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Continuous Infusion of Interleukin-1 β in Rats Induces a Profound Fall in Plasma Levels of Cholesterol and Triglycerides

Ad R.M.M. Hermus, C.G.J. Sweep, Pierre N.M. Demacker, Mike J.M. van der Meer, Peter W.C. Kloppenborg, and Jos W.M. van der Meer

During infectious diseases, striking alterations in plasma concentrations of cholesterol (hypcholesterolemia) and triglycerides (hypertriglyceridemia) may occur. It has been suggested that interleukin-1 is a mediator of these alterations. We studied the effects of continuous administration of recombinant human interleukin-1 β (rhIL-1 β) on plasma levels of cholesterol and triglycerides. A total of 42 rats were equipped with minipumps loaded with either rhIL-1 β (delivery rate of 0.5, 2.0, or 4.0 μ g/day i.p. for 1 week) or saline. After 1 day of treatment with rhIL-1 β , plasma cholesterol levels had not changed. On day 2 a remarkable decrease of plasma cholesterol levels was observed in rats treated with 2.0 μ g rhIL-1 β /day (1.49 ± 0.13 versus 2.23 ± 0.08 mmol/l, $p < 0.005$; rhIL-1 β versus saline) or 4.0 μ g rhIL-1 β /day (1.46 ± 0.04 versus 2.18 ± 0.04 mmol/l, $p < 0.0005$). This decrease persisted until the end of the experiment and occurred in all major lipoprotein fractions. Triglycerides in plasma (and in very low density lipoprotein) decreased almost concomitantly with plasma cholesterol, although to a lesser degree. Infusion of 2.0 μ g rhIL-1 β /day did not affect either cholesterol esterification or total postheparin lipolytic activity in plasma. Long-term infusion with 4.0 μ g rhIL-1 β /day induced prolonged fever, whereas at the lower doses temperatures were elevated only the first 2 days. rhIL-1 β at a dose of 2.0 and 4.0 μ g/day induced a transient decrease of food intake and a suppression of body weight gain. Restriction of food consumption to the level observed in the 2.0 μ g rhIL-1 β experiment caused only a small decrease of plasma cholesterol level and had no effects on plasma concentrations of triglycerides. Therefore, it is unlikely that the decline in triglyceride levels during rhIL-1 β infusion was caused by a decrease in food intake. Diminished food consumption also cannot completely explain the profound decline of cholesterol levels during rhIL-1 β administration. Whether IL-1 plays a role as a mediator of the lowering of plasma cholesterol levels during infections remains to be determined. (*Arteriosclerosis and Thrombosis* 1992;12:1036-1043)

KEY WORDS • interleukin-1 • cholesterol • triglycerides • lipids • rats

During infectious diseases, striking alterations in the plasma concentrations of cholesterol and triglycerides may occur in both humans and laboratory animals. Except for studies of rabbits,^{1,2} most reports show that plasma cholesterol levels are decreased during the febrile phase of bacterial infections.³⁻⁸ The mechanism causing hypcholesterolemia during infectious diseases is unknown. In contrast, plasma levels of triglycerides are usually elevated in bacterial, viral, and parasitic infections, particularly during recovery.⁸⁻¹² In animal models of infection a decrease in the catabolism of circulating triglyceride-rich lipoproteins has been observed.¹³

Other studies have emphasized the role of increased hepatic synthesis of such particles during infection.^{14,15} Cerami and coworkers (Kawakami and Cerami¹⁶ and Kawakami et al¹⁷) were the first to suggest that macrophage factors are important mediators of the hypertriglyceridemia of infection. Their hypothesis was based on the observation that endotoxin-stimulated mouse macrophages secrete a factor that decreases the activity of adipose tissue lipoprotein lipase (LPL) as well as the synthesis and storage of lipids in cultured fat cells.^{16,17} In later studies this macrophage factor was identified as tumor necrosis factor (TNF).¹⁸ Further indirect evidence for the concept that TNF is an important mediator of the hypertriglyceridemia of infection came from a series of studies conducted by Feingold and Grunfeld. These researchers showed that administration of TNF to rodents produces hypertriglyceridemia mainly by increased hepatic synthesis of triglyceride-rich lipoproteins.¹⁹⁻²³

Interleukin-1 (IL-1) is a cytokine that shares with TNF a variety of important biological effects.²⁴ Increased levels of both cytokines have been measured in biological fluids of patients suffering from infections.^{25,26} Although the effects of TNF have been extensively studied, only a few reports have focused on the effects of IL-1 on lipid metabolism. Recently it was demonstrated

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that a single bolus injection of IL-1 causes hypertriglyceridemia in rats.^{27,28} These short-term studies demonstrate that IL-1, like TNF, is a potential mediator of the hypertriglyceridemia of infection. Until now no data have been available with respect to the effects of long-term administration of IL-1 on plasma cholesterol and triglyceride levels in rats. Although IL-1 is likely released in a pulsatile fashion during most infections, long-term administration of IL-1 is of interest because in some infections and especially in chronic inflammation, IL-1 may be released in a more continuous fashion. Such a long-term study is also relevant because rapid development of tolerance with respect to a number of biological effects of IL-1 occurs during long-term administration of this peptide.^{29,30} In this study we show that long-term intraperitoneal administration of relatively low doses of IL-1 to rats for 1 week caused a profound decrease of plasma cholesterol levels as well as a moderate decline in triglyceride levels.

Methods

Test Materials

Recombinant human IL-1 β (rhIL-1 β) was kindly provided by Dr. D. Boraschi (Sclavo, Siena, Italy). The preparation had a specific activity of 10⁹ units/mg protein on D10.G4.1 cells, corresponding to an activity of 10⁵ units/ μ g versus the interim IL-1 β reference reagent 86/552. According to the specifications of the suppliers, endotoxin contamination was negligible (<1.2 ng lipopolysaccharide/mg IL-1). rhIL-1 β was diluted in sterile pyrogen-free saline.

Animals

Male albino Wistar rats (Cpb:WU) were obtained from a local breeding facility. They were housed in an artificially lighted room (lights on at 7 AM and off at 7 PM) in individual Plexiglas cages. Rats were fed with commercial rat chow (RMH-TM, Hope Farms, Woerden, The Netherlands) and tap water ad libitum. The diet contained 22% protein, 4.8% fat, 5.1% fiber, and 61.7% other carbohydrates. At the start of the experiments rats were 10 weeks old and weighed 200–220 g. All experimental procedures were in accordance with institutional guidelines concerning the care and use of laboratory animals.

Experimental Protocols

Long-term administration of rhIL-1 β . To accommodate the animals to the stress of the experimental procedures, they were handled daily by the experimenter starting 1 week before venous cannulation. Body weight was measured daily at 8 AM, and food and water intake was recorded by weighing the residual food and water for individual cages. Body temperature was measured serially two times a day between 8:30 and 9 AM and between 1 and 2:30 PM in conscious hand-held rats by insertion of a thermal probe into the rectum. The probe was connected to a digital temperature monitor (Digital DT100, Elbatron, Kerckdriel, The Netherlands). Mean daily temperature for each rat was determined by averaging the morning and afternoon rectal temperatures. Blood was collected from freely moving rats by means of an indwelling cannula. Rats were cannulated as described by Steffens.³¹ Under anesthesia with pen-

tobarbital (60 mg/kg body wt i.p., Apharma, Arnhem, The Netherlands) and atropine (0.125 mg/kg body wt i.m., Pharmachemie, Haarlem, The Netherlands), a Silastic cannula (0.5 mm i.d., 0.94 mm o.d., Dow-Corning Corp., Midland, Mich.) was inserted into the right external jugular vein under sterile conditions and passed down to the atrium. The distal end of the cannula was tunneled subcutaneously and exteriorized through a stab wound in the skin of the head, where it was connected to a hooked stainless steel tube. This assembly was anchored to the skull with three stainless steel screws and acrylic cement. During cannulation, the rats were continuously exposed to a gas flow of O₂/N₂O. The cannula was filled with a 0.9% NaCl solution containing 500 IU/ml heparin (Thromboliquine, Organon Teknika, Boxtel, The Netherlands) and polyvinylpyrrolidone (1 g/ml, Merck, Darmstadt, FRG).

Seven to nine days after cannulation, rats were equipped with intraperitoneal osmotic minipumps (2 to 4 μ M). The osmotic pumps (1 μ l/hr, model 2001, Alzet Corp., Palo Alto, Calif.) were loaded before implantation with rhIL-1 β dissolved in sterile, pyrogen-free physiological saline or with saline alone, subsequently equilibrated, and immersed in saline for 3–4 hours at 37°C according to the instructions of the manufacturer. Three separate experiments, each including 14 animals, were performed. In each experiment one group of rats ($n=7$) was continuously infused at a rate of 0.5, 2.0, or 4.0 μ g rhIL-1 β /day, and a control group ($n=7$) received osmotic pumps filled with 0.9% pyrogen-free saline. The indwelling cannula and the osmotic pump were well tolerated by the animals with no signs of discomfort or infection.

Blood samples for determination of plasma cholesterol and triglyceride levels were collected daily from freely moving, nonfasted rats by means of the jugular cannula between 10 AM and noon for 10 days starting 2 days before implantation of the pumps. Blood samples were collected in prechilled tubes containing dry lithium-heparin additive (Vacutainer, 30 USP units/tube, Becton Dickinson, Etten-Leur, The Netherlands). In the 2.0 μ g rhIL-1 β experiment additional blood was sampled for lipoprotein analysis, for determination of the ratio of unesterified to total cholesterol in plasma, and for determination of the rate of cholesterol esterification in vitro on day 5 after implantation of the minipumps. On day 7 of this experiment postheparin plasma samples were obtained in EDTA (1 mg/ml) 15 minutes after intravenous injection of 100 IU heparin/kg body wt (Thromboliquine, Organon Teknika). Blood samples were gently shaken and spun for 10 minutes at 1,500g (4°C). Plasma was separated, and the red blood cells were resuspended in sterile physiological saline and returned to each rat. Plasma samples were apportioned into aliquots and stored at -20°C until assayed.

Effects of food restriction on plasma levels of cholesterol and triglycerides. To investigate whether the rhIL-1 β -induced changes in plasma cholesterol and triglyceride levels could be explained solely by the decrease in food intake observed in the rhIL-1 β -infused rats, a fourth experiment was performed. In this experiment rats equipped with a jugular cannula underwent the same surgical procedure on day 0 as the rats in the first three

experiments but without implantation of osmotic minipumps. One group of animals ($n=6$) received food ad libitum, whereas another group ($n=7$) was fed daily with the same amount of food that had been consumed on that particular day of the experiment by rats treated with 2.0 μg rhIL-1 β /day (food-restricted group). Plasma levels of cholesterol and triglycerides as well as food intake and body weight were measured daily for 1 week.

Analytical Methods

Plasma levels of triglycerides and cholesterol were determined by enzymatic methods by using a BM/Hitachi 717 automatic analyzer system and Boehringer Mannheim (Mannheim, FRG) diagnostic kits No. 1058550 (for triglycerides) and No. 1040839 (for cholesterol). The very low density lipoprotein (VLDL) plus intermediate density lipoprotein (IDL) ($d < 1.019$ g/ml), low density lipoprotein (LDL, $1.019 < d < 1.070$ g/ml), and high density lipoprotein (HDL, $d > 1.070$ g/ml) fractions were isolated by sequential ultracentrifugation for 18 hours at 168,000g. By previous density gradient ultracentrifugation and electrophoretic studies, the density of 1.070 g/ml was found to give an accurate separation of LDL from HDL in rat plasma. LPL and hepatic lipase (HL) in postheparin plasma samples were measured selectively by a method that uses a gum arabic-stabilized emulsion of triolein and [^3H]triolein.³² With the substrate containing 0.1 M NaCl and an optimal amount of dialyzed human serum to provide activating apolipoprotein C-II, total triglyceride lipase activity (LPL+HL) was measured. HL was determined in the same substrate at a sodium chloride concentration of 1 M with omission of the serum activator. LPL was determined by subtraction. The assays were performed by incubating 10 μl postheparin plasma with 250 μl of either substrate for 30 minutes at 30°C. The rate of cholesterol esterification by lecithin:cholesterol acyltransferase (LCAT) was measured by estimating the decrease in unesterified cholesterol during incubation of plasma at 37°C for 1 and 3 hours.³³ Plasma unesterified cholesterol was measured by an enzymatic method with diagnostic kit No. 310328 from Boehringer Mannheim. All incubations were performed in duplicate with undiluted plasma.

Statistical Analysis

In each experiment a few rats had to be excluded because of blood clotting within the cannulas. All data are presented as mean \pm SEM of five to seven rats. Comparisons between treatment groups were made by analysis of variance (ANOVA) with repeated measurements to analyze the effects of the treatment course. Only when the ANOVA revealed a significant difference between both groups was a comparison of the groups at specific time points evaluated further by Student's *t* test for unpaired observations.

Results

Results of Long-term Treatment of Rats With rhIL-1 β

Physical discomfort. Signs of physical discomfort including piloerection and decreased physical activity were observed in animals infused with 4.0 μg rhIL-1 β /day starting a few hours after implantation of the

pumps. This visually observed uneasiness gradually diminished during the first day of the treatment period and had disappeared by the end of day 1. Infusion with 2.0 μg rhIL-1 β /day also induced signs of discomfort that were less pronounced than those induced by the 4.0 μg rhIL-1 β dose. Treatment of rats with 0.5 μg rhIL-1 β /day or with saline did not perceptibly distress the animals.

Plasma levels of cholesterol and triglycerides (Figure 1). Figure 1A shows that from day 2 after implantation of the minipumps, plasma cholesterol levels in rats treated with 0.5 μg rhIL-1 β /day were slightly but not significantly lower than those in rats treated with saline. After 1 day of treatment with 2.0 or 4.0 μg rhIL-1 β /day, plasma cholesterol levels had not changed. From day 2 onward a remarkable decrease in plasma cholesterol levels was observed in rhIL-1 β -treated rats, which persisted until the end of the experiment (Figures 1C and 1E; ANOVA days 2–7, 2.0 μg rhIL-1 β experiment, $p < 0.0005$; 4.0 μg rhIL-1 β experiment, $p < 0.0005$). Plasma cholesterol levels in rhIL-1 β -treated rats were significantly decreased from day 2 (2.0 μg rhIL-1 β experiment, 1.49 ± 0.13 versus 2.23 ± 0.08 mmol/l, $p < 0.005$; 4.0 μg rhIL-1 β experiment, 1.46 ± 0.04 versus 2.18 ± 0.04 mmol/l, $p < 0.0005$; rhIL-1 β versus saline) up to and including day 7 (2.0 μg rhIL-1 β experiment, 1.71 ± 0.07 versus 2.09 ± 0.04 mmol/l, $p < 0.005$; 4.0 μg rhIL-1 β experiment, 1.57 ± 0.05 versus 2.15 ± 0.05 mmol/l, $p < 0.0005$; rhIL-1 β versus saline).

Treatment with rhIL-1 β also induced a decrease in plasma levels of triglycerides (Figures 1B, 1D, and 1F; ANOVA days 1–7, 0.5 μg rhIL-1 β , $p < 0.10$; 2.0 μg rhIL-1 β , $p < 0.05$; 4.0 μg rhIL-1 β , $p < 0.05$). In contrast with the decrease in cholesterol level, which persisted until the end of the infusion, the decline in triglyceride levels was diminishing at the end of the infusion period. Statistically significant differences in plasma levels of triglycerides were observed on days 1–4 during treatment with 2.0 μg rhIL-1 β /day and on days 1–3 and 5–7 during treatment with 4.0 μg rhIL-1 β /day. Although blood samples were not taken from previously fasted rats, chylomicrons appeared to be absent, as evidenced by agarose gel electrophoresis (data not shown).

Suppression of food intake and daily body weight change (Figure 2). In saline-treated rats there was a slight reduction in food intake 1 day after implantation of the minipumps. Long-term treatment of rats with 0.5 μg rhIL-1 β /day did not affect daily food intake. Analysis of repeated measures (days 1–7) revealed a depressive effect on food consumption associated with treatment with the higher doses of rhIL-1 β (2.0 μg rhIL-1 β , $p < 0.05$; 4.0 μg rhIL-1 β , $p < 0.0005$). One day after implantation of the pumps, the decrease in food intake was maximal (2.0 μg rhIL-1 β , 4.4 ± 1.6 versus 11.6 ± 1.7 g, rhIL-1 β versus saline, $p < 0.05$; 4.0 μg rhIL-1 β , 5.5 ± 0.9 versus 13.7 ± 0.9 g, $p < 0.0005$). Food intake remained significantly lower in rhIL-1 β -treated than in control animals up to and including days 3 (2.0 μg) and 5 (4.0 μg).

There was only a small (0.5 μg rhIL-1 β , -3.4 ± 1.8 g; 2.0 μg rhIL-1 β , -7.1 ± 1.9 g) or no (4.0 μg rhIL-1 β) decrease in body weight on the first day of saline infusion (Figure 2). Infusion of rats with 0.5 μg rhIL-1 β /day did not notably affect daily body weight change compared with saline infusion. Continuous infusion of

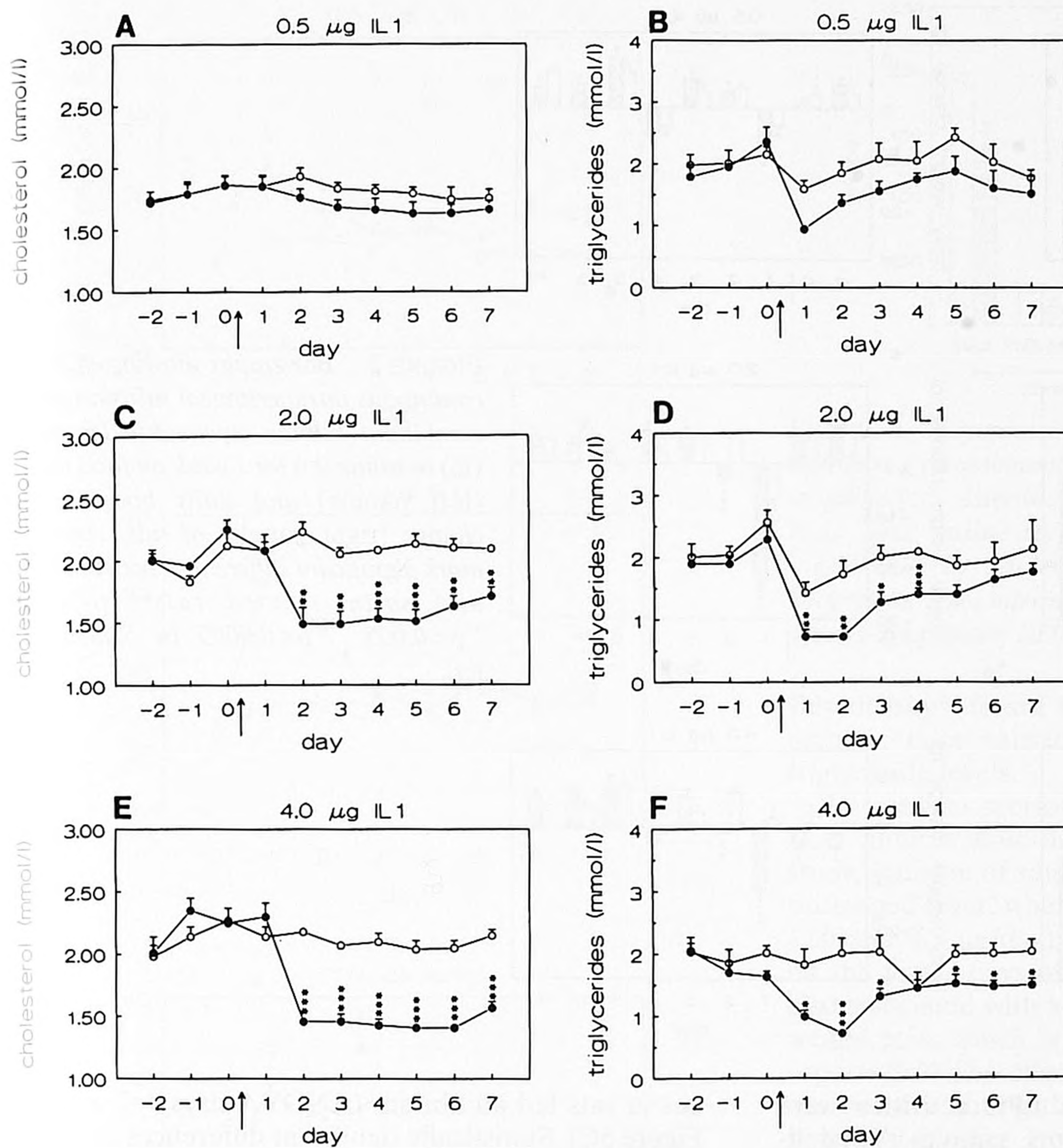


FIGURE 1. Line plots showing effects of continuous infusion of different doses of recombinant human interleukin-1 β (IL1) (0.5 (panels A and B), 2.0 (panels C and D), and 4.0 (panels E and F) $\mu\text{g}/\text{day}$; ●) or saline (○) for 1 week on the plasma concentrations of cholesterol (left panels) and triglycerides (right panels) in rats. Blood samples were taken daily between 10 AM and noon. Osmotic minipumps containing IL1 or saline were implanted intraperitoneally between 2 and 4 PM on day 0 (arrow). Data are presented as mean \pm SEM of five to seven rats. Asterisks mark significant differences between IL1- and saline-treated rats: * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$ by Student's *t* test.

2.0 or 4.0 μg rhIL-1 β /day reduced body weight gain (ANOVA days 1–7, 2.0 μg , $p < 0.0005$; 4.0 μg , $p < 0.05$). Weight loss was already maximal on the first day of infusion. Daily body weight change in rhIL-1 β -treated rats reached approximately initial levels by the third day of infusion. Thereafter the rate of daily body weight increase was essentially the same for both rhIL-1 β - and saline-treated groups of animals. Continuous infusion of rhIL-1 β at doses of 0.5, 2.0, and 4.0 $\mu\text{g}/\text{day}$ into rats had no effect on total daily fluid intake (data not shown).

Rectal temperature changes (Figure 3). Saline-treated rats maintained a virtually constant rectal temperature throughout the entire infusion period. Infusion of rhIL-1 β produced an increase in rectal temperature (ANOVA days 1–7, 0.5 μg , $p < 0.005$; 2.0 μg , $p < 0.05$; 4.0 μg , $p < 0.05$). Temperature peaked 1 day after implantation of pumps infusing rhIL-1 β at a rate of 0.5 and 2.0 $\mu\text{g}/\text{day}$ but had returned to values of saline-infused rats on day 3. From then on values of rhIL-1 β -treated rats and saline-treated rats were similar. The mean maximal increase in temperature induced by 2.0 μg rhIL-1 β /day (+1.7°C) was significantly greater ($p < 0.05$) than that induced by infusion of 0.5 μg rhIL-1 β /day (+0.9°C). At a dose of 4.0 μg rhIL-1 β /day, rectal temperature also peaked on day 1 (+1.3°C) and then gradually declined but remained elevated throughout the whole experimental period. Post hoc analysis of the individual groups

in this experiment revealed that rectal temperatures were significantly elevated on days 1, 2, 3, 4, and 7.

Lipoprotein analysis. Figure 4 shows the lipoprotein profile in plasma on day 5 of treatment with 2.0 μg rhIL-1 β /day or saline. The decrease in total plasma cholesterol (–32.8%) was accounted for by a decrease of cholesterol in all major lipoprotein fractions (VLDL+IDL, 0.13 ± 0.02 versus 0.25 ± 0.04 mmol/l, $p < 0.05$; LDL, 0.36 ± 0.04 versus 0.54 ± 0.03 mmol/l, $p < 0.005$; and HDL, 0.38 ± 0.01 versus 0.56 ± 0.02 mmol/l, $p < 0.0005$; rhIL-1 β versus saline). The decrease in plasma levels of triglycerides was reflected by a decrease in VLDL+IDL triglycerides.

Determination of cholesterol esterification rate. The percentage of unesterified cholesterol to total cholesterol in plasma was not different between rats treated with 2.0 μg rhIL-1 β /day ($35.4 \pm 1.0\%$) or with saline ($32.6 \pm 1.9\%$). The cholesterol esterification rate measured during incubation of plasma for 1 and 3 hours also did not differ significantly between both groups: at 1 hour the rate of cholesterol esterification was 0.056 ± 0.008 mmol/l \cdot hr $^{-1}$ in the rhIL-1 β group and 0.057 ± 0.007 mmol/l \cdot hr $^{-1}$ in the control group; at 3 hours these values were 0.060 ± 0.010 and 0.062 ± 0.012 mmol/l \cdot hr $^{-1}$, respectively.

Total lipolytic, HL, and LPL activities in postheparin plasma samples. On day 7 of the 2.0 μg rhIL-1 β exper-

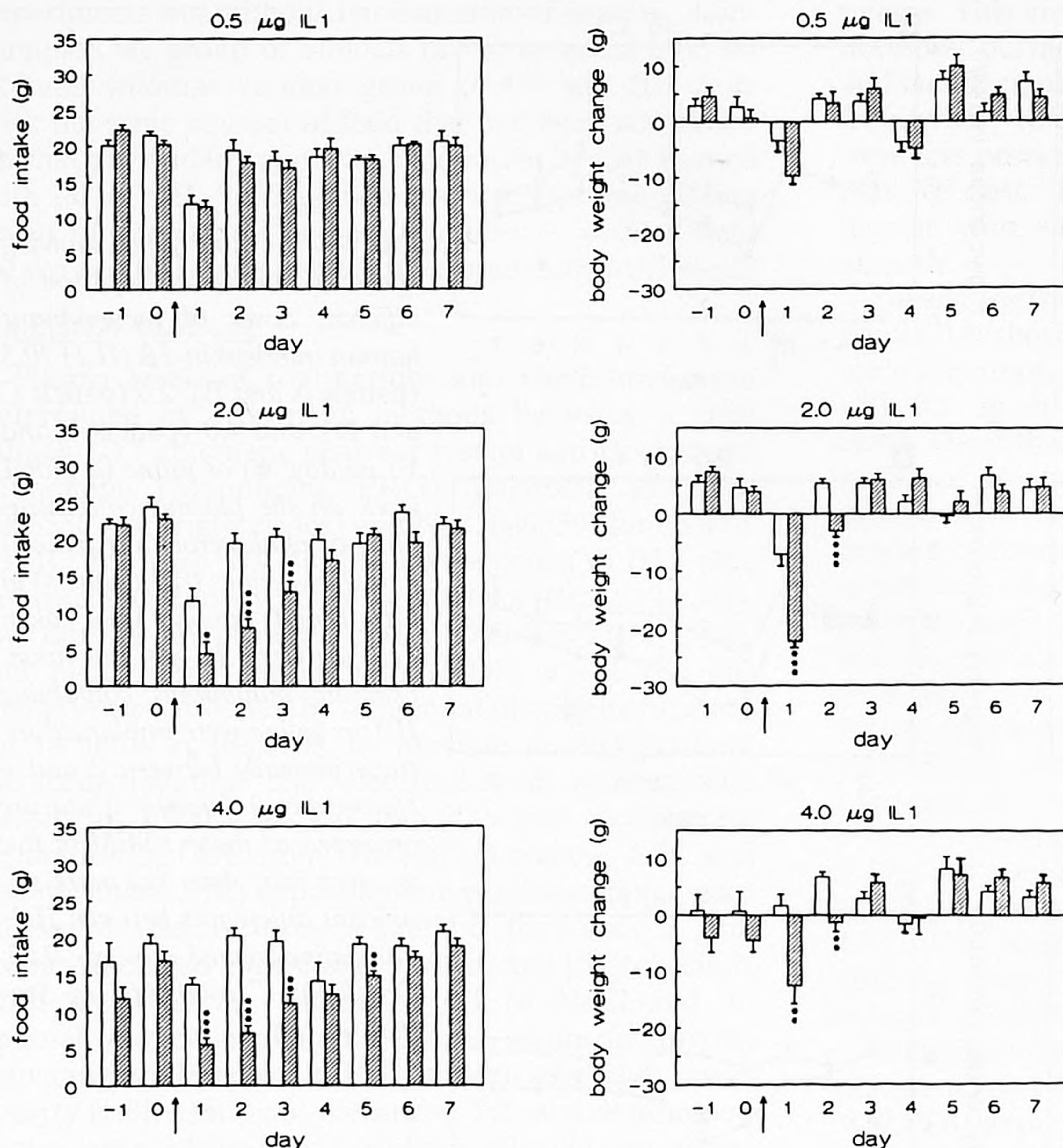


FIGURE 2. Bar graphs showing effects of continuous intraperitoneal infusion of recombinant human interleukin-1 β (IL1) (\square) or saline (\square) for 1 week on food intake (left panels) and daily body weight change (right panels) of rats. Asterisks mark significant differences between IL1- and saline-treated rats: * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$ by Student's *t* test.

iment, total lipolytic, HL, and LPL activities were determined in postheparin plasma samples. Total lipolytic activity did not differ significantly between both groups (41.9 ± 2.1 versus 43.2 ± 1.2 μmol free fatty acids [FFA]/hr \cdot ml $^{-1}$ plasma, rhIL-1 β versus saline). rhIL-1 β treatment caused a significant decrease in HL activity (24.7 ± 1.7 versus 29.8 ± 0.5 μmol FFA/hr \cdot ml $^{-1}$ plasma, rhIL-1 β versus saline, $p < 0.05$), whereas rhIL-1 β caused a significant increase in LPL activity (17.2 ± 0.8 versus 13.4 ± 1.1 μmol FFA/hr \cdot ml $^{-1}$ plasma, rhIL-1 β versus saline, $p < 0.05$). During rhIL-1 β infusion the ratio of HL to LPL was significantly decreased (1.45 ± 0.10 versus 2.29 ± 0.19 , $p < 0.005$).

Effects of Food Restriction on Plasma Levels of Cholesterol and Triglycerides

As expected, the food intake of the food-restricted rats mimicked that of the rats given 2.0 μg rhIL-1 β /day (Figure 5A). Rats that were fed the same amount of food as that consumed by rhIL-1 β -treated rats showed a significantly different pattern of body weight change in comparison with control rats fed ad libitum (Figure 5B; ANOVA days 1–7, $p < 0.0005$). On day 1 food-restricted rats showed a body weight decrease of 16.4 ± 1.5 g while control rats fed ad libitum lost 3.0 ± 2.0 g (food-restricted versus control rats, $p < 0.0005$). On day 2 food-restricted rats lost 5.7 ± 1.0 g and control rats gained 2.8 ± 1.1 g ($p < 0.0005$). Thereafter, daily body weight gain was no longer decreased in the food-restricted rats.

Food restriction induced a slight but significant decrease in plasma cholesterol levels compared with val-

ues in rats fed ad libitum (ANOVA days 1–7, $p < 0.05$; Figure 5C). Statistically significant differences in plasma cholesterol levels between both groups of rats were observed on day 3 (1.62 ± 0.04 versus 1.88 ± 0.06 mmol/l, rhIL-1 β versus saline, $p < 0.05$) and day 4 (1.62 ± 0.06 versus 1.89 ± 0.05 mmol/l, rhIL-1 β versus saline, $p < 0.05$). Restriction of food intake had no effects on plasma concentrations of triglycerides (Figure 5D).

Discussion

The present study demonstrates that continuous infusion of rhIL-1 β in doses as low as 2.0 and 4.0 μg /day profoundly lowers plasma cholesterol concentrations in rats. Infusion of 0.5 μg rhIL-1 β /day did not significantly change plasma cholesterol levels. rhIL-1 β was infused for 7 days by osmotic minipumps implanted in the peritoneal cavity. Previously it has been shown that under these conditions IL-1 remains bioactive and bioavailable for at least 1 week.²⁹ The cholesterol-lowering effect of the higher doses of IL-1 persisted until the end of the study. Interestingly, the decrease in plasma cholesterol levels was not observed before the second day of treatment with IL-1. Lipoprotein analysis on day 5 of the 2.0 μg rhIL-1 β experiment revealed that the decrease in the total plasma cholesterol level was due to a decrease in cholesterol levels in all lipoprotein fractions.

Our study also shows that triglyceride levels in plasma obtained from nonfasted rats were significantly decreased during long-term administration of 2.0 and 4.0 μg rhIL-1 β /day and that at the end of the infusion

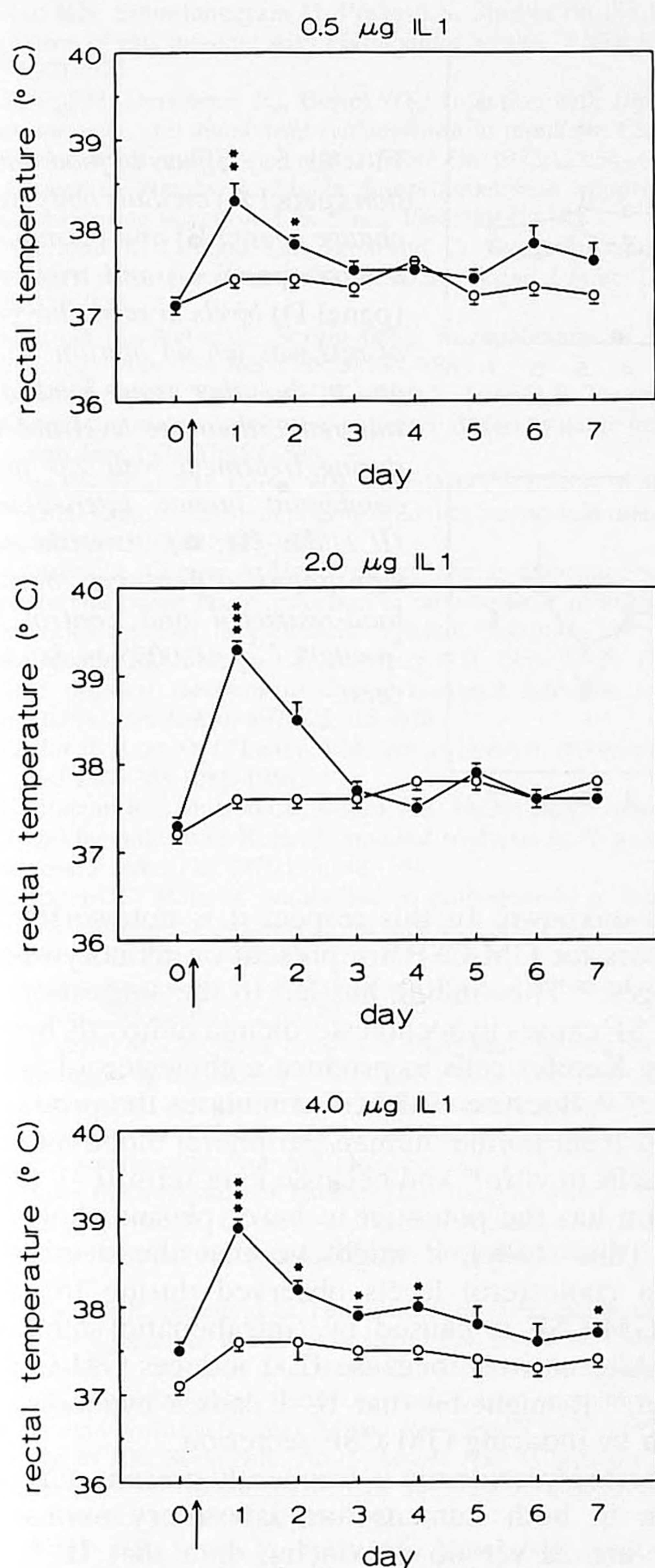


FIGURE 3. Line plots showing effects of continuous infusion of recombinant human interleukin-1 β (IL1) (●) or saline (○) for 1 week on rectal temperature of rats. Asterisks mark significant differences between IL1- and saline-treated rats: * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$ by Student's *t* test.

period this effect was diminishing. In contrast, Feingold et al²⁷ showed that short-term intravenous administration of 1 μ g IL-1 rapidly increased serum triglyceride levels in rats. This elevation in triglyceride levels persisted for 17 hours and was accompanied by an increase of hepatic triglyceride secretion and de novo fatty acid synthesis, whereas the clearance of triglyceride-rich lipoproteins was unaffected by IL-1. In that study IL-1 had no effect on serum cholesterol levels. Argiles et al,²⁸ using an even higher dose of IL-1 (20 μ g), also found an increase in triglyceride levels after short-term administration of the cytokine in rats, but these workers failed to demonstrate any effect on hepatic lipogenesis. In contrast with both studies with rats, Del Rey and

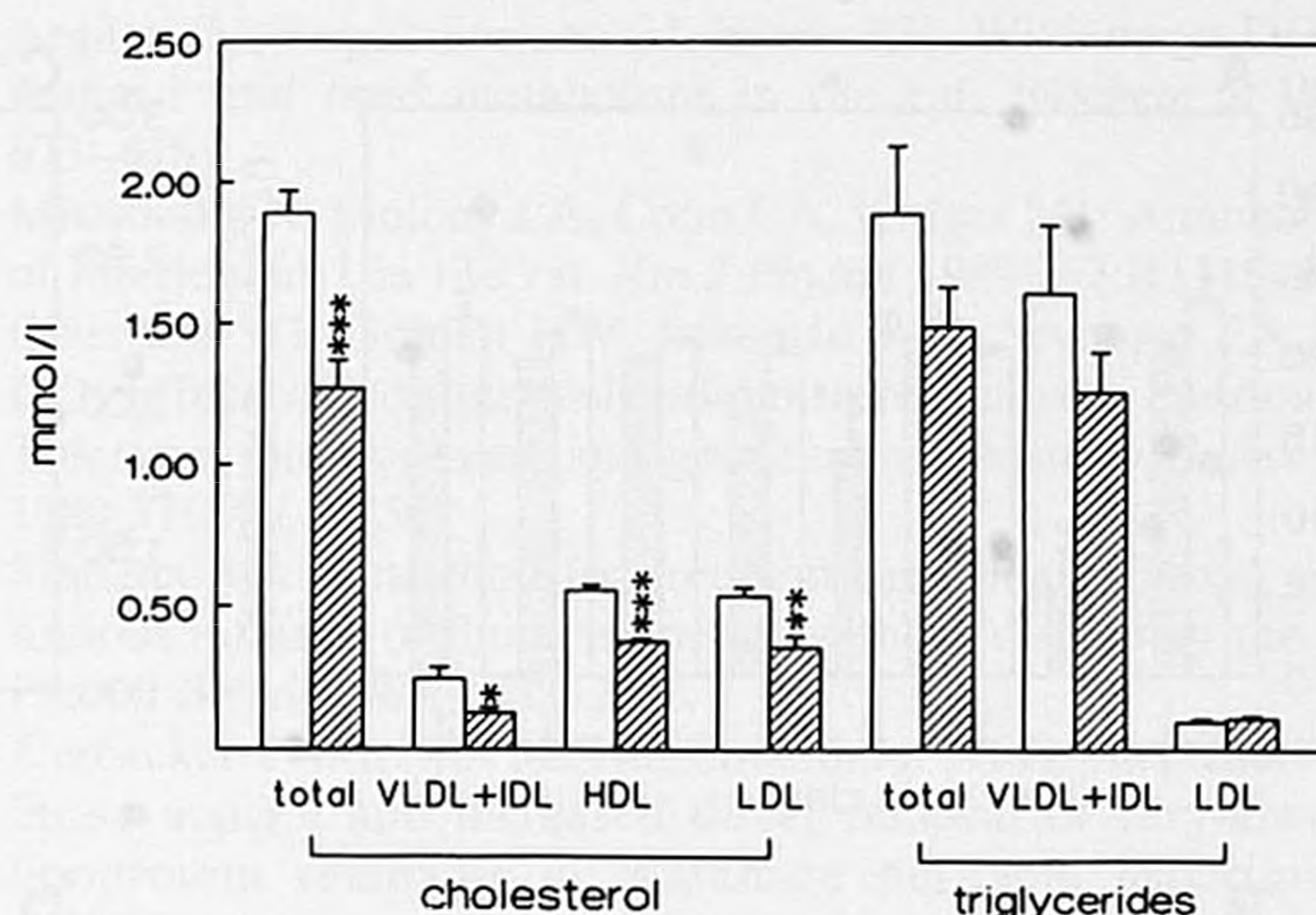


FIGURE 4. Lipoprotein profile in rats on day 5 of treatment with 2.0 μ g recombinant human interleukin-1 β (IL1)/day (▨) or saline (□). Asterisks mark significant differences between IL1- and saline-treated rats: * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$ by Student's *t* test. VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein.

Besedovsky³⁴ found that injection of 0.25 μ g IL-1 in diabetic mice caused a marked reduction in serum triglyceride levels.

We confirm previous findings that administration of IL-1 induces dose-dependent fever in rats.³⁵ In our study, infusion of rats with 4.0 μ g rhIL-1 β /day induced prolonged fever, whereas at the lower doses of rhIL-1 β (2.0 and 0.5 μ g/day) temperatures were elevated only on the first 2 days of infusion. rhIL-1 β treatment was also associated with a decrease in food intake and body weight gain, which is in good agreement with previous reports.³⁶⁻³⁸ The effect of rhIL-1 β on body weight gain was present during the first 2 days of infusion but had been lost by day 3 of treatment. This is in line with studies demonstrating that tolerance to the anorectic effects of IL-1 develops within a few days.^{29,30}

In fasting rats plasma cholesterol and triglyceride levels are lower than in rats with unrestricted access to food.³⁹ To clarify whether the effects of IL-1 on plasma cholesterol and triglyceride levels could be explained solely by the reduction of food intake during rhIL-1 β treatment, we investigated the effects of food consumption restricted to the same level as in the 2.0 μ g rhIL-1 β experiment on plasma cholesterol and triglyceride concentrations. This experiment revealed that such a reduction of food intake had no effect on plasma triglyceride concentrations, whereas plasma cholesterol levels were moderately lowered. Therefore, it can be concluded that it is highly unlikely that the decrease in plasma triglyceride levels observed during long-term IL-1 infusion is caused by decreased food intake and that the observed decrease in food intake cannot completely explain the profound decrease of plasma cholesterol levels during IL-1 administration.

Intravenous injection of lipopolysaccharide or TNF causes a decrease in the activity of the LCAT enzyme in nonhuman primates.^{40,41} It has been suggested that this decrease in LCAT activity after TNF administration is causally related to the occurrence of hypocholesterolemia after injection of this cytokine in nonhuman primates. However, it is unlikely that in our study a decrease in LCAT activity was the cause of the hypocholesterolemia during IL-1 treatment because the ra-

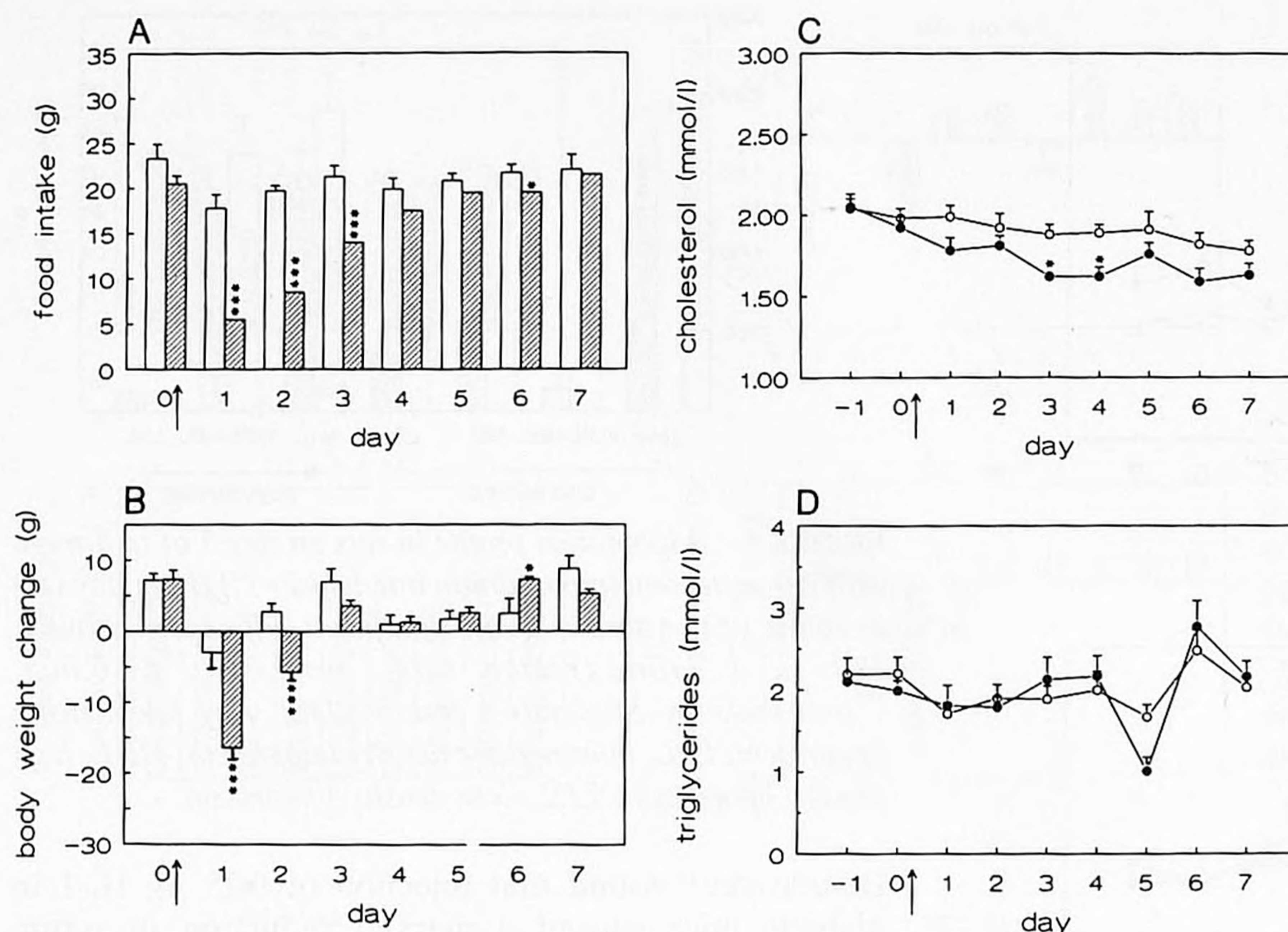


FIGURE 5. Effects of food restriction (panel A) on daily body weight change (panel B) and plasma cholesterol (panel C) and triglyceride (panel D) levels in rats. One group of rats was fed ad libitum (\square , \circ), and in the other group food intake was reduced to the level observed during treatment with $2.0 \mu\text{g}$ recombinant human interleukin- 1β (IL1)/day (\blacksquare , \bullet). Asterisks mark significant differences between food-restricted and control rats: * $p < 0.05$, *** $p < 0.0005$ by Student's t test.

ratio of free cholesterol to esterified cholesterol in the plasma of rats infused with $2.0 \mu\text{g}$ rhIL- 1β /day was not significantly different from control rats, which suggests a normal cholesterol esterification rate in vivo. Indeed, cholesterol esterification rates determined in vitro were similar for rhIL- 1β -treated and control rats.

Thus, other mechanisms causing hypocholesterolemia and hypotriglyceridemia should be considered. In patients with sepsis, plasma concentrations of apolipoprotein B, the major structural protein of cholesterol-carrying lipoproteins in humans, are decreased.⁵ Other investigators have shown that IL-1 decreases the secretion of apolipoprotein B by Hep G2 cells.⁴² Therefore it might be that hypocholesterolemia during long-term IL-1 treatment is caused by impaired secretion of apolipoprotein B by hepatocytes. With respect to the metabolism of triglycerides, we found that although small reciprocal changes were observed in the activities of LPL and HL, the total postheparin lipolytic activity in plasma on day 7 of the $2.0 \mu\text{g}$ rhIL- 1β experiment was similar in rhIL- 1β - and saline-treated rats. With the assumption that the clearance of triglycerides in plasma is largely determined by this lipolytic activity, it seems unlikely that the decrease in triglyceride levels during IL-1 treatment is caused by an increased clearance of triglyceride-rich lipoproteins. We suggest that the decrease in triglyceride levels is due to a diminished secretion of nascent VLDL.

A decrease in plasma cholesterol level has also been observed during treatment with other cytokines. Administration of macrophage colony-stimulating factor (M-CSF) to nonhuman primates and normocholesterolemic and hypercholesterolemic rabbits results in decreases in plasma cholesterol levels.⁴³ In humans treatment with M-CSF also induces a sharp decrease in plasma cholesterol levels.⁴⁴ Hypocholesterolemia in humans is also observed during treatment with granulocyte-macrophage colony-stimulating factor (GM-CSF).^{45,46} The mechanism of the hypocholesterolemia after administration of these hematopoietic growth fac-

tors is unknown. In this respect it is noteworthy that receptors for GM-CSF are present on monocytes/macrophages.⁴⁷ This finding has led to the suggestion that GM-CSF causes hypocholesterolemia indirectly by stimulating Kupffer cells to produce a cholesterol-lowering factor.^{45,48} Because GM-CSF stimulates the production of IL-1 from normal human peripheral blood mononuclear cells in vitro⁴⁹ and because long-term IL-1 administration has the potential to lower plasma cholesterol levels (this study), it might be that the decrease of plasma cholesterol levels observed during treatment with GM-CSF is caused by (intrahepatic) release of IL-1. Alternatively, because IL-1 induces GM-CSF secretion,⁵⁰ it might be that IL-1 causes hypocholesterolemia by inducing GM-CSF secretion.

Hypertriglyceridemia is frequently present during infection in both humans and laboratory animals.⁸⁻¹² There are as yet no convincing data that IL-1 does mediate the hypertriglyceridemia of infection. Except for studies of rabbits,^{1,2} most reports of cholesterol metabolism during bacterial infections agree that plasma cholesterol levels are decreased during the febrile phase of bacterial infections.³⁻⁸ Whether IL-1 released from the inflammatory site or produced locally by endotoxin-stimulated Kupffer cells plays a role as a mediator in the lowering of plasma cholesterol levels during infections remains to be investigated.

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