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(54) **COMPOSITIONS LIPOSOMIQUES ET LEURS METHODES  
D'UTILISATION**

(54) **LIPOSOMAL COMPOSITIONS AND METHODS OF USING  
THEM**

(57) L'invention porte sur le transport inverse du cholestérol des tissus périphériques vers le foie chez un mammifère avec, concurremment, régulation des concentrations de lipoprotéines athérogènes du plasma, notamment des concentrations de lipoprotéines à faible densité. La méthode et le mode de fonctionnement des dispositifs consistent à administrer une quantité efficace de plusieurs accepteurs constitués de plusieurs phospholipides essentiellement exempts de stérol. On peut, facultativement, doser périodiquement des concentrations de lipoprotéines athérogènes en procédant à un dosage au cours de la période de traitement pour évaluer les concentrations de lipoprotéines athérogènes et obtenir un profil de ces lipoprotéines avant d'ajuster l'administration en fonction de ce profil. Les liposomes de grande dimension sont plus grands que les ouvertures d'une couche endothéliale bordant les sinusoides hépatiques du foie, de telle sorte que ces liposomes sont, selon l'un des modes envisageables, trop grands pour pénétrer à travers ces ouvertures. Les quantités thérapeutiquement efficaces se situent dans la plage comprise entre environ 10 et 1600 mg de phospholipides par kg de poids corporel par dose. L'invention porte sur une composition pharmaceutique et sur un kit destiné à mobiliser le cholestérol périphérique et la sphingomyéline entrant dans le foie du sujet, cette composition et ce kit étant essentiellement constitués de liposomes d'un calibre supérieur aux ouvertures de la couche endothéliale revêtant les sinusoides hépatiques du foie. L'invention porte également sur la régulation des gènes se rapportant au cholestérol et d'autres composés.

(57) The present invention provides a liposomal composition, method of using a liposomal composition, and devices and modes of operation of the devices and of the compositions, and kits related thereto. The invention provides for the reverse transport of cholesterol from peripheral tissues to the liver in a warm blooded mammal while controlling plasma atherogenic lipoprotein concentrations, including LDL concentrations. The method and mode of operation of the devices includes the step of administering an effective amount of a multiplicity of acceptors comprised of phospholipids substantially free of sterol. The method optionally includes the step of periodically assaying atherogenic lipoprotein concentrations with an assay during the treatment period to assess atherogenic lipoprotein concentrations and obtain an atherogenic lipoprotein profile, and adjusting the administration in response to said profile. The large liposomes are dimensioned larger than fenestrations of an endothelial layer lining hepatic sinusoids in the liver so that the liposomes are too large to readily penetrate the fenestrations of one variant. The therapeutically effective amounts are in the range of about 10 mg to about 1600 mg phospholipid per kg body weight per dose. A pharmaceutical composition and related kit for mobilizing peripheral cholesterol and sphingomyelin that enters the liver of a subject consisting essentially of liposomes of a size and shape larger than fenestrations of an endothelial layer lining hepatic sinusoids in the liver is also provided. The invention also provides for control of cholesterol related genes and other compounds.

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<b>(21) International Application Number:</b> PCT/US96/16388 <b>(22) International Filing Date:</b> 11 October 1996 (11.10.96)  <b>(30) Priority Data:</b> 60/005,090      11 October 1995 (11.10.95)      US Not furnished      11 October 1996 (11.10.96)      US  <b>(71)(72) Applicant and Inventor:</b> WILLIAMS, Kevin, Jon [US/US]; 425 Wister Road, Wynnewood, PA 19096-(US).  <b>(74) Agent:</b> ZIARNO, Witold, A.; Michael, Best & Friedrich, 100 East Wisconsin Avenue, Milwaukee, WI 53202-4108 (US).		<b>(81) Designated States:</b> AU, CA, CN, JP, MX, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the          claims and to be republished in the event of the receipt of          amendments.</i>

**(54) Title:** LIPOSOMAL COMPOSITIONS AND METHODS OF USING THEM**(57) Abstract**

The present invention provides a liposomal composition, method of using a liposomal composition, and devices and modes of operation of the devices and of the compositions, and kits related thereto. The invention provides for the reverse transport of cholesterol from peripheral tissues to the liver in a warm blooded mammal while controlling plasma atherogenic lipoprotein concentrations, including LDL concentrations. The method and mode of operation of the devices includes the step of administering an effective amount of a multiplicity of acceptors comprised of phospholipids substantially free of sterol. The method optionally includes the step of periodically assaying atherogenic lipoprotein concentrations with an assay during the treatment period to assess atherogenic lipoprotein concentrations and obtain an atherogenic lipoprotein profile, and adjusting the administration in response to said profile. The large liposomes are dimensioned larger than fenestrations of an endothelial layer lining hepatic sinusoids in the liver so that the liposomes are too large to readily penetrate the fenestrations of one variant. The therapeutically effective amounts are in the range of about 10 mg to about 1600 mg phospholipid per kg body weight per dose. A pharmaceutical composition and related kit for mobilizing peripheral cholesterol and sphingomyelin that enters the liver of a subject consisting essentially of liposomes of a size and shape larger than fenestrations of an endothelial layer lining hepatic sinusoids in the liver is also provided. The invention also provides for control of cholesterol related genes and other compounds.

## LIPOSOMAL COMPOSITIONS AND METHODS OF USING THEM

### BACKGROUND OF THE INVENTION

Several human conditions are characterized by distinctive lipid compositions of tissues, cells, membranes, and extracellular regions or structures. For example, in atherosclerosis, cholesterol (unesterified, esterified, and oxidized forms) and other lipids accumulate in cells and in extracellular areas of the arterial wall and elsewhere. These lipids have potentially harmful biologic effects, for example, by changing cellular functions, including gene expression, and by narrowing the vessel lumen, obstructing the flow of blood. Removal of these lipids would provide numerous substantial benefits. Moreover, cells, membranes, tissues, and extracellular structures will benefit in general from compositional alterations that include increasing resistance to oxidation and oxidative damage, such as by increasing the content and types of anti-oxidants, removing oxidized material, and increasing the content of material that is resistant to oxidation. In aging, cells have been shown to accumulate sphingomyelin and cholesterol, which alter cellular functions. These functions can be restored in vitro by removal of these lipids and replacement with phospholipid from liposomes. A major obstacle to performing similar lipid alterations in vivo has been disposition of the lipids mobilized from tissues, cells, extracellular areas, and membranes. Natural (e.g., high-density lipoproteins) and synthetic (e.g., small liposomes) particles that could mobilize peripheral tissue lipids have a substantial disadvantage: they deliver their lipids to the liver in a manner that disturbs hepatic cholesterol homeostasis, resulting in elevations in plasma concentrations of harmful lipoproteins, such as low-density lipoprotein (LDL), a major atherogenic lipoprotein. There exist a need for a better method to manipulate the lipid content and composition of peripheral tissues, cells, membranes, and extracellular regions in vivo.

The intravenous administration of cholesterol-poor phospholipid vesicles (liposomes) or other particles that transport cholesterol and other exchangeable material from lipoproteins and peripheral tissues, including atherosclerotic arterial lesions, to the liver produces substantial derangements of hepatic cholesterol homeostasis, such as enhanced hepatic secretion of apolipoprotein-B, and suppression of hepatic LDL receptors. The hepatic derangements lead to increase plasma concentrations of LDL and other atherogenic lipoproteins. Increased concentrations of LDL or other atherogenic lipoproteins will

accelerate, not retard, the development of vascular complications. Deranged hepatic cholesterol homeostasis can also be manifested by abnormal regulation of genes, such as a gene for the LDL receptor, a gene for HMG-CoA reductase, a gene for cholesterol 7-alpha hydroxylase, and a gene regulating a function involved in cholesterol homeostasis. There exists a need for methods and compounds that can produce a removal of cholesterol and other exchangeable material, from peripheral cells, tissues, organs, and extracellular regions, and that can produce a delivery of material, such as phospholipids, to cells, tissues, or organs, extracellular regions without harmfully disrupting hepatic cholesterol homeostasis and plasma concentrations of atherogenic lipoproteins.

By way of example, atherosclerosis, a major killer in Western countries, is characterized by the accumulation of cholesterol and cholesteryl ester in cells and in extracellular areas of the arterial wall and elsewhere. There exists a need for a better method to manipulate the lipid content and composition of peripheral tissues, cells, membranes, and extracellular regions *in vivo*. There further exists a need for methods or compounds that can produce removal of cholesterol from cellular and extracellular regions of arteries, but without provoking a rise in the plasma concentration of LDL.

The invention described herein provides methods and compositions related to the removal of cholesterol from arteries, whole controlling plasma concentrations of LDL. The present invention addresses these needs so that diseases and detrimental medical conditions can be treated, controlled or eliminated.

This invention provides methods and compositions that relate to the "reverse" transport of lipids and other exchangeable material from peripheral tissues to the liver *in vivo* while controlling plasma LDL concentrations. There exists a need for a method of, treatment, and a pharmaceutical composition for forcing the reverse transport of lipids from peripheral tissues to the liver *in vivo* while controlling plasma LDL concentrations; of regulating hepatic parenchymal cell cholesterol content and metabolism in a cell having at least one gene selected from the group consisting of a gene for an LDL receptor, a gene for HMG-CoA reductase, a gene for cholesterol 7-alpha-hydroxylase, and a gene regulating a function involved in cholesterol homeostasis; and, homeostasis thereof; suppressing hepatic expression of a cholesterol ester transfer protein gene *in vivo*, whereby plasma LDL and HDL are controlled as a result of said administration; suppressing the rise in plasma LDL concentrations after administration of an agent having small acceptors of cholesterol or other

lipids; of diagnosing a side-effect of reverse transport of cholesterol from peripheral tissues to the liver *in vivo* accompanying parenteral administration of a multiplicity of large liposomes and small liposomes during a treatment period, whereby a side effect of administration of said liposomes is diagnosed and effectively regulated; and, diagnosing and treating a side-effect of reverse transport of lipids from peripheral tissues to the liver *in vivo* accompanying parenteral administration of a multiplicity of large liposomes and small liposomes during a treatment period. There further exists a need for a system in which patients will have a decreased risk of developing atherosclerosis and/or cellular changes from aging; an improved method of reducing the lipid content of lesions.

The invention described herein provides methods and compositions related to the removal of cholesterol and other exchangeable material from peripheral tissues, and otherwise altering peripheral tissue lipids, while controlling plasma concentrations of LDL and other atherogenic lipoproteins and avoiding harmful disruptions of hepatic cholesterol homeostasis. Specific genes in both the peripheral tissues and in the liver are controlled by these methods and compositions. There exists a need for better methods to manipulate the lipid content and composition of peripheral tissues, cells, membranes, and extracellular regions *in vivo*, particularly in regard to diseases and processes involving oxidation and oxidative damage. Moreover, currently available artificial particles for intravenous administration contain significant amounts of oxidized material (Helbock *et al.* **Pediatrics** 91:83-87, 1993), which contributes to their unsuitability for these purposes.

There further exists a need for methods or compounds that can produce a removal of cholesterol and other exchangeable material, including oxidized materials, from peripheral cells, tissues, organs, and extracellular regions, and that can produce a delivery of anti-oxidants to cells, tissues, organs, and extracellular regions, but without harmfully disrupting hepatic cholesterol homeostasis, including hepatic gene expression and regulation.

The invention described herein provides methods and compositions related to the removal of cholesterol and other exchangeable material from peripheral tissues, and otherwise altering peripheral tissue composition, to reduce or avoid oxidation and its effects and products, while controlling plasma concentrations of LDL and other atherogenic lipoproteins and avoiding harmful disruption of hepatic cholesterol homeostasis. It is an object of the invention of the present invention to solve the problems articulated above and other problems in the art.

Renal failure, both acute and chronic, is a major health problem. Current treatments for these conditions include hemodialysis, peritoneal dialysis, renal transplantation, and treatment of the underlying renal disease when possible. A major, widely recognized drawback to all methods of treatment of renal failure is accelerated atherosclerosis, which leads to heart attacks, strokes, claudication, and many other complications. Renal patients also undergo accelerated aging. There exists a need to reduce or eliminate atherosclerosis in patients with renal failure and reduce the rate of aging. These specific complications are treated with lipid-lowering drugs, LDL apheresis, angioplasty, coronary bypass surgery, carotid endarterectomy, other vascular reconstructive surgery, heart transplantation, and restoration of renal function when possible. Nevertheless, these methods are at best only partially effective and are often extremely invasive. There exists a need for a simple, effective, non-invasive or minimally invasive approach to reduce atherosclerosis or slow its development in patients with renal disease.

The intravenous administration of cholesterol-poor phospholipid vesicles (liposomes) or other particles to transport cholesterol from peripheral tissues, including atherosclerotic arterial lesions, to the liver produces substantial derangements in hepatic cholesterol homeostasis, such as enhanced hepatic secretion of apolipoprotein-B, the major protein of atherogenic lipoproteins, and suppression of hepatic LDL receptors (see, for example, Spady *et al.* **J. Lipid Res.** 26:465-472, 1985; Williams *et al.* **Proc. Natl. Acad. Sci. USA** 85:242-246, 1988; Williams *et al.* **J. Biol. Chem.** 265:16741-16744, 1990; Dixon & Ginsberg **J. Lipid Res.** 34:167-179, 1993; Tanka *et al.* **Atherosclerosis** 114:73-82, 1995; and citations therein). The hepatic derangements lead to increased plasma concentrations of LDL and other atherogenic lipoproteins. Increased concentrations of LDL or other atherogenic lipoprotein will accelerate, not retard, the development of vascular complications. Deranged hepatic cholesterol homeostasis can also be manifested by abnormal regulation of other genes, such as a gene for the LDL receptor, a gene for HMG-CoA reductase, a gene for cholesterol 7-alpha hydroxylase, and a gene regulating a function involved in cholesterol homeostasis. There exists a need for methods or compounds that can produce a removal of cholesterol and other exchangeable material from peripheral cells, tissues, organs, and extracellular regions, but without harmfully disrupting hepatic cholesterol homeostasis.

The invention described herein provides methods and compositions related to the removal of cholesterol and other lipids from peripheral tissues, and otherwise altering peripheral tissue

lipids, in patients with renal disease, while controlling plasma concentrations of LDL and other atherogenic lipoproteins and avoiding harmful disruptions of hepatic cholesterol homeostasis.

### **SUMMARY OF THE INVENTION**

The present invention provides a pharmaceutical composition, devices, modes of operation of devices, kit, and method of forcing the reverse transport of cholesterol from peripheral tissues to the liver *in vivo* while controlling plasma LDL concentrations. The method includes the step of administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period. The method optionally includes the step of periodically assaying plasma LDL concentrations with an assay during the treatment period to assess plasma atherogenic lipoprotein concentrations and obtain an atherogenic lipoprotein profile, and adjusting the administration in response to said profile. The large liposomes are dimensioned larger than fenestrations of an endothelial layer lining hepatic sinusoids in the liver so that the liposomes are too large to readily penetrate the fenestrations. The therapeutically effective amounts are in the range of about 10 mg to about 1600 mg phospholipid per kg body weight per dose. A pharmaceutical composition and related kit for mobilizing peripheral cholesterol and sphingomyelin that enters the liver of a subject consisting essentially of liposomes of a size and shape larger than fenestrations of an endothelial layer lining hepatic sinusoids in the liver is also provided.

The present invention provides a pharmaceutical composition consisting essentially of large liposomes comprised of phospholipids substantially free of sterol. The composition forces the reverse transport of cholesterol from peripheral tissues to the liver *in vivo*. The invention further provides a method of treating atherosclerosis in a subject comprising the step of administering a liposome composition to the subject. The liposome composition is selected from the group consisting of unilamellar liposomes and multilamellar liposomes and the liposomes have an average diameter of about 50-150 nanometers. LDL levels in said subject do not increase with utilization of the method.

The invention also provides a method of controlling cholesterol metabolism in hepatic parenchymal cells in a subject *in vivo* through cell-cell communication from Kupffer cells to the parenchymal cells. The method includes the step of administering a liposome composition

to a subject. The liposome composition is selected from the group consisting of large unilamellar liposomes and large multilamellar liposomes, and the liposomes having an average diameter of about 50-150 nanometers. Similarly, LDL levels in the subject do not increase. In variants, the liposome composition is given periodically, given more than once, or given in repeated doses.

The liposomes have diameters larger than about 50 nm, diameters larger than about 80 nm, and diameters larger than about 100 nm in different variants. Administration is selected from the group of parenteral administration, intravenous administration, intra-arterial administration, intramuscular administration, subcutaneous administration, transdermal administration, intraperitoneal administration, intrathecal administration, via lymphatics, intravascular administration, including administration into capillaries and arteriovenous shunts, rectal administration, administration via a chronically indwelling catheter, and administration via an acutely placed catheter, and given in about 10 to about 1600 mg/kg/dose of the liposome composition. The liposomes are phospholipids selected from the group consisting of phosphatidyl choline, phosphatidyl glycerol, palmitoyl-oleoyl phosphatidyl choline, combinations thereof, and derivatives thereof.

The present invention provides an improved mode of operation of an apparatus for angioplasty or cardiac catheterization, apparatus for angioplasty and cardiac catheterization, and method of angioplasty or cardiac catheterization. The improved mode of operation includes the mode of operation involving the administration of a therapeutically effective amount of a lipid acceptor during angioplasty or cardiac catheterization of a subject with the apparatus or component thereof. The lipid acceptor is selected from the group consisting of a large liposome comprised of phospholipids substantially free of sterol and small acceptors. The effective period of time is in the range of about less than 1 minute to about two years from the time of the angioplasty or cardiac catheterization. The improved angioplasty or cardiac catheterization apparatus includes means for administering a therapeutically effective amount of a lipid acceptor, and optional co-administration means for administering the lipid acceptor and a diagnostic agent. The improved mode of operating an angioplasty or cardiac catheterization apparatus includes administering a therapeutically effective amount of a lipid acceptor from the apparatus or component thereof into a vessel of a subject by administration means disposed on said apparatus. The invention further provides a method of diagnosing a side-effect of reverse transport of cholesterol from peripheral tissues to the liver *in vivo*

accompanying parenteral administration of a multiplicity of large liposomes and small liposomes during a treatment period. The method includes the step of periodically assaying plasma atherogenic lipoprotein concentrations with an assay to obtain an assayed atherogenic lipoprotein concentration. The objects and features of the present invention other than those specifically set forth above, will become apparent in the detailed description of the invention.

A method of regulating cholesterol related genes, enzymes and other compounds, pharmaceutical compositions and a kit related thereto are provided. Exemplary genes that are regulated include a gene for an LDL receptor, a gene for HMG-CoA reductase, a gene for cholesterol 7-alpha-hydroxylase, and a gene regulating a function involved in cholesterol homeostasis. The method comprises the step of parenterally administering a therapeutically effective amount of a lipid acceptor. The lipid acceptor in one variant includes a multiplicity of large liposomes comprised of phospholipids substantially free of sterol during a treatment period. The method includes the steps of periodically assaying plasma LDL concentrations with an assay during a period of time to assess said plasma LDL and to obtain an LDL profile, and adjusting the parenteral administration in response to the LDL profile. The method further includes the step of enhancing tissue penetration of a cholesterol acceptor and enhancing extraction of tissue cholesterol and other exchangeable material with co-administration of an effective amount of a compound selected from the group consisting of a small acceptor of cholesterol, an amphipathic compound, and a drug that increases endogenous small acceptors of cholesterol.

Generally the compositions described herein include large liposomes of a size and shape larger than fenestrations of an endothelial layer lining hepatic sinusoids in said liver, whereby said liposomes are too large to readily penetrate said fenestrations. Therapeutically effective amounts of said compositions include in the range of 10 mg to 1600 mg phospholipid per kg body weight per dose. The large liposomes are selected from the group consisting of uni-lamellar liposomes and multi-lamellar liposomes. In variants, the liposomes have diameters larger than about 50 NM, diameters larger than about 80 NM, and diameters larger than about 100 NM.

The present invention provides compositions for, a method of suppressing the rise in plasma concentrations of atherogenic lipoproteins after administration of an agent having small acceptors of cholesterol, other lipids or compounds. The method includes the step of co-administering an effective amount of a multiplicity of an agent having large liposomes that

include phospholipids substantially free of sterol with the administration of the agent having the small acceptors. The atherogenic lipoproteins include LDL, VLDL, IDL,  $\beta$ -VLDL, Lp(a), a lipoprotein containing apolipoprotein-B, oxidized lipoproteins, and modified lipoproteins. The agent having small acceptors consists essentially of small acceptors and in which the agent having large liposomes consists essentially of large liposomes. In a variant, co-administration of the agent having large liposomes is simultaneous with the administration of the agent having small acceptors. Optionally, co-administration of the agent having large liposomes is separated in time from the administration of the agent having small acceptors by an effective time period. An improved pharmaceutical composition for reducing the size of arterial lesions that enters the liver of a subject is also provided the improvement comprises an anti-oxidant and derivatives thereof. The invention also provides an improved mode of operation of liposomes utilizing the improvements described herein.

The present invention further provides various methods, systems and compositions for forcing the reverse transport of cholesterol from peripheral tissues to the liver in vivo while controlling plasma LDL concentrations, and other significant components of living biological systems. The method comprises the step of parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period whereby said liposomes pick-up said cholesterol during said treatment period. The method optionally includes the step of periodically assaying plasma LDL concentrations with an assay during said treatment period to assess said plasma LDL concentrations and obtain an LDL profile, and adjusting said parenteral administration in response to said LDL profile.

Exemplary assays are selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma, a precipitation assay of plasma, and an immuno turbidometric assay of plasma.

The methods optionally include the step of enhancing tissue penetration of a cholesterol acceptor by co-administration of an effective amount of a compound, said compound selected from the group consisting of a small acceptor of cholesterol and a drug that increases endogenous small acceptors of cholesterol. The small acceptor is selected from the group consisting of a high-density lipoprotein, a phospholipid protein complex having a group selected from the group consisting of apoA-I, apoA-II, apoA-IV, apoE, synthetic

fragments thereof, natural fragments thereof, an amphipathic protein, and an amphipathic peptide, said protein substantially free of phospholipid, small phospholipid liposomes, and a small cholesterol acceptor. This includes an agent that raises physiologic HDL concentrations, said agent selected from the group consisting of nicotinic acid, ethanol, a fibric acid, a cholesterol synthesis inhibitor, a drug that increases HDL concentrations, and derivatives thereof. The invention further provides a method of, and composition for regulating hepatic parenchymal cell cholesterol content and gene expression by the steps described herein.

The present invention provides an improved dialysis apparatus for the treatment of a subject, improved mode of operation of a dialysis apparatus and improved method of dialysis. The improvement includes means for and a mode of operation for administering a therapeutically effective amount of a lipid acceptor during the treatment of a subject, and actuation of the means during operation of the dialysis apparatus. The lipid acceptor is selected from the group consisting of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol and small acceptors. The means for administering said agent is selected from the group consisting of means for extracorporeal administration and means for intracorporeal administration. The dialysis includes hemodialysis, peritoneal dialysis, and rectal dialysis, and the agent is added directly to blood or blood plasma of a subject in one variant.

Liposome compositions utilized herein also pick up and are useful in removing undesirable components in addition to cholesterol which improves the dialysis. Accordingly, an assay of the undesirable components, which may include lipids and other exchangeable material, is used to determine the effectiveness of the treatment. The method, mode of operation and apparatus provide for the control of plasma LDL concentrations, plasma concentrations of atherogenic lipoproteins and hepatic cholesterol homeostasis.

It is an object of the present invention to provide for better methods to manipulate the lipid content and composition of peripheral tissues, cells, membranes, and extracellular regions *in vivo*, particularly in regard to diseases and processes involving oxidation and oxidative damage. It is a further object of the present invention to provide for methods or compounds that can produce a removal of cholesterol and other exchangeable material, including oxidized materials, from peripheral cells, tissues, organs, and extracellular regions, and that can produce a delivery of anti-oxidants to cells, tissues, organs, and extracellular

regions, but without harmfully disrupting hepatic cholesterol homeostasis, including hepatic gene expression and regulation.

It is an object of the invention to provide better methods to manipulate the lipid content and composition of peripheral tissues, cells, membranes, and extracellular regions *in vivo*.

It is a further object of the invention to regulate and control deranged hepatic cholesterol homeostasis as manifested by abnormal regulation of genes, such as a gene for the LDL receptor, a gene for HMG-CoA reductase, a gene for cholesterol 7-alpha hydroxylase, and a gene regulating a function involved in removal of cholesterol and other exchangeable material from peripheral cells, tissues, organs, and extracellular regions, but without harmfully disrupting hepatic cholesterol homeostasis, including hepatic gene expression and regulation.

It is an object of the invention to provide a simple, effective, non-invasive or minimally invasive approach, method, device, and mode of operation of the device to reduce re-stenosis or slow its development in patients who undergo mechanical or surgical revascularization procedures.

It is a further object of the invention to provide a method, device, and mode of operation of a device to manipulate the lipid content and composition of the arterial wall before, during, and after revascularization procedures, to reduce re-stenosis. It is yet a further object of the invention to provide for a method to change LDL composition and size.

It is yet another object of the invention to provide a method, compound, device and mode of operation of a device that can produce a removal of cholesterol and other exchangeable material from peripheral cells, tissues, organs, and extracellular regions without harmfully disrupting hepatic cholesterol homeostasis.

It is an object of the invention to provide for better methods to manipulate the lipid content and composition of peripheral tissues, cells, membranes, and extracellular regions *in vivo*.

It is a further object of the invention to provide for methods and compounds that can produce removal of cholesterol from cellular and extracellular regions of arteries, but without provoking a rise in the plasma concentration of LDL.

It is an object of the present invention to provide a better method to manipulate the lipid content and composition of peripheral tissues, cells, membranes, and extracellular regions *in vivo*.

**11**

It is a further object of the invention to provide methods or compounds that can produce a removal of cholesterol and other exchangeable material, from peripheral cells, tissues, organs, and extracellular regions, and that can produce a delivery of material, such as phospholipids, to cells, tissues, or organs, extracellular regions, but without harmfully disrupting hepatic cholesterol homeostasis and plasma concentrations of atherogenic lipoproteins.

*The objects and features of the present invention, other than those specifically set forth above, will become apparent in the detailed description of the invention set forth below.*

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 is a side cross-sectional view of a lipoprotein and a liposome;

FIG. 2 illustrates a table of hepatic mRNA content (pg/ $\mu$ g) for CETP, HMG-CoAR, LDL receptors, and 7 $\alpha$ -hydroxylase; and LDL ChE;

FIGS. 3 and 4 illustrate plasma LDL cholesteryl ester concentrations in response to injections of LUVs, SUVs or saline over time in one variant;

FIG. 5 illustrates LDL receptor mRNA levels in liver in response to injections of LUVs, SUVs or saline over time;

FIG. 6 illustrates HMG-CoA reductase mRNA levels in liver in response to injection of LUVs, SUVs, or saline;

FIG. 7 Illustrates cholesteryl ester transfer protein mRNA levels in liver in response to injection of LUVs, SUVs, or saline;

FIG. 8 illustrates 7-alpha hydroxylase mRNA levels in liver in response to injections of LUVs, SUVs, or saline;

FIG. 9 illustrates key points about LUVs and atherosclerosis;

FIG. 10 illustrates plasma LDL unesterified cholesterol concentrations in response to injections of LUVs, SUVs or saline over time;

FIG. 11 illustrates plasma LDL esterified cholesterol concentrations in response to injections of LUVs, SUVs or saline over time;

FIG. 12 illustrates LDL esterified cholesterol concentrations in response to injections of LUVs, SUVs or saline;

FIG. 13 illustrates plasma VLDL esterified cholesterol concentrations in response to injections of LUVs, SUVs or saline;

FIGS. 14 and 15 illustrate HDL esterified cholesterol concentrations in response to injections of LUVs, SUVs or saline;

FIG. 16 illustrates the time course of cholesterol mobilization following an LUV injection into control or apoE KO mice;

FIG. 17 illustrates the time course of LUV clearance in control mice and apoE mice;

FIG. 18 illustrates that the compositions and methods of the present invention are effective in humans;

FIG. 19 illustrates a perspective view of an improved hemodialysis system of the present invention and improved method of hemodialysis;

FIG. 20 illustrates a perspective view of an improved peritoneal dialysis system 2000 and method of peritoneal dialysis;

FIG. 21 illustrates a perspective view of a variant of an improved peritoneal dialysis system with assaying means 2100 and method of peritoneal dialysis and analysis of spent fluid;

FIG. 22 illustrates a perspective view of an improved cardiac catheterization and/or angioplasty system 2200 and method of cardiac catheterization and/or angioplasty;

FIG. 23 illustrates a perspective view of a variant of an improved cardiac catheterization and/or angioplasty system 2300 and method of cardiac catheterization and/or angioplasty;

FIG. 24 illustrates a graph of hepatic lipid contents in response to injections of LUVs, SUVs, or saline;

FIG. 25 illustrates plasma free cholesterol concentrations following repeated injections of SUVs or LUV (300 mg/kg) in NZW rabbits;

FIG. 26 illustrates plasma cholesterol ester concentrations following repeated injections of SUVs or LUV (300 mg/kg) in NZW rabbits;

FIG. 27 illustrates alternations in plasma components after repeated injections of SUVs; and,

FIG. 28 illustrates an agarose gel electrophoresis of whole plasma following repeated injections of LUVs, SUVs, or saline.

### **DETAILED DESCRIPTION OF THE INVENTION**

FIG. 1 illustrates a schematic illustration of the structure of a normal lipoprotein 100 and a unilamellar liposome 200. Lipoprotein 100 and liposome 200 are comprised of a phospholipid molecule 300. Phospholipid molecules generally have polar head 500 and a fatty acyl chains 400. Molecule 600 represents a molecule of unesterified cholesterol. Lipoprotein 100 is comprised of a hydrophobic core 102 composed mainly of triglycerides and cholesteryl esters surrounded by a monolayer of phospholipid molecules 300 with their fatty acyl side chains 400 facing the hydrophobic core 102 and their polar heads 500 facing the surrounding aqueous environment (not shown). Unesterified cholesterol 600 is found largely within the phospholipid monolayer. Apolipoprotein 700 is disposed within phospholipid molecules 300. Artificial triglyceride emulsion particles have essentially identical structures, either with or without protein.

Liposome 200 is comprised of phospholipid molecules 300 forming a phospholipid bilayer, e.g. one lamella, either with or without protein, in which fatty acyl side chains 400 face each other, the polar head groups 500 of the outer leaflet face outward to the surrounding aqueous environment (not shown), and the polar head groups 500 of the inner leaflet face inward to the aqueous core 202 of the particle 200. Depending on the composition of particle 200, phospholipid bilayers can have a large capacity for unesterified cholesterol and other

exchangeable material and components thereof. As illustrated in FIG. 1 there is no sterol. Typically, such liposomes can pick up unesterified cholesterol from other lipid bilayers, such as cell membranes, and from lipoproteins. Liposomes also pick up proteins and donate phospholipids and other exchangeable material and components thereof. Liposomes can also have multilamellar structures, in which the bilayers are contained within the environment encapsulated by an outer bilayer to form multiple lamellae. The multiple lamellae can be arranged concentrically, like the layers of an onion, or in another variant non-concentrically.

FIGS. 3 and 4 illustrate plasma LDL cholesteryl ester concentrations in response to injections of LUVs, SUVs or saline over time. Rabbits were intravenously injected on days 1, 3 and 5 as indicated by arrows 302, 304, and 306 respectively, with a bolus of 300 mg of phosphatidylcholine per kg of body weight or a matched volume of saline. The phosphatidylcholine was pharmaceutical grade egg PC, in the form of either large unilamellar vesicles (LUVs) having diameters of approximately 100 NM (preferably  $\simeq$  120 NM) prepared by extrusion (LUVs were measured at about 120 NM ( $123 \pm 35$  NM and the extrusion membrane had pores of about 100 NM in diameter) or small unilamellar vesicles with diameters of approximately 30 NM (preferably 35 NM) prepared by sonication. (SUVs were measured in the range of  $34 \pm 30$  NM.) Blood was drawn just before each injection and on the sixth day at sacrifice. Plasma LDL cholesteryl ester concentrations were determined by a gel filtration assay of the plasma with an in-line enzymatic assay for cholesteryl ester. Means  $\pm$  SEMs are shown in FIG. 3. Animals infused with SUVs showed significantly higher plasma concentrations of LDL cholesteryl ester at days 3, 5, and 6 compared to either LUV-infused or saline infused animals. FIGS. 2-8, 10-15, 24 and 28 illustrate data from the same experiment in which injections were made on days 1, 3, and 5 and then livers were taken. Gel filtration was done of plasma to measure lipid contents of individual lipoprotein classes.

FIG. 2 illustrates a table of hepatic mRNA content (pg/ $\mu$ g) for CETP, HMG-CoA R (hydroxy methylglutaryl coenzyme A reductase), LDL receptors, and cholesterol 7  $\alpha$ -hydroxylase; and LDL ChE (low density lipoprotein cholesteryl ester) for the rabbits given saline (HEPES buffered saline) (rabbits 1-4), LUVs (rabbits 5-8), and SUVs (rabbits 10-12) for the experiment described for FIGS. 3 and 4. Rabbit 13 is the "Mix" rabbit.

FIG. 4 shows an animal labeled as mix. "Mix" refers to a single animal that received SUVs on day 1, 3 and 5, but also one injection of LUVs on day 3. Before this injection of LUVs, the plasma concentration of LDL cholesteryl ester rose, but after the injection of LUVs, the LDL concentration fell, despite continued injections of SUVs.

FIG. 5 illustrates LDL receptor mRNA levels in liver in response to injections of LUVs, SUVs or saline over time. The rabbits described above were sacrificed at day 6, and samples of liver were snap-frozen in liquid nitrogen. mRNA was extracted, and rabbit mRNA for the LDL receptor was quantified by an internal standard/RNase protection assay (Rea T.J. et al. J. Lipid Research 34:1901-1910, 1993 and Pape M.E., Genet. Anal. 8:206-312, 1991). Means  $\pm$  SEMs are shown in FIG. 5. Animals infused with SUVs showed significant suppression of hepatic LDL receptor mRNA compared to LUV-infused or saline-infused animals. Suppression of hepatic LDL receptor mRNA reflects parenchymal cell overload with sterol, and is a potentially harmful alteration from normal hepatic cholesterol homeostasis. In contrast, LUV-infused animals showed the highest levels of hepatic LDL receptor mRNA, though the increase above that seen in the saline-infused animals did not reach statistical significance. The liver from the "Mix" animal described above showed a value of 5.28 pg LDL receptor mRNA/microgram which is closer to the average value in the saline group than in the SUV group. Thus, LDL receptor mRNA was stimulated by the single injection of LUVs despite repeated injections of SUVs.

FIG. 6 illustrates HMG-CoA reductase mRNA levels in liver in response to injections of LUVs, SUVs, or saline. The experimental details are those as referenced above. Animals infused with SUVs showed significant suppression of hepatic HMG-CoA reductase mRNA compared to LUV-infused or saline infused animals. Suppression of hepatic HMG-CoA reductase mRNA reflects parenchymal cell overload with sterol, which can be a potentially harmful alteration from normal hepatic cholesterol homeostasis. In contrast, LUV-infused animals showed the highest levels of hepatic HMG-CoA reductase mRNA, though the increase above that seen in the saline-infused animals did not reach statistical significance.

The "mix" animal showed a value of 0.50 pg HMG-CoA reductase mRNA/microgram, which is essentially identical to the average value in the saline group (0.51) and substantially higher than the value in the SUV group (0.27). Thus, HMG-CoA reductase mRNA was stimulated to its normal value by the single injection of LUVs, despite repeated injections of SUVs.

FIG. 7 illustrates cholesteryl ester transfer protein mRNA levels in liver in response to injection of LUVs, SUVs, or saline. The experimental details are those as referenced above. Animals infused with LUVs showed significant suppression of hepatic CETP mRNA compared to SUV infused or saline infused animals. Suppression of CETP mRNA produce changes in the plasma lipoprotein profile usually associated with reduced risk of atherosclerosis. The "mix" animal showed a value of 3.18 pg CETP mRNA/microgram, which is closer to the average value in the LUV group than in the SUV or saline groups. Thus, CETP mRNA was suppressed by the single injection of LUV's despite repeated injections of SUVs.

FIG. 8 illustrates cholesterol 7-alpha hydroxylase mRNA levels in liver in response to injections of LUVs, SUVs, or saline. The experimental details are those as reference

above. Animals infused with SUVs showed suppression of hepatic 7-alpha hydroxylase mRNA compared to LUV infused or saline infused animals. Suppression of 7-alpha hydroxylase can be a potentially harmful alteration from normal hepatic homeostasis. In contrast, LUV-infused animals showed the highest levels of hepatic 7-alpha hydroxylase mRNA, though the increase above that seen in the saline infused animals did not reach statistical significance. The "mix" animal showed a value of 0.51 pg 7-alpha hydroxylase mRNA/microgram, which is higher than the average value in the SUV group. Thus, 7-alpha-hydroxylase mRNA was stimulated by the single injection of LUVs, despite repeated injections of SUVs.

FIG. 10 illustrates unesterified cholesterol concentrations in whole plasma in response to injections of LUVs, SUVs, or saline over time. The experimental details are those as referenced above. As indicated by this figure, LUVs and SUVs significantly raised the plasma concentrations of unesterified cholesterol indicating mobilization of tissue stores. The LUVs raised the concentration of unesterified cholesterol more than did the SUVs.

FIG. 11 illustrates esterified cholesterol concentrations in whole plasma in response to injections of LUVs, SUVs or saline over time. The experimental details are those as referenced above. SUVs raised the plasma concentrations of cholesteryl ester on days 3, 5, and 6. FIG. 12 duplicates the information contained in FIG. 3.

FIG. 13 illustrates plasma VLDL esterified cholesterol concentrations in response to injections of LUVs, SUVs, or saline. SUVs increased the plasma concentration of VLDL cholesteryl ester over that seen in the saline or LUV treated groups. The "mix" animal showed a plasma VLDL cholesteryl ester concentration at day 6 of 2.4 mg/dl, which is lower than the average value in the SUV group. The experimental details are those as referenced above.

FIGS. 14 and 15 illustrate HDL esterified cholesterol concentrations in response to injections of LUVs, SUVs, or saline. The experimental details are those as referenced above as in FIG. 2. Suitable phospholipid can be obtained from Avanti Polar Lipids, Nippon Oil and Fat in Japan and Princeton Lipids, as well as other suppliers. LUVs are made through an extruder that is commercially available. SUVs caused a small but statistically significant rise in HDL cholesteryl ester concentrations on days five and six.

FIG. 16 illustrates the time course of cholesterol mobilization following an LUV injection into control or apoE KO (knock-out) mice commercially available from Jackson Laboratories, in Bar Harbor, Maine. Control (C57/BL6) and apolipoprotein E knock-out mice were injected at time zero with a single bolus of 300 mg LUV phospholipid/kg body weight. The LUVs contained a tracer amount of labeled cholesteryl hexadecylether, which remains on the liposomes after injection into a mouse. Displayed data are for concentrations of total cholesterol, i.e. esterified plus unesterified, in whole plasma. The rise in both sets of animals indicated that LUVs mobilize cholesterol into the plasma, even in the presence of a severe genetic hyperlipidemia.

FIG. 17 illustrates the time course of LUV clearance in control mice and apoE mice. The experimental details are as described in FIG. 16. The clearance of LUVs from the plasma is unimpaired in the apoE knock-out mice, indicating mobilization (FIG. 16) and disposal (FIG. 17) of cholesterol even in the presence of a severe genetic hyperlipidemia. This indicates the usefulness of this preparation in hyperlipidemias.

FIG. 18 illustrates exemplary applications for the compositions and methods of the present invention in humans. The therapeutic targets of the compositions and methods presented herein are lipid-rich, rupture prone plaques, critical stenosis, post-angioplasty restenosis, atherosclerosis in general, and any membrane, cell, tissue, organ, and extracellular

region and/or structure, in which compositional and/or functional modifications would be advantageous.

FIG. 19 illustrates a perspective view of an improved hemodialysis system of the present invention and improved method of hemodialysis. Blood is taken from a site for circulatory access (shown here as arm 1900) and transported into a cell-plasma separator 1910. The plasma is then transported to a dialysis chamber 1920 and is divided into at least two compartments that are separated by a semi-permeable membrane 1930. One side of the membrane 1930 is the patient's plasma 1940 and on the other side is the dialysate 1950. Selected molecules exchange across the membrane 1930 depending on the characteristics of the membrane (charge, pore size, etc.). The device 1960 comprises a device for adding lipid acceptors to the dialysate and for sampling the dialysate to allow assays of cholesterol, phospholipid, and other components, such as acceptors, specific lipoproteins, specific components, and to monitor treatment. Extraction of plasma cholesterol or other extractable material comprises several possibilities: 1) acceptors are disposed in the dialysate that do not cross membrane 1930 into plasma; 2) the acceptors do cross membrane 1930 and are either left in the plasma and returned to the patient or are separated from plasma before it is returned to the patient; and/or 3) immobilized acceptors on a sheet (such as membrane 1930 itself), on beads, and/or on the walls of the chamber 1920. Plasma thus treated is returned to the patient, usually after having being re-mixed with the blood cells. As noted, cholesterol acceptors can be added at any stage, as an example, a device 1970 comprises acceptors and for adding acceptors to plasma shortly before its return into the patient is also illustrated in FIG. 19. It is further understood that contaminating cellular material, such as platelets, in the plasma will also become cholesterol depleted in endogenous lipids and enriched in

phospholipid. It is further understood that all acceptors mentioned throughout this application may accept molecules in addition to cholesterol and may donate material as well.

The cellular concentrate from the cell-plasma separator 1910 can then be treated in any of several ways before being returned to the patient: 1) returned to the patient with no further treatment (this includes being mixed with plasma that has been treated as above); 2) transferred to a second dialysis chamber (not shown) in which the dialysate contains cholesterol acceptors to lipid deplete the cells of endogenous lipids, such as cholesterol, before their return to the patient; 3) mixed with a suspension or solution of lipid acceptors to lipid deplete the cells of endogenous lipids, then either returned to the patient with the acceptors or option 1) and option 2) above can be performed with all cell types together, or after further separation into specific cell types (for example, purified platelets could be lipid depleted of endogenous lipids, such as cholesterol, and enriched in liposomal lipids). Options 2) and 3) can be performed with periodic assays of cellular cholesterol, phospholipid, fluidity, viscosity, fragility, cell composition and/or cell function. Devices 1960, 1970 include an apparatus that allows for the periodic sampling of cells during treatment. As with plasma, lipid acceptors can be added at any stage of the treatment. All fluids, e.g. plasma and concentrated cells, are moved by gravity, mechanically, by manual manipulation (a syringe), or with pumps as needed. Of course, it is understood that blood can be drawn for processing from any appropriate part of the body.

FIG. 20 illustrates a perspective view of an improved peritoneal dialysis system 2000 and method of peritoneal dialysis. Patient's abdomen 2010 (FIGS. 20-21) receives peritoneal dialysate 2020 stored in container 2030 into the peritoneal cavity through incision 2040 by way of channel 2050. Lipid acceptors and/or cholesterol acceptors 2060 are optionally disposed in container 2070. In another variant, lipid acceptors are added to dialysate 2020;

added to container 2030 in concentrated form shortly before infusion; added as shown to the stream of fluid entering the peritoneal cavity; or infused by a separate portal of entry into the patient by any effective route. Throughout this application, it is understood that all acceptors may accept molecules in addition to cholesterol and may donate material such as phospholipids and anti-oxidants.

FIG. 21 illustrates a perspective view of a variant of an improved peritoneal dialysis system with assaying means 2100 and method of peritoneal dialysis and analysis of spent fluid. Container 2110 accepts spent fluid from abdomen 2010 by way of channel 2120. The device 2110 provides access to diagnostic samples of spent dialysate to allow for assay of cholesterol, phospholipid, and other parameters as described herein showing the efficacy of the treatments described. Optionally, assay syringe 2130 is inserted by way of access portal 2140 into channel or tube 2120, or into container 2110, and optional pumps (not shown) are used to move the various fluids to appropriate locations for assay thereof.

FIG. 22 illustrates a perspective view of an improved cardiac catheterization and/or angioplasty system 2200 and method of cardiac catheterization and/or angioplasty. Patient 2210 undergoes cardiac catheterization and/or angioplasty. The patient intravenously receives effective doses of lipid acceptors or cholesterol acceptors 2230 co-administered with said treatment(s) from container 2220. Intraarterial access of a catheter for coronary angiography and/or angioplasty allows for ready co-administration of cholesterol acceptors and administration of diagnostic agents such as cholinergic agents, to assess vascular function.

FIG. 23 illustrates a perspective view of a variant of an improved cardiac catheterization and/or angioplasty system 2300 and method of cardiac catheterization and/or angioplasty. Catheterization and/or angioplasty catheter 2310 has apertures 2320 that allow for the egress of cholesterol acceptors therefrom. In a variant, catheter 2310 has a permeable

membrane that allow for the egress for cholesterol acceptors therefrom. Phantom arrows 2330 indicate egress sites for cholesterol acceptors and/or diagnostic agents. Sites 2340 indicate entry sites for the acceptors or agents. The balloon on the device 2300 can be replaced or supplemented with other devices or can form an inner balloon layer disposed within an outer balloon layer. The acceptors are disposed between the inner and outer flexible balloon layers. Upon expansion of said inner balloon layer a force is exerted against the fluid or gel-like acceptors forcing the acceptors out of the sites 2320, and into direct contact (forcefully) against arterial lesions more locally directing the treatment. It will be appreciated that this variant of the invention provides for maximal penetration of the acceptors into the arterial lesions. The infusions can be accomplished by gravity, manual manipulation of a syringe, or by mechanical infusion pump 2350. The same method and system can be utilized with standard vascular imaging techniques or vessels that include the femorals, carotids, and mesenteric vessels by way of example.

Patient 2210 undergoes cardiac catheterization and/or angioplasty. The patient intravenously receives effective doses of cholesterol or lipid acceptors 2230 co-administered with said treatments(s) from container 2220. Intraarterial access of a catheter for coronary angiography and/or angioplasty allows for ready co-administration of lipid or cholesterol acceptors and administration of diagnostic agents such as cholinergic agents, to assess vascular function.

Container 2110 accepts spent fluid from abdomen 2010 by way of channel 2120. The device 2110 provides access to diagnostic samples of spent dialysate to allow for assay of cholesterol, phospholipid, and other parameters as described herein showing the efficacy of the treatments described. Optionally, assay syringe 2130 is inserted by way of access portal

2140 into channel or tube 2120, and optional pumps (not shown) are used to move the various fluids to appropriate locations for assay thereof.

FIG. 24 illustrates a graph of hepatic lipid contents in response to injections of LUVs, SUVs, or saline. The experimental details are as outlined above. Liver samples were assayed for contents of several lipids: cholesterol ester (CE); triglyceride (TG); unesterified cholesterol (Chol); phosphatidylethanolamine (PE); and phosphatidylcholine (PC), which are displayed in units of  $\mu\text{g}$  (micrograms) lipid/mg. Lower values of PE and PC in the SUV-treated animals were produced; thus, the Chol:phospholipid ratios in these animals was higher than in the other groups.

FIG. 25 illustrates cholesterol ester concentrations following repeated injections of SUVs or LUVs (30 mg/kg) in NZW rabbits (New Zealand White rabbits). The arrows indicate times of phospholipid injection here on days 0, 3 and 5. For a given phospholipid dose, LUVs promote a greater rise in plasma free cholesterol concentrations.

FIG. 26 illustrates plasma free cholesterol concentrations following repeated injections of SUV or LUV (300 mg/kg) in NZW rabbits in the same experiment as in FIG. 25, arrows indicate times of phospholipid injection. Repeated injections of LUV, unlike SUV, do not provoke a dramatic rise in CE concentrations in plasma.

The rise in plasma CE concentrations that results from the delivery of excess cholesterol to the liver may be the consequence of two processes. It may involve an over production of CE-rich particles or an impaired clearance of CE-rich lipoproteins. Over production of CE-rich particles that occurs following SUV infusions may result in the plasma or in the liver. In plasma, LCAT acts on small unilamellar phospholipid vesicles or on phospholipid enriched HDL generating CE which may be subsequently transferred by CETP onto LDL. The results with gel filtration of plasma from animals treated with SUVs indicate

that CE is carried mostly or substantially on LDL. Also, in plasma, removal of apoE from VLDL by SUVs will slow the clearance of VLDL, thereby favoring a more efficient conversion into LDL. In the liver, the increased delivery of cholesterol to hepatocytes during cholesterol mobilization stimulates an over secretion of apoB, CE-rich lipoproteins.

In a variant, the rise in plasma CE concentrations observed is the result of an impaired clearance of CE rich atherogenic lipoproteins. Intravenously administered liposomes that acquire apoE compete with LDL for LDL-receptor mediated uptake. The delivery of excess cholesterol to the liver down regulates LDL receptors. The processes responsible for an increase in plasma CE concentrations are different between the two liposome preparations. LUVs, unlike SUVs, do not provoke a rise in plasma CE concentrations. LUVs are superior preparations for mobilizing tissue cholesterol without harmful side effects.

The method and composition of the present invention also provides enrichment of HDL cholesterol esters by SUVs. One contributing process is the stimulation of lecithin cholesterol acyl transferase (LCAT) and other processes related thereto. The ability of SUVs to increase HDL cholesterol ester is the result of stimulation of LCAT and other processes related thereto. LCAT need phospholipid and cholesterol to generate cholesteryl ester and lysophosphatidylcholine; liposomes can supply extra phospholipid. The present invention also provides for alterations in lipoprotein (LDL, HDL, etc.) composition and function by LUVs and/or SUVs and/or other acceptors.

The liposome compositions described herein and methods utilizing same also include the liposomes picking up endogenous apoE and hence blocking cellular uptake of LDL. The liposomes pick up apolipoproteins, such as apoE and apoA-I, and that this alters or enhances their functions. For example, the uptake of endogenous apoA-I enhances the ability of liposomal derived phospholipid to pick up cholesterol, and the uptake of endogenous apoE

would allow the liposomes to block certain pathways for arterial uptake of lipoproteins. All of this is in the context of controlling LDL levels and hepatic gene expression and cholesterol homeostasis.

LUVs and SUVs deliver cholesterol to different regulatory pools within the liver. This conclusion is supported by the differences in hepatic gene responses and CETP mRNA is suppressed: the LDL receptor mRNA is unaffected or increased by LUVs but suppressed by SUVs; and CETP is suppressed by LUVs, but unaffected by SUVs. Further, it is understood that the arterial lesions referenced herein include, by way of example, critical stenoses.

The key points about LUVs and atherosclerosis are illustrated in FIG. 9. The practical benefits of using LUVs as a treatment for atherosclerosis are that they are straight forward to manufacture, and non-toxic even at very high doses. Mechanistically, LUVs promote reverse cholesterol transport *in vivo* without provoking a rise in LDL concentration, and LUVs are an optimal preparation.

The compositions that are used herein can direct clearance away from hepatic parenchymal cells. And the various methods described herein are utilized with slow infusions of the compositions described, so that hepatic cells are not cholesterol overloaded even if clearance by parenchymal cells occurs. Further, HDL is also controlled by CETP gene suppression.

As described herein assays are performed by: assaying fasting plasma triglyceride to estimate VLDL concentrations; assaying plasma cholesterol (free and ester, or total minus free = ester); precipitating LDL (& VLDL) with polyanions-cations; assaying the supernatant which is HDL; and computing LDL's (whole plasma value minus VLDL - HDL) sterol (or sterol ester) in whole plasma. Liposomes will precipitate with polyanions-cations; or optionally assaying the ester which liposomes mostly lack. Other assays include

electrophoresis, chromatography, immune assays, electron microscopic assays, functional assays, structural assays, and compositional assays.

In the dialysate of the present invention, any liposomes or emulsions could be used as long as it's a cholesterol acceptor and either it does not raise LDL or it is not returned to the patient's circulation. In either case, one would need to assay plasma LDL and the plasma concentration of the acceptors, and plasma concentrations of other atherogenic lipoproteins.

With respect to the methods that require delivering the cholesterol to the liver at a slow rate, or in low doses administration might permit small acceptors, such as SUVs, to be used without LUVs provided LDL levels as levels of other atherogenic lipoproteins are monitored and regulated. To avoid disrupting hepatic cholesterol homeostasis, the entrapped drug as described herein need not be given at low doses, but rather the encapsulating liposome or emulsion is given in low doses; the drug could be present at high amounts within a small number of liposomes or a small mass of liposomal lipid.

Alterations in HDL size, composition and function can be accomplished by administering high or even truly low doses of large and/or small liposomes that have little or no sterol. Liposomes without sterol, when given in low doses are easily broken apart by HDL and HDL apolipoproteins and then pieces are incorporated into the HDL fraction of plasma enriching it in phospholipid. Such small doses, e.g. 10-100 mg/kg/dose, even of SUVs without LUVs or drugs to lower LDL levels, are unlikely to raise plasma LDL levels, although periodic monitoring would be prudent.

Also, the method as disclosed herein of altering LDL composition without increasing LDL concentration would be to enrich the composition with phospholipids, like POPC (palmitoyloleoylphosphatidylcholine), that are resistant to oxidation, enrich the composition

with anti-oxidants, deplete unesterified cholesterol, and reduce cellular or arterial uptake of oxidized LDL by phospholipid enrichment.

Liposomes up to about 1000 NM or so would work in the present invention. Larger liposomes would also work but extraction of tissue lipoprotein may be less efficient. It is further possible to concentrate or dry compositions of the present invention. These preparations are then diluted or reconstituted at the time of therapy or administration. In this variant, a two component kit comprising the active material and a diluent is provided. Inclusion of phosphatidyl glycerol (PG) to make the liposomes negatively charged, or charge other components of the composition, to prevent aggregation during storage is also provided.

Figure 27 illustrates alterations in plasma components after repeated injections of SUVs. Watanabe Heritable Hyperlipidemic (WHHL) rabbits were given intravenously 1000 mg of SUV phospholipid per kg of body weight, or the equivalent volume of saline, on Monday, Wednesday, & Friday of each week for three weeks (nine doses total). Three days after the final dose, blood samples were taken, and plasma components were fractionated by size by passage over a Superose-6 gel-filtration column. Eluents were read by an in-line spectrophotometer. The tracing on the right is from a saline-injected rabbit, and shows VLDL around fractions #17-18, and LDL around fraction #27. The tracing on the left is from an SUV-injected rabbit, and shows VLDL with persistent liposomes around fraction #16, and LDL-sized particles around fraction #25. The tracings indicate an increase in the amount of LDL-sized particles after repeated injections of SUVs, consistent with an increase in LDL, which is a harmful effect. Because WHHL rabbits have a genetic lack of LDL receptors, this result indicates that SUVs disrupt hepatic cholesterol homeostasis not just by suppressing LDL receptors (Figure 5), but also by mechanisms independent of LDL receptors (Figure 27). SUVs avoid both LDL receptor-dependent and - independent disruptions.

Figure 28 illustrates an agarose gel electrophoresis of whole plasma following repeated injections of LUVs, SUVs, or saline. Experimental details are referenced in Figures 2-8 & elsewhere herein. Four- $\mu$ L plasma samples from two rabbits in each group at day 6 were electrophoresed through 1 % agarose then stained for lipids with Sudan black. O: origin.  $\beta$ : migration of an LDL standard. The SUV-mediated increase in LDL concentration is illustrated by the darker but otherwise unremarkable  $\beta$ -band in those lanes. SUVs in plasma exhibited a mobility ahead of LDL, owing to their acquisition of plasma proteins, chiefly from HDL. In contrast, plasma LUVs exhibited essentially the same mobility as freshly prepared, protein-free vesicles, i.e., just above the origin (O), indicating a substantial absence or reduction of acquired proteins on the LUVs.

Based on the electrophoretic mobilities in Figure 28, quantification of the acquisition of protein by LUVs versus SUVs was obtained. LUVs and SUVs were incubated with human HDL in vitro for 4 hours at 37°C, then separated from the HDL by gel filtration chromatography and assayed for protein and phospholipid. LUVs acquired 1.09  $\mu$ g of protein per mg of liposomal phospholipid, whereas SUVs acquired 40.4  $\mu$ g/mg, i.e., almost 40 times as much. Thus, the two types of liposomes exhibit a striking quantitative difference in protein adsorption. SUVs, but not LUVs, avidly strip apoE from VLDL, thereby slowing its clearance from plasma and favoring its conversion to LDL. In addition, adsorbed proteins play a role in directing the SUVs into a hepatic metabolic pool that disrupts hepatic cholesterol homeostasis, whereas LUVs are not directed into such a pool. Liposomes, emulsions, or any other particles or compounds that extract tissue lipids but do not acquire large amounts of plasma proteins behave similarly to LUVs in these regards.

Specific vascular genes affected by cholesterol loading of cells include genes for prolyl-4-hydroxylase; hnRNP-K; osteopontin (there may be a role for oxidized lipids in

provoking arterial calcifications); and Mac-2. The methods of regulating these genes described herein effect restoration of normal vascular or arterial function. Elevated expression of prolyl-4-hydroxylase (an enzyme in the synthesis of collagen, a component of fibrotic plaques) and hnRNP-K (identified in pre-mRNA metabolism and cell cycle progression) messages were found in aortic smooth muscle cells after cholesterol feeding. These would normalize after the liposome treatments described herein. Other genes or enzymes that are abnormal with cholesterol-loading and should normalize with liposome treatment as described herein include osteopontin, nitric oxide synthase (NOS), adhesion molecules, chemoattractants, tissue factor, PAI-1 (plasminogen activator inhibitor), tPA (tissue plasminogen activator) and Mac-2 (Ramaley et al. 1995). Other genes affected by cholesterol, cholesterol loading, oxidized lipids would also be corrected.

Many examples of small acceptors such as SUVs, apolipoprotein-phospholipid disks, and HDL are commercially available and can be used in the invention. Kilsdonk EP et al. Cellular cholesterol efflux mediated by cyclodextrins, J. Biol. Chem. 270:17250-17256, 1995. By way of further example, another small acceptor includes the cyclodextrins. Small acceptors (specifically HDL) shuttle cholesterol from cells to liposomes. Cyclodextrins and also other small acceptors can shuttle cholesterol and other exchangeable material from cultured cells to LUVs, which substantially increases the removal and donation of material between cells and LUVs.

Examples of anti-hyperlipidemic drugs include fibric acid derivatives, HmG CoA reductase inhibitors, Niacin, probucol, bile acid binders, other drugs and combinations thereof. Anti-hyperlipidemic treatments also include LDL, apheresis, ileal bypass, liver transplantation and gene therapy.

The data presented in this application support three possible explanations for the difference in metabolic response to LUVs *versus* SUVs. The three mechanisms act separately or in combination. First, LUVs are taken up largely by Kupffer cells, whereas SUVs are primarily directed towards hepatic parenchymal cells. This is partly a mechanical consequence of hepatic architecture: hepatic endothelial fenestrae are oval openings of about 100x115nm, through which SUVs of 30-nm diameter or so can readily pass and gain access to parenchymal cells. Large particles, such as large liposomes, of sufficient diameter will not pass easily, and are cleared instead by the macrophage Kupffer cells that line the liver sinusoids. While SUVs also have access to Kupffer cells, their sheer number (~ 10 times as many SUVs as LUVs per mg of phospholipid) appears to saturate the reticuloendothelial system, and so parenchymal cells predominate in their clearance. Other methods to direct artificial particles away from parenchymal cells are also available, such as by changing the particle structure or composition, including charge and specific ligands for cell-specific binding.

Cholesterol clearance pathways mediated by parenchymal *versus* Kupffer cells have distinct metabolic consequences. Direct delivery of cholesterol to parenchymal cells by SUVs suppresses sterol-responsive messages (Figures 5, 6, & 8). Delivery of cholesterol to Kupffer cells can be followed by gradual transfer of lipid to parenchymal cells, for example, via the extensions of Kupffer cells that reach down through the space of Disse to make physical contact with parenchymal cells. The rate of sterol delivery to the parenchymal cells by transfer from Kupffer cells can be slower than by direct uptake; the chemical form of the sterol may be altered by the Kupffer cells before transfer; there is other cell-cell communication; and, based on other pathways for lipid transfer amongst liver cells, the

process of transfer from Kupffer to parenchymal cells may be regulated, whereas SUV clearance does not appear to be.

The second contributing explanation for the difference in metabolic response to LUVs *versus* SUVs is based solely on differences in the kinetics of their delivery of cholesterol to the liver. LUVs are cleared from plasma somewhat more slowly than are SUVs, and thereby produce a relatively constant delivery of cholesterol mass to the liver from the time of injection until the bulk of injected material is cleared. SUVs are cleared more rapidly, thereby delivering a large bolus of cholesterol mass to the liver several hours after each injection, which is followed by the sustained rise in plasma concentrations of cholesteryl ester and atherogenic lipoproteins. The slow, steady delivery by LUVs avoids disrupting hepatic cholesterol homeostasis, while the more rapid uptake of SUV cholesterol overwhelms the ability of the liver to maintain homeostasis, thereby provoking suppression of hepatic LDL receptors. Other methods to deliver artificial particles or their components to the liver at a proper rate are also available, such as by changing the particle structure or composition, including charge and specific ligand for cell-specific binding.

The third contributing explanation is based on the striking quantitative difference in protein adsorption between the two types of vesicles (Figure 28), which, in that particular experiment, was a result of their distinct surface curvatures. Thus, SUVs, but not LUVs, would avidly strip apoE from VLDL, thereby showing its clearance from plasma and favoring its conversion to LDL. SUVs that acquire apoE will compete with VLDL, LDL, and other particles for receptor mediated uptake by the liver. Also, adsorbed apoproteins can play a role in directing phospholipid vesicles to different hepatic metabolic pools. Other methods to reduce protein uptake by artificial particles are also available, such as by changing the particle structure or composition, including charge and specific ligands for cell-specific binding.

Overall, given the observation that cholesteryl ester and LDL concentrations do not increase after delivery of large amounts of cholesterol and other exchangeable material to the liver by LUVs, it was apparent that delivery was to a specific metabolic pool or pools with unique properties that do not increase plasma concentrations of atherogenic lipoproteins or harmfully disturb hepatic cholesterol homeostasis, including the regulation of genes and other functions. Thus, these inventions can be regarded in part as a unique delivery system that brings original particle components, such as phospholipid, plus material acquired by the particles, such as cholesterol, to a specific delivery site for harmless disposal and other additional benefits. The delivery system with these characteristics will be useful in any situation whatsoever in which control of hepatic cholesterol homeostasis, hepatic phospholipid homeostasis, and hepatic metabolism in general is advantageous.

For example, in a situation in which it is desirable to modify erythrocyte lipids, a straightforward approach would be to administer artificial particles that can donate and remove the appropriate lipids. If SUVs are used for this purpose, however, they will transport cholesterol and other material to the liver in a harmful manner, to the wrong pool and/or at the wrong rate, and this will cause increases in plasma concentrations of atherogenic lipoproteins, which is an undesirable side-effect that would preclude this approach. In contrast, the use of large liposomes or other particles with similar properties will result in the proper delivery of original and acquired material, to the proper pool(s) at a proper rate, so that the desired effect (modification of erythrocyte lipids) can be achieved without harmful increases in plasma concentrations of atherogenic lipoproteins.

As another example, it can be desirable to modify infectious agents, such as bacteria, fungi, and viruses, using the compositions and method described herein. Administration of large liposomes or other particles with similar properties will remove and donate exchangeable

materials to and from these infectious agents, and then the administered particles will be delivered to the proper pool(s), so that the desired effect can be achieved without harmful increases in plasma concentrations of atherogenic lipoproteins.

As another example, a valuable therapy may provoke an increase in plasma concentrations of atherogenic lipoproteins as an unwanted side-effect. Administration of large liposomes or other particles with similar properties will alter this response through the delivery of lipids and other material to the proper hepatic metabolic pool. The data with the "Mix" animal provides a specific example of this effect (Figure 4).

There are several mechanisms for affecting arterial uptake, accumulation, and retention of lipoproteins. Liposomes can pick up apoE from atherogenic lipoproteins, thereby reducing lipoprotein binding to arterial cells and also competing for binding to arterial cells. Finally, alterations in LDL size and/or composition affect its binding to extracellular matrix and affect subsequent, harmful alterations within the arterial wall, for example, susceptibility to oxidation or enzymatic modifications.

The action or mode of operation of large acceptors, such as large liposomes, can be aided by small acceptors, and *vice-versa*, and this applies to both endogenous (*e.g.*, HDL) and exogenous (*e.g.*, apoprotein-phospholipid complexes) small acceptors. Large acceptors penetrate poorly into the interstitial space and appear to inefficiently approach the cell surface under certain circumstances. These effects impede their uptake and donation of exchangeable material from membranes, cells, tissues, organs, and extracellular regions and structures. Small acceptors do penetrate well into the interstitial space and are able to approach the cell surface, thereby allowing efficient uptake of exchangeable material. Small acceptors have major disadvantages, however. They have a very limited capacity to acquire or donate material (even though the initial rate of acquisition or donation is rapid, until their capacity

becomes saturated) and, once they have acquired material, they deliver it to the liver in a way that disrupts hepatic cholesterol homeostasis.

Large acceptors and small acceptors together, however, synergistically overcome each other's drawbacks through at least three mechanisms. First, the large acceptors act as a sink (or supply) for exchangeable material, while the small acceptors act as a shuttle that siphons material from peripheral stores to the large acceptors and in the other direction. Thus, for example, the small acceptors penetrate tissue, acquire (and/or donate) material from the tissue, and their capacity becomes at least partly saturated. They leave the tissue and encounter the large acceptors in the plasma, at which point the small acceptors are stripped of tissue lipids. The capacity of the small acceptors is thereby restored, so that when they return to the tissue, they can acquire (and/or donate) more material. This cycle can continue many times. Second, the large acceptors can re-model some small acceptors. For example, large acceptors can donate phospholipid to HDL, which increases the capacity of HDL acquire tissue cholesterol and other material. Third, as noted elsewhere, the presence of large acceptors can block or reduce the harmful disruptions in hepatic cholesterol homeostasis caused by the small acceptors.

Large liposomes avoid raising plasma concentrations of atherogenic lipoproteins in general, not just LDL. This list includes all lipoproteins that contain apolipoprotein B (apoB), such as LDL, IDL, VLDL, Lp(a),  $\beta$ -VLDL, and remnant lipoproteins.

Immune cells are also the targets for depletion using the methods and modes of operation disclosed herein. It is understood that administration of an HMG-CoA reductase inhibitor, pravastatin, to cardiac transplant recipients reduced their natural-killer-cell cytotoxicity *in vitro*, reduced episodes of rejection accompanied by hemodynamic compromise, reduced coronary vasculopathy, reduced plasma LDL levels (and increased HDL

levels), and significantly enhanced one-year survival. The effect on survival was dramatic: in the control group, 22% died in the first year, whereas only 6% died in the pravastatin-treated group.

Immunologic effects of HMG-CoA reductase inhibitors have been reported *in vitro*. These reported immunologic effects include the regulation of DNA in cycling cells, the inhibition of chemotaxis by monocytes, the regulation of natural-killer-cell cytotoxicity, and the inhibition of antibody-dependent cellular cytotoxicity. Regulation of such inhibitors results from changes in circulating lipids or other effects and by utilization of the methods and modes of operation disclosed herein.

HMG-CoA reductase catalyzes an early step in cholesterol biosynthesis and is crucial in the synthesis of molecules besides cholesterol. Adding cholesterol to immune cells treated with HMG-CoA reductase inhibitors does not restore function, although the addition of mevalonate does. Although this suggests that cholesterol depletion is not directly responsible for the immune effects, the use of liposomes or other acceptors to remove cholesterol from cells increases endogenous consumption of mevalonate, as the cells try to make more cholesterol. To impede the ability of the immune or other cells to make up their cholesterol loss by picking up more LDL or other lipoproteins, the methods and treatment described herein are also be done in conjunction with therapies to lower plasma cholesterol concentrations (including HMG-CoA reductase inhibitors, fibric acids, niacin, bile acid binders, LDL-pheresis, etc.).

These processes include enhancement of cholesterol removal and reduction of cholesterol influx. Levels of HDL, the apparent natural mediator of cholesterol removal from peripheral cells, increased in a treated group of patients, and LDL levels were decreased. The administration of HMG-CoA reductase inhibitors *in vivo* usually causes very tiny changes in

reductase enzyme activity: cells simply make more enzyme to overcome the presence of the inhibitor. They also make more LDL receptors (especially in the liver) and so LDL levels fall.

The invention further provides for additives to PD (peritoneal dialysis solutions) that reduce the accelerated atherosclerosis that occurs in renal failure.

Chemotaxis of monocytes is an important early event in atherosclerotic lesion development: monocytes become attracted to abnormal arterial lipid deposits, and to cellular products made in response to the presence of these deposits, enter the vessel wall, transform into macrophages, internalize the lipid by phagocytosis and/or endocytosis, and become a major component of the so-called lipid-rich foam cells of human atherosclerotic lesions. Thus, inhibition of monocyte chemotaxis is important for atherosclerosis as well and can be accomplished using the methods disclosed herein. Both cellular and humoral immunity seem to be affected by reductase inhibition: cardiac rejection accompanied by hemodynamic compromise has often been associated with humoral rejection (i.e., that occurring without producing marked lymphocytic infiltration in endomyocardial-biopsy specimens).

Pravastatin may interact with cyclosporine [an important immunosuppressive drug], which blocks the synthesis of interleukin-2 in stimulated T-lymphocytes. The addition of interleukin-2 restored the natural-killer-cell cytotoxicity and partly restored the antibody-dependent cytotoxicity that were inhibited in lovastatin-treated in vitro cell cultures. A synergy between cyclosporine and pravastatin explains increased immunosuppression in recipients of cardiac transplants, whereas patients without transplants who receive HMG-CoA reductase inhibitors for hypercholesterolemia do not have clinical immunosuppression.

Thus, the use of safe cholesterol acceptors with other immunosuppressives, such as cyclosporine &/or glucocorticoids (which can also suppress IL-2) is also contemplated by this invention.

It is also appreciated that the invention utilizes derivatives of various compounds described herein.

Pathological specimens from patients with cardiac transplants who have severe coronary vasculopathy have been reported to have a high cholesterol content. Therefore, early cholesterol lowering with pravastatin may play a part in decreasing the incorporation of cholesterol into the coronary arteries of the donor heart. Large liposomes or other cholesterol acceptors are used to accomplish the same effect, quickly and directly, alone or in combination, therewith.

Immune modulations is important in many conditions, not just cardiac transplantation. Areas in which the above approaches could be used also include transplantations of other organs, autoimmune diseases (in which the body's immune system mistakenly attacks the body's own tissues), some infections (in which the immune reaction becomes harmful), and any other situation in which immune modulation would be helpful.

With respect to infections, modification of the lipid content and composition of foreign objects in the body (such as infectious agents) while maintaining normal hepatic cholesterol homeostasis should also be mentioned.

Oxidized lipids alter tissue function and cause damage, including decreased EDRF, and increased adhesion molecules, cell damage, and macrophage chemotaxis.

There are interactions between LUVs and small acceptors, such as HDL, apoprotein phospholipid complexes, and cyclodextrins. Liposomes remodel HDL into a better acceptor by donating extra phospholipid, and the small acceptors act as a shuttle, carrying cholesterol

efficiently from cells to liposomes. LUVs do not elevate LDL concentrations and do not suppress hepatic LDL receptor gene expression. The medical utility for LUVs includes restoring EDRF secretion by endothelial cells. High cholesterol levels inhibit endothelial release of EDRF not through cholesterol, but through an oxidized derivative of cholesterol. Because HDL itself restores EDRF release, perhaps through the removal of cholesterol or of oxidized lipids, then liposomes would be able to do the same (the HDL ferries cellular oxidized lipids to liposomes, for example).

The invention provides a method and mode of operation for modifying cellular lipids, including oxidized lipids, without provoking a rise in LDL concentrations or harmfully disturbing hepatic homeostasis. Thus, the LUVs, presumably acting in concert with endogenous (or exogenous) small acceptors of cholesterol (such as HDL), pull oxidized lipids out of peripheral tissues and deliver them to the liver for disposal. Oxidized lipids have a wide range of harmful biological effects, including suppression of EDRF release, induction of cell adhesion molecules, cellular damage, chemotaxis of macrophages, and so forth.

Oxidized lipids and their harmful effects include decrease endothelial C-type ANF; increased endothelial PAI-1 and decreased tPA and decreased endothelial thrombomodulin. Liposomes enhance or participate in this effect. These changes impair the body's ability to dissolve clots. The methods disclosed herein assist in ameliorating these harmful effects of oxidized lipids. HDL acts in part by transporting enzymes that inactivate biologically active oxidized lipids.

It is understood that oxidized LDL inhibits endothelial secretion of C-type natriuretic peptide (CNP). It is the lipid component of oxidized LDL that mediates this effect. Most importantly, HDL blocks the action of oxidized LDL, presumably by picking up oxidized lipids (e.g., oxidized cholesterol). Coincubation with high-density lipoprotein (HDL), which

alone had no effect on CNP release, significantly prevented Ox-LDL-induced inhibition of CNP secretion by endothelial cells (ECs). Analysis by thin-layer chromatography demonstrated that oxysterols, including 7-ketocholesterol, in Ox-LDL were transferred from Ox-LDL to HDL during coincubation of these two lipoproteins. These results indicate that Ox-LDL suppresses CNP secretion from ECs by 7-ketocholesterol or other transferable hydrophilic lipids in Ox-LDL, and the suppressive effect of Ox-LDL is reversed by HDL.

Whatever molecule HDL picks up, the presence of liposomes or other acceptors around as described herein will allow it to do a better job, because of remodeling of HDL by liposomes & shuttling of oxidized lipids by HDL from tissues to liposomes (i.e., the liposomes continuously strip the HDL). Liposomes with an exogenous small acceptor will also work.

It is further understood that transferable lipids in oxidized low-density lipoprotein stimulate plasminogen activator inhibitor-1 and inhibit tissue-type plasminogen activator release from endothelial cells. As above, it is the lipids in oxidized LDL, such as oxidized forms of cholesterol, that produce the effect. It is understood that oxidized low density lipoprotein reduced thrombomodulin transcription in cultured human endothelial cells. It is appreciated that oxidized lipids play a role in atherosclerosis, and enzymes on HDL that inactivate oxidized lipids may contribute to a protective effect. It is contemplated that the methods and compositions disclosed herein will help this proposed mechanism as well, for example, by removing end-products of these enzymes, by otherwise altering HDL, and by providing an additional platform for enzyme transport and action.

As such the use of large liposomes to remove harmful lipids in general (here, oxidized lipids) from peripheral tissues, either directly or via HDL, which would extract the lipids first, possibly inactivate them, then deliver them or their break-down products to liposomes in the

circulation is described. Direct methods to assess oxidation and oxidative damage in vivo include for lipids, assays for 8-epiPGF<sub>2</sub>alpha; for DNA, assess 8-oxo-2' deoxyguanosine; generally assess anti-oxidant enzymes in tissues; and assess anti-oxidants levels, such as vitamin E, vitamin C, urate, and reduced/oxidized glutathione.

Methods relating to and modes for effecting the reverse lipid transport, from cells, organs, & tissues, including transport of extracellular material, and any exchangeable material in general are described herein. This covers not just cholesterol, but also sphingomyelin, oxidized lipids, lysophosphatidylcholine, proteins, and also phospholipid donation. Some effects of oxidized material include increased calcification in arterial cells as described above and below.

Three potential differences between large versus small liposome to explain their different effects on LDL and apoB levels include: fenestral penetration (LUV <<SUV); rate of clearance (LUV < SUV, so that LUVs produce a slow, sustained cholesterol delivery to the liver that may be less disruptive); and protein adsorption (LUV << SUV).

Unesterified cholesterol increases tissue factor expression by macrophages. This is extremely important, because it is macrophage-derived tissue factor that makes the material released by unstable, rupturing plaques such a powerful stimulus for a clot to form that then blocks the vessel leading to a heart attack. The methods and modes of operation and compositions of the invention act upon the expression of tissue factor.

Poor absorption of proteins by large liposomes affects LDL levels and/or atherosclerosis by the following mechanisms: 1) acquisition of apoE from VLDL by small liposomes impairs the removal of VLDL from the circulation, thereby allowing it to be more efficiently converted into atherogenic LDL; ii) absorbed proteins on small liposomes direct

these particles into the wrong metabolic pool within the liver. Polyacrylamide gel electrophoresis shows that liposomes (actually small liposomes) increase the size of LDL. Liposomes are used to alter LDL size, composition and structure to decrease its atherogenicity.

Other properties of LDL could be changed by administration of liposomes. For example, liposomes reduce surface unesterified cholesterol; reduce surface sphingomyelin; replace surface phospholipids with POPC which is poorly oxidized; supplement the LDL with antioxidants that were added to the liposomes before administration. These changes would substantially alter arterial entry, retention, modification and atherogenicity of LDL.

The side-effects controlled are focused on hepatic cholesterol metabolism, hepatic expression of genes involved in cholesterol metabolism, and plasma concentrations of cholesterol-rich atherogenic lipoproteins that contain apolipoprotein B (chiefly, LDL). Reverse transport of sphingomyelin, for example, changes hepatic cholesterol metabolism (cellular sphingomyelin affects the intracellular distribution of cholesterol, and hence its regulatory effects; also sphingomyelin is a precursor to ceramide, which mediates intracellular signaling), though large liposomes appear to avoid any problems in the area. The same holds true for reverse transport of oxidized forms of cholesterol (they are even more potent than unoxidized cholesterol in suppressing LDL receptor gene expression). Cyclodextrins do not pick up phospholipids.

Liposomes pick up any exchangeable lipid (actually, any exchangeable amphipathic or hydrophobic material, which includes lipid or protein or anything else with these characteristics). This includes sphingomyelin, oxidized or modified lipids, such as oxidized sterols and phospholipids. Typically, such liposomes can pick up unesterified cholesterol and other exchangeable material from other lipid bilayers, such as cell membranes, and from

lipoproteins. Liposomes also pick up proteins and donate phospholipids. During and after these modifications, the liposomes are removed from the plasma, chiefly by the liver. Throughout this application, we will refer to this general process as "reverse lipid transport", although it is understood that any exchangeable material in tissues, blood, or liposomes could participate. Specific examples of exchangeable material include unesterified cholesterol, oxidized forms of cholesterol, sphingomyelin, and other hydrophobic or amphipathic material.

These molecules accumulate in atherosclerosis and mediate harmful effects (e.g., cholesterol, oxidized cholesterol, and other material, such as lysophospholipids) or in aging (e.g., sphingomyelin). For example, oxidized lipids, particularly sterols, alter many peripheral tissue functions, including stimulating calcification by arterial cells in atherosclerosis & stimulating endothelial plasminogen activator inhibitor-1 release by endothelial cells; other oxidized lipid products include lysophospholipids that stimulate endothelial expression of adhesion molecules that attract macrophages into lesions, and sphingomyelin accumulates in some cell-culture models of aging and, with cholesterol, may account for some of the cellular changes. Other changed, such as oxidation, may also mediate or accelerate aging. Many of these molecules have been shown to be picked up by liposomes in vitro (e.g., cholesterol, sphingomyelin, & probably oxidized cholesterol) and many by HDL (cholesterol, oxidized cholesterol by liposomes) but it is likely that they pick up these other molecules as well. In terms of total mass, however, the bulk of the acquired material is unesterified cholesterol, with proteins in second place. Alternatively, by acquiring unesterified cholesterol, the liposomes may reduce the amount of oxidized cholesterol that develops, because there will be less starting material.

The effective periods of time described herein should not be interpreted to exclude very long courses of treatment, lasting years, for example. Nor should it exclude repeated courses of treatment separated by weeks, months, or years.

Side effects include overload of the liver with cholesterol or other materials acquired by the liposomes; with subsequent alterations in hepatic function, such as suppression of LDL receptors, stimulation of intrahepatic cholesterol esterification, stimulation of intrahepatic cholesterol esterification, stimulation of hepatic secretion of atherogenic lipoproteins that contain apolipoprotein-B, and impaired uptake of atherogenic lipoproteins by the liver from plasma.

As used herein the word, "endogenous" indicates that the HDL arises from within the body, and is not itself administered. HDL and related acceptors can, however, be administered.

The data indicates another difference between large and small liposomes in vivo. Before injection, the liposomes that are used in our experiments were essentially electrically neutral, indicated by a failure to migrate rapidly through a gel of agarose when an electric field is applied. (This does not imply that charged liposomes or other particles could not be used. The small liposomes pick up proteins and other material, and become electrically charged: they now rapidly migrate through agarose gels when an electric field is applied. Agarose gels of plasma samples we had stored from the three groups of rabbits were run. The small liposomes became more mobile LDL in these gels. The large liposomes were substantially less mobile, indicating a lower charge density, reflecting a lower protein content.

Two explanations for the difference between large and small liposomes exist: 1) small ones penetrate through hepatic endothelial fenestrae while large ones do not (thus, large ones go to Kupffer cells and small ones go to hepatic parenchymal cells and cause problems); 2)

large liposomes are known to be cleared by the liver somewhat more slowly than are small liposomes (the reason is not known), and so may not overwhelm the liver as easily. The data on charge density provides an explanation in part: less protein, therefore slower or altered hepatic uptake. The delivery of cholesterol to the liver by LUVs is actually more efficient than by SUVs, per mg of phospholipid. One difference is that the delivery by LUVs is steady over a long period after the injection, whereas the delivery by SUVs peaks then falls.

Some of the composition described herein include egg phosphatidylcholine; synthetic phosphatidylcholines that are not crystalline at body temperature (e.g., they contain at least one double bond) yet are resistant to oxidation (e.g., they do not have many double bonds, such as 1-palmitoyl, 2-oleyl phosphatidylcholine, abbreviated POPC); other natural or synthetic phospholipids alone or in mixtures; any of the preceding supplemented or replaced with hydrophobic or amphipathic material that still allows a liposomal or micellar structure. An extruder is certainly not the only conceivable method for making large liposomes or even particularly LUVs. Other methods known to practitioners in the field are available or can be adapted to make large liposomes in general and LUVs in particular.

As used herein, a dose includes from 10 to 1600 mg of phospholipid, in the form of large liposomes, per kg of body weight. Other acceptable rates described herein can be determined empirically by the response of plasma LDL concentrations.

Where there is a change in membrane composition, as well as function, one can use an assay of membrane composition or an assay of tissue composition. Compositional assays should include lipids, proteins, and other components.

HDL can pick up oxidized material, and HDL-associated enzymes may inactivate oxidized material.

The separations in time will depend on the actual dose of material, its effects on hepatic cholesterol homeostasis, and whether cholesterol-lowering agents are being concurrently administered. Thus, for doses of about 300 mg of small liposomes per kg of body weight, slight disruptions will occur after even a single dose, and single administrations of higher doses may cause even more disruptions. Exemplary separations in time include one day to one month, but the precise schedules would have to be determined by monitoring hepatic cholesterol metabolism and plasma levels of LDL and other atherogenic lipoproteins.

The major macrophages that would be involved in liposomal clearance would be Kupffer cells in the liver and macrophages in the bone marrow or spleen. The catabolism here would be the so-called alternative pathway for initiating the conversion of cholesterol into bile acids (macrophages are known to have at least one cholesterol-catabolizing enzyme), or would be transfer of sterol (enzymatically altered or not) to other cells, such as hepatic parenchymal cells that would then dispose of the molecules.

The methods described herein also control effects of cellular aging.

The invention includes means for assessing the efficacy of liposomal therapy by performing assays of oxidation in vitro and in vivo, assays of oxidative susceptibility of plasma components, and assays of the ability of altered HDL to inhibit oxidation (by binding oxidative products and/or through its paroxinase or other anti-oxidant components), and the ability of HDL or plasma or serum or blood to mobilize cholesterol and other exchangeable material.

Large liposomes may cause the mobilization of some material that is trapped between cells as well (this is the extracellular space). This extracellular material causes problems a) when it contacts cells or platelets, altering their function and b) by simply taking up space.

Estimate rates of cholesterol mobilization can be empirically determined. It is appreciated that the kinetics of liposomal clearance is different in different species (the  $t_{1/2}$  of LUVs in mice is about 8h, but in rabbits it is about 24h, and in humans it is longer). Thus, rates calculated may vary from species to species. Based on my data on injection of 300mg of SUVs into rabbits, the peak rate of liposomal cholesterol removal from plasma was between 3h and 6h after the injection. At that point, the liposomes had raised plasma unesterified cholesterol by just over 2mmol/L; assuming a total plasma volume of 90mL in a 3-kg rabbit, the total liposomal cholesterol at that point was 180 $\mu$ moles; the  $t_{1/2}$  for SUVs in these rabbits was about h, so roughly 10% is removed in 3h; thus, the peak rate of liposomal cholesterol removal was about 2  $\mu$ moles/h/kg, and this caused a subsequent rise in plasma cholesteryl ester concentrations. Notice that at other time periods after the injection, the rate of liposomal cholesterol removal from plasma was less. Note also that the liver is the predominant organ for clearance, but not the sole organ for clearance.

It has been calculated that a single injection of 300 mg LUVs/kg into 20-22-g mice mobilized about 2400 nmoles of cholesterol in the first 24h after injection. In contrast to the data with SUVs in rabbits, the mobilization of cholesterol during the first 24h in the mice injected with LUVs was quite steady. This calculates to about 4.7  $\mu$ moles/h/kg over this first 24-h period, which is actually more than the above figure of 2  $\mu$ moles/h/kg, which was a peak rate. It is not fair comparison, because the clearance of LUVs in mice is three times as fast as in rabbits. If we take 4.7 divided by 3, we get 1.6  $\mu$ moles/h/kg, which is less than 2, but

these are imperfect estimates. Human rates can be empirically determined. It is clear, however, that LUVs deliver their cholesterol at a steady rate, whereas SUVs make a brief, rapid push of lipid into the liver. At body temperature, the most desirable liposomes are fluid within the confines of the bilayer, which is called the liquid crystalline state. Less desirable are liposomes in the gel state, which is less fluid.

It is understood that unesterified cholesterol stimulates macrophages to express more tissue factor, a substance known to provoke blood clots. This explains the presence of abundant tissue factor in rupture-prone plaques, which, when they rupture, expose tissue factor to plasma and provoke a clot that can occlude the vessel, causing a heart attack. This would be another example of an abnormal cellular function that may be reversed by removal of cholesterol by liposomes.

Several human conditions are characterized by distinctive lipid compositions of tissues, cells, membranes and/or extracellular regions. For example, in atherosclerosis, cholesterol (unesterified, esterified, and oxidized forms) and other lipids accumulated in cells and in extracellular areas of the arterial wall and elsewhere. These lipids have potentially harmful biologic effects, for example, by changing cellular functions and by narrowing the vessel lumen, obstructing the flow of blood. Removal of the lipids would provide numerous, substantial benefits. Moreover, cells, membranes, tissues and extracellular structures would benefit from composition and alteration that include increasing resistance to oxidation and oxidative damages, such as by increasing the content and types of anti-oxidants, removing oxidized material, and increasing the content of material that is resistant to oxidation. In aging, cells have been shown to accumulate sphingomyelin and cholesterol, which alter cellular functions. These functions can be restored *in vitro* by removal of these lipids and replacement with phospholipid from liposomes. A major obstacle to performing similar lipid

alterations *in vivo* has been disposition of the lipids mobilized from tissues, cells, extracellular areas, and membranes. Natural (*e.g.*, high-density lipoproteins) and synthetic (*e.g.*, small liposomes) particles that could mobilize peripheral tissue lipids have a substantial disadvantage: they delivery their lipids to the liver in a manner that disturbs hepatic cholesterol homeostasis, resulting in elevations in plasma concentrations of harmful lipoproteins, such as low-density lipoprotein (LDL), a major atherogenic lipoprotein.

The invention described herein provides methods and compositions related to the "reverse" transport of cholesterol and other materials and compounds from peripheral tissues to the liver *in vivo* while controlling plasma LDL concentration.

Agarose gel electrophoreses of plasma samples from the last a set of rabbits injected with LUVs, SUVs, or saline (these agarose gels separate particles by their charge, which is not the same from one type of particle to another) were performed. Freshly made SUVs migrate very slowly through agarose, which indicates that freshly made liposomes have very little charge. After injection into animals or after co-incubation with plasma or lipoproteins, SUVs pick up proteins from lipoproteins. These proteins give more charge to the SUVs and substantially enhance their migration through agarose gels. SUVs after exposure to plasma migrate faster through these gels than does LDL.

The gels showed a substantial difference between LUVs and SUVs. As expected, the SUVs migrated ahead of LDL in these gels. The LUVs, however, migrated almost exactly where freshly made, protein-free liposomes migrate. This result indicates that LUVs, unlike SUVs, do not readily pick up proteins from circulating lipoproteins.

There is a direct verification of this difference between the liposomes. Human HDL (which has most of the proteins that liposomes pick up) was incubated with either LUVs or SUVs, then the liposomes were reisolated, and assayed their protein-to-phospholipid ratios.

Per amount of liposomal phospholipid, the SUVs picked up about 40 times as much protein as did the LUVs. This difference appears to arise because of the difference in surface curvature: SUVs are smaller, so their surface is more tightly curved, thus under greater strain, proteins can more easily insert.

There are three most likely metabolic effects of the difference in protein uptake between the two types of liposomes are as follows:

1. VLDL has two metabolic fates: it can be removed from plasma before it is fully converted to LDL by lipolytic enzymes, or it can be fully converted into circulating LDL. SUVs strip apoE off VLDL, thereby slowing its clearance from plasma and favoring its conversion to LDL. In contrast, LUVs leave apoE on VLDL, and so LDL concentrations in plasma would not rise.

2. Absorbed apoproteins might play a role in directing liposomes to different hepatic metabolic pools.

Here are some ways to assay effect on oxidation in vivo: Catella F, Reilly MP, Delanty N, Lawson JA, Moran N, Meagher E, FitzGerald GA. Physiological formation of 8-epi-PGF<sub>2</sub> alpha in vivo is not affected by cyclooxygenase inhibition. Adv Prostaglandin Thromboxane Leukot Res. 23:233-236, 1995. These authors describes 8-epi-PGF<sub>2</sub>alpha, which is an end-product of lipid oxidation. This molecule can be used, they suggest, as a measure of lipid oxidative flux in an animal. It is superior to other commonly used measure of oxidation in vivo, such as anti-oxidant levels (which are affected by diet), thiobarbituric acid reactive substances (some sugars interfere with this assay), and short-lived oxidative intermediates (these do not indicate total flux of material being oxidized). Administration of LUVs, by removing oxidized lipids from the periphery, would lower total oxidative flux in vivo, and 8-epi-PGF<sub>2</sub>alpha would be a suitable way to measure this; Cadet J, Ravanat JL,

Buchko GW, Yeo HC, Ames BN. Singlet oxygen DNA damage: chromatographic and mass spectrometric analysis of damage products. *Methods Enzymol.* 234:79-88, 1994. they describe 8-oxo-2'-deoxyguanosine, which is an end-product of DNA oxidation. As above, this molecule can be used as a measure of DNA oxidative flux in an animal. Administration of LUVs would lower DNA oxidative flux in vivo, and this is a suitable way to measure this; and, Xia E, Rao G, Van Remmen H, Heydari AR, Richardson A. Activities of antioxidant enzymes in various tissues of male Fischer 344 rats are altered by food restriction. *J Nutr.* 125(2):195-201, 1995. Antioxidant enzymes in tissues were measured, to indicate de-oxidant capacity. LUVs help this. Anti-oxidant levels (vitamin E, ascorbate, urate); oxidized and reduced glutathione; and many other measures can be used to assess peripheral oxidation and oxidative damage. Again, these and other measures would be coupled with LUV administration, to assess efficacy of the therapy.

Other particles that mimic the properties of large liposomes will act similarly, to mobilize peripheral lipids and other exchangeable materials, and deliver exchangeable materials, while avoiding harmful disruptions in hepatic cholesterol homeostasis. For example, these would include emulsion particles that are too large to penetrate hepatic endothelial fenestrae, of a composition and structure that is taken up by the liver slowly, and/or a composition and structure that does not readily acquire specific endogenous proteins. Such emulsions could be made with or without proteins, and could be made from phospholipid and a neutral lipid, such as triglycerides or another neutral lipid.

The invention also provides a pharmaceutical composition comprised or consisting essentially of liposomes dimensioned and of a composition so that the liposomes are taken up slowly by the liver.

The invention also includes a method of forcing the reverse transport of cholesterol from peripheral tissues to the liver *in vivo* while controlling plasma LDL concentrations comprising the step of parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period, whereby the liposomes pick-up the cholesterol during the treatment period. The method includes the optional step of enhancing tissue penetration of a cholesterol acceptor and increasing extraction of tissue cholesterol and other exchangeable material by co-administration of an effective amount of a compound. The compound is selected from the group consisting of a small acceptor of cholesterol and a drug that increases endogenous small acceptors of cholesterol. In a variant, co-administration of the compound is simultaneous with the parenteral administration of the large liposomes. In another variant, co-administration of the compound is separated in time from the parenteral administration of the therapeutically effective amount of a multiplicity of the large liposomes by an effective time period. The effective time period is in the range of about 1 minute to about two weeks.

In another aspect the invention includes an improved method of reducing the lipid content of arterial lesions comprising the steps of inducing the reverse transport of cholesterol from peripheral tissues to the liver *in vivo* by administering a therapeutically effective amount of an agent to a subject. The agent is selected from the group consisting of large liposomes comprised of phospholipids substantially free of sterol and small acceptors; periodically monitoring plasma LDL concentrations of the subject to obtain an LDL concentration profile; adjusting the therapeutically effective amount of the agent responsive to the LDL concentration profile; and, administering a pharmaceutical agent to the subject. The agent is selected from the group consisting of compounds to lower LDL concentrations, small acceptors, and compounds to raise HDL concentrations, responsive to the LDL concentration

profile, whereby the reduction in lipid content of the arterial lesions is effectively treated and monitored over a treatment period. The arterial lesions comprise lipid rich, rupture prone, type IV and type V arterial lesions. Plaque rupture, thrombosis, and tissue infarction are greatly reduced.

In yet another aspect the invention provides for an improved method of assessing the efficiency of a treatment for reducing the lipid content of arterial lesions. the lesions coming into contact with plasma and a component thereof comprising the steps of inducing the reverse transport of cholesterol from peripheral tissues to the liver *in vivo* by administering a therapeutically effective amount of an agent to a subject. The agent is selected from the group consisting of large liposomes comprised of phospholipids substantially free of sterol and small acceptors; and, periodically monitoring the plasma component with an assay. The assay is selected from the group consisting of an assay for plasma unesterified cholesterol and phospholipid, an assay of bile acids and cholesterol in stool, an assay of bile acids and cholesterol in bile, an assay of hepatic gene expression in a liver biopsy, an assay of gene expression in peripheral blood leukocytes, the gene comprising a gene involved in cholesterol metabolism, an assay of plasma LDL concentration, and a vascular imaging technique. The vascular imaging technique is selected from the group consisting of cardiac catheterization, magnetic resonance imaging, ultrasound, ultrafast CT and a radionuclide assay which optionally includes a stress-thallium scan.

The invention also includes a method of beneficially altering arterial function, blood platelet function, and controlling plasma LDL concentrations and hepatic cholesterol homeostasis *in vivo* comprising the step of parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period with or without administration of other agents. The other

agents optionally include small acceptors and LDL lowering agents. Optionally the method includes the step of taking a measurement of arterial function. The measurement is selected from the group consisting of a measurement of endothelial-derived relaxing factor, a measurement of intracellular calcium concentration in arterial cells, a measurement of arterial cell proliferation, an assay of arterial enzymes, an assay in the presence of calcium channel blockers, an assay of arterial uptake, accumulation and retention of lipoproteins, an assay of arterial accumulation of liposomes, an assay of arterial retention of liposomes, an assay of gene products, and an assay of arterial cell functions. The measurement of endothelial-derived relaxing factor is selected from the group consisting of a functional determination of endothelial-dependent arterial relaxation, chemical determination of production of said endothelial relaxing factor, and an assay of nitric oxide synthase.

A method of beneficially altering blood platelet function while controlling plasma LDL concentrations, arterial function, hepatic cholesterol homeostasis and said platelet function *in vivo* is also included. The method comprises the step of parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period, said liposomes administered with or without other agents. The method optionally includes the step of taking a measurement of arterial function. The measurement selected from the group consisting of a measurement of endothelial-derived relaxing factor, a measurement of intracellular calcium concentration in arterial cells, a measurement of arterial cell proliferation, an assay of arterial enzymes, and an assay of gene products. The measurement of endothelial relaxing factor is selected from the group consisting of a functional determination of endothelial-dependant arterial relaxation and chemical determination of production of said endothelial relaxing factor.

Also included is a method of catabolizing cholesterol with macrophages *in vivo* and also affecting a plasma component or structural aspects of an artery, comprising the step of administering an effective amount of liposomes to a subject substantially free of cholesterol and being of a size and composition such that the liposomes are capable of being taken up by the macrophages and capable of being catabolized by the macrophages. The cholesterol is mobilized by the liposomes resulting in the liposomes being taken up by the macrophages and catabolized. The method also can include the step of periodically monitoring the plasma component with an assay. The assay is selected from the group consisting of an assay for plasma unesterified cholesterol and phospholipid, an assay of plasma cholesterol ester transfer protein activity, an assay of bile acids and cholesterol in stool, an assay of hepatic gene expression in a liver biopsy, an assay of gene expression in a peripheral blood leukocytes, the gene comprising a gene involved in cholesterol metabolism, an assay of plasma LDL concentration, and a vascular imaging technique.

In yet another aspect the invention includes a method of delivering a drug *in vivo* and avoiding harmful disruptions of hepatic cholesterol homeostasis, comprising the steps of entrapping the drug with an agent. The agent is selected from the group consisting of a cholesterol poor liposome, a cholesterol free liposome, an emulsion, a liposome primarily taken up slowly by hepatic parenchymal cells, an emulsion primarily taken up slowly by hepatic parenchymal cells. The agent is selected from the group consisting of an agent with a protein and an agent without protein to obtain an entrapped drug. The method also includes the step of administering a therapeutically effective amount of the entrapped drug for a treatment period. The step of administering comprises the step of slowly infusing said entrapped drug. In variants, the step of administering comprises the step of administering small doses of the agent, appropriately separated in time, to avoid harmful disruptions in

hepatic cholesterol homeostasis, and includes using low doses of said agent, whereby disrupting hepatic cholesterol homeostasis is avoided.

A method of controlling plasma LDL levels, hepatic cholesterol homeostasis, arterial enzymes, arterial function, and platelet function, and altering platelet hormone production is also provided. The method includes the step of parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period. The effective amount is administered in a dosage and the dosage is selected from a single dose and repeated doses. The method optionally includes the step of diagnosing the efficacy of the administration by taking a measurement of the hormone production and regulating the effective amount in response to the measurement. The measurement of hormone production is an assay selected from the group consisting of an assay for thromboxanes, an assay for prostacyclines, an assay of prostaglandins, an assay for leukotrienes, and an assay for derivatives thereof.

In yet a further aspect the invention provides a method of increasing plasma HDL concentrations, while controlling plasma LDL levels, hepatic cholesterol homeostasis, and hepatic gene expression. The method comprises the step of parenterally administering a therapeutically effective amount of a first agent. The first agent comprising a multiplicity of small liposomes to raise HDL concentrations for a treatment period. The method then includes the step of co-administering a second agent. The second agent includes large liposomes comprised of phospholipids substantially free of sterol for a treatment period. The effective amount is administered in a dosage selected from a single dose and repeated doses. The co-administration acts to prevent the small liposomes from stimulating harmful changes in hepatic cholesterol homeostasis and an increase in plasma LDL. In a variant, the first agent consists essentially of small liposomes and the second agent consists essentially of large

liposomes. The method also includes the step of diagnosing the efficacy of the administration by taking a measurement of plasma HDL and LDL levels before, during and after the treatment period.

A method of controlling plasma LDL levels, and hepatic cholesterol homeostasis *in vivo* while altering cell membrane composition and function is also described herein. The method includes the step of parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period. The effective amount administered in a dosage selected from a single dose and repeated doses. The method includes the step of co-administering a small acceptor selected from the group consisting of a small acceptor of cholesterol, an acceptor of sphingomyelin, an acceptor of lysophosphatidylcholine, and an acceptor of a lipid. The method can optionally include the step of diagnosing the efficacy of the administration by performing a measurement selected from the group consisting of a measurement of membrane fluidity, a measurement of transmembrane ion flux, said ions selected from the group consisting of calcium ions, sodium ions, and potassium ions, an assay of membrane fragility, and an assay of membrane function.

In a further embodiment, the invention includes a pharmaceutical composition for mobilizing peripheral cholesterol and sphingomyelin that enters the liver of a subject consisting essentially of liposomes selected from the group of uni-lamellar liposomes, multi-lamellar liposomes, combinations thereof, and derivatives thereof, and a pharmaceutical composition for reducing the size of arterial lesions that enters the liver of a subject consisting essentially of a multiplicity of non-liposomal particles for cholesterol depletion of peripheral tissues while avoiding harmful disruptions of hepatic cholesterol homeostasis. The particles

are selected from the group of particles substantially free of cholesterol and particles free of cholesterol.

Non-liposomal particles are selected from the group consisting of triglyceride-phospholipid emulsions. The emulsions include emulsions that are not taken up rapidly by hepatic parenchymal cells, emulsions that are not taken up to a large extent by parenchymal cells, and triglyceride-phospholipid-protein emulsions.

Also included in the invention is a pharmaceutical composition for reducing the size of arterial lesions that enters the liver of a subject consisting essentially of a drug entrapped within an agent. The agent is selected from the group consisting of a cholesterol poor liposome, a cholesterol free liposome, an emulsion, a liposome primarily taken up slowly by hepatic parenchymal cells, and an emulsion primarily taken up slowly by hepatic parenchymal cells. The agent is selected from the group consisting of an agent with a protein and an agent without protein.

The invention also provides for a pharmaceutical composition for increasing plasma HDL concentrations, while controlling plasma LDL levels, hepatic cholesterol homeostasis, and hepatic gene expression, comprising a first agent which comprises a multiplicity of small liposomes to raise HDL concentrations, and a second agent which comprises large liposomes comprised of phospholipids substantially free of sterol.

In yet another aspect a method of controlling cholesterol metabolism in hepatic parenchymal cells in a subject in vivo through cell-cell communication from Kupffer cells to the parenchymal cells is included. The method includes the steps of administering a liposome composition to the subject. The liposome composition is selected from the group consisting of large unilamellar liposomes and large multilamellar liposomes. The liposomes have an average diameter of about 50-150 nanometers. The LDL levels in the subject do not increase.

The method also includes the step of diagnosing the efficacy of the control of cholesterol metabolism by assaying an indicator in the subject. The indicator is selected from the group consisting of plasma LDL concentrations of the subject, hepatic gene expression of said subject, sterol excretion controlling cholesterol metabolism in hepatic parenchymal cells in the subject, and sterol excretion in bile of the subject; and adjusting the administration in response to the assay.

The present invention further provides a mode of operation of arterogenic lipoproteins, cellular structures, and extracellular structures that is altered by the compositions described herein through which beneficial physiological effects are obtained.

Cellular and organism aging and oxidation can be reversed or slowed utilizing the methods and devices described herein.

While only a few, preferred embodiments of the invention have been described hereinabove, those of ordinary skill in the art will recognize that the embodiment may be modified and altered without departing from the central spirit and scope of the invention. Thus, the preferred embodiment described hereinabove is to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims, rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced herein.

CLAIMS**I CLAIM:**

1. A pharmaceutical composition consisting essentially of:  
large liposomes comprised of phospholipids substantially free of sterol,  
whereby said composition forces the reverse transport of cholesterol from peripheral tissues to the liver *in vivo*.
2. A composition for controlling cholesterol related genes, enzymes and other compounds while forcing the reverse transport of lipids peripheral tissues to the liver consisting essentially of large liposomes sized and shaped to interact with molecules interacting with said genes, enzymes or other compounds, said large liposomes being substantially free of sterol.
3. A pharmaceutical composition for mobilizing peripheral cholesterol and sphingomyelin that enters the liver of a subject consisting essentially of liposomes of a size and shape larger than fenestrations of an endothelial layer lining hepatic sinusoids in said liver, whereby said liposomes are large enough not to penetrate most of said fenestrations and interact with hepatic parenchymal cells in said liver.
4. A pharmaceutical kit for mobilizing peripheral cholesterol and sphingomyelin comprising: a first container having a pharmaceutical composition for reducing the size of arterial lesions that enters the liver of a subject consisting essentially of liposomes of a size and shape larger than fenestrations of an endothelial layer lining hepatic

sinusoids in said liver, whereby said liposomes are large enough not to penetrate most of said fenestrations and interact with hepatic parenchymal cells; and

a second container having a compound, said compound selected from the group consisting of a small acceptor of cholesterol and a drug that increases endogenous small acceptors of cholesterol.

5. The kit in accordance with claim 4 in which said small acceptor is selected from the group consisting of a high-density lipoprotein, a phospholipid protein complex having a group selected from the group consisting of apoA-I, apoA-II, apoA-IV, apoE, synthetic fragments thereof, natural fragments thereof, an amphipathic protein, and an amphipathic peptide, said protein substantially free of phospholipid, small phospholipid liposomes, and a small cholesterol acceptor; said drug including an agent that raises endogeneous HDL concentrations, said agent selected from the group consisting of nicotinic acid, ethanol, a fibric acid, a cholesterol synthesis inhibitor, a drug that increases HDL concentrations, an amphipathic compound, and derivatives thereof.
6. An improved pharmaceutical composition for reducing the size of arterial lesions that enters the liver of a subject, said composition consisting essentially of liposomes, in which said improvement comprises an anti-oxidant and derivatives thereof.
7. The composition of claim 6 further comprising means for providing for the control and regulation of atherogenic lipoprotein concentrations.

8. The composition in accordance with claim 6 further comprising, in combination, an agent for controlling plasma concentrations of atherogenic lipoproteins, said agent selected from the group consisting of large liposomes, anti-hyperlipidemic drugs, and concentration lowering compounds other than large liposomes and anti-hyperlipidemic drugs.
9. The composition in accordance with claim 7 in which said means regulates an atherogenic lipoprotein selected from the group consisting of LDL, VLDL, IDL,  $\beta$ -VLDL, Lp(a), a lipoprotein containing apolipoprotein-B, oxidized lipoproteins, and modified lipoproteins.
10. The pharmaceutical composition of claim 6 in which said anti-oxidant is selected from the group consisting of vitamin E, tocopherol, butyl hydroxy toluene (BHT), EDTA, chelators, ascorbate, probucol, carotenoids, derivatives thereof, a material resistant to oxidation, a material inhibiting oxidation, and combinations and derivatives thereof.
11. The composition in accordance with claim 6 in which said tocopherol is added at about or above 0.1 mole %.
12. The composition in accordance with claim 6 in which said tocopherol is added at below about 0.1 mole %.

13. The composition in accordance with claim 6 in which said ascorbate is added at about or above 0.1 mole %.
14. The composition in accordance with claim 6 in which said ascorbate is added at below about 0.1 mole %.
15. An improved pharmaceutical composition for modifying membranes, cells, tissues, extracellular structures, including reducing the size, instability, and other properties of arterial lesions that enters the liver of a subject while simultaneously avoiding harmful disruptions of cholesterol homeostasis, said improvement comprising palmitoyl-oleyl-phosphatidylcholine.
16. The improved composition of claim 15 in which said composition comprises large liposomes.
17. An improved pharmaceutical composition for modifying membranes, cells, tissues, extracellular structures, including reducing the size, instability, and other properties of arterial lesions that enters the liver of a subject while simultaneously avoiding harmful disruptions of cholesterol homeostasis, said improvement comprising a double bond in a second fatty acyl chain of components of said composition, whereby said composition is more fluid and more readily accepts cholesterol and other material, and donates material to said membranes, cells, tissues, and extracellular structures.

18. The improved composition of claim 17 in which said composition comprises large liposomes.
19. An improved pharmaceutical composition for modifying membranes, cells, tissues, extracellular structures, including reducing the size, instability, and other properties of arterial lesions that enters the liver of a subject while simultaneously avoiding harmful disruptions of cholesterol homeostasis, said improvement comprising PEG-liposomes in an effective amount, whereby said composition is more fluid and more readily accepts cholesterol and other accepted material, and donates donated material to said membranes, cells, tissues, and extracellular structures.
20. The improved composition of claim 19 in which said composition comprises large liposomes.
21. The composition of claim 19 in which said accepted material is selected from the group consisting of sphingomyelin and oxidized lipids.
22. The composition of claim 19 in which said donated material is selected from the group consisting of phospholipid, phosphatidylcholine, and anti-oxidants.
23. An improved dialysis apparatus for the treatment of a patient in which said improvement comprises means for administering a therapeutically effective amount of a lipid acceptor during the treatment of a subject at or during a suitable period of time.

24. The apparatus of claim 23 in which the lipid acceptor is selected from the group consisting of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol and small acceptors.
25. The apparatus of claim 23 in which said means for administering is selected from the group consisting of means for extracorporeal administration of said lipid acceptor and means for intracorporeal administration of said lipid acceptor.
26. The apparatus of claim 23 in which said means for administering said lipid acceptor is a means other than said means selected from the group consisting of means for extracorporeal administration and means for intracorporeal administration.
27. An improved mode of operating a dialysis apparatus in which said improvement comprises a lipid acceptor administration mode for administering or emitting a therapeutically effective amount of a lipid acceptor by way of a lipid acceptor administration means.
28. The improved mode of operating a dialysis apparatus of claim 27 in which the lipid acceptor is selected from the group consisting of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol and small acceptors.

29. The improved mode of operating a dialysis apparatus of claim 27 in which said administration means is selected from the group consisting of means for extracorporeal administration and means for intracorporeal administration.
30. The apparatus of claim 27 in which said administration means is a means other than said means selected from the group consisting of means for extracorporeal administration and means for intracorporeal administration.
31. An improved method of angioplasty or cardiac catheterization in which said improvement comprises the step of:  
administering a therapeutically effective amount of a lipid acceptor during angioplasty or cardiac catheterization of a subject.
32. The improved method of claim 31 in which said lipid acceptor is selected from the group consisting of a large liposome comprised of phospholipids substantially free of sterol and small acceptors.
33. The method in accordance with claim 31 in which said administration of said lipid acceptor occurs at or over an effective period of time.
34. The method in accordance with claim 31 in which said administration of said lipid acceptor occurs simultaneously with said angioplasty.

35. The method in accordance with claim 33 in which said effective period of time is in the range of about 1 minute to about two years from the time of said angioplasty.
36. An improved angioplasty apparatus in which said improvement comprises: lipid acceptor administration means for administering a therapeutically effective amount of a lipid acceptor.
37. The improved angioplasty apparatus of claim 36 further comprising co-administration means for administering said lipid acceptor and a diagnostic agent.
38. An improved mode of operating an angioplasty or cardiac catheterization apparatus in which said improvement comprises a mode of operation for administering a therapeutically effective amount of a lipid acceptor from said apparatus or component thereof into a vessel.
39. The improved mode of claim 38 in which said lipid acceptor is selected from the group consisting of a large liposome comprised of phospholipids substantially free of sterol and small acceptors.
40. A mode of altering the normal mode of processing of atherogenic lipoproteins in a warm blooded mammal comprising the step of administering a liposome composition to said mammal, said liposome composition selected from the group consisting of unilamellar liposomes and multilamellar liposomes, said liposomes

having an average diameter of about 50-150 nanometers, in which LDL levels in said subject do not increase.

41. A method of controlling cholesterol metabolism in hepatic parenchymal cells in a warm blooded mammal *in vivo* through cell-cell communication from Kupffer cells to said parenchymal cells, comprising the step of administering a liposome composition to said mammal, said liposome composition selected from the group consisting of large unilamellar liposomes and large multilamellar liposomes, said liposomes having an average diameter of about 50-150 nanometers, in which LDL levels in said mammal do not increase.
42. The mode in accordance with claim 40 in which the liposome composition is given periodically.
43. The method in accordance with claim 40 in which the liposome composition is given more than once.
44. The mode in accordance with claim 40 in which the liposomes have diameters larger than about 50 nm.
45. The mode in accordance with claim 40 in which the liposomes have diameters larger than about 80 nm.

46. The mode in accordance with claim 40 in which the liposomes have diameters larger than about 100 nm.
47. The mode in accordance with claim 40 in which administration is selected from the group of parenteral administration, intravenous administration, intra-arterial administration, intramuscular administration, subcutaneous administration, transdermal administration, intraperitoneal administration, intrathecal administration, via lymphatics, intravascular administration, including administration into capillaries and arteriovenous shunts, rectal administration, administration via a chronically indwelling catheter, and administration via an acutely placed catheter.
48. The mode in accordance with claim 40 in which about 10 to about 1600 mg/kg/dose of said liposome composition is administered.
49. The mode in accordance with claim 40 in which the liposome composition is given in repeated doses.
50. The mode in accordance with claim 40 in which the liposomes are phospholipids substantially free of sterol and in the range of about 50-150 nm in approximate diameter.
51. The mode in accordance with claim 40 in which the liposomes are phospholipids substantially free of sterol.

52. The mode in accordance with claim 40 in which the liposomes are phospholipids selected from the group consisting of phosphatidyl choline, phosphatidyl glycerol, palmitoyl-oleoyl phosphatidyl choline, combinations thereof, and derivatives thereof.
53. A method of regulating hepatic parenchymal cell cholesterol content, said cell having at least one gene selected from the group consisting of a gene for an LDL receptor, a gene for HMG-CoA reductase, a gene for cholesterol 7-alpha-hydroxylase, and a gene regulating a function involved in cholesterol homeostasis; and, homeostasis thereof, comprising the step of:
- administering an effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol during a period of time.
54. The method in accordance with claim 53 further comprising the steps of periodically assaying a property with an assay, said property selected from the group consisting of concentration, composition, and characteristic of atherogenic lipoproteins in plasma with an assay during said treatment period to assess said plasma atherogenic lipoprotein and to obtain a atherogenic lipoprotein profile, and adjusting said administration in response to said profile.
55. The method in accordance with claim 54 in which said assay is selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma, a precipitation assay of plasma, an electrophoretic assay, an electron microscopic

assay, an immuno-turbidometric assay, and an assay of lipoprotein composition, size or function.

56. The method in accordance with claim 53 further comprising the step of enhancing tissue penetration of a cholesterol acceptor and enhancing extraction of tissue cholesterol and other exchangeable material with co-administration of an effective amount of a compound, said compound selected from the group consisting of a small acceptor of cholesterol, an amphipathic compound, and a drug that increases endogenous small acceptors of cholesterol.
57. The method in accordance with claim 56 in which said small acceptor is selected from the group consisting of a high-density lipoprotein, a phospholipid protein complex having a group selected from the group consisting of apoA-I, apoA-II, apoA-IV, apoE, synthetic fragments thereof, natural fragments thereof, an amphipathic protein, and an amphipathic peptide, said protein substantially free of phospholipid, small phospholipid liposomes, and a small cholesterol acceptor; said drug including an agent that raises endogenous HDL concentrations, said agent selected from the group consisting of nicotinic acid, ethanol, a fibric acid, a cholesterol synthesis inhibitor, a drug that increases HDL concentrations, and derivatives thereof.
58. The method in accordance with claim 56 in which said co-administration of said compound is simultaneous with said administration of said large liposomes.

59. The method in accordance with claim 56 in which said co-administration of said compound is separated in time from said parenteral administration of said therapeutically effective amount of a multiplicity of said large liposomes by an effective time period.
60. The method in accordance with claim 59 in which said effective time period is in the range of about 1 minute to about two weeks.
61. The method in accordance with claim 53 in which the effective amount is in the range of about 10 to about 1600 mg/kg/dose.
62. The method in accordance with claim 53 in which the liposomes are given in repeated doses.
63. The method in accordance with claim 53 further comprising the step of controlling the expression of said genes by said parenteral administration of a multiplicity of said large liposomes.
64. The method in accordance with claim 53 in which said parenchymal cell functions in a system having hepatic sinusoids, an endothelial layer lining said hepatic sinusoids, and fenestrations; and, in which the large liposomes are of a size and shape larger than said fenestrations of said endothelial layer lining said hepatic sinusoids or in which said large liposomes optionally having other properties that prevent said large liposomes from

penetrating said fenestrations, whereby said large liposomes have properties to prevent the ready penetration of said fenestrations.

65. The method in accordance with claim 53 in which the large liposomes are selected from the group consisting of uni-lamellar liposomes and multi-lamellar liposomes.
66. The method in accordance with claim 53 in which the liposomes have diameters larger than about 50 nm.
67. The method in accordance with claim 53 in which the liposomes have diameters larger than about 80 nm.
68. The method in accordance with claim 53 in which the liposomes have diameters larger than about 100 nm.
69. The method in accordance with claim 53 in which administration is selected from the group of intravenous administration, intra-arterial administration, intramuscular administration, subcutaneous administration, transdermal administration, intrathecal administration, via lymphatics, intravascular administration, rectal administration, via a chronically indwelling catheter or other device, via an acutely placed catheter or other device, and intraperitoneal administration.

70. A method of suppressing hepatic expression of a cholesterol ester transfer protein gene in a warm blooded mammal *in vivo* comprising the step of:
- parenterally administering an effective amount of a multiplicity of large liposomes comprised of phospholipids, said phospholipids selected from the group of phospholipids substantially free of sterol and phospholipids containing sterol, for an effective period of time,
- whereby plasma LDL and HDL are controlled as a result of said administration.
71. The method in accordance with claim 70 further comprising the step of periodically assaying plasma LDL concentrations with an assay during said treatment period to assess said plasma LDL.
72. The method in accordance with claim 71 in which said assay is selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma, a precipitation assay of plasma, an electrophoretic assay, an electron microscopic assay, an immuno-turbidometric assay of plasma, and an assay of lipoprotein composition, size or function.
73. The method in accordance with claim 70 in which the liposomes are given periodically.
74. The method in accordance with claim 70 in which the effective amount is in the range of about 10 mg/kg/dose to about 1600 mg/kg/dose.

75. The method in accordance with claim 70 further comprising the step of enhancing tissue penetration of a cholesterol acceptor and enhancing extraction of tissue cholesterol and other exchangeable material with co-administration of an effective amount of a compound, said compound selected from the group consisting of a small acceptor of cholesterol, an amphipathic compound, and a drug that increases endogenous small acceptors of cholesterol.
76. The method in accordance with claim 75 in which said small acceptor is selected from the group consisting of a high-density lipoprotein, a phospholipid protein complex having a group selected from the group consisting of apoA-I, apoA-II, apoA-IV, apoE, synthetic fragments thereof, natural fragments thereof, an amphipathic protein, and an amphipathic peptide, said protein substantially free of phospholipid, small phospholipid liposomes, and a small cholesterol acceptor; said drug including an agent that raises physiologic HDL concentrations, said agent selected from the group consisting of nicotinic acid, ethanol, a fibric acid, a cholesterol synthesis inhibitor, a drug that increases HDL concentrations, and derivatives thereof.
77. The method in accordance with claim 75 further comprising the step of periodically assaying plasma LDL concentrations with an assay to assess said plasma LDL.
78. The method in accordance with claim 77 in which said assay is selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma,

a precipitation assay of plasma, an electrophoretic assay, an electron microscopic assay, and a immuno-turbidometric assay of plasma.

79. The method in accordance with claim 75 in which said co-administration of said compound with the administration of said large liposomes is simultaneous.
80. The method in accordance with claim 75 in which said co-administration of said compound is separated in time from said parenteral administration of said therapeutically effective amount of a multiplicity of said large liposomes by an effective time period.
81. The method in accordance with claim 80 in which said separation in time is in the range of about 1 minute to about two weeks.
82. The method in accordance with claim 70 in which the large liposomes are of a size and shape larger than fenestrations of an endothelial layer lining hepatic sinusoids or in which said large liposomes optionally having other properties that prevent said large liposomes from penetrating said fenestrations, whereby said large liposomes have properties to prevent the ready penetration of said fenestrations.
83. The method in accordance with claim 70 in which the large liposomes are selected from the group consisting of uni-lamellar liposomes and multi-lamellar liposomes.

84. The method in accordance with claim 70 in which the liposomes have diameters larger than about 50 nm.
85. The method in accordance with claim 70 in which the liposomes have diameters larger than about 80 nm.
86. The method in accordance with claim 70 in which the liposomes have diameters larger than about 100 nm.
87. A method of controlling hepatic expression of a gene while forcing the reverse transport of cholesterol from peripheral tissues to the liver, comprising the step of:  
parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period, said effective amount administered in a dosage, said dosage selected from a single dose and repeated doses.
88. A method of controlling plasma LDL levels, hepatic cholesterol homeostasis, arterial enzymes, platelet function, and hepatic gene expression, comprising the step of:  
parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period, said effective amount administered in a dosage, said dosage selected from a single dose and repeated doses.

89. The method in accordance with claim 88 further comprising the step of diagnosing the efficacy of said administration by taking a measurement of enzyme activity, said measurement selected from the group consisting of a measurement of an arterial lipase activity, a measurement of arterial triglyceride lipase activity, a cholesterol esterase activity, a measurement of lysophospholipase activity, a measurement of an arterial lipid esterase activity, a measurement of arterial ACAT activity, a measurement of endothelial-derived relaxing factor, a measurement of intracellular calcium concentration in arterial cells, a measurement of arterial cell proliferation, an assay of arterial enzymes, an assay in the presence of calcium channel blockers, a measurement of nitric oxide synthesis, an assay of arterial uptake of liposomes, an assay of arterial uptake, accumulation and retention of lipoproteins, an assay of arterial retention of liposomes, an assessment of platelet function, an assessment of arterial function, an assessment of gene function, and a measurement of an effect of a biologically active substrate of said enzyme.
90. The method in accordance with claim 89 in which the substrate is lysophosphatidylcholine.
91. The method in accordance with claim 53 in which said atherogenic lipoprotein is selected from the group consisting of LDL, VLDL, IDL, Lp(a),  $\beta$ -VLDL and a lipoprotein that comprises apolipoprotein B.
92. The method in accordance with claim 41 in which said parenchymal cell functions in a system having hepatic sinusoids, an endothelial layer lining said hepatic

sinusoids, and fenestrations; and, in which the large liposomes have properties that provide for the slow and steady delivery of cholesterol to the liver, and reduce protein uptake.

93. The method in accordance with claim 70 further comprising the steps of periodically assaying a CETP property, said property selected from the group consisting of plasma CETP concentration and CETP activity and adjusting said effective amount in response thereto.
94. The method in accordance with claim 70 in which an atherogenic lipoprotein selected from the group consisting of LDL, VLDL, IDL, Lp(a),  $\beta$ -VLDL and a lipoprotein that comprises apolipoprotein B, is controlled.
95. The method in accordance with claim 70 further comprising the steps of periodically assaying a property, said property selected from the group consisting of concentration, composition, and characteristic of atherogenic lipoproteins in plasma with an assay during said treatment period to assess said plasma atherogenic lipoprotein and to obtain a atherogenic lipoprotein profile, and adjusting said parenteral administration in response to said profile.
96. The method in accordance with claim 75 further comprising the steps of periodically assaying a CETP property, said property selected from the group consisting of plasma CETP concentration and CETP activity and adjusting said effective amount in response thereto.

97. The method in accordance with claim 75 in which an atherogenic lipoprotein selected from the group consisting of LDL, VLDL, IDL, Lp(a),  $\beta$ -VLDL and a lipoprotein that comprises apolipoprotein B, is controlled.
98. The method in accordance with claim 75 further comprising the steps of periodically assaying a property, said property selected from the group consisting of concentration, composition, and characteristic of atherogenic lipoproteins in plasma with an assay during said treatment period to assess said plasma atherogenic lipoprotein and to obtain a atherogenic lipoprotein profile, and adjusting said parenteral administration in response to said profile.
99. The method in accordance with claim 63 in which said gene is selected from the group consisting of genes controlling the hepatic expression of 7-alpha hydroxylase, hepatic expression of an LDL receptor, hepatic expression of HMG Co A reductase, and derivatives thereof.
100. A method of forcing the reverse transport of cholesterol from peripheral tissues to the liver *in vivo* while controlling plasma LDL concentrations comprising the step of:  
parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period whereby said liposomes pick-up said cholesterol during said treatment period.

101. The method in accordance with claim 100 further comprising the step of periodically assaying plasma LDL concentrations with an assay during said treatment period to assess said plasma LDL concentrations and obtain an LDL profile, and adjusting said parenteral administration in response to said LDL profile.
102. The method in accordance with claim 101 in which said assay is selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma, a precipitation assay of plasma, an immuno-turbidometric assay of plasma, an electrophoretic assay, an electron microscopic assay, a function assay, a compositional assay, and a structural assay.
103. The method in accordance with claim 100 in which the large liposomes are of a size and shape larger than fenestrations of an endothelial layer lining hepatic sinusoids in said liver,  
whereby said liposomes are too large to readily penetrate said fenestrations.
104. The method in accordance with claim 100 in which the therapeutically effective amount is in the range of about 10 mg to about 1600 mg phospholipid per kg body weight per dose.
105. The method in accordance with claim 100 in which the liposomes are given periodically during said treatment period.

106. The method in accordance with claim 100 in which the large liposomes are selected from the group consisting of uni-lamellar liposomes and multi-lamellar liposomes.
107. The method in accordance with claim 100 in which the liposomes have diameters larger than about 50 nm.
108. The method in accordance with claim 100 in which the liposomes have diameters larger than about 80 nm.
109. The method in accordance with claim 100 in which the liposomes have diameters larger than about 100 nm.
110. The method in accordance with claim 100 in which parenteral administration is selected from the group of intravenous administration, intra-arterial administration, intramuscular administration, subcutaneous administration, transdermal administration, intraperitoneal administration, intrathecal administration, via lymphatics, intravascular administration, including administration into capillaries and arteriovenous shunts, rectal administration, administration via a chronically indwelling catheter, and administration via an acutely placed catheter.
111. The method in accordance with claim 100 further comprising the step of enhancing tissue penetration of a cholesterol acceptor and increasing extraction of tissue cholesterol and other exchangeable material by co-administration of an effective

amount of a compound, said compound selected from the group consisting of a small acceptor of cholesterol and a drug that increases endogenous small acceptors of cholesterol.

112. The method in accordance with claim 111 in which said small acceptor is selected from the group consisting of a high-density lipoprotein, a phospholipid protein complex having a group selected from a group consisting of apoA-I, apoA-II, apoA-IV, apoE, synthetic fragments thereof, natural fragments thereof, an amphipathic compound, including amphipathic compounds that are not a protein, an amphipathic protein, and an amphipathic peptide, said protein substantially free of phospholipid, small phospholipid liposomes, and a small cholesterol acceptor; said drug including an agent that raises physiologic HDL concentrations, said agent selected from the group consisting of nicotinic acid, ethanol, a fibric acid, a cholesterol synthesis inhibitor, a drug that increases HDL concentrations, and derivatives thereof.

114. A method of beneficially altering arterial function, blood platelet function, and controlling plasma LDL concentrations and hepatic cholesterol homeostasis in a warm blooded mammal comprising the step of:

parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period with or without administration of other agents, said other agents optionally including small acceptors and LDL lowering agents.

115. The method in accordance with claim 114 further comprising the step of taking a measurement of arterial function, said measurement selected from the group consisting of a measurement of endothelial-derived relaxing factor, a measurement of intracellular calcium concentration in arterial cells, a measurement of arterial cell proliferation, an assay of arterial enzymes, an assay in the presence of calcium channel blockers, an assay of arterial uptake, accumulation and retention of lipoproteins, an assay of arterial accumulation of liposomes, an assay of arterial retention of liposomes, an assay of gene products, and an assay of arterial cell functions.
116. A method of beneficially altering blood platelet function while controlling plasma LDL concentrations, arterial function, hepatic cholesterol homeostasis and said platelet function in a mammal comprising the step of:  
parenterally administering an effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period, said liposomes administered with or without other agents.
117. The method in accordance with claim 116 further comprising the step of taking a measurement of platelet function, said measurement selected from the group consisting of a measurement of a ratio of cholesterol to phospholipid in said platelets, a measurement of platelet reactivity, a measurement of platelet metabolic markers, a measurement of platelet calcium fluxes, a measurement of intracellular calcium, a measurement of platelet aggregability, a measurement of platelet granule release, and a measurement of platelet hormone synthesis and release.

118. A method of forcing the reverse transport of cholesterol from peripheral tissues to the liver in a warm blooded mammal and delivering said cholesterol to said liver while controlling plasma LDL concentrations, comprising the step of:
- delivering the cholesterol to the liver at a sufficiently slow rate so that hepatic cholesterol homeostasis is free of substantial disruption by administration of an agent, said agent selected from the group consisting of large liposomes and small acceptors to a subject.
119. The method in accordance with claim 118 in which the large liposomes are chemical compositions of liposomes of a size, function or composition so that said liposomes are cleared slowly by the liver.
120. The method in accordance with claim 119 in which the step of delivering comprises slowly infusing said liposomes.
121. The method in accordance with claim 118 in which the step of delivering comprises administering small doses of said liposomes, separated in time, to avoid increasing said LDL concentration.
122. The method in accordance with claim 118 further comprising the step of periodically assaying said plasma LDL concentrations with an assay to obtain an assayed LDL concentration, said assay selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma, and a precipitation assay having

a component, said component selected from the group consisting of polyanions, divalent cations, and antibodies, an ultracentrifugal assay of plasma, a precipitation assay, a immuno-turbidometric assay, and an electrophoretic assay to determine the level of a therapeutically effective amount of each of said liposomes.

123. A method of forcing the reverse transport of cholesterol from peripheral tissues to the liver *in vivo* and delivering said cholesterol primarily to hepatic Kupffer cells rather than hepatic parenchymal cells so that hepatic cholesterol homeostasis is not harmfully disrupted and controlling said homeostasis and effecting a plasma component, comprising the step of:

administering an effective amount of liposomes, said liposomes selected from the group of liposomes being substantially incapable of penetrating endothelial fenestrae of said liver and incapable of substantially interacting with hepatic parenchymal cells and said liposomes being of a size, composition or shape so that said liposomes are directed away from said hepatic parenchymal cells.

124. The method in accordance with claim 123 further comprising the step of periodically monitoring said plasma component with an assay, said assay selected from the group consisting of an assay for plasma unesterified cholesterol and phospholipid, an assay of bile acids and cholesterol in stool, an assay of bile acids and cholesterol in bile, an assay of hepatic gene expression in a liver biopsy, an assay of hepatic gene expression in peripheral blood leukocytes, said gene comprising a gene involved in cholesterol metabolism, an assay of plasma LDL concentration, and a vascular imaging technique.

125. A method of forcing the reverse transport of cholesterol from a body part, said body part selected from the group of organs, peripheral tissues, cells, and platelets *in vivo*, and modifying the lipid composition of membranes, said cells, tissues, organs, and other extracellular structures, comprising the step of:  
administering for a treatment period a therapeutically effective amount of a multiplicity of non-liposomal particles for cholesterol depletion of peripheral tissues while avoiding harmful disruptions of hepatic cholesterol homeostasis, said particles being selected from the group of a particle substantially free of cholesterol and particles free of cholesterol.
126. The method in accordance with claim 125 in which said non-liposomal particles are selected from the group consisting of triglyceride-phospholipid emulsions, said emulsions selected from the group of emulsions that are not taken up rapidly by hepatic parenchymal cells, emulsions that are not taken up to a large extent by parenchymal cells, triglyceride-phospholipid-protein emulsions, and emulsions taken up slowly by the liver.
127. A method of controlling plasma LDL levels *in vivo* and lowering blood viscosity, comprising the step of:  
parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids a substantially free of sterol for a treatment period, said effective amount administered in a dosage, said dosage selected from a single dose and repeated doses.

128. The method in accordance with claim 127 further comprising the steps of taking a measurement, said measurement selected from the group consisting of a measurement of blood flow in a carotid artery, measurement of blood flow in a coronary artery, a measurement of blood flow in a lower limb, an ultrasound measurement of blood flow in other vessels, an MRI measurement of blood flow, a radioisotope tracer measurement of blood flow, and a measurement of blood viscosity.
129. A method of controlling plasma LDL levels *in vivo* and reducing the sphingomyelin to phosphatidylcholine ratio in a cell membrane and cell aging, comprising the step of:
- parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period, said effective amount administered in a dosage, said dosage selected from a single dose and repeated doses.
130. The method in accordance with claim 129 further comprising the step of periodically assaying said cells with an assay, said assay selected from the group consisting of an assay of sphingomyelin, an assay of phosphatidylcholine, an assay of membrane function, and an assay of cellular function.
131. A method of controlling hepatic secretion of apolipoprotein-B while forcing the reverse transport of cholesterol from peripheral tissues to the liver, comprising the step of:

parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period, said effective amount administered in a dosage, said dosage selected from a single dose and repeated doses.

132. A method of controlling plasma LDL levels and hepatic cholesterol homeostasis and gene expression and blocking uptake of atherogenic lipoproteins by cells of the arterial wall of a subject, comprising the step of:

parenterally administering a therapeutically effective amount of a multiplicity of liposomes, said liposomes selected from the group consisting of large liposomes comprised of phospholipids substantially free of sterol and small acceptors for a treatment period, said therapeutic amount given in a dosage, said dosage selected from a single dose and repeated doses.

133. The method in accordance with claim 132 further comprising the step of diagnosing the efficacy of said administration by performing an assay, said assay selected from the group consisting of an assay of arterial uptake and retention of lipoproteins, an assay of uptake of lipoproteins of said subject by cells in culture, said lipoproteins disposed in whole plasma, whole blood, and plasma fractions from said subject, an assay of arterial function, an assay of oxidized lipid in an artery or plasma, an assay of oxidized lipids, and an assay of lesion size.

134. A method of controlling plasma LDL levels, hepatic cholesterol homeostasis, and hepatic gene expression *in vivo* while altering plasma HDL, comprising the step of:

parenterally administering a therapeutically effective amount of a multiplicity of liposomes, said liposomes selected from the group consisting essentially of large liposomes comprised of phospholipids substantially free of sterol and acceptors for a treatment period, said effective amount administered in a dosage, said dosage selected from a single dose and repeated doses.

135. The method in accordance with claim 134 in which said small acceptors are selected from the group consisting of a small acceptor of cholesterol, an acceptor of sphingomyelin, an acceptor of lysophosphatidylcholine, an acceptor of a protein, and an acceptor of a lipid.

136. The method in accordance with claim 134 further comprising the step of diagnosing the efficacy of said administration by performing an assay, said assay selected from the group consisting of an assay of HDL, an assay of HDL unesterified cholesterol, an assay of HDL cholesteryl ester, an assay of HDL phospholipid, an assay of HDL protein, an assay of protein species, an assay of HDL size, a functional assay of the ability of HDL to extract cholesterol from cells, and a functional assay of the ability of HDL to alter cell membrane composition, and a functional assay of whole plasma to determine the ability of said whole plasma to extract cholesterol from cells *in vitro*.

137. A method of suppressing the rise in plasma concentrations of atherogenic lipoproteins after administration of an agent having small acceptors of cholesterol, other lipids or compounds in a warm blooded mammal comprising the step of:
- co-administering an effective amount of a multiplicity of an agent having large liposomes comprised of phospholipids substantially free of sterol with said administration of said agent having said small acceptors.
139. The method in accordance with claim 137 in which said atherogenic lipoproteins are selected from the group consisting of LDL, VLDL, IDL,  $\beta$ -VLDL, Lp(a), a lipoprotein containing apolipoprotein-B, oxidized lipoproteins, and modified lipoproteins.
140. The method in accordance with claim 137 in which said agent having small acceptors consists essentially of small acceptors and in which said agent having large liposomes consists essentially of large liposomes.
141. The method in accordance with claim 137 in which said co-administration of said agent having large liposomes is simultaneous with said administration of said agent having small acceptors.
142. The method in accordance with claim 137 in which said co-administration of said agent having large liposomes is separated in time from said administration of said agent having small acceptors by an effective time period.

143. The method in accordance with claim 142 in which said effective time period is in the range of about 1 minute to about two weeks.
144. The method in accordance with claim 137 in which atherogenic lipoprotein concentrations are controlled.
145. The method in accordance with claim 144 in which the atherogenic lipoprotein concentrations are optionally controlled through adjusting a dosage, size or composition of liposomes.
146. An improved method of dialysis in which said improvement comprises the step of:  
  
administering a therapeutically effective amount of an agent, said agent selected from the group consisting of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol, small acceptors, an acceptor of cholesterol, and an acceptor of lipid, during operation of a dialysis device.
147. The method in accordance with claim 146 in which said dialysis is selected from the group consisting of hemodialysis, peritoneal dialysis, and rectal dialysis.
148. The method in accordance with claim 147 in which said agent is added directly to blood or blood plasma.

149. The method in accordance with claim 146 in which said administration of said agent is selected from the group consisting of extracorporeal administration and intracorporeal administration.
150. The method in accordance with claim 146 in which said agent is added directly to dialysate of said patient, said dialysate optionally including extracorporeal dialysate, intraperitoneal dialysate, and intrarectal dialysate.
151. The method in accordance with claim 146 in which plasma component concentrations and properties are periodically assayed using an assay, said components selected from the group consisting of LDL, HDL, unesterified cholesterol, phospholipid, IDL, VLDL, Lp(a),  $\beta$ VLDL, liposome acceptors, apolipoprotein-B, and cholesteryl ester.
152. The method in accordance with claim 151 further comprising the step of administering LDL lowering agents or optionally adjusting a liposome dosage or liposome size where LDL or other atherogenic lipoproteins are found to be at inappropriate levels.
153. The method in accordance with claim 151 further comprising the step of administering HDL raising agents where HDL or other anti-atherogenic lipoproteins are found not to be at appropriate levels in response to results of said assay.

154. The method in accordance with claim 146 in which said patient has cells, and further comprising the steps of treating said patient's cells, together or after separation of said cells into erythrocytes, leukocytes, and platelets, extracorporeally with liposomes, and periodically assaying a component, said component selected from the group consisting of cellular cholesterol, phospholipid, fluidity, fragility, gene expression, hormone secretion, an ion flux, and cell function.
155. The method in accordance with claim 146 in which said amount is adjusted where said cellular fragility increases, or in accordance with an altered cell function or property.
156. The method in accordance with claim 150 in which said dialysate is assayed for cholesterol or assayed for other exchangeable material to determine the effectiveness of said treatment.
157. The method in accordance with claim 146 said amount is regulated as a function of the rate of removal of cholesterol or other exchangeable material.
158. The method in accordance with claim 146 in which said acceptor is an acceptor of sphingomyelin.
159. The method in accordance with claim 146 in which said acceptor of lipid is selected from the group consisting of an acceptor of an oxidized lipid, an emulsion, and protein-phospholipid complex.

160. The method in accordance with claim 146 further comprising the step of minimizing a rise in plasma LDL levels.
161. An improved method of dialysis treatment of a patient in which said improvement comprises the step of admixing a dialysate with a therapeutically effective amount of an agent, said agent selected from the group consisting of a multiplicity of small liposomes so that said small liposomes do not substantially enter the circulation of a subject in large amounts.
162. The method in accordance with claim 161 in which said small liposomes are admixed so as to minimize a rise in plasma LDL or other atherogenic proteins.
163. The method in accordance with claim 146 in which plasma component concentrations and properties are periodically assayed using an assay, said assay being selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma, a precipitation assay of plasma, and an immuno-turbidometric assay of plasma.
164. The method in accordance with claim 163 in which the large liposomes are of a size and shape larger than fenestrations of an endothelial layer lining hepatic sinusoids in a liver,  
whereby said liposomes are too large to readily penetrate said fenestrations.

165. The method in accordance with claim 146 in which the therapeutically effective amount is in the range of about 10 mg to about 1600 mg phospholipid per kg body weight per dose.
166. The method in accordance with claim 146 in which the large liposomes are given periodically during said treatment period.
167. The method in accordance with claim 146 in which the large liposomes are selected from the group consisting of uni-lamellar liposomes and multi-lamellar liposomes.
168. The method in accordance with claim 146 in which the liposomes have diameters larger than about 50 nm.
169. The method in accordance with claim 146 in which the liposomes have diameters larger than about 80 nm.
170. The method in accordance with claim 146 in which the liposomes have diameters larger than about 100 nm.
171. A method of regulating hepatic parenchymal cell cholesterol content, said cell having at least one gene selected from the group consisting of a gene for an LDL receptor, a gene for HMG-CoA reductase, a gene for cholesterol 7-alpha-hydroxylase,

and a gene regulating a function involved in cholesterol homeostasis; and, homeostasis thereof, comprising the step of:

administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol during a treatment period.

172. The method in accordance with claim 171 further comprising the steps of periodically assaying a property, said property selected from the group consisting of concentration, composition, and characteristic of atherogenic lipoproteins in plasma with an assay during said treatment period to assess said plasma atherogenic lipoprotein and to obtain a atherogenic lipoprotein profile, and adjusting said parenteral administration in response to said profile.

173. The method in accordance with claim 172 in which said assay is selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma, a precipitation assay of plasma, an electrophoretic assay, an electron microscopic assay, an immuno-turbidometric assay, and an assay of lipoprotein composition, size or function.

174. The method in accordance with claim 171 further comprising the step of enhancing tissue penetration of a cholesterol acceptor and enhancing extraction of tissue cholesterol and other exchangeable material with co-administration of an effective amount of a compound, said compound selected from the group consisting

of a small acceptor of cholesterol, an amphipathic compound, and a drug that increases endogenous small acceptors of cholesterol.

175. The method in accordance with claim 174 in which said small acceptor is selected from the group consisting of a high-density lipoprotein, a phospholipid protein complex having a group selected from the group consisting of apoA-I, apoA-II, apoA-IV, apoE, synthetic fragments thereof, natural fragments thereof, an amphipathic protein, and an amphipathic peptide, said protein substantially free of phospholipid, small phospholipid liposomes, and a small cholesterol acceptor; said drug including an agent that raises endogenous HDL concentrations, said agent selected from the group consisting of nicotinic acid, ethanol, a fibric acid, a cholesterol synthesis inhibitor, a drug that increases HDL concentrations, and derivatives thereof.
176. The method in accordance with claim 174 in which said co-administration of said compound is simultaneous with said administration of said large liposomes.
177. The method in accordance with claim 174 in which said co-administration of said compound is separated in time from said parenteral administration of said therapeutically effective amount of a multiplicity of said large liposomes by an effective time period.
178. The method in accordance with claim 177 in which said effective time period is in the range of about 1 minute to about two weeks.

179. The method in accordance with claim 171 in which the therapeutically effective amount is in the range of about 10 to about 1600 mg/kg/dose.
180. The method in accordance with claim 171 in which the liposomes are given in repeated doses.
181. The method in accordance with claim 171 further comprising the step of controlling the expression of said genes by said parenteral administration of a multiplicity of said large liposomes.
182. The method in accordance with claim 171 in which said parenchymal cell functions in a system having hepatic sinusoids, an endothelial layer lining said hepatic sinusoids, and fenestrations; and, in which the large liposomes are of a size and shape larger than said fenestrations of said endothelial layer lining said hepatic sinusoids or in which said large liposomes optionally having other properties that prevent said large liposomes from penetrating said fenestrations, whereby said large liposomes have properties to prevent the ready penetration of said fenestrations.
183. The method in accordance with claim 171 in which the large liposomes are selected from the group consisting of uni-lamellar liposomes and multi-lamellar liposomes.
184. The method in accordance with claim 171 in which the liposomes have diameters larger than about 50 nm.

185. The method in accordance with claim 171 in which the liposomes have diameters larger than about 80 nm.
186. The method in accordance with claim 171 in which the liposomes have diameters larger than about 100 nm.
187. The method in accordance with claim 171 in which administration is selected from the group of intravenous administration, intra-arterial administration, intramuscular administration, subcutaneous administration, transdermal administration, intrathecal administration, via lymphatics, intravascular administration, rectal administration, via a chronically indwelling catheter or other device, via an acutely placed catheter or other device, and intraperitoneal administration.
188. A method of suppressing hepatic expression of a cholesterol ester transfer protein gene in a warm blooded mammal comprising the step of:
- administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids, said phospholipids selected from the group of phospholipids substantially free of sterol and phospholipids containing sterol, for an effective period of time,
- whereby plasma LDL and HDL are controlled as a result of said administration.
189. The method in accordance with claim 188 further comprising the step of periodically assaying plasma LDL concentrations with an assay during said treatment period to assess said plasma LDL.

190. The method in accordance with claim 189 in which said assay is selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma, a precipitation assay of plasma, an electrophoretic assay, an electron microscopic assay, an immuno-turbidometric assay of plasma, and an assay of lipoprotein composition, size or function.
191. The method in accordance with claim 188 in which the liposomes are given periodically.
192. The method in accordance with claim 188 in which the therapeutically effective amount is in the range of about 10 mg/kg/dose to about 1600 mg/kg/dose.
193. The method in accordance with claim 188 further comprising the step of enhancing tissue penetration of a cholesterol acceptor and enhancing extraction of tissue cholesterol and other exchangeable material with co-administration of an effective amount of a compound, said compound selected from the group consisting of a small acceptor of cholesterol, an amphipathic compound, and a drug that increases endogenous small acceptors of cholesterol.
194. The method in accordance with claim 193 in which said small acceptor is selected from the group consisting of a high-density lipoprotein, a phospholipid protein complex having a group selected from the group consisting of apoA-I, apoA-II, apoA-IV, apoE, synthetic fragments thereof, natural fragments thereof, an amphipathic

protein, and an amphipathic peptide, said protein substantially free of phospholipid, small phospholipid liposomes, and a small cholesterol acceptor; said drug including an agent that raises physiologic HDL concentrations, said agent selected from the group consisting of nicotinic acid, ethanol, a fibric acid, a cholesterol synthesis inhibitor, a drug that increases HDL concentrations, and derivatives thereof.

195. The method in accordance with claim 193 further comprising the step of periodically assaying plasma LDL concentrations with an assay during said treatment period to assess said plasma LDL.
196. The method in accordance with claim 195 in which said assay is selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of plasma, a precipitation assay of plasma, an electrophoretic assay, an electron microscopic assay, and an immuno-turbidometric assay of plasma.
197. The method in accordance with claim 193 in which said co-administration of said compound with the administration of said large liposomes is simultaneous.
198. The method in accordance with claim 193 in which said co-administration of said compound is separated in time from said parenteral administration of said therapeutically effective amount of a multiplicity of said large liposomes by an effective time period.

199. The method in accordance with claim 198 in which said separation in time is in the range of about 1 minute to about two weeks.
200. The method in accordance with claim 188 in which the large liposomes are of a size and shape larger than fenestrations of an endothelial layer lining hepatic sinusoids or in which said large liposomes optionally having other properties that prevent said large liposomes from penetrating said fenestrations, whereby said large liposomes have properties to prevent the ready penetration of said fenestrations.
201. The method in accordance with claim 188 in which the large liposomes are selected from the group consisting of uni-lamellar liposomes and multi-lamellar liposomes.
202. The method in accordance with claim 188 in which the liposomes have diameters larger than about 50 nm.
203. The method in accordance with claim 188 in which the liposomes have diameters larger than about 80 nm.
204. The method in accordance with claim 188 in which the liposomes have diameters larger than about 100 nm.

205. A method of controlling hepatic expression of a gene while forcing the reverse transport of cholesterol from peripheral tissues to the liver in a warm blooded mammal, comprising the step of:

parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period, said effective amount administered in a dosage, said dosage selected from a single dose and repeated doses.

206. A method of controlling plasma LDL levels, hepatic cholesterol homeostasis, arterial enzymes, platelet function, and hepatic gene expression in an animal, comprising the step of:

parenterally administering a therapeutically effective amount of a multiplicity of large liposomes comprised of phospholipids substantially free of sterol for a treatment period, said effective amount administered in a dosage, said dosage selected from a single dose and repeated doses.

207. The method in accordance with claim 206 further comprising the step of diagnosing the efficacy of said administration by taking a measurement of enzyme activity, said measurement selected from the group consisting of a measurement of an arterial lipase activity, a measurement of arterial triglyceride lipase activity, a cholesterol esterase activity, a measurement of lysophospholipase activity, a measurement of an arterial lipid esterase activity, a measurement of arterial ACAT activity, a measurement of endothelial-derived relaxing factor, a measurement of intracellular calcium concentration in arterial cells, a measurement of arterial cell

proliferation, an assay of arterial enzymes, an assay in the presence of calcium channel blockers, a measurement of nitric oxide synthesis, an assay of arterial uptake of liposomes, an assay of arterial uptake, accumulation and retention of lipoproteins, an assay of arterial retention of liposomes, an assessment of platelet function, an assessment of arterial function, an assessment of gene function, and a measurement of an effect of a biologically active substrate of said enzyme.

208. The method in accordance with claim 207 in which the substrate is lysophosphatidylcholine.
209. A composition for controlling cholesterol related genes, enzymes and other compounds while forcing the reverse transport of lipids peripheral tissues to the liver consisting essentially of large liposomes sized and shaped to interact with molecules interacting with said genes, enzymes or other compounds, said large liposomes being substantially free of sterol.
210. The method in accordance with claim 206 in which said atherogenic lipoprotein is selected from the group consisting of LDL, VLDL, IDL, Lp(a),  $\beta$ -VLDL and a lipoprotein that comprises apolipoprotein B.
211. The method in accordance with claim 206 in which said parenchymal cell functions in a system having hepatic sinusoids, an endothelial layer lining said hepatic sinusoids, and fenestrations; and, in which the large liposomes have properties that

provide for the slow and steady delivery of cholesterol to the liver, and reduce protein uptake.

212. The method in accordance with claim 206 further comprising the steps of periodically assaying a CETP property, said property selected from the group consisting of plasma CETP concentration and CETP activity and adjusting said effective amount in response thereto.
213. The method in accordance with claim 206 in which an atherogenic lipoprotein selected from the group consisting of LDL, VLDL, IDL, Lp(a),  $\beta$ -VLDL and a lipoprotein that comprises apolipoprotein B, is controlled.
214. The method in accordance with claim 206 further comprising the steps of periodically assaying a property, said property selected from the group consisting of concentration, composition, and characteristic of atherogenic lipoproteins in plasma with an assay during said treatment period to assess said plasma atherogenic lipoprotein and to obtain a atherogenic lipoprotein profile, and adjusting said parenteral administration in response to said profile.
215. The method in accordance with claim 206 further comprising the steps of periodically assaying a CETP property, said property selected from the group consisting of plasma CETP concentration and CETP activity and adjusting said effective amount in response thereto.

216. The method in accordance with claim 215 in which an atherogenic lipoprotein selected from the group consisting of LDL, VLDL, IDL, Lp(a),  $\beta$ -VLDL and a lipoprotein that comprises apolipoprotein B, is controlled.
217. The method in accordance with claim 206 further comprising the steps of periodically assaying a property, said property selected from the group consisting of concentration, composition, and characteristic of atherogenic lipoproteins in plasma with an assay during said treatment period to assess said plasma atherogenic lipoprotein and to obtain a atherogenic lipoprotein profile, and adjusting said parenteral administration in response to said profile.
218. The method in accordance with claim 206 in which said gene is selected from the group consisting of genes controlling the hepatic expression of 7-alpha hydroxylase, hepatic expression of an LDL receptor, hepatic expression of HMG Co A reductase, and derivatives thereof.
220. A method of diagnosing a side-effect of reverse transport of cholesterol from peripheral tissues to the liver *in vivo* accompanying parenteral administration of a multiplicity of large liposomes and small liposomes during a treatment period, comprising the step of:
- periodically assaying plasma atherogenic lipoprotein concentrations with an assay to obtain an assayed atherogenic lipoprotein concentration, said assay selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B, a gel filtration assay of plasma, an ultracentrifugal assay of

plasma, and a precipitation assay having a component, said component selected from the group consisting of polyanions, divalent cations, and antibodies, an assay of triglyceride in plasma, and an immunoturbidometric assay of plasma, to determine the level of a therapeutically effective amount of each of said liposomes,

whereby a side effect of administration of said liposomes is diagnosed and effectively regulated.

221. The method in accordance with claim 220 in which said atherogenic lipoprotein is LDL.

222. The method in accordance with claim 220 further comprising the step of adding an emulsion and an agent to increase reverse transport of cholesterol from peripheral tissues to the liver.

223. The method in accordance with claim 220 in which said agent is selected from the group consisting of protein-phospholipid complexes and HDL.

224. A method of diagnosing and treating a side-effect of reverse transport of cholesterol from peripheral tissues to the liver in a warm blooded animal accompanying parenteral administration of a multiplicity of large liposomes and small liposomes during a treatment period, comprising the steps of:

periodically assaying plasma atherogenic protein concentrations with an assay to obtain an assayed atherogenic protein concentration, said assay selected from the group consisting of an assay of plasma esterified cholesterol, an assay of plasma apolipoprotein-B,

a gel filtration assay of plasma, an ultracentrifugal assay of plasma, and a precipitation assay having a component, said component selected from the group consisting of polyanions, divalent cations, and antibodies, an assay of triglyceride in plasma, an immunoturbidometric assay of plasma, to determine the level of a therapeutically effective amount of each of said liposomes, and an electrophoretic assay, and an electron microscopy assay; and, adjusting said therapeutically effective amount of each of said small and large liposomes in response to said assayed LDL concentration during said treatment period.

225. The method in accordance with claim 224 in which said atherogenic lipoprotein is LDL.

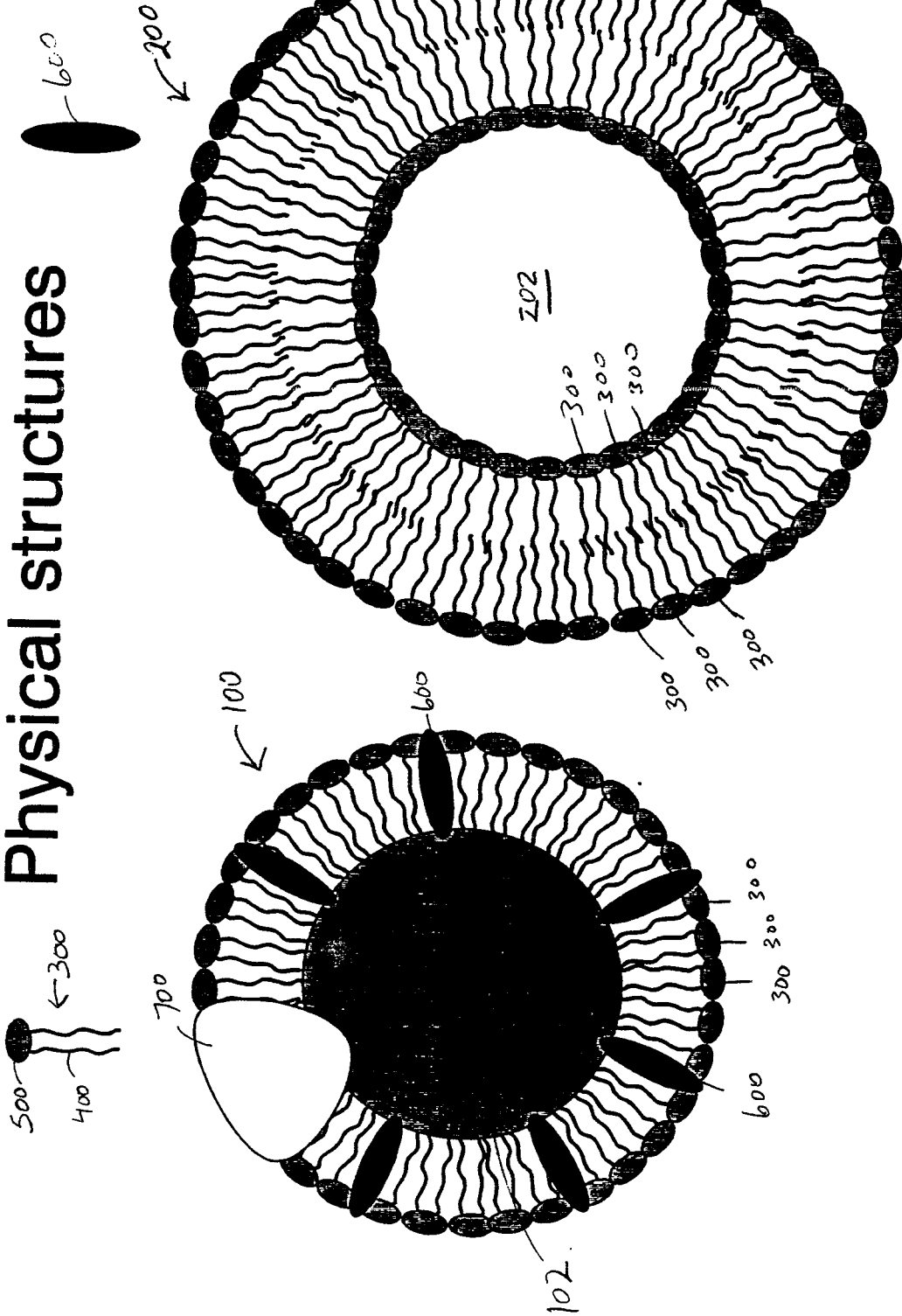
226. The method in accordance with claim 224 further comprising the steps of co-administering a pharmaceutical agent to lower said atherogenic protein concentration with said liposome administration, and adjusting the administration of said agent responsive to said assayed LDL concentration.

227. The method in accordance with claim 224 in which said atherogenic lipoprotein is LDL.

228. The method in accordance with claim 224 further comprising the step of adding an emulsion and an agent to increase reverse transport of cholesterol from peripheral tissues to the liver.

229. The method in accordance with claim 224 in which said agent is selected from the group consisting of protein-phospholipid complexes and HDL.

# Physical structures



Liposome

Lipoprotein

Fig. 1

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2/28

PCT/US96/16388

LUV-SUV #2		Hepatic mRNA content (pg/ug)										
Rabbit #	Treatment	HMG-CoA R			LDL R			7a-hydroxylase	LDL ChE, day 1	LDL ChE, day 3	LDL ChE, day 5	LDL ChE, day 6
		CETP										
1	(A) PBS	2.87	0.54		4.27		0.56	7.4	7.1		5.2	6.5
2	(A) PBS	5.63	0.55		5.38		0.39	16.1	11.8		6.2	9.7
3	(A) PBS	5.34	0.39		8.93		0.74	8.5	8.9		4.4	8.7
4	(A) PBS	5.04	0.55		5.49		0.82	14.1	14.1		8.8	8.6
	Mean	4.72	0.51		6.02		0.63	11.53	10.48		6.15	8.38
	SEM	0.63	0.04		1.01		0.10	2.12	1.55		0.96	0.67
5	(B) LUV	3.15	0.58		7.23		0.63	25.3	14.9		13.6	10.5
6	(B) LUV	3.02	0.47		8.15		0.58	14.0	15.9		10.8	8.2
7	(B) LUV	2.52	0.58		4.81		0.83	28.3	22.5		21.3	22.4
8	(B) LUV	2.68	0.58		7.37		0.94	17.5	21.8		13.4	9.5
	Mean	2.84	0.55		6.89		0.75	21.28	18.78		14.78	12.65
	SEM	0.15	0.03		0.72		0.08	3.33	1.96		2.27	3.28
	t vs. PBS	2.910	0.939		0.703		0.919	2.473	3.318		3.506	1.275
13	SUV + LUV	3.18	0.50		5.28		0.51	11.9	34.0		20.1	22.2
10	(C) SUV	5.64	0.38		3.98		0.30	21.1	45.3		15.3	46.3
11	(C) SUV	3.39	0.29		3.67		0.42	10.0	36.3		59.6	42.7
12	(C) SUV	3.00	0.13		3.34		0.63	17.8	31.8		45.5	22.3
	Mean w/o #3	4.01	0.27		3.68		0.45	16.30	37.80		40.13	37.10
	SEM w/o #3	0.82	0.07		0.18		0.10	3.29	3.97		13.07	7.47
	t vs. PBS	0.986	2.903		2.295		1.304	1.220	6.414		2.594	3.829
	t vs. LUV	1.397	3.660		4.328		2.301	1.063	4.296		1.912	2.995
	Mean w/ #3	3.80	0.33		4.07		0.47	15.20	36.85		35.13	33.38
	SEM w/ #3	0.62	0.08		0.42		0.07	2.57	2.96		10.51	6.46
	t vs. PBS	1.041	2.091		1.781		1.389	1.103	7.890		2.746	3.846
	t vs. LUV	1.512	2.753		3.369		2.554	1.445	6.085		1.893	2.858

Fig. 2

3/28

**Plasma LDL cholesteryl ester concentrations  
in response to injections of LUVs, SUVs, or saline**

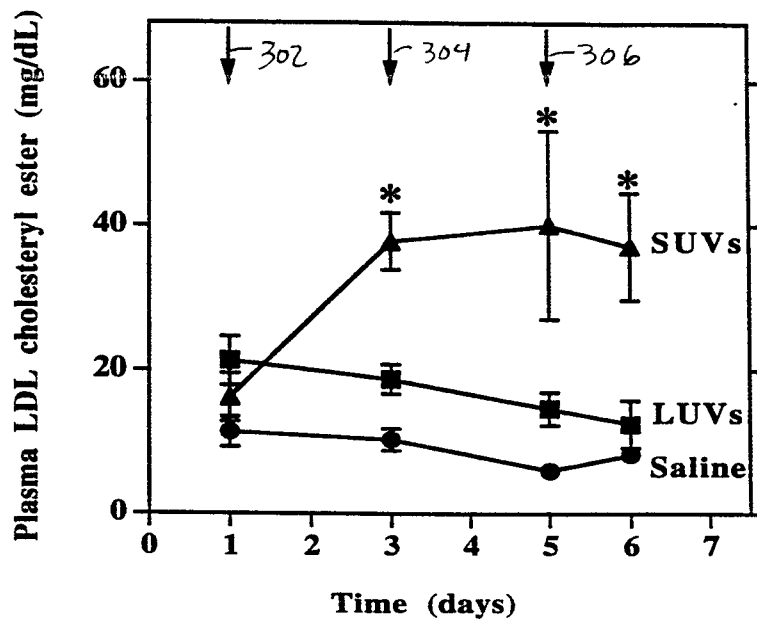
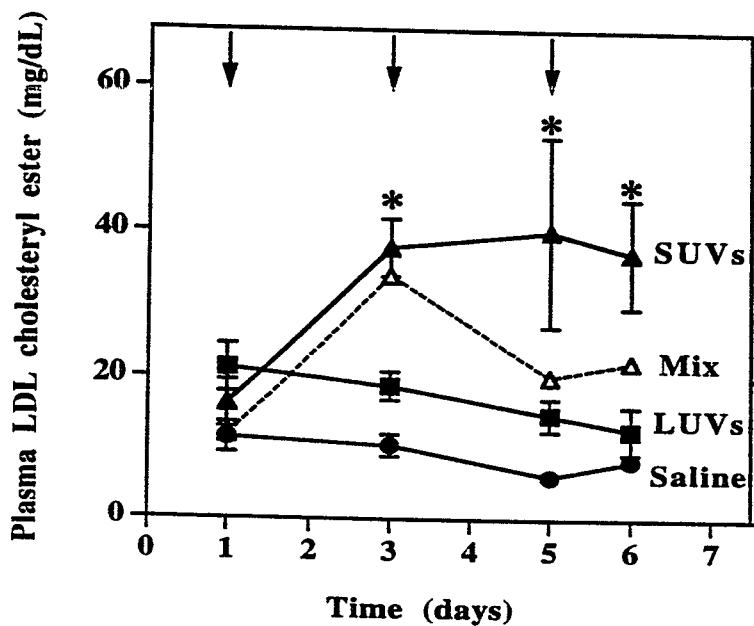


Fig. 3

**Plasma LDL cholesteryl ester concentrations  
in response to injections of LUVs, SUVs, or saline**



"Mix" received SUVs on days 1, 3, & 5,  
but also a dose of LUVs on day 3.  
All other animals received single injections  
on days 1, 3, & 5 (indicated by arrows).

**Fig. 4**

5/28

**LDL receptor mRNA levels in liver  
in response to injections of LUVs, SUVs, or saline**

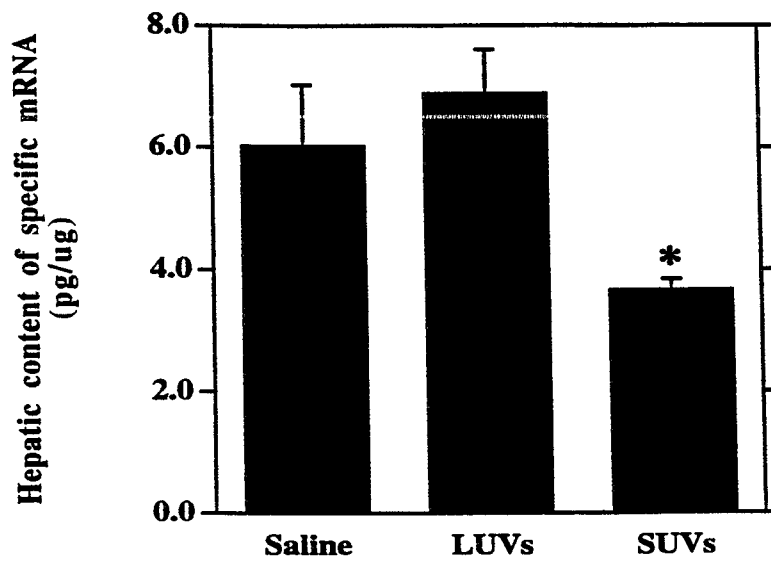


Fig. 5

6/28

**HMG-CoA reductase mRNA levels in liver  
in response to injections of LUVs, SUVs, or saline**

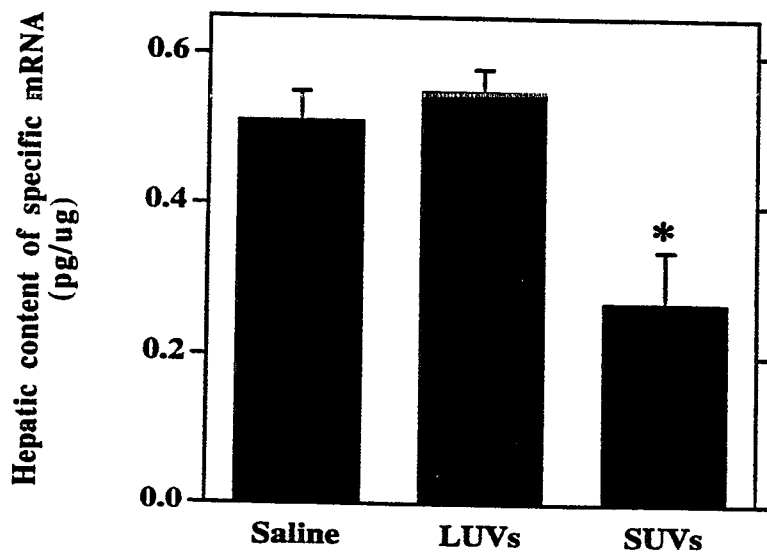


Fig. 6

7/28

**Cholesteryl ester transfer protein mRNA levels in liver  
in response to injections of LUVs, SUVs, or saline**

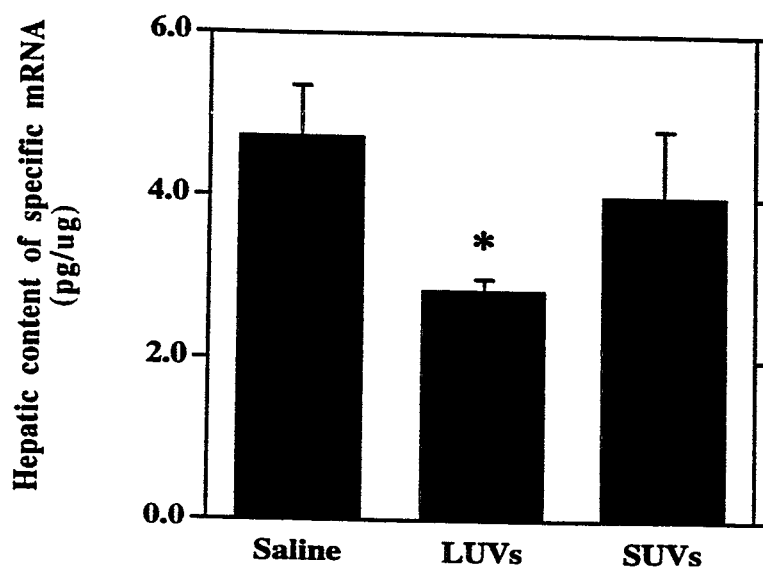
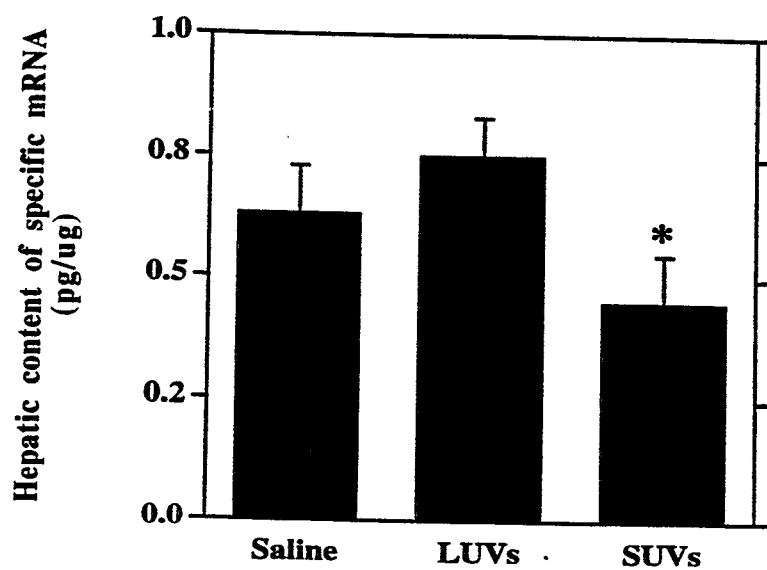


Fig. 7

8/28

**7-alpha hydroxylase mRNA levels in liver  
in response to injections of LUVs, SUVs, or saline**



\* Indicates column of interest

Fig. 8

9/28

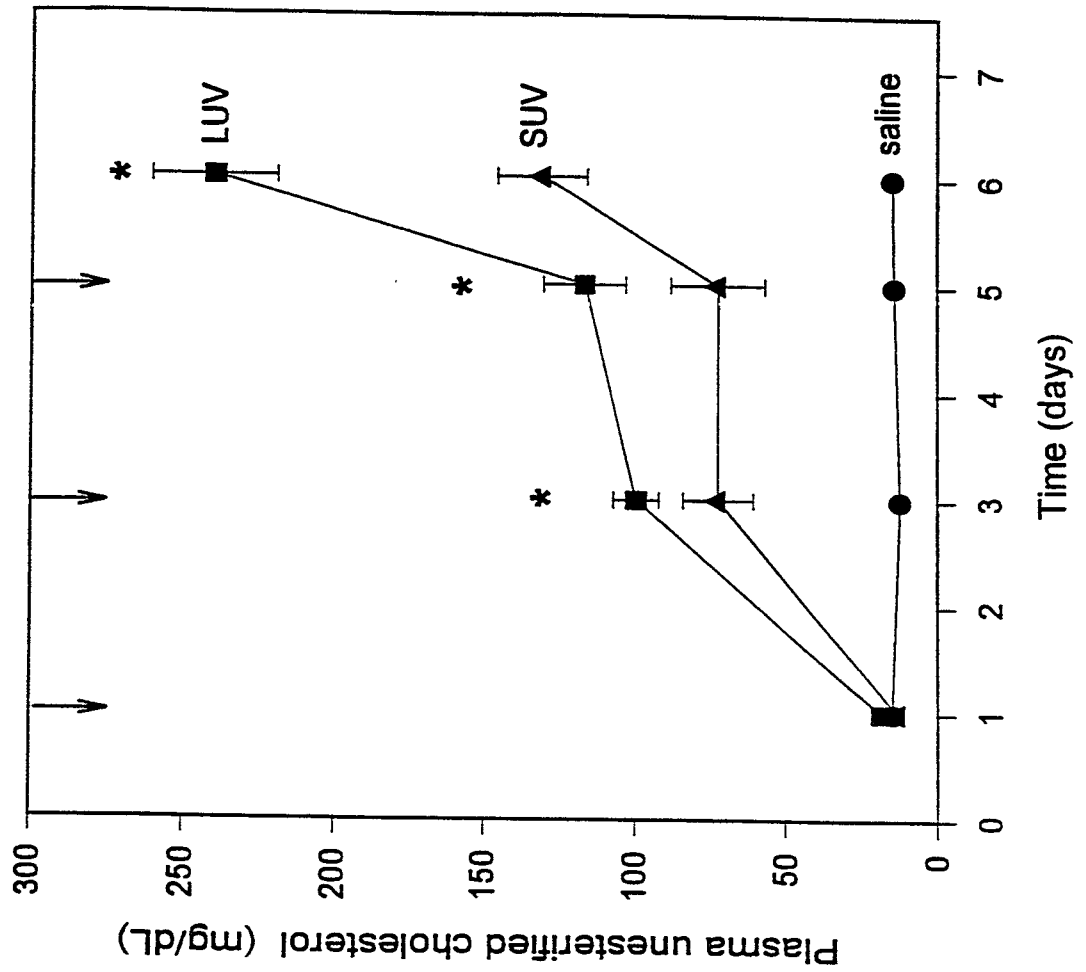
## **Key points about LUV and atherosclerosis**

- 1) Practical:**      **Straight forward to manufacture**  
**Non-toxic at very high doses**
- 2) Mechanistic:**      **Liposomes promote reverse cholesterol transport *in vivo***  
**LUV are the optimal preparation**

**Fig. 9**

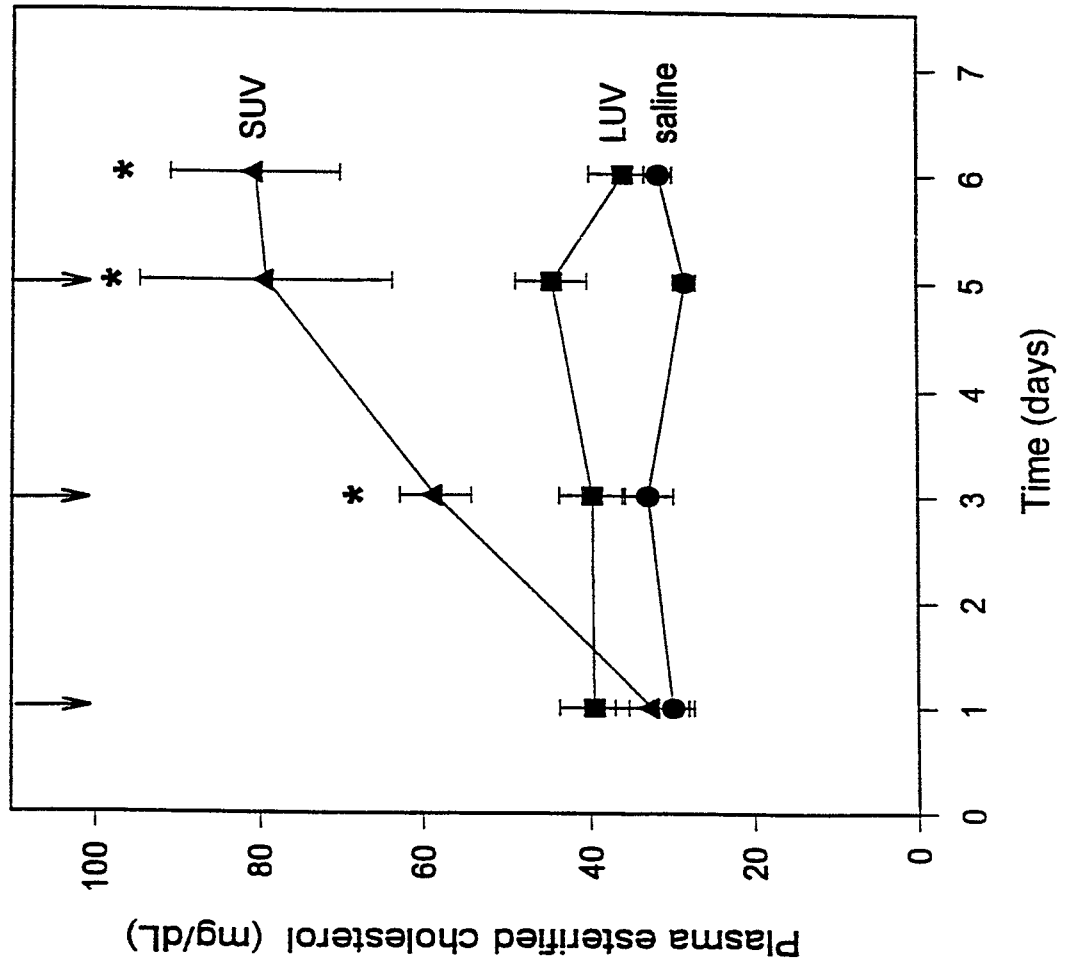
Fig. 10

Plasma unesterified cholesterol concentrations  
in response to injections of LUV, SUV, or saline



11/28

**Fig. 11**  
**Plasma esterified cholesterol concentrations**  
**in response to injections of LUV, SUV, or saline**

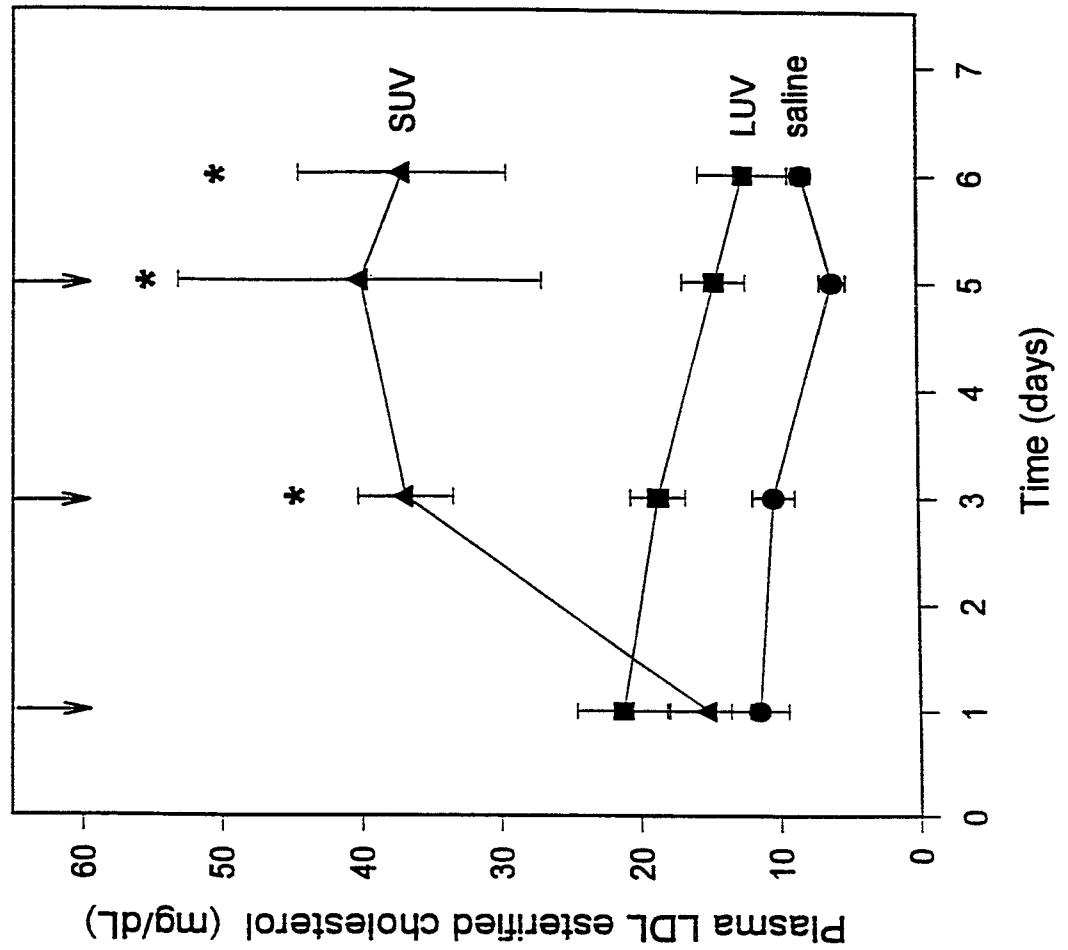


WO 97/13501

PCT/US96/16388

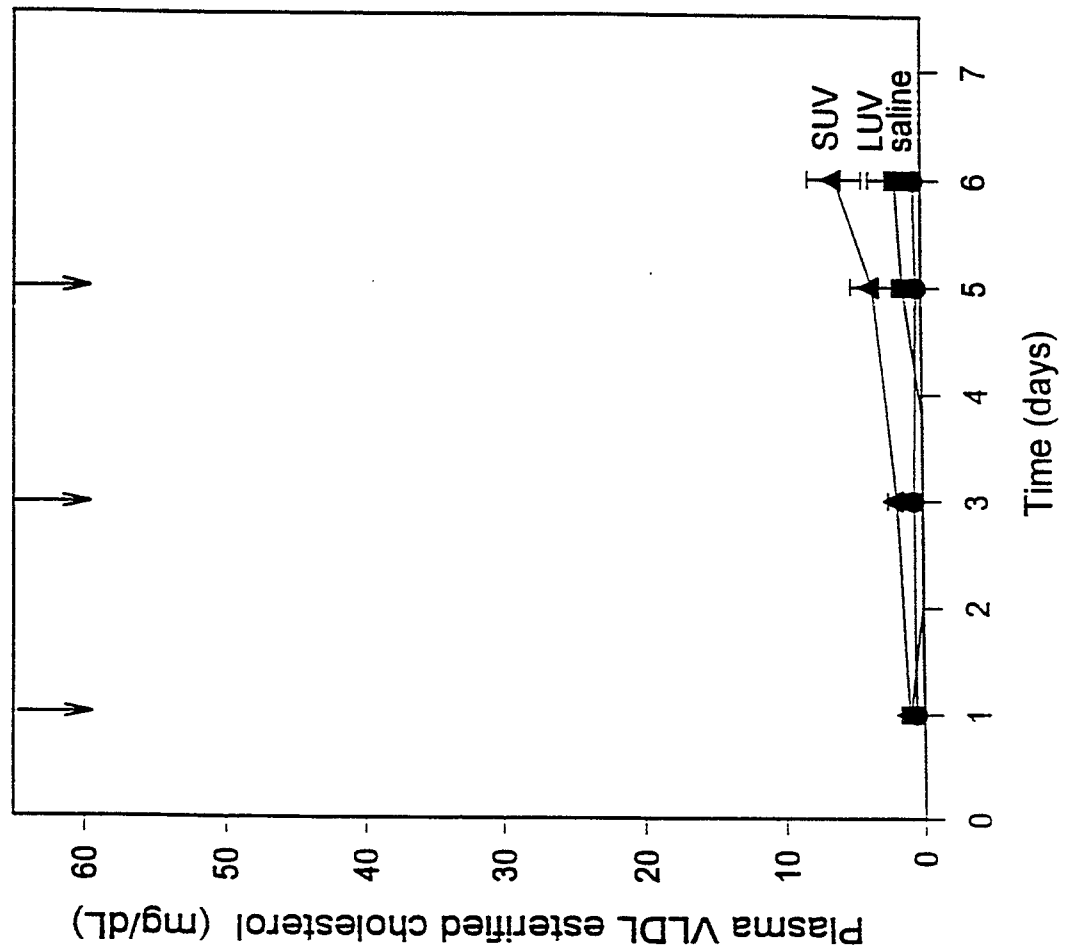
12/28

**Fig. 12**  
**LDL esterified cholesterol concentrations**  
**in response to injections of LUV, SUV, or saline**



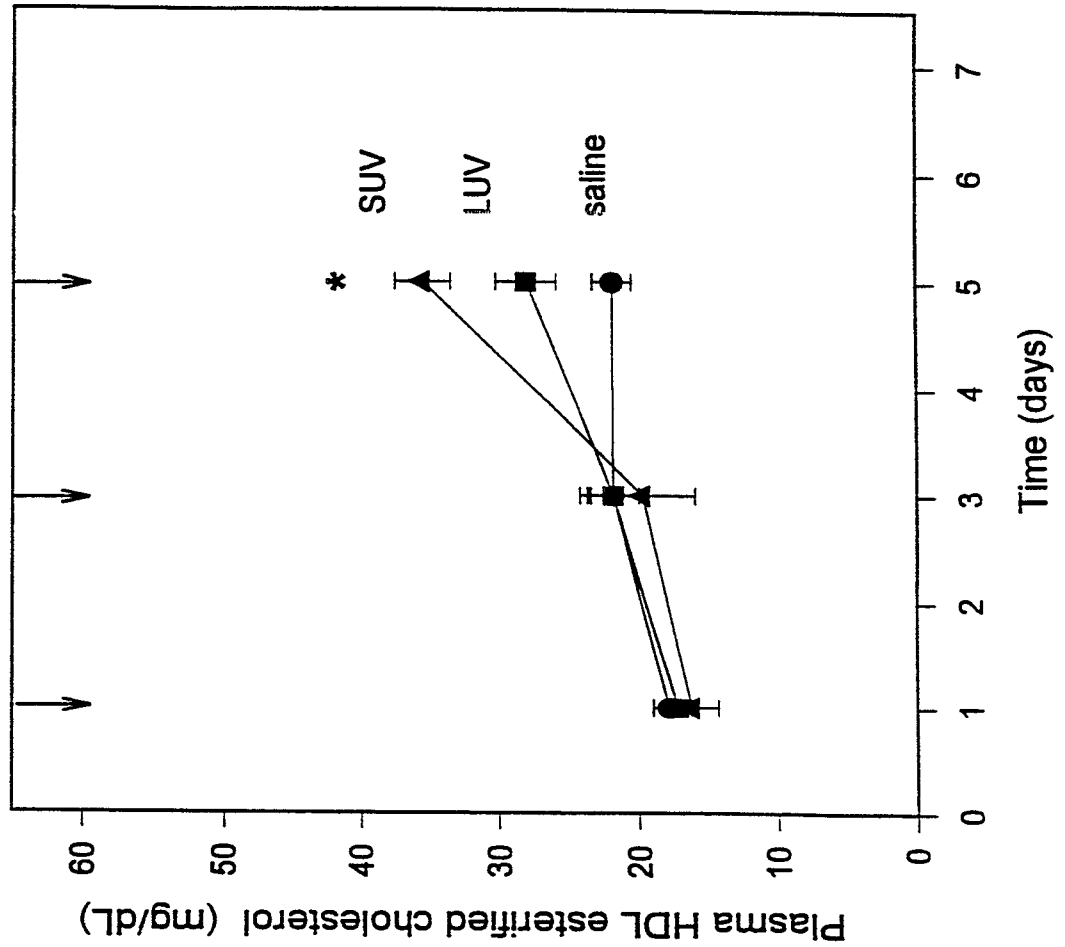
13/28

**Fig. 13**  
**VLDL esterified cholesterol concentrations**  
**in response to injections of LUV, SUV, or saline**



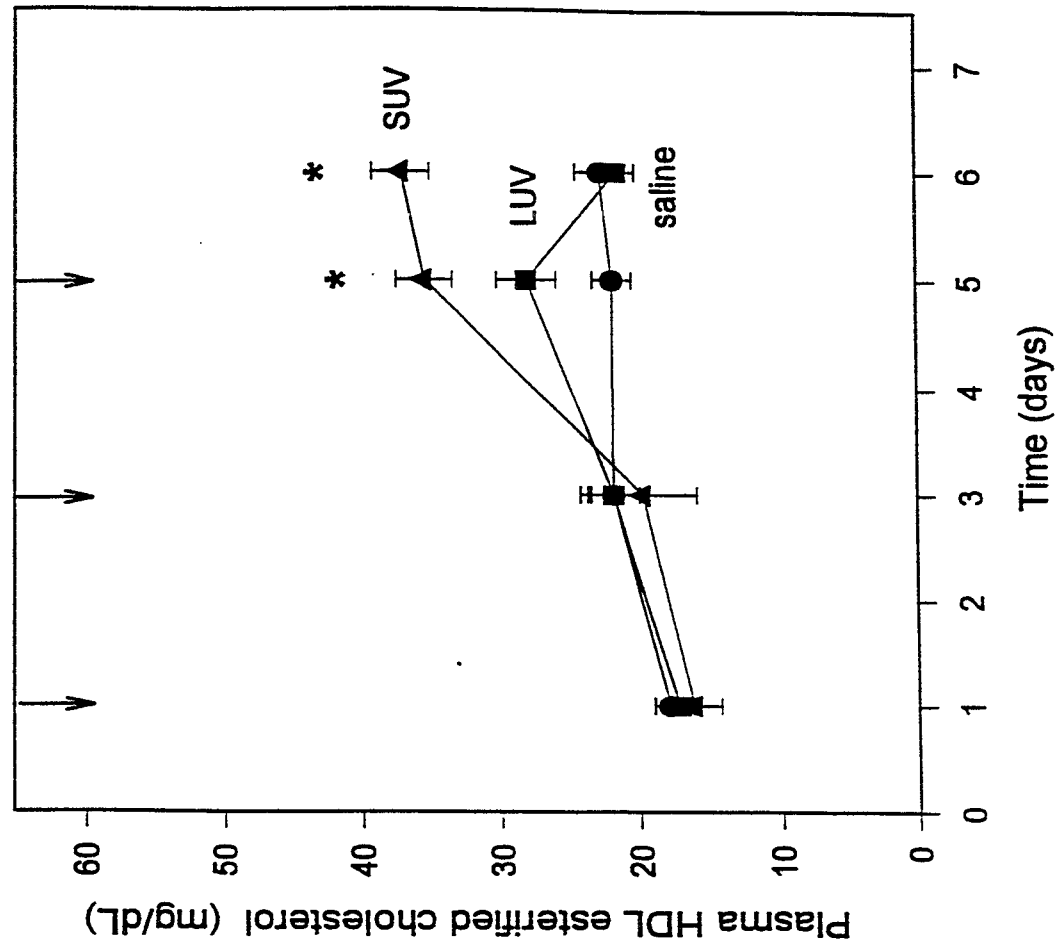
14/28

**Fig. 14**  
**HDL esterified cholesterol concentrations**  
**in response to injections of LUV, SUV, or saline**

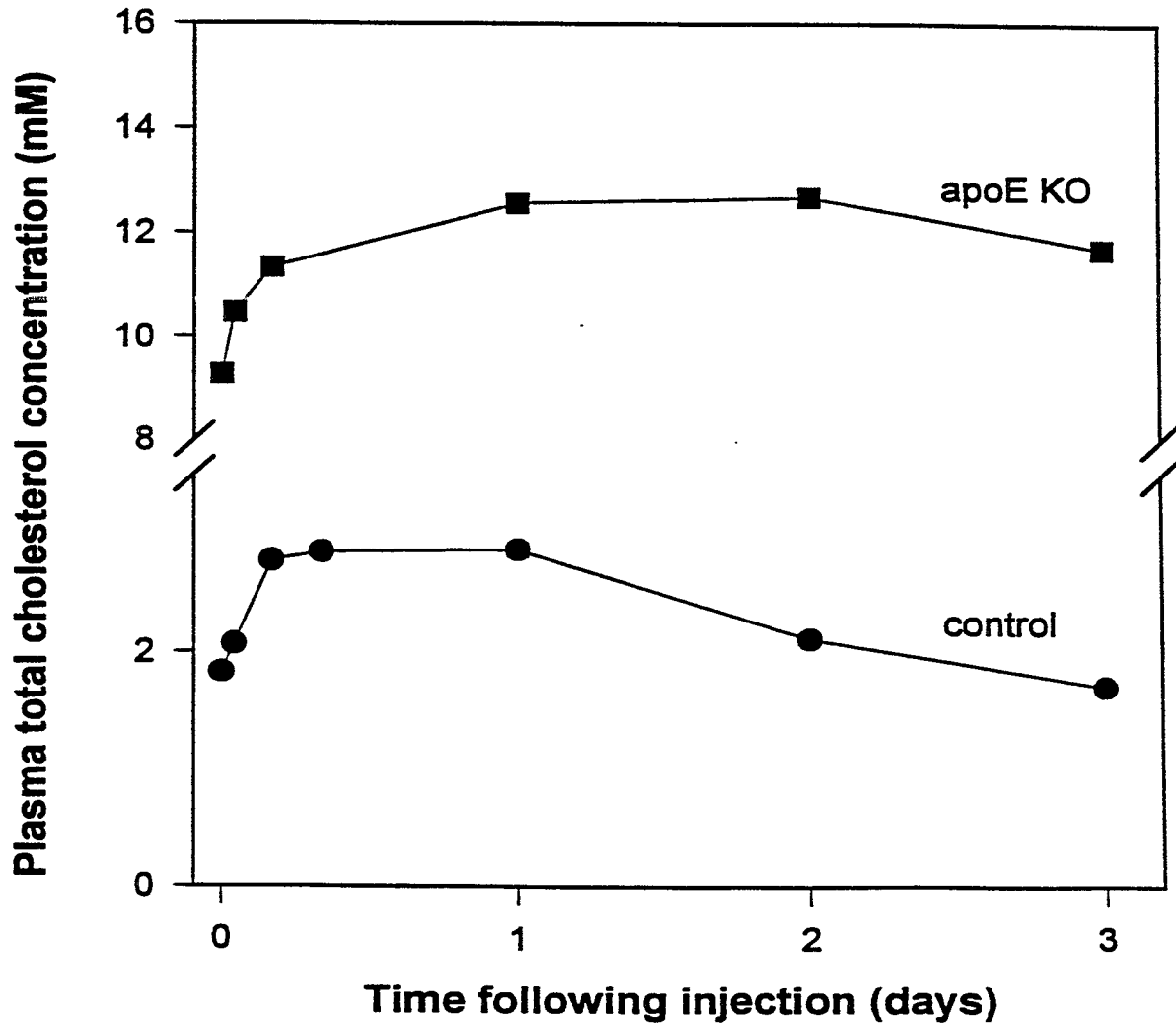


15/28

**Fig. 15**  
**HDL esterified cholesterol concentrations**  
**in response to injections of LUV, SUV, or saline**



**Time course of cholesterol mobilization following an LUV injection into control or apoE KO mice**



**Fig. 16**

### Time course of LUV clearance in control C57BL6 and ApoE KO mice

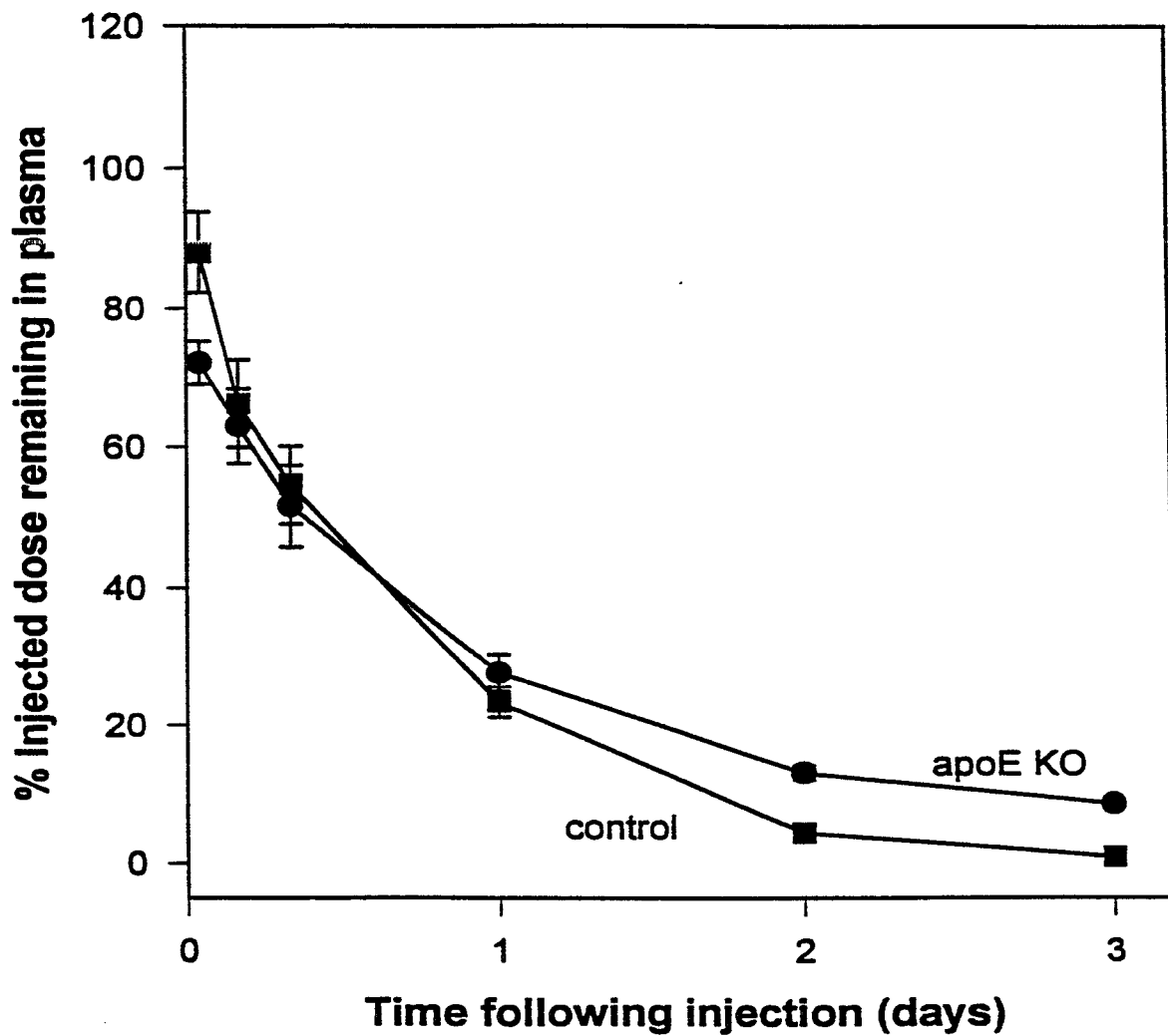


Fig. 17

18/28

- Effectiveness in humans

- Therapeutic targets

Lipid-rich, rupture-prone plaques  
Critical Stenosis  
Post-angioplasty re-stenosis  
Atherosclerosis in general

Fig. 18

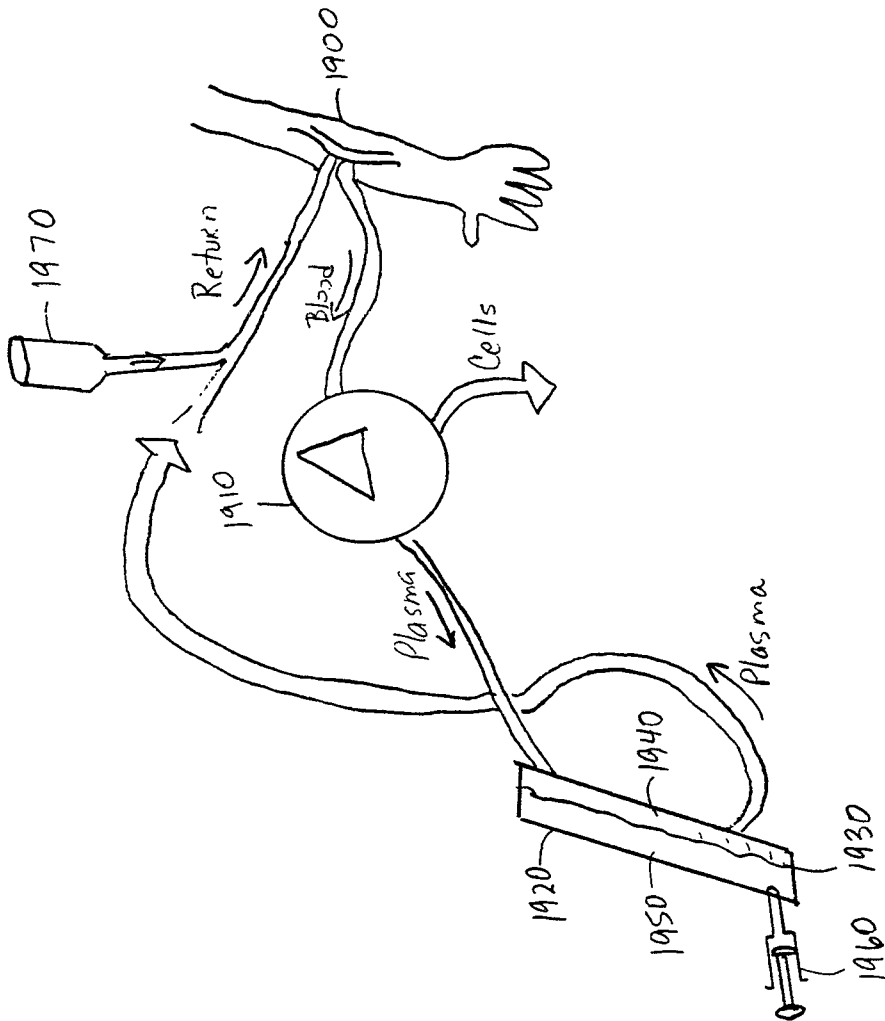


Fig. 19

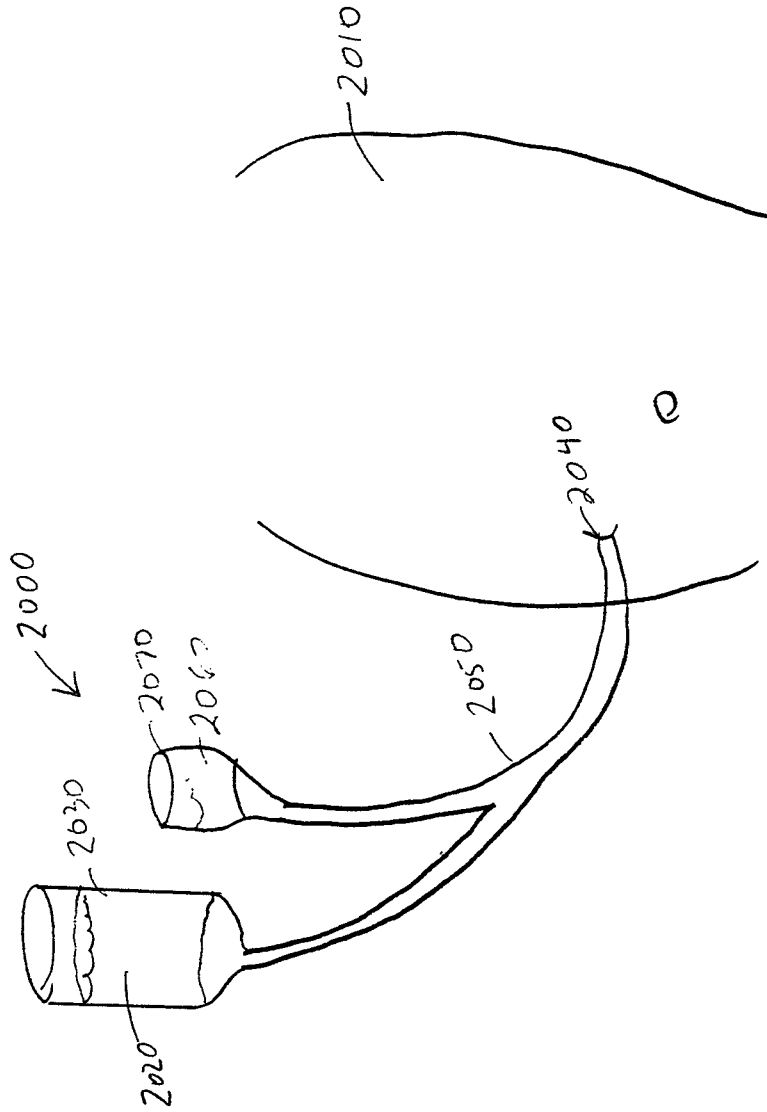


Fig. 20

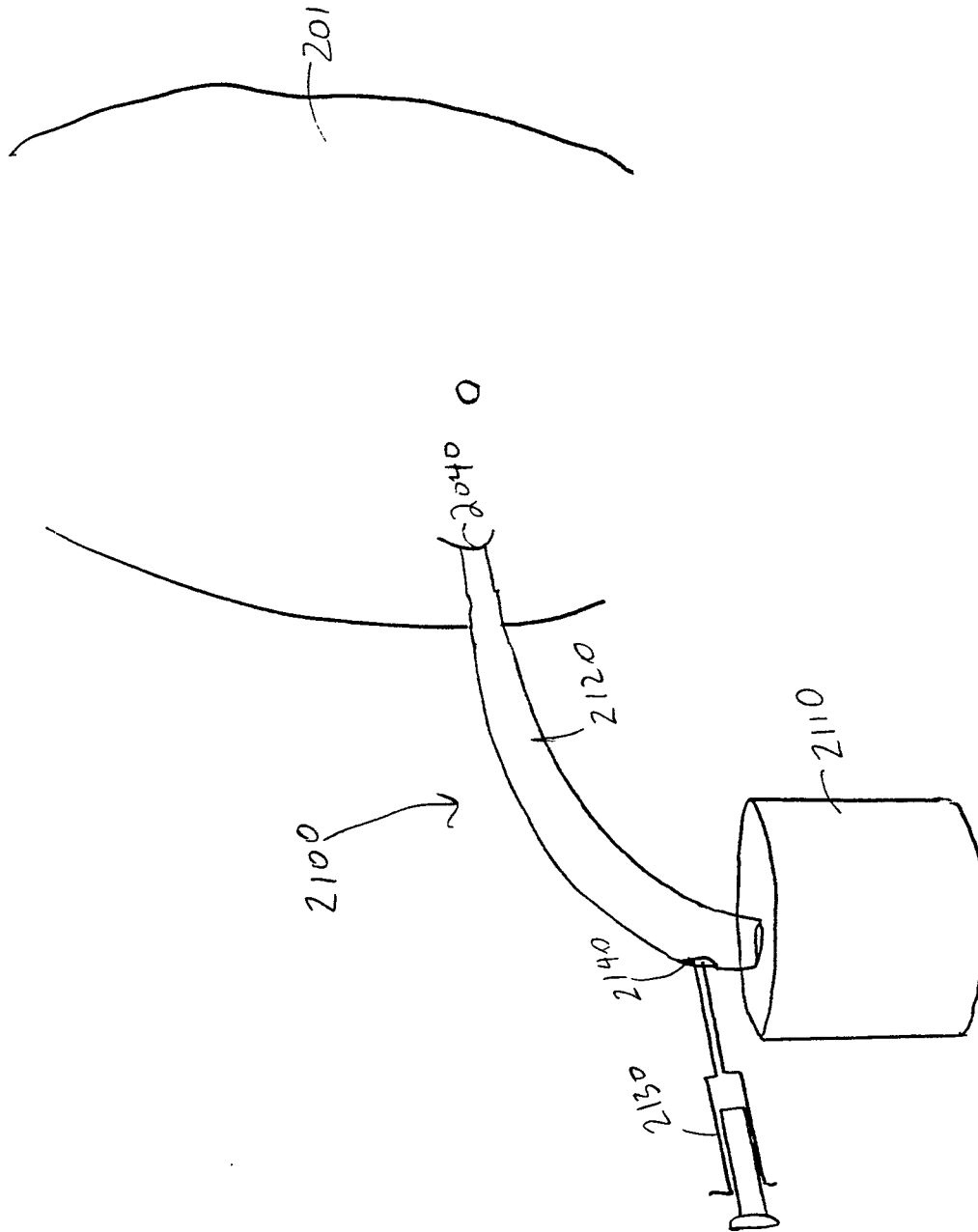


Fig. 21

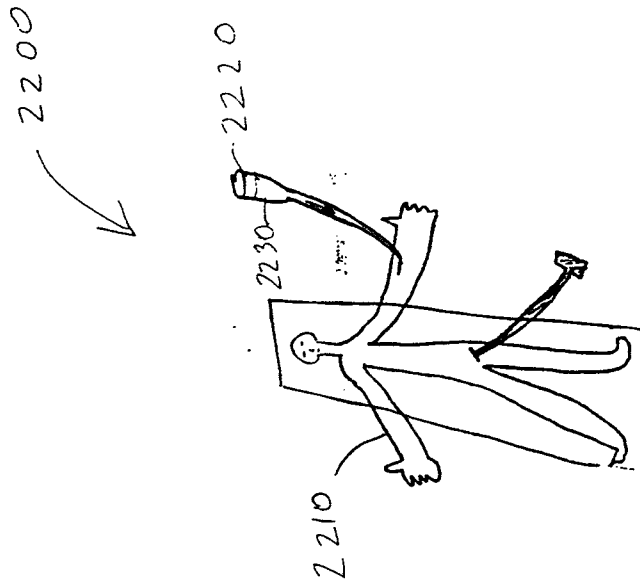


Fig. 22

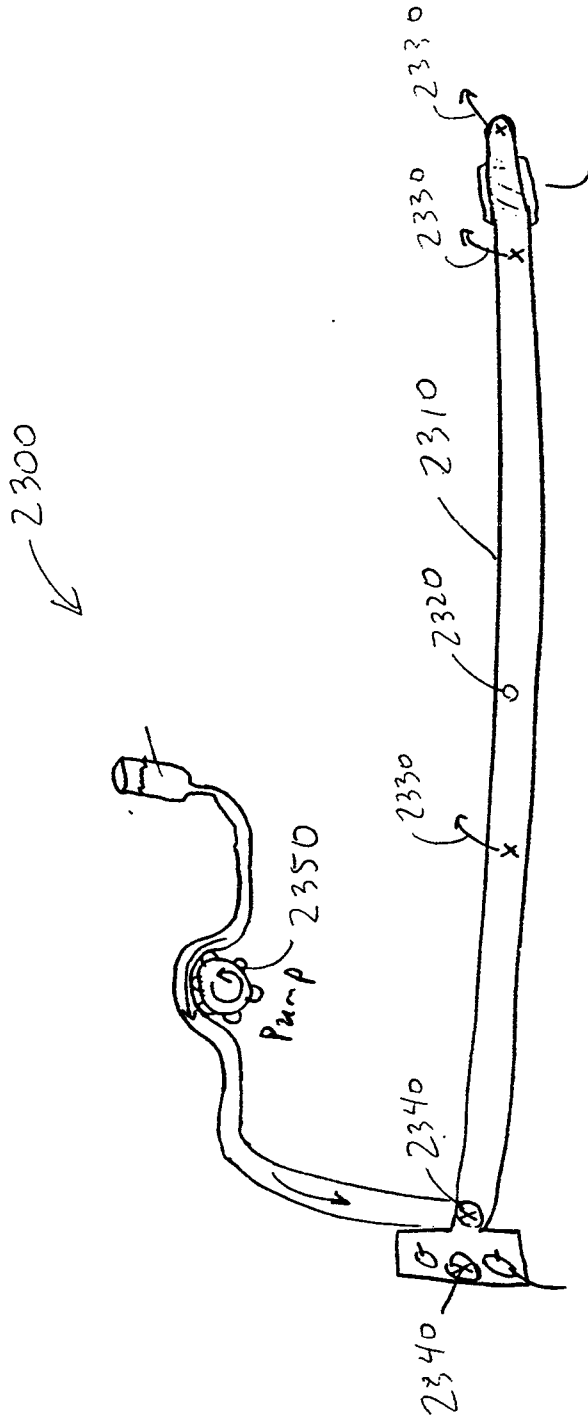


Fig. 23

WO 97/13501

24/28

PCT/US96/16388

**hepatic lipid contents in response to injections of  
LUVs, SUVs, or saline**

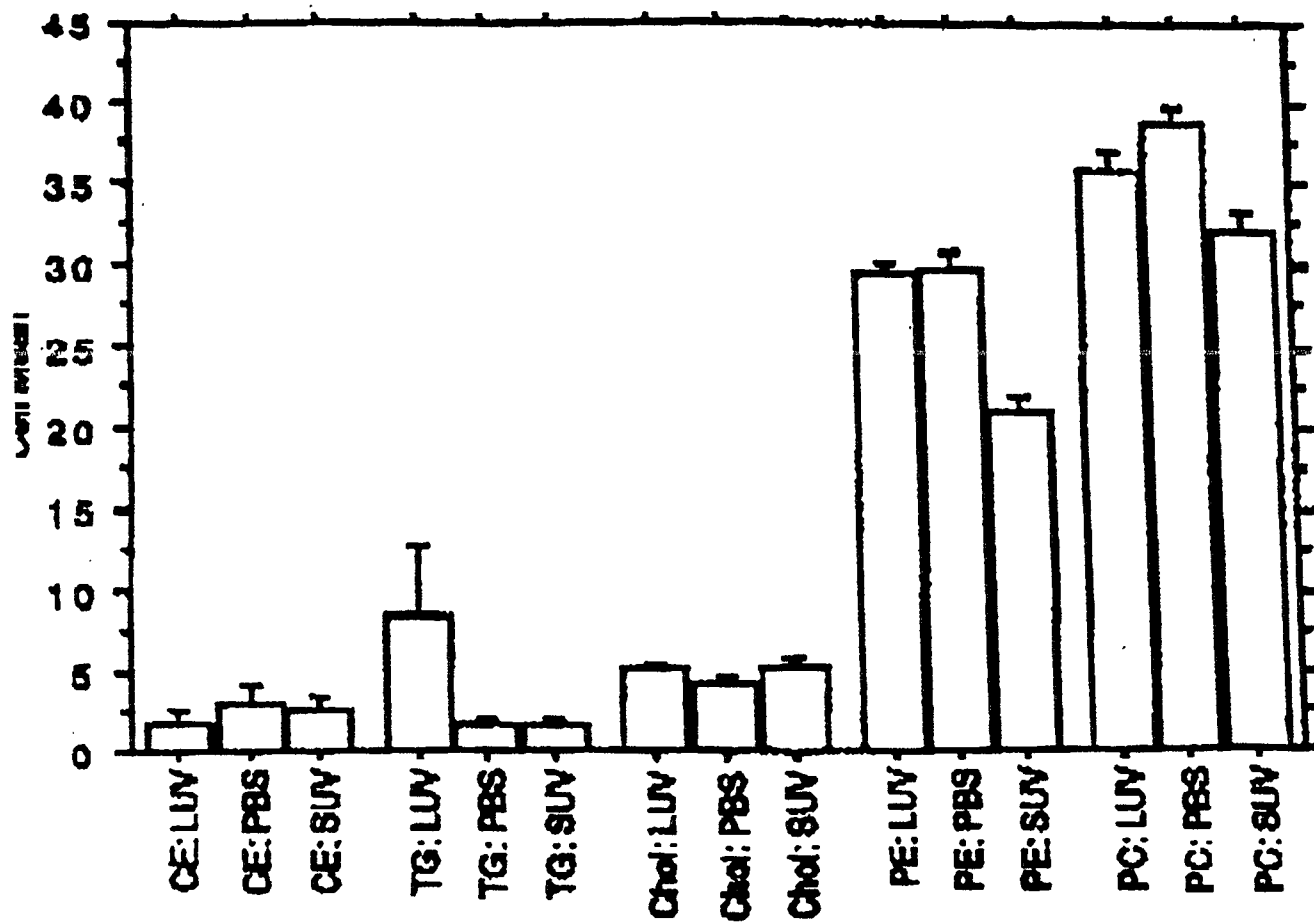


Fig. 24

25/28

# Plasma free cholesterol concentrations

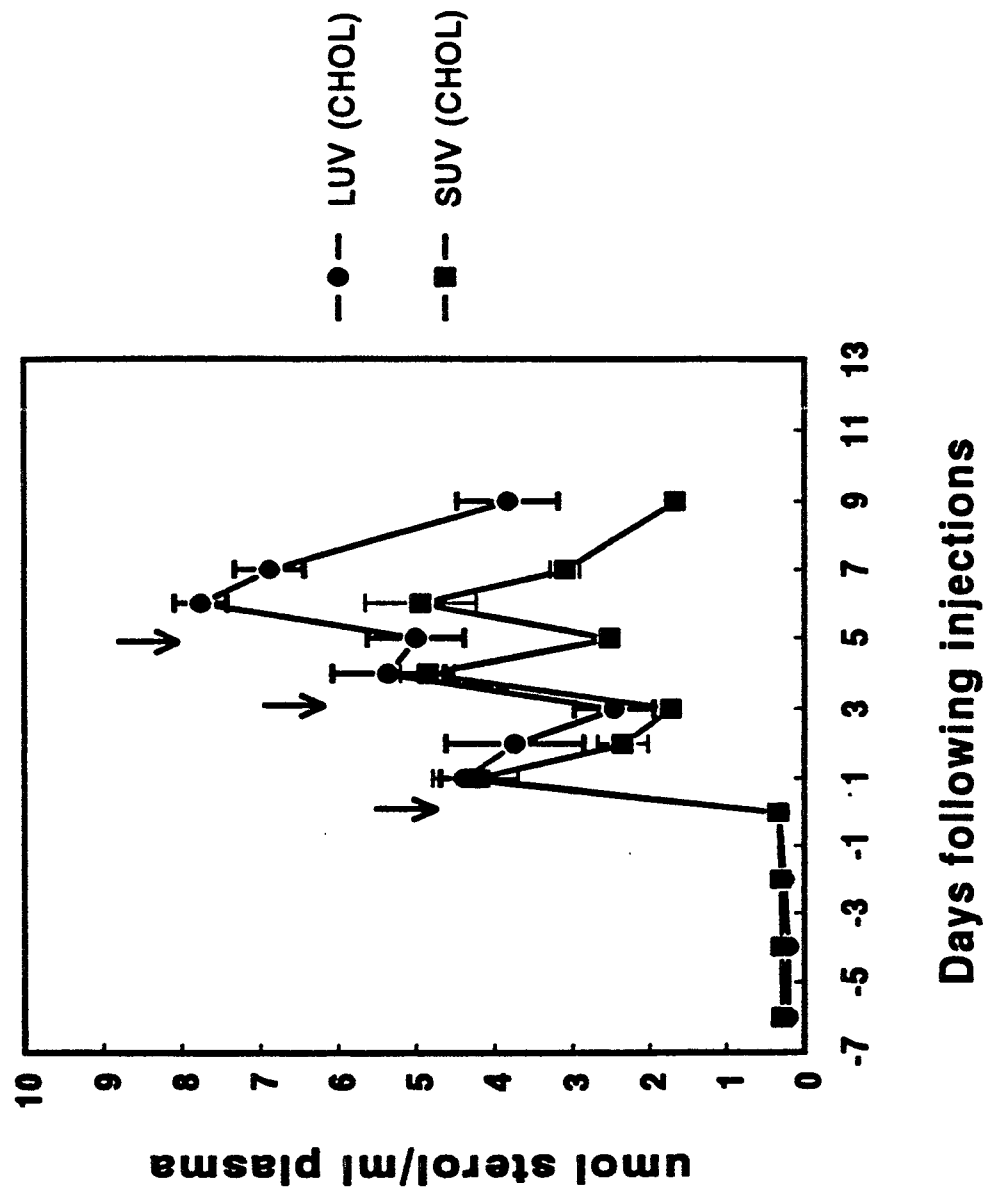


Fig. 25

26/28

# Plasma cholesterol ester concentrations

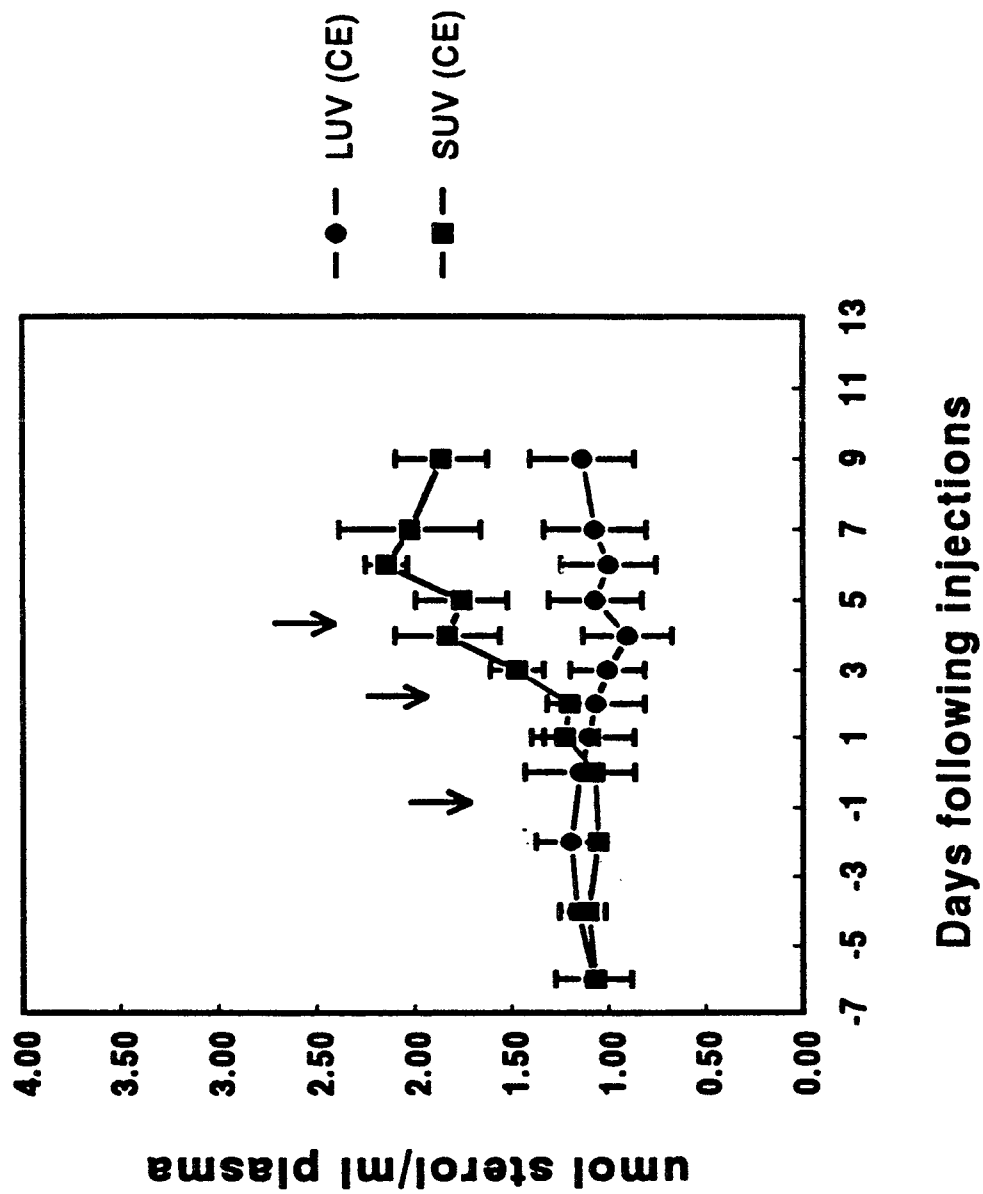


Fig. 26

WO 97/13501

27/28

PCT/US96/16388

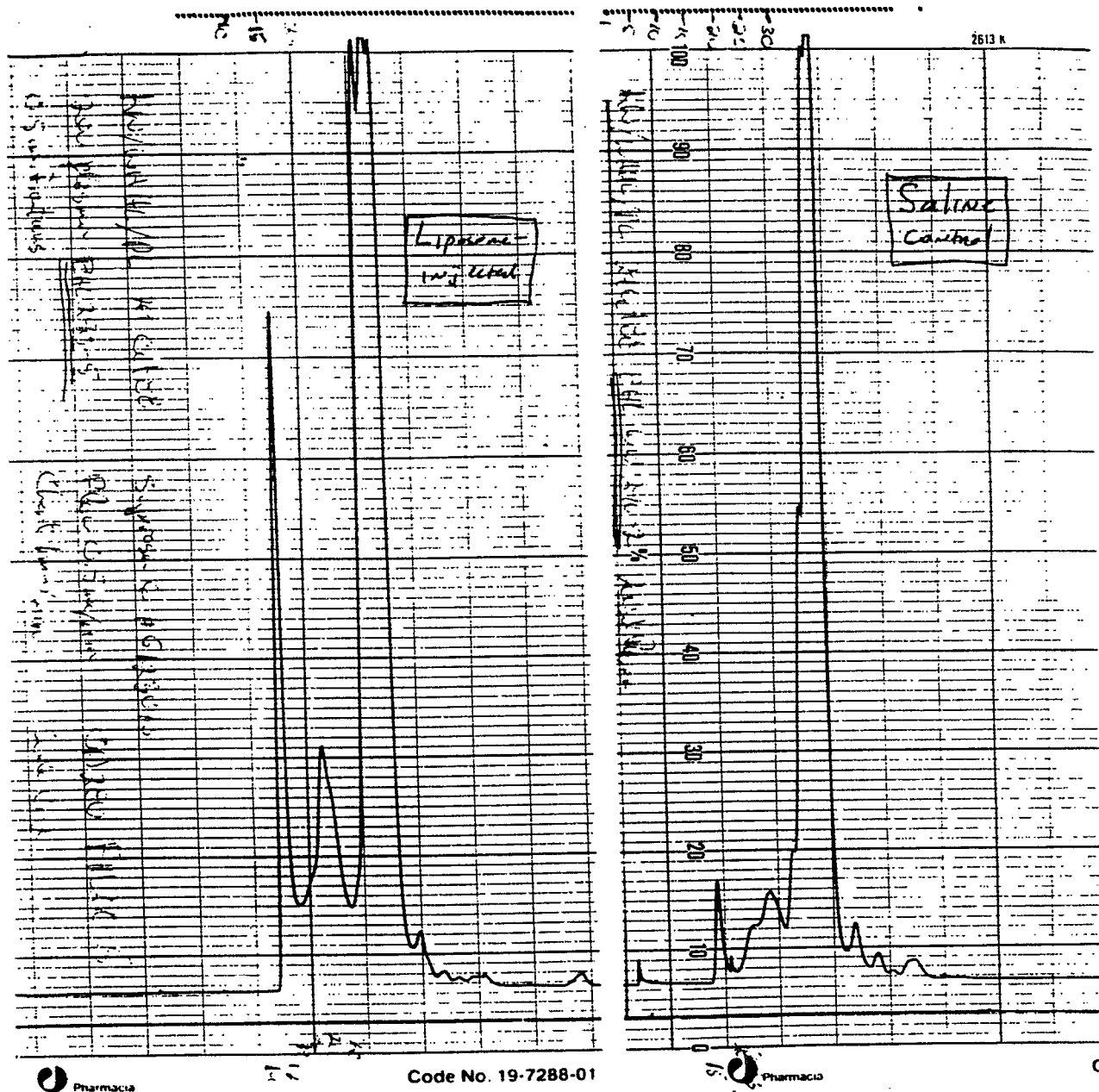


Fig. 27

28/28

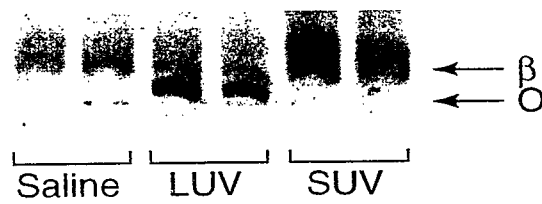


Fig. 28