Insulin Deficiency Alters Cellular Cholesterol Metabolism in Murine Macrophages

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SUMMARY

Mice made insulin deficient by the injection of streptozocin develop hyperglycemia and hypertriglyceridemia with triglyceride-rich, very-low-density lipoproteins (VLDLs). Thioglycolate-elicited peritoneal macrophages freshly isolated from insulin-deficient mice have increased activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, which is reflected in a greater rate of cholesterol synthesis by these macrophages. In contrast, thioglycolate-elicited macrophages from control mice with diet-induced hypertriglyceridemia had normal levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Cell surface receptors responsible for VLDL uptake are decreased in macrophages isolated from insulin-deficient mice, although receptors for acetylated low-density lipoproteins are not altered. Insulin treatment of insulin-deficient mice lowers plasma glucose and triglyceride concentrations. Additionally, insulin treatment returns the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase and the rate of cholesterol synthesis in thioglycolate-elicited macrophages to normal while increasing the number of receptors responsible for VLDL uptake. It is suggested that the increases in 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and the rate of cholesterol synthesis in macrophages isolated from insulin-deficient mice are secondary to the reduction in the number of receptors responsible for VLDL uptake induced by insulin deficiency. These alterations in the cholesterol metabolism of macrophages occurring with insulin deficiency may have important implications for the atherosclerotic process in diabetes mellitus. DIABETES 1986; 35:764–70.

Patients with diabetes mellitus have an increased incidence of morbidity and mortality due to atherosclerotic disease.[1] Although there are many metabolic abnormalities in diabetes that might contribute to atherosclerosis (e.g., hyperlipidemia, glucosylation), the effects of insulin deficiency on cholesterol metabolism in cells involved in the atherosclerotic process have not been fully explored. Studies of experimental diet-induced atherosclerosis have shown that the earliest morphologic changes to be observed are a monocytic invasion of the vessel wall and the conversion of monocytes to lipid-laden macrophages (foam cells).[2,3] Given the important role the macrophage appears to play in atherosclerosis, the present study was undertaken to examine the effects of insulin deficiency on cholesterol metabolism in macrophages. The experimental approach used streptozocin-induced insulin deficiency in mice to examine the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (mevalonate: $NADP^+$ oxidoreductase: EC 1.1.1.34), the rate of cholesterol synthesis, and the activities of lipoprotein receptors in thioglycolate-elicited peritoneal macrophages isolated from control, insulin-deficient, and insulin-treated animals. Measurements were also made in control mice fed an experimental diet to induce hypertriglyceridemia.

The results of these experiments show that insulin deficiency is associated with a marked increase in HMG CoA reductase activity and in the rate of cholesterol synthesis in thioglycolate-elicited macrophages, even though circulating plasma lipid levels are increased. However, HMG CoA reductase activity and the rate of cholesterol synthesis were normal in thioglycolate-elicited macrophages from mice with diet-induced hypertriglyceridemia. Insulin deficiency also caused a decrease in the number of surface receptors responsible for very-low-density lipoprotein (VLDL) uptake without changing the expression of other lipoprotein receptors. Insulin treatment corrected the hyperglycemia and the hyperlipidemia in these animals. In addition, insulin treatment returned the activity of HMG CoA reductase and the rate of cholesterol synthesis in macrophages to normal while increasing the expression of receptors responsible for VLDL uptake.

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TABLE 1
Fasting plasma glucose, cholesterol, and triglyceride values in mice

Group	*N*	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Control	43	194 ± 4	99 ± 6	166 ± 12
Insulin deficient	85	552 ± 13*	102 ± 4	249 ± 19†
Insulin treated	49	68 ± 4*	117 ± 4†	139 ± 8‡
Sucrose diet	14	242 ± 4*	97 ± 8	202 ± 15‡

*$P < .001$ compared with control; †$P < .01$ compared with control; ‡$P < .05$ compared with control.

MATERIALS AND METHODS

Materials. Carrier-free Na ^{125}I (13–17 mCi/μg), 3-hydroxy-3-methyl[3-^{14}C]glutaryl CoA (58 mCi/mmol), [2-^{14}C]acetate (54 mCi/mmol), DL-[2-^{3}H]mevalonic acid lactone (1.28 Ci/mmol), and [1α,2α(*n*)-^{3}H]cholesterol (44 Ci/mmol) were purchased from Amersham (Arlington Heights, IL). Silica gel thin-layer chromatography (TLC) plates were from Whatman Chemical Separation (Clifton, NJ). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were obtained from Gibco (Grand Island, NY). Streptozocin was a kind gift of Dr. William Dulin of Upjohn (Kalamazoo, MI). Male Swiss-Webster mice (25–30 g) were obtained from Simonsen Labs (Gilroy, CA). A pelleted sucrose/corn oil diet (66% sucrose, 12% corn oil, 22% casein, supplemented with vitamins) was obtained from Teklad Test Diets (Madison, WI). All other chemicals were from Sigma (St. Louis, MO) or from J. T. Baker Chemical (Phillipsburg, NJ).

Lipoproteins. Blood was drawn from fasted, normal human volunteers into tubes containing EDTA (1 mg/ml). Very-low-density lipoproteins ($\rho < 1.006$ g/ml) and low-density lipoproteins (LDLs; $\rho = 1.019$–1.063 g/ml) were isolated from plasma by sequential ultracentrifugation in a 50.2 Ti rotor at 45,000 rpm for 24 h with solid KBr for density adjustment.[4] Each fraction was then washed by recentrifugation. Low-density lipoproteins were acetylated by the addition of acetic anhydride.[5] The lipoproteins were iodinated by the iodine monochloride method of MacFarlane,[6] extensively dialyzed against 0.15 M NaCl, 0.05 M Tris-HCl (pH 7.4), and sterilized by filtration.

Cells. Insulin deficiency was induced in mice by the intraperitoneal injection of streptozocin (215 mg/kg) diluted in 0.05 M Na citrate (pH 4.5). Three days after the injection of streptozocin or carrier, some of the animals were started on daily subcutaneous injections of 0.5 U of ultralente insulin. Ten days after the injection of streptozocin or carrier, animals were fasted for 5 h; blood was obtained from the tail vein for measurement of glucose,[7] triglyceride,[8] and cholesterol,[9] and then food was restored. (Mice treated with insulin were not fasted because severe hypoglycemia developed during food withdrawal.) A second control group of mice was fed a sucrose/corn oil diet for 7 days before fasting blood samples or peritoneal macrophages were obtained. Animals were injected with 2 ml of thioglycolate medium intraperitoneally, and the following day peritoneal exudative cells were harvested in phosphate-buffered saline. Cell yields were not statistically different among the groups and averaged 22 ± 1 × 10^{6}, 20 ± 5 × 10^{6}, 30 ± 6 × 10^{6}, and 30 ± 5 × 10^{6} cells per mouse for control, insulin-deficient, insulin-treated, and diet-treated groups, respectively. The differential counts of the peritoneal exudative cells were also similar among the groups with counts averaging between 49 ± 3% polymorphonuclear leukocytes, 8 ± 2% lymphocytes, and 43 ± 4% macrophages for control and 53 ± 6% polymorphonuclear leukocytes, 9 ± 4% lymphocytes, and 37 ± 3% macrophages for insulin-deficient groups. The cells were collected by centrifugation (700 × *g* × 5 min), suspended in DMEM with 10% fetal bovine serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin, and then plated into plastic Petri dishes at a density of 4 × 10^{6} cells in 35 × 10-mm dishes or 12 × 10^{6} cells in 60 × 15-mm dishes. Nonadherent cells were removed by washing 2 h after plating, and the adherent cells were used immediately for experimentation. As assessed by light microscopy after staining with Wright's stain, or with α-naphthylbutyrate esterase, and by the ability to phagocytize latex particles and antibody-coated red blood cells, >99% of the adherent cells from control, insulin-treated, and diet-treated mice were macrophages, whereas 85–90% of the adherent cells from insulin-deficient mice were macrophages with 10–15% polymorphonuclear leukocytes. Approximately 20% of the cells initially seeded adhered to the dishes; there were no differences in plating efficiencies among the groups.

Cholesterol synthesis. After nonadherent cells were removed by washing, macrophages (60 × 15-mm dishes) were incubated in serum-free media with 5 μCi/ml of [2-^{14}C]acetate at 37°C for 1 h. The reaction was stopped by placing the cells in ice and rapidly washing three times with ice-cold, phosphate-buffered saline. The cells were scraped off the dish with a rubber policeman and [1,2-^{3}H]cholesterol (40,000 dpm) was added for an internal standard. An aliquot was taken for determination of cell protein,[10] and the remainder saponified in ethanolic KOH at 80°C for 2 h. The nonsaponifiable sterols were extracted with hexane, dried under N_2, and resolved by TLC on Whatman LKD plates with toluene:ethyl acetate (4:1) as the developing solvent. Cholesterol ($R_f = 0.47$) was visualized with I_2 vapor, scraped into scintillation vials, and counted in a toluene-based scintillation mixture.

HMG CoA reductase. Macrophages (60 × 15-mm dishes) were washed three times with ice-cold, phosphate-buffered saline, scraped off the dish with a rubber policeman, suspended in 0.25 M sucrose, 0.3 mM EDTA, 0.1 M potassium phosphate, and 5 mM dithiothreitol, and disrupted by sonication. An aliquot of the postnuclear homogenate (1000 × *g* × 10 min) was preincubated at 37°C for 10 min and then incubated in a final volume of 0.2 ml containing 0.1 M potassium phosphate (pH 7.5), 3 mM NADP, 20 mM glucose-6-phosphate, 1 U glucose-6-phosphate dehydrogenase, 10

TABLE 2
Lipid composition of very-low-density lipoproteins

Group	*N*	Triglyceride/ protein	Cholesterol/ protein	Triglyceride/ cholesterol
Control	7	4.88 ± 0.38	1.05 ± 0.15	5.30 ± 0.85
Insulin deficient	9	6.85 ± 0.45*	1.48 ± 0.09	4.75 ± 0.46
Insulin treated	6	6.10 ± 0.39	1.39 ± 0.05	5.14 ± 0.44
Sucrose diet	5	7.42 ± 1.48*	1.87 ± 0.06†	3.99 ± 0.86†

*$P < .05$ compared with control; †$P < .01$ compared with control.

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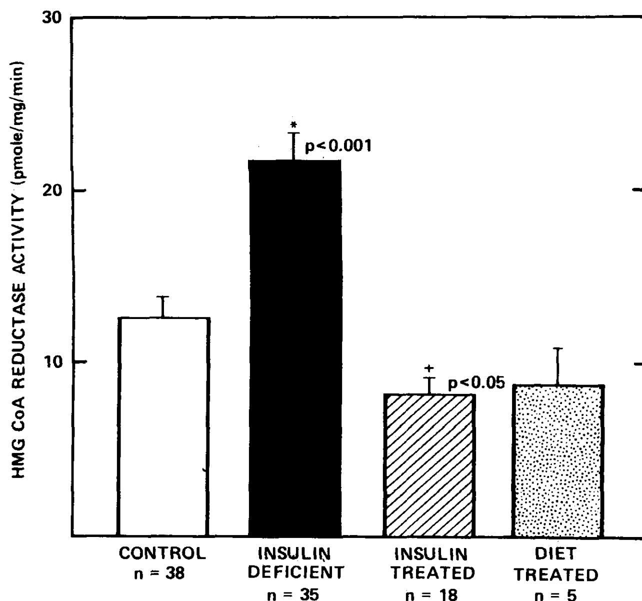

FIGURE 1. Activity of HMG CoA reductase in macrophages isolated from control, insulin-deficient, insulin-treated, and diet-treated mice. Mice were injected intraperitoneally with diluent (control) or with 215 mg/kg of streptozocin (insulin deficient). Three days after injection with streptozocin, some animals were started on subcutaneous insulin therapy (insulin treated). Some control mice were fed a sucrose/corn oil diet for 7 days (diet treated). Ten days after injection with streptozocin or diluent, mice were injected with thioglycolate medium. Next day, macrophages were isolated from peritoneal exudates by attachment on plastic Petri dishes. Immediately after isolation, macrophages were washed, disrupted by sonication, and postnuclear fraction assayed for HMG CoA reductase activity. *N* denotes number of dishes of macrophages tested in 8 separate experiments. Insulin-deficient, insulin-treated, and diet-treated groups are compared with control for statistical analysis.

nM EDTA, 5 mM dithiothreitol, and [3-^{14}C]HMG CoA (14 μM) at 37°C for 20 min.[11] The reaction was stopped by addition of 30 μl 5 N HCl, [2-^{3}H]mevalolactone (40,000 dpm) was added as an internal standard to monitor recovery, and 200 μg of mevalolactone was added as carrier. The mixtures were incubated for 30 min at 37°C to complete lactonization and then plated on Whatman TLC plates with toluene:acetone (1:1, vol:vol) as the developing solvent. Mevalolactone (R_f = 0.6) was visualized with I_2 vapor, scraped into scintillation vials, and counted in a toluene-based scintillation mixture. All assays were done at two different protein concentrations as a test for linearity. Blank values were obtained from incubations in the absence of protein.

Lipoprotein binding. The cells (35 × 10-mm dishes) were washed with DMEM and then incubated in a final volume of 1 ml of ^{125}I-lipoproteins of various concentrations and DMEM with 10% lipoprotein-deficient fetal bovine serum at 4°C for 2 h. A 10- to 100-fold excess of unlabeled lipoproteins was added to parallel dishes to determine nonspecific binding. The incubation was terminated by washing four times with 1 ml of 0.15 M NaCl, 0.05 M Tris-HCl, 5 mM $CaCl_2$, and 2 mg/ml of bovine serum albumin, followed by one wash with 2 ml of 0.15 M NaCl, 0.05 M Tris-HCl, and 5 mM $CaCl_2$. The cells were then dissolved in 1 ml of 0.05 N NaOH, and the amounts of ^{125}I-lipoproteins bound by the cells were determined in a gamma scintillation spectrophotometer. An aliquot of the cells was taken for determination of cellular protein content.[10]

Values were corrected for activity observed in control dishes containing no cells.

Statistics. Statistical comparisons were made by use of analysis of variance, Student's *t* test for unpaired samples, or the Wilcoxon signed-rank test. All data are expressed as the mean ± SEM.

RESULTS

Fasting plasma glucose and lipid levels. Ten days after the induction of insulin deficiency by the injection of streptozocin, mice were markedly hyperglycemic ($P < .001$) compared with controls (Table 1). Plasma cholesterol levels were not changed in the insulin-deficient mice, but plasma triglyceride concentrations were 50% higher ($P < .01$). When insulin-deficient mice were treated daily with subcutaneous injections of long-acting insulin, these metabolic abnormalities were reversed. Plasma glucose and triglyceride levels were lowered below control values ($P < .001$ and $P < .05$, respectively); however, plasma cholesterol concentrations were increased slightly (18% higher, $P < .01$). The reasons for the elevation of cholesterol are unclear, but it was probably because the measurements in the insulin-treated mice were made in the nonfasting state and/or because of the presence of counterregulatory hormones (e.g., glucocorticoids and catecholamines) in the animals that tended to be hypoglycemic. Because the insulin-deficient mice were hypertriglyceridemic in addition to being hyperglycemic, normal animals were fed an experimental diet consisting of sucrose/corn oil in an effort to create a normoglycemic, hypertriglyceridemic control group. Diet-treated mice had a

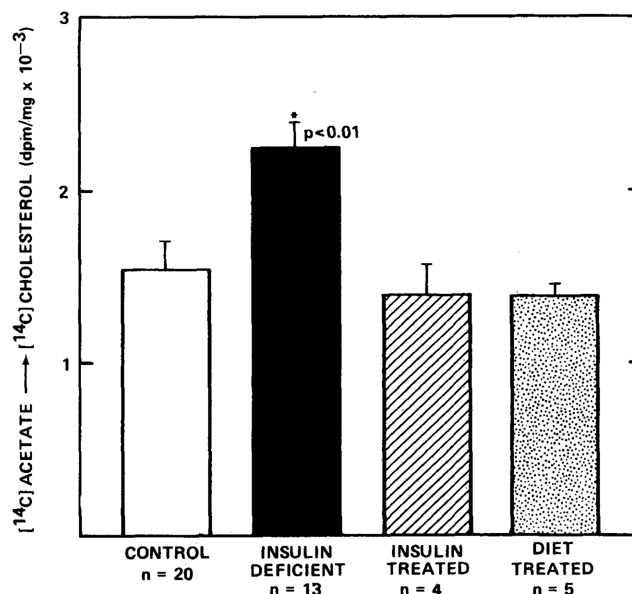

FIGURE 2. Cholesterol synthesis in macrophages isolated from control, insulin-deficient, insulin-treated, and diet-treated mice. Macrophages were isolated from animals as described in Figure 1. Immediately after isolation, macrophages were washed and incubated in serum-free medium with 5 μCi/ml of [2-^{14}C]acetate at 37°C for 1 h. Incubation was terminated by placing cells on ice, cellular lipids were saponified, and nonsaponifiable sterols were extracted and separated on thin-layer chromatography. Area corresponding to cholesterol was scraped from chromatogram and its radioactivity was determined. *N* denotes number of dishes tested in 5 separate experiments. Statistical comparisons were made between control and each experimental group.

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TABLE 3
Ability of nonlipoprotein cholesterol to inhibit HMG CoA reductase and cholesterol synthesis in macrophages

Group	HMG CoA reductase activity (pmol · mg⁻¹ · min⁻¹)	Cholesterol synthesis (dpm/mg)
Control		
Cholesterol present	51.39 ± 3.77	1730 ± 65
Cholesterol absent	3.25 ± 1.09	472 ± 10
Insulin deficient		
Cholesterol present	59.37 ± 3.77	2153 ± 162
Cholesterol absent	4.15 ± 0.78	562 ± 45

Macrophages were isolated from control and insulin-deficient mice as described in Figure 1. After isolation, macrophages were incubated in DMEM supplemented with 10%-lipoprotein-deficient media and cholesterol (30 μg/ml) or ethanol carrier for 16 h at 37°C. At the end of the incubation, HMG CoA reductase activity and cholesterol synthesis were assayed as described in Figures 1 and 2.

small but statistically significant ($P < .001$) increase in fasting plasma glucose compared with controls. Plasma triglyceride levels in diet-treated mice were statistically increased (22%, $P < .05$) compared with controls but were lower than observed in insulin-deficient mice.

The experimental groups were further characterized by evaluating the lipid compositions of their circulating VLDL (Table 2). To obtain sufficient quantities of VLDL for analysis, each experimental observation was performed on VLDL isolated from the pooled plasma of three mice. The VLDL from insulin-deficient mice had increased triglyceride-to-protein ratios compared with controls ($P < .05$); insulin therapy corrected this increase to almost normal. The cholesterol-to-protein ratios of VLDL did not differ among control, insulin-deficient, or insulin-treated mice. The VLDL from diet-treated mice had increased triglyceride-to-protein ratios ($P < .05$), as well as significantly increased cholesterol-to-protein ratios ($P < .01$). The triglyceride-to-cholesterol ratio of VLDL was decreased in the diet-treated mice but did not differ among any of the other groups.

HMG CoA reductase activity and cholesterol synthesis in macrophages. After 10 days of insulin deficiency, thioglycolate-elicited peritoneal macrophages were harvested from the experimental animals, and HMG CoA reductase activity was measured in the freshly isolated cells (Figure 1). Thioglycolate-elicited macrophages from insulin-deficient mice had levels of HMG CoA reductase activity that were 75% greater than controls ($P < .001$), even though circulating cholesterol values were similar in the two groups. This increase in HMG CoA reductase activity was not due to a toxic effect of streptozocin because macrophages from insulin-deficient mice treated with insulin had HMG CoA reductase activity that was actually lower than that of controls ($P < .05$). Furthermore, the increase in HMG CoA reductase activity in macrophages from insulin-deficient mice does not appear to be due to the presence of endogenous hypertriglyceridemia or circulating triglyceride-rich VLDL because HMG CoA reductase activity in thioglycolate-elicited macrophages from diet-treated mice was similar to control values.

To show that the effects of insulin deficiency on HMG CoA reductase activity reflected changes in cellular cholesterol

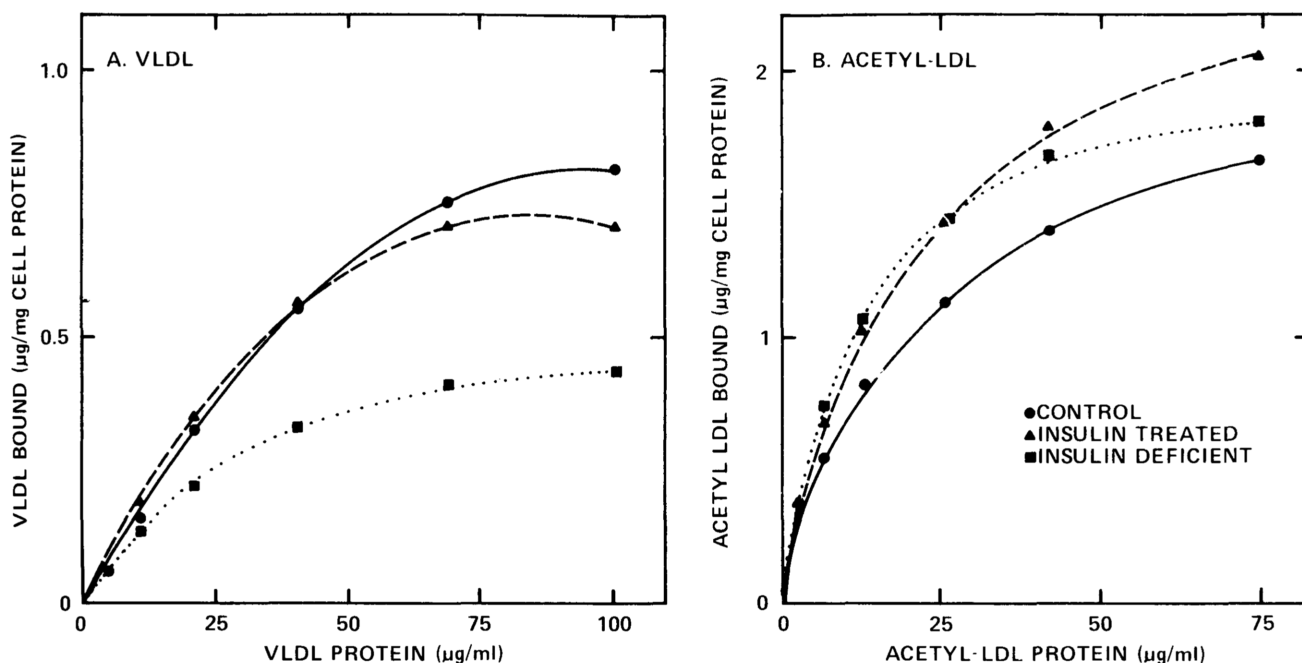

FIGURE 3. Binding of ^{125}I-VLDL (*A*) and ^{125}I-acetyl LDL (*B*) to macrophages isolated from control (●), insulin-deficient (■), and insulin-treated (▲) mice. Macrophages were isolated from mice as described in Figure 1. Indicated concentration of ^{125}I-VLDL or ^{125}I-acetyl LDL were incubated with monolayers of freshly isolated macrophages in final volume of 1 ml of Dulbecco's modified Eagle's medium and 10%-lipoprotein-deficient serum at 4°C for 2 h, and amounts of ^{125}I-VLDL or ^{125}I-acetyl LDL bound by the cells were determined. Specific binding of ^{125}I-lipoprotein has been corrected by subtracting amounts of ^{125}I-VLDL or ^{125}I-acetyl LDL bound in parallel dishes containing 2 mg/ml of unlabeled VLDL or unlabeled acetyl LDL. Each *point* represents mean of duplicate dishes. Data are representative of 5 separate experiments.

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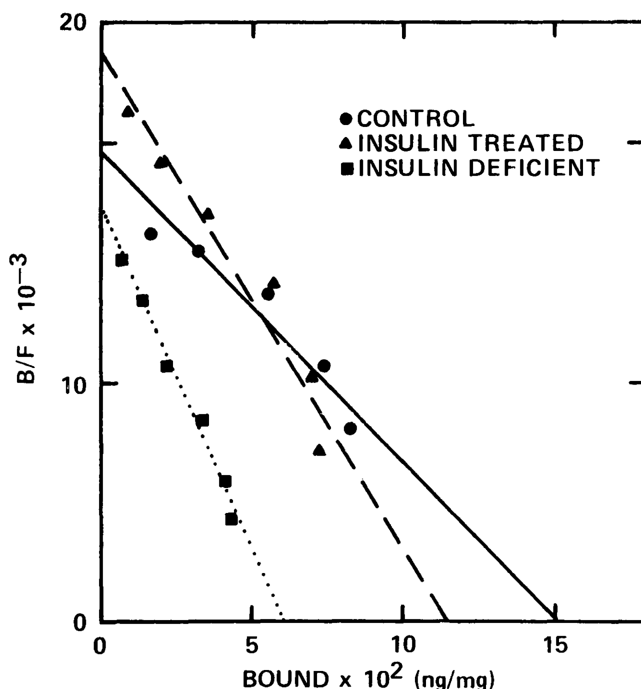

FIGURE 4. Scatchard plots of ^{125}I-VLDL binding to macrophages isolated from control (●), insulin-deficient (■), and insulin-treated (▲) mice. Experimental data from Figure 3*A* are plotted. B/F, bound/free.

synthesis, the incorporation of [^{14}C]acetate into [^{14}C]cholesterol was measured (Figure 2). Insulin deficiency was associated with a 46% increase in cholesterol synthesis in the freshly isolated macrophages ($P < .01$). This increased rate of cholesterol synthesis was returned to normal by insulin therapy. The sucrose/corn oil diet had no effect on cholesterol synthesis in macrophages.

Because alterations in plasma lipid levels and lipoprotein composition could not explain the increase in HMG CoA reductase activity and cholesterol synthesis in insulin deficiency, other possibilities were considered: *1*) insulin deficiency might cause the loss of the normal feedback inhibition of HMG CoA reductase by cholesterol; or *2*) insulin deficiency might affect the expression of cell surface lipoprotein receptors. To examine whether HMG CoA reductase activity in macrophages isolated from insulin-deficient mice could be inhibited by cholesterol, thioglycolate-elicited macrophages were incubated in the presence or absence of nonlipoprotein cholesterol (Table 3). Cholesterol clearly inhibited HMG CoA reductase activity and cholesterol synthesis similarly in macrophages from insulin-deficient and control mice, establishing that insulin deficiency does not alter the regulation of HMG CoA reductase by cholesterol.

Lipoprotein binding. To explore the possibility that insulin deficiency might alter the expression of cell surface lipoprotein receptors, the specific binding of ^{125}I-acetylated LDL, ^{125}I-VLDL, and ^{125}I-LDL was assessed. As previously reported,[12] murine macrophages specifically bound ^{125}I-acetylated LDL and ^{125}I-VLDL (Figure 3). No specific binding of ^{125}I-LDL could be detected under the present conditions in the freshly isolated preparations of macrophages. Thioglycolate-elicited macrophages from insulin-deficient mice demonstrated less binding of ^{125}I-VLDL than controls, and this was returned to normal with insulin therapy (Figure 3*A*). Similar amounts of binding of ^{125}I-acetylated LDL were observed in macrophages from control, insulin-deficient, and insulin-treated mice (Figure 3*B*). When the data from the ^{125}I-VLDL binding curves were transformed into Scatchard plots (Figure 4), it appeared that insulin deficiency was associated with an ~50% decrease in the number of receptors responsible for VLDL uptake without significant changes in receptor affinity. In five separate experiments, the number of receptors responsible for VLDL uptake decreased from 781 ± 189 ng VLDL bound per milligram of cell protein in control mice to 422 ± 81 ng VLDL bound per milligram of cell protein in insulin-deficient mice ($P < .05$); control values were returned by insulin treatment (881 ± 263 ng VLDL bound per milligram of cell protein). The K_d for VLDL binding was statistically similar among the groups (58 ± 9, 33 ± 5, and 34 ± 13 μg VLDL protein per milliliter for control, insulin-deficient, and insulin-treated mice, respectively.

DISCUSSION

Cholesterol synthesis is dependent on the activity of its rate-limiting enzyme HMG CoA reductase, which is predominantly controlled by feedback inhibition from intracellular cholesterol.[13] In addition to the major effect of intracellular cholesterol, several hormones appear to modulate HMG CoA reductase activity. In particular, whether insulin and conversely insulin deficiency influence HMG CoA reductase activity and, hence, cellular cholesterol synthesis has been debated. In vitro studies in cultured fibroblasts have shown that insulin increases HMG CoA reductase activity and the rate of cholesterol synthesis.[14] In vivo studies in insulin-deficient animals have usually found that hepatic HMG CoA reductase activity and the rate of cholesterol synthesis are decreased and that insulin therapy restores these activities to normal.[15–20] Paradoxically, insulin deficiency is associated with increased intestinal HMG CoA reductase activity and increased rate of cholesterol synthesis.[17–20] In parallel to the increases in the rate of intestinal cholesterol synthesis, insulin-deficient animals develop hyperlipidemia due to increased circulating intestinal lipoproteins.[21]

In the present study, insulin deficiency was associated with an increase in circulating triglyceride-rich lipoproteins. The receptor-mediated uptake of these triglyceride-rich lipoproteins would be expected to lead to a decrease in cellular HMG CoA reductase activity and a decreased rate of cholesterol synthesis. However, the results showed an increase in HMG CoA reductase activity and an increased rate of cholesterol synthesis in freshly isolated thioglycolate-elicited macrophages, and this was normalized by insulin therapy. There are several possibilities that might explain these findings. First, insulin deficiency may have caused a change in the population of peritoneal exudative cells. The available evidence suggests that this is unlikely because similar numbers and types of peritoneal cells, as well as plating efficiencies, were observed in control, insulin-deficient, insulin-treated, and diet-treated mice. Nonetheless, the possibility that insulin deficiency caused a qualitative change in the

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population of macrophages obtained or that the small number of adherent neutrophils present in the insulin-deficient dishes caused the alterations in cholesterol metabolism cannot be entirely excluded. Second, insulin deficiency might alter the feedback inhibition of HMG CoA reductase by cholesterol. Because the addition of exogenous cholesterol to macrophages isolated from insulin-deficient mice inhibited HMG CoA reductase activity, it appears that insulin deficiency does not directly affect the ability of intracellular cholesterol to regulate HMG CoA activity. Third, insulin deficiency may have altered the composition of lipoproteins in vivo and this change secondarily increased HMG CoA reductase activity and the rate of cholesterol synthesis. Triglyceride-rich, cholesterol-poor particles have been reported to have a reduced ability to suppress cholesterol synthesis and may even increase HMG CoA reductase activity.[22,23] Indeed, in the present studies insulin-deficient mice accumulated triglyceride-rich VLDL in their plasma. However, mice fed a sucrose/corn oil diet also accumulated triglyceride-rich VLDL in their plasma, but in contrast to insulin deficiency, thioglycolate-elicited macrophages isolated from the diet-treated mice had low levels of HMG CoA reductase activity. Therefore, it appears that insulin deficiency causes hypertriglyceridemia and an alteration in the composition of VLDL, but these changes in lipoprotein levels and composition are not responsible for the increase in HMG CoA reductase activity. Fourth, it is possible that insulin deficiency altered the receptor-mediated uptake of lipoproteins by macrophages.

Murine macrophages express several different receptors that recognize specific lipoproteins. Unlike human monocyte macrophages that actively express LDL receptors,[24,25] murine macrophages possess few if any LDL receptors.[26] Although no specific LDL binding was observed, it is important to note that human lipoproteins were used as ligands in the present study. Because human LDL is a relatively low affinity ligand for the LDL receptor compared with apoprotein-E–containing lipoproteins,[27-29] the presence of LDL receptors on murine macrophages cannot be excluded by these studies. As opposed to normal LDL, chemically modified LDLs (e.g., acetylated and malondialdehyde) are actively taken up by macrophages via the "scavenger receptor" with a resultant accumulation of cellular cholesteryl esters.[23,24] In addition to acetylated LDL, murine macrophages possess another receptor that mediates the uptake of several different VLDLs, including cholesterol-rich β-VLDL, hypertriglyceridemic VLDL, and normal VLDL.[12,30-34] The uptake of β-VLDL results in the cellular accumulation of cholesterol esters, whereas the uptake of hypertriglyceridemic or normal VLDL predominantly causes the cellular accumulation of triglyceride with some cholesteryl ester accumulation. Of the different lipoprotein receptors that have been examined, insulin has been noted to increase the expression of LDL receptors in cultured fibroblasts in vitro[35] and in mononuclear cells in vivo.[36] In contrast, insulin has been reported to have no effects on the scavenger receptor of monocyte macrophages in vitro.[37,38] The only previous study directly examining the effects of insulin deficiency on lipoprotein receptor expressions reported that insulin deficiency did not alter β-VLDL binding to rat liver.[39] The present studies show that in vivo insulin deficiency causes a decrease and insulin therapy an increase in the number of receptors responsible for the uptake of VLDL by thioglycolate-elicited macrophages, whereas the expression of other lipoprotein receptors remains unchanged. Because human lipoproteins were used to assess receptor binding, these changes in VLDL binding might actually reflect alterations in LDL receptor expression. In addition to the changes in the number of cell surface lipoprotein receptors, the receptor-mediated uptake of lipoproteins can be altered by changes in the affinities of lipoproteins for their receptors. Although the affinities of the lipoproteins isolated from the insulin-deficient mice were not assessed in this study, the low lipolytic activity in insulin-deficient animals[40] would be expected to result in lipoprotein particles that would accentuate the deficit in receptor-mediated lipoprotein uptake in vivo.[41]

These alterations in the expression of receptors responsible for VLDL uptake may well explain the changes observed in cellular cholesterol metabolism in insulin deficiency. Because the intracellular concentration of cholesterol is predominantly dependent on the receptor-mediated uptake of circulating lipoproteins,[42] the decrease in the number of receptors responsible for VLDL uptake will result in a lower uptake of cholesterol by the macrophage. This decrease in lipoprotein-derived cholesterol will then lead secondarily to the increase in HMG CoA reductase activity and the increased rate of cholesterol synthesis observed in thioglycolate-elicited macrophages from insulin-deficient mice. The possibility that the decreased expression of lipoprotein receptors is secondary to the increase in HMG CoA reductase activity and cholesterol synthesis is unlikely in view of the observed inhibition of HMG CoA reductase activity and cholesterol synthesis by exogenous cholesterol. Likewise, the changes in lipoprotein composition occurring in insulin deficiency would not cause the decrease in lipoprotein receptors because triglyceride-rich particles are less able to regulate receptor activity downward.[22]

The significance of the changes in the cholesterol metabolism of macrophages observed in the present study may relate to the accelerated atherosclerosis seen in patients with diabetes mellitus. The foam cells characteristic of atherosclerotic lesions appear to be lipid-laden macrophages derived from blood-borne monocytes.[2,3] Thus, the macrophage seems to play an important role in the atherosclerotic process; however, it is unclear whether the macrophage contributes to atherosclerosis or functions to protect the integrity of the vessel. The insulin-deficiency–induced decrease in the expression of receptors responsible for VLDL uptake could impair the ability of macrophages to clear lipoproteins from the vessel wall and thus may contribute to atherogenesis in diabetes mellitus.

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