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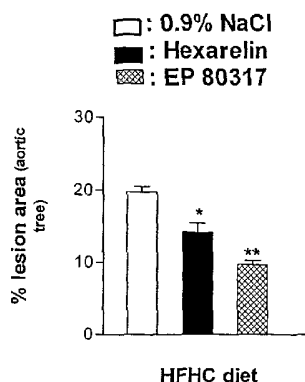
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(54) Title: GROWTH HORMONE-RELEASING PEPTIDES IN THE TREATMENT OF PREVENTION OF ATHEROSCLEROSIS AND HYPERCHOLESTEROLEMIA

ApoE-null mice, 12 weeks treatment with GHRPs



*, $p < 0.01$ and **, $p < 0.001$ compared with 0.9% NaCl; #, $p < 0.05$ compared with HEX

(57) Abstract: According to the invention there is provided a method of treatment or prophylaxis of atherosclerosis, hypercholesterolemia or a cardiovascular disease associated with atherosclerosis, which method comprises administration of one or more Growth Hormone Releasing Peptides (GHRPs) to a patient in need of such treatment or prophylaxis. There are also provided methods of reducing blood plasma cholesterol levels, as well as methods of modulating the expression of the scavenger receptor CD36 and genes involved in cellular cholesterol efflux.

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GROWTH HORMONE-RELEASING PEPTIDES IN THE TREATMENT OF PREVENTION OF
ATHEROSCLEROSIS AND HYPERCHOLESTEROLEMIA

Field of the Invention

The present invention relates to the use of Growth Hormone Releasing Peptides (GHRPs) in the treatment of and prophylaxis of atherosclerosis, hypercholesterolemia and related diseases.

Background and Prior Art

Atherosclerosis is a multifactorial disease developing preferentially in subjects presenting biochemical risks factors including smoking, hypertension, diabetes mellitus, hypercholesterolemia, elevated plasma low density lipoprotein (LDL) and triglycerides, hyperfibrinogenemia and hyperglycemia, among others. Atherosclerotic lesions develop over a number of decades in humans, leading to complications such as coronary and cerebral ischemic and thromboembolic diseases and myocardial and cerebral infarction. To date, cardiovascular disease is the leading cause of morbidity and mortality in industrialised countries and progresses steadily in emerging countries, with coronary atherosclerosis being the main underlying pathology. Currently, therapy of atherosclerosis is not completely efficient to prevent disease development and complication.

Atherosclerosis develops through the sequential interplay of at least three pathological processes: foam cell differentiation, inflammatory reaction and cell proliferation.

In humans and in various animal models, one of the earliest detectable events in the first of these processes is the recruitment of mononuclear phagocytes and lymphocytes to the intact endothelial lining of large arteries. Enhanced adhesion and accumulation of blood monocytes into the intima is then accompanied by a change in cell phenotype, where they transform into macrophages. The latter engulfs lipids and stores them as cytoplasmic droplets, thus becoming "foam cells". The formation of such foam cells is a key stage in the development of early fatty streak lesions, and hence is a central event in atherogenesis.

The type B scavenger receptor CD36, which is known to have multiple functions, is recognised to play a key role in scavenging oxidized low density lipoproteins into monocytes/macrophages.

Growth hormone releasing peptides (GHRPs), which consist of a family of small synthetic peptides modelled from Met-enkephalin, possess potent and dose dependent growth hormone-releasing activity and significant prolactin and corticotropin-releasing effects (see *Horm. Res.* 51(suppl. 3), 9 (1999)).

The neuroendocrine activities of GHRPs are mediated by a specific G protein-coupled receptor identified as Ghrelin receptor expressed in hypothalamus and pituitary gland (see *Horm. Res.* 56(suppl. 1), 93 (2001)). However, GHRPs also provide a cardioprotective effect against cardiac ischemia in growth hormone (GH) deficient or aged rats. This protective activity is not coupled to any apparent stimulation of the somatotropic function, suggesting a direct myocardial action of these peptides (see *Eur. J. Pharmacol.* 334, 201 (1997)).

A study of GHRP binding sites in the cardiovascular system found that hexarelin, a hexapeptide member of the GHRP family, binds to a glycosylated membrane protein of 84 kD distinct from the Ghrelin receptor (see *Circ. Res.*

85, 796 (1999)). This protein was later identified as the CD36 receptor (see *Circ. Res.* 90, 844 (2002)).

None of the above-mentioned prior art suggests that GHRPs, or their synthetic analogues, can be employed to inhibit the lipid-scavenging function of the receptor CD36, thereby providing utility in the treatment and prevention of atherosclerosis, hypercholesterolemia and related diseases.

Disclosure of the Invention

The present inventors have found that a prolonged treatment (12 weeks) with GHRPs interferes with the CD36 scavenger receptor function and expression, thereby reducing the uptake of oxidised LDL (oxLDL) and the accumulation of lipids and cholesterol, and consequently, reducing the formation of fatty streak lesions in ApoE-null mice.

The ApoE-null mouse was selected as an experimental model of atherosclerosis as it features the progressive series of atherogenic events seen in human, including increased adhesive interactions between leukocyte and endothelium, conversion of monocyte-derived macrophages into foam cells with lesions distributing throughout the arterial tree, and late development of more advanced lesions (fibrous plaques).

ApoE-null mice show very high levels of plasma cholesterol as a result of impaired clearance of cholesterol-enriched lipoproteins and, as for humans, high fat high cholesterol (HCHF) diet exacerbates disease progression and markedly enhances plasma cholesterol levels (see *J. Clin. Invest.* 105(8), 1049-1056 (2000) and *J. Clin. Invest.* 105, 1039-1041 (2000)). Evidence accumulates to support that fatty streaks in anatomical sites prone to atheromatous plaque development precede mature lesions in humans,

although all fatty streaks do not progress to atheromas (see *Arterioscler. Thromb. Vasc. Biol.* 19, 2364-2367 (1999)). In both human and mice, T lymphocyte and foam cells are found in fatty streaks and immunological processes have been shown to be similar over the years in both species.

The present inventors have found that GHRPs reduce total plasma cholesterol and non-HDL cholesterol, and increase HDL cholesterol in ApoE-deficient mice fed with a HFHC diet from 6 weeks old. These favourable changes in plasma lipids were associated with a significant decrease in fatty streak lesion area in the ApoE-null mice treated with GHRPs, as compared with controls.

The present inventors have also found that GHRPs regulate CD36 expression in peritoneal macrophages from ApoE-null mice in mice fed a HFHC diet for 12 weeks.

The present inventors have further found using cultured macrophages from differentiated monocytic THP-1 cells, that treatment with GHRPs contributes to decrease lipid storage in macrophages, with a concomitant increase in gene expression of nuclear receptor LXR α and ABCA1 transporter, two proteins involved in cellular cholesterol efflux.

GHRPs therefore appear to prevent cholesterol accumulation in macrophages and foam cell formation, thereby reducing fatty streak development. Although not wishing to be bound by theory, it is for this reason that the present inventors believe that GHRPs may be efficient to prevent the development of atherosclerotic plaques and cardiovascular disease linked to atherosclerotic processes such as coronary artery disease, myocardial infarction and strokes.

Thus, according to a first aspect of the present invention, there is provided a method of treatment or prophylaxis of atherosclerosis or hypercholesterolemia, thereby preventing the development of and/or treating a cardiovascular disease associated with atherosclerosis, which method comprises administration of one or more GHRPs to a patient in need of such treatment or prophylaxis.

The term “cardiovascular disease associated with atherosclerosis” includes references to diseases that are medically linked to atherosclerosis in that they are a consequence of atherosclerotic lesions. Cardiovascular diseases associated with atherosclerosis that may be mentioned include coronary artery disease, myocardial infarction and strokes.

In one embodiment of the first aspect of the invention, there is provided a method of preventing the development of atherosclerotic plaques, hypercholesterolemia or a cardiovascular disease associated with atherosclerosis, which method comprises the administration of one or more GHRPs to a patient at risk of developing such plaques, hypercholesterolemia or cardiovascular diseases. This embodiment of the invention encompasses treatment of patients with GHRPs in order to prevent the formation of atherosclerotic plaques or their precursors (e.g. fatty streaks).

Without wishing to be bound by theory, it is believed that this embodiment of the invention prevents fatty streak formation and atherosclerosis development through modulating negatively CD36 expression in macrophages, which leads to inhibition of oxLDL internalization, thus breaking the cycle of foam cell formation and the feed-forward loop of oxLDL-induced PPAR γ and CD36 expression (see *Nature Med.* 7, 41-47 (2001)), in addition to reducing total plasma cholesterol and non-HDL cholesterol.

In a second embodiment of the first aspect of the invention, there is provided a method of treating pre-existing atherosclerosis or hypercholesterolemia, thereby treating and/or preventing the development of a cardiovascular disease associated with atherosclerosis, which method comprises administration of one or more GHRPs to a patient who has atherosclerosis, hypercholesterolemia and/or a cardiovascular disease associated with atherosclerosis. This embodiment of the invention encompasses treatment of patients with GHRPs in order to reduce the size of pre-existing atherosclerotic plaques or their precursors (e.g. fatty streaks).

According to a second aspect of the invention, there is provided a method of reducing the total blood plasma cholesterol level of a patient in need of such cholesterol level reduction, which method comprises administering to said patient one or more GHRPs. In one embodiment of this method, GHRPs are administered to the patient in order to reduce plasma levels of non-HDL cholesterol.

According to a third aspect of the invention, there is provided a method of negatively modulating (reducing) CD36 expression, which method comprises administering one or more GHRPs to a patient who would benefit from negative modulation of CD36 expression.

In a preferred embodiment of this aspect of the invention, there is provided a method of negatively modulating CD36 expression in (monocyte-derived) macrophages, which method comprises administering one or more GHRPs to a patient who would benefit from such negative modulation of CD36 expression in macrophages.

According to a fourth aspect of the invention, there is provided a method of increasing expression of genes involved in cellular cholesterol efflux, which

method comprises administering one or more GHRPs to a patient who would benefit from increased expression of such genes.

Genes involved in cellular cholesterol efflux that may be mentioned include those for nuclear receptor LXR α and ABCA1 transporter.

Decreased expression of CD36 and increased expression of LXR α and ABCA1 transporter may take place simultaneously. As such, an embodiment of the invention relates to a method of modulating the key regulators of net cellular flux of cholesterol (e.g. to and from macrophages), by negative modulation of CD36 expression and/or function and by increasing expression of genes involved in cellular cholesterol efflux, which method comprises administering one or more GHRPs to a patient who would benefit from such modulation of net cellular cholesterol flux. As will be appreciated by those skilled in the art, negative modulation of CD36 expression and/or function results in reduced uptake of oxLDL by scavenger receptor CD36.

When used herein, the term “*expression*”, unless otherwise indicated, refers to the production of mRNA by transcription of the relevant gene and/or, particularly, to production of protein *via* gene transcription and then mRNA translation. Similarly, the term “*expression of genes*” refers to production of protein *via* gene transcription and then mRNA translation and/or, particularly, to the production of mRNA by transcription of the relevant gene.

Patients:

- (i) in need of blood cholesterol level reduction;
- (ii) who would benefit from negative modulation of CD36 expression;

- (iii) who would benefit from increased expression of genes involved in cellular cholesterol efflux; and
- (iv) who would benefit from reduced uptake of oxLDL by scavenger receptor CD36 and from increased expression of genes involved in cellular cholesterol efflux

include those that have blood cholesterol levels that are likely to cause or exacerbate cardiovascular disease or dysfunction. Such patients include:

- (a) those having coronary heart disease and those at risk of developing it because of multiple risk factors (including obesity, smoking, hypertension, diabetes mellitus and a family history of premature coronary heart disease);
- (b) those with familial conditions characterised by very high plasma concentrations of cholesterol and/or triglycerides;
- (c) those with hyperlipidemia not secondary to underlying diseases (such as hypothyroidism, nephrotic syndrome, hepatic disease or alcoholism);
- (d) those with elevated LDL-cholesterol; and
- (e) those under dietary hypolipidemic intervention (complementary treatment).

When used herein, the term "GHRPs" includes references to (synthetic) peptide compounds that can activate the pituitary-hypothalamus axis (see *Neuroscience* 53, 303 (1993)) and act directly on the pituitary somatotroph by a non-Growth Hormone Releasing Hormone (non-GHRH), non-opiate and

non-somatostatin secretory pathway. The term also includes references to structurally related (peptidomimetic) compounds that are: (a) capable of inhibiting GH release by other GHRPs and themselves have no effect on GHRH agonism; and/or (b) devoid of GH-releasing activity and have no effect on GHRH agonism. Examples of GHRPs (including their antagonists) are described in: "*Xenobiotic Growth Hormone Secretagogues*", Eds. B. Bercu & R. F. Walker, pp. 9-28 and 85-102, Springer-Verlag, New York (1996); *J. Ped. End. Metab.* 8, 311-313 (1996); *Acta Paediatr. Suppl.* 423, 85-87 (1997); *Life Sci.* 50, 1149-1155 (1992); *Eur. J. Endocrinol.* 136, 445-460 (1997); *J. Pediatr. Endocrinol.* 6, 21-31 (1993); *Ann. Rep. Med. Chem.* 28, 177-186 (1993); *Endocrinology* 106, 663-667 (1980); *ibid.* 108, 31-39 (1981); *ibid.* 114, 1531-1536 and 1537-1545 (1984); *Front. Neuroendocrinol.* 19(1), 47-72 (1998); *Cell. Mol. Life Sci.* 54(12), 1316-1329 (1998); WO 89/07110; WO 89/07111; WO 93/04081; WO 96/15148; US 4,411,890; and US 6,025,471.

In respect of all of the above-mentioned aspects of the invention, GHRPs that may be mentioned include those of the hexarelin (HEX) family, such as HEX (His-(D)-(Me)Trp-Ala-Trp-(D)-Phe-Lys-NH₂) and EP80317 (Haic-(D)-(Me)Trp-(D)-Lys-Trp-(D)-Phe-Lys-NH₂), the latter peptide being devoid of GH-secreting activity *in vivo*. Preferred GHRPs include Hexarelin and EP80317. Particularly preferred GHRPs include those, like EP80317, that are devoid of GH-secreting activity *in vivo*.

The methods of the present invention, such as the method of the first aspect of the invention, preferably include the step of systemic administration of GHRP to afford a protective amount of drug in circulation. In a most specific embodiment, Hexarelin, EP80317 and any GHRP analog is exogenously administered. The methods of the present invention may be used to treat animals or, preferably, humans.

Further aspects of the present invention include methods according to any of the above-mentioned aspects of the invention, except that, in place of GHRPs, related synthetic analogs of HEX and EP80317 and any ligands which bind to the receptor(s) of those compounds or of GHRPs are employed. Ligands which bind to the receptor(s) of GHRPs include CD36 ligands, which may be identified using *in silicio* techniques involving molecular modelling of the (GHRP) binding site on CD36.

Further aspects of the invention also include:

- 1) The use of growth hormone releasing peptides of Hexarelin family, of derived peptidomimetics and of CD36 ligands in the prevention and treatment of atherosclerosis and hypercholesterolemia.
- 2) The use of GHRP derivatives, of derived peptidomimetics, and of CD36 ligands which modulate the expression of scavenger receptor B (CD36) in the prevention of the development of atherosclerotic lesions and in the prevention of heart attacks and strokes associated with coronary artery disease and hypercholesterolemia.
- 3) The use of GHRP derivatives and of derived peptidomimetics which modulate the expression of the ATP-binding cassette ABCA1 transporter and scavenger receptor B (CD36) in the prevention of the development of atherosclerotic lesions and in the prevention of heart attacks and strokes associated with coronary artery disease and hypercholesterolemia.
- 4) A pharmaceutical composition containing a compound as defined in points 1) to 3) above, to be administered exogenously.

In one embodiment of aspect 1) above, there is provided the use of growth hormone releasing peptides of Hexarelin family, of derived peptidomimetics as CD36 ligands in the prevention and treatment of atherosclerosis and hypercholesterolemia.

Further, in an embodiment of aspect 3) above, there is provided the use of GHRP derivatives and of derived peptidomimetics which modulate the expression of the ATP-binding cassette ABCA1 transporter in the prevention of the development of atherosclerotic lesions and in the prevention of heart attacks and strokes associated with coronary artery disease and hypercholesterolemia.

In the treatment of atherosclerosis, hypercholesterolemia or cardiovascular diseases associated with atherosclerosis, GHRPs may be administered in combination with other agents that have different mechanisms of action and that have beneficial effects on blood lipid levels.

Thus, in a yet further aspect of the invention there is provided a combination product comprising:

- (i) a GHRP; and
- (ii) an agent that produces beneficial effects on blood lipid levels,

wherein each of components (i) and (ii) is formulated in admixture with a pharmaceutically-acceptable adjuvant, diluent or carrier.

The combination product according to this aspect of the invention provides for the administration of a GHRP in conjunction with an agent that produces beneficial effects on blood lipid levels, and may thus be presented either as

separate formulations, wherein at least one of those formulations comprises a GHRP and at least one comprises an agent that produces beneficial effects on blood lipid levels, or may be presented (i.e. formulated) as a combined preparation (i.e. presented as a single formulation including a GHRP and an agent that produces beneficial effects on blood lipid levels).

Thus, there is further provided:

- (1) a pharmaceutical formulation including a GHRP and an agent that produces beneficial effects on blood lipid levels, in admixture with a pharmaceutically-acceptable adjuvant, diluent or carrier; and
- (2) a kit of parts comprising components:
 - (a) a pharmaceutical formulation including a GHRP in admixture with a pharmaceutically-acceptable adjuvant, diluent or carrier; and
 - (b) a pharmaceutical formulation including an agent that produces beneficial effects on blood lipid levels in admixture with a pharmaceutically-acceptable adjuvant, diluent or carrier,

which components (a) and (b) are each provided in a form that is suitable for administration in conjunction with the other.

When used herein, the term “*an agent that produces beneficial effects on blood lipid levels*” includes references to pharmaceutically-active agents that are not GHRPs and that, upon administration to patients, have the effect of lowering of total cholesterol and/or triglyceride levels in the blood, of lowering of LDL cholesterol levels in the blood and/or of increasing HDL

cholesterol levels in the blood. As such, this term includes references to any of the following active agents:

- (I) statins (i.e. inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase), such as atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pravastatin, rosuvastatin and simvastatin;
- (II) fibrates (i.e. derivatives of fibric acids and related compounds), such as beclobrate, bezafibrate, ciprofibrate, clinofibrate, clofibrate, clofibrade, etofylline clofibrate, fenofibrate, gemfibrozil, pirifibrate, plafibrade, simfibrate and tocofibrate;
- (III) bile-acid binding resins, such as colesevelam, colestipol, colestyramine and divistyramine;
- (IV) nicotines (i.e. nicotinic acid and its derivatives), such as acipimox, binifibrate, etofibrate, niceritrol, nicofibrate, pirozadil, ronifibrate, sorbinicate and tocoferil nicotinate;
- (V) omega-3-triglycerides, such as omega-3-acid ethyl esters and omega-3-marine triglycerides;
- (VI) acyl-coenzyme A:cholesterol O-acyl transferase (ACAT) inhibitors, such as avasimibe;
- (VII) PPAR γ agonists, such as pioglitazone;
- (VIII) cholesterol absorption inhibitors, such as ezetimibe; and
- (IX) lipase inhibitors, such as orlistat.

Preferred agents that produces beneficial effects on blood lipid levels include statins, such as those specifically mentioned at (I) above.

It will be appreciated that this aspect of the invention includes embodiments where the combination product additionally comprises one or more further GHRPs and/or one or more further agents that produce beneficial effects on blood lipid levels. As for the essential components of the combination product, each further GHRP or agent that produces beneficial effects on blood lipid levels may be formulated separately or in combination with any one of the other components of the combination product.

This invention will be described herein below, referring to specific embodied examples and appended figures, which purpose is to illustrate the invention rather than limit its scope.

Brief description of the drawings

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

Figure 1

GHRPs prevent fatty streak formation in ApoE deficient mice fed a HFHC diet for 12 weeks (from 6 weeks old). Effects of HEX (100 µg/kg per day) and of EP80317 (300 µg/kg per day), administered subcutaneously for 12 weeks (from 6 weeks old).

A: Lesion area (% of total aorta area) on ApoE-null mouse aortas were reduced by 28 and 51% following treatment with HEX and EP80317,

respectively, as compared to controls treated with 0.9% NaCl. The open bar represents vehicle (0.9% NaCl) treated mice, the solid bar, HEX treatment and the cross-hatched bar, EP80317 treatment. Asterisk indicates $P < 0.05$ (**, $P < 0.01$) compared with vehicle, and # indicates $P < 0.05$ compared with HEX.

B: Photograph of representative mice aortas from vehicle- (top), HEX- (middle) and EP80317-treated (bottom) ApoE-null mice stained with Oil Red O (obtained from Sigma-Aldrich).

Figure 2

GHRPs reduced total plasma cholesterol and non-HDL cholesterol, and tended to increase HDL cholesterol in ApoE-null mice fed a HFHC diet for 12 weeks (from 6 weeks old). Total plasma cholesterol and non-HDL cholesterol were decreased by 30 and 31%, respectively, in EP80317-treated ApoE-null mice under HFHC diet as compared to controls. HDL cholesterol increased by 65 and 73% in ApoE-null mice under HFHC treated with HEX and EP80317, respectively.

A: Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (open bar), HEX, 100 $\mu\text{g/kg}$ per day (solid bar) or EP80317 300 $\mu\text{g/kg}$ per day (cross-hatched bar) on total plasma cholesterol in mice fed a HFHC or a normal diet.

B: Effects of treatments on plasma triglycerides.

C: Effects of treatments on HDL cholesterol.

D: Effects of treatments on non-HDL cholesterol.

Asterisk indicates $P < 0.01$ compared with vehicle, and # indicates $P < 0.01$ compared with HEX.

Figure 3

GHRPs reduced oxLDL-induced peritoneal macrophage accumulation in wild type and in ApoE-null mice fed a HFHC diet for 12 weeks (from 6 weeks old.

A: Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (open bar), HEX, 100 $\mu\text{g/kg}$ per day (solid bar) or EP80317, 300 $\mu\text{g/kg}$ per day (cross-hatched bar) on oxLDL (250 μg i.p., < 6 nmol MDA/mg lipoprotein)-induced accumulation of macrophages in the peritoneal cavity in wild type C57BL/6 mice and CD36-null mice fed a HFHC diet. Peritoneal macrophage accumulation in wild type mice tended to be reduced by 37%.

B: Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (open bar), HEX, 100 $\mu\text{g/kg}$ per day (solid bar) or EP80317, 300 $\mu\text{g/kg}$ per day (cross-hatched bar) on oxLDL-induced accumulation of macrophages in the peritoneal cavity in ApoE-null mice fed either a HFHC or a normal diet. Peritoneal macrophage accumulation was reduced by 39% in mice fed a normal diet.

Figure 4

GHRPs modulate CD36 and ABCA1 protein levels in peritoneal macrophages from ApoE-null mice fed a HFHC diet for 12 weeks. Figure 4 shows Western blot analysis on ox-LDL (250 μg i.p., < 6 nmol MDA/mg lipoprotein)-elicited peritoneal macrophages collected from ApoE-null mice fed an HFHC diet and treated with GHRPs (either HEX, 100 $\mu\text{g/kg}$ per day or EP80317, 300 $\mu\text{g/kg}$ per day) for 12 weeks (from 6 weeks old). Relative protein levels for **A**, CD36 and **B**, ABCA1 are normalised to β -actin and expressed as % of change

compared to vehicle (0.9% NaCl)-treated controls set at 100%. Data represent the mean of two independent experiments.

Figure 5

GHRPs did not modulate the growth curve in ApoE-null mice fed a HFHC diet (or a normal diet) for 12 weeks (from 6 weeks old).

Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (diamond), HEX, 100 µg/kg per day (square) or EP80317, 300 µg/kg per day (triangle) on weight of mice fed:

A: a HFHC diet; or

B: a normal diet.

Figure 6

GHRPs did not modulate food intake in ApoE-null mice fed a HFHC diet (or a normal diet) for 12 weeks (from 6 weeks old).

Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (diamond), HEX, 100 µg/kg per day (square) or EP80317, 300 µg/kg per day (triangle) on food intake in mice fed:

A: a HFHC diet; or

B: a normal diet.

Figure 7

A: GHRPs reduce lipid accumulation in differentiated human macrophages. To induce lipid accumulation, human monocytic THP-1 cells were differentiated to macrophages with 5 ng/mL PMA for 48 hrs and then treated as indicated for 24 hrs with 10 μ M HEX or EP80317 in the presence of phorbol myristate acetate (PMA). Non-differentiated cells (no PMA) were also analysed. Lipid accumulation was quantified by photometry at 510 nm following extraction of Oil Red O from stained cells. Lipid staining was reduced by 35% and 28% in macrophages treated with HEX and EP80317, respectively, compared to untreated cells.

B: GHRPs reduced lipid accumulation in peritoneal macrophages isolated from ApoE-null mice fed a HFHC diet from 13 to 18 weeks old. EP80317 (300 μ g/kg) s.c. has been administered from 13 to 18 weeks old. Lipid staining (assessed by Oil Red O extraction) was reduced by 57% (compared to peritoneal macrophages isolated from ApoE-null mice fed a HFHC diet from 13 weeks old, but not treated with EP80317).

Figure 8

GHRPs increase expression of genes involved in cellular cholesterol removal.

A: PMA-differentiated THP-1 macrophages were treated with HEX or EP80317 as described in relation to Figure 7, and mRNA expression was analysed for selected genes by RT-PCR. The procedure involves extraction of total RNA from cells, which RNA is then reverse transcribed into cDNA and amplified in a PCR reaction with specific primers. Primers used are:

LXR α forward CCTGTCAGAAGAACAGATCCGC

LXR α reverse TCTTCAGCAGGGCAATCTGGTCC;

ABCA1 forward GGTC AATGGAAGGTTTCAGGTGC

ABCA1 reverse GGAGTCGCTTTTTGCTCTGGGAGAGG;

GAPDH forward GGTCTTACTCCTTGGAGGCCATGT

GAPDH reverse GACCCCTTCATTGACCTCAACTACA.

B: Measurement of signal intensity from the experiment described in **A** was performed using an Alpha Imager analysis system and results expressed as fold response compared to untreated differentiated cells. GAPDH was used as a control for data normalisation.

Figure 9

GHRPs prevent or delay the development or modulate the regression of fatty streaks in ApoE-null mice fed on a HFHC diet starting at 6 weeks of age for a period of 12 weeks.

The effects of EP80317 (300 µg/kg per day) administered subcutaneously for 8 weeks (from 10 to 18 weeks of age), 6 weeks (from 12 to 18 weeks of age) and 4 weeks (from 14 to 18 weeks of age) were documented, as well as the effect of hexarelin (100 µg/kg per day) given for 4 weeks (from 14 to 18 weeks of age).

A: Lesion area (% total aorta area) on ApoE-null aortas were reduced by 39.2% following treatment with EP80317 for 8 weeks as compared to controls treated with 0.9% NaCl.

B: Lesion area (% total aorta area) on ApoE-null aortas were reduced by 31.3% following treatment with EP80317 for 6 weeks as compared to controls treated with 0.9% NaCl.

C: Lesion area (% total aorta area) on ApoE-null aortas were reduced by 8% and 36% following treatment with hexarelin and EP80317, respectively, for 4 weeks as compared to controls treated with 0.9% NaCl.

In Figure 9, the open bars represent vehicle (0.9% NaCl) treated mice, the solid bar, hexarelin treatment and the cross-hatched bars, EP80317 treatment.

Example 1

Growth hormone-releasing peptides prevent fatty streaks formation

Severe morbidity results as a consequence of the arterial disease atherosclerosis and complications of the disease, such as myocardial infarction and strokes, remain a common cause of mortality in Western Society (see *J. Hematotherapy & Stem Cell Res.* 10(1), 27-42 (2001)). Current management strategies of atherosclerosis include life-style interventions such as healthy dietary and exercise recommendations and, mainly, the use of lipid lowering drug therapy, in addition to blood pressure control and use of antiplatelet drugs. Among hypolipemic drugs, hepatic hydroxymethylglutaryl-coenzyme A reductase inhibitors or statin drugs interfere with hepatic cholesterol metabolism which leads to a compensatory increase in LDL receptors and cholesterol clearance via these receptors. In contrast, fibrates and nicotinic acid mainly reduce circulating VLDL whereas bile acid binding resins interfere with bile acid reabsorption, thereby increasing fecal excretion of cholesterol. However, statins, used in monotherapy or in association with another lipid-lowering drug, have been associated with serious adverse effects, myopathy and rhabdomyolysis (see *Texas Heart Institute J.* 28, 142-145 (2001)). For instance, cerivastatin therapy has been associated with hundreds of myopathies and some dozens of deaths (see *Toxicol. Lett.* 128, 159-168 (2002)). Hence, use of these drugs should be revisited (see *Can. Fam. Phys.* 48, 486-489 (2002)).

To determine whether GHRPs reduce atherosclerosis development, we have used the ApoE deficient mice strain and their C57BL/6 control littermates to assess the effects of prolonged (12 weeks) GHRPs treatment on fatty streak formation in mice fed a an enriched lipid diet. The surface area of oil red-O staining aortas of mice has been used as an index of plaque development and changes in the plasma levels of lipids as an index of hypolipemic effect. Taken together, these end points served to evaluate the anti-atherosclerotic effect of GHRPs *in vivo*.

Methods

Drugs

HEX and EP80317 were a generous gift of Dr. R. Deghenghi, Europeptides, Argenteuil, France. HEX and EP80317 stock solutions were prepared in sterile 0.9% NaCl.

Animals

CD36-deficient and ApoE-deficient mice, as well as their control littermates were raised in the animal facilities of the Université de Montréal. The ApoE deficient mouse features the progressive series of atherogenic events seen in human, including increased adhesive interactions between leukocyte and endothelium, conversion of monocyte-derived macrophages into foam cells with lesions distributing throughout the arterial tree, and late development of more advanced lesions (fibrous plaques). ApoE-null mice show very high levels of plasma cholesterol as a result of impaired clearance of cholesterol-enriched lipoproteins and, as for humans, HCHF diet exacerbates disease progression and markedly enhances plasma cholesterol levels (see *J. Clin. Invest.* 105(8), 1049-1056 (2000) and *J. Clin. Invest.* 105, 1039-1041 (2000)). However, complex lesions as seen in humans are not observed except for the innominate artery in mice ~ 42 weeks old, where loss of continuity of the

fibrous cap, rupture of xanthomas at the shoulders of lesions and intraplaque haemorrhage are seen (see *Arterioscler. Thromb. Vasc. Biol.* 20, 2587-2592 (2000)). Evidence accumulates to support that fatty streaks in anatomical sites prone to atheromatous plaque development precede mature lesions in humans, although all fatty streaks do not progress to atheromas (see *Arterioscler. Thromb. Vasc. Biol.* 19, 2364-2367 (1999)). - In both human and mice, T lymphocyte and foam cells are found in fatty streaks and immunological processes have been shown to be similar over the years in both species.

The animals were housed in cages (less than 5 per cage) and fed a normal chow diet and water ad libitum. At 6 weeks of age, male C57BL/6, CD36 and ApoE-null mice were assigned to 1 of 3 groups (n=12 mice per group): Group 1 received daily injections of 0.9% NaCl (1 μ L/g); Group 2, HEX (100 μ g/kg, 1 μ L/g), and Group 3, EP80317 (300 μ g/kg, 1 μ L/g). All three groups of mice were fed a HFHC diet (purchased from Research Diet Inc. (D.12108)) from 6 weeks of age. Daily s.c. treatment with HEX, EP80317 or vehicle was begun at 6 weeks old and continued for 12 weeks. Four days before sacrifice, 6 mice from Groups 1 to 3 were injected i.p. with oxLDL (minimally oxidized, 3-6 nmol/mg protein, as determined by Thiobarbituric Acid Reactive Substances (TBARS) assay). At 18 weeks old, mice were fasted overnight, anaesthetised with ketamine-xylazine (90:10 mg/kg) two hours after the s.c. administration of the morning dose of the drug under study. Blood (1 mL) was taken from the heart and put into EDTA pre-coated microcontainers (BD, Franklin Lakes, NJ, USA). Mice were killed, the hearts were perfused with 20 mL 0.9% NaCl. The peritoneal cavity was washed with 3 mL of heparinised saline (10 units/mL) in mice injected i.p. with oxLDL. A hemacytometer and stained cytospin preparation (Diff Quick stain, Dade Diagnostics of P.R. Inc., Aguada, PR) were used to determine the total and differential leukocyte numbers, respectively, for the peritoneal cavity lavage fluid. The entire aorta

from the heart, extending 5-10 mm after bifurcation of the iliac arteries and including the subclavian right and left common carotid arteries, was removed, dissected, and evaluated for lesion development by en face oil red-O staining and morphometry of scanned images using the software Scion Image (Scion Corp., Fredrick, Maryland). The animal study protocol was reviewed and approved by the institutional Animal Ethics Committee of the Université de Montréal and conducted in accordance with the Canadian Council on Animal Care guidelines for use of experimental animals.

Plasma lipid analysis

Total plasma cholesterol, triacylglycerol and HDL cholesterol were determined using enzymatic kits (Sigma Chemicals). Appropriate standards and controls were included in each assay.

Statistical analysis

Data are expressed as mean $\pm$ SEM. Comparisons between groups were performed using a one-way analysis of variance (ANOVA) followed by pair-wise multiple comparisons using the Student-Newman-Keuls method. Differences were considered significant at $p < 0.05$.

Results

The most striking observation is a reduction of lesion area in ApoE-deficient mice, by 28 and 51% following treatment with HEX (100 μ g/kg) and EP80317 (300 μ g/kg) daily, respectively, in mice fed a HFHC diet (Figure 1). CD36-deficient mice and their wild type C57BL/6 control littermates did not develop significant fatty streak lesions on HFHC diet (12 weeks, from 6 weeks old).

Reduced lesion area was accompanied with a decrease in total plasma cholesterol (30%), as well as in non-HDL plasma cholesterol (31%), in ApoE-deficient mice fed a HFHC diet and treated daily with EP80317 (300 µg/kg) daily for 12 weeks (from 6 weeks old), as compared to controls and to ApoE-null mice treated with HEX (100 µg/kg) daily (Figure 2A and Figure 2D). In contrast, HDL cholesterol tended to be increased in ApoE-null mice under HFHC diet (12 weeks, from 6 weeks old) treated with HEX (65%) or EP80317 (73%) as compared to (0.9% NaCl-administered) controls on HFHC diet (Figure 2C). Plasma triglycerides did not change significantly (Figure 2B). GHRPs did not modulate total plasma cholesterol levels in CD36-deficient or C57BL/6 controls fed, from 6 weeks old, a HFHC diet for 12 weeks (data not shown).

EP80317 reduced oxLDL-induced peritoneal macrophage accumulation by 37% and 39 %, respectively, in wild type C57BL/6 mice fed a HFHC diet and ApoE-null mice fed a normal chow diet (Figure 3A and Figure 3B). EP80317 did not modulate macrophage accumulation in CD36-null mice (Figure 3A). CD36 protein expression was reduced to 57 and 27% of controls, respectively, in peritoneal macrophages harvested from HEX- and EP80317-treated ApoE-null mice fed a HFHC diet (Figure 4).

GHRP therapy did not affect the growth curve in ApoE-deficient mice fed a HFHC diet (Figure 5A) or a normal diet (Figure 5B), nor the food intake in these mice (Figure 6A and Figure 6B).

Discussion

In the present study, we have assessed the contribution of GHRPs in the protective effect against atherosclerosis development. The major findings are:

- 1) that a prolonged treatment with GHRPs protects mice fed a HFHC diet from developing fatty streak lesions;
- 2) these protective effects of GHRPs are associated with a favourable modulation in plasma lipids, inasmuch as total plasma cholesterol and non-HDL cholesterol are reduced, and HDL plasma cholesterol is increased, in ApoE-null mice fed a HFHC diet;
- 3) the atheroprotective effects of GHRPs are associated with a negative modulation of CD36 expression and/or function and increased mRNA levels of LXR α and ABCA1 transporter in macrophages.

These findings suggest that GHRP therapy, through reducing atherosclerosis development, may afford protection against heart attacks and strokes. Thus, GHRPs and their synthetic analogs would appear to be potentially helpful for the treatment or prophylaxis of coronary cardiovascular diseases. For example, they will be useful to prevent hypercholesterolemia and atherosclerosis, thereby reducing the complications associated with the disease. Currently, only few drugs have the capacity to achieve these goals, and these have been associated with potentially severe adverse effects.

Example 2

Growth hormone-releasing peptides modulate the development of fatty streaks

To determine whether GHRPs are able to delay the development or to modulate the regression of fatty streaks, we have used the ApoE-deficient mice to assess the effect of the duration of the treatment with GHRPs on the progression of fatty streaks after the initiation of atherosclerotic lesions induced by a HFHC diet starting at 6 weeks of age.

Methods

Drugs

Solutions of HEX and EP80317 were prepared as described in respect of Example 1 above.

Animals

Male ApoE-null mice (n=35) were housed in cages (less than 5 per cage). After weaning until 4 weeks of age, the mice were fed a normal chow diet and water ad libitum. Starting at 6 weeks of age, the mice were fed a HFHC diet (purchased from Research Diet Inc. (D.12108)). The animals were segregated in 3 groups.

Group 1 (n=10) was subdivided into 2 subgroups, receiving, from 10 to 18 weeks of age (i.e. for 8 weeks), daily injections of EP80317 (300µg/kg) (n=5) or 0.9% NaCl (n=5).

Group 2 (n=10) was subdivided into 2 subgroups receiving, from 12 to 18 weeks of age (i.e. for 6 weeks), daily injections of EP80317 (300µg/kg/per day) (n=5) or 0.9% NaCl (n=5).

Group 3 (n=15) was subdivided into 3 subgroups, receiving, from 14 to 18 weeks of age (i.e. for 4 weeks), EP80317 (300µg/kg/per day) (n=5), or hexarelin (100µg/kg per day) (n=5) or 0.9% NaCl (n=5).

Mice were euthanised at 18 weeks of age. The entire aortas from the heart extending 5-10 mm after bifurcation of the iliac arteries and including the subclavian right and left common carotids were removed, dissected and evaluated for lesion development by en face oil red-O staining and

morphometry of scanned images using the software Scion image (Scion Corp., Frederick, Maryland).

Results

A significant reduction of the lesion area by 31.3% and 39.2% was observed in the ApoE-null mice aorta following the daily treatment with EP80317 (300 µg/kg) for 6 and 8 weeks, respectively. In addition, a reduction of 36%, although not significant (due to larger variability in the control group), of the lesion area of ApoE-null aortas could be observed following the daily treatment with EP80317 (300 µg/kg) for a period of 4 weeks as compared to controls treated with NaCl.

No significant difference in terms of percentage of the lesion area in the aorta could be observed following the treatment with hexarelin (8%) for a period of 4 weeks as compared to controls treated with 0.9% NaCl.

Discussion

In the present study, we have assessed the contribution of a GHRP derivative, EP80317, not only for its protective effect but also for its curative effect on atherosclerosis lesion development.

EP80317 may delay the development of fatty streaks induced by the HFHC diet. This peptide might also modulate the regression of these fatty streaks in the aortas of ApoE-null mice in which the atherosclerotic process has been induced by a HFHC diet starting at 6 weeks of age.

Claims

1. A method of treatment or prophylaxis of atherosclerosis, hypercholesterolemia or a cardiovascular disease associated with atherosclerosis, which method comprises administration of one or more GHRPs to a patient in need of such treatment or prophylaxis.
2. A method as claimed in Claim 1, which comprises preventing the development of atherosclerotic plaques, hypercholesterolemia or a cardiovascular disease associated with atherosclerosis by administering one or more GHRPs to a patient at risk of developing such plaques, hypercholesterolemia or cardiovascular diseases.
3. A method as claimed in Claim 1, which comprises treating pre-existing atherosclerosis, hypercholesterolemia or a cardiovascular disease associated with atherosclerosis by administering one or more GHRPs to a patient who has atherosclerosis, hypercholesterolemia or a cardiovascular disease associated with atherosclerosis.
4. A method as claimed in any one of Claims 1 to 3 wherein the cardiovascular disease associated with atherosclerosis is coronary artery disease, myocardial infarction or stroke.
5. A method of reducing the total blood plasma cholesterol level of a patient in need of such cholesterol level reduction, which method comprises administering to said patient one or more GHRPs.
6. A method of reducing the blood plasma level of non-HDL cholesterol in a patient in need of such non-HDL cholesterol level reduction, which method comprises administering to said patient one or more GHRPs.

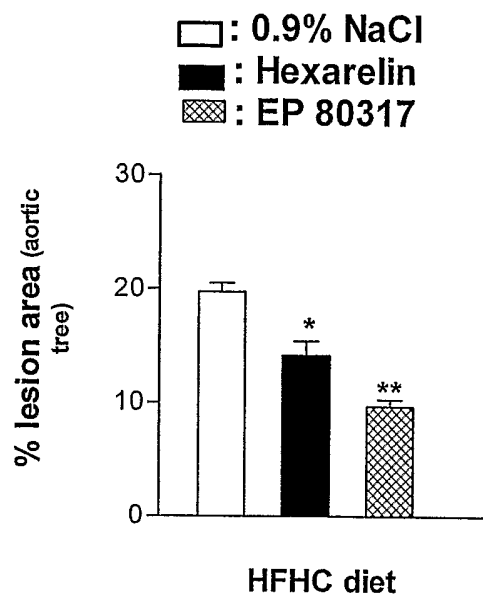
7. A method of negatively modulating CD36 expression, which method comprises administering one or more GHRPs to a patient who would benefit from negative modulation of CD36 expression.
8. A method of increasing expression of genes involved in cellular cholesterol efflux, which method comprises administering one or more GHRPs to a patient who would benefit from increased expression of such genes.
9. A method as claimed in Claim 8, wherein the genes involved in cellular cholesterol efflux are those for nuclear receptor LXR α and/or ABCA1 transporter.
10. A method for decreasing oxLDL cholesterol uptake by macrophages by modulating CD36 expression and/or function, which method comprises administering one or more GHRPs to a patient who would benefit from reduction in the uptake of oxidised lipoprotein by macrophages.
11. A method as claimed in any one of the preceding claims, wherein the one or more GHRPs are hexarelin (His-(D)-(Me)Trp-Ala-Trp-(D)-Phe-Lys-NH₂) or EP80317 (Haic-(D)-(Me)Trp-(D)-Lys-Trp-(D)-Phe-Lys-NH₂).
12. The use of one or more GHRPs for the manufacture of a medicament for the treatment or prophylaxis of atherosclerosis, hypercholesterolemia or a cardiovascular disease associated with atherosclerosis.

13. Use as claimed in Claim 12, wherein the medicament is for preventing the development of atherosclerotic plaques, hypercholesterolemia or a cardiovascular disease associated with atherosclerosis.
14. Use as claimed in Claim 12, wherein the medicament is for treating pre-existing atherosclerosis, hypercholesterolemia or a cardiovascular disease associated with atherosclerosis.
15. Use as claimed in any one of Claims 12 to 14 wherein the cardiovascular disease associated with atherosclerosis is coronary artery disease, myocardial infarction or stroke.
16. The use of one or more GHRPs for the manufacture of a medicament for reducing the total blood plasma cholesterol level of a patient in need of such cholesterol level reduction.
17. The use of one or more GHRPs for the manufacture of a medicament for reducing the blood plasma level of non-HDL cholesterol in a patient in need of such non-HDL cholesterol level reduction.
18. The use of one or more GHRPs for the manufacture of a medicament for the negative modulation of CD36 expression in a patient who would benefit from such modulation of expression.
19. The use of one or more GHRPs for the manufacture of a medicament for increasing expression of genes involved in cellular cholesterol efflux in a patient who would benefit from increased expression of such genes.

20. Use as claimed in Claim 19, wherein the genes involved in cellular cholesterol efflux are those for nuclear receptor LXR α and/or ABCA1 transporter.
21. The use of one or more GHRPs for the manufacture of a medicament for the modulation of oxidised lipoprotein uptake by macrophages in a patient who would benefit from such modulation.
22. Use as claimed in any of Claims 12 to 21, wherein the one or more GHRPs are hexarelin (His-(D)-(Me)Trp-Ala-Trp-(D)-Phe-Lys-NH₂) or EP80317 (Haic-(D)-(Me)Trp-(D)-Lys-Trp-(D)-Phe-Lys-NH₂).
23. The use of growth hormone releasing peptides of Hexarelin family, of derived peptidomimetics and of CD36 ligands in the prevention and treatment of atherosclerosis and hypercholesterolemia.
24. The use of GHRP derivatives, of derived peptidomimetics, and of CD36 ligands which modulate the expression of scavenger receptor B (CD36) in the prevention of the development of atherosclerotic lesions and in the prevention of heart attacks and strokes associated with coronary artery disease and hypercholesterolemia.
25. The use of GHRP derivatives and of derived peptidomimetics which modulate the expression of the ATP-binding cassette ABCA1 transporter and scavenger receptor B (CD36) in the prevention of the development of atherosclerotic lesions and in the prevention of heart attacks and strokes associated with coronary artery disease and hypercholesterolemia.

26. A pharmaceutical composition containing a compound as defined in Claims 23 to 25 above, to be administered exogenously.

ApoE-null mice, 12 weeks treatment with GHRPs



*, $p < 0.01$ and **, $p < 0.001$ compared with 0.9% NaCl; #, $p < 0.05$ compared with HEX

Figure 1A

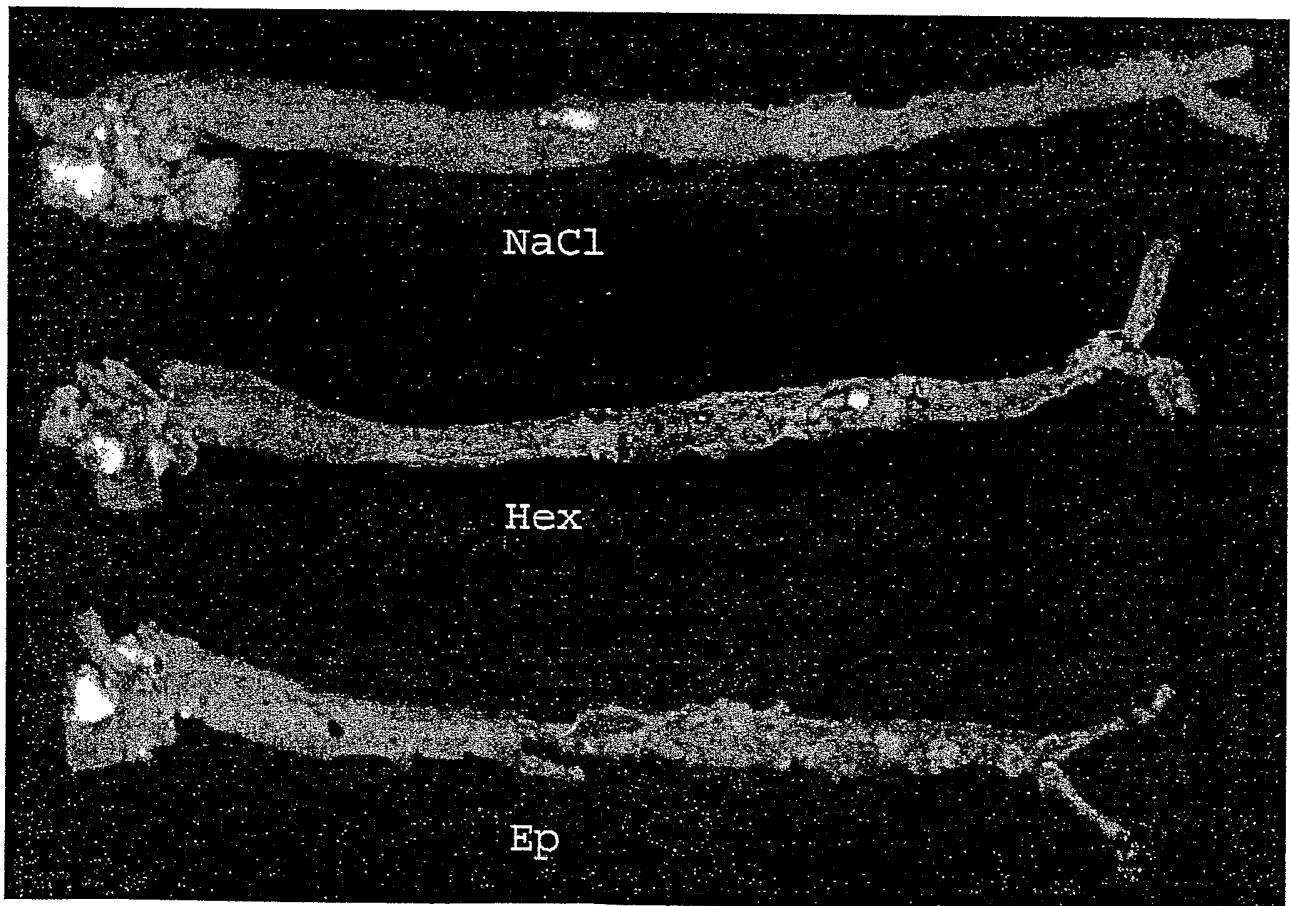
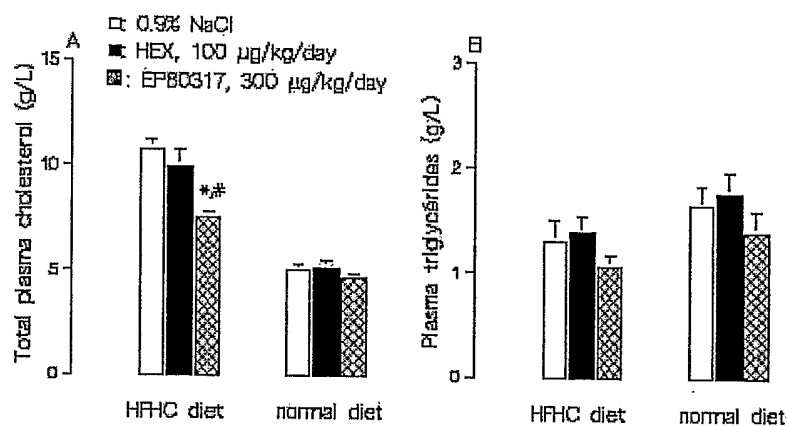
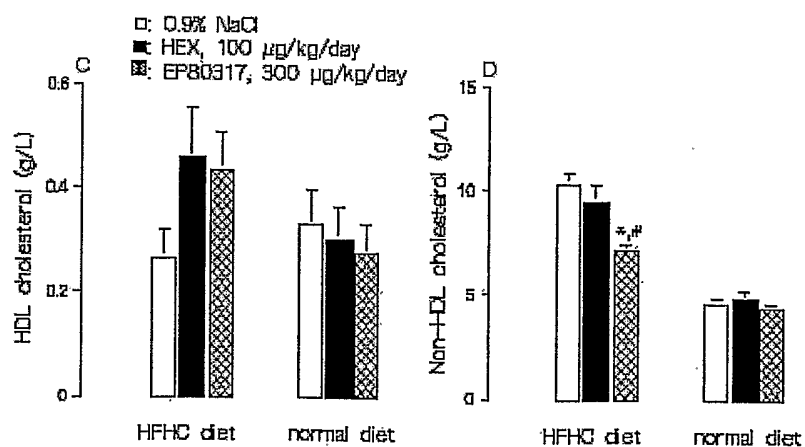


FIGURE 1B

ApoE null mice, 12 weeks treatment with GHRPs



*, $p < 0.01$ compared with 0.9% NaCl; #, $p < 0.01$ compared with HEX



*, $p < 0.01$ compared with 0.9% NaCl; #, $p < 0.01$ compared with HEX

Figure 2

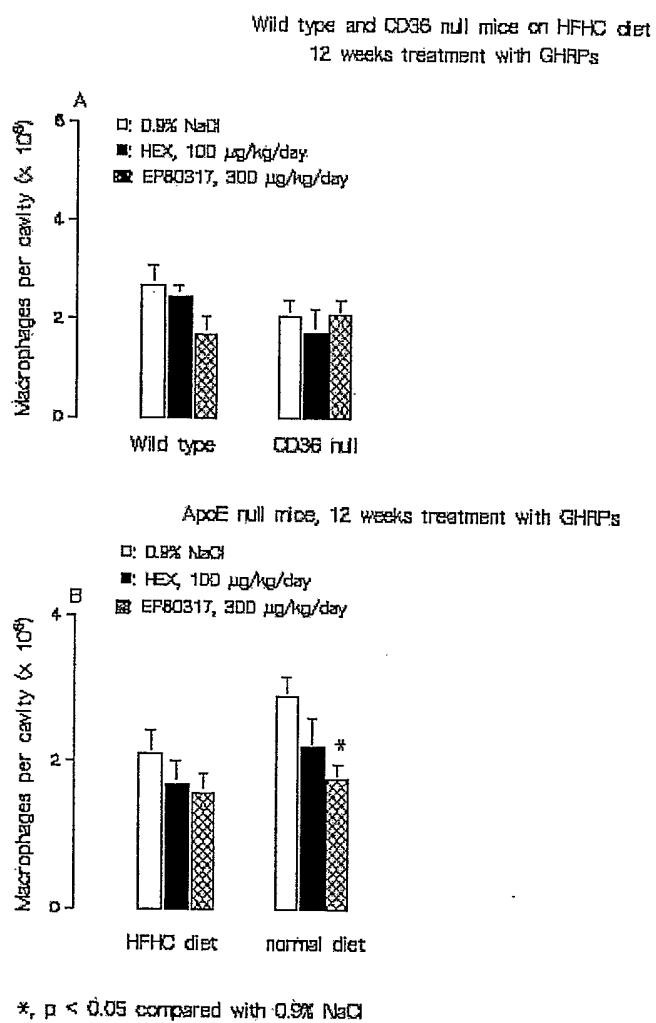


Figure 3

Western blot, ApoE-null 12 weeks treatment with GHRPs

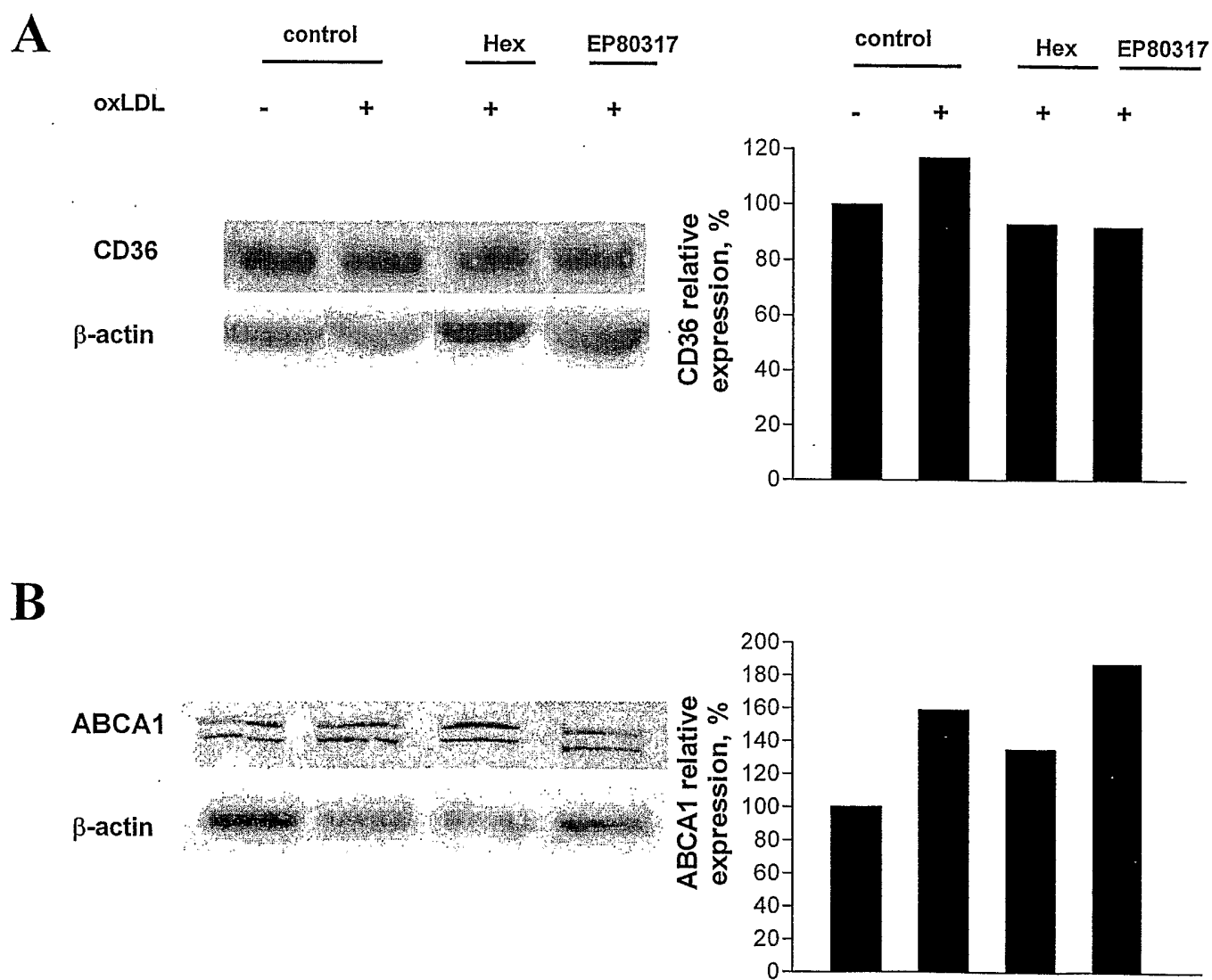
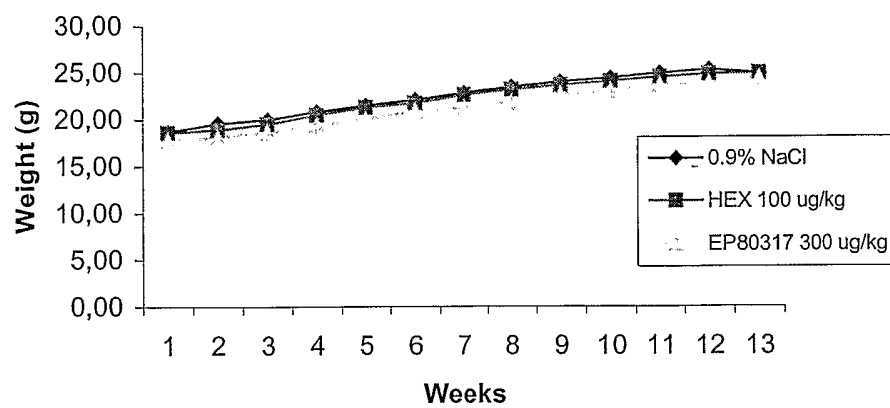
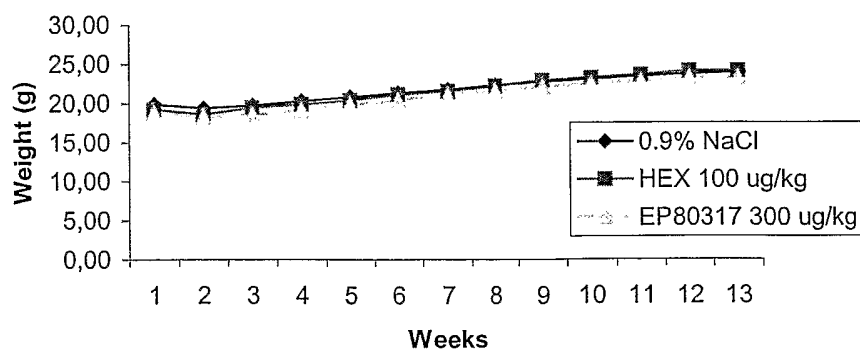
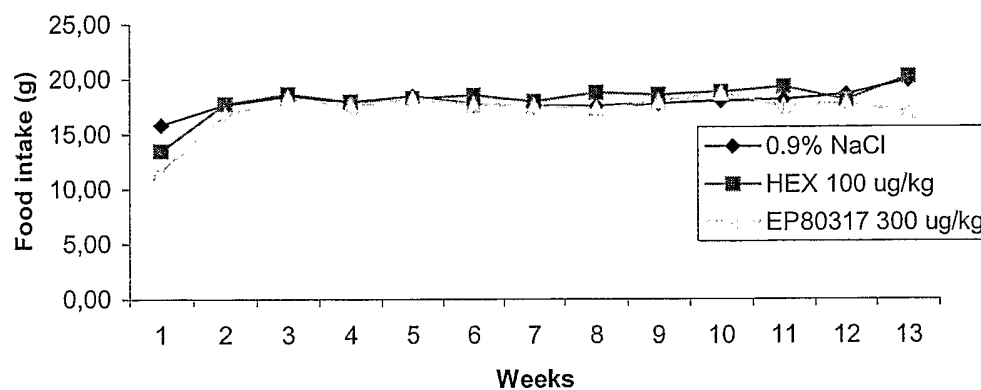
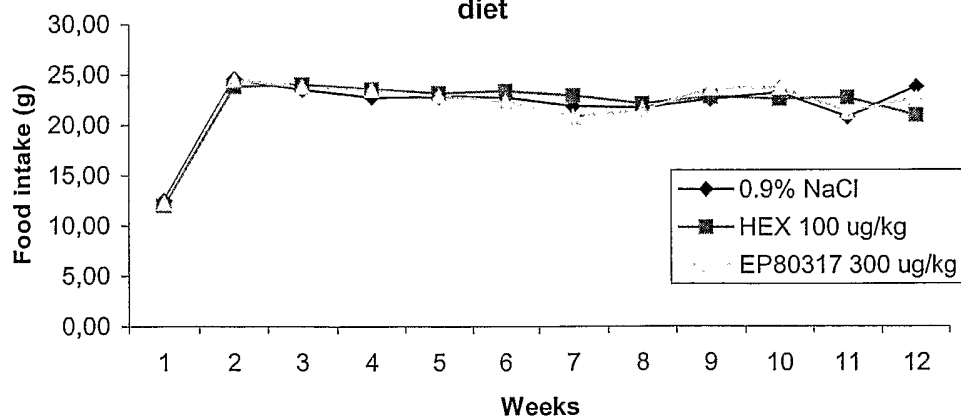


Figure 4

A. Variation in weight of ApoE null mice on lipid diet**B. Variation in weight of ApoE null mice on normal diet****Figure 5**

A. Food intake per week in ApoE null mice on lipid diet**B. Food intake per week in ApoE null mice on normal diet****Figure 6**

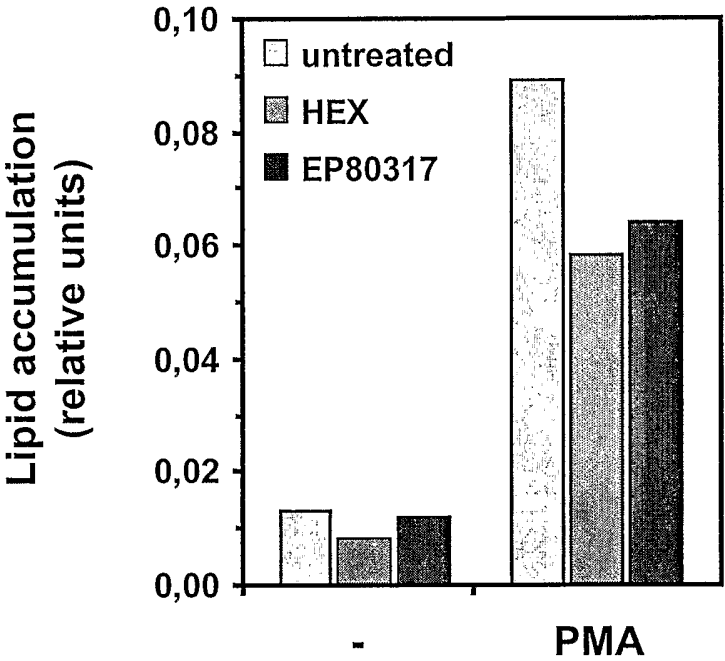


Figure 7A

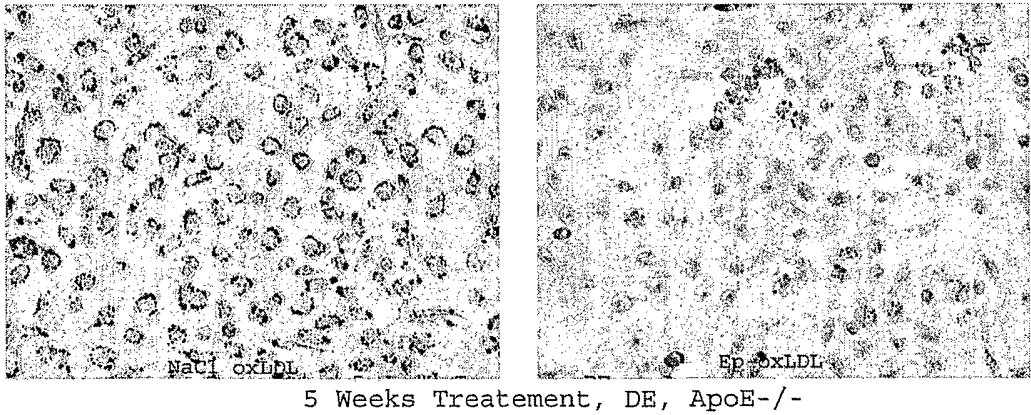


Figure 7B

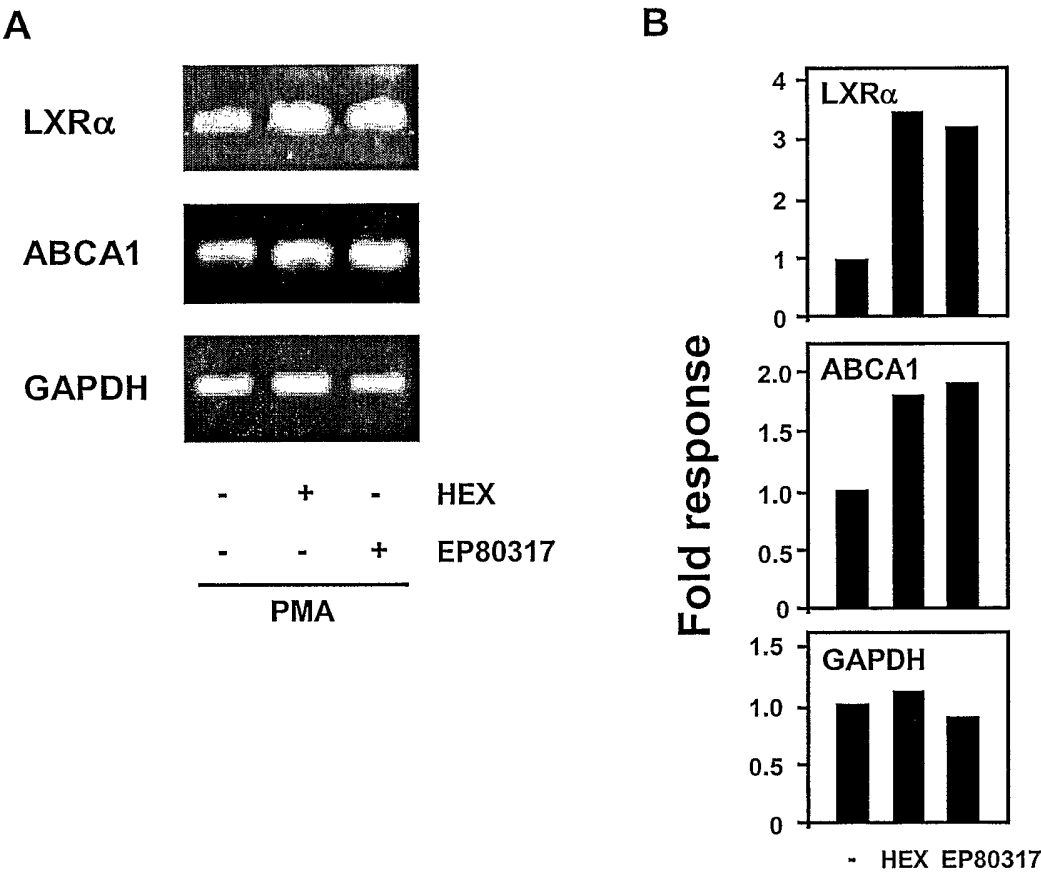
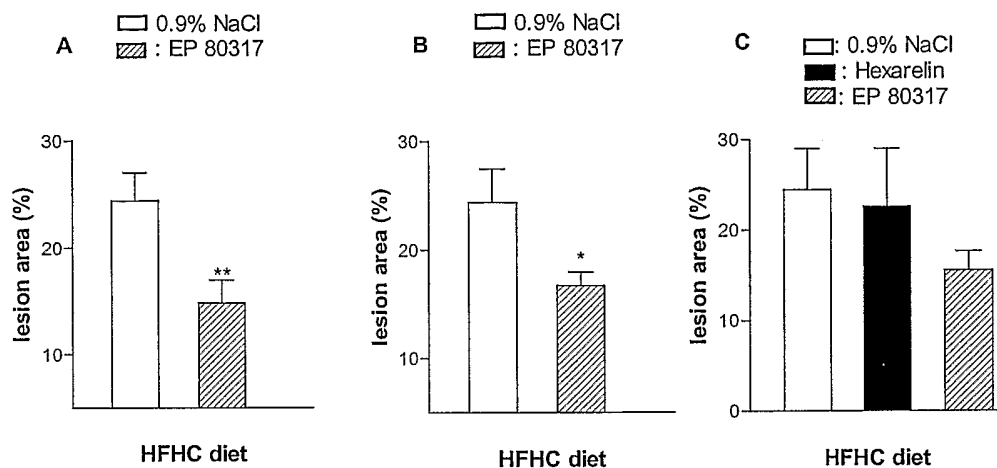


Figure 8

**Figure 9**

A: 8 weeks (from 10 to 18 weeks of age) treatment with EP80317 or 0.9% NaCl solution.

B: 6 weeks (from 12 to 18 weeks of age) treatment with EP80317 or 0.9% NaCl solution.

C: 4 weeks (from 14 to 18 weeks of age) treatment with EP80317 or 0.9% NaCl solution.

The symbols * and ** indicate $P < 0.05$ and $P < 0.02$, respectively, compared to vehicle.

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB 03/03669

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/08 A61K38/25 A61P9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 23097 A (SAHLTECH AB ;ANGELIN BO (SE); RUDLING MATS (SE)) 27 April 2000 (2000-04-27) claims page 4, paragraph 3 ---	1-5, 11-16, 22,26
X	WO 98 22124 A (GENNARO COLONNA VITO DE ;BOGHEN MUNY (IT); BERTI FERRUCCIO (IT); M) 28 May 1998 (1998-05-28) claims examples page 26, paragraph 1 ---	1-4, 12-15, 22,24-26
X	WO 00 67770 A (SAHLTECH AB ;BOLLANO ENTELA (SE); ISGAARD JOERGEN (SE); OMEROVIC E) 16 November 2000 (2000-11-16) claims ---	1,3,12, 14,15,26
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* & * document member of the same patent family

Date of the actual completion of the international search

14 November 2003

Date of mailing of the international search report

28/11/2003

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INTERNATIONAL SEARCH REPORT

Internal Application No

PCT/GB 03/03669

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 09537 A (UNIV TULANE ;BOWERS CYRIL Y (US); LIANG YONGWU (US); MOMANY FRANK) 24 February 2000 (2000-02-24) claims page 24, paragraph 3 ---	1,3,5,6, 12,14, 16,17,26
X	BROGLIO FABIO ET AL: "Effects of acute hexarelin administration on cardiac performance in patients with coronary artery disease during by-pass surgery" EUROPEAN JOURNAL OF PHARMACOLOGY, vol. 448, no. 2-3, 19 July 2002 (2002-07-19), pages 193-200, XP002261551 ISSN: 0014-2999 abstract page 198, left-hand column, last paragraph ---	1,3,4, 12,14, 15,26
X	WALKER RICHARD F ET AL: "Effects of stimulated growth hormone secretion on age-related changes in plasma cholesterol and hepatic low density lipoprotein messenger RNA concentrations" MECHANISMS OF AGEING AND DEVELOPMENT, vol. 75, no. 3, 1994, pages 215-226, XP009021106 ISSN: 0047-6374 abstract; figure 2 ---	1,2,4,5, 11-13, 16,22, 23,26
X	MARLEAU S ET AL: "Effect of growth hormone releasing peptides (GHRPs) on monocyte/macrophage scavenger receptors (SR) B (CD36) expression and monocyte trafficking" INFLAMMATION RESEARCH, vol. 50, no. Supplement 3, September 2001 (2001-09), page S154 XP009021107 5th World Congress on Inflammation;Edinburgh, Scotland; September 22-26, 2001 ISSN: 1023-3830 abstract	7,10,11, 18,21
A	BODART V ET AL: "CD36 mediates the cardiovascular action of growth hormone-releasing peptides in the heart" CIRCULATION RESEARCH, vol. 90, no. 8, 3 May 2002 (2002-05-03), pages 844-849, XP002261552 ISSN: 0009-7330 abstract -----	7,10,11, 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 03/03669

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-11 and 23-25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 8,19,23,24 (all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box I.2

Claims Nos.: 8,19,23,24 (all partially)

Present claims 8 and 19 relate to a genes defined by reference to a desirable characteristic or property, namely to genes involved in cellular cholesterol efflux.

The claims cover all genes having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such genes. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the genes LXRA α and ABCA1 as defined in claims 9 and 20.

Present claims 23 and 24 relate to a compounds defined by reference to a desirable characteristic or property, namely to CD36 ligand.

The claims cover all compounds having this characteristic or property, whereas the application does not provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any such compound. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the GHRPs and derived peptidomimetics.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

information on patent family members

Internal: Application No

PCT/GB 03/03669

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0023097	A	27-04-2000	AU 764779 B2	28-08-2003
			AU 1428800 A	08-05-2000
			CA 2342794 A1	27-04-2000
			EP 1124571 A1	22-08-2001
			JP 2002527486 T	27-08-2002
			WO 0023097 A1	27-04-2000
			US 6559289 B1	06-05-2003
WO 9822124	A	28-05-1998	AU 5142498 A	10-06-1998
			WO 9822124 A1	28-05-1998
WO 0067770	A	16-11-2000	AU 4790800 A	21-11-2000
			EP 1173190 A1	23-01-2002
			WO 0067770 A1	16-11-2000
WO 0009537	A	24-02-2000	AU 5671099 A	06-03-2000
			EP 1159292 A2	05-12-2001
			JP 2002525265 T	13-08-2002
			WO 0009537 A2	24-02-2000
			US 2002151501 A1	17-10-2002
			US 6468974 B1	22-10-2002