Effects of Insulin on Cholesterol Synthesis in Type II Diabetes Patients

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OBJECTIVE — To evaluate the effects of intensive insulin therapy and subsequent optimized metabolic control on daily urinary mevalonic acid (MVA) excretion, an index of whole-body cholesterol synthesis, and the acute effects of insulin on plasma MVA concentrations in type II diabetes.

RESEARCH DESIGN AND METHODS — Ten (five men and five postmenopausal women) nonobese, normolipidemic (total cholesterol <6.2 mmol/l, triglycerides <2.82 mmol/l), type II diabetic patients in poor metabolic control (HbA_{1c} >10%, fasting plasma glucose >11 mmol/l) and receiving sulfonylurea treatment were selected. The 24-h urinary MVA excretion and plasma lipid values were determined before and after intensive insulin therapy. The acute effects of insulin on plasma MVA concentrations were also evaluated during a 3-h euglycemic hyperinsulinemic clamp study.

RESULTS — Urinary MVA excretion rates (μmol/24 h) were 1.82 ± 0.21 in control subjects and 2.49 ± 0.35 ($P < 0.01$ vs. control subjects) and 1.78 ± 0.28 in patients before and after intensive insulin therapy, respectively. Total cholesterol, low-density-lipoprotein (LDL) cholesterol, and triglycerides decreased by 9, 8, and 12%, respectively, after blood glucose optimization. Acute insulin infusion during the euglycemic clamp studies reduced mean plasma MVA concentrations at 120 and 180 min by 29 and 38%, respectively ($P < 0.01$ for both vs. baseline).

CONCLUSIONS — Our study demonstrates that in nonobese, normolipidemic, type II diabetic patients under poor metabolic control, an increased cholesterol synthesis is normalized by insulin therapy. Hyperinsulinemia in the presence of euglycemia acutely decreases the circulating levels of MVA, the immediate product of hydroxymethylglutaryl-CoA reductase activity and an index of whole-body cholesterol synthesis.

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apo, apolipoprotein; FFA, free fatty acids; FPG, fasting plasma glucose; GC, gas chromatography; HDL, high-density lipoprotein; HMG-CoA, hydroxymethylglutaryl-CoA; IRI, immunoreactive insulin; LDL, low-density lipoprotein; MVA, mevalonic acid; MVL, mevalonolactone; MS, mass spectrometry; PPG, postprandial glucose; VLDL, very-low-density lipoprotein.

Type II diabetes is associated with accelerated atherosclerosis, and the risk of coronary artery disease is three- to fourfold increased when compared with the nondiabetic population (1–3).

Among the atherogenic risk factors, high or borderline high total cholesterol has been reported in 70% of adults with diagnosed and 77% of undiagnosed type II diabetes in the U.S. population (4). The improvement in glycemic control induced by sulfonylureas or insulin therapy reduces low-density lipoprotein (LDL) cholesterol by 8–10%, while plasma high-density lipoprotein (HDL) cholesterol concentrations increase by about 10–12% (5–8). Multiple abnormalities of very-low-density lipoprotein (VLDL) and LDL metabolism in type II diabetes have been discussed. Turnover studies have indicated increased VLDL synthesis and both decreased conversion of VLDL to LDL and impaired LDL clearance (9,10). Insulin therapy reduced the VLDL production rate and increased the fractional catabolic rate of LDL (11). Only a few studies have addressed the possibility that an increased cholesterol synthesis might contribute to the elevated cholesterol plasma concentrations. Using the sterol balance, a few studies reported a slight increase in cholesterol synthesis in type II diabetic patients under less-than-optimal metabolic control (12–14). Improved glycemic control after insulin treatment decreased cholesterol synthesis in one study (12) but not in others (13,14). In another study in patients with well-controlled diabetes, the cholesterol synthesis estimated by sterol balance was comparable with that of normolipidemic subjects and significantly increased only in those with hypertriglyceridemia (15). On the contrary, a recent study (16) demonstrates normal plasma lathosterol concentrations (an index of cholesterol synthesis) in type II diabetic patients under poor metabolic control and a clear-cut increase after a few days of optimized glucose con-

trol with a continuous intravenous insulin infusion.

Mevalonic acid (MVA), an intermediate in cholesterol biosynthesis and the immediate product of hydroxymethylglutaryl-CoA (HMG-CoA) reductase, is a normal constituent of human plasma (19). Plasma and urinary MVA concentrations correlate with cholesterol production rates in normal subjects (17,20,24) as well as in patients with different lipid disorders, namely, hypertriglyceridemia, homozygous and heterozygous familial hypercholesterolemia (17,18), abetalipoproteinemia, and homozygous hypobetalipoproteinemia (23). We have recently demonstrated that hyperinsulinemia in the presence of euglycemia acutely decreases MVA plasma concentrations in normal subjects (27).

The aim of our study in type II diabetes was to investigate *1*) the effects of intensive insulin therapy and subsequent optimized metabolic control on MVA daily urinary excretion, an index of whole-body cholesterol synthesis, and *2*) the acute effects of insulin on plasma MVA concentrations.

RESEARCH DESIGN AND METHODS

Patients

Ten normolipidemic (total cholesterol <6.2 mmol/l; triglycerides <2.82 mmol/l) type II diabetic patients (five men and five postmenopausal women), 52.2 ± 6.8 years of age, body mass index 25.8 ± 0.7 kg/m^2, with a mean duration of diabetes of 11.3 ± 2.4 years were selected from our outpatient population. All patients had responded adequately in terms of glucose control to glyburide therapy for at least 1 year. At the time of study, all patients had unsatisfactory blood glucose levels (defined as fasting plasma glucose >11 mmol/l and HbA_{1c} >10%) for 1 month despite repeated dietary and exercise counseling and maximal glyburide treatment (15 mg/day given as two doses). All patients were maintained on an isocaloric diet containing 55% carbohydrate (half refined and half complex), 25% fat (total cholesterol <300 mg/day and polyunsaturated fat/saturated fat <1), and 20% protein for at least 1 month before entering the study. They had neither proteinuria nor microalbuminuria. Hepatic function and thyroid tests were in the normal range in all participants. They were taking no medications known to affect cholesterol metabolism. Four patients were being treated with angiotensin-converting enzyme inhibitors for hypertension. All subjects gave informed consent for the investigation.

Experimental design

Patients were admitted to the metabolic unit with the above characteristics and fed the standardized diet described above. They were weighed daily to maintain a constant weight throughout the study. One week after admission (poor metabolic control [period 1]), blood and 24-h urine samples were collected for measurement of fasting plasma glucose (FPG), postprandial glucose (PPG), fasting insulin, free fatty acids (FFA), total cholesterol, HDL cholesterol, triglycerides, apolipoprotein (apo) A-1, apo B-100, and the 24-h urinary MVA excretion. Daily mevalonate excretion was also determined in 10 normal subjects matched for age and sex who had been following the above-described diet for at least 1 month. On completion of period 1, glyburide administration was stopped, and all patients received intensive insulin therapy using multiple daily injections until excellent glycemic control (FPG and PPG <8 and <10 mmol/l, respectively) was achieved and maintained for 3 weeks (good metabolic control [period 2]). The same measurements performed at the end of period 1 were repeated at the end of period 2; moreover, on two subsequent days, the plasma MVA physiological variations were evaluated in the fasting state under saline infusion at 9:00 and 10:30 A.M. and 12:00 P.M. and a 3-h euglycemic hyperinsulinemic clamp study was performed starting at 9:00 A.M. after an overnight fast.

Insulin clamp

Insulin (1 mU · kg^{-1} · min^{-1}) was infused to obtain steady-state plasma insulin levels of ~600 pmol/l. Euglycemia was maintained with a variable 20% dextrose infusion throughout the study. Glucose levels were measured every 5 min during the 3-h study. The amount of glucose metabolized (mg · kg^{-1} · min^{-1}) was calculated using previously described methods (28). Venous blood samples were drawn immediately before and every 60 min thereafter for the determination of apo A-1 and apo B-100, FFA, and MVA concentrations. Aliquots of plasma samples were then ultracentrifuged to separate VLDL (d < 1.006 g/ml) and LDL (1.006 < d < 1.063 g/ml) for the determination of VLDL cholesterol, VLDL triglycerides, and LDL cholesterol.

Assays

Glucose concentrations were determined using a Beckman glucose analyzer (Beckman, Fullerton, CA). Insulin was measured using a double-antibody radioimmunoassay technique (Pharmacia Kit, Uppsala, Sweden). Blood HbA_{1c} was determined by chromatography (normal value < 6%) (Boheringer Mannheim, Mannheim, Germany). Serum cholesterol and triglycerides were determined in whole plasma and lipoproteins by enzymatic colorimetric methods using commercially available kits (Boehringer Mannheim) (29,30). Plasma HDL cholesterol concentration was determined after precipitation of apo B–containing lipoproteins with sodium phosphotungstate/magnesium chloride as described previously (31). Total plasma FFA and apo A-1 and apo B-100 levels were determined by enzymatic and immunoturbidimetric assays, respectively (Boehringer Mannheim). The plasma concentrations and 24-h urine excretion of MVA were quantified using an extension of our previously described method (26). The MVA was extracted (from 1 ml of plasma or from 100 μl of urine) with addition of 5 ng [$^2H_3$]mevalonolactone (MVL) to each sample as internal standard. Essentially,

Table 1—*Effects of optimized metabolic control in type II diabetic patients on diet, weight, HbA_{1c}, FPG, PPG, IRI, FFAs, lipids, lipoproteins, and apolipoproteins*

	Period 1	Period 2
Diet (kcal/day)	1,580 ± 81	1,565 ± 87.6
Weight (kg)	70.5 ± 2.85	70.6 ± 2.72
HbA_{1c} (%)	10.7 ± 0.4	7.8 ± 2.7†
FPG (mmol/l)	13.8 ± 0.7	7.6 ± 0.3†
PPG (mmol/l)	18.7 ± 0.7	8.9 ± 0.5†
IRI (pmol/l)	74.4 ± 14	153.4 ± 57.9*
FFAs (μmol/l)	646 ± 137	458 ± 64†
Total cholesterol (mmol/l)	5.3 ± 0.3	4.8 ± 0.2
LDL cholesterol (mmol/l)	3.78 ± 0.2	3.47 ± 0.3
HDL cholesterol (mmol/l)	1.2 ± 0.1	1.3 ± 0.2
Triglycerides (mmol/l)	1.6 ± 0.1	1.4 ± 0.1
Apo A-1 (mg/dl)	94.3 ± 16.8	98.7 ± 23.7
Apo B-100 (mg/dl)	75.5 ± 23.2	73.2 ± 16.7

Data are means ± SD.
*$P < 0.01$, †$P < 0.001$ vs. period 1. LDL cholesterol determined by Friedewald's formula.

plasma was diluted with 0.1 mol/l phosphate buffer, pH 7.4 (1:1.5 vol/vol), and incubated with Dowex 50 (H^+) for 1 h at 30°C to convert free MVA to MVL. After the removal of resin, the nonpolar lipids were extracted in hexane (1 ml). For urine samples, extraction with hexane was avoided. MVL from the aqueous solution was extracted into CH_2Cl_2-isopropanol (9:1 vol/vol) (3 × 5 ml), which was loaded into an Lc-Si silica cartridge for the purification. The lactone was finally reconverted to free MVA by incubation with 100 μl diisopropylethylamine-water (1:1.5 vol/vol) for 1 h at room temperature. Then, the samples were desiccated by a freezer dry vacuum pump, and MVA was converted to its 3,5-bis(trifluoroethyl)-benzyl ester by addition of acetonitrile (20 μl), diisopropylethylamine (10 μl), and 3,5-bis(trifluoroethyl)benzyl bromide-acetonitrile (1:1 vol/vol). After 30 min at room temperature, the reagents were removed under a stream of nitrogen. The trimethylsilyl ether derivative was then prepared by incubation with 30 μl bis(trimethylsilyl)trifluoroacetamide for 18 h at room temperature. Excess reagent was removed under nitrogen, and the samples were reconstituted in *n*-octane for gas chromatography (GC)–mass spectrometry (MS).

Derivatives were chromatographed on a 35 m–long, 0.20 mm inner diameter fused silica capillary column coated with SPB 1 stationary bonded phase, operating at 100–250°C with a temperature gradient of 20°C/min. The GC column was routed into the ion source of a VG Biotech-Quattro mass spectrometer. The amount of the derived MVA was measured by chemical ionization-MS using CH_4 as reactant gas, operating the spectrometer in the single-ion mode to enhance the sensitivity and the accuracy of the analysis. With our new modified method, recovery through the assay monitored by adding 0.04 mCi [^{3}H]MVL to some normal samples was 75.7 ± 2.8% (n = 25). The limit of detection is 10 pg derivatized MVA. The intra- and interassay coefficients of variation were 5.3 and 6.6%, respectively. All MVA concentrations were assayed in duplicate.

Statistical analysis

Values are expressed as means ± SD. The significance of differences between means was calculated by the Student's paired or unpaired *t* test using the Statview statistical package (Abacus Concepts, Berkeley, CA).

RESULTS — There were statistically significant improvements in glycemic control in the diabetic patients after intensive glycemic management. The initial mean HbA_{1c}, FPG, PPG, immunoreactive insulin (IRI), and FFA concentrations were 10.7 ± 0.4%, 13.8 ± 0.7 mmol/l, 18.7 ± 0.7 mmol/l, 74.4 ± 14.0 pmol/l, and 646 ± 137 mmol/l, respectively. After the initiation of insulin treatment, HbA_{1c}, FPG, PPG, IRI, and FFA concentrations decreased significantly as shown in Table 1 ($P < 0.001$). There were no variations in body weight and caloric intake during the study. Total cholesterol, LDL cholesterol, and triglycerides decreased by about 9, 8, and 12%, respectively, while HDL cholesterol increased by about 8% and apolipoprotein concentrations did not change (Table 1). The 24-h urinary MVA excretion in the diabetic patients during period 1 was significantly higher (2.49 ± 0.35 μmol/24 h) than in control subjects (1.82 ± 0.21 μmol/24 h) ($P < 0.01$). The achievement of an optimized metabolic control (period 2) significantly ($P < 0.01$) reduced the 24-h urinary MVA output to a level (1.78 ± 0.28 μmol/24 h) not different from those of control subjects (Fig. 1).

The mean basal MVA plasma values were similar during the clamp study (5.04 ± 0.66 ng/ml), during the saline infusion (5.16 ± 0.76 ng/ml), and in the normal range for our laboratory (2.11–9.63 ng/ml). Under saline infusion, MVA concentrations slightly decreased from 9:00 A.M. to 12:00 P.M. (mean percentage decrease, 13%; $P < 0.005$ vs. baseline) (Fig. 2). Data on lipids, lipoproteins, and apolipoproteins during the clamp studies are reported in Table 2. With increasing insulin levels, mean plasma FFA decreased significantly from 415 ± 98 μmol/l at baseline to 84 ± 31 μmol/l at 180 min ($P < 0.001$). Slight and significant reductions were noted for VLDL cholesterol at 120 and 180 min ($P < 0.001$) and for VLDL triglycerides at 60, 120, and 180 min ($P < 0.01$). LDL cholesterol, HDL cholesterol, and apo A-1 and apo B-100 showed no significant

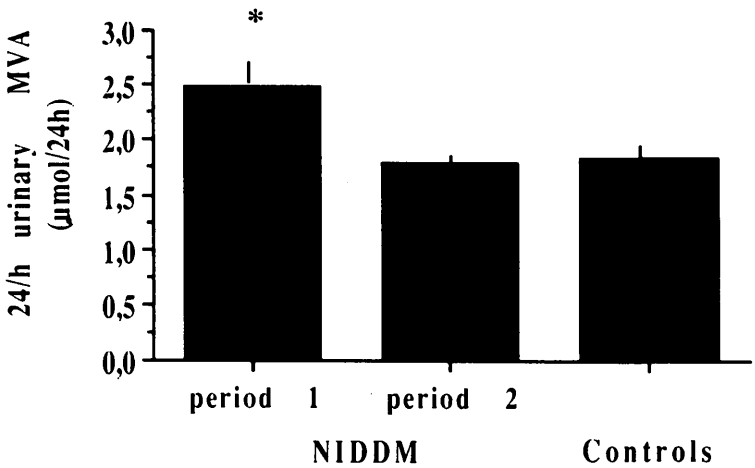

Figure 1—*Effects of optimized metabolic control on 24-h urinary MVA excretion in type II diabetic patients compared with control subjects.* *P < *0.01 vs. controls.*

changes. MVA concentrations were 4.12 ± 0.5, 3.58 ± 0.36, and 3.1 ± 0.24 ng/ml at 60, 120, and 180 min, respectively. All were significantly different from baseline ($P < 0.01$) with a decrease of 29 and 38% at 120 and 180 min, respectively (Fig. 3). Mean MVA values at 180 min during the clamp study (3.1 ± 0.24 ng/ml) were significantly lower ($P < 0.01$) than during saline infusion (4.44 ± 0.58 ng/ml), and the percentage of MVA change at 180 min during the clamp study (−38%) was significantly higher ($P < 0.01$) than during saline infusion (−13%) (Fig. 2). The mean value of metabolized glucose was 3.93 ± 0.42 $mg \cdot kg^{-1} \cdot min^{-1}$.

CONCLUSIONS— Although atherosclerosis involving arterial accumulation of cholesterol is a common and serious complication of diabetes mellitus, the effect of insulin on cholesterol metabolism is largely unknown. Assuming that 24-h urinary MVA excretion is an index of whole-body cholesterol synthesis, we undertook the present study to explore whether defects in sterol synthesis can be identified in type II diabetic patients. Moreover, we sought to determine whether intensive insulin treatment affects cholesterol synthesis. Finally, we explored the acute effects of insulin on MVA plasma concentrations. By studying a selected group of normolipidemic type II diabetic patients under strict metabolic ward conditions, we found that intensive insulin treatment normalized the increased cholesterol synthesis.

There have been only a few studies on the direct effect of insulin on cholesterol synthesis in type II diabetes. Bennion and Grundy (12) studied four obese Pima Indians and reported an 18.5% increase in cholesterol synthesis during uncontrolled hyperglycemia. Saudek and Brach (13), who studied 5 nonobese type II diabetic patients, and Abrams et al. (14), who studied 14 moderately obese diabetic patients, reported similar increases in cholesterol synthesis with no effect on insulin treatment. Finally, Briones et al. (15) studied a group of patients with well-controlled type I diabetes and type II diabetes and observed increased cholesterol synthesis only in those with hypertriglyceridemia. The normolipidemic patients in the above-cited study had a mean cholesterol synthesis comparable with that of control subjects. Because of the potential confounding effects of obesity, hypertriglyceridemia, and hypercholesterolemia on cholesterol synthesis, we selected a group of patients of normal weight who despite poor metabolic control were not hyperlipidemic.

Our data demonstrate a clear-cut increase (+29%) in cholesterol synthesis in our patients under poor metabolic control and a normalization of the cholesterol synthesis after intensive insulin treatment. A number of differences between our study and the previous ones may help to understand the different results. Subjects from previous studies were either obese (12) or dyslipidemic (13–15), two possible causes of enhanced cholesterol production not necessarily related to diabetes. One study reported that there was also a diminished caloric intake during the study (12).

The methods used to assess cho-

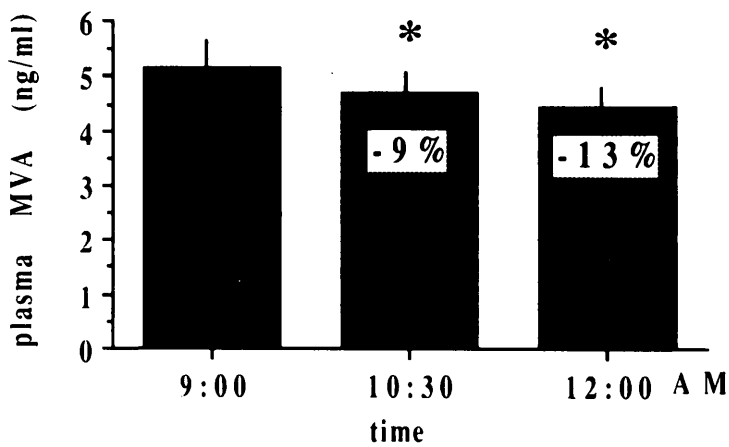

Figure 2—*Plasma MVA during saline infusion in type II diabetic patients studied from 9:00 A.M. to 12:00 P.M. Normal range for our laboratory: 2.11–9.63 ng/ml.* *P < *0.005 vs. 9:00 A.M.*

Table 2—*IRI, FFAs, lipoproteins, and apolipoproteins in type II diabetic patients during the clamp study*

	0 min	60 min	120 min	180 min
IRI (pmol/l)	81 ± 22.2	436 ± 64.2*	533.2 ± 76*	532 ± 66*
FFA (μmol/l)	415 ± 98	160 ± 42*	123 ± 23*	84 ± 31*
VLDL cholesterol (mmol/l)	0.72 ± 0.1	0.66 ± 0.1	0.64 ± 0.1*	0.63 ± 0.1*
VLDL triglycerides (mmol/l)	1.32 ± 0.2	1.20 ± 0.2†	1.16 ± 0.1†	1.13 ± 0.1†
LDL cholesterol (mmol/l)	2.84 ± 0.3	2.85 ± 0.3	2.84 ± 0.3	2.69 ± 0.2
HDL cholesterol (mmol/l)	1.02 ± 0.07	1.03 ± 0.07	1.01 ± 0.08	1.01 ± 0.07
Apo A-1 (mg/dl)	95.7 ± 8	94.7 ± 8.4	98 ± 8.2	96.1 ± 7.1
Apo B-100 (mg/dl)	76.2 ± 5.2	76.2 ± 6.8	75.8 ± 7	72.8 ± 6.2

Date are means ± SD.
*$P < 0.01$, †$P < 0.001$ vs. baseline.

lesterol synthesis rates deserve special comments. Cholesterol intake/balance methods (sterol balance), although accurate in assessing cholesterol formation rates over periods of weeks, require reliance on comprehensive stool collections as well as data on food intake and/or adherence to a test diet. The sterol balance technique cannot measure diurnal variations or other short-term regulatory influences because it requires a prolonged steady state. In abetalipoproteinemia and homozygous hypobetalipoproteinemia, for example, sterol balance techniques gave lower rates of whole-body cholesterol synthesis than MVA urinary determinations (23). The magnitude of this difference appeared to be largely attributable to malabsorption of endogenously synthesized cholesterol secreted into bile (23). Plasma MVA concentrations correlate with cholesterol production rates evaluated by sterol balance, and incorporation of deuterated water (17,18,20) correlates with HMG-CoA reductase activity in human hepatocytes (24) and is decreased by HMG-CoA reductase inhibitors (18,26). Urinary excretion of MVA varies in parallel with plasma concentration in normal subjects (32) and is reduced in heterozygous familial hypercholesterolemia by administration of HMG-CoA reductase inhibitors (22). Finally, our own method of MVA determination by GC-MS (26) closely parallels the radioenzymatic assay (25). The differences between our results and those of Feillet et al. (16) are difficult to explain. Both MVA and lathosterol are well-established indexes of cholesterol synthesis rates but differ in being the respective products of early and late steps on the cholesterol-biosynthetic pathway. Plasma levels of these two compounds show circadian variations, and we believe that the 24-h urinary mevalonate excretion reflects the daily cholesterol synthesis more accurately than a single fasting plasma determination of MVA or lathosterol (18,21–23,32). In a previous study, plasma lathosterol concentrations were similar in 52 patients with well-controlled type II diabetes and in nondiabetic control subjects (33). Perhaps, differences in patient selection and/or in the protocol adopted might have determined the differing results.

Our study, in which patients were neither obese nor dyslipidemic and in which their weight was held constant, provides evidence that uncontrolled diabetic hyperglycemia is an independent cause of increased cholesterol production. The mechanism responsible for this effect might be the perturbed LDL metabolism in type II diabetes. Kissebah et al. (34) and Howard et al. (9) found that in moderately severe diabetic subjects, the fractional catabolic rate of LDL apo B was reduced. Whatever the cause of this abnormality in LDL catabolism, this would lead to decreased inhibition of HMG-CoA reductase activity inside the cells and elevated rates of endogenous cholesterogenesis. The finding by Taskinen et al. (11) and, to a minor extent, by Howard et al. (9) of a direct LDL–apo B synthesis in type II diabetic subjects under poor metabolic control (not all LDL–apo B could be accounted for by the delipidation of VLDL) gives indirect support to the hypothesis of an increased cholesterogenesis, presumably in the liver. Questions about the effects of insulin on the sterol metabolism were raised because normalization of urine mevalonate excretion af-

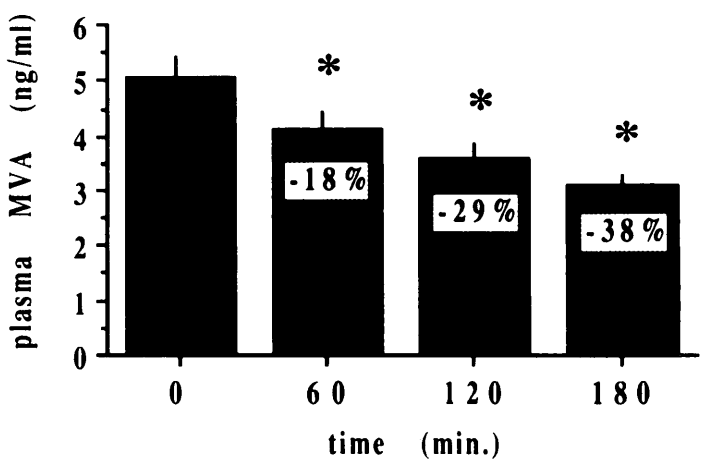

Figure 3—*Plasma MVA concentrations during clamp study in type II diabetic patients.* *P < *0.01 vs. baseline.*

ter blood glucose optimization was achieved with insulin therapy. A previous study failed to demonstrate any difference in the individual nocturnal peaks of MVA concentrations between patients with well-controlled type I diabetes and normal subjects (21). This finding supports our hypothesis that insulin-treated diabetic patients that are normolipidemic and maintained in good metabolic control do not have increased cholesterogenesis. The effects of insulin on HMG-CoA reductase activity and on cholesterol synthesis are yet to be clarified. Under certain circumstances, insulin increases hepatic HMG-CoA reductase activity in rats (35–38), but the increases in the rate of cholesterol synthesis are either absent (35) or relatively minor (36). HMG-CoA reductase activity and de novo cholesterogenesis increase in the gut of streptozotocin diabetic rats and decrease after insulin therapy (39). Animal models resembling human type II diabetic patients have increased liver cholesterogenesis (40).

In two in vivo animal studies in which cholesterol synthesis was assessed by urinary MVA excretion, the induction of diabetes increased urinary MVA output sixfold, with increased intestinal and liver HMG-CoA reductase activity (40–42). Most significantly, insulin administration resulted in a 57% decrease in urinary MVA excretion which, however, remained higher than normal (42). It is apparent from the animal models thus far studied that insulin therapy reduces the increased cholesterogenesis commonly found during uncontrolled hyperglycemia. Therefore, the reduction of whole-body cholesterogenesis, which we observed in patients under tight metabolic control, was not unexpected. To clarify further the effect of insulin on MVA, we also performed in our patients a 3-h hyperinsulinemic clamp study. Basal MVA values were in the normal range for our laboratory, and the slight decrease after saline infusion reflected the physiological fall throughout the morning related to the diurnal rhythm of this compound in normal subjects (17,27). The clear-cut reduction observed during the clamp studies confirms previous observations in normal subjects (27). The decreased VLDL cholesterol concentrations may further support the hypothesis of a reduction of the hepatic cholesterol synthesis.

The mechanisms involved in the insulin-induced decrease of mevalonate concentrations have not been explored in the present study. One possibility is that a decrease in fatty acids arriving at the liver had decreased acetyl-CoA for mevalonate synthesis (43). A direct relationship between FFA and MVA concentrations was denied in a report aimed at evaluating the role of growth hormone in nocturnal lipolysis (21).

Another possible explanation resides in the ability of insulin to stimulate in vivo LDL catabolism (44). Because insulin therapy in type II diabetic patients increases LDL–apo B fractional catabolic rate and reduces the elevated direct input into this fraction (11), we suggest insulin-enhanced LDL receptor activity, thus inhibiting HMG-CoA reductase activity and reducing daily MVA excretion to normal values. According to previous studies (27,44), we did not observe any significant change of LDL cholesterol during the clamp study, owing to the long half-life of LDL particles in relation to the duration of insulin infusion.

Our study demonstrates that nonobese type II normolipidemic diabetic patients under poor metabolic control have an increased cholesterol synthesis that is normalized by insulin therapy. Hyperinsulinemia in the presence of euglycemia acutely decreases the circulating levels of MVA, the immediate product of HMG-CoA reductase activity, and an index of whole-body cholesterol synthesis.

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