOS, respectively, and for the ILS group, adherence (percentage of individuals meeting assigned goal of ≤7% of their baseline weight) was 49.9 and 37.5%, respectively. As previously reported, weight, fasting insulin, and fasting glucose fell in both the metformin and the ILS groups by 1 year and the reductions, except for fasting glucose, persisted until EOS. CRP, tPA, and fibrinogen levels did not differ at baseline by intervention group and did not change significantly during follow-up in the placebo group. There were significant changes in biomarkers between intervention groups as follows.

CRP

Compared with placebo, CRP fell at 1 year by 33 and 8% in the ILS and metformin groups, respectively. At EOS, CRP in the ILS group rose somewhat but remained 20% lower than the baseline value; in the metformin group, the CRP was unchanged at EOS from its 1 year value. In both ILS and metformin groups, CRP values at EOS were significantly lower than the corresponding placebo group values.

tPA

tPA levels fell by 22% at 1 year in the ILS group and by 18% in the metformin group versus 7% in the placebo group and then rose similarly in both intervention groups at EOS to levels that were still significantly lower than the corresponding placebo value.

Fibrinogen

Fibrinogen levels fell significantly in the ILS group at 1 year compared with those in the placebo and metformin groups but showed no differences between groups at EOS, as both placebo and metformin groups showed a fall in levels.

Comparison of Weight, Glucose, Insulin, and Biomarker Values in Those With and Without Incident Diabetes

To determine whether the values of these three biomarkers differed in participants who developed diabetes among the intervention groups, the participants were divided into those who developed diabetes versus those who did not at each of the follow-up time points. At 1 year, 37, 80, and 123 participants in the ILS, metformin, and placebo groups, respectively, developed diabetes; 141, 216, and 293, respectively, had been diagnosed with diabetes at EOS. Table 2 shows the absolute biomarker values by presence/absence of diabetes at each time point. Weight was consistently greater in those with diabetes compared with those without in the ILS group only, whereas fasting glucose and insulin were greater in participants who had developed diabetes in all three intervention groups. All three biomarkers were numerically higher in each of the intervention subgroups that developed diabetes than their counterparts without diabetes; differences were statistically significant at EOS for tPA for all groups, for tPA in the ILS group at 1 year, and for fibrinogen in the placebo group.

Effect of Interventions by Presence or Absence of Incident Diabetes on Biomarker Change

Figure 1 shows the biomarker changes from baseline for the three intervention groups according to the presence or absence of a diabetes diagnosis before the assessment.

CRP

There were no changes in CRP levels among participants either with or without diabetes in the placebo group. Among those who had not developed diabetes in the metformin and ILS groups, there was a significant reduction in CRP at both 1 year and EOS, whereas there were no changes in CRP in either ILS or metformin group participants who had developed diabetes.

tPA

The tPA value was reduced overall and among those without diabetes in the placebo group at 1 year but was significantly increased above baseline at EOS, with the elevation accounted for by the

Table 2—Characteristics by diabetes or nondiabetes status and by intervention group

	Placebo		Metformin		Lifestyle	
	Diabetes	Nondiabetes	Diabetes	Nondiabetes	Diabetes	Nondiabetes
n						
Year 0.5	38	987	10	1,018	9	1,037
Year 1	123	898	80	934	37	985
EOS	293	595	216	644	141	786
Weight (kg)						
Year 0.5	**95.8 (94.8, 96.9)**	**93.8 (93.6, 94.1)**	93.2 (91.3, 95.1)	91.8 (91.6, 92.0)	**93.7 (91.4, 96.1)**	**87.2 (86.8, 87.5)**
Year 1	94.3 (93.6, 94.9)	93.7 (93.4, 94.0)	**92.3 (91.5, 93.0)**	**91.3 (91.0, 91.6)**	**89.0 (87.8, 90.2)**	**87.1 (86.7, 87.5)**
EOS	95.0 (94.3, 95.7)	94.3 (93.8, 94.8)	92.7 (91.9, 93.5)	92.5 (92.1, 93.0)	**91.4 (90.4, 92.4)**	**89.9 (89.4, 90.5)**
0′ Glucose (mmol/L)						
Year 0.5	**7.01 (6.85, 7.17)**	**5.89 (5.85, 5.92)**	**6.92 (6.63, 7.21)**	**5.68 (5.65, 5.71)**	**7.13 (6.84, 7.42)**	**5.63 (5.60, 5.66)**
Year 1	**6.58 (6.46, 6.70)**	**5.86 (5.82, 5.91)**	**6.23 (6.12, 6.34)**	**5.64 (5.60, 5.67)**	**6.17 (6.01, 6.33)**	**5.60 (5.57, 5.64)**
EOS	**6.80 (6.68, 6.91)**	**5.98 (5.90, 6.06)**	**6.31 (6.22, 6.41)**	**5.82 (5.76, 5.87)**	**6.82 (6.70, 6.95)**	**5.81 (5.76, 5.86)**
0′ Insulin (pmol/L)						
Year 1	**197 (182, 212)**	**160 (156, 165)**	**160 (146, 176)**	**138 (134, 142)**	**178 (153, 207)**	**121 (118, 125)**
EOS	**181 (172, 191)**	**157 (151, 163)**	**163 (153, 173)**	**144 (139, 149)**	**185 (171, 199)**	**131 (127, 136)**
CRP (mg/L)						
Year 0.5	4.21 (3.51, 5.06)	3.56 (3.42, 3.71)	**4.86 (3.30, 7.16)**	**2.99 (2.86, 3.13)**	2.86 (1.83, 4.47)	2.79 (2.66, 2.92)
Year 1	3.67 (3.30, 4.08)	3.45 (3.31, 3.61)	3.26 (2.85, 3.72)	2.88 (2.75, 3.01)	2.85 (2.31, 3.51)	2.35 (2.24, 2.46)
EOS	3.69 (3.42, 3.99)	3.45 (3.26, 3.65)	3.03 (2.76, 3.34)	2.86 (2.70, 3.03)	3.11 (2.74, 3.53)	2.78 (2.63, 2.95)
tPA (ng/dL)						
Year 1	10.9 (10.4, 11.4)	10.6 (10.4, 10.8)	9.7 (9.2, 10.3)	9.1 (9.0, 9.3)	**10.2 (9.3, 11.0)**	**8.8 (8.6, 8.9)**
EOS	**12.4 (12.0, 12.8)**	**11.5 (11.2, 11.8)**	**11.2 (10.7, 11.7)**	**10.5 (10.2, 10.8)**	**11.5 (11.0, 12.1)**	**10.6 (10.3, 10.8)**
Fibrinogen (μmol/L)						
Year 1	397 (386, 408)	389 (385, 393)	387 (374, 400)	379 (375, 383)	386 (366, 406)	374 (371, 378)
EOS	**385 (377, 394)**	**375 (369, 380)**	377 (367, 386)	367 (362, 372)	377 (365, 390)	371 (366, 376)

Data are expressed as mean (95% CI) except for fasting insulin and CRP, which are expressed as geometric mean with adjustment for baseline levels. Data in bold indicate significant difference between the diabetes versus nondiabetes levels within the treatment group ($P < 0.05$).

subgroup with diabetes. There were reductions in tPA among participants both without and with diabetes at 1 year in the ILS and metformin groups. At this time, the fall in tPA was significantly greater in those without diabetes compared with those with diabetes in the ILS group, but there were no differences between those with or without diabetes in the metformin group. In both metformin and ILS groups, tPA levels remained significantly lower than baseline at EOS among those without diabetes, although they trended upward from the 1-year values. For those with diabetes at EOS, there was no change in tPA levels from baseline in the metformin group, and there was a significant increase above baseline in the ILS group.

Fibrinogen

At 1 year, fibrinogen levels did not change in the placebo or metformin groups but decreased significantly in ILS participants due to reductions in those who had not developed diabetes. At EOS, fibrinogen levels were reduced in those without diabetes in all three groups, but there were no changes from baseline among participant subgroups that had developed diabetes.

Effect of Weight, Fasting Insulin, and Glucose on Time-Related Biomarker Differences According to Presence/Absence of Incident Diabetes

CRP, tPA, and fibrinogen levels are strongly influenced by weight, insulin resistance, and glycemia measures (7). We next used mixed modeling to assess the contributory influences of intervention- and time-related changes in weight, fasting insulin as a surrogate marker of insulin resistance, and fasting glucose on the differences in biomarker responses between those with versus without incident diabetes (Table 3). There was no interaction between the interventions and the effect of incident diabetes on CRP, tPA, or fibrinogen time-related changes. Significant effects of the presence of diabetes on the biomarker response to the interventions in the basic model (model 1) are shown by emboldened text in Table 3. Additional adjustment for metformin or lifestyle adherences did not change the diabetes effect in model 1. Although the R^2 in model 1 was small in each case, the addition of weight, insulin resistance, or glycemia variables to model 1 accounted for the diabetes effect on CRP and tPA responses to interventions as indicated by a loss of significance of the diabetes effect noted in model 1 as follows.

CRP Change

The estimated diabetes effect for CRP change was not influenced by baseline or change in insulin resistance in any intervention group (model A3, Table 3). In contrast, weight partly accounted for the effect of developing diabetes on CRP changes in the placebo and metformin groups and fully accounted for it in the ILS group (model A4). Baseline and changes in glucose also completely accounted for the effect of diabetes on time-related CRP change (model A5).

tPA Change

Baseline and changes in insulin and weight partially accounted for the effect of diabetes on the tPA change over time (models B3 and B4), and glucose accounted for most of the effect of

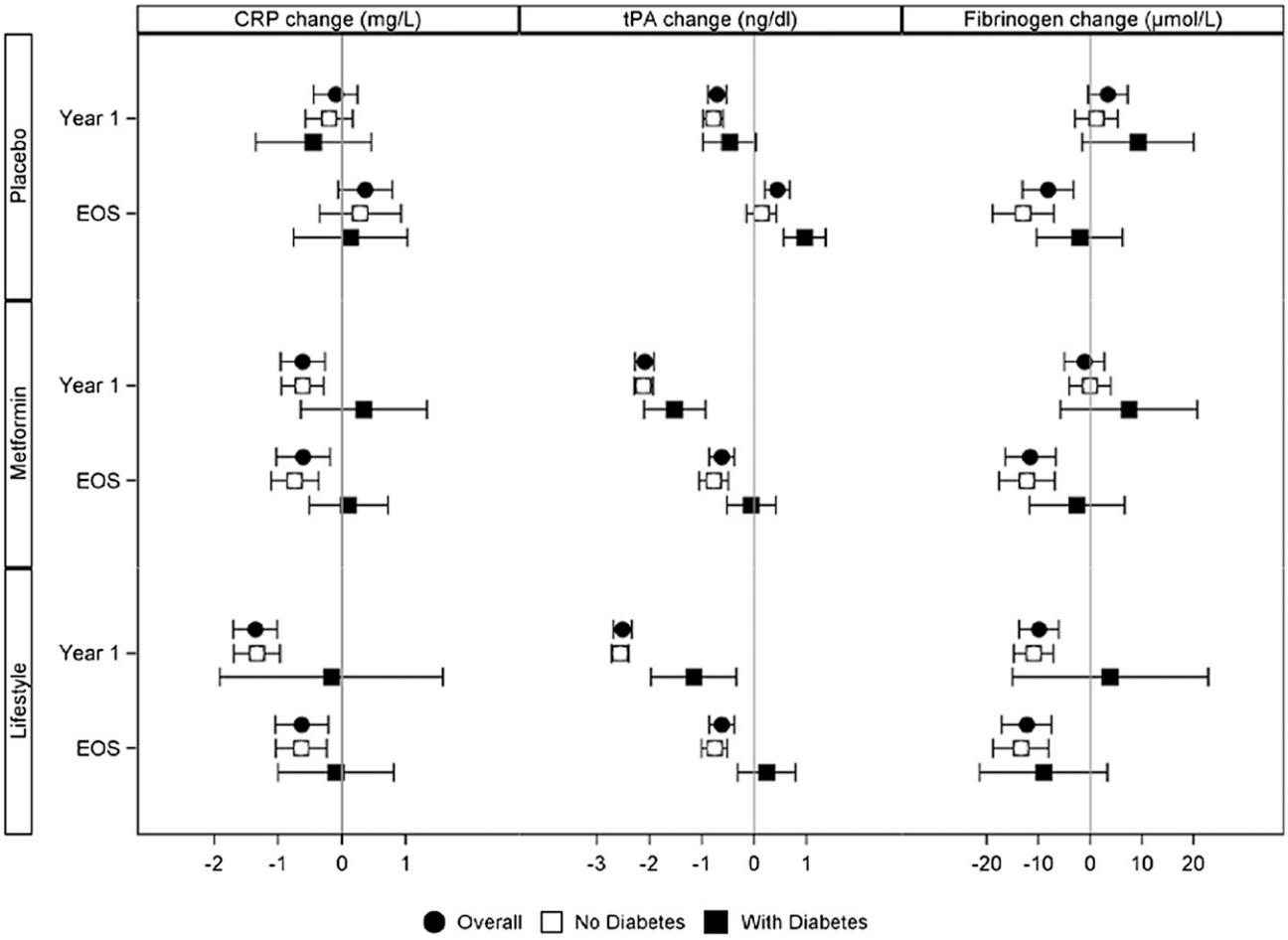

Figure 1—Mean (95% CI) change from baseline in CRP, fibrinogen, and tPA according to DPP intervention group, diabetes status, and time of assessment.

diabetes on tPA change in all three intervention groups (model B2).

Fibrinogen Change

Incident diabetes had no significant effect on fibrinogen changes in the metformin or ILS groups in this analysis (model C1). There was a significant effect in the placebo group that was only partly accounted for by glucose (model C2).

CONCLUSIONS

We previously showed that ILS and metformin interventions lowered CRP levels but not fibrinogen compared with placebo after 1 year of follow-up in subjects with IGT in the DPP (6), and we now show that lowered CRP and tPA levels at 1 year persisted after 3.4 years of follow-up. Previous studies have demonstrated beneficial effects of lifestyle change or metformin on CRP or tPA, but few have evaluated these effects beyond 1 year, and none have compared the these effects in a long-term controlled clinical trial. Baseline CRP levels correlated strongly with body weight in the DPP (7), and the effect of ILS to reduce CRP by 33% after 1 year is likely to be related to the weight change in the ILS (6). Weight loss in the ILS reached its nadir at 6 months through 1 year and then began to increase, though remaining significantly reduced from baseline at EOS. This pattern closely resembled the CRP response to the ILS intervention. Interestingly, CRP levels continued to fall from 6 to 12 months despite no further weight reduction after 6 months, suggesting that the benefit associated with ILS intervention or weight reduction on CRP levels extends beyond the period of active weight loss. The less robust initial CRP reduction in the metformin group at 1 year may be related to the smaller degree of weight loss achieved with this intervention, but the CRP reduction remained stable through the EOS in these participants as compared with that in the ILS group, which tended to attenuate. Some studies of metformin treatment have not noted an associated fall in CRP, and this may reflect the absence of significant weight loss in short-term studies (10). The similarity of CRP levels in the ILS and metformin groups at the EOS is of interest considering the fact that mean weight was significantly lower and the physical activity level considerably higher at this time point in the ILS than the metformin group (1). This suggests that metformin may have long-term effects on CRP beyond weight reduction. The fact that CRP levels remained lower than baseline values for an average of 3.4 years is consistent with a durable anti-inflammatory effect of both ILS and metformin treatment in the DPP.

The Finnish Diabetes Prevention Study (FDPS) reported that lifestyle intervention lowered levels of plasminogen

Table 3—Adjusted effect of diabetes status on CRP, fibrinogen, and tPA by treatment group

	Placebo		Metformin		Lifestyle	
	Diabetes β	Diabetes R^2	Diabetes β	Diabetes R^2	Diabetes β	Diabetes R^2
Outcome: CRP						
Model A1: baseline CRP	**−0.09 (−0.16, −0.02)**	**0.28%**	**−0.10 (−0.19, −0.01)**	**0.29%**	**−0.13 (−0.25, −0.01)**	**0.27%**
Model A2: A1 + 0′ glucose	−0.04 (−0.11, 0.04)	0.04%	−0.05 (−0.14, 0.04)	0.07%	−0.031 (−0.16, 0.09)	0.01%
Model A3: A1 + 0′ insulin	**−0.09 (−0.17, −0.01)**	**0.26%**	**−0.12 (−0.22, −0.02)**	**0.34%**	**−0.13 (−0.26, −0.002)**	**0.27%**
Model A4: A1 + weight	−0.06 (−0.13, 0.01)	0.13%	−0.08 (−0.16, 0.01)	0.18%	−0.01 (−0.13, 0.10)	0.00%
Model A5: fully adjusted	−0.04 (−0.13, 0.04)	0.06%	−0.07 (−0.16, 0.03)	0.11%	−0.02 (−0.16, 0.11)	0.01%
Outcome: tPA						
Model B1: baseline tPA	**−0.60 (−0.99, −0.21)**	**0.50%**	**−0.68 (−1.1, −0.24)**	**0.53%**	**−1.1 (−1.6, −0.56)**	**0.98%**
Model B2: B1 + 0′ glucose	−0.23 (−0.64, 0.18)	0.07%	−0.22 (−0.67, 0.23)	0.05%	−0.31 (−0.86, 0.24)	0.07%
Model B3: B1 + 0′ insulin	**−0.48 (−0.88, −0.08)**	**0.33%**	**−0.52 (−0.95, −0.08)**	**0.32%**	**−0.80 (−1.3, −0.26)**	**0.53%**
Model B4: B1 + weight	**−0.44 (−0.82, −0.05)**	**0.29%**	**−0.51 (−0.92, −0.09)**	**0.34%**	**−0.52 (−1.0, −0.01)**	**0.24%**
Model B5: B1 + fully adjusted	−0.14 (−0.54, 0.27)	0.03%	−0.16 (−0.59, 0.27)	0.03%	−0.11 (−0.64, 0.42)	0.01%
Outcome: Fibrinogen						
Model C1: baseline fibrinogen	**−10 (−19, −2.1)**	**0.35%**	−9.0 (−18, 0.05)	0.24%	−7.4 (−19, 4.3)	0.10%
Model C2: C1 + 0′ glucose	**−8.8 (−17, −0.03)**	**0.22%**	−5.4 (−15, 3.9)	0.08%	−4.7 (−17, 7.7)	0.03%
Model C3: C1 + 0′ insulin	**−10 (−19, −1.9)**	**0.35%**	−8.7 (−18, 0.49)	0.22%	−5.8 (−18, 6.1)	0.06%
Model C4: C1 + weight	**−9.5 (−18, −1.1)**	**0.29%**	−8.2 (−17, 0.88)	0.20%	−5.6 (−18, 6.5)	0.05%
Model C5: C1 + fully adjusted	**−8.5 (−17, 0.41)**	**0.21%**	−5.1 (−15, 4.3)	0.07%	−5.7 (−18, 6.9)	0.05%

The adjusted effect of diabetes on CRP, fibrinogen, and tPA over time are assessed separately for each treatment group in a series of five mixed models that include demographics (age, sex, and race/ethnicity), year of assessment, and the baseline value of the outcome (series 1); baseline and change in fasting glucose (series 2); baseline and change in fasting insulin (series 3); baseline and change in weight (series 4); and baseline levels and changes in fasting glucose, fasting insulin, and weight (series 5). Estimates of the diabetes effect in bold indicate significant β for diabetes at $\alpha = 0.05$.

activator inhibitor 1 (PAI-1), a key endogenous fibrinolysis inhibitor, by 31% after 1 year, persisting for 3 years in a small subgroup, while fibrinogen values were not affected (5). Similarly, we found that tPA, which was used as a surrogate measure of PAI-1 (11), fell by 21% in the ILS group and 18% in the metformin group at 1 year. tPA then tended to rise toward baseline values, suggesting an attenuation of the effects of these interventions. The similarities in response of tPA to the two interventions argues against weight reduction being the sole determinant of these changes since the amount of weight reduction in ILS was greater than with metformin. tPA remained significantly lower than the baseline values at EOS, suggesting that there was a persisting favorable change in coagulation balance in both active intervention groups. No net effect of either intervention on fibrinogen was noted, as has previously been reported (5,6). How long these effects persist was not tested in this study. We chose to focus on the masked phase of the DPP to avoid the confounding effects of the alteration of the interventions that occurred with the launching of the long-term DPP outcome study.

A new finding in this study not apparent in the overall response of the DPP cohort was the lack of durable effects of ILS or metformin intervention on biomarker levels in participants who developed diabetes versus those who did not. One obvious explanation for this is that those who developed diabetes were less successful with the interventions than those who did not. This is likely the case for ILS, where the amount of weight reduction was shown to be inversely proportional to the incidence of diabetes (12). Because those who developed diabetes in the DPP did not on average lose weight, and since weight is a powerful determinant of CRP levels, this could explain why CRP did not fall with the ILS intervention over time in this subgroup. In support of this, when baseline and change in weight were entered into a model assessing the influence of diabetes development on time-related CRP changes in the ILS group, the effect of diabetes was completely accounted for. Furthermore we did not find evidence that developing diabetes in the placebo group was accompanied by an increase in CRP from baseline or that CRP was higher in those with diabetes versus without diabetes. This suggests that the major factor underlying subclinical inflammation in newly developed diabetes is overweight.

The diminished tPA response to ILS in the subgroup that developed diabetes was partly accounted for by weight and fasting insulin, in keeping with our previous observation that baseline tPA levels in DPP independently associate with each of these two variables (7). In the FDPS, the reduction in PAI-1 was largely accounted for by weight reduction. There remained a significant independent effect of diabetes development on the lack of reduction in tPA levels in the ILS groups, mostly accounted for by entering fasting glucose into the model. Hyperglycemia has been shown to increase PAI-1 expression through activation of the hexosamine pathway (13).

The lack of a reduction in CRP and tPA levels in those who developed diabetes in the metformin arm is less easy to explain on the basis of reduced adherence to treatment since the major mechanism by which metformin slows diabetes development is thought to involve inhibition of hepatic glucose production, which is not directly associated with CRP or tPA/PAI-1 levels. However, the effect of metformin to reduce

diabetes development in the DPP is also related to weight reduction and possibly to improvement in insulin sensitivity (14). Participants who developed diabetes in the metformin group were, however, of similar weight to those without diabetes, although fasting insulin levels were higher in those with diabetes. In the multivariate model, in contrast to the ILS group, weight only partly accounted for the lack of a time-related decrease in CRP in the metformin subgroup with diabetes, with insulin having no effect. Thus there remained a significant residual independent effect of diabetes development on the lack of a CRP response to metformin that was accounted for by fasting glucose.

The effect of diabetes development on change in tPA in the metformin group showed some similarities but also some differences from that in the ILS group. Weight and insulin each partly accounted for the attenuation in reduction of tPA levels related to incident diabetes and glucose explained most of the residual effect in the two groups. However, unlike in the placebo and lifestyle groups where tPA levels increased above baseline at the EOS, tPA levels were no different from baseline in the metformin group. Metformin appeared to prevent this progressive rise in tPA despite the development of diabetes. This may be related to its known effect to inhibit PAI-synthesis through a tumor necrosis factor-α–dependent pathway (15,16). We also considered whether the temporary 1-week discontinuation of metformin during the final 6-month sampling period to evaluate the effect of a metformin washout on diabetes development could have influenced results (9). It is possible that tPA levels may have increased during the metformin washout, but since this would have been transient, affected both those with and without diabetes, and constituted no more than a 5% fraction of the entire metformin cohort, the potential confounding was considered minimal. Similarly even though the 20 excess cases of diabetes directly attributable to the washout may have behaved aberrantly with respect to their effect on biomarkers, they represented less than 10% of cases with incident diabetes in the metformin group. Other limitations of the study as a whole include the fact that there were fewer participants at the end of the study than at the beginning and that the timing of sampling for the EOS biomarker measurements and that of the rest of the data collection could have been up to 7 months apart, although 89% of samples were collected halfway through that period.

Importantly our findings demonstrate that over the 3.4-year period of follow-up, tPA, but not CRP or fibrinogen, levels progressively increase from baseline in both the placebo and ILS but not metformin groups in those who develop diabetes and that prevention of diabetes by ILS and metformin is accompanied by lowered CRP and tPA levels. The progressive increase in tPA values in the placebo and ILS subgroups with diabetes resembles the pattern described previously in this setting for more traditional cardiovascular risk factors (17), consistent with the appearance of dysfunctional fibrinolysis and enhanced cardiovascular risk early on in those progressing to diabetes. In this respect, tPA may be more closely linked to diabetes development than CRP, which is more strongly related to weight change. The effect of metformin treatment to prevent the increase of tPA noted in the other two subgroups with diabetes provides support for the use of metformin at the earliest stages of diabetes development, perhaps even if it is not able to prevent the emergence of diabetes.

In summary, we show that in subjects with IGT receiving metformin or lifestyle interventions who do not develop diabetes, there are durable reductions in biomarkers of inflammation and coagulant imbalance. In contrast, those individuals who develop diabetes do not experience these apparently beneficial effects and may rapidly show unfavorable changes. Our analysis suggests that failure to lose weight among those who developed diabetes explains the lack of reduction of CRP. Lack of weight loss or failure of insulin sensitivity to improve were important but not sufficient explanations to account for the lack of reduction of tPA in those who progress to diabetes. This could mean that rising glucose levels per se or other associated metabolic factors could interfere with the beneficial effects of lifestyle or metformin interventions on tPA. It is also possible that subjects who develop diabetes may inherently not respond as well to these interventions. Whatever the explanation, the higher CRP or tPA levels in those who develop diabetes despite ILS or metformin intervention is consistent with the notion that ongoing inflammatory and procoagulant activities accompany diabetes development and that prevention of diabetes through lifestyle or metformin intervention ameliorates these processes.

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