Intralipid Infusion Abolishes Ability of Human Serum to Cholesterol-load Cultured Macrophages

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Intralipid is widely used for intravenous alimentation and contains triglyceride-emulsion particles and phospholipid liposomes. After infusion, triglyceride-emulsion particles resemble chylomicron remnants and thus may be atherogenic. On the other hand, Intravenous infusion of phospholipid liposomes produces regression of experimental atherosclerosis and abolishes the ability of hypercholesterolemic rabbit plasma to cholesterol-load cultured macrophage foam cells. To determine the net effect of Intralipid infusion on cellular cholesterol balance, J-774 macrophages were incubated for 18 hours with human serum obtained before or during an infusion of 6-hour infusion of 10% Intralipid. Compared to serum-free medium, pre-infusion serum increased cellular unesterified cholesterol by 76% and cholesterol ester by 78%. In contrast, serum obtained after the 6-hour infusion reduced cellular unesterified cholesterol by 23% and cholesterol ester by 15%. Serum obtained 18 hours after the end of the infusion still showed impaired cholesterol-loading ability. Mouse peritoneal macrophages incubated with these serum samples behaved similarly. Compared to pre-infusion serum, postinfusion serum inhibited cellular uptake of 125I-low density lipoprotein and 125I-very low density lipoprotein by 90% and 90%, respectively, and also enhanced the efflux of cellular cholesterol by 48%. We conclude that the ability of human serum to cause cholesterol accumulation in cultured macrophages is abolished by an infusion of Intralipid. This effect is mediated by a reduction in cholesterol uptake by the cells and by an increase in cell cholesterol efflux. Similar events occur in the arterial wall. Intralipid infusion might inhibit foam cell formation in vivo.

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Atherosclerosis involves the accumulation of cholesterol ester droplets in arterial wall macrophages and smooth muscle cells, which, because of their appearance on light microscopy, have been called "foam cells." The lipoproteins responsible for cholesteryl ester accumulation include low density lipoprotein (LDL), 

β-very low density lipoprotein (VLDL), or modified forms of these particles. It has also been suggested that the remnant particles formed after lipolysis of dietary chylomicrons may contribute to atherogenesis. Chylomicron remnants have been shown to cause cholesteryl ester accumulation in cultured macrophages.

Regression of atherosclerosis has been produced in experimental animals by intravenous infusion of dispersed phospholipid. Infusion of phospholipid has been shown to result in the appearance of vesicular lipoproteins in plasma. These vesicular lipoproteins remove cholesterol from other lipoproteins and tissues and may transport this cholesterol to the liver. In addition, the vesicular particles acquire apolipoprotein (apo) E, bind to LDL receptors, and competitively reduce receptor-mediated uptake of atherogenic lipoproteins by cultured macrophages. As a consequence of these effects on cellular cholesterol efflux and influx, a single infusion of phospholipid abolishes the ability of hypercholesterolemic rabbit plasma to cause cholesterol accumulation in cultured macrophage foam cells.

Intralipid, which is in common clinical use for intravenous feeding, also results in the appearance of vesicular lipoproteins in the plasma of human subjects, and has, therefore, been suggested as a potential treatment for human atherosclerosis. However, Intralipid is a mixture of phospholipid liposomes and large, nonvesicular triglyceride-emulsion particles, whose effects on atherogenesis are unknown. These large triglyceride-emulsion particles can acquire cholesteryl ester and apo E from plasma lipoproteins, and thereby resemble dietary chylomicrons. Thus, the emulsion particles or their remnants could cause cholesteryl ester accumulation in arterial wall macrophages.

To determine the net effect of Intralipid on macrophage cholesterol balance, we infused Intralipid into six normal volunteers and then determined the ability of their serum to promote cholesterol loading or efflux in
cultured macrophages. Our results show that Intralipid infusion results in a marked reduction in cholesterol loading of cultured macrophages.

Methods

Subjects

Six normal healthy male volunteers were admitted to the Clinical Research Center after an overnight fast. They each received an infusion of 10% Intralipid at a rate of 300 mg triglyceride per kg of body weight per hour for 6 hours. Samples of serum (20 ml) were collected before the infusion (t=0 hours) and at 15 minutes, 3, 6, 8, and 24 hours after the beginning of the infusion. Serum unesterified and esterified cholesterol, \(^{14}\)C phospholipid, \(^{3}H\) triglyceride (Sigma kit 335, St. Louis, MO), and fatty acid (Wako kit 990-75401, Osaka, Japan) concentrations were determined by enzymatic methods using commercial kits. The experimental protocols were approved by the Institutional Review Board of Columbia Presbyterian Medical Center, and informed consent was obtained from each subject.

Materials

Dulbecco’s modified Eagle’s medium (DMEM), penicillin (10 000 U/ml), streptomycin (100 000 μg/ml), and glutamine (200 mM) solutions were obtained from Gibco Laboratories (Grand Island, NY), and fetal bovine serum (lot #116164) was obtained from Hydcole Laboratories (Logan, UT). The Falcon tissue culture plasticware used in these studies was purchased from Fisher Scientific Co. (Pittsburgh, PA). Intralipid (10% and 20%) solutions were obtained from Kabi Vitrum Inc. (Alameda, CA). Na\(^{14}\)H (carrier-free in 0.1N NaOH), [1.2,\(^{14}\)C]-acetic acid sodium salt, [7-\(^{3}H\)] cholesterol were purchased from New England Nuclear (Boston, MA). Essential fatty acid-free BSA (A6003) and egg phosphatidylcholine (P-2772) were obtained from Sigma Chemical Company (St. Louis, MO). Compound 58035 (3-[deoxy(methyl)silyl]-N-[2-(4-methylphenyl)-1-phenyl-ethyl] propanamide) was generously provided by Sandoz Incorporated (East Hanover, NJ).

Lipoproteins, Liposomes, and Triglyceride Emulsions

VLDL (d<1.006 g/ml), LDL (d=1.019 to 1.063 g/ml), and high density lipoproteins (HDL\(_{3}\), d=1.125 to 1.210 g/ml) were isolated from fresh, fasted human plasma by preparative ultracentrifugation.\(^{20}\) \(^{3}H\)-labeled LDL and \(^{14}\)C-labeled VLDL were prepared by a modification of the iodine monochloride method\(^{21}\) the specific activities ranged between 150 and 300 cpm/g of protein. The majority (69%) of the radioactivity in VLDL was in apo B, 21% was in apo C, and 8% was in apo E. \(^{3}H\)-CE-HDL\(_{3}\) was prepared as described.\(^{22}\) \(^{3}H\)-cholesteryl ester-LDL (\(^{3}H\)-CE-LDL) was then prepared by incubation of \(^{3}H\)-CE-HDL\(_{3}\) with unlabeled LDL in the presence of partially purified CE transfer protein, followed by ultracentrifugal re-isolation of the LDL. The final preparation of \(^{3}H\)-CE-labeled LDL contained 99% of the \(^{3}H\)-radioactivity in cholesteryl ester and 1% in free cholesterol. The specific activity was 350 to 450 cpm/μg of cholesteryl ester.

A 1.2% (wt/vol) dispersion of chromatographically pure egg phosphatidylcholine (Sigma Chemical Company, Catalog #P-2772) in physiologic saline (0.9%) was prepared by ultrasonic irradiation at 0°C under argon for a total of 40 minutes, followed by centrifugation to remove fragments of titanium shed by the sonicator probe.\(^{22}\) The liposomes were then sterilized by passage through a 0.45 μm Millex GS filter. Liposomes were always used within 24 hours of preparation.

Triglyceride-emulsion particles were purified from Intralipid by separation from the liposome-rich fraction by preparative ultracentrifugation. Three ml of solution, d=1.006 g/ml, was layered over 2 ml of 10% or 20% Intralipid, then ultracentrifuged at 4°C for 20 minutes at 25 000 rpm in an SW 50.1 rotor. The triglyceride-emulsion particles floated to the upper layer and contained 90% of the initial triglyceride mass. Excess vesicular phospholipid remained in the infranatant, and accounted for 40% and 19.5% of initial phospholipid mass in 10% and 20% Intralipid, respectively.

Cells

Monolayer cultures of J-774 murine macrophage-like cells were grown and maintained in DMEM supplemented with 10% (vol/vol) fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and glutamine (2 mM). For each experiment, the cells were plated in 35 mm dishes at a density of 10\(^6\) cells/dish. The cells were used in two forms: nonloaded or cholesterol-loaded cells. Cholesterol-loaded cells were prepared by incubation with 600 μg of LDL\(^{24}\) protein/ml in DMEM medium containing 10% heat-inactivated fetal bovine serum for 18 hours, followed by three washes with phosphate-buffered saline (PBS).\(^{24}\) Similarly, cholesterol-labeled cells were prepared by incubation with 10\(^6\) cpm/ml of \(^{3}H\)-CE-HDL (50 μg of LDL protein/ml) for 18 hours. Cell viability was determined by 0.04% trypan blue exclusion\(^{25}\) and was always greater than 90%.

Monolayer cultures of mouse peritoneal macrophages (MPM) were harvested from unstimulated female Swiss-Webster mice (25 to 35 g) in PBS, collected by centrifugation, and were plated under similar conditions to those described for the J-774 cells, except that 10% lipoprotein-deficient serum was substituted for 10% fetal calf serum to up-regulate LDL receptor activity. The average protein content per dish was 1.0 mg for J-774 cells and 0.25 mg for MPM.

Cells were incubated for 18 hours in DMEM supplemented with a 1% bovine serum albumin (BSA) (control medium) or in control medium supplemented with 10% heat-inactivated serum derived from the volunteers before, during, or after infusion with Intralipid. To avoid flotation of the large triglyceride-phospholipid particles, cells were incubated on a rocker platform (Bellco Biotechnology, Vineland, NJ). After the incubations, cells were scraped into the media, and media and cells were separated by low speed centrifugation. Cells were then washed with PBS three times and aliquots were removed for determination of cellular protein,\(^{26}\) cholesterol and cholesteryl ester,\(^{27,28}\) triglycerides, and phospholipid.\(^{22}\) In separate experiments, cells were also incubated with liposomes or
isolated triglyceride-emulsion particles in the presence of fasted, pre-infusion human serum.

**Measurement of Cellular Uptake and Degradation of LDL and VLDL**

\(^{125}\text{I}-\)labeled LDL or \(^{128}\text{I}-\)labeled VLDL (10 μg of protein/ml) was incubated with cells for 5 hours at 37°C in the presence of pre-infusion sera, post-infusion sera, or pre-infusion sera supplemented with liposomes, Intralipid, or triglyceride-emulsion particles. Cell-association of these \(^{125}\text{I}-\)labeled lipoproteins was determined by measuring total cellular \(^{125}\text{I}-\)radioactivity. Degradation of \(^{129}\text{I}-\)lipoproteins was determined by measuring the release of TCA-soluble, chloroform-insoluble radioactivity into the media.\(^3\)\(^\text{2}\)\(^\text{3}\) Cell-specific degradation was calculated by subtracting spontaneous degradation measured in cell-free wells from the total degradation measured in the presence of cells. Spontaneous degradation was less than 7.5 ng \(^{3}\text{H}-\)lipoprotein per 35 mm tissue culture well. Cell association was also determined for \(^{3}\text{H}-\)cholesterol ester-labeled LDL by measuring cellular \(^{3}\text{H}-\)radioactivity.

**Measurement of Cellular Retention of \(^{3}\text{H}-\)Cholesterol**

Cellular cholesterol efflux was studied by incubating \(^{3}\text{H}-\)cholesterol-labeled cells for 18 hours with media containing various sera or emulsions. At the end of the incubation, total cellular \(^{3}\text{H}-\)radioactivity was measured.\(^2\)\(^\text{2}\) Thin-layer chromatography (hexane/diethyl ether/glacial acetic acid at a volume ratio of 70:30:1) was used to separate cellular \(^{3}\text{H}-\)cholesterol counts from \(^{3}\text{H}-\)cholesterol ester counts. \(^{14}\text{C}-\)cholesterol served as an internal standard to correct for losses during the chromatography procedure.

**Measurement of Cholesterol Synthesis**

Cells were incubated in the presence of the various sera for 18 hours, were washed and then incubated for 2 hours with 1.2 \(^{14}\text{C}-\)sodium acetate (1.25 mM, 10 μCi/ml), as previously described.\(^2\) At the end of the incubation, the incorporation of radioactivity into cellular unesterified cholesterol and into the sterol moiety of cellular cholesteryl ester was determined by thin-layer chromatography.

**Statistical Analyses**

Each separate experiment was performed in triplicate. The results are the means ± the SEM.

**Results**

**Serum Lipids and Cellular Cholesterol Content after Intralipid Infusion**

The mean serum lipid levels after Intralipid infusion into the six subjects are shown in Figure 1. The infusions of Intralipid elevated serum concentrations of triglyceride, phospholipid, free fatty acids, and unesterified cholesterol. Serum cholesteryl ester concentration was not affected. Changes in serum lipids were most pronounced during or shortly after the end of the 6-hour infusion and declined toward baseline values 18 hours after the end of the infusion (Figure 1).

To determine the effect of these alterations on the ability of serum to cholesterol-load cultured macrophages, we incubated J-774 cells for 18 hours in serum-free control medium or in control medium supplemented with 10% serum obtained from the volunteers before, during, or after Intralipid infusion. Compared to control cells incubated without serum, normal fasted serum (time=...
human serum in mouse peritoneal macrophages and in J-774 cells preloaded with cholesterol. Incubation of mouse peritoneal macrophages with pre-infusion serum resulted in a cellular cholesteryl ester content of 24±9 nmol/mg (mean±SEM, n=3); cells incubated with serum obtained at 6 hours and 24 hours contained only 4±2 and 9±4 nmol/mg, respectively. Preloaded J-774 cells incubated with pre-infusion serum, 6-hour serum, or 24-hour serum contained 126±5, 93±8, and 110±6 nmol cholesteryl ester/mg, respectively. Thus, under several conditions, incubation with postinfusion sera resulted in lower cellular contents of cholesteryl ester than with normal, pre-infusion sera.

**Mechanisms for Decreased Cellular Accumulation of Cholesterol and Cholesteryl Ester**

Potential mechanisms contributing to decreased cell cholesterol content include decreased cholesterol influx, increased cholesterol efflux, or decreased cholesterol synthesis. Experiments were performed to determine a possible contribution of each of these mechanisms to the decrease in cell cholesterol mass after incubations with postinfusion serum.

Cellular uptake of cholesteryl ester-rich lipoproteins was measured by incubating J-774 macrophages for 5 hours at 37°C with medium supplemented with the various serum samples and either [125I]-LDL (10 μg protein/ml medium) or [125I]-VLDL (10 μg protein/ml). Cell association (not shown) and degradation (Figure 4) of [125I]-LDL and [125I]-VLDL were substantially reduced in postinfusion serum samples. The time course of decreased lipoprotein degradation was parallel to the decrease in free and esterified cholesterol that results from Intralipid infusion (Figure 2). A similar pattern was seen with cellular uptake of [3H-cholesteryl ester]-LDL (data not shown).

Parallel studies conducted in separate subjects using the same infusion protocol demonstrated that, from...
0 hours to 24 hours, total serum apo B levels varied by less than 5%, and LDL contained 89.6% to 97.9% of total serum apo B. Thus, there were only small differences in the dilution of the specific activity of the ^125^I-LDL that was added to the various serum samples. With nearly constant specific activity, the decrease in ^125^I-LDL degradation shown in Figure 4A reflects a true decrease in the entry of LDL mass into the cells. In contrast, the amount of apo B on VLDL rose from 4.4% of total serum apo B at 0 hours to 9.1% at 6 hours. Thus, in Figure 4B, degradation of ^125^I-VLDL in 6-hour serum was 22% of the value in pre-infusion serum. Correcting for the difference in dilution of specific activity yielded a true value of (22% (9.1/4.4)) = 46% for the ratio of VLDL degradation in 6-hour versus 0-hour serum. These results demonstrate a markedly reduced uptake and degradation of LDL and VLDL mass from human serum after infusion of Intralipid.

Effects of Intralipid infusion on cellular cholesterol efflux were measured by prelabeling J-774 macrophages with cholesterol (with ^3H-CE-LDL), incubating the cells with the serum samples for 18 hours, then determining the amount of cellular free and esterified cholesterol radioactivity. Compared to serum-free controls, pre-infusion sera caused only a moderate reduction in cellular retention of ^3H-free cholesterol and ^3H-cholesterol ester (Figure 5). Intralipid infusion markedly enhanced the ability of human serum to promote cellular loss of cholesterol radioactivity (Figure 5). Maximum efflux was seen in the 6-hour serum samples. Sera obtained at 24 hours still promoted greater loss of ^3H-cholesterol ester than did the pre-infusion sera. Thus, Intralipid infusion promoted substantial losses of cellular cholesterol radioactivity.

Cellular cholesterol biosynthesis after incubation with the serum samples was assessed by the conversion of ^14C-acetate into ^14C-cholesterol. The reductions in cell cholesterol mass induced by postinfusion serum samples were accompanied by an increase in cell cholesterol synthesis, from 148±15 cpm/mg cell protein with pre-infusion serum to 415±17 cpm/mg cell protein with sera obtained 6 hours after start of the infusions (mean±SEM, n=5). Thus, cell cholesterol synthesis acted to compensate for, not contribute to, the reduction in cellular cholesterol mass.

In summary, the loss of the cholesterol-loading ability of human serum after infusion of Intralipid was accompanied by substantially impaired entry of LDL and VLDL into macrophages and substantially enhanced efflux of cellular cholesterol stores.

Quantitation of Contributions of Reduced Cholesterol Influx and Enhanced Cholesterol Efflux

To quantitate the contributions of reduced cholesterol influx and enhanced cholesterol efflux, we examined changes in cellular cholesteryl ester mass during a 5-hour incubation with the acyl coenzyme A:cholesterol acyl transferase (ACAT) inhibitor, Sandoz-58035. This inhibitor of cholesterol esterification blocks entry of any endogenous or exogenous cholesterol into cellular stores of cholesteryl ester. Thus, with no influx into cellular cholesteryl ester stores in the presence of the inhibitor, changes in cellular cholesteryl ester mass provide a direct measurement of the effects of efflux alone.

In the presence of 58035 (Figure 6, hatched bars), pre-infusion serum (0 hours) and serum obtained 6 hours after infusion reduced cellular cholesteryl ester mass by 16 nmol/mg and 21 nmol/mg, respectively, compared to serum-free control (dashed line). Thus, the efflux of cellular cholesteryl ester mass was 5 nmol/mg greater in 6-hour postinfusion serum than in pre-infusion serum.
In the absence of 58035 (Figure 6, open bars), influx and efflux occurred together, and changes in cellular cholesterol ester mass resulted from the sum of these two effects. During a 5-hour incubation without 58035, pre-infusion serum increased cellular cholesterol ester by 13 nmol/mg, and 6-hour serum decreased cholesterol ester by 1.0 nmol/mg, compared to control, yielding a total difference of 14 nmol/mg. Of this 14 nmol/mg difference, 5 nmol/mg (36%) was the result of the difference in efflux between pre- and postinfusion sera (Figure 6, hatched bars). Therefore, the remaining 9 nmol/mg (64%) was the result of the difference in cholesterol influx between the two sera.

We conclude that the major mechanism by which Intralipid infusion reduced cholesterol ester mass in cultured macrophages was by blockage of cholesterol influx into the cells. Enhancement of cholesterol efflux played a quantitatively smaller role. Cellular free cholesterol content remained unchanged throughout the experiment. Incubation of serum obtained 6 hours after infusion or pre-infusion serum (0 hours) with macrophages resulted in 77±6 and 79±5 nmol, respectively, free cholesterol/mg cell protein (n=3). Thus, since cellular free cholesterol mass remained unchanged, the changes in esterified cholesterol reflect net cholesterol flux.

**Independent Contributions of Liposomes and Triglyceride Phospholipid Emulsion Particles**

Intralipid contains triglyceride-poor phospholipid vesicles, which are known to block cholesterol influx and promote cholesterol efflux from macrophages and triglyceride-emulsion particles, whose effects are unknown. We therefore sought to determine the effects of triglyceride-emulsion particles on macrophage cholesterol balance. Liposomes, Intralipid, and triglyceride-emulsion particles were added to human serum in vitro. J-774 macrophages were incubated for 18 hours in DMEM with 1% BSA (control medium) or in control medium supplemented with the mixtures of serum and exogenous lipid dispersions. All experimental media contained 10% serum. The amounts of the different exogenous lipid dispersions were matched so that the concentration of exogenous phospholipid was always 120 μg/ml of medium. The concentrations of exogenous triglyceride in the media varied from zero (liposomes) to 2500 μg (washed Intralipid, which contained only triglyceride-emulsion particles and no liposomes).

All dispersions impaired the ability of human serum to cause cholesterol and cholesteryl ester accumulation in the cultured macrophages (Figure 7A). The preparations with higher contents of triglyceride were slightly more effective. All preparations reduced LDL entry into the cells (Figure 7B) and also enhanced cellular cholesterol loss (Figure 7C). Thus, both liposomes and triglyceride-emulsion particles may independently contribute to the reduction in serum cholesterol-loading ability after infusion of Intralipid.

**Discussion**

We have shown that a single, 6-hour infusion of 10% Intralipid into normal volunteers abolished the ability of their serum to cause accumulation of unesterified and esterified cholesterol mass in cultured J-774 cells and mouse peritoneal macrophages. Moreover, postinfusion serum actually reduced cholesteryl ester stores in cholesteryl ester-loaded J-774 foam cells. The direction and magnitude of the effect of Intralipid-modified serum on macrophage cholesterol stores was surprising, since a major component of Intralipid is triglyceride-phospholipid emulsion particles, which accumulate apo E and cholesteryl ester from lipoproteins, and therefore have the potential to behave like atherogenic chylomycron remnants. Our data suggest, however, that the major effect of Intralipid infusion in normolipidemic subjects reflects reduced uptake of CE-rich endogenous lipoproteins complemented by a lesser effect of increased cholesterol efflux. Overall, our results suggest that Intralipid infusions might inhibit foam cell formation in vivo. However, different results might be obtained if Intralipid was infused into subjects with impaired clearance of chylomicrons.

In an earlier study, Williams et al. showed that a single infusion of phospholipid liposomes was able to abolish the cholesterol-loading ability of hypercholesterolemic rabbit plasma. The present investigation extends these earlier findings by showing that a similar effect can be produced in humans by infusing Intralipid. Normal human lipoproteins differ substantially from those seen in hypercholesterolemic rabbits. In addition, the current study used 10% Intralipid, a clinically available mixture of liposomes and triglyceride-emulsion particles. We demonstrated that the addition of either phospholipid liposomes or triglyceride-emulsion particles to human serum in vitro also abolished cellular cholesterol accumulation, suggesting that the emulsion component of Intralipid, like liposomes, can have favorable effects on macrophage cholesterol balance.

The effect of Intralipid and its components on cellular cholesterol balance was predominantly associated with blockage of the uptake of LDL and VLDL, and secondarily...
through promotion of cellular cholesterol efflux. Liposomes were previously shown to block cellular uptake of cholesteryl ester-rich lipoproteins through two mechanisms.12 First, infused liposomes acquired apo E, and thereby competitively blocked receptor-mediated lipoprotein uptake. Second, the liposomes reduced the apo E content of the cholesteryl ester-rich lipoproteins, thereby directly reducing their receptor affinity.

Similarly, it has been demonstrated that triglyceride-phospholipid emulsion particles acquire apo E from endog-

ous lipoproteins in vitro during co-incubation.14,15 In studies in vivo, Intralipid infusion increased total plasma apo E by 40%,11,16,30 and two-thirds of the total plasma apo E was on the triglyceride-rich particles.18,33 In the current study, blockage of cellular uptake of LDL by Intralipid, liposomes, and triglyceride-phospholipid particles presumably involved uptake of apo E by the exogenous particles, followed by direct competition with LDL for cell surface receptors. Competition between Intralipid emulsion particles and endogenous lipoproteins for cellular uptake is further supported by our data showing uptake of triglyceride mass by the cultured macrophages (Figure 3) and by the blockage of 125I-LDL uptake after addition of triglyceride-emulsion particles to serum in vitro (Figure 7).

Three additional factors may have also contributed to decreased cholesterol uptake by the cells. First, LDL loses substantial amounts of cholesteryl ester by neutral lipid transfer in the presence of Intralipid added to serum in vitro15 and after infusion in vivo.33,34 Thus, each LDL particle internalized by the cultured macrophages after Intralipid infusion delivered less total sterol. Second, it is possible that triglyceride uptake by the cells may have inhibited ACAT and thereby reduced cellular accumulation of cholesteryl ester. Support for this concept comes from the recent observation that chylomicron remnants increased 1H-oleate incorporation into macrophage triglycerides, while decreasing its incorporation into macrophage cholesteryl ester.7 Finally, it has been shown that binding and uptake of LDL is reduced by alterations in the phospholipid composition of cultured cell35 and by alterations in the surface lipids of LDL.30

Intralipid and its components also affected macrophage cholesterol balance by promoting cellular cholesterol efflux. Previous studies have shown that cholesterol efflux from cells in the presence of lipoproteins37,38,39 or liposomes38,40 is mediated by desorption of unesterified cholesterol from the plasma membrane, followed by hydrolysis of intracellular stores of cholesteryl ester. In our study, the mechanism of enhanced cholesterol efflux into postinfusion serum samples presumably involved increased removal of cellular unesterified cholesterol by cholesterol-poor, exogenous acceptor particles and by phospholipid-enriched, cholesterol-depleted endogenous lipoproteins.9

By adding isolated Intralipid components to serum in vitro, we showed that triglyceride-emulsion particles, like phospholipid liposomes, can contribute to enhanced efflux. Per unit mass of phospholipid, the triglyceride-emulsion particles were the better acceptor of unesterified cholesterol, consistent with previous demonstrations that substantial amounts of unesterified cholesterol can partition into the particle triglyceride core.11 Concerning alterations in the surface lipid composition of endogenous lipoproteins, parallel studies with the same Intralipid infusion protocol demonstrated that, at the end of a 6-hour infusion, LDL and HDL increased their phospholipid contents by 30% and 20%, respectively, and lost 20% and 12%, respectively, of their unesterified cholesterol.33,34 This drop in the ratio of free cholesterol to phospholipid would presumably make these lipoproteins better promoters of cellular cholesterol efflux.
An associated effect of infusing Intralipid was the accumulation of triglyceride in cells incubated with postinjection serum. Nevertheless, in all cases, the total amount of macrophage neutral lipid (cholesterol ester plus triglyceride) was lower after incubation in postinjection serum than in pre-injection serum. Moreover, accumulation of triglyceride is not likely to be harmful. Unlike cholesterol ester, triglyceride that has accumulated in cultured macrophages is rapidly removed in the presence of a fatty acid acceptor, such as albumin. Relative to this point, enuphathmatic in vivo rapidly regresses after reduction of the serum concentration of triglyceride, and there are no reports of eruptive xanthomatous or triglyceride-rich xanthomat in patients treated with Intralipid.

Compared to pre-injection serum, the serum samples that were obtained 18 hours after the end of the Intralipid infusion were still associated with substantial reductions in cellular cholesterol ester, yet caused little elevation in cellular triglyceride content. The predominant exogenous particle in the circulation 18 hours after an injection of Intralipid is liposomes. These liposomal particles would be expected to reduce cellular sterol without increasing cellular triglyceride.

Our studies suggest that Intralipid infusion into normolipemic humans with normal triglyceride clearance may favorably affect the cholesterol balance in arterial wall foam cells.

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