

# Effect of Oxidized Lipids in the Diet on Oxidized Lipid Levels in Postprandial Serum Chylomicrons of Diabetic Patients

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**OBJECTIVE** — To determine whether humans with type 2 diabetes have increased levels of oxidized fatty acids in their serum chylomicron fraction after the ingestion of dietary oxidized fatty acids.

**RESEARCH DESIGN AND METHODS** — The study was performed on 31 male type 2 diabetic patients and 24 age-matched control subjects. Among the diabetic patients, 22 had poor glycemic control, defined as HbA<sub>1c</sub> >10% (normal value <7.7%). Nine patients had good glycemic control (HbA<sub>1c</sub> ≤10). Heated corn oil containing low or high levels of oxidized fatty acids was used as a test meal. At 2.5 h after the test meal, 50-ml blood samples were obtained from all subjects, and the chylomicron fraction ( $S_f > 1,000$ ) was isolated. The degree of oxidation in chylomicrons was determined by measuring conjugated dienes. For determining the postprandial levels of triglycerides and of oxidized lipids in serum chylomicrons over an extended time period, blood samples were obtained at 0, 2.5, 5.0, and 7.5 h for isolation of chylomicrons and determination of fatty acid oxidation.

**RESULTS** — We found that at 2.5 h after the consumption of the test meal containing either a low or high oxidized fatty acid content, conjugated dienes in serum chylomicrons in diabetic subjects in poor glycemic control were increased compared with those in control subjects. Diabetic patients in good glycemic control had similar levels of oxidized lipid in their chylomicrons when compared with control subjects. Additionally, in diabetic patients in poor glycemic control, the levels of oxidized lipids in chylomicrons remained elevated for an extended postprandial period.

**CONCLUSIONS** — In diabetic subjects with poor glycemic control, dietary oxidized lipids induce an exaggerated and sustained increase in the levels of oxidized lipids in chylomicrons when compared with either control subjects or diabetic patients with good glycemic control. These increased postprandial levels of potentially atherogenic oxidized lipids may contribute to the accelerated atherosclerosis associated with diabetes.

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Epidemiological studies have provided convincing evidence that diabetes is an important risk factor for cardiovascular disease. Alterations in plasma lipid levels and other known risk factors such as hypertension occur frequently in the diabetic population, but these changes do not

totally account for the increased risk of coronary artery disease (1–3). Thus, the mechanism by which diabetes per se increases the risk of atherosclerosis remains speculative.

Extensive studies have demonstrated that lipid oxidation converts lipoproteins to

a more atherogenic form and that oxidized lipoproteins may play an important role in atherosclerosis (4,5). Moreover, it has been shown that serum from both humans with diabetes (6–8) and animals with experimentally induced diabetes (9–11) contains more lipid peroxidation products. Thus, diabetes may be associated with the increased presence of potentially atherogenic oxidized serum lipoproteins. The origin of the increased levels of oxidized lipoproteins in diabetes is not clear, and limited data exist on how and where the oxidation of lipoproteins occurs in vivo. It has been suggested that the nonenzymatic glycation of serum lipoproteins may increase their susceptibility to oxidation (12,13).

Studies in our own laboratory and others have shown that in rodents (11,14,15) and humans (16,17) oxidized lipids in the diet are absorbed by the small intestine and incorporated into chylomicrons. In rodents, oxidized dietary fatty acids are also incorporated into the endogenous serum VLDL + LDL fraction (11). These results indicate that oxidized lipids in the diet are absorbed by the small intestine and are transported in chylomicrons to the circulation where they contribute to the total body pool of oxidized lipids. Recently we have further demonstrated that oxidized lipids in the diet are delivered to the liver, incorporated into VLDL, and resecreted into the circulation, thereby providing a mechanism by which dietary oxidized lipids can affect endogenous lipoproteins (18).

As observed in control animals (14,15), diabetic rodents also absorb dietary oxidized lipids, transport them in chylomicrons to the circulation, and package them into endogenous lipoproteins (11). Furthermore, we have shown that at all levels of dietary oxidized lipids, the quantities of oxidized lipids in the serum VLDL + LDL lipoprotein fraction, when expressed as nanomoles of oxidized lipid per milligram of cholesterol, were markedly increased in the diabetic rats compared with control subjects (11). Moreover, after the administration of an oxidized meal, the quantity of oxidized lipids in mesenteric lymph chylomicrons, expressed as nanomoles of oxidized lipid

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

per milligram of triglyceride, was greater in the diabetic rats, indicating increased absorption of oxidized lipids by diabetic animals (11). Thus, in both diabetic and control rats, the quantity of oxidized lipids in the diet determines the levels of oxidized lipids in serum lipoproteins and in mesenteric lymph, but the effect of the diet is more pronounced in diabetic animals. These results indicate that in diabetic animals, oxidized lipids are absorbed more efficiently and that this increased absorption of dietary oxidized lipids may play a role in the elevated levels of oxidized lipids in the lipoproteins of diabetic animals.

In the present study, we have examined whether humans with type 2 diabetes have increased levels of oxidized lipids in postprandial serum chylomicrons after ingestion of oxidized fats.

## RESEARCH DESIGN AND METHODS

### Study subjects

The study was performed on 31 male type 2 diabetic patients attending the Veterans Affairs Outpatient Clinics and 24 sex-, weight-, serum triglyceride-, and serum cholesterol-matched control subjects selected from employees at the Veterans Affairs Medical Center. Blood was drawn from each subject after a 12-h fast (0 time) for measurement of glycated hemoglobin (HbA<sub>1c</sub>) and fasting serum triglyceride and cholesterol levels. Diabetic patients were selected for the study on the basis of normal fasting serum triglyceride levels (<2.3 mmol/l) and were divided into 2 groups based on HbA<sub>1c</sub> measurements to assess glycemic control. There were 22 patients with poor control, defined as HbA<sub>1c</sub> >10% (normal value <7.7%). In this group, 11 patients were treated with insulin, and 1 patient was treated with insulin plus metformin. Four patients received metformin plus glipizide and two patients received metformin plus tolbutamide and troglitazone. One patient was on glyburide, one was on metformin plus tolbutamide, and two were on metformin plus glyburide. Nine patients had HbA<sub>1c</sub> in the normal or modestly elevated range (HbA<sub>1c</sub> <10). In this group, two patients were treated with insulin, two were treated with tolbutamide, two received metformin plus glipizide, and three were treated only with diet. None of the subjects was on antioxidant therapy. All subjects were nonsmokers, were moderately active, consumed a typical American

diet, and had normal fasting triglyceride levels (<2.3 mmol/l). None of the subjects had congestive heart failure or gastrointestinal disorders. None was taking bile resin binders or acarbose. This study was approved by the Committee on Human Research at University of California, San Francisco, California.

### Experimental diets

Corn oil ( $\alpha$ -tocopherol-depleted), purchased from ICN Biochemicals (Irvine, CA), was used as a test meal. To obtain oil with different oxidized lipid content, the oil was heated at 100°C (1–3 h), which resulted in conjugated diene levels ranging from 40 to 200  $\mu$ mol/mmol triglyceride, resembling levels commonly found in foods (19–24). Oil containing 40–99  $\mu$ mol conjugated dienes/mmol triglyceride was designated as oil containing low levels of oxidized lipids and was added to mashed potatoes to obtain the diet with low levels of oxidized lipids. Oil containing 100–200  $\mu$ mol conjugated dienes/mmol triglyceride was designated as oil containing high levels of oxidized lipids and was added to mashed potatoes to obtain the diet with high levels of oxidized lipids. Maximally oxidized oil containing 200  $\mu$ mol conjugated dienes/mmol triglyceride typically yielded 220–350  $\mu$ mol lipid peroxides/mmol triglyceride when measured with methylene blue derivative reagent and 4–10  $\mu$ mol TBARS/mmol triglyceride.

### Study protocol

After a 12-h fast, subjects were given a corn oil (1 ml/kg body weight) diet containing either low or high oxidized lipid content. In the experimental diet, oxidized corn oil was mixed with 100 g of carbohydrate (mashed potatoes) that contained no other fat. In the control group, 11 subjects received a diet containing low levels of oxidized lipids and 13 subjects received a diet with high levels of oxidized lipids. In the diabetic patient group with poor glycemic control, 8 patients received a diet containing low levels of oxidized lipids and 14 patients received a diet with high levels of oxidized lipids. In the diabetic patient group with good glycemic control, four patients received a diet containing low levels of oxidized lipids and five patients received a diet with high levels of oxidized lipids. The average levels of oxidized lipids were similar in both the low and high oxidized diets that were administered to control and diabetic subjects (low oxidized lipid diet: control subjects 58.70  $\pm$  4.89 vs. dia-

betic patients 67.38  $\pm$  7.93  $\mu$ mol/mmol triglyceride in oil, NS; high oxidized lipid diet: control subjects 162.50  $\pm$  10.98 vs. diabetic patients 158.69  $\pm$  7.97  $\mu$ mol/mmol triglyceride in oil, NS). The subjects tolerated the test meal well, and none had gastrointestinal symptoms. At 2.5 h, 50-ml blood samples were obtained from all subjects for determination of triglycerides and conjugated dienes in the chylomicron fraction ( $S_f > 1,000$ ). The degree of oxidation was expressed as micromoles of conjugated dienes per deciliter of serum or as micromoles of conjugated dienes per millimoles of triglyceride in the chylomicron fraction. The postprandial time interval (2.5 h) for chylomicron isolation was chosen on the basis of our previous observation that chylomicron triglyceride concentration peaked at 2.5 h (16). For determining the postprandial levels of triglycerides and of oxidized lipids in serum chylomicrons not only at 2.5 h, but over an extended time period, blood samples were obtained at 0, 2.5, 5.0, and 7.5 h from six control and seven diabetic subjects in poor glycemic control (HbA<sub>1c</sub> >10) who had received a test meal containing corn oil with high oxidized fatty acid content. Relative areas under the clearance curves (measured above baseline) spanning the postprandial time period of 7.5 h were measured in square centimeters using MacDraft software on a Macintosh computer. The subjects were not permitted to consume any food for the duration of the test period. Water was allowed ad libitum.

### Chylomicron isolation

The chylomicron fraction ( $S_f > 1,000$ ) in the postprandial serum was isolated as described by us previously (16). Briefly, EDTA was added to serum samples until the concentration was 0.5  $\mu$ mol/l. The serum was then overlaid with saline and centrifuged in a swinging bucket rotor (SW 40; Beckman, Palo Alto, CA) for 60 min at 25,000 rev/min at 12°C. The chylomicron fraction, which may also contain small amounts of large VLDL, was removed using a tube cutter, extracted (25), and analyzed immediately for fatty acid oxidation.

### Determination of fatty acid oxidation

Lipid oxidation in oils and the isolated serum chylomicron fraction was determined by measuring conjugated dienes using second-derivative ultraviolet spectroscopy in a Perkin Elmer 555 Spectrophotometer (Perkin Elmer, San Jose, CA)

**Table 1—Clinical characteristics of control subjects and diabetic patients with poor and good glycemic control**

	Control subjects	Diabetic patients	
		Poor control	Good control
n	24	22	9
Age	52.1 ± 2.9	58.5 ± 2.5	63.6 ± 4.3*
BMI	26.2 ± 0.6	27.1 ± 0.6	26.5 ± 1.0
HbA <sub>1c</sub> (%)	ND	13.33 ± 0.55	7.96 ± 0.55†
Triglyceride (mmol/l)	1.61 ± 0.18	1.66 ± 0.29	1.57 ± 0.59
Cholesterol (mmol/l)	5.51 ± 0.33	5.18 ± 0.24	5.38 ± 0.39
Chylomicron triglycerides (μmol/dl)§	17.60 ± 1.84	30.05 ± 2.40†	26.61 ± 3.07‡

Data are means ± SEM. \*P < 0.05 when compared with control subjects; †P < 0.001 when compared with control subjects; ‡P < 0.02 when compared with poor control. §Postprandial chylomicron triglycerides were measured 2.5 h after the administration of the test meal. ND, no data.

using the method of Corongiu et al. (26) as reported by us previously (16). Oxidized linoleic acid was used as a standard. This method measures lipid hydroperoxy and hydroxy fatty acids, which are the major lipid peroxidation products derived from polyunsaturated fatty acids (27). Since both the control and diabetic groups were administered similar oils after a fasted state, it is unlikely that there were other interfering conjugated diene-containing substances in the isolated chylomicron fraction at 2.5 h after the administration of the oxidized meal. It should be noted that this method does not yield an absolute measurement of lipid peroxides, but rather has been used to compare lipid oxidation between groups. Conjugated dienes were not measured on whole fasting serum samples because of the extremely low diene levels and large quantities of interfering substances, including lipid soluble bile pigments, carotenes, and glucose. These interfering substances are not present in significant quantities in the purified chylomicron fraction. Oxidation in oils was confirmed by measuring TBARS (10), and lipid peroxides directly with methylene blue color reagent, using cumene hydroperoxide as a standard (28).

**Analytical methods**

Total serum cholesterol (kit #352-20; Sigma, St. Louis, MO) and triglycerides (kit #339-20; Sigma) were determined by enzymatic assays. HbA<sub>1c</sub> was measured at the Veterans Affairs Medical Center Clinical Laboratory using IMx glycated hemoglobin assay package from Abbot Laboratories (Abbot Park, IL).

**Statistical analyses**

For data presented in Table 1, statistical significance among the three subject groups

was determined for each category of measurement by one-way analysis of variance. Calculations were performed using Primer Biostatistics Software for Macintosh (1988).

In Figs. 1 and 2, for each type of diet, the levels of significance among the control and diabetic subject groups with respect to the presence of oxidized lipids in chylomicrons were calculated by one-way analysis

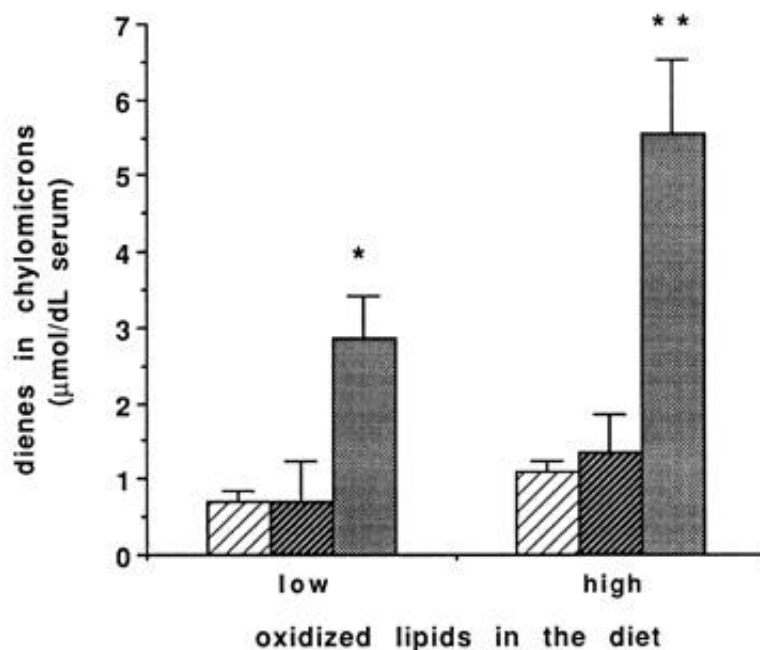
of variance. Calculations were performed with the Bonferoni multiple comparison test using InStat Statistical Software for Macintosh. In Fig. 2, statistical differences in chylomicron oxidation between two groups (low oxidized fat and high oxidized fat diet) were calculated using Student's t test.

In Fig. 3, the postprandial chylomicron triglyceride values and oxidized lipid levels in the two subject groups (normal subjects and poorly controlled diabetic patients) were compared using analysis of variance with repeated measurements. Calculations were performed using JMP statistical software for Macintosh version 3.2.1.

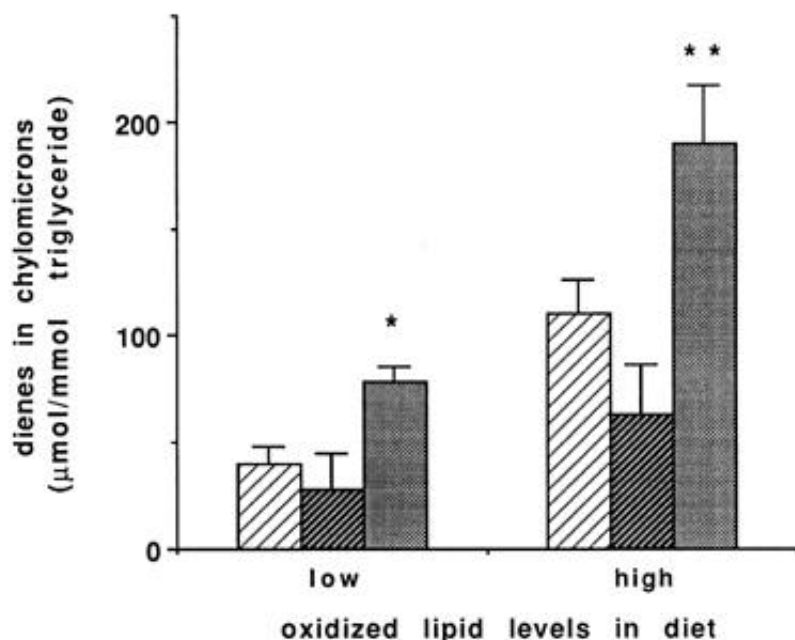
**RESULTS**

**Subject characteristics**

Table 1 summarizes the clinical characteristics of the diabetic and control subjects. In the fasted state, serum triglyceride and cholesterol levels were not significantly different between the control group and either diabetic group. No differences were expected for these categories, since subjects were



**Figure 1—Lipid oxidation in postprandial serum chylomicron fraction.** Control subjects (diabetic patients in good and poor glycemic control) were administered diets containing either low or high amounts of lipid peroxides; 2.5 h after consumption of the meal, chylomicrons were isolated and conjugated dienes were measured to determine oxidation. All data represent means ± SEM. \*For the low oxidized lipid diet, < 0.01 when control subjects are compared with diabetic patients in poor glycemic control and < 0.05 when diabetic patients in good control are compared with diabetic patients in poor control. \*\*For the high oxidized lipid diet, 0.01 when control subjects are compared with diabetic patients in poor glycemic control and 0.05 when diabetic patients in good control are compared with diabetic patients in poor control. The difference between control subjects and diabetic patients in good control is not significant.



**Figure 2**—Lipid oxidation in postprandial serum chylomicron fraction. Control subjects ( ) and diabetic patients with good ( ) and poor ( ) control were administered diets containing either low or high amounts of lipid peroxides; 2.5 h after consumption of the test meal, chylomicrons were isolated and conjugated dienes were measured to determine oxidation. All data represent means  $\pm$  SEM. \*For the low oxidized lipid diet,  $P < 0.05$  when control subjects are compared with diabetic patients in poor glycemic control and  $P < 0.05$  when diabetic patients in good control are compared with diabetic patients in poor control. \*\*For the high oxidized lipid diet,  $P < 0.05$  when control subjects are compared with diabetic patients in poor glycemic control and  $P < 0.01$  when diabetic patients in good control are compared with diabetic patients in poor glycemic control. The difference between control subjects and diabetic patients in good control is not significant. When control subjects fed the low oxidized fat diet were compared with control subjects fed the high oxidized lipid diet,  $P < 0.002$ . When diabetic subjects in poor glycemic control fed the low oxidized fat diet were compared with diabetic subjects in poor glycemic control fed the high oxidized lipid diet,  $P < 0.01$ . In well-controlled diabetic subjects, the difference did not reach statistical significance.

selected to minimize differences in serum lipids. The levels of triglycerides in chylomicrons measured above the baseline at 2.5 h after the consumption of the test meal were significantly higher in both groups of diabetic patients when compared with control subjects. The difference in HbA<sub>1c</sub> levels between the two diabetic groups was highly significant ( $P < 0.001$ ), since the subjects were selected on the basis of HbA<sub>1c</sub>.

### The incorporation of dietary oxidized lipids into postprandial chylomicrons

Figure 1 shows the effect of oxidized lipids in the diet on the levels of oxidized lipids in chylomicrons in control subjects and diabetic patients in good or poor glycemic control. Diabetic subjects in poor glycemic control fed the low oxidized lipid test meal had a threefold increase in the levels of oxidized lipids in postprandial chylomicrons compared with control subjects (control

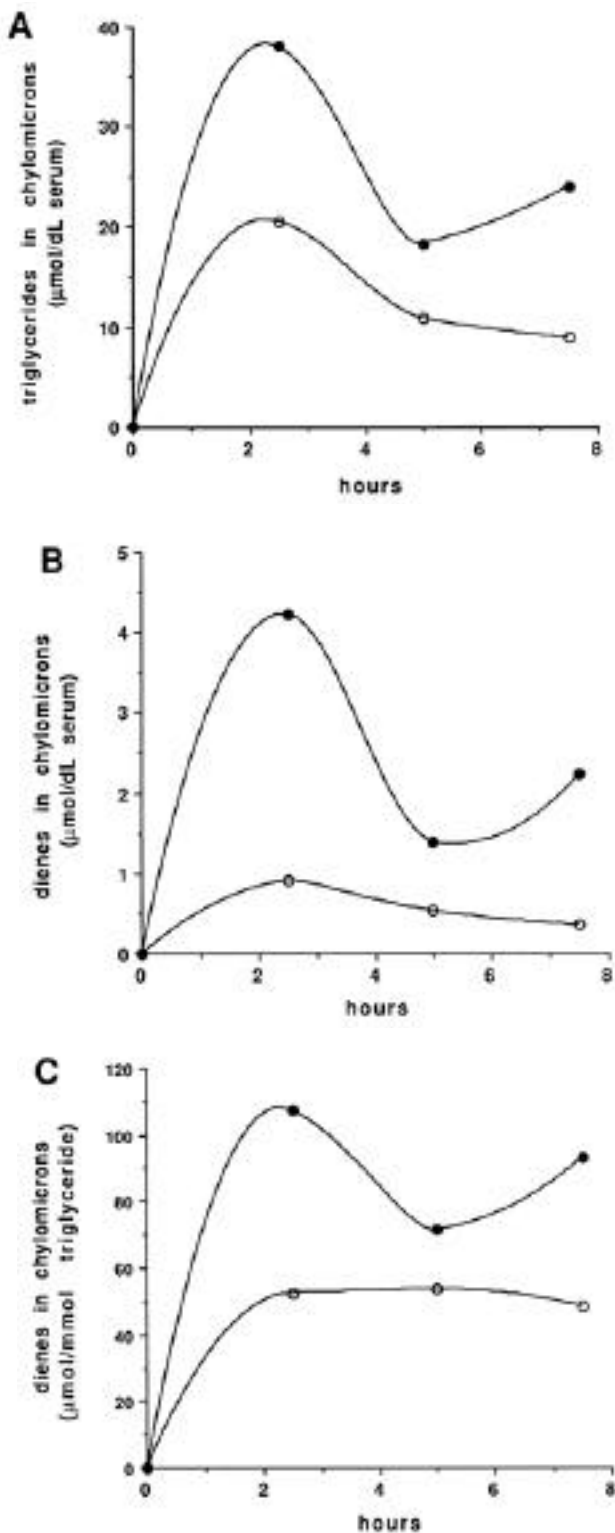
subjects:  $0.69 \pm 0.14$  vs. diabetic patients:  $2.85 \pm 0.57$   $\mu\text{mol/dl}$  serum,  $P < 0.01$ ). Moreover, after consumption of a test meal containing high levels of oxidized lipid, this difference between control subjects and poorly controlled diabetic patients was even greater (fivefold increase) (control subjects:  $1.09 \pm 0.14$  vs. diabetic patients:  $5.55 \pm 0.97$   $\mu\text{mol/dl}$  serum,  $P < 0.01$ ). In contrast, in the well-controlled diabetic patients, the levels of oxidized lipids in postprandial chylomicrons were similar to those of control subjects and significantly lower ( $P < 0.05$ ) than those in poorly controlled diabetic patients. Thus, at both low and high levels of oxidized lipid intake, the quantity of oxidized lipids in chylomicrons is increased in poorly controlled diabetic patients, but not in well-controlled diabetic patients, suggesting that this increase is dependent on the glycemic control of the diabetic patient. It should be noted that 12 patients in the poorly controlled diabetic

patient group were treated with insulin and 10 received oral medication; however, the mode of treatment did not affect the oxidized lipid levels in postprandial chylomicrons (insulin treated:  $5.73 \pm 1.24$  vs. oral medication treated:  $5.54 \pm 1.4$   $\mu\text{mol/dl}$  serum, NS).

As reported by other investigators (29,30), we also observed that postprandial chylomicron triglyceride concentrations were increased in diabetic subjects (Table 1). Because the increase of oxidized lipid levels in chylomicrons in the poorly controlled diabetic subjects (Fig. 1) could be due to increased postprandial serum triglyceride levels, we have also expressed our data as micromoles of conjugated dienes per millimole of chylomicron triglyceride (Fig. 2).

When the levels of oxidized lipids in chylomicrons were expressed in this fashion, the levels of oxidized lipids in postprandial chylomicrons were increased in nearly 100% in the diabetic patients, compared with control subjects, regardless of the quantity of oxidized lipid in the diet (low oxidized lipid diet: control subjects  $40.17 \pm 7.39$  vs. diabetic patients  $78.28 \pm 6.72$   $\mu\text{mol/mmol}$  triglyceride,  $P < 0.05$ ; high oxidized lipid diet: control subjects  $108.70 \pm 13.05$  vs. diabetic patients  $198.74 \pm 20.74$   $\mu\text{mol/mmol}$  triglyceride,  $P < 0.05$ ). In diabetic patients in good glycemic control, the levels of oxidized lipids in postprandial chylomicrons, when expressed per millimole of triglyceride, were similar or less than those in control subjects and significantly lower ( $P < 0.05$ ) than those observed in poorly controlled diabetic subjects, regardless of whether they were fed a low or high oxidized lipid test meal (Fig. 2). Thus, the increase in oxidized lipids in chylomicrons is observed in poorly controlled diabetic patients even when expressed per millimole of triglyceride, suggesting that the increase in postprandial triglyceride is not the sole basis for the increased oxidized lipids present in postprandial chylomicrons.

Moreover, Fig. 2 shows that the quantity of oxidized lipid in the diet significantly influences the levels of oxidized lipids in postprandial chylomicrons in both control and diabetic subjects in poor glycemic control. After ingestion of the high oxidized lipid diet, the levels of oxidized lipids in chylomicrons increased more than twofold in both control and diabetic subjects when compared with those observed on the low oxidized diet (control



**Figure 3**—Time course of postprandial serum chylomicron triglycerides and dienes. Seven diabetic patients in poor glycemic control (●) and six control subjects (○) were administered corn oil-containing high oxidized lipid levels. Triglyceride levels measured at indicated times are shown in **A**. The quantity of conjugated dienes expressed per deciliter of serum and the quantity of dienes expressed per millimole of triglyceride are shown in **B** and **C**, respectively. The two subject groups, when compared by analysis of variance with repeated measurements, differed significantly with regard to triglyceride levels ( $P = 0.011$ ), conjugated diene quantities in deciliter of serum ( $P = 0.001$ ), and conjugated diene quantities when expressed per millimole of triglyceride concentration ( $P = 0.027$ ).

subjects: low oxidized lipid diet  $40.17 \pm 7.39$  vs. high oxidized lipid diet  $108.70 \pm 13.05$   $\mu\text{mol}/\text{mmol}$  triglyceride,  $P < 0.001$ ; diabetic subjects: low oxidized lipid diet  $78.28 \pm 14.74$  vs. high oxidized lipid diet  $189.81 \pm 26.74$   $\mu\text{mol}/\text{mmol}$  triglyceride,  $P < 0.02$ ). While the trend is similar in the diabetic group in good glycemic control, because of the small number of subjects, the increase in oxidized lipids in chylomicrons did not reach statistical significance.

We also observed (Fig. 3A) that diabetic patients in poor glycemic control had significantly increased postprandial chylomicron triglyceride levels for extended time periods compared with control subjects ( $P = 0.011$ ). When we examined six control subjects, the postprandial levels of chylomicron triglycerides peaked at 2–3 h ( $20.56 \pm 6.08$   $\mu\text{mol}/\text{dl}$ ) after the administration of the test meal, and by 7.5 h the triglyceride concentration in the chylomicron fraction had decreased to  $8.91 \pm 2.70$   $\mu\text{mol}/\text{dl}$ . In six diabetic patients with poor glycemic control, triglycerides also peaked at 2.5 h ( $38.05 \pm 8.07$ ), but the triglyceride concentration in the chylomicron fraction remained significantly elevated at 7.5 h after the administration of the test meal ( $24.06 \pm 4.08$   $\mu\text{mol}/\text{dl}$ ). Figure 3B shows that there was also a significant increase in the levels of conjugated dienes in the chylomicron fraction of diabetic patients after the ingestion of oxidized oil containing 100–150  $\mu\text{mol}$  conjugated dienes/ $\text{mmol}$  triglyceride ( $P = 0.003$ ). The areas under the clearance curves spanning the postprandial time period of 7.5 h were used to estimate the subjects' relative exposure to the oxidized lipids contained in chylomicrons and chylomicron remnants. The area under the curve of conjugated dienes in the chylomicron fraction (Fig. 3B) was increased approximately threefold in the poorly controlled diabetic patients ( $4.31$  vs.  $16.24$   $\mu\text{mol} \cdot \text{dl}^{-1} \cdot \text{h}^{-1}$ ). Moreover, as shown in Fig. 3C, this difference between control subjects and diabetic patients in poor glycemic control persists even when data are expressed per millimole of triglyceride. Thus, in poorly controlled diabetic patients, oxidized lipids in the diet result in an increase in oxidized lipids in the circulation for an extended postprandial time period.

**CONCLUSIONS**— The results of the present manuscript demonstrate that in type 2 diabetic patients in poor glycemic control, the levels of oxidized lipids in post-

prandial chylomicrons is increased compared with control subjects or diabetic patients in good glycemic control. Moreover, in poorly controlled diabetic patients, the ingestion of dietary oxidized lipids results in increased levels of oxidized chylomicrons in the circulation for an extended period of time (Fig. 3B). This increase in the levels of oxidized lipids in chylomicrons of poorly controlled diabetic patients could be due to alterations in the clearance and/or an increase in the absorption of oxidized dietary lipids. The marked increase in the levels of dienes in chylomicrons (threefold) undoubtedly reflects an abnormality in clearance. However, it is unlikely that clearance alone accounts for the increase. When our results were expressed as micromoles of conjugated dienes per millimole of triglyceride basis, there was still a 100% increase in chylomicron dienes in the poorly controlled diabetic patients (Figs. 2 and 3C). Previously, we have demonstrated in rodents that the metabolism of oxidized fatty acids in chylomicrons was virtually identical to that of nonoxidized fatty acids in chylomicrons (15,18), suggesting that if the increase was simply due to a clearance defect, one would not see an increase in dienes when expressed per millimole of triglyceride. Moreover, our studies in rats have directly demonstrated an increase in the absorption of oxidized lipids in diabetic animals with poor glycemic control (11). The mesenteric lymph drainage of diabetic rats contained 100% more oxidized lipid than did that of control subjects (11). Based on these observations, we thus hypothesize that both abnormalities in clearance and in absorption may account for the increased conjugated dienes found in the chylomicrons of poorly controlled diabetic patients.

Postprandial chylomicrons in the circulation are present as a mixture of chylomicrons and their remnants (31). Chylomicron remnants generated postprandially can potentially deposit cholesterol in the vessel wall (32), and it has been demonstrated that prolonged presence of chylomicron remnants in the circulation is a risk factor for arteriosclerotic disease (33). Thus, the presence of chylomicron remnants in the circulation of diabetic patients may increase the atherogenic risk of diabetic patients with poor glycemic control, and the atherogenic risk may be exaggerated when chylomicron remnants are oxidized (34,35). Recently, we have demonstrated in rabbits that oxidized fatty acids in

the diet accelerate atherosclerosis in cholesterol-fed rabbits (36). Rabbits were fed a cholesterol diet containing either 5% nonoxidized corn oil or 5% oxidized corn oil. Rabbits fed the oxidized diet had increased levels of oxidized fatty acids in their serum  $\beta$ -VLDL fraction. Most importantly, feeding a diet enriched in oxidized lipid resulted in a 100% increase in fatty streak lesions in the aorta. Additionally, rabbits fed the oxidized diet also had a >100% increase in total cholesterol in the pulmonary artery that was due primarily to an increase in cholesteryl ester.

There is abundant evidence that because of the popularity of processed and fried foods and the wide-spread fast food industry, the typical Western diet contains large quantities of oxidized fat (19–24). Moreover, an increased use of polyunsaturated fats in the diet has been recommended because this reduces total and LDL cholesterol levels. However, depending on storage and preparation, polyunsaturated fatty acid-containing foods may contain oxidized fatty acids, and these oxidized fatty acids may contribute to accelerated atherosclerosis observed in patients with diabetes.

In summary, the present study demonstrates that after an oxidized lipid-containing meal, in poorly controlled type 2 diabetic patients, the levels of oxidized lipids in the postprandial serum chylomicrons were increased. These increased levels of potentially atherogenic oxidized lipids in these diabetic patients may indicate a need for the modification of the diet to reduce the intake of oxidized lipids.

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