Effect of Metformin on Postprandial Lipemia in Patients With Fairly to Poorly Controlled NIDDM

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OBJECTIVE — To quantify the effect of metformin on the metabolism of triglyceride (TG)-rich lipoprotein of intestinal origin in patients with non-insulin-dependent diabetes mellitus (NIDDM) who had responded to sulfonylurea but still had fasting hyperglycemia.

RESEARCH DESIGN AND METHODS — Sixteen patients with NIDDM who had demonstrated a fall in fasting plasma glucose concentration >2.2 mmol/l in response to glipizide treatment but continued to have fasting plasma glucose concentrations >8.3 mmol/l were studied. Fasting glucose, GHb, lipid and lipoprotein concentrations were determined, and resistance to insulin-mediated glucose disposal was estimated by measuring the steady-state plasma glucose (SSPG) concentration at the end of a 180-min infusion of somatostatin, glucose, and insulin. In addition, plasma glucose, insulin, and TG concentrations were measured at frequent intervals from 0800 to 2400, with patients eating breakfast at 0800 and lunch at 1200. Vitamin A was also given at lunch, and the retinyl ester content in plasma and in chylomicron (Svedberg flotation constant [S_f] > 400) and the chylomicron remnant (S_f 20–400) fractions were used to quantify the concentration of postprandial intestinal TG-rich lipoprotein from 1200 to 2400.

RESULTS — Fasting plasma glucose concentrations (6.8 ± 0.4 vs. 10.5 ± 0.4 mmol/l), GHb levels (7.9 ± 0.3 vs. 10.8 ± 0.5%), and day-long plasma glucose concentrations were all significantly lower after metformin treatment ($P < 0.001$), which was associated with a significant ($P < 0.001$) fall in SSPG concentration (11.0 ± 0.9 to 9.6 ± 0.6 mmol/l). In addition, postprandial concentrations of glucose, insulin, free fatty acids, and TG were lower ($P < 0.001$) following metformin treatment. Postprandial retinyl ester concentrations were also lower in plasma by 33 ± 5.7% ($P < 0.001$) and in both the chylomicron (32 ± 7.2%, $P < 0.001$) and chylomicron remnant (26 ± 7.0%, $P < 0.005$) fractions.

CONCLUSIONS — Addition of metformin to sulfonylurea-treated patients with NIDDM with less than optimal glycemic control was associated with improved glycemic control, lower postprandial insulin and TG concentrations, and a decrease in postprandial concentration of TG-rich lipoproteins of intestinal origin. All of these changes might be expected to decrease risk of coronary heart disease.

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CHD, coronary heart disease; NIDDM, non-insulin-dependent diabetes mellitus; TG, triglyceride; CRC, Clinical Research Center; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; S_f, Svedberg flotation constant; HPLC, high-performance liquid chromatography; SSPG, steady-state plasma glucose; SSPI, steady-state plasma insulin; FFA, free fatty acid.

The importance of coronary heart disease (CHD) in the clinical course of individuals with non-insulin-dependent diabetes mellitus (NIDDM) is clear (1). However, the commonly measured risk factors can only account for a minor fraction of the excess CHD in these patients (1). We have recently shown (2,3) that postprandial concentrations of triglyceride (TG)-rich lipoproteins of intestinal origin are higher in patients with NIDDM, either untreated or treated with sulfonylurea compounds, and that this difference was independent of fasting plasma TG concentration. Given evidence that postprandial concentrations of TG-rich lipoproteins of intestinal origin may predispose nondiabetic individuals to develop CHD (4–8), it seemed reasonable to speculate that this would also be true of patients with NIDDM. In this context, we have previously emphasized that, although fasting plasma TG concentration decreased when the patients with NIDDM were treated with metformin, the fall in the postprandial TG response was of even greater magnitude (9–11). However, in these earlier studies, we did not specifically determine the effect of metformin on postprandial increases in the concentration of TG-rich lipoproteins of intestinal origin. Furthermore, although a great deal is known concerning the metabolic effects of metformin (12), we were not aware of any published data concerning the effect of metformin treatment on metabolism of lipoproteins of intestinal origin. Given the possibility that these particles may play a central role in the accelerated atherogenesis of patients with NIDDM, we initiated this study, in which we quantified the effect of metformin on the metabolism of TG-rich lipoproteins of intestinal origin in patients with NIDDM

in fair to poor glycemic control on sulfonylurea compounds.

RESEARCH DESIGN AND METHODS

The study was approved by the Stanford Human Subjects Committee, and each research subject gave written informed consent before entering the Clinical Research Center (CRC). Sixteen glipizide-treated patients (10 males and 6 females) with NIDDM were studied. Patients were 57 ± 3 years of age (mean ± SD), had a body mass index of 28.0 ± 4.0 kg/m^2, a mean duration of diabetes of 7 ± 6 years, and, aside from NIDDM, were in good general health. All patients had demonstrated an initial fall in fasting plasma glucose concentration >2.2 mmol/l in response to glipizide (40 mg/day) treatment but continued to have fasting plasma glucose concentrations >8.3 mmol/l (range: 8.3–12.2 mmol/l) on maximal sulfonylurea treatment. Before entering this study, all subjects had been seen by a physician at weekly intervals for at least 12 weeks, and they had been on a combined diet and sulfonylurea treatment program during this period. At the end of this period they were admitted to the CRC, and the following studies and laboratory analyses were performed after 12 h of overnight fasting.

Fasting lipid and lipoprotein concentrations

On three occasions, blood was obtained in EDTA tubes for measurement of fasting plasma TG, cholesterol, and lipoprotein TG and cholesterol concentrations. These samples were subjected to sequential density ultracentrifugation (2) to separate very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) fractions at densities below 1.006, at 1.019, and at 1.063 mg/dl, respectively, and the TG (13) and cholesterol (14) concentrations of the various fractions were determined.

Postprandial glucose, insulin, TG, and TG-rich intestinal lipoproteins

On the day these measurements were made, subjects consumed identical isocaloric test meals containing (as percent of total calories) 15% protein, 45% fat, and 40% carbohydrate. Subjects were given breakfast at 0800 (20% of daily calories) and lunch at 1200 (40% of daily calories). After breakfast and lunch, the subjects consumed only water or noncaloric decaffeinated beverages until the study ended at 2400. Blood was drawn at 1-h intervals from 0800 until 1800, and then at 2-h intervals until 2400 for determination of plasma glucose (15), insulin (16), free fatty acid (FFA) (17), and TG concentrations. Note that the assay used for insulin does not distinguish between proinsulin and insulin.

At 1200, 60,000 U/m^2 body surface area of vitamin A (Aquasol) was given with lunch. Blood was drawn into tubes containing EDTA and 120 μmol/l butylated hydroxytoluene at 1-h intervals from 0800 to 1800 and every 2 h from 1800 to 2400. All samples for retinyl ester determination were shielded from light with aluminum foil. Fresh plasma (3 ml) from each time point was overlayed with 2.2 ml 0.9% NaCl and ultracentrifuged for 100,000 *g* × 44 min at $d < 1.006$ g/ml and 15°C in a 50.3 rotor with 20,000 rpm to float lipoprotein particles of Svedberg flotation constant (S_f) > 400 (predominantly chylomicrons). The infranatant from the original separation was overlayed with 1.15% KBr and subjected to ultracentrifugation using the same rotor with 39,000 rpm at $d = 1.006$ g/ml and 10°C for 100,000 *g* × 15 h; the top layer obtained was defined as the S_f 20–400 fraction (containing both VLDL and chylomicron remnants), and the infranatant was defined as the $S_f < 20$ fraction (VLDL and chylomicron-free fraction). Samples were processed as quickly as possible in the laboratory under subdued light.

Aliquots of plasma, $S_f > 400$, S_f 20–400, and $S_f < 20$ lipoproteins fractions were extracted by chloroform:methanol = 2:1 (Folch's solution) using high-performance liquid chromatography (HPLC)-grade solvent. A known quantity of retinyl acetate (250–500 ng) was added to each sample before extraction as an internal standard. Extracted material was dried under a nitrogen stream, reconstituted in Folch's solution, and separated and quantitated on HPLC at 326 nm, using a reverse phase Supelcosil LC-8 column (25 × 4.6 mm internal diameter) with 100% methanol as the mobile phase at a flow rate of 1.75 ml/min. Standard curves were created for retinyl palmitate, with the concentrations of this compound being calculated by using a molar extinction coefficient of 52,275 at 326 nm. The interassay coefficient of variance of plasma vitamin A in our laboratory is 8%. Isolated lipoprotein fractions have a smaller interassay variance of 5%. The interassay variance of 10 repeated injections of retinyl acetate is 2%. As we have demonstrated before (2), when vitamin A is given with lunch at 1200, 4 h after a standard breakfast, the peak appearance of vitamin A in plasma occurs 2–4 h postlunch, and the majority of vitamin A is present in the $S_f > 20$ fraction throughout the 12-h period of measurement, with very little present in the $S_f < 20$ fraction until the end of the study. Retinyl palmitate concentrations in $S_f > 400$ and S_f 20–400 fractions were measured directly, as well as by calculating the differences between the retinyl palmitate concentrations measured in plasma and $S_f < 400$ (infranatant of plasma free of $S_f > 400$ particles) and those in $S_f < 400$ and $S_f < 20$ (infranatant of plasma free of both $S_f > 400$ and S_f 20–400 particles).

To rule out the possibility that metformin-induced changes in postprandial lipemia might be due to a decrease in intestinal fat absorption, 72-h fecal fat collections were obtained in the first six patients studied (Stanford University Hospital Clinical Laboratory). The procedure was omitted when it became apparent that metformin treatment was not associated with any evidence of fat malabsorption.

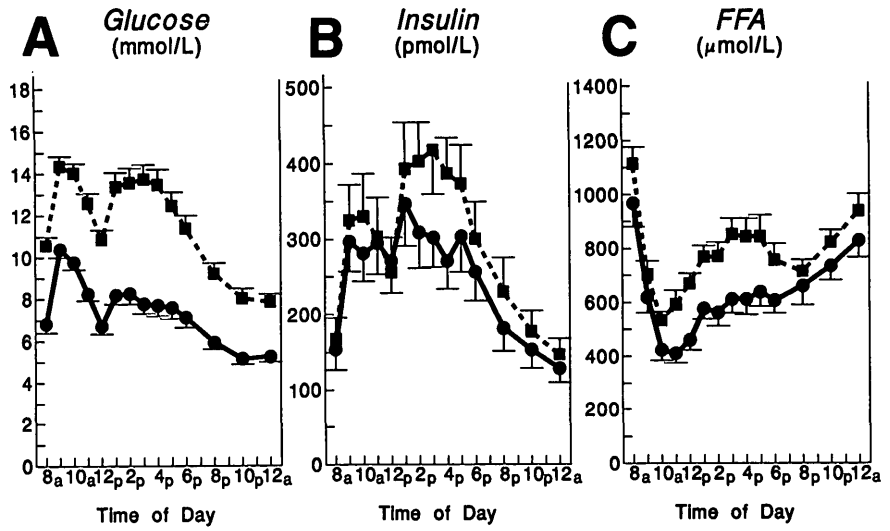

Figure 1—*Mean ± SE plasma glucose (A), insulin (B), and FFA (C) concentrations in patients with NIDDM before (■) and after (●) metformin treatment (P < 0.001).*

Resistance to insulin-mediated glucose disposal

The ability of insulin to promote glucose uptake was estimated by a modification (18) of the insulin suppression test originally described by our laboratory (19). After an overnight fast, intravenous catheters were placed in each arm of the subjects. Blood was sampled from one arm for measurement of plasma glucose and insulin concentrations, and test substances were administered in the other arm. Somatostatin was administered at 250 μg/h in a solution containing 2.5% (wt/vol) human serum albumin by a Harvard infusion pump to suppress endogenous insulin secretion. Insulin and glucose were simultaneously infused at 25 $mU \cdot m^{-2} \cdot min^{-1}$ and 120 $mg \cdot m^{-2} \cdot min^{-1}$, respectively. Blood was sampled every 30 min until 150 min into the study and then every 10 min until 180 min had elapsed. Insulin concentrations typically plateaued by 60 min and glucose by 120 min. Somatostatin inhibits endogenous insulin secretion, as well as the secretion of all other hormones modulating glucose homeostasis. Under these conditions, the average of the four values obtained between 150 and 180 min represent the steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations achieved during the infusion. Because SSPI concentrations are similar in all subjects, the higher the SSPG, the more resistant the individual is to insulin-mediated glucose disposal.

Following completion of the baseline studies outlined above, patients were started on 850 mg of metformin a day, given with breakfast, and discharged from the CRC. They were seen at weekly intervals, and the metformin dose was increased until either excellent glycemic control was achieved (fasting plasma glucose concentration <6.1 mmol/l) or the maximum dose of metformin had been reached (2.55 g/day, given as one 850 mg tablet three times a day, with breakfast, lunch, and dinner). If fasting plasma glucose concentration fell below 5.6 mmol/l, the metformin dose was reduced to 1.7 g a day, given as one 850 mg tablet with breakfast and dinner, whereas the sulfonylurea was kept unchanged. The patients were then readmitted to the CRC after at least 8 weeks of treatment (mean 10.7 ± 0.8 weeks), and the baseline measurements were repeated. Concentration of GHb was assessed by affinity chromatography using minicolumns at baseline and at weekly intervals after the initiation of drug therapy (20).

Statistical analysis

Data are expressed as the mean ± SE, and statistical evaluation was performed with the Statistical Analysis System program (SAS Institute, Cary, NC) using general linear models procedure. To evaluate the effect of metformin treatment, values before and after treatment were compared by either a paired Student's *t* test or two-way analysis of variance followed by the Bonferroni multiple comparison test (21,22).

RESULTS — The mean (± SD) dose of metformin used in the study was 2.3 ± 0.4 g/day. Six of the 16 patients described symptoms consistent with hypoglycemia during the treatment period, and in two instances it was necessary to decrease the metformin dose. Although most patients noted feelings of abdominal bloating, cramping, and fullness with initiation of metformin treatment, the symptoms were mild, tended to subside with time, and all patients completed the study. Drug compliance was monitored at each visit by pill counts and was >90% in all patients. Finally, weight did not change (83.3 ± 2.2 kg before and 83.8 ± 2.1 kg after) during the metformin treatment period.

Plasma glucose concentrations from 0800 to 2400 are shown in Fig. 1*A*. It is apparent that day-long plasma glucose concentrations were significantly lower after metformin treatment ($P < 0.001$), particularly from 1200 to 1600. GHb concentrations were also lower after metformin treatment (7.9 ± 0.3 vs. 10.8 ± 0.5%, $P < 0.001$). Metformin treatment was also associated with significantly lower day-long plasma insulin (Fig. 1*B*, $P < 0.001$) and FFA (Fig. 1*C*, $P < 0.001$) concentrations. As with the glucose response, decrease in plasma insulin concentration associated with metformin treatment was greater in magnitude after lunch than after breakfast.

SSPI and SSPG concentrations are seen in Fig. 2. SSPI concentrations were

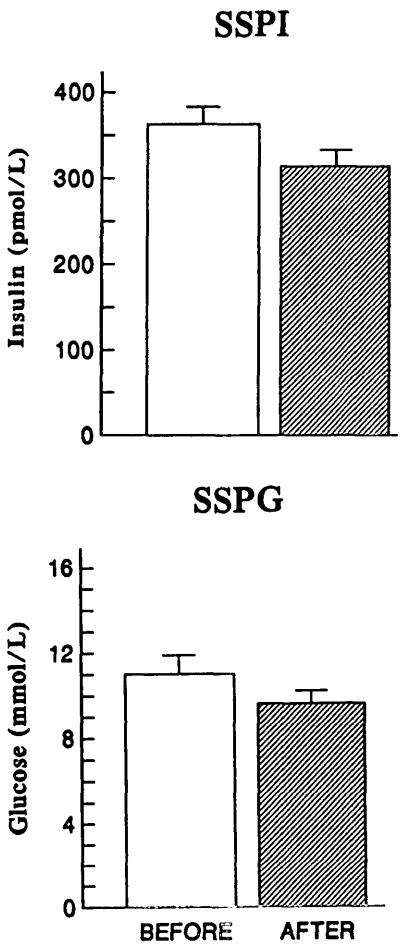

Figure 2—*Mean ± SE SSPI and SSPG concentrations in patients with NIDDM before (□) and after (▨) metformin treatment (P < 0.001).*

actually somewhat lower when patients were studied after metformin treatment. However, despite this, the SSPG concentrations were also significantly lower in metformin-treated patients. Thus, insulin-mediated glucose uptake improved after treatment with metformin.

Fasting lipid and lipoprotein concentrations before and after metformin treatment are listed in Table 1. These results show that both plasma TG and cholesterol concentrations were significantly lower in association with metformin administration and that this was entirely due to decreases in VLDL TG and VLDL cholesterol. Finally, the ratio of total:HDL cholesterol was also significantly lower after metformin treatment.

Figure 3 shows postprandial concentrations of TG in plasma (Fig. 3*A*) and in the $S_f > 400$ (Fig. 3*B*) and S_f 20–400 (Fig. 3*C*) fractions. It is obvious from these data that TG concentrations were significantly lower ($P < 0.001$) following metformin treatment in all three categories. Also, the effect of metformin on daylong plasma TG concentrations was greater after lunch compared with breakfast. It should be noted that TG concentrations in plasma were measured from 0800 to 2400, whereas TG concentrations in the $S_f > 400$ and S_f 20–400 fractions were only measured from 1200 to 2400.

Figure 4 shows the effect of metformin on postprandial TG-rich lipoproteins of intestinal origin. These data show that the retinyl ester concentration was significantly lower ($P < 0.001$) after metformin treatment of patients with NIDDM in the plasma and in both the $S_f > 400$ (chylomicron) and S_f 20–400 (chylomicron remnant) lipoprotein fractions.

CONCLUSIONS — Earlier studies of the TG-lowering effect of metformin have tended to focus on the changes in fasting plasma TG concentration (23,24). Recent results from our group have emphasized that postprandial TG concentrations were also lower after metformin treatment and that the decrease in plasma TG concentrations was accentuated as the day progressed (9–11). These data suggested that metformin treatment was associated with lower postprandial levels of TG-rich lipoproteins of intestinal origin, and the current results provide direct experimental evidence in support of this possibility. Obviously, the strength of this conclusion is based on the premise that measurement of postprandial retinyl ester concentration provides an accurate estimate of TG-rich lipoproteins of intestinal origin. For this to be the case, it is essential that little, if any, exchange of retinyl palmitate between lipoproteins of intestinal origin and other lipoproteins occurs. The fact that this exchange process may confound the interpretation of results based on the use of vitamin A to estimate postprandial lipemia has been recently emphasized (25), and we have previously presented (2) evidence that the experimental protocol used in this study is such that essentially all of the retinyl ester remains in the $S_f > 20$ fraction throughout the 12-h study. We believe this is primarily due to our decision to give the vitamin A with the noon meal, following a standard breakfast, rather than with breakfast as is usually done. In any event, it appears that determination of retinyl palmitate provided an accurate measure of postprandial changes in the level of TG-rich lipoprotein of intestinal origin in this study.

As discussed earlier, it is not clear

Table 1—*Lipid and lipoprotein concentrations before and after metformin treatment*

	Before treatment (mmol/l)	After treatment (mmol/l)	*P*
Plasma TG	2.63 ± 0.36	2.07 ± 0.23	0.02
VLDL TG	2.12 ± 0.35	1.62 ± 0.23	0.03
IDL TG	0.15 ± 0.01	0.14 ± 0.01	NS
LDL TG	0.23 ± 0.01	0.20 ± 0.01	0.001
HDL TG	0.14 ± 0.01	0.13 ± 0.01	NS
Plasma cholesterol	4.90 ± 0.17	4.64 ± 0.17	0.002
VLDL cholesterol	1.15 ± 0.19	0.80 ± 0.12	0.005
IDL cholesterol	0.34 ± 0.05	0.37 ± 0.05	NS
LDL cholesterol	2.37 ± 0.17	2.41 ± 0.17	NS
HDL cholesterol	1.05 ± 0.05	1.07 ± 0.05	NS
Plasma/HDL cholesterol	4.85 ± 0.28	4.47 ± 0.24	0.01

Data are means ± SE; NS, not significant.

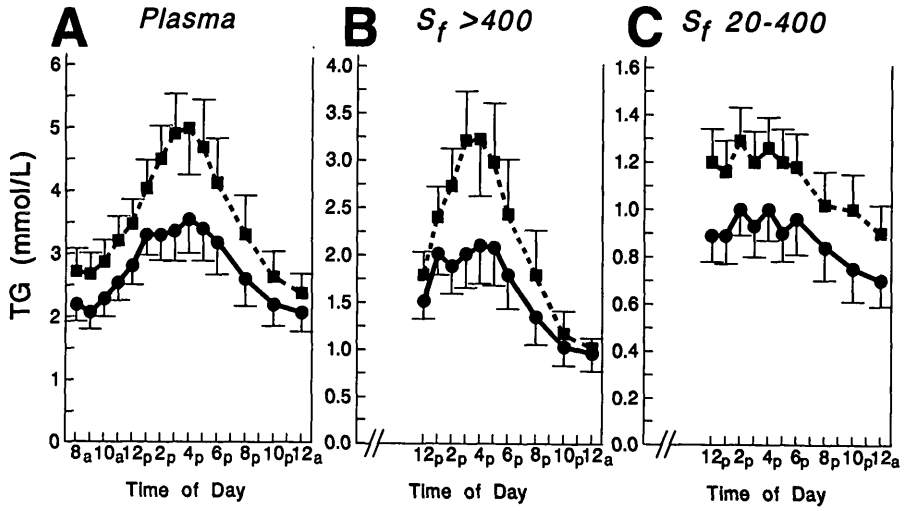

Figure 3—*Mean ± SE TG concentrations in plasma (A) and in the S_f > 400 (B) and S_f 20–400 (C) lipoprotein fractions in patients with NIDDM before (■) and after (●) metformin treatment (P < 0.001).*

why patients with NIDDM are at increased risk for CHD. Given published evidence of the association between postprandial lipemia and CHD in nondiabetic individuals (4–8) and the results of earlier animal studies by Zilversmit and colleagues (26–28) emphasizing the atherogenic potential of TG-rich lipoproteins of intestinal origin, it seems reasonable to postulate that the elevated levels of these particles described in patients with NIDDM (2) may help explain the accelerated atherogenesis seen in these individuals. As such, the marked fall in the postprandial concentration of TG-rich lipoproteins of intestinal origin associated with metformin treatment is of significant clinical relevance. Indeed, we are unaware of any other therapeutic intervention previously shown to be as effective as metformin therapy in lowering postprandial lipemia in patients with NIDDM.

In addition to having lower postprandial concentrations of TG-rich lipoproteins, metformin-treated patients had lower fasting and postprandial plasma glucose, insulin, and FFA concentrations, as well as lower fasting plasma TG concentrations. Insulin-mediated glucose disposal also improved following treatment. Thus, the therapeutic benefit of metformin in this group of patients with poorly controlled NIDDM was not limited to the decrease in postprandial concentration of TG-rich intestinal lipoproteins. Given evidence that increased insulin (29) and TG (30,31) concentrations may contribute to the added risk of CHD in patients with NIDDM, metformin treatment may help decrease the major cause of morbidity and mortality in patients with NIDDM. Finally, the finding that both ambient plasma glucose and insulin concentrations were lower after metformin treatment is consistent with the notion that resistance to insulin-mediated glucose disposal improved in these patients. Indeed, the data in Fig. 2 indicate that this was the case. However, the improvement in in vivo insulin action was modest in magnitude and consistent with results of a previous study of ours in which metformin was added to sulfonylurea treatment (11). Because lowering plasma glucose will enhance insulin action per se (32), we are not persuaded that the improved glycemic control seen in metformin-treated patients can be explained entirely by enhanced insulin-mediated glucose disposal. Indeed, it is possible that improved glycemic control, for whatever reason, accounted for the improved in vivo insulin action. On the other hand, it is also quite possible that, consistent with previous studies of hypertriglyceridemia in rats (33), the decrease in ambient insulin concentration associated with metformin treatment led to a decrease in hepatic VLDL TG secretion and plasma TG concentration.

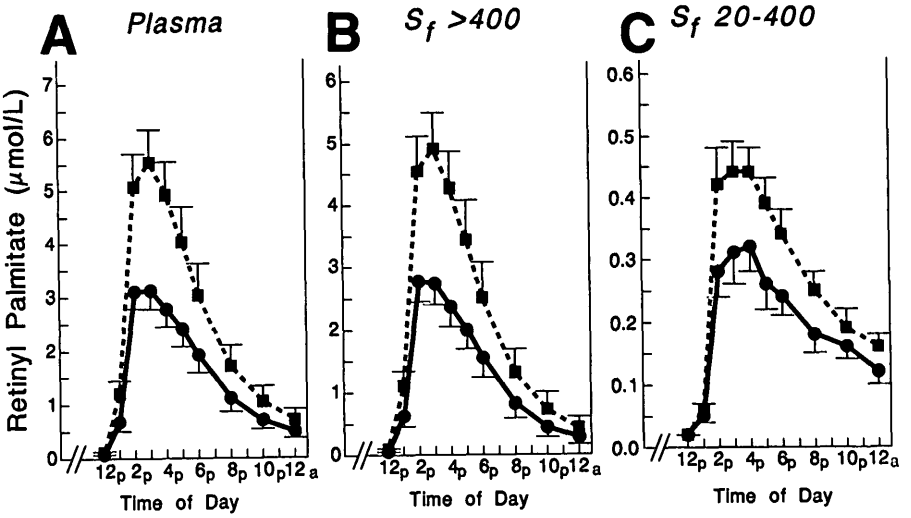

Figure 4—*Mean ± SE retinyl palmitate concentrations in plasma (A) and in the S_f > 400 (B) and S_f 20–400 (C) lipoprotein fractions in patients with NIDDM before (■) and after (●) metformin treatment (P < 0.001).*

In conclusion, the addition of metformin to the treatment program of sulfonylurea-treated patients with uncontrolled NIDDM was associated with lower day-long plasma glucose, insulin, FFA, and TG concentration and a decrease in insulin resistance. In addition, metformin treatment was associated with a substantial decrease in the postprandial concentration of TG-rich lipoproteins of intestinal origin. All of these changes should help reduce risk of microvascular and macrovascular complications in patients with NIDDM.

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