Effect of Metformin Treatment on Multiple Cardiovascular Disease Risk Factors in Patients With Type 2 Diabetes Mellitus

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In light of the conflicting results of the recent United Kingdom Prospective Study (UKPDS), where diabetic patients on metformin monotherapy had lower all-cause mortality and the addition of metformin in sulfonylurea-treated patients was associated with an increased risk of diabetes-related death, we sought to compare the effects on cardiovascular disease (CVD) risk factors of metformin monotherapy with metformin treatment when added to a sulfonylurea compound in patients with type 2 diabetes. Thirty-one volunteers with type 2 diabetes mellitus, 16 on dietary therapy and 15 on sulfonylurea monotherapy (SU), were treated with metformin for 12 weeks. Measurements were made of (1) fasting plasma glucose, hemoglobin A_{1c} (HbA_{1c}), lipid, remnant lipoprotein cholesterol (RLP-C) levels, and low-density lipoprotein (LDL) particle size; (2) daylong plasma glucose, insulin, free fatty acid (FFA), triglyceride (TG), and RLP-C concentrations; and (3) fasting levels of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble E-selectin (sE-selectin). Fasting plasma glucose concentrations decreased to a similar degree after treatment with metformin in both the metformin monotherapy group (12.45 ± 0.48 *v* 9.46 ± 0.47 mmol/L, *P* < .001) and the combined SU and metformin therapy group (14.09 ± 0.51 *v* 10.57 ± 0.85 mmol/L, *P* = .001). Fasting plasma lipid concentrations and LDL particle size did not significantly change in either treatment group, whereas fasting RLP-C concentrations were significantly lower in the metformin monotherapy group (0.43 ± 0.09 *v* 0.34 ± 0.07 mmol/L, *P* = .02). Daylong concentrations of plasma glucose, FFA, TG, and RLP-C were lower to a similar degree in both treatment groups, whereas daylong plasma insulin concentrations were unchanged. Fasting plasma sVCAM-1 levels were significantly lower in both the metformin monotherapy group (484 ± 19 *v* 446 ± 18 ng/mL, *P* = .02) and the combined SU and metformin therapy group (496 ± 29 *v* 456 ± 31 ng/mL, *P* = .05), whereas fasting plasma sICAM-1 and sE-selectin levels were essentially unchanged. Administration of metformin, either as monotherapy or in combination with a sulfonylurea drug, improved glycemic control and led to a decrease in several CVD risk factors in patients with type 2 diabetes.

RESULTS OF a substudy analysis of the United Kingdom Prospective Study (UKPDS) demonstrated that improved glycemic control in overweight patients treated with metformin alone was associated with a decreased risk of combined diabetes-related end points, all-cause deaths, and myocardial infarction compared with the conventionally treated patients.[1] However, the same analysis indicated that addition of metformin in sulfonylurea (SU)-treated patients was associated with an increased risk of diabetes-related death and all-cause death compared with those continued on SU alone. The UKPDS investigators addressed this apparent paradox by pointing out that patients receiving combined SU and metformin were older, with worse glycemic control, less overweight, and were monitored for a shorter period. In addition, they indicated that the reported differences in outcome were based on a relatively small number of end points, and that an epidemiological analysis of the entire UKPDS population did not find an association of diabetes-related deaths with combined SU and metformin therapy.

Since the publication of these findings, 2 epidemiological studies have shown increased mortality in patients with type 2 diabetes treated with combined SU and metformin as compared with metformin monotherapy.[2,3] However, the diabetes in the patients receiving combined treatment with SU and metformin in these studies was of longer duration and the glycemia less well controlled. Thus, it seemed possible that these differences might have contributed to the increased mortality in those patients receiving combined SU and metformin, as differentiated from an adverse effect of adding metformin to patients being treated with SU compounds.

Adding metformin to the treatment program of patients with type 2 diabetes who remain hyperglycemic despite treatment with a SU compound has been clearly shown to be effective,[4-7] and the results of the UKPDS have demonstrated how important it is to obtain glycemic control in patients with type 2 diabetes.[1,8] The present study is an attempt to respond to questions that have been raised concerning the safety of administering metformin to patients who are in poor glycemic control on SU treatment. For this purpose, we compared the metabolic effects of initiating treatment with metformin in 2 groups of volunteers with type 2 diabetes in relatively poor glycemic control, matched for other relevant clinical variables, but differing in that one group was not receiving any antihyperglycemic agent, while the other was treated with a SU drug. In particular, we assessed the impact of metformin treatment on multiple cardiovascular disease (CVD) risk factors in these 2 patient groups.

MATERIALS AND METHODS

The study participants were recruited from the San Francisco Bay area by advertisements in local newspapers indicating our interest in studying risk factors for CVD in hyperglycemic patients with type 2

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Submitted March 1, 2003; accepted July 9, 2003.

Supported by Research Grants from the National Institutes of Health (RR-00070 and DK 30732).

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0026-0495/04/5302-0041$30.00/0

doi:10.1016/j.metabol.2003.07.020

Table 1. Baseline Characteristics of the 31 Subjects With Type 2 Diabetes Mellitus

Characteristic	Dietary Therapy Alone (n = 16)	Sulfonylurea Monotherapy* (n = 15)	*P*
Age (yr)	58 ± 2	56 ± 3	.69
Sex, male/female (n)	10/6	12/3	.28
Body mass index (kg/m^2)	29.7 ± 2.3	29.5 ± 1.1	.91
Systolic blood pressure (mm Hg)	147 ± 4	137 ± 4	.08
Diastolic blood pressure (mm Hg)	81 ± 2	81 ± 2	.89
Known duration of diabetes (yr)	5.3 ± 1.5	4.5 ± 0.6	.65
Subjects on antihypertensive therapy (n)	8	10	.35
Subjects on lipid-lowering therapy (n)	5	6	.61
Fasting plasma glucose (mmol/L)	12.45 ± 0.48	14.09 ± 0.51	.03
HbA_{1c} (%)	11.0 ± 0.6	13.1 ± 0.8	.05

NOTE. Data are mean ± SE, n, or percent.

*Twelve of the 15 SU-treated patients were receiving glipizide and 3 were receiving glyburide.

diabetes mellitus. The Stanford Human Subjects Committee approved the experimental protocol, and each volunteer gave written informed consent. The study was performed at the General Clinical Research Center of Stanford University Medical Center. Thirty-one volunteers with type 2 diabetes, 22 men and 9 women, were studied. The main inclusion criteria were fasting plasma glucose concentration greater than 9.5 mmol/L on dietary therapy alone or SU monotherapy (glipizide or glyburide 10 to 20 mg/day), body mass index (BMI) less than 40.0 kg/m^2, and no apparent CVD. All subjects were required to have normal complete blood count, liver function, and serum creatinine level. Baseline characteristics of the 31 volunteers are shown in Table 1, and it can be seen that the 2 groups were similar in terms of age, sex distribution, BMI, and resting blood pressure. The 2 groups were also comparable in terms of the known duration of diabetes and treatment with antihypertensive and lipid-lowering drugs. Although both groups were in poor glycemic control, the SU-treated volunteers had somewhat higher fasting plasma glucose concentrations.

After enrollment, study participants met with a research dietitian and were instructed on a weight maintenance diet with similar macronutrient composition. Volunteers were admitted to the General Clinical Research Center for baseline metabolic studies after at least 4 weeks of glycemic stability on either diet alone or their usual SU dose. After an over night fast, blood samples were drawn for measurement of glucose, hemoglobin A_{1c} (HbA_{1c}), and lipid concentrations as described previously.[4,7] In addition, low-density lipoprotein (LDL) particle size was determined by gradient gel electrophoresis.[9] Remnant lipoprotein (RLP) particles were isolated by an immunoseparation method, and RLP cholesterol (RLP-C) concentration was quantified by determining the cholesterol content of these particles with a highly sensitive assay.[10-12] Fasting blood samples were also drawn for measurement of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble E-selectin (sE-selectin).[13]

In addition, from 8 AM to 4 PM, hourly measurements were made of plasma glucose, insulin, and free fatty acid (FFA) concentrations,[4,7] and 2 hourly measurements were made of plasma triglyceride (TG) and RLP-C concentrations. On the day these measurements were made, patients consumed isocaloric test meals containing 15% protein, 45% fat, and 40% carbohydrate. Subjects were given breakfast at 8 AM (20% of daily calories) and lunch at noon (40% of daily calories).

Following completion of the baseline studies, patients were given metformin 500 mg twice per day, and seen at weekly intervals for the next 4 weeks, and every 2 weeks thereafter. At these visits patients were questioned concerning any side effects, and the dose of metformin was gradually increased to a total of 2 g (1g twice per day). All patients tolerated this dose of metformin, except for 2 patients who were maintained on 1.5 g/d as a result of gastrointestinal symptoms. After 12 weeks of metformin treatment patients were readmitted to repeat all baseline measurements.

Summary statistics are expressed as mean ± SE. Baseline characteristics of the 2 groups were compared using unpaired *t* test and chi-square test. The effect of metformin treatment within each group was compared using paired *t* test, 2-way analysis of variance (ANOVA), and Wilcoxon signed-rank test. Effect of metformin treatment between groups was compared using unpaired *t* test and Mann-Whitney test.

RESULTS

Neither blood pressure nor weight changed significantly after metformin treatment in either group. The effect of metformin on glycemic control in the 2 groups is shown in Table 2. It is apparent that both fasting plasma glucose and HbA_{1c} concentrations decreased significantly ($P < .01$) in both groups. If anything, the decrements in both variables were somewhat greater in those in whom the metformin was added to the SU compound, and in this group the improvement in fasting plasma glucose and HbA_{1c} concentrations were significantly correlated ($r = 0.70$, $P = .005$).

Fasting lipid concentrations in the two experimental groups, before and after the addition of metformin, are also presented in Table 2. Baseline values were quite similar in the 2 groups, and did not change significantly following the administration of metformin. The only exception to this generalization was that baseline RLP-C concentrations were somewhat higher in the SU-naive patients ($P = .07$), and decreased ($P = .02$) in association with the administration of metformin. It should be noted that baseline RLP-C concentrations were lower in SU-treated patients prior to the addition of metformin, and did not change when it was added. It was also of interest that the significant decrements in HbA_{1c} concentration seen in both groups were unrelated to changes in the lipid variables measured.

The changes in daylong plasma glucose, insulin, and FFA concentrations associated with metformin administration are illustrated in Fig 1. These results demonstrate that daylong plasma glucose and FFA concentrations fell significantly in response to the administration of metformin in both groups ($P < .05$), whereas insulin concentrations did not change with metformin treatment. Similar to the situation regarding treatment-related decreases in fasting plasma glucose concentration, the fall in the daylong postprandial glucose concentration in

Table 2. Effect of Metformin Treatment on Fasting Metabolic Variables

Variable	Metformin Monotherapy (MET) (n = 16)			Combined Sulfonylurea and Metformin Therapy (SU+MET) (n = 15)			MET *v* SU+MET†
	Before	After*	*P*	Before	After*	*P*	*P*
Fasting glucose (mmol/L)	12.45 ± 0.48	9.46 ± 0.47	<.001	14.09 ± 0.51	10.57 ± 0.85	.001	.58
HbA_{1c} (%)	11.0 ± 0.6	9.2 ± 0.5	<.001	13.1 ± 0.8	10.7 ± 0.7	.002	.30
Total cholesterol (mmol/L)	4.90 ± 0.21	4.78 ± 0.22	.39	4.85 ± 0.24	4.63 ± 0.15	.18	.64
Triglycerides (mmol/L)	2.26 ± 0.33	2.19 ± 0.29	.65	1.93 ± 0.26	1.61 ± 0.15	.13	.34
HDL cholesterol (mmol/L)	0.97 ± 0.04	0.97 ± 0.04	.97	1.01 ± 0.05	0.97 ± 0.05	.20	.48
LDL cholesterol (mmol/L)	3.03 ± 0.19	2.87 ± 0.18	.32	2.87 ± 0.32	2.92 ± 0.14	.71	.85
RLP cholesterol (mmol/L)	0.43 ± 0.09	0.34 ± 0.07	.02	0.24 ± 0.02	0.23 ± 0.02	.66	.06
LDL particle diameter (Å)	253 ± 2	256 ± 2	.10	254 ± 3	254 ± 3	1.00	.28
Small dense LDL particle (%)	56	50	.56	60	60	1.00	.73

NOTE. Data are mean ± SE, or percent of patients. Subjects with LDL particle diameter <256 Å were classified as having small dense LDL particles.
*Baseline variables compared with those measured after 12 weeks of metformin treatment.
†Changes in variables in the MET group compared with those in the SU+MET group.

patients treated with combined SU and metformin was correlated with the decrease in HbA_{1c} concentration ($r = 0.80$, $P = .001$).

Daylong plasma TG and RLP-C concentrations before and after metformin treatment are illustrated in Fig 2. These results indicate that daylong plasma concentrations of both TG and RLP-C were lower after the addition of metformin, and the decrease in the postprandial accumulation of TG-rich lipoproteins was similar in both groups.

Table 3 shows the effect of metformin treatment on plasma concentrations of sICAM-1, sVCAM-1, and sE-selectin. The levels of each of these adhesion molecules were similar at

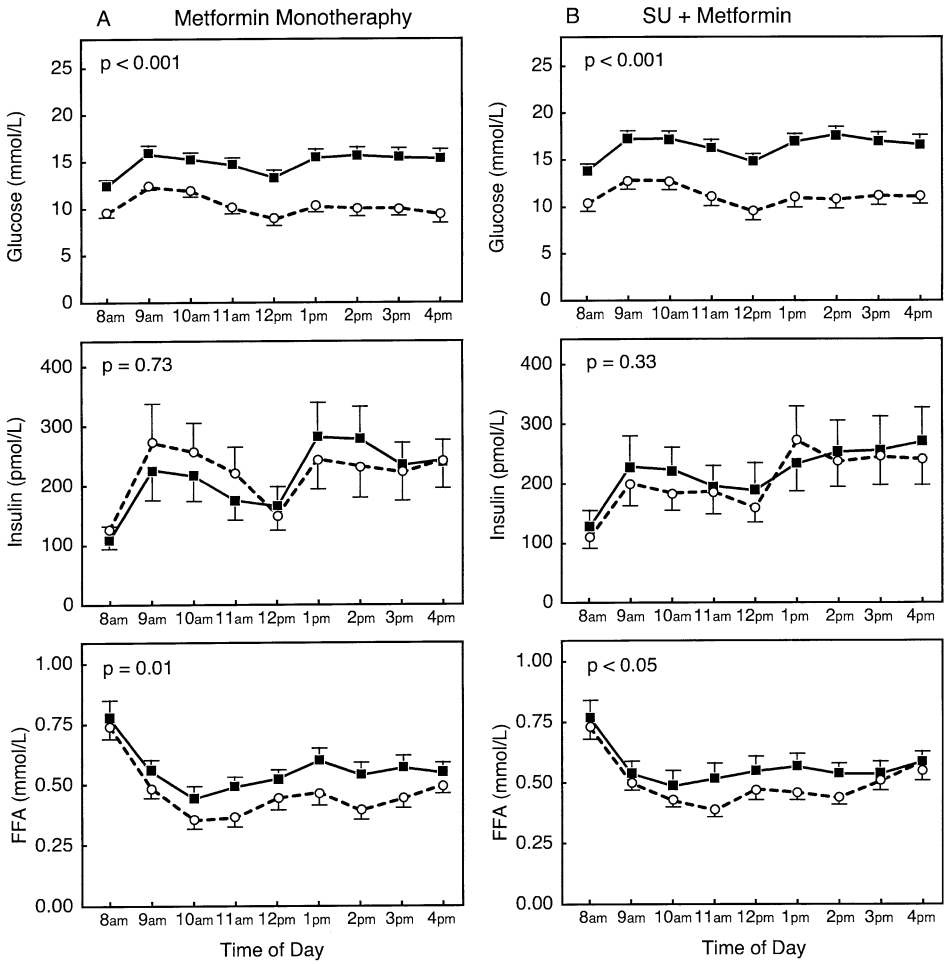

Fig 1. Plasma glucose, insulin, and free fatty acid (FFA) concentrations, before (■) and after (○) metformin treatment, measured at hourly intervals from 8 AM to 4 PM. Breakfast was served at 8 AM and lunch at 12 PM. Data are presented as mean ± SE.

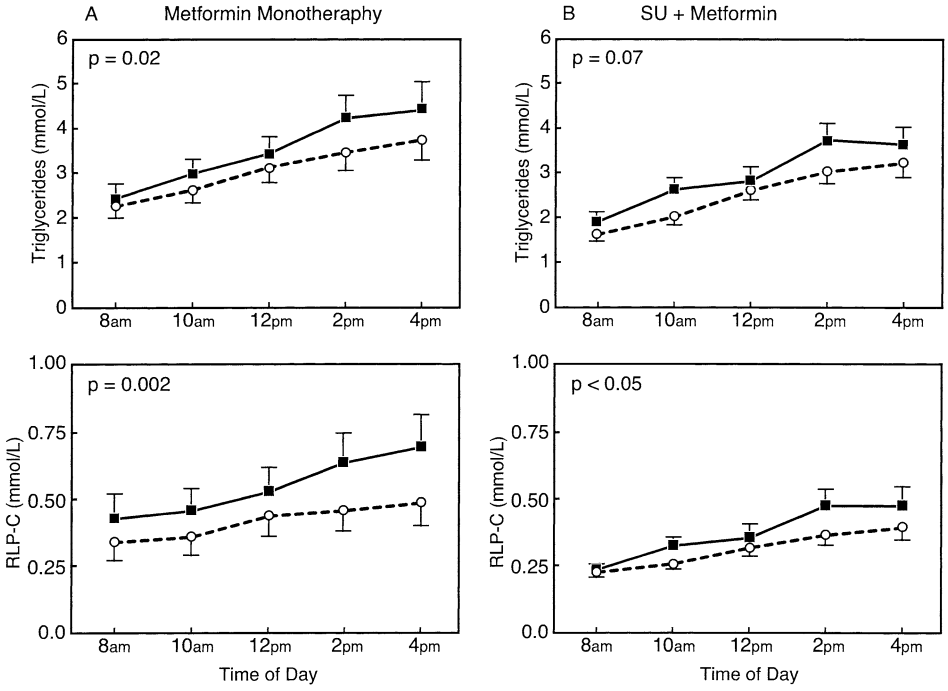

Fig 2. Plasma triglyceride and remnant lipoprotein cholesterol (RLP-C) concentrations, before (■) and after (○) metformin treatment, measured at 2 hourly intervals from 8 AM to 4 PM. Breakfast was served at 8 AM and lunch at 12 PM. Data are presented as mean ± SE.

baseline in the 2 groups. Although sICAM-1 and sE-selectin levels did not change with treatment, sVCAM-1 levels were significantly lower after metformin treatment in both the monotherapy group (484 ± 19 *v* 446 ± 18 ng/mL, $P = .02$) and the combined SU and metformin group (496 ± 29 *v* 456 ± 31 ng/mL, $P = .05$).

DISCUSSION

Perhaps the most appropriate way to begin discussing our results is to make a clear statement that our findings do not offer an answer to the question whether or not the long-term benefits of metformin treatment vary as a function of its use as monotherapy versus its impact when added to a SU compound in patients with type 2 diabetes in less than optimal glycemic control. There is no doubt that its addition will effectively lower plasma glucose concentrations in SU-treated patients that remain hyperglycemic, but whether or not this benefit is rendered moot because all cause mortality is increased in patients receiving combined SU-metformin treatment can only be definitively answered by a well-planned prospective, intervention trial.

On the other hand, our findings do have some clinical relevance in the context of the fact that patients with type 2 diabetes are 2 to 3 times more likely to develop CVD than are nondiabetic subjects.[14] The conventional CVD risk factors most commonly seen in patients with type 2 diabetes include hyperglycemia, elevated TG and low high-density lipoprotein (HDL) cholesterol concentrations, and hypertension.[15] It is apparent from our results that the effect of metformin treatment on these variables was similar in the 2 groups of patients. Specifically, the improvement in glycemic control in patients with type 2 diabetes was comparable when metformin was administered as monotherapy, or when added to the treatment program of patients poorly controlled on SU compounds alone. Furthermore, neither blood pressure nor fasting plasma lipid concentrations changed significantly following the administration of metformin to either experimental group. Thus, there is no reason on the basis of these data to predict that the administration of metformin to SU-treated patients would lead to an adverse outcome.

More recently, it has been emphasized that the conventional risk factors evaluated above cannot entirely account for the increased CVD risk in patients with type 2 diabetes.[16] With this in mind, we also compared the effect of adding metformin to the 2 experimental groups on three additional CVD risk factors: (1) LDL particle size; (2) the postprandial accumulation of

Table 3. Effect of Metformin Treatment on the Concentrations of Soluble Cellular Adhesion Molecules

	Metformin Monotherapy (n = 16)			Combined Sulfonylurea and Metformin Therapy (n = 15)		
Variable	Before	After	*P*	Before	After	*P*
sE-selectin (ng/mL)	62 ± 9	59 ± 9	.25	58 ± 6	55 ± 7	.67
sICAM-1 (ng/mL)	237 ± 14	218 ± 13	.15	205 ± 12	194 ± 14	.60
sVCAM-1 (ng/mL)	484 ± 19	446 ± 18	.02	496 ± 29	456 ± 31	.05

NOTE. Data are mean ± SE.

TG-rich remnant lipoproteins; and (3) plasma concentrations of soluble cellular adhesion molecules. In 2 of these instances, the results have provided additional information concerning the potential utility of metformin in decreasing CVD risk in patients with type 2 diabetes. Perhaps the most clinically relevant of these new findings was the observation that postprandial RLP-C concentrations were lower following metformin treatment. There is considerable evidence as to the atherogenicity of the postprandial accumulation of TG-rich lipoproteins,[17] and we had previously demonstrated that postprandial lipemia was decreased in metformin-treated patients.[18] The results in Fig 2 extend these earlier findings and show that metformin treatment led to a decrease in the daylong accumulation of RLP-C as determined by a specific method for isolating and quantifying chylomicron and very-low-density lipoprotein remnant particles.[10-12] The results in Fig 2 also show that the decrease in postprandial TG and RLP-C concentrations associated with metformin treatment was seen in both experimental groups.

The second change with metformin treatment that might be anticipated to reduce CVD risk was the decrease in the plasma concentration of sVCAM-1. Several publications have described a relationship between an increase in the plasma concentration of soluble cellular adhesion molecules and CVD risk.[19-21] Reports have also been published demonstrating that the plasma concentrations of one or more cellular adhesion molecules are increased in patients with type 2 diabetes.[22,23] Moreover, elevated levels of sE-selectin have been shown to fall in response to aggressive treatment of hyperglycemia with insulin.[24] To the best of our knowledge, evidence that sVCAM-1 concentrations decrease in response to improved glycemic control with metformin treatment has not been previously reported. Again, it should be noted that the fall in sVCAM-1 concentrations was at least as pronounced in the group of patients in whom metformin was added to their prior SU-treatment program as in those treated with metformin alone.

Although the discussion to this point has focused on comparing the effects of metformin when given as either monotherapy or combined with a SU compound, it seems worthwhile to address as well the more general issue of the relationship between metformin treatment and CVD risk in patients with type 2 diabetes. In this context, metformin appears to offer many therapeutic advantages. In addition to its well-recognized effectiveness as an antihyperglycemic agent, metformin has the additional benefit of being the drug associated with the least weight gain when glycemic control is improved in patients with type 2 diabetes. If the recent finding that plasma insulin concentrations predict CVD in type 2 diabetes[25] is confirmed, metformin has the advantage of improving glycemic control without increasing plasma insulin concentrations.[7] Given recent evidence that chronic elevations in FFA concentrations may predict increased risk of CVD[26] as well as downregulate glucose-stimulated insulin secretion,[27] the fact that daylong plasma FFA concentrations decrease following metformin administration[7] is of considerable interest. There is also evidence that endothelial-dependent vasodilation improves in metformin-treated patients,[28] a finding consistent with the present demonstration of a decrease in sVCAM-1 concentrations and our previous finding that asymmetric dimethylarginine concentrations fall in response to metformin treatment.[29] Finally, although we could not demonstrate any change in fasting lipid concentrations, the decrease in postprandial TG and RLP-C concentrations in the current study shows that metformin treatment can also decrease CVD risk factors associated with abnormal lipoprotein metabolism.

In conclusion, we have attempted to evaluate the hypothesis that the adverse outcomes that have been reported to occur in patients with type 2 diabetes treated with combined SU and metformin, as compared to metformin alone,[1-3] were due to worsening of multiple CVD risk factors. The results presented do not support this hypothesis. Not only were the metabolic effects of metformin comparable, when used as sole antihyperglycemic agent or in combination with a SU compound, the clinical benefits were considerable in both instances. On the other hand, it should be pointed out that 12 of the 15 SU-treated patients were receiving glipizide, and similar results might not have been achieved with other compounds in this class. Finally, as emphasized at the outset of the discussion, the fact that we could not discern any evidence of an adverse effect on several CVD risk of the use of combined SU and metformin treatment, should not be extrapolated to imply that these results negate the epidemiological evidence suggesting that all cause mortality may be increased in patients treated in this manner.

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