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Phosphoglycan Messengers and Their Medical Uses (SLE)

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(54) Title: PHOSPHOGLYCAN MESSENGERS AND THEIR MEDICAL USES

(57) Abstract: Phosphoglycan messengers (PGMs) which are carbohydrate derivatised phosphatidyl cyclitols are disclosed, together with the finding that these substances are biologically active, especially in modulating plasma cholesterol, plasma triglycerides and/or high density lipoprotein levels and/or modulating the LDL:HDL ratio, and for the treatment of lipodystrophy or dyslipidemia. These compounds are distinct from GPI-anchors disclosed in the prior art as these GPI-anchors are protein linked and are not biologically active.

Phosphoglycan Messengers and Their Medical Uses

Field of the Invention

The present invention relates to phosphoglycan messengers (PGMs) and their medical uses, and in particular to PGMs comprising one or more lipid moieties, and in particular to their use in treating dyslipidemia and related conditions.

Background of the Invention

Glycosylphosphatidylinositols (GPIs) are essential components in the plasma membrane of cells (Thomas et al, 1990) including malaria parasites, both as membrane anchors for proteins and as the sole class of free glycolipids (Gerold et al,1997), and, in their role of precursors of IPGs, they may also play a role in insulin signal transduction (Saltiel & Cuatrecasas, 1986). Binding of insulin to its receptor leads to phosphatidylinositol-specific phospholipase cleavage of GPI and generation of two extracellular signals, diacyl glycerol and inositol phosphoglycans (IPGs).

Inositol phosphoglycans (IPGs) which resemble the polar core glycan head of free GPIs, mimic several insulin actions and have been suggested to constitute a unique insulin second messenger system (Rademacher et al, 1994; Varela-Nieto et al, 1996). IPG are released in a biologically active form by the cleavage of GPI precursors by the action of the enzyme GPI-PLD. In mammalian cells, two chemically and functionally distinct classes of IPGs denominated IPG-A and IPG-P, are tissue-specific (Kunjara et al, 1994). IPG-A mediators mimic the lipogenic activity of insulin in adipocytes and inhibit cAMP-dependent protein kinase (Caro et al, 1997).

IPG-P mediators mimic the glycogenic activity of insulin in skeletal muscle and stimulate pyruvate dehydrogenase phosphatase (PDH). WO98/11116 and WO98/11117 (Rademacher Group Limited) disclose the purification, isolation and characterisation of IPG-P and IPG-A from human tissue. Prior to these applications, it had not been possible to isolate single components from the tissue derived IPG fractions, much less in sufficient quantities to allow structural characterisation.

Although IPGs have been isolated from different sources including mammals, yeast and Trypanosomes, and have been extensively tested for their insulin mimetic activity *in vitro*, they have so far only been tested *in vivo* in streptozotocin (STZ)-diabetic rats (Huang et al, 1993; Fonteles et al, 1996). IPG-P from malaria parasites has been shown to have insulin mimetic activities *in vitro* (Caro et al, 1996). No IPGs or IPG-related compounds from any source have been studied in murine models of Type 2 diabetes. GPIs purified from malaria parasites have been tested *in vivo* only in normal mice pretreated with thioglycollate (Schofield & Hackett, 1993) in experiments demonstrating that some components of parasitized erythrocytes trigger the release of toxins such as TNF from activated macrophages.

Summary of the Invention

The present invention is based on the finding that phosphoglycan messengers (PGMs), and in particular PGMs comprising lipid groups, are biologically active and therefore have therapeutic utility in the treatment of conditions characterised by dyslipidemia or lipodystrophy. The results disclosed herein show that PGMs of the present invention can be used to treat

conditions characterised by abnormal or elevated levels of cholesterol, plasma triglycerides, and/or high density lipoproteins (HDL), especially elevated levels or amounts of these substances, or an abnormal LDL: HDL ratio. The PGMs of the present invention can be contrasted with the GPI-anchors purified in prior art studies as the latter are protein linked.

These observations arose from the experiments described herein in which GPIs and IPGs obtained from malaria parasites were studied in murine models of Type 2 diabetes. Experiments in which the hypoglycaemic effect of *Plasmodium yoelii* GPIs and IPGs on STZ-diabetic mice are also disclosed and other insulin mimetic activities are explored *in vitro*, showing that malaria parasites can be used as a source of biologically active GPIs.

Thus, in contrast to the prior art view that GPIs are biologically inactive and merely serve as the precursor of biologically active IPGs (Rademacher et al, 1994; Varela-Nieto et al, 1996), or else induce the production of malaria toxins such as TNF, the experiments described herein demonstrate for the first time that PGMs comprising lipid groups, such as GPIs, are active molecules in their own right and may be useful in the treatment of various medical conditions ameliorated by PGM second messengers, for example as anti-dyslipidaemic agents for treating lipidic disorders associated with Type 1 or Type 2 diabetes, cardiovascular disorders and their complications such as coronary artery disease (CAD) other than associated with diabetes, hyperlipidemia, the treatment of systemic lupus erythematosis (SLE), especially atherosclerotic lesions. These and other uses

are discussed in more detail below.

Accordingly, in a first aspect, the present invention provides a substance which is a carbohydrate derivatised phosphatidyl cyclitol. In one preferred embodiment, the substance comprises one or more lipid moieties attached to the cyclitol group or the phosphatidyl group via one or more ester and/or ether linkages. Preferably, the substance has one or more properties selected from modulating plasma cholesterol, plasma triglycerides and/or high density lipoprotein levels or modulating, and in particular lowering, the LDL:HDL ratio. The substance may also have one or more properties selected from reducing blood glucose in a diabetic *ob/ob* and *db/db* murine model, regulating lipogenesis, stimulating pyruvate dehydrogenase phosphatase, inhibiting cAMP dependent protein kinase, inhibiting fructose-1,6-bisphosphatase, and inhibiting glucose-6-phosphatase. These and other properties of the PGMs are discussed below. As mentioned above, preferred substances are devoid of protein.

In a further aspect, the present invention provides one or more of the substances described above for use in method of medical treatment, e.g. as discussed above. In particular, the results show that the PGMs disclosed herein can be used to treat (either prophylactically or therapeutically) conditions characterised by dyslipidemia or lipodystrophy. The results disclosed herein show that PGMs of the present invention can be used to treat conditions characterised by abnormal or elevated levels of cholesterol, plasma triglycerides and/or high density lipoproteins (HDL), especially elevated levels or amounts

of these substances, or an abnormal LDL: HDL ratio.

As set out above, as PGMs comprising lipids were previously thought to be the precursor of biologically active IPGs or to stimulate the release of toxins, the finding that they can be used in the treatment of medical disorders requiring the administration of PGMs such as GPIs and IPGs is highly surprising.

In a further aspect, the present invention provides the use of one or more of the above substances for the preparation of a medicament for the treatment of a condition ameliorated by administration of a PGM second messenger. Examples of such conditions are described below.

In a further aspect, the present invention provides an isolated PGM as obtainable from malaria parasites. In one embodiment, the PGMs are obtainable using a sequence of steps comprising:

(a) making an extract by heat and acid treatment of red blood cells parasitized with *Plasmodium yoelii*;

(b) after centrifugation and charcoal treatment, allowing the resulting solution to interact overnight with an AG1-X8 (formate form) anion exchange resin;

(c) collecting a fraction having A-type IPG activity obtained by eluting the column with 50 mM HCl;

(d) neutralising to pH4 (pH not to exceed 7.8) and lyophilising the fraction to isolate the substance;

(e) descending paper chromatography using 4/1/1 butanol/ethanol/water as solvent;

(f) purification using high-voltage paper electrophoresis in pyridine/acetic acid/water; and,

(g) purification using Dionex anion exchange chromatography, or purification and isolation using Vydac HPLC chromatography.

The experiments described herein demonstrate for the first time *Plasmodium* GPI administered to diabetic mice normalised levels of plasma cholesterol and plasma triglycerides, and lowered the LDL:HDL ratio.

Embodiments of the present invention will now be described in more detail by way of example and not limitation with reference to the accompanying figures.

Brief Description of the Figures

Figure 1. Effect of *Plasmodium* GPI (10 nmole/mouse i.v.) on total plasma cholesterol (A) and plasma triglycerides (B) in STZ-diabetic mice. Values are Means ± S.E.M.; n=6-7, *p<0.05, **p<0.01 vs saline.

Figure 2. Effect of *Plasmodium* GPI (10 nmole/mouse i.v.) on plasma HDL cholesterol.

Figure 3. Inhibitory effect of different concentrations of IPGs eluted from a cellulose column on the activity of PKA. IPG-A water (--▵--), IPG-A acid (--▲--); IPG-P water (○); IPG-P acid (●). Values are the mean of two experiments.

Figure 4. Effect of different concentrations of IPG-P acid fraction eluted from a cellulose column on PDH-phosphatase, n=2.

Figure 5. High pH anion exchange chromatograms of IPG-A,

showing separation of sugars in Carbopac™ PA 10 column (a and b) and separation of sugar alditols in Carbopac™ MA1 column (c). IPG-A hydrolysed in 6N HCl for 24 hours (a and c); IPG-A hydrolysed in 4N HCl for 4 hours (b).

Figure 6. High pH anion exchange chromatograms of IPG-P, showing separation of sugars in Carbopac™ PA 10 column (a and b) and separation of sugar alditols in Carbopac™ MA1 column (c). IPG-P hydrolysed in 6N HCl for 24 hours (a and c); IPG-P hydrolysed in 4N HCl for 4 hours (b).

Detailed Description

Phosphoglycan Messengers (PGMs)

In the present application, "phosphoglycan messengers" or PGMs denote a class of substances comprising inositol phosphoglycans (IPGs) and glycosyl phosphatidyl inositols (GPIs). IPGs are examples of a family of PGMs which may comprise lipidic groups, whereas GPIs are examples of a family of PGMs comprising one or more lipidic groups attached to the cyclitol. As discussed above, free IPGs are produced from GPIs by cleavage by enzymes such as GPI-PLD which removes the lipid groups from the parent GPI. In contrast, the GPI anchors of the prior art are protein linked.

In a preferred embodiment, the present invention concerns PGM substances which are carbohydrate derivatised phosphatidyl cyclitols. In these PGMs, the phosphatidyl groups comprise one or more lipid moieties attached to the cyclitol group or the phosphatidyl group via one or more ester and/or ether linkages.

Preferably, the cyclitol is *chiro*-inositol, *myo*-inositol,

or derivatives thereof, such as substituted derivatives thereof, including pinitol (*3-O*-methyl-*chiro*-inositol).

In some embodiments, the lipid moieties are linked to the phosphatidyl group. Preferably this group is linked to one or two oxygen linked lipid moieties. The lipid moieties can be selected from the group consisting of diacyl, dialkyl, acyl-alkyl, lyso-acyl, lyso-alkyl, lyso, acyl or alkyl lipids. Examples of lipidic moieties include 1-*O*-(C16:0)*lyso*-alkylglycerol; (C16:0))*lyso*-acylglycerol; (C18:0))*lyso*-acylglycerol; (C20:0))*lyso*-acylglycerol; (C22:0))*lyso*-acylglycerol; ceramide, (C16:0)fatty acid-(C18:1)sphingosine; ceramide, (C16:0)fatty acid-(C18:0)sphinganine; ceramide, (C24:0)fatty acid-(C18:1)sphingosine; ceramide, (C24:0)fatty acid-(C18:0)sphinganine; 1-*O*-(C16:0)alkyl-2-*O*-(C16:0)acylglycerol; 1-*O*-(C16:0)alkyl-2-*O*-(C18:2)acylglycerol; 1-*O*-(C16:0)alkyl-2-*O*-(C18:1)acylglycerol; 1-*O*-(C16:0)alkyl-2-*O*-(C18:0)acylglycerol; (C16:0)-alkyl-(C16:0)acyl-glycerol (AAG) and (C16:0)mono(lyso)-alkyl-glycerol (MAG).

Preferably, the carbohydrate group is a hexose, and more preferably is selected from glucosamine, galactosamine, galactose, mannose, glucose, fucose or xylose including substituted derivatives thereof.

Preferably, the substances of the invention have one or more properties selected from lowering blood glucose, lowering plasma cholesterol or plasma triglycerides and/or normalising the ratio of low: high density lipoproteins (LDL:HDL ratio), for example by reducing the level of low density lipoprotein. These properties can

be readily assessed in a suitable animal model such as a diabetic *ob/ob* and *db/db* mice fed with a high fat diet or in STZ-diabetic mice. The substances may also have one or more of the properties attributed to IPGs in the prior art, such as regulating lipogenesis, stimulating pyruvate dehydrogenase phosphatase, inhibiting cAMP dependent protein kinase, inhibiting fructose-1,6-bisphosphatase and/or inhibiting glucose-6-phosphatase.

IPG-A mediators modulate the activity of a number of insulin-dependent enzymes such as cAMP dependent protein kinase (inhibits), adenylate cyclase (inhibits) and cAMP phospho-diesterases (stimulates). In contrast, IPG-P mediators modulate the activity of insulin-dependent enzymes such as pyruvate dehydrogenase phosphatase (stimulates) and glycogen synthase phosphatase (stimulates). The IPG-A mediators mimic the lipogenic activity of insulin on adipocytes, whereas the IPG-P mediators mimic the glycogenic activity of insulin on muscle. Both IPG-A and IPG-P mediators are mitogenic when added to fibroblasts in serum free media. The ability of the mediators to stimulate fibroblast proliferation is enhanced if the cells are transfected with the EGF-receptor. IPG-A mediators can stimulate cell proliferation in the chick cochleovestibular ganglia.

Soluble IPG fractions having IPG-A and IPG-P activity have been obtained from a variety of animal tissues including rat tissues (liver, kidney, muscle, brain, adipose, heart) and bovine liver. IPG-A and IPG-P biological activity has also been detected in human liver and placenta, malaria parasitized RBC and mycobacteria. The ability of an anti-inositolglycan antibody to inhibit

insulin action on human placental cytotrophoblasts and BC3H1 myocytes or bovine-derived IPG action on rat diaphragm and chick cochleovestibular ganglia suggests cross-species conservation of many structural features. However, it is important to note that although the prior art includes these reports of IPG-A and IPG-P activity in some biological fractions, the purification or characterisation of the agents responsible for the activity is not disclosed.

IPG-A substances are cyclitol-containing carbohydrates, also containing Zn^{2+} ion and optionally phosphate and having the properties of regulating lipogenic activity and inhibiting cAMP dependent protein kinase. They may also inhibit adenylate cyclase, be mitogenic when added to EGF-transfected fibroblasts in serum free medium, and stimulate lipogenesis in adipocytes.

IPG-P substances are cyclitol-containing carbohydrates, also containing Mn^{2+} and/or Zn^{2+} ions and optionally phosphate and having the properties of regulating glycogen metabolism and activating pyruvate dehydrogenase phosphatase. They may also stimulate the activity of glycogen synthase phosphatase, be mitogenic when added to fibroblasts in serum free medium, and stimulate pyruvate dehydrogenase phosphatase.

Methods for obtaining IPG-A and IPG-P and for determining characteristic activites of these substances and GPIs are set out in Caro et al, 1997, and in WO98/11116 and WO98/11117.

Drug Formulation

The PGMs of the invention may be derivatised in various

ways. As used herein "derivatives" of the PGMs includes salts, coordination complexes with metal ions such as Mn^{2+} and Zn^{2+}, esters such as *in vivo* hydrolysable esters, free acids or bases, hydrates, prodrugs or lipids, coupling partners.

Salts of the PGM compounds of the invention are preferably physiologically well tolerated and non toxic. Many examples of salts are known to those skilled in the art. Compounds having acidic groups, such as phosphates or sulfates, can form salts with alkaline or alkaline earth metals such as Na, K, Mg and Ca, and with organic amines such as triethylamine and Tris (2-hydroxyethyl)amine. Salts can be formed between compounds with basic groups, e.g. amines, with inorganic acids such as hydrochloric acid, phosphoric acid or sulfuric acid, or organic acids such as acetic acid, citric acid, benzoic acid, fumaric acid, or tartaric acid. Compounds having both acidic and basic groups can form internal salts.

Esters can be formed between hydroxyl or carboxylic acid groups present in the compound and an appropriate carboxylic acid or alcohol reaction partner, using techniques well known in the art.

Derivatives which as prodrugs of the PGM compounds are convertible *in vivo* or *in vitro* into one of the active PGMs. Typically, at least one of the biological activities of compound will be reduced in the prodrug form of the compound, and can be activated by conversion of the prodrug to release the compound or a metabolite of it.

Other derivatives include coupling partners of the compounds in which the compounds is linked to a coupling partner, e.g. by being chemically coupled to the compound or physically associated with it. Examples of coupling partners include a label or reporter molecule, a supporting substrate, a carrier or transport molecule, an effector, a drug, an antibody or an inhibitor. Coupling partners can be covalently linked to compounds of the invention via an appropriate functional group on the compound such as a hydroxyl group, a carboxyl group or an amino group. Other derivatives include formulating the compounds with liposomes.

Pharmaceutical Compositions

The PGMs described herein or their derivatives can be formulated in pharmaceutical compositions, and administered to patients in a variety of forms, in particular to treat conditions which are ameliorated by the administration of inositol phosphoglycan second messengers.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant or an inert diluent. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. Such compositions and preparations generally contain at least 0.1wt% of the compound.

Parenteral administration includes administration by the following routes: intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraocular, transepithelial, intraperitoneal and topical (including dermal, ocular, rectal, nasal, inhalation and aerosol), and rectal systemic routes. For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, solutions of the compounds or a derivative thereof, e.g. in physiological saline, a dispersion prepared with glycerol, liquid polyethylene glycol or oils.

In addition to one or more of the compounds, optionally in combination with other active ingredient, the compositions can comprise one or more of a pharmaceutically acceptable excipient, carrier, buffer, stabiliser, isotonicizing agent, preservative or anti-oxidant or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. orally or parenterally.

Liquid pharmaceutical compositions are typically formulated to have a pH between about 3.0 and 9.0, more preferably between about 4.5 and 8.5 and still more preferably between about 5.0 and 8.0. The pH of a composition can be maintained by the use of a buffer such as acetate, citrate, phosphate, succinate, Tris or

histidine, typically employed in the range from about 1 mM to 50 mM. The pH of compositions can otherwise be adjusted by using physiologically acceptable acids or bases.

Preservatives are generally included in pharmaceutical compositions to retard microbial growth, extending the shelf life of the compositions and allowing multiple use packaging. Examples of preservatives include phenol, meta-cresol, benzyl alcohol, para-hydroxybenzoic acid and its esters, methyl paraben, propyl paraben, benzalconium chloride and benzethonium chloride. Preservatives are typically employed in the range of about 0.1 to 1.0 % (w/v).

Preferably, the pharmaceutically compositions are given to an individual in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. Typically, this will be to cause a therapeutically useful activity providing benefit to the individual. The actual amount of the compounds administered, and rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980. By way of example, and the compositions

are preferably administered to patients in dosages of between about 0.01 and 100mg of active compound per kg of body weight, and more preferably between about 0.5 and 10mg/kg of body weight .

The composition may further comprise one or more other pharmaceutically active agents, either further compounds of the invention, inositol phosphoglycans, growth factors such as insulin, NGF or other growth factors listed below, or other drugs, e.g. those in use for the treatment of diabetes or other conditions set out below.

Medical Uses

The PGM compounds of the invention can be used in the preparation of medicaments for the treatment (either prophylactically or therapeutically) conditions characterised by dyslipidemia or lipodystrophy. The results disclosed herein show that PGMs of the present invention can be used to treat conditions characterised by abnormal or elevated levels of cholesterol, plasma triglycerides, and/or high density lipoproteins (HDL), especially elevated levels or amounts of these substances, or an abnormal LDL: HDL ratio.

Lipoproteins are the form in which lipids are transported in mammals and are characterised by their densities as follow: 1.006-1.019, very low density (VLDL); 1.019-1.063, low density (LDL); 1.063-1.21, high density (HDL); and >1.21, very high density (VHDL). Lipoproteins range in molecular weight from about 200 kD to about 10,000 kD and have lipid contents between about 4 and 95%.

Examples of these conditions include lipidic disorders associated with Type 1 or Type 2 diabetes, cardiovascular

disorders and their complications such as coronary artery disease (CAD), e.g. other than that associated with diabetes, hyperlipidemia, the treatment of systemic lupus erythematosis (SLE), especially atherosclerotic lesions.

Abbreviations

NIDDM, Non-insulin-dependent diabetes mellitus.

P. yoelii, Plasmodium yoelii.

TNF, Tumour necrosis factor.

IPGs, Inositol phosphoglycans.

GPI, Glycosylphosphatidyl inositol.

PGM, Phosphoglycan messenger.

PKA, cAMP-dependent-protein kinase A.

STZ, Streptozotocin.

HPAE, High-performance Anion Exchange Chromatography.

PAD, Pulsed Amperometric Detection.

PDH, Pyruvate dehydrogenase.

FBPase, Fructose-1,6-Bisphosphatase.

G6Pase, Glucose-6-phosphatase.

Materials and Methods

Streptozotocin, activated charcoal, ascorbic acid, ammonium molybdate, *myo*-inositol and bovine serum albumin (BSA) were obtained from Sigma, Poole, UK. D-[3-^{3}H] glucose (18 Ci/mmol) was obtained from Amersham Pharmacia, Bucks, UK. Collagenase and insulin were obtained from (Boehringer Mannheim GmbH, Germany). Silica gel 60 HPTLC aluminium sheets were obtained from (Merck, Darmstadt, Germany). 50% Sodium hydroxide was obtained from (Fisher Scientific, Loughborough, UK). Ion exchange resin AG1-X8 (HO^-, 20-50 mesh) was obtained from Bio-Rad Laboratories (Hemel Hempsted, UK). Cellulose microcrystalline was from (Merck, Germany).

MonoStandard™ was obtained from Dionex Corporation, Sunnyvale, CA, USA. All other materials were of high purity and were obtained from BDH.

Mice

The normal (C57BlxBalb/c) F_1 mice and obese diabetic (C57BL/Ks - *db/db*) were bred in our animal colony from parental strains obtained from the National Institute for Medical Research, Mill Hill, London, UK. Wistar rats (120-150 g) and obese diabetic (C57BL/6J-*ob/ob*) mice were obtained from Harlan Olac Ltd, Bicester, UK. Male mice 8-12 weeks old were used, when both their blood glucose and insulin levels were markedly raised. Mice were allowed to acclimatize for at least 7 days before being used. All animals had free access to water and were fed ad libitum with normal laboratory chow.

Induction of diabetes with Streptozotocin (STZ)

Mice were made diabetic by 3 daily i.p. injections of 100mg/kg bw STZ dissolved in 0.01N citrate buffer (pH 4.5) within 5 min of solubilization. Control groups of mice received injections of equivalent volumes of sodium citrate buffer. Blood glucose rose to 15-20 mmol/l and remained stable for 3 weeks. The syndrome induced in mice or rats by STZ injection closely resembles that observed in patients with Type 1 diabetes.

Parasites

The lethal YM line of *P. yoelii* strain 17X (from Dr. A. Holder, NIMR, London, UK), was maintained in (C57BLxBalb/c) F_1 mice by blood passage of parasitized red blood cells. Mice were bled 5-7 days after intravenous infection with 10^4 parasites and parasitaemia was determined from blood films stained with Giemsa.

Extraction and purification of GPIs

Plasmodium yoelii was maintained by blood passage of 10^4 parasitised red cells in F_1 mice. Mice were bled 5-7 days after infection. Parasitized blood (>90% parasitaemia) was washed x 3 with sterile saline by centrifugation at 3000 rpm at 4°C, and the pellet was lysed by incubation in 0.01 % saponin for 3 minutes at room temperature. Parasites were washed x 3 with sterile saline by centrifugation at 3000 rpm at 4°C. The pellet was suspended in 5 ml saline, sonicated for 12 seconds and freeze dried. GPIs were extracted following a procedure described before with some modifications (Gerold et al, 1994). Briefly, parasites (9×10^{10}) from 10 mice (0.1 gram dry weight) were extracted twice with 10 ml of Chloroform: Methanol: Water (10:10:3)(CMW) and centrifuged for 15 minutes at 1800 rpm. The CMW extracted GPIs were pooled, dried in Speed-Vac and suspended in 5 ml of water-saturated n-butanol. An equal volume of water was added, thoroughly mixed, and centrifuged for 10 minutes at 1800 rpm. The organic top layer was removed by aspiration and the bottom layer of water was back-extracted with an equal volume of water-saturated n-butanol. The organic phases were pooled and dried. Non-lipidic material was removed following a procedure described before with some modifications (Zawadski et al, 1998). Briefly GPIs were dried and suspended in 1 ml of 5% 1-propanol containing 0.1M ammonium acetate and applied to C_8 Bond Elute™ cartridge equilibrated in 5% 1-propanol containing 0.1M ammonium acetate. The cartridge was then washed with 10 ml 5% 1-propanol containing 0.1M ammonium acetate. GPIs were eluted with 10 ml of 40% 1-propanol and 10 ml of 60% 1-propanol. Eluates containing glycolipids were pooled

and dried in a rotary evaporator.

Isolation of inositol phosphoglycans (IPGs)

Plasmodium yoelii IPGs were extracted from parasitized erythrocytes as described before (Caro et al, 1996) with some modifications. Briefly, mice infected with the lethal YM line of *P. yoelii* were bled 5 -7 days after infection into tubes containing heparinised saline. Parasitized erythrocytes were pelleted and washed twice with saline by centrifugation at 3000 rpm at 4°C for 20 min. Parasitized erythrocytes (>90% parasitaemia) containing 20-30 $x10^9$ parasites were homogenised and boiled for 5 minutes in 25 ml of a solution of 50 mM formic acid, 1 mM EDTA and 1mM 2-mercaptoethanol. The extract was centrifuged at 18,000 x g for 90 minutes at 4°C. The supernatant was stirred for 10 minutes on ice with charcoal (10 mg/ml) and centrifuged at 18,000 x g for 30 minutes at 4°C. The supernatant was then diluted with 10 volumes of distilled water, adjusted to pH 6 with 10% NH_4OH, and shaken overnight at 4°C with AG1X8 ion exchange resin (formate form). The resin was poured into a column and washed with 2 bed volumes of water and 2 bed volumes of 1 mM HCl (pH 3). The resin was sequentially eluted with 5 bed volumes of 10 mM HCl (pH 2) to yield IPG-P, followed by 5 bed volumes of 50 mM HCl (pH 1.3) to yield IPG-A respectively. The two fractions were concentrated and freeze dried twice to remove residual HCl and stored at -20°C. IPGs were injected i.v. in 0.2 ml saline. Control preparations derived from the same number of normal red cell ghosts were made as described above.

Cellulose column chromatography

Plasmodium yoelii IPGs eluted from the anion exchange

resin were subjected to cellulose chromatography. IPGs were dissolved in 1 ml of a solvent containing n-butanol, ethanol, and water [4:1:1] (B:E:W) and applied slowly onto a cellulose column (1 ml) pre-equilibrated in B:E:W [4:1:1]. The column was sequentially eluted with 5 ml of B:E:W, 5 ml methanol, 5 ml water, and 5 ml 50 mM HCl (pH 1.3). Different fractions were concentrated and freeze dried.

Metabolic labelling of *P. yoelii* schizonts

Inositol-free MEM tissue culture medium was used. The medium was supplemented with 0.001% (w/v) Para-amino benzoic acid; L-methionine, 14.92 mg/ml; L-glutamine, 292 mg/ml; L-cystine, 24.02 mg/ml; L-arginine 126.4 mg/ml; L-leucine, 52.46 mg/ml, 5% foetal calf serum and 5.5 mM glucose. Parasites were obtained from 4 infected 12 wk old male (C57/Bl6 x Balb/c)F_1 mice. Parasitaemias were 90-95% comprising mainly trophozoites and schizonts in roughly equal number. Parasitized RBCs (PRBCs) were collected aseptically in heparanized tissue culture medium and washed once by centrifugation at 2500 rpm for 10 minutes. The pellet was resuspended in 10 ml culture medium (1 x10^9 parasites/ml) and incubated with 250 µCi of *myo*-[^{3}H]inositol in 25 ml Falcon TC flask for 3 hours at 37°C with 5% CO_2. Giemsa-stained blood smears showed that the majority of the late trophozoites had transformed into schizonts. Parasites were viable during the course of labelling. Labelled PRBCs were washed and lysed with saponin and GPIs were extracted from labelled PRBCs as described above.

Thin layer chromatography

GPIs were dissolved in CMW (10:10:3) and applied to silica gel HPTLC plates. These were developed twice in a

solvent containing Chloroform: Acetone: Methanol: glacial acetic acid: Water (50:20:10:10:5). GPIs were detected using orcinol and ninhydrin reagents. Biologically active glycolipids that remained at the origin, were scraped and eluted with 2 x 20 ml of methanol, filtered and dried using a rotary evaporator. Control preparations derived from the same number of normal red cell ghosts were made as described earlier. Radioalabelled GPIs were monitored by fluorography after HPTLC sheets were sprayed with En3H enhancer (New England Nuclear) and exposed to BioMax Mj Film (Kodak) at -80°C for 10 days.

Assay of inorganic phosphate

Inorganic phosphate in malaria GPIs and IPGs was determined following the standard procedure with some modifications (Bartlett, 1958). Disodium hydrogen phosphate was used as standard (0-2µM). Briefly, 10 µl of samples and standards were dried and hydrolysed with 90 µl perchloric acid (70%) at 180°C for 45 minutes. Distilled water (600 µl), 5% ammonium molybdate (50 µl) and 10% ascorbic acid (50 µl) were added and incubated at 95°C for 15 minutes. Absorbance was measured at 830 nm in an OPTI max™ microplate reader (Molecular Device Corporation, Sunnyvale, CA, USA).

Assay of hexosamines

The hexosamine content of *Plasmodium yoelii* of malaria GPIs and IPGs were measured following a procedure described before (Bosworth et al, 1994). Briefly, 100 µl samples were deacetylated with 100 µl 5.5 N HCl at 105°C and adjusted to pH 3 with 100 µl 6M potassium acetate. 100 µl of 10 % acetic acid and 50 µl $NaNO_2$ were added to the mixture on ice. After 30 minutes 100 µl of ammonium

sulfamate was added. Fluorescence was developed after coupling with 100 µl of 0.8 % (w/v) 3,5-diaminobenzoic acid. To the mixture, 20 µl of 12 N HCl was added and diluted five fold in water. Fluorescence was measured at 514 nm, the excitation wavelength being 422 nm.

Acid hydrolysis of GPIs

Plasmodium yoelii GPIs were hydrolysed at 100°C in Teflon-lined screw-capped tubes using100 µl of 4N HCl for 4 hours or 6N HCl for 24 hours. Hydrolysates were centrifuged at 2000 rpm for 20 minutes at 4°C and the supernatants dried in a rotary evaporator. 100 µl of methanol was added and dried twice and the samples were dissolved in 200-400 µl water and filtered through a 0.2 mm PTFE filter. Sugars released by acid hydrolysis were separated by strong anion exchange HPLC on Carbopak TM PA10 (4 x 250 mm, Dionex) and Carbopak TM MA1 (4 x 250 mm, Dionex) analytical columns. Retention times were compared with a set of authentic standards.

Acid hydrolysis of IPGs

Plasmodium yoelii IPGs were hydrolysed at 100°C in Teflon-lined screw-capped tubes using 100 µl of 4N HCl for 4 hours or 6N HCl for 24 hours. Hydrolysates were centrifuged at 2000 rpm for 20 minutes at 4°C and the supernatants were dried in a Speed Vac. To remove residual acid, 100 µl of methanol was added and dried, this treatment was repeated twice. Samples were dissolved in 200-300 µl water and passed through a 0.2 µm PTFE filtered (Whatman, NJ, USA) and stored at -20°C.

Carbohydrate chromatography

Dionex 500 HPLC system carbohydrate analyser from Dionex Corporation, Sunnyvale, CA, USA. It consists of an

eluant degassing module, GP40 gradient pump module, AS 50 Autosampler, ED40 amperometric detector with a working gold electrode (Ag/Ag Cl reference electrode) and PeakNet chromatography workstation software was used.

Monosaccharides and monosaccharide-alditols of *Plasmodium yoelii* IPGs hydrolysates were analysed by HPLC using Carbopac™ PA10 and Carbopac™ MA1 columns respectively. An AminoTrap guard column was used to eliminate any possible interference of amino acids and peptides.

Carbopak™ PA10 column chromatography

A Carbopak™ PA10 column (4 x 250 mm) with a guard column (4 x 50 mm) was used for the separation of sugars as recommended by the supplier (Weitzhandler et al, 1996). Before injecting each sample, columns were regenerated by elution with 200 mM sodium hydroxide for 15 minutes and were equilibrated for 15 minutes with 18 mM sodium hydroxide at a flow rate of 1 ml/minute. The monosaccharides were resolved by isocratic elution with 18 mM sodium hydroxide for 20 minutes. Standard carbohydrate detection waveform (E1/2/3= +0.05/ +0.75/ -0.15 V and Time1/2/3 = 0.0-0.4/ 0.41-0.6/ 0.61-1 s) was applied to the ED40 working electrode for pulsed amperometric detection (PAD). 10 µl of a cocktail of monosaccharide standards, MonoStandard™ (fucose, galactosamine, glucosamine, galactose, glucose, mannose) was used for calibration.

Carbopak™ MA1 column chromatography

A Carbopak™ MA1 column (4 x 250 mm) with a guard column (4 x 50 mm) was used for the separation of monosaccharide-alditol (*chiro*-inositol and *myo*-inositol) as recommended by the supplier. 612 mM NaOH was used for regeneration and re-equilibration of columns.

Chiro-inositol and *myo*-inositol were resolved using an isocratic system of NaOH 612 mM (0.4 ml/minute) for 30 minutes. ED40 operating parameters were E1= +0.05 V, E2= +0.65 V, E3= -0.10 V. *Myo*-inositol, *chiro*-inositol, mannitol, fructose and glucose were used to calibrate the column.

Lipogenesis assay

Male Wistar rats (120-150 g) were killed by cervical dislocation and adipocytes were obtained from the epididymal adipose tissue and prepared by digestion with collagenase according to the method of Rodbell (1964) with some modifications. Fat pads from two rats were dissected and placed in Krebs Ringer Hepes (KRH) buffer containing 9.2 mM Hepes, 2.2 mM $NaH_2PO_4.2H_2O$, 10 mM $NaHCO_3$, 132 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4.7H_2O$, 2.5 mM $CaCl_2.6H_2O$, 2% BSA and 5 mM glucose, pH 7.4. Fat pads were finely minced with scissors and incubated in10 ml KRH buffer containing 20 mg collagenase for 20-30 minutes at 37°C in a shaking water bath with continuous gassing with 95% O_2 and 5% CO_2.

Lipogenesis was determined as the incorporation of [3-^{3}H] glucose into toluene-extractable lipids. Briefly, into a 96-multiwell plate, 100 µl of adipocyte suspension (3.5 x 10^5/ml) was incubated for 30 minutes at 37°C in a CO_2 incubator with 2 µl of various concentrations of GPIs or IPGs. Lipogenesis was initiated by the addition of 100 µl KRH containing 0.2 µCi D-[3-^{3}H] glucose, and the incubation continued for 2 hours. Adipocytes were harvested onto glass-fibre filter mats using a cell harvester, and rinsed with 5 mM glucose in 0.154M NaCl. 3 ml of a toluene-based scintillation cocktail were added to each filter disc for counting the radioactivity

incorporated into lipids.

Effects on pyruvate dehydrogenase phosphatase (PDH)

HPTLC-purified GPI was sonicated in 100 µl of water and tested for its effect on bovine heart PDH-phosphatase. GPIs were sonicated in water before assay. Different fractions of IPGs eluted from cellulose columns were tested for their effects on bovine heart PDH-phosphatase. 2-16 µl of IPGs (containing 1-2 nmol phosphate/ml) were used to stimulate PDH phosphatase. Activated PDH was determined spectrophotometrically following the procedure described earlier (Caro et al, 1997) by measuring the rate of production of NADH at 340nm (Jasco V560 spectrophotometer, Jasco corporation, Tokyo, Japan).

Inhibition of cAMP-dependent protein kinase activity (PKA)

GPIs were sonicated in dilution buffer. The ability of GPIs and IPGs to inhibit PKA activity was determined using a colourimetric assay kit and a standard PKA preparation (Pierce, Rockford, IL, USA). Kemptide labelled with a fluorescent probe was used as PKA substrate. Phosphorylated Kemptide was detected by measuring absorbance at 570nm in OPTI max™ microplate reader (Molecular Device Corporation, Sunnyvale, CA, USA).

Measurement of plasma cholesterol and triglycerides

Blood was collected from the trunk after decapitation into heparinised tubes. Plasma was separated by centrifugation and frozen -20°C. Plasma was assayed for total cholesterol and total triglycerides using kits from Sigma diagnostic. HDL cholesterol was assayed after precipitation of LDL cholesterol using phosphotungistic

acid supplied by Sigma Diagnostics. Results, in mmol/l, are expressed as means ± SEM.

Statistical analysis

Statistical significance was assessed using ANOVA or Student's t-test for unpaired samples as appropriate. Values of $P<0.05$ were considered to be statistically significant.

Results

Effect of GPIs on total cholesterol and plasma triglycerides

The effects of *Plasmodium* GPI on plasma cholesterol and triglycerides level in STZ-diabetic mice are shown in Figures 1A and 1B. 6 hours after injection of GPI (10 nmole/mouse, i.v.) there was a significant drop in total plasma cholesterol compared to saline treated mice (Figure 1A, $p<0.01$, $n=7$). 6 hours after injection of GPI (10 nmole/mouse, i.v.) there was a significant drop in plasma triglycerides compared to saline treated mice(Figure 1B, $p<0.05$, $n=6$).

Effect of *Plasmodium yoelii* GPI on plasma cholesterol in STZ-diabetic mice

The effects of *Plasmodium yoelii* GPI on plasma cholesterol and the ratio of LDL:HDL lipids is shown in Figure 2 and Table 1.

Table 1:

Treatment	Total Cholesterol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	Ratio of LDL/HDL
STZ-mice + GPI	2.460	2.456	0.004	0.002
STZ-mice + saline	3.838	3.025	0.813	0.269
Normal F1 mice	2.725	2.506	0.219	0.087

Chemical composition of *Plasmodium yoelii* GPIs

Initial characterisation of the total biologically active *Plasmodium yoelii*-derived GPIs anion revealed the presence of phosphorus and hexosamines. Using a Dionex-HPLC chromatography with Carbopac TM MA1 column on acid hydrolysates of GPIs after 24h hydrolysis in 6N HCl demonstrated the presence of *myo*-inositol and glycerol. Analysis on Carbopac TM PA10 column showed the presence of glucosamine, galactose, mannose, glucose and galactosamine. *Plasmodium yoelii* GPI was found to be labelled with H^3-*myo*-inositol.

Effect of *Plasmodium yoelii* IPGs on cAMP-dependent protein kinase activity (PKA)

The ability of *Plasmodium yoelii* IPGs to inhibit PKA activity was determined after fractionation of IPGs on cellulose chromatography columns (Figure 3). Different concentrations (0.001-7 µM) of acid and water eluates were tested. Both IPG-P and IPG-A induced a dose-related inhibition of cAMP-dependent protein kinase

activity, with IPG-P being more inhibitory. Acid eluates of IPG-A and IPG-P inhibited PKA by more than 95%. 50% inhibitory dose concentrations (IC_{50}) for the acid eluates of IPG-A and IPG-P were 0.9 and 0.09 μM respectively.

Effect of *Plasmodium yoelii* IPGs on lipogenesis

IPG-P and IPG-A eluted from the anion exchange resin were subjected to cellulose chromatography column (1 ml) and eluted with different solvents (refer to the method section) and 1 μM of IPGs (equivalent of phosphate) was used for the lipogenesis assay. IPG-P from mammalian sources normally has no significant lipogenic activities. *Plasmodium* IPG-P increased lipogenesis in adipocytes by 20-30% from basal (Table 2, with methanol fraction stimulation was statistically significant, $p<0.01$). In the presence of a maximal dose of insulin (10^{-8} M), IPG-P increased lipogenesis by 20-30% (Table 1, with acid fraction stimulation was statistically significant, $p<0.01$). However, different fractions of IPG-A had no significant lipogenic activity (Table 2).

Table 2. Effect of IPG-A and IPG-P fractions (1μM) eluted from a cellulose column on lipogenesis in absence of insulin and in presence of a maximal concentration of insulin (10^{-8}M). Values are mean of percentage stimulation ± SD, *$p<0.01$ vs basal, n=4.

Fraction	IPG-P (1µM)		IPG-A (µM)	
	(-) insulin	(+) insulin	(-) insulin	(+) insulin
B:E:W	13.1 ± 4.6	20.2 ± 6.0	-21.2 ± 2.3	-20.5 ± 3.4
Methanol	23.9 ± 0.4*	23.0 ± 5.4	-3.4 ± 0.4	-6.4 ± 1.3
Water	31.9 ± 6.8	30.9 ± 7.0	4.4 ± 0.5	1.8 ± 0.4
Acid	21.6 ± 5.9	30.2 ± 1.7*	2.2 ± 0.2	5.7 ± 0.7

Effects on pyruvate dehydrogenase phosphatase (PDH)

The ability of *Plasmodium yoelii* IPGs to stimulate PDH was determined after fractionation of IPGs on cellulose chromatography columns. Only IPG-P (acid fraction) stimulated PDH-phosphatase. Different concentrations of IPG-P (0.006, 0.0125 and 0.02 µM) stimulated PDH-phosphatase by 60, 100 and 130% above the base line value and at 0.05 µM by 101% (Figure 4). 50% effective dose concentration of IPG-P (ED_{50}) was 0.005 µM. IPG-A fractions had no effect on PDH phosphatase (data not shown).

Chemical composition of *Plasmodium yoelii* IPGs

Initial characterisation of the total biologically active *Plasmodium*-derived molecules recovered from the anion-exchange resin revealed the presence of phosphorus and hexosamines. Thus, the *Plasmodium yoelii* IPGs from

15 infected mice contained 0.568 µmole and 0.265 µmole inorganic phosphate in IPG-A and IPG-P respectively. Hexosamine concentrations in IPG-A and IPG-P were 0.117 µmole and 0.106 µmole respectively. Using a Dionex-HPLC chromatography with Carbopac TM PA10 column on acid hydrolysates of IPG-A (Figure 5a) and IPG-P (Figure 6a) after 24 h hydrolysis in 6N HCl demonstrated the presence of galactosamine, glucosamine and glucose (Figure 5a). However, there was a difference in the composition of sugar released after 4 h hydrolysis of IPGs in 4N HCl acid (Figures 5b and 6b). After 4 h hydrolysis of IPG-A, galactosamine, galactose, mannose and glucose were released (Figure 5b). However, after 4 h hydrolysis of IPG-P, in addition of galactosamine, galactose, mannose and glucose, galactosamine was also released. (Figure 6b). Analysis on Carbopac™ MA1 column showed the presence of *myo*-inositol and glycerol in IPG-A (Figure 5c) and (Figure 6c) after hydrolysis in 6N HCl for 24h.

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The references mentioned herein are all incorporated by reference.

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Claims:

1. A substance which is a carbohydrate derivatised phosphatidyl cyclitol.

2. The substance of claim 1, wherein the substance comprises one or more lipid moieties attached to the cyclitol group or the phosphatidyl group via one or more ester and/or ether linkages.

3. The substance of claim 1 or claim 2, wherein the substance has one or more properties selected from modulating plasma cholesterol, plasma triglycerides and/or high density lipoprotein levels and/or modulating the LDL:HDL ratio.

4. The substance of claim 3 further having one or more properties selected reducing properties selected from reducing blood glucose in a diabetic *ob/ob* or *db/db* murine model, regulating lipogenesis, stimulating pyruvate dehydrogenase phosphatase, inhibiting cAMP dependent protein kinase, inhibiting fructose-1,6-biphosphatase, and/or inhibiting glucose-6-phosphatase.

5. The substance of any one of claims 1 to 4, wherein the cyclitol is *chiro*-inositol, *myo*-inositol or derivatives thereof.

6. The substance of claim 5, wherein the cyclitol is pinitol or a derivative thereof.

7. The substance of any one of claims 1 to 6, wherein the phosphatidyl group has one oxygen linked lipid moieties.

8. The substance of any one of claims 1 to 6, wherein the phosphatidyl group has two oxygen linked lipid moieties.

9. The substance of claim 7 or claim 8, wherein the lipid(s) attached phosphatidyl group is or are diacyl, dialkyl, acyl-alkyl, alkyl-acyl, lysol-acyl, lysol-alkyl, acyl-lysol or alkyl-lysol.

10. The substance of any one of claims 1 to 9, wherein the carbohydrate group is a hexose.

11. The substance of claim 10, wherein the carbohydrate is glucosamine, galactosamine, galactose, mannose or glucose.

12. A substance which is a carbohydrate derivatised phosphatidyl cyclitol which is devoid of protein.

13. The substance of any one of the preceding claims for use in method of medical treatment.

14. The substance of claim X wherein the substance is used for treatment of lipodystrophy or dyslipidemia.

15. Use of a substance of any one of claims 1 to 12 for the preparation of a medicament for the treatment of a condition characterised by an abnormal level or amount of cholesterol, plasma triglycerides or high density lipoproteins (HDL).

16. The use of claim 15, wherein the abnormal level or amount is an elevated level or amount.

17. The use of claim 15 or claim 16, wherein the substance normalises the ratio of low density: high density lipoproteins (LDL: HDL).

5

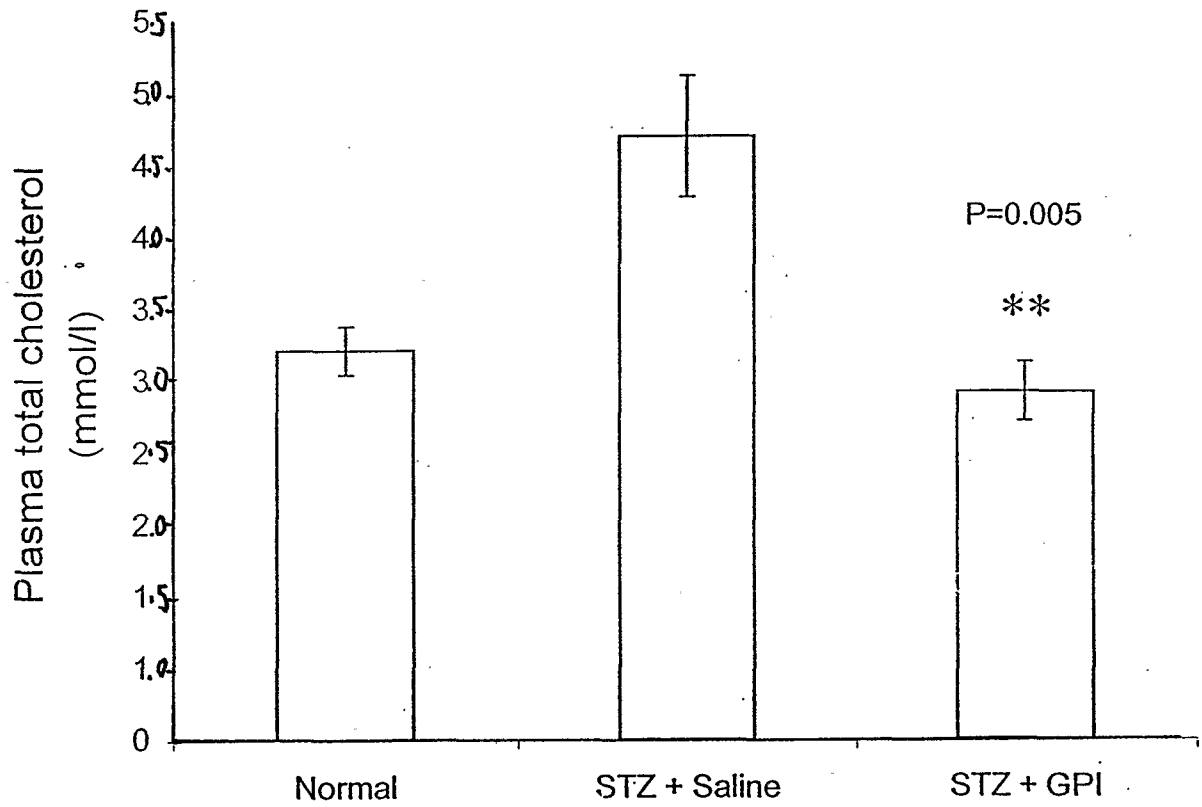

Plasma total cholesterol
(mmol/l)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
P=0.005
**
Normal
STZ + Saline
STZ + GPI

Fig 1A

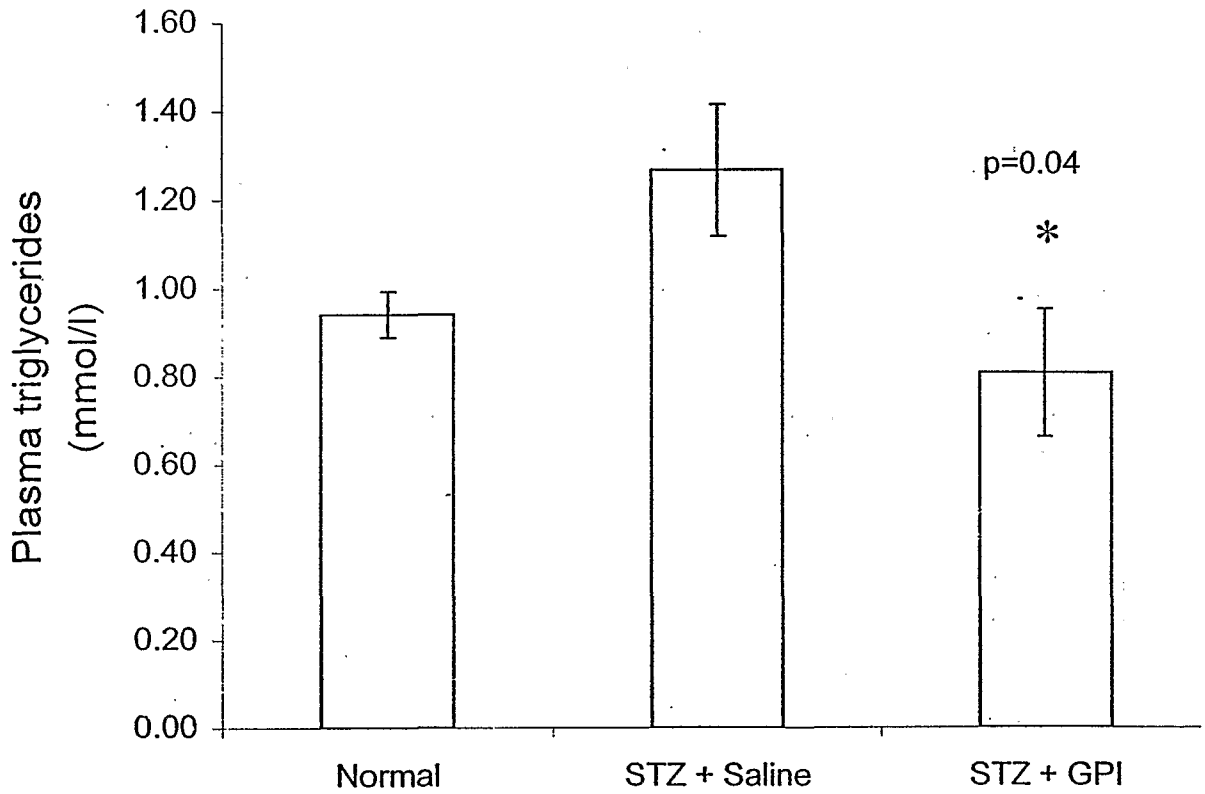
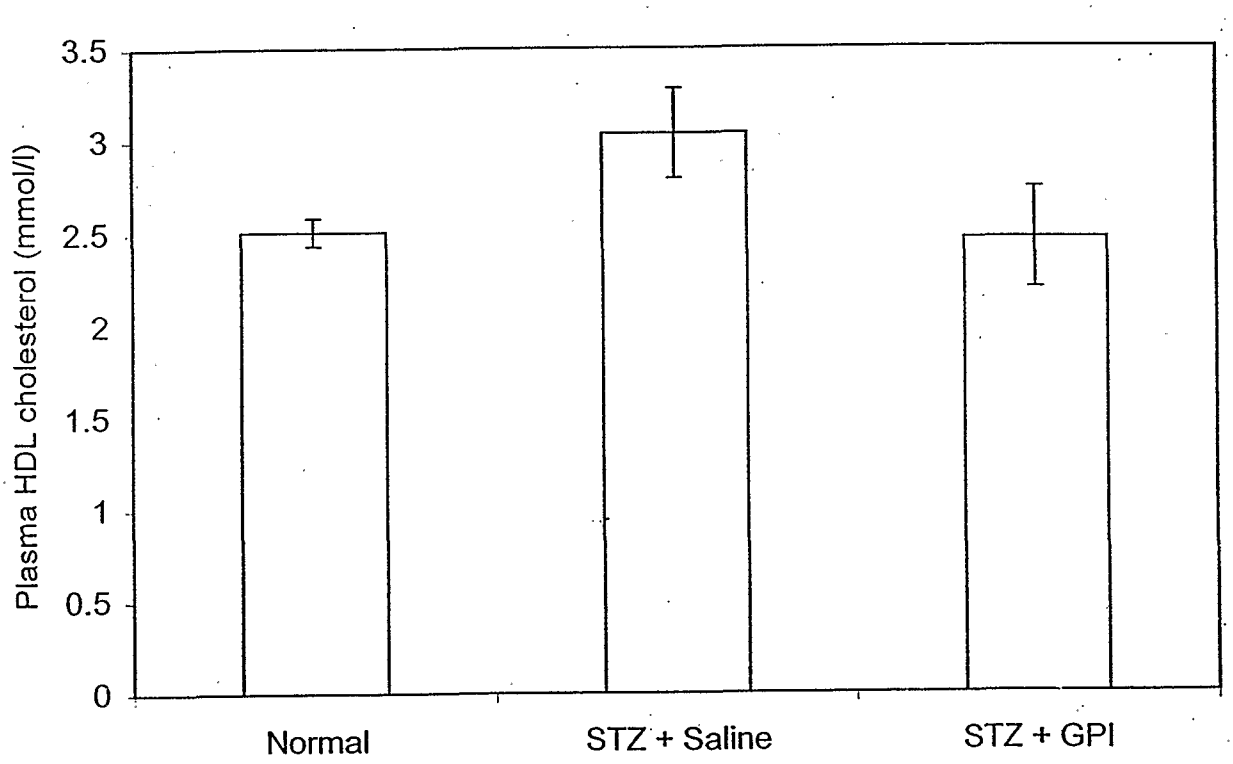

Plasma triglycerides (mmol/l)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
p=0.04
*
Normal
STZ + Saline
STZ + GPI

Fig 1B


Plasma HDL cholesterol (mmol/l)
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3
2.5
2
1.5
1
0.5
0
Normal
STZ + Saline
STZ + GPI

Fig 2

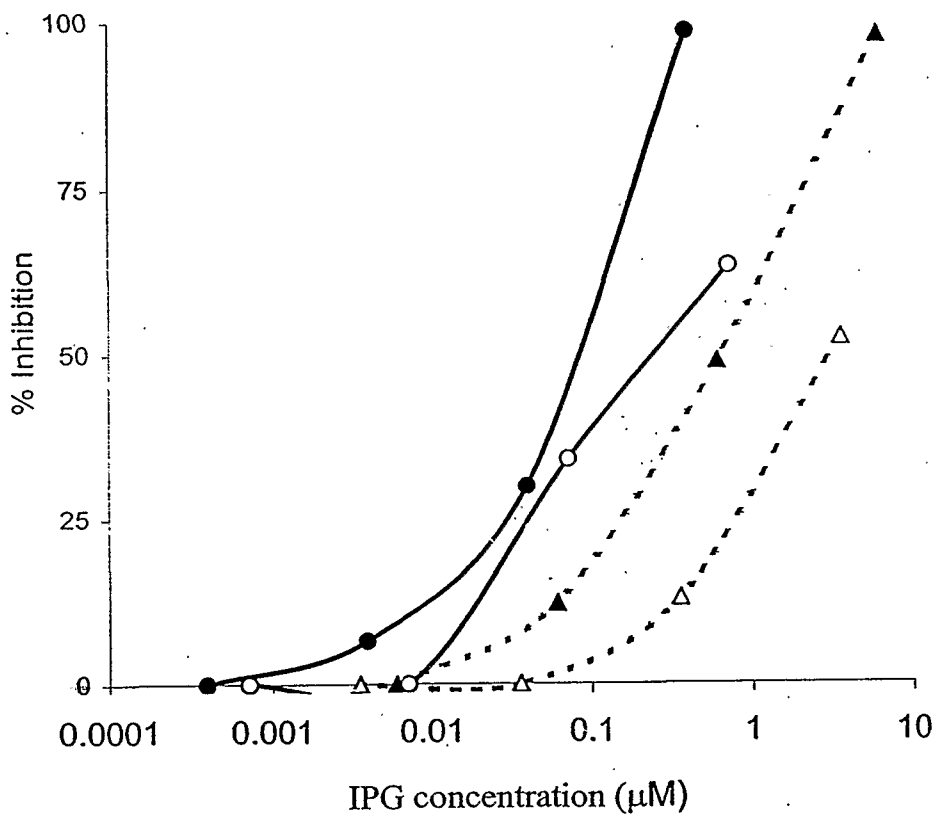
100
75
50
25
0
% Inhibition
0.0001
0.001
0.01
0.1
1
10
IPG concentration (μM)

Fig 3

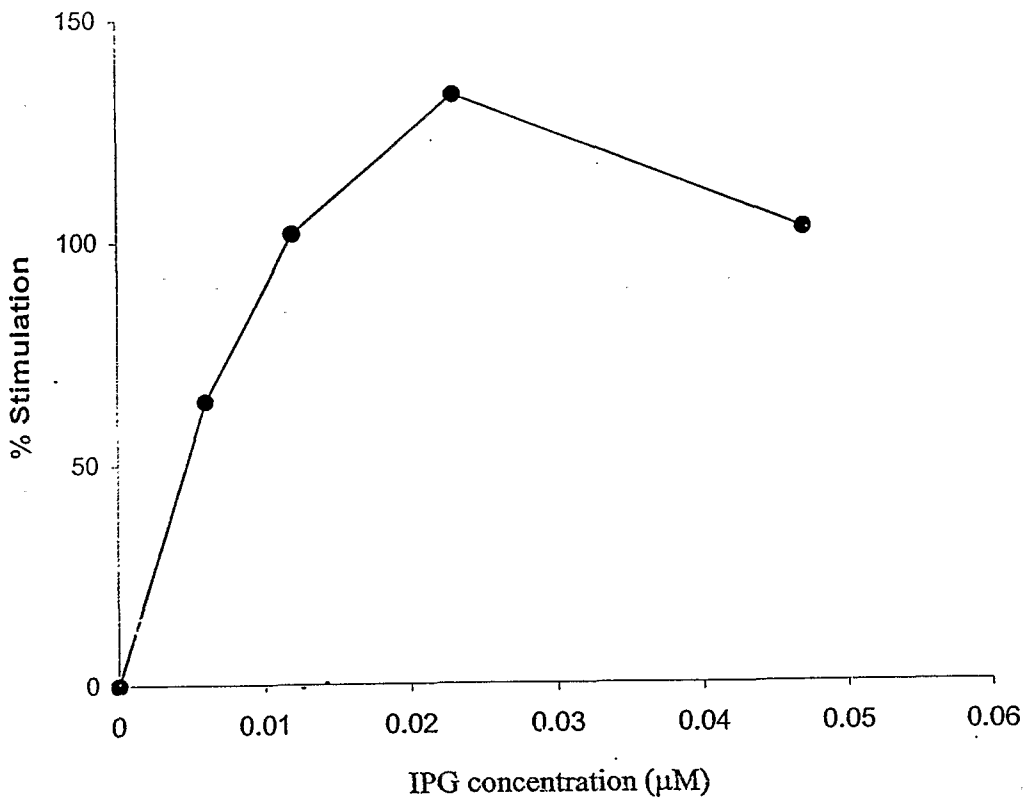
150
100
50
0
% Stimulation
0
0.01
0.02
0.03
0.04
0.05
0.06
IPG concentration (μM)

Fig 4

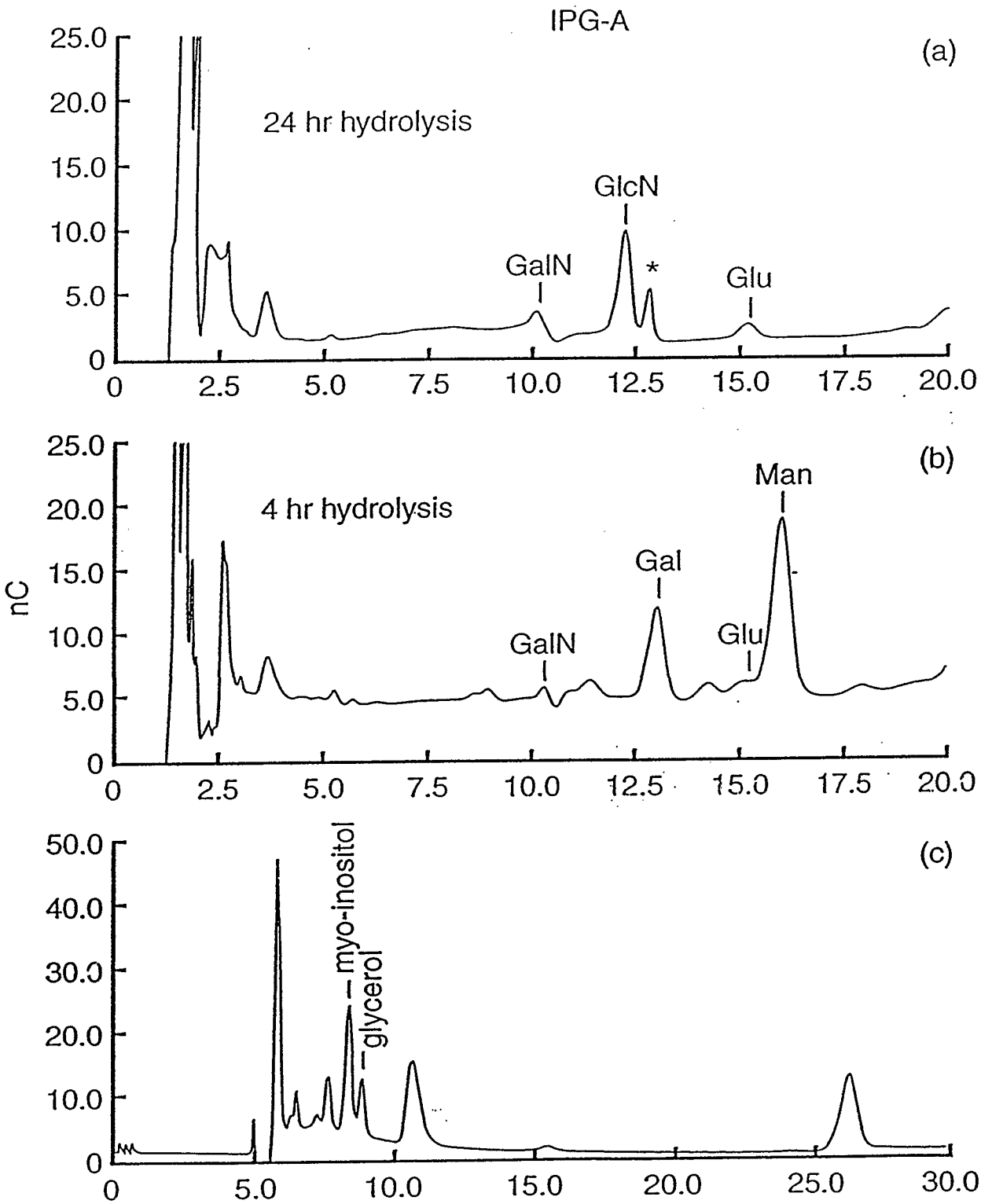
IPG-A
(a)
24 hr hydrolysis
GlcN
GalN
*
Glu
(b)
4 hr hydrolysis
Man
Gal
GalN
Glu
nC
(c)
myo-inositol
glycerol

Fig 5

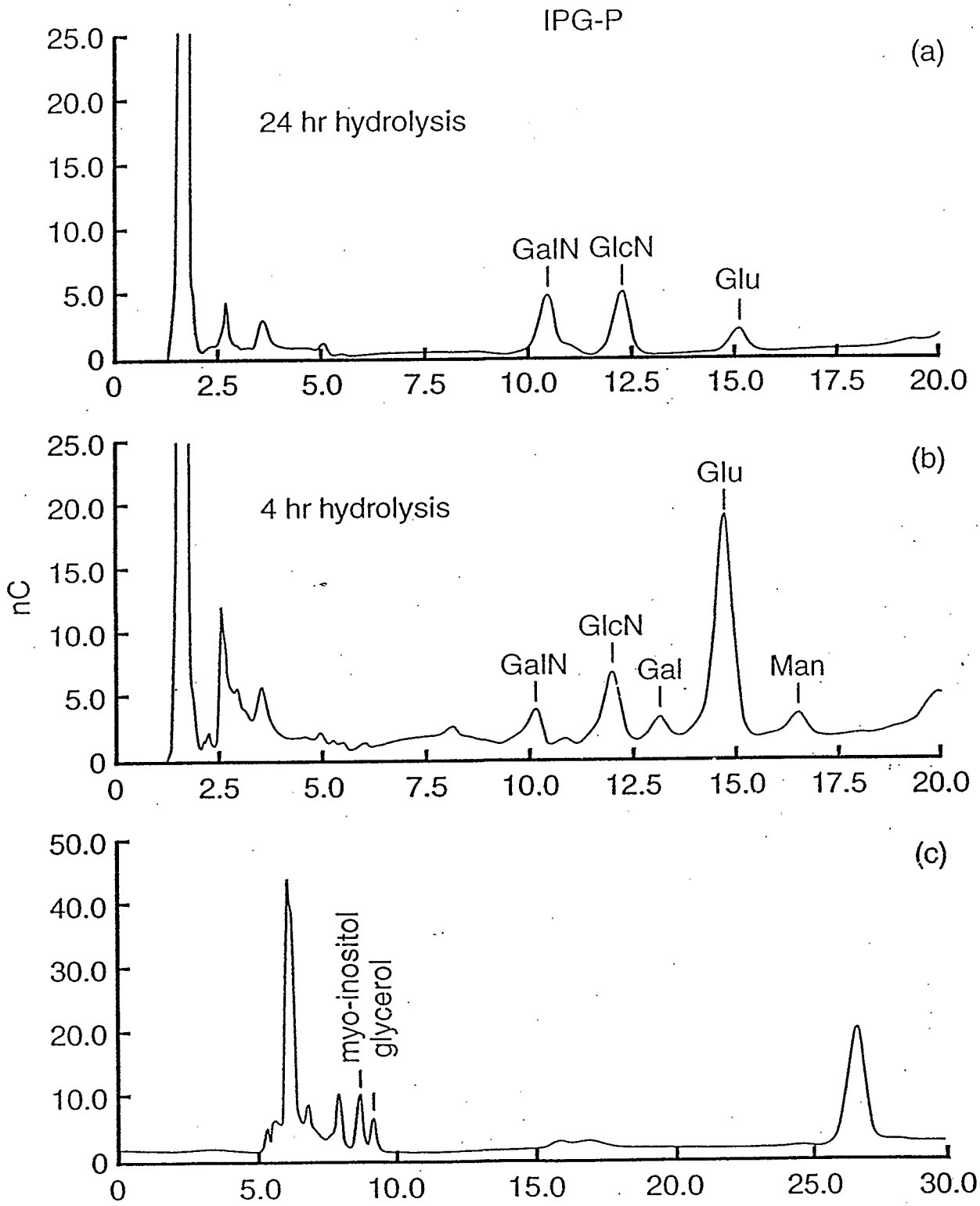
IPG-P
(a)
24 hr hydrolysis
GalN GlcN
Glu
(b)
4 hr hydrolysis
Glu
GlcN
GalN
Gal
Man
nC
(c)
myo-inositol
glycerol

Fig 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/02775

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07G3/00 C07H1/08 A61K31/70 A61P3/00

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 C07G C07H A61K A61P

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, 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	GIGG R ET AL: "SYNTHESIS OF GLYCOSYLPHOSPHATIDYLINOSITOL ANCHORS" GLYCOPEPTIDES AND RELATED COMPOUNDS, DEKKER, NEW YORK,, US, 1997, pages 327-392, XP000897779 page 369 -page 380 ---	1-14
X	P. GEROLD ET AL.: "Glycosylphosphatidylinositols synthesised by asexual erythrocytic stages of the malaria parasite, Plasmodium falcoparum" J. BIOL. CHEM., vol. 269, 1994, pages 2597-2606, XP002176134 abstract ---	1-14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2001	25/09/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	de Nooy, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/02775

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	S. COTTAZ ET AL.: "Parasite glycoconjugates. Part 3. Synthesis of substrate analogues of early intermediates in the biosynthetic pathway of glycosylphosphatidylinositol membrane anchors" J. CHEM. SOC. PERKIN TRANS. 1, 1995, pages 1673-1678, XP002176135 the whole document	1-14
X	C.J.J. ELIE ET AL.: "Synthesis of 1-O-(1,2-di-O-palmitoyl-SN-glycero-3-phosphoryl)-2-O-alfa-D-mannopyranosyl-D-myo-inositol: fragment of mycobacterial phospholipids" TETRAHEDRON, vol. 45, no. 11, 1989, pages 3477-3486, XP002176136 page 3477 -page 3479	1-14
X	MARTIN-LOMAS M ET AL: "The solution conformation of glycosyl inositols related to inositolphosphoglycan (IPG) mediators" TETRAHEDRON: ASYMMETRY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 11, no. 1, January 2000 (2000-01), pages 37-51, XP004202370 ISSN: 0957-4166 structures 4 and 5	1-14
X	SCHOFIELD L ET AL: "REGULATION OF HOST CELL FUNCTION BY GLYCOSYLPHOSPHATIDYLINOSITOLS OF THE PARASITIC PROTOZOA" IMMUNOLOGY AND CELL BIOLOGY, CARLTON, AU, vol. 74, no. 6, 1996, pages 555-563, XP001007048 page 555	1-14
X	US 4 839 466 A (SALTIEL ALAN R) 13 June 1989 (1989-06-13) figure 15	1-14
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/02775

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 11116 A (RADEMACHER THOMAS WILLIAM ;CARO HUGO (GB); HOEFT RADEMACHER LIMITE) 19 March 1998 (1998-03-19) cited in the application abstract page 1	15-17

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-14 (in part)

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, it appears impossible to execute a meaningful search and/or to issue a complete search report over the whole breadth of the above mentioned claims. Consequently, the search was directed to the use of the claimed compounds in the preparation of a medicament for the treatment of conditions caharacterised by an abnormal level or amount of cholesterol, plasma triglycerides or high density lipoproteins (HDL).

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

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