Effect of Postprandial Hypertriglyceridemia and Hyperglycemia on Circulating Adhesion Molecules and Oxidative Stress Generation and the Possible Role of Simvastatin Treatment

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Adhesion molecules, particularly intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin, have been associated with cardiovascular disease. Elevated levels of these molecules have been reported in diabetic patients. Postprandial hypertriglyceridemia and hyperglycemia are considered risk factors for cardiovascular disease, and evidence suggests that postprandial hypertriglyceridemia and hyperglycemia may induce an increase in circulating adhesion molecules. However, the distinct role of these two factors is a matter of debate. Thirty type 2 diabetic patients and 20 normal subjects ate three different meals: a high-fat meal, 75 g of glucose alone, and a high-fat meal plus glucose. Glycemia, triglyceridemia, plasma nitrotyrosine, ICAM-1, VCAM-1, and E-selectin were assayed during the tests. Subsequently, diabetic subjects took simvastatin 40 mg/day or placebo for 12 weeks. The three tests were performed again at baseline, between 3 and 6 days after starting the study, and at the end of each study. High-fat load and glucose alone produced an increase of nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels in normal and diabetic subjects. These effects were more pronounced when high fat and glucose were combined. Short-term simvastatin treatment had no effect on lipid parameters, but reduced the effect on adhesion molecules and nitrotyrosine, which was observed during every different test. Long-term simvastatin treatment was accompanied by a lower increase in postprandial triglycerides, which was followed by smaller variations in ICAM-1, VCAM-1, E-selectin, and nitrotyrosine during the tests. This study shows an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on ICAM-1, VCAM-1, and E-selectin plasma levels, suggesting oxidative stress as a common mediator of such effects. Simvastatin shows a beneficial effect on oxidative stress and the plasma levels of adhesion molecules, which may be ascribed to a direct effect in addition to the lipid-lowering action of the drug. *Diabetes* 53: 701–710, 2004

Patients with diabetes have an increased risk of cardiovascular disease (CVD). Recently, much attention has been paid to evidence that abnormalities of the postprandial state are important contributing factors to the development of atherosclerosis, even in diabetes (1). In nondiabetic subjects, there is evidence that postprandial hypertriglyceridemia is a risk factor for CVD (2), whereas in diabetic subjects, postprandial hyperglycemia has been recently proposed as an independent risk factor for CVD (3). Since in diabetic patients the postprandial phase is characterized by the simultaneous increase of plasma triglycerides and glucose (1), the distinct role and relative importance of these two factors in the pathogenesis of CVD in diabetes is a matter of debate.

The adhesion of circulating leukocytes to endothelial cells plays an important role in the initiation of atherosclerosis (4). Cellular adhesion molecules are poorly expressed by the resting endothelium, but they are upregulated during atherogenesis (5). Soluble forms of some cellular adhesion molecules can be found in plasma, and it has been suggested that elevated plasma levels of some cellular adhesion molecules may be an index of endothelial activation (6) or even a molecular marker of early atherosclerosis (7).

Circulating levels of some cellular adhesion molecules, particularly intracellular adhesion molecule (ICAM)-1, vascular cellular adhesion molecule (VCAM)-1, and E-selectin, have been found to be increased in diabetic patients (8–15). Hyperglycemia has been shown to condition, even acutely (13–15), the concentration of these cellular adhesion molecules. However, hyperlipemia has also been reported to be associated with increased levels of cellular adhesion molecules (16–18), and evidence also suggests that postprandial hyperlipemia may condition an increase of these molecules (19). Moreover, it has been suggested that oxidative stress may be the mediator of the effect of both hyperglycemia and hypertriglyceridemia (12–13,19). In particular, the mechanism underlining free radical generation during an acute increase of both glycemia and

TABLE 1
Baseline characteristics of the normal and diabetic subjects

	Control subjects	Diabetic subjects
n	20	30
Sex (M/F)	12/8	22/18
Age (years)	53.5 ± 2.5	54.3 ± 2.6
BMI (kg/m^2)	28.4 ± 2.1	29.7 ± 2.3
Duration of disease (years)	—	5.9 ± 2.7
Fasting glucose (mmol/l)	4.8 ± 0.2	$11.1 \pm 2.2*$
HbA$_{1c}$ (%)	5.8 ± 0.2	$7.8 \pm 0.3*$
Resting systolic blood pressure (mmHg)	118.3 ± 7.5	122.4 ± 6.5
Resting diastolic blood pressure (mmHg)	78.4 ± 2.1	82.2 ± 3.1
Total cholesterol (mmol/l)	4.5 ± 0.6	$7.5 \pm 0.8*$
Triglycerides (mmol/l)	0.9 ± 0.2	$3.7 \pm 0.4*$
HDL cholesterol (mmol/l)	1.4 ± 0.2	$0.9 \pm 0.3*$
LDL cholesterol (mmol/l)	2.5 ± 0.3	$3.6 \pm 0.4*$
Nitrotyrosine (μmol/l)	0.23 ± 0.5	$0.56 \pm 0.3*$
ICAM-1 (ng/ml)	144.6 ± 22.7	$225.5 \pm 18.4*$
VCAM-1 (ng/ml)	630.8 ± 11.5	$934.6 \pm 32.7*$
E-selectin (ng/ml)	35.8 ± 18.5	$75.9 \pm 12.3*$

Data are means $\pm$ SE. $*P < 0.001$ vs. control.

triglyceridemia seems to be mediated by the induction of NADPH activity (20,21).

In our previous study (22), we demonstrated that postprandial hyperglycemia and postprandial hypertriglyceridemia may have a direct and cumulative effect in producing an endothelial dysfunction and nitrotyrosine generation. Nitrotyrosine is a suitable marker of peroxynitrite and nitrosative stress generation (23), and increased nitrotyrosine plasma level has been found in the plasma of diabetic patients (24,25).

The aim of this study was primarily to evaluate whether postprandial hypertriglyceridemia and hyperglycemia play a distinct role in producing an increase of cellular adhesion molecule plasma levels and if this phenomenon is accompanied by oxidative stress generation, as evaluated by nitrotyrosine formation. To demonstrate this hypothesis, ICAM-1, VCAM-1, and E-selectin plasma levels were measured after a fat-rich meal associated or not with the simultaneous administration of an oral glucose tolerance test (OGTT) in both diabetic patients with moderate hyperlipidemia and normal subjects. The secondary objective was to evaluate the possible effect of simvastatin treatment. Therefore, the diabetic patients were retested after 3–6 days or 3 months of simvastatin treatment. The objective of the short-term simvastatin treatment was to evaluate a possible direct effect of the drug on cellular adhesion molecule plasma levels, possibly through an inhibition of oxidative stress because statins can decrease nitrotyrosine production (24,25), whereas the long-term treatment was aimed to also evaluate the effect of decreased postprandial hypertriglyceridemia (17).

RESEARCH DESIGN AND METHODS

Thirty type 2 diabetic patients and 20 healthy subjects were recruited. The clinical characteristics of the groups are reported in Table 1. All subjects were nonsmokers and had a normal resting electrocardiogram and no personal history of vascular disease. None of the subjects were taking aspirin, lipid-lowering agents, or supplemental vitamins. Diabetes was treated in 18 patients with diet alone and with sulfonylureas in the remaining 12 patients. All subjects were recommended to consume their habitual diet during the entire period of the study. Written consent was obtained from all subjects, with local ethics committee approval.

Study design. At the end of a 4-week placebo run-in period, blood samples were taken, and fasting glycemia, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, nitrotyrosine, and HbA$_{1c}$ were measured. Therefore, diabetic patients and control subjects ate three different menus in randomized order on different days, i.e., a high-fat meal, 75 g glucose alone, or a high-fat meal plus 75 g glucose. Blood samples were drawn at 0, 1, 2, 3, and 4 h, and the following variables were assayed: glycemia, triglyceridemia, and nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels. Studies began at 8:00 A.M., after a 12- to 14-h overnight fast.

Subsequently, a double-blind, crossover, placebo-controlled study was started in diabetic patients. Diabetic subjects were randomly assigned to take simvastatin 40 mg/day or placebo for 12 weeks. The washout period between the simvastatin treatment and placebo period was 3 months. At baseline and at the end of each period, the following parameters were measured: fasting glycemia, serum total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, HbA$_{1c}$, nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels. The three meal tests described above were performed at baseline, between 3 and 6 days after the start of both placebo and simvastatin treatment and at the end of each study. The scheme of the protocol is illustrated in Fig. 1.

High-fat load. The standardized high-fat meal consisted of whipping cream and contained 75 g of fat, 5 g of carbohydrates, 6 g of proteins per square meter of body surface area. The corresponding caloric intake was 700 kcal/m^2.
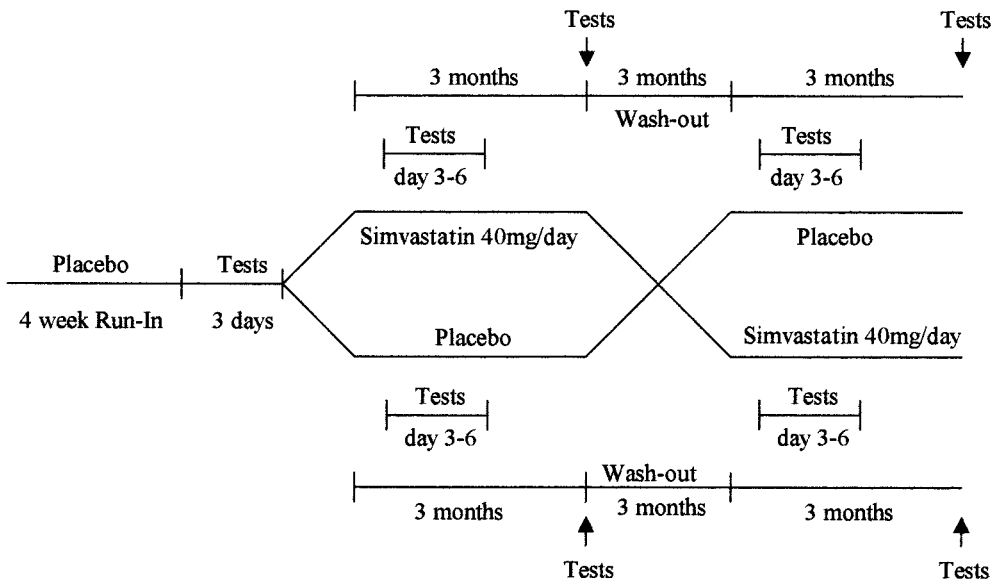

FIG. 1. Scheme of the simvastatin trial.

FIG. 2. Effects of a high-fat load, OGTT, and high-fat load plus OGTT on glycemia, triglycerides, ICAM-1, VCAM-1, and E-selectin levels and nitrotyrosine plasma levels in healthy, normal control subjects. Bars indicate SE. ○, high fat; ●, OGTT; △, high fat + OGTT.
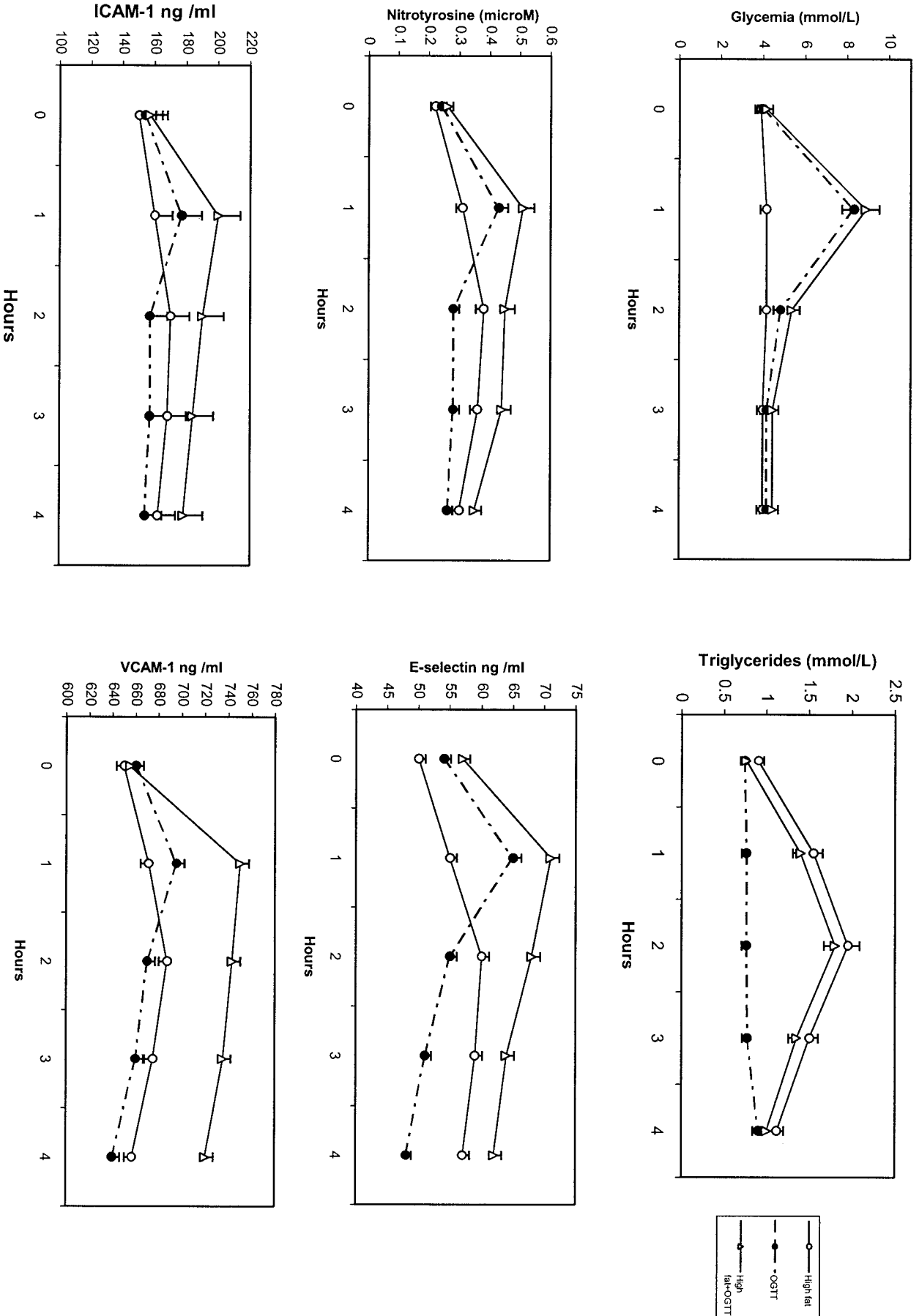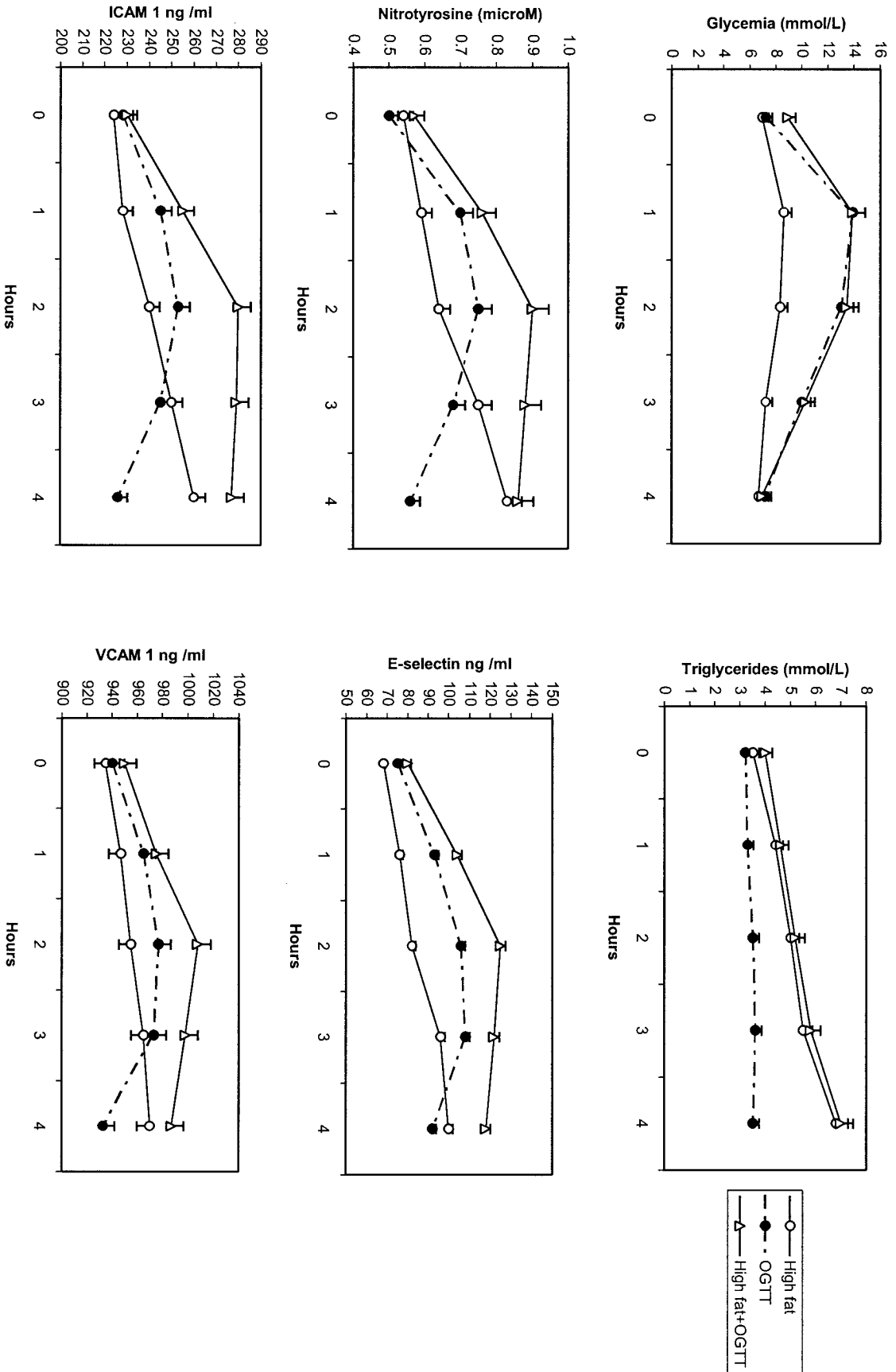
FIG. 3. Effects of a high-fat load, OGTT, and high-fat load plus OGTT on glycemia, triglycerides, ICAM-1, VCAM-1, and E-selectin levels and nitrotyrosine plasma levels in type 2 diabetic patients. Bars indicate SE. ○, high fat; ●, OGTT; △, high fat + OGTT.

TABLE 2
Effects of short- and long-term simvastatin treatment in diabetic patients

	3–6 days		3 months	
	Placebo	Simvastatin	Placebo	Simvastatin
BMI (kg/m^2)	29.8 ± 2.2	29.7 ± 2.1	29.5 ± 2.2	29.4 ± 2.3
Fasting glucose (mmol/l)	11.3 ± 2.0	11.4 ± 2.3	11.2 ± 3.2	10.8 ± 2.4
HbA$_{1c}$ (%)	7.8 ± 0.2	7.8 ± 0.3	7.8 ± 0.5	7.9 ± 0.5
Resting systolic blood pressure (mmHg)	119.1 ± 7.0	120.4 ± 6.5	125.3 ± 7.1	123.2 ± 7.5
Resting diastolic blood pressure (mmHg)	80.4 ± 2.1	81.2 ± 3.1	84.6 ± 2.1	82.4 ± 3.7
Total cholesterol (mmol/l)	7.4 ± 0.6	7.5 ± 0.8	7.5 ± 0.9	5.5 ± 0.6*
Triglycerides (mmol/l)	3.5 ± 0.5	3.7 ± 0.4	3.6 ± 0.5	2.3 ± 0.4*
HDL cholesterol (mmol/l)	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.4	1.2 ± 0.2†
LDL cholesterol (mmol/l)	3.6 ± 0.5	3.5 ± 0.4	3.5 ± 0.4	2.6 ± 0.6†
Nitrotyrosine (μmol/l)	0.55 ± 0.3	0.41 ± 0.2†	0.57 ± 0.4	0.32 ± 0.3*‡
ICAM-1 (ng/ml)	218.3 ± 15.1	189.2 ± 13.4†	220.2 ± 17.7	165.2 ± 11.1*‡
VCAM-1 (ng/ml)	949.4 ± 33.7	899.1 ± 27.8†	940.6 ± 29.0	820.2 ± 18.5*‡
E-selectin (ng/ml)	77.5 ± 11.3	59.8 ± 10.1†	79.2 ± 13.4	45.9 ± 10.0*‡

Data are means ± SE. *$P < 0.001$ vs. placebo; †$P < 0.05$ vs. placebo; ‡$P < 0.001$ vs. simvastatin short-term treatment.

Biochemical measurements. Cholesterol and triglycerides were measured enzymatically (Roche Diagnostics, Basel, Switzerland). HDL cholesterol was estimated after precipitation of apolipoprotein B with phosphotungstate/magnesium (26). LDL cholesterol was calculated after lipoprotein separation (27). Plasma glucose was measured by the glucose-oxidase method and HbA$_{1c}$ by high-performance liquid chromatography. Nitrotyrosine plasma concentration was assayed by enzyme-linked immunosorbent assay (24). Serum samples for ICAM-1, VCAM-1, and E-selectin were stored at −80°C until the time of the assay. Serum concentration was determined in duplicate with commercially available immunosorbent kits (British Bio-technology, Abington, Oxon, U.K.). Intra- and interassay coefficients of variation were 3.5 and 5.6, respectively.

Statistical analysis. The Kolmogorov-Smirnov algorithm was used to determine whether each variable had a normal distribution. Comparisons of baseline data among the groups were performed using the unpaired Student's t test. The paired Student's t test was used for comparison of the various parameters before and after treatment with simvastatin or placebo. The changes in variables during the tests were assessed by two-way ANOVA with repeated measures. If differences reached statistical significance, post hoc analyses with two-tailed paired t test was used to assess differences at individual time periods in the study, using Bonferroni correction for multiple comparisons. Statistical significance was defined as $P < 0.05$.

RESULTS

Baseline cholesterol, LDL cholesterol, triglycerides, nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels were increased in diabetic patients, whereas HDL cholesterol levels were reduced (Table 1). As compared with preprandial values, serum triglycerides were increased from 1 to 3 h during tests 1 and 3 in normal subjects ($P < 0.001$ vs. baseline) (Fig. 2) and from 1 to 4 h in diabetic patients ($P < 0.001$ vs. baseline) (Fig. 3). Glycemia increased at 1 h in normal subjects ($P < 0.001$ vs. baseline) (Fig. 2) and at 1, 2, and 3 h in diabetic patients during tests 2 and 3 ($P < 0.001$ vs. baseline) (Fig. 3), whereas triglycerides remained unchanged during test 2 (Figs. 2–3).

High-fat load alone produced an increase in nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels from 1 to 3 h in normal subjects ($P < 0.001$ vs. baseline) (Fig. 2) and from 1 to 4 h diabetic patients ($P < 0.001$ vs. baseline) (Fig. 3). The increase in glycemia during the OGTT was accompanied by a significant change in nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels during the studies ($P < 0.001$ vs. baseline) (Figs. 2–3).

The combination of high fat and a glucose load, i.e., test 3, produced an increase in nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels ($P < 0.001$ vs. baseline) (Figs.

2–3) that was even more pronounced than that with either nutrient taken alone ($P < 0.01$) (Figs. 2–3) in both normal and diabetic subjects. The increase in nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels was maximal at 1 h in normal subjects (Fig. 2), whereas in diabetic patients such variations were observed throughout the test, with a maximum at 2 h (Fig. 3).

Short-term simvastatin treatment had no effect on lipid parameters in diabetic patients (Table 2). However, a significant improvement in basal nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels was observed (Table 2). Such treatment was able to reduce the effect on nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels, which was observed during every different test, even though postprandial hypertriglyceridemia was not affected ($P < 0.001$ vs. placebo) (Figs. 4–6).

Long-term simvastatin treatment reduced fasting total cholesterol, LDL cholesterol, and triglycerides and raised HDL cholesterol in diabetic patients, whereas BMI and HbA$_{1c}$ remained unchanged throughout the study (Table 2). Long-term simvastatin treatment was accompanied by a significant improvement of fasting nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels (Table 2). When the high-fat load test was performed, the increase in postprandial triglycerides was lower compared with placebo ($P < 0.01$) (Figs. 4 and 6) and the increase in nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels was still significant, but significantly smaller ($P < 0.01$) (Figs. 4 and 6). During the glucose challenge, triglycerides remained unchanged and nitrotyrosine, ICAM-1, VCAM-1 and E-selectin plasma level increases were smaller than those in the placebo period ($P < 0.01$) (Fig. 5), although they were still significant. The combined load of a high-fat meal and glucose resulted in a smaller increase in triglycerides after simvastatin treatment ($P < 0.01$) (Figs. 4–6). Again, nitrotyrosine, ICAM-1, VCAM-1, and E-selectin plasma levels increased in this test, and the observed variations were smaller than those at the end of the placebo period ($P < 0.01$) (Figs. 4–6).

DISCUSSION

This study confirms that ICAM-1, VCAM-1, and E-selectin plasma levels are increased in type 2 diabetic patients.
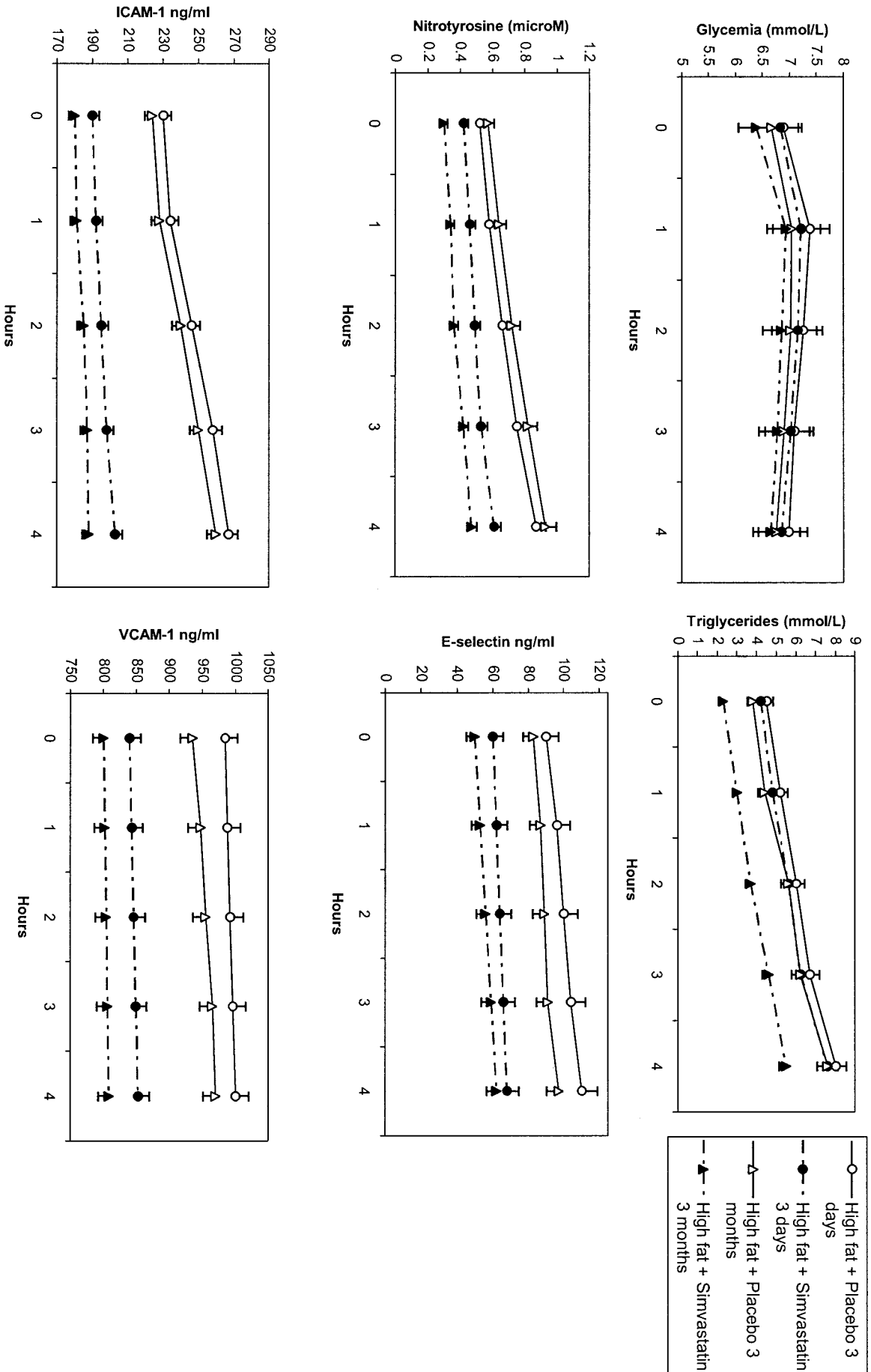
FIG. 4. Effects of a high-fat load on glycemia, triglycerides, ICAM-1, VCAM-1, and E-selectin levels and nitrotyrosine plasma levels in type 2 diabetic patients after short- and long-term simvastatin treatment. Bars indicate SE. ○, high fat + placebo at 3 days; ●, high fat + simvastatin at 3 days; △, high fat + placebo at 3 months; ▲, high fat + simvastatin at 3 months.
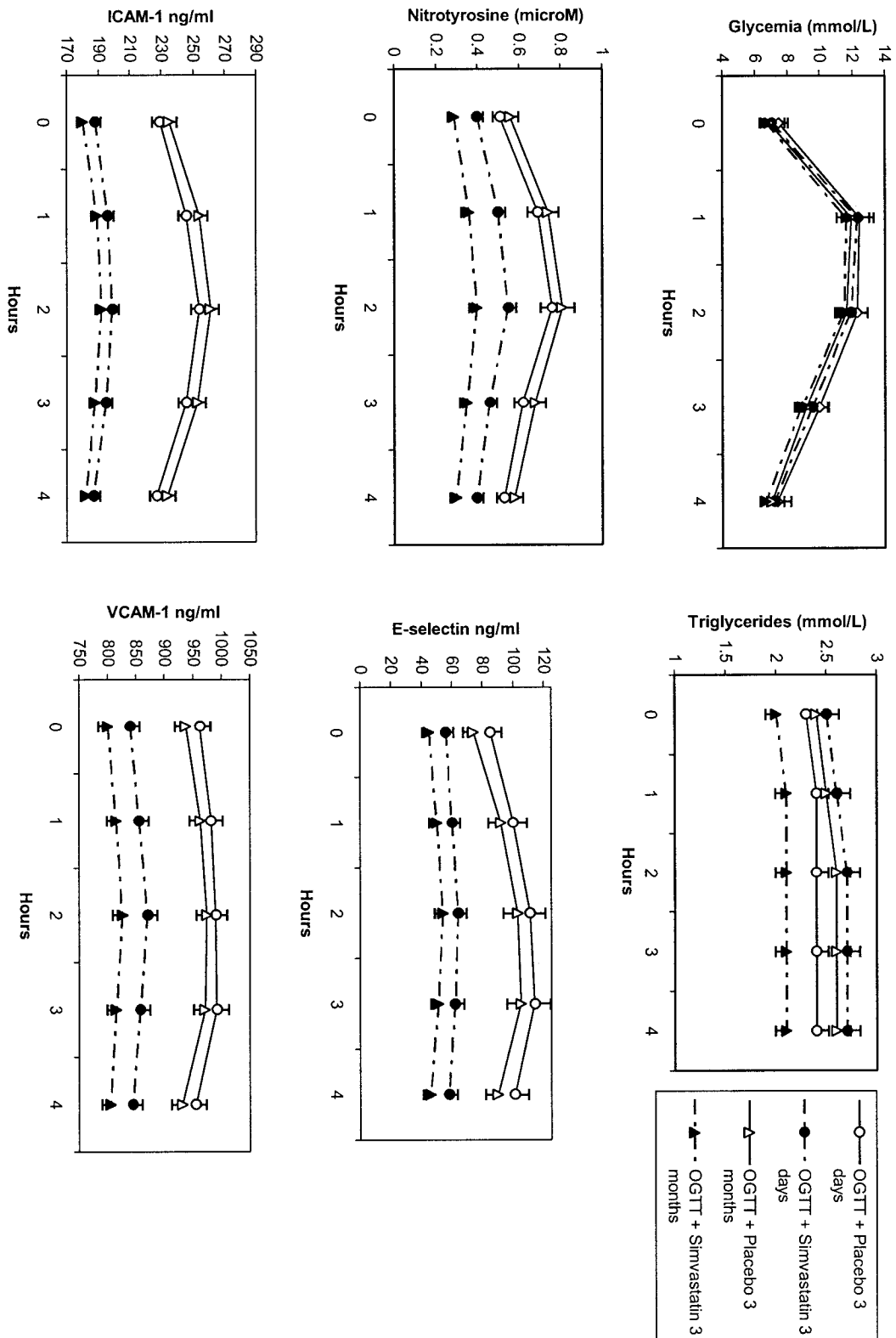
FIG. 5. Effects of an OGTT on glycemia, triglycerides, ICAM-1, VCAM-1, and E-selectin levels and nitrotyrosine plasma levels in type 2 diabetic patients after short- and long-term simvastatin treatment. Bars indicate SE. ○, OGTT + placebo at 3 days; ●, OGTT + simvastatin at 3 days; △, OGTT + placebo at 3 months; ▲, OGTT + simvastatin at 3 months.

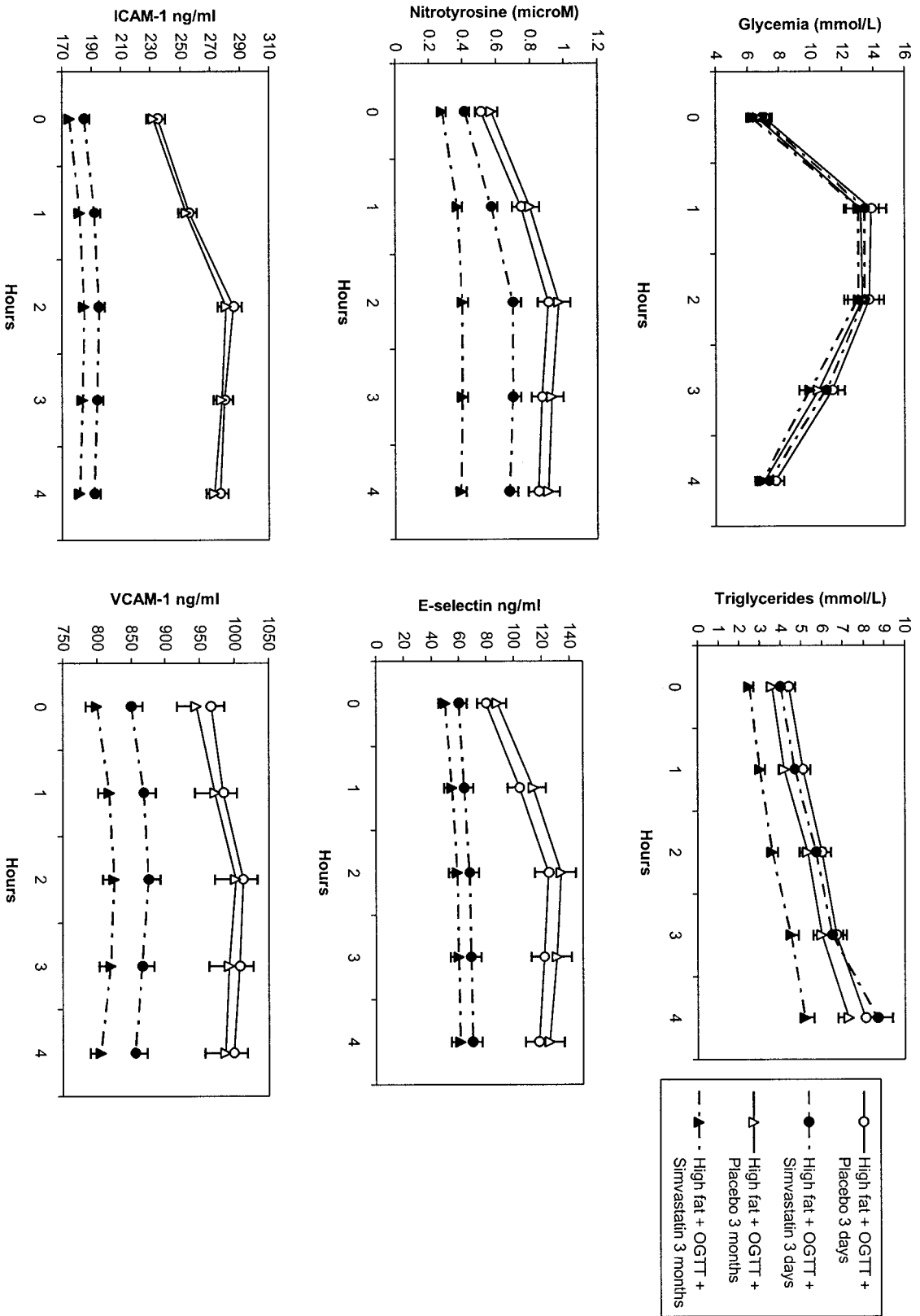FIG. 6. Effects of a high-fat load plus OGTT on glycemia, triglycerides, ICAM-1, VCAM-1, and E-selectin levels and nitrotyrosine plasma levels in type 2 diabetic patients after short- and long-term simvastatin treatment. Bars indicate SE. ○, high fat + OGTT + placebo at 3 days; ●, high fat + OGTT + simvastatin at 3 days; △, high fat + OGTT + placebo at 3 months; ▲, high fat + OGTT + simvastatin at 3 months.

However, for the first time, our data show that postprandial hyperglycemia and hypertriglyceridemia have an independent and cumulative effect in determining an acute rise of these molecules in the plasma. Our data also suggest that oxidative stress may be the common mediator of this phenomenon, since the increase of cellular adhesion molecules during both postprandial hyperglycemia and hypertriglyceridemia is accompanied by a significant nitrotyrosine increase.

Postprandial hypertriglyceridemia may represent an independent predictor of CVD in nondiabetic patients (2) and a predictor of carotid intima-media thickness in patients with type 2 diabetes (28). However, recent studies (1) support the hypothesis that postprandial hyperglycemia is also a risk factor for CVD. The notion that postprandial hypertriglyceridemia and hyperglycemia may be important factors for the development of CVD is supported by evidence that both induce an increase of the adhesion molecules ICAM-1, VCAM-1, and E-selectin (12–13,19), which have been shown to predict the development of atherosclerosis in diabetes (29–31).

Meal absorption is a complex phenomenon, and postprandial hyperlipidemia and hyperglycemia are simultaneously present in the postabsorptive phase, particularly in diabetic subjects and in subjects with impaired glucose tolerance. Therefore, a specific and direct role of hyperglycemia, independent of the concomitant hyperlipidemia, has been frequently questioned. Our study clearly shows that on both cellular adhesion molecules and nitrotyrosine, hyperglycemia has an earlier effect during the postprandial period, whereas hypertriglyceridemia has a delayed but lasting effect, and that the combination of both has an early and lasting effect. Moreover, when hyperglycemia and hypertriglyceridemia were simultaneously present, there was a greater increase in cellular adhesion molecules compared with that observed during either hyperglycemia or hypertriglyceridemia alone. These data suggest that postprandial hyperglycemia and hypertriglyceridemia have an independent but cumulative effect on favoring the development of atherosclerosis and that their combination may favor an atherogenetic postprandial profile for >4 h.

The independent role of hyperglycemia is also supported by the long-term simvastatin study in diabetic patients. Consistent with other studies, after that treatment, ICAM-1, VCAM-1, and E-selectin plasma levels are ameliorated in basal conditions (32–34) and the level of postprandial triglycerides after a high-fat load is significantly reduced (35). Consequently, there is a smaller increase in ICAM-1, VCAM-1, and E-selectin plasma levels during the fat load. In this condition, the independent role of hyperglycemia is pointed out by the demonstration that even in the presence of diminished postprandial hypertriglyceridemia, ICAM-1, VCAM-1, and E-selectin plasma levels increased during the OGTT.

It has been suggested that statins may have beneficial effects independently of their lipid-lowering effect (36). To examine this effect as well as its influence on the results of long-term simvastatin treatment, wherein postprandial hypertriglyceridemia is expected to decrease by treatment, we also performed a short-term trial. We found that a 3- to 6-day simvastatin treatment can ameliorate ICAM-1,

VCAM-1, and E-selectin plasma levels in diabetic patients, a phenomenon independent of the lipid-lowering effect of the drug, as the plasma lipid concentration was unchanged after this brief treatment. Such an effect is conceivably related to the reduction of oxidative stress because nitrotyrosine was significantly decreased, supporting the recent finding (37) that simvastatin may work as an intracellular antioxidant. Since it has been suggested that nitrotyrosine production may influence the overgeneration of adhesion molecules in hyperglycemia (38–39), it should be hypothesized that the reduction of nitrotyrosine during the simvastatin trial may play an important role in reducing cellular adhesion molecule plasma levels. Interestingly, nitrotyrosine has recently been shown as an independent risk factor for CVD (25).

Our data are different from the results by Dandona et al. (40), who recently reported that 80 mg of simvastatin in 1 week of treatment was not able to suppress the oxygen species generation and nuclear factor-κB activation in leukocytes from normal subjects. A possible explanation may be that normal subjects have a balanced production of free radicals that is not influenced by statin treatment; this is efficacious in the short term, when the inflammatory pathways are activated as in diabetic patients (38).

From a clinical point of view, these data suggest that the larger reduction of cellular adhesion molecules, and possibly the best approach to the prevention of CVD, in type 2 diabetes with combined hyperlipemia may be obtained by correcting both hyperglycemia and hyperlipemia. This goal may be very relevant because an association between the risk of future myocardial infarction and raised ICAM-1 plasma concentration has been found, at least, in nondiabetic patients (7,41), and an increased risk of cerebral infarct associated with increased ICAM-1 level has been demonstrated in diabetic patients (42).

In conclusion, the direct role of hyperglycemia in determining an increase of adhesion molecules in type 2 diabetes, although suggested, was questionable in previous studies because the possible concomitant role of hyperlipemia was not evaluated. In our opinion, this study clearly demonstrates that hyperglycemia is, by itself, a sufficient stimulus to increase adhesion molecules independently of hyperlipemia.

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