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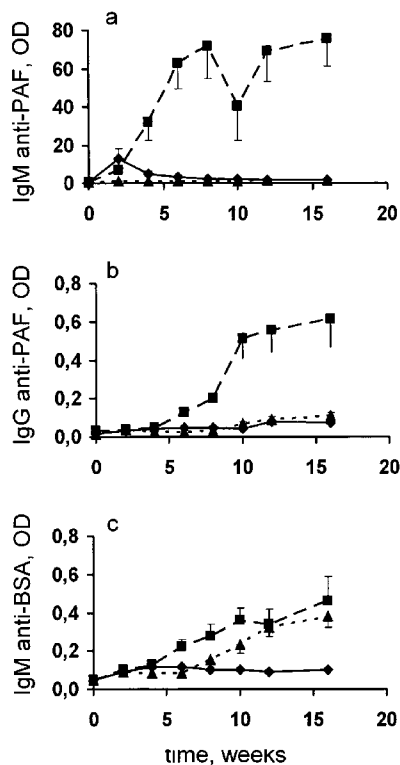
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(54) Title: DIAGNOSTIC AND THERAPEUTIC METHODS AND COMPOSITIONS FOR METABOLIC DISEASE

Figure 1



(57) Abstract: The present invention relates to a method for the immunization or prophylaxis against, or the treatment of, metabolic diseases in a mammal, the method comprising the step of administering to the mammal a pharmaceutical composition comprising at least one platelet activating factor (PAF) conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative. The metabolic disease may, for example, be a condition selected from the group consisting of metabolic syndrome, insulin resistance, glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS). Also provided is a method for diagnosing metabolic disease, or assessing a patient's risk of developing or progression of metabolic disease, the method comprising the steps of - (a) assessing the patient's level of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative; and (b) diagnosing metabolic disease or determining the patient's level of risk of developing or progression of metabolic disease based on the assessed levels of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.

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DIAGNOSTIC AND THERAPEUTIC METHODS AND COMPOSITIONS FOR METABOLIC DISEASE

FIELD OF THE INVENTION

5 The present invention relates to the treatment, prevention and diagnosis of metabolic diseases.

BACKGROUND OF THE INVENTION

10 The listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

15 The metabolic syndrome is the clustering of a number of symptoms that relates to the consequences of disturbances in energy metabolism, that is the metabolism of lipids, carbohydrates and proteins. Obesity, insulin resistance, diabetes, hypertension and hyperlipidemia are the components of the syndrome. Several definitions of the metabolic syndrome exist. The NECP/ATP III criteria (*Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III)*). JAMA, 2001. **285**(19), 2486-97) is one definition, according to which at least three of the following five criteria should be fulfilled: Blood pressure >130/85 mmHg or antihypertensive treatment, fasting plasma glucose > 6.1 mmol/l, serum triglycerides >1.7 mmol/l, waist circumference > 102 cm in men and >88 cm in women, HDL-cholesterol < 1.0 mmol/l in men and <1.3 in women. The individual components of the syndrome are themselves associated to increased morbidity and mortality, especially for premature cardiovascular disease (CVD), in individuals suffering from metabolic syndrome this risk is greatly increased (Bonora, E., *The metabolic syndrome and cardiovascular disease*. Ann Med, 2006. **38**(1), 64-80). All components of the metabolic syndrome have been associated to chronic systemic inflammation (Cirillo, P., Y.Y. Sautin, J. Kanellis, D.H. Kang, L. Gesualdo, T. Nakagawa, and R.J. Johnson, *Systemic inflammation, metabolic syndrome and progressive renal disease*. Nephrol Dial Transplant, 2009. **24**(5) 1384-7).

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Polycystic ovary syndrome (PCOS) is reported to affect from 5% up to 20 % of women in child-bearing ages (Lindholm, A., L. Andersson, M. Eliasson, M. Bixo, and I. Sundstrom-Poromaa, *Prevalence of symptoms associated with polycystic ovary syndrome*. Int J Gynaecol Obstet, 2008. **102**(1) 39-43; Teede, H., A. Deeks, and L. Moran, *Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan*. BMC Med. **8**(1) 41). Women are diagnosed as having PCOS if they are positive for at least two of the following symptoms: oligoovulation or anovulation, excess androgen activity or the presence of polycystic ovaries.

In addition to the short term consequence of infertility, the pathophysiology of PCOS also involves disturbances in energy metabolism, with symptoms similar to the metabolic syndrome (Lindholm, A., L. Andersson, M. Eliasson, M. Bixo, and I. Sundstrom-Poromaa, *Prevalence of symptoms associated with polycystic ovary syndrome*. Int J Gynaecol Obstet, 2008. **102**(1), 39-43). Thus, patients suffering from PCOS do not only suffer the well known fertility related morbidities, but also suffers the same health problems as other victims of the metabolic syndrome not having the PCOS, including increased risk for CVD etc (Wild, S., T. Pierpoint, P. McKeigue, and H. Jacobs, *Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study*. Clin Endocrinol (Oxf), 2000. **52**(5), 595-600). Like metabolic syndrome, PCOS has been associated to chronic systemic inflammation (Gonzalez, F., N.S. Rote, J. Minium, A.L. Weaver, and J.P. Kirwan, *Elevated circulating levels of macrophage migration inhibitory factor in polycystic ovary syndrome*. Cytokine. 2010. epub July 1).

Diabetes mellitus is a group of diseases resulting in elevated levels of plasma glucose. Diabetes is currently defined (WHO/ADA) as symptoms of diabetes plus:

random plasma glucose concentration above 11.1 mmol/L [200mg/dl], or

fasting plasma glucose above 7.0 mmol/L [126mg/dl], or

2-h plasma glucose concentration after 75 g anhydrous glucose in an oral glucose, tolerance test above 11.1 mmol/L [200mg/dl].

In the absence of symptoms, diabetes should not be diagnosed on a single glucose measurement but needs confirmation. There are two main types of diabetes, Type 1 (T1DM) and Type 2 (T2DM). T1DM is an autoimmune disease where pancreatic beta cells are destroyed, and the patients are thus dependent on exogenous

insulin administration. T1DM is characterized by elevated glucose levels and low insulin levels, as the pancreas is unable to secrete insulin in response to the elevation in plasma glucose.

In diabetes, especially in T2DM, there is a relation between elevated markers
5 for ongoing systemic inflammatory processes and disease development (Devaraj, S.,
U. Singh, and Jialal, *Human C-reactive protein and the metabolic syndrome*. *Curr Opin
Lipidol*, 2009. **20**(3), 182-91), indicating that inflammatory processes are important in
diabetes. Before T2DM has developed, the patients are going through a period of pre-
diabetes. In this period insulin resistance has developed, but fasting plasma glucose is
10 normal due to an increased secretion of insulin. Insulin resistance is diagnosed as
elevated fasting insulin levels with normal fasting glucose levels, or as increased
HOMA-IR, the product of fasting glucose and fasting insulin levels. Also insulin
resistance in pre-diabetic individuals is associated to low-grade systemic inflammation.

Platelet activating factor (PAF) antibodies anti-PAF has been shown to be
15 related to the risk of developing cardiovascular disease (WO 00/002046). Low levels of
antibodies directed to PAF-conjugates have been shown to be related to an increased
risk of developing cardiovascular disease (WO 2009/056826). Active immunization with
PAF-conjugates and passive immunization with antibodies directed to PAF-conjugates
have been suggested for the prevention and treatment of cardiovascular disease (WO
20 2009/056826).

However, there is currently no information in the art showing that anti-PAF is of
any importance in the pathophysiology of the metabolic syndrome, diabetes, insulin
resistance or PCOS. Neither has anti-PAF been suggested for the prevention or
treatment of metabolic diseases.

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SUMMARY OF THE INVENTION

The present invention is based on the surprising findings that low levels of
antibodies reactive with a PAF-conjugate are related to an increased risk of developing
metabolic diseases. The present inventors have surprisingly found that the
progression of metabolic diseases, such as insulin resistance, can be reduced by
30 administration of a composition that increases the levels of anti-PAF antibodies.

Accordingly, a first aspect of the present invention provides a composition
comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF
derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF

derivative, or conjugate of a PAF derivative, for use in the immunization or prophylaxis against, or the prevention or treatment of, metabolic diseases in mammals.

To put it another way, the first aspect of the present invention provides for the use of a composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, in the manufacture of a medicament for the immunization or prophylaxis against, or the prevention or treatment of, metabolic diseases in mammals.

Thus, according to the first aspect of the present invention, there is provided a method for the immunization or prophylaxis against, or the treatment of, metabolic diseases in a mammal, the method comprising the step of administering to the mammal a pharmaceutical composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative. The method may thus include administration of a therapeutically effective amount of a composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, is administered to the mammal

According to the first aspect of present invention, any mammal may be treated, although in one embodiment the mammal may be a human.

According to the first aspect of present invention, any metabolic disease may be addressed. Exemplary metabolic diseases include a condition selected from the group consisting of metabolic syndrome, insulin resistance, glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

According to the first aspect of present invention the composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, may, for example, be a pharmaceutical composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, and may optionally include an adjuvant. Any suitable adjuvant, for example aluminium hydroxide, may be used.

According to the first aspect of present invention the antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative,

may, for example, comprise a polyclonal antibodies, or a monoclonal antibody, with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.

The first aspect of present invention may provide, for example, for the therapeutic treatment of a mammal suffering from metabolic disease, or for the prophylactic treatment of a mammal facing the risk of developing metabolic disease. The mammal may be identified as being of risk of developing metabolic disease by a method according to the second aspect of the present invention, as discussed further below.

Accordingly, in one embodiment, the first aspect of the invention provides the use of at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation, for example a monoclonal antibody, with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, in the manufacture of a medicament for immunization and prophylaxis, prevention or treatment of mammals, including humans, against metabolic diseases, such as metabolic syndrome, insulin resistance (IRS), glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS). The medicament is intended to provide immunization having immunogenic or therapeutic properties against metabolic diseases.

In another embodiment, the first aspect of the invention provides a method for immunization and treatment of a mammal, including a human, against metabolic diseases, such as metabolic syndrome, insulin resistance (IRS), glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS), the method comprising the step of administering to the mammal a pharmaceutical composition comprising at least PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation, for example a monoclonal antibody, with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative. The pharmaceutical composition is intended to provide immunization having immunogenic or therapeutic properties against metabolic diseases.

In another embodiment, the first aspect of the invention provides the use of one or more of the PAF conjugates, PAF derivatives, or conjugates of a PAF derivative, as defined in relation to the preceding aspects of the invention, in the manufacture of a pharmaceutical composition, optionally in combination with an adjuvant, for

immunotherapy or therapy for the prevention, prophylaxis and/or treatment of metabolic diseases.

In another embodiment, the first aspect of the invention provides a method of prophylactic or therapeutic treatment of a mammal, which may be a human being, suffering from metabolic disease or facing the risk of developing metabolic disease, whereby a therapeutically effective amount of at least one PAF conjugates, PAF derivatives, or conjugates of a PAF derivative, or an antibody preparation, for example a monoclonal antibody, with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, is administered.

A second aspect of the present invention provides a method for diagnosing metabolic disease, or assessing a patient's risk of developing or progression of metabolic disease, the method comprising the steps of –

- (a) assessing the patient's level of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative; and
- (b) diagnosing metabolic disease or determining the patient's level of risk of developing or progression of metabolic disease based on the assessed levels of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.

The method of the second aspect of the present invention may assess the level of all of the patient's antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, or may comprise the assessment of a particular isotype, such as the patient's level of IgM, IgG or IgA antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.

Typically, although not necessarily, the patient's level of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative are assessed by analysis of an *ex vivo* sample taken from the patient. Thus, the sample may be a blood, plasma or serum sample that has been obtained from the patient.

The method of the second aspect of the present invention may be employed to diagnose metabolic disease, or assess a patient's risk of developing or progression of metabolic disease, in any mammalian patient, although in one embodiment the patient is human.

Lower levels of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative are indicative of the presence of metabolic disease and/or the risk of developing or progression of metabolic disease. Accordingly, in the method of the second aspect of the present invention, the patient's level of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative may correlate negatively with the patient's risk of developing or progression of the metabolic disease.

The method of the second aspect of the present invention may be employed to diagnose any metabolic disease, or assess a patient's risk of developing or progression of any metabolic disease. Exemplary metabolic diseases include a condition selected from the group consisting of metabolic syndrome, insulin resistance, glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

Accordingly, the second aspect of the invention provides a method of diagnosing the presence or absence of antibodies, for example IgM, IgG or IgA antibodies, related to increased or decreased risk of developing metabolic diseases, using PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.

The second aspect of the present invention also provides for the use of PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative in a method for diagnosing metabolic disease and/or for assessing a patient's risk of developing or progression of metabolic disease in which the patient's levels of antibodies (for example, all antibodies, or a particular isotype, such as IgM, IgG or IgA antibodies) with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, are assessed.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have surprisingly found that immunization with a PAF conjugate, which gives rise to increased serum levels of anti-PAF antibodies, protects hyperlipidemic mice from developing the metabolic disease of insulin resistance.

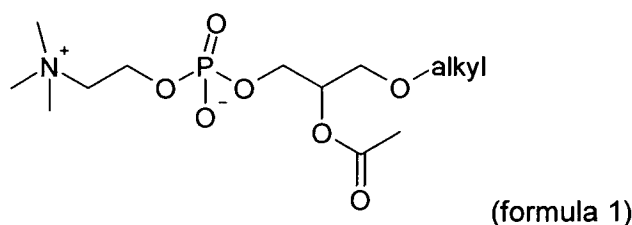
The invention relates to pharmaceutical compositions comprising a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, or an antibody preparation, for example a monoclonal antibody, with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, and the use of these

compositions in the treatment, prophylaxis or prevention of metabolic diseases, such as metabolic syndrome, insulin resistance (IRS), glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

- 5 Furthermore, the invention also relates to the use of PAF conjugate, a PAF derivative, or conjugate of a PAF derivative or said antibody preparation, for example monoclonal antibody, to produce a pharmaceutical composition optionally with an adjuvant.

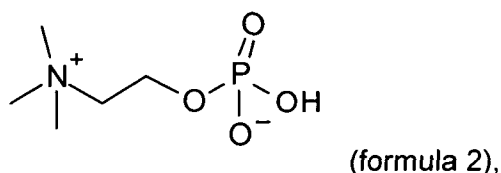
- 10 Furthermore the invention relates to diagnosing the absence, presence and/or levels of antibodies, for example IgM, IgG or IgA antibodies, reactive with a PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, related to increased or decreased risk of displaying or developing metabolic diseases.

By PAF is meant 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine according to formula 1.



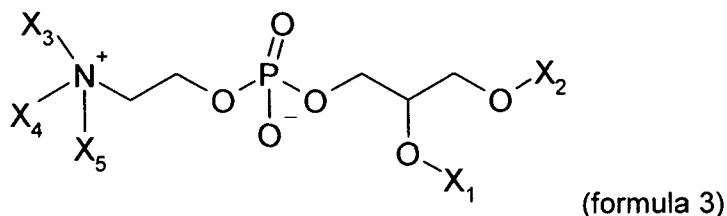
- 15 Preferably the alkyl group is a palmityl (hexadecyl) group or an oleyl (octadecyl) group.

For the avoidance of doubt, phosphorylcholine (PC) itself, according to formula 2,



- 20 is not included within the scope of the term of the "PAF derivative" according to the present invention.

In one option, a minimum requirement for a molecule to be considered as a PAF derivative is the presence of a PC moiety linked, via its phosphate group, to a substituted glycerol-based moiety, having the general formula shown by formula 3,



where X_1 and X_2 can each, independently, be any substituent. For example, X_1 and X_2 can each independently be selected from C_2 - C_{25} acyl or alkyl, and further may each independently be saturated, unsaturated, hydroxylated and/or oxidised. Thus, for example, X_1 and/or X_2 may each independently be $-(CO)$ -alkyl, wherein the alkyl group may be C_2 - C_{25} alkyl, and further may be saturated, unsaturated, hydroxylated and/or oxidised. In one embodiment, X_1 is $-(CO)-CH_3$ and/or X_2 is C_2 - C_{25} alkyl. X_3 , X_4 and X_5 may, each independently, be selected from C_1 to C_6 (such as C_1 , C_2 , C_3 , C_4 , C_5 , or C_6) alkyl, in one embodiment at least one, two, or all three of X_3 , X_4 and X_5 are methyl.

10 Preferably, the identity of the X_1 , X_2 , X_3 , X_4 and X_5 substituents is such that the derivative is reactive with antibodies that are reactive to PAF, or to a PAF conjugate such as a PAF-BSA conjugate of the type used in the examples of this application.

PC clearly does not possess a substituted glycerol-based moiety as shown above in formula 3, and so is not a PAF derivative according to the present invention.

15 Likewise, naturally-occurring phospholipids, such as phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol, which do not possess a PC moiety as defined by formula 3, are also not PAF derivatives according to the present invention.

Where X_3 , X_4 and X_5 are methyl, and then in one embodiment it may be preferred that X_1 and X_2 are not independently both $-(CO)$ -alkyl.

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Optionally, the term PAF derivative may, or may not, be construed to exclude phosphatidylcholine.

In one embodiment, PAF derivatives according to formula 3 may be identical to PAF at only four positions selected from X_1 , X_2 , X_3 , X_4 and X_5 (and, therefore, differ from PAF at one position selected from X_1 , X_2 , X_3 , X_4 and X_5). Thus, PAF derivatives according to formula 3 may be identical to PAF only (a) at positions X_1 , X_2 , X_3 , and X_4 (b) at positions X_1 , X_2 , X_3 , and X_5 ; (c) at positions X_1 , X_2 , X_4 and X_5 ; (d) at positions X_1 , X_3 , X_4 and X_5 ; and/or (e) at positions X_2 , X_3 , X_4 and X_5

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In another embodiment, PAF derivatives according to formula 3 may be identical to PAF at only three positions selected from X₁, X₂, X₃, X₄ and X₅ (and, therefore, differ from PAF at two positions selected from X₁, X₂, X₃, X₄ and X₅). Thus, PAF derivatives according to formula 3 may be identical to PAF only (a) at positions X₁, X₂, and X₃; (b) at positions X₁, X₂, and X₄; (c) at positions X₁, X₂ and X₅; (d) at positions X₂, X₃, and X₄; (e) at positions X₂, X₃ and X₅; and/or (f) at positions X₃, X₄ and X₅.

In another embodiment, PAF derivatives according to formula 3 may be identical to PAF at only two positions selected from X₁, X₂, X₃, X₄ and X₅ (and, therefore, differ from PAF at three positions selected from X₁, X₂, X₃, X₄ and X₅). Thus, PAF derivatives according to formula 3 may be identical to PAF only (a) at positions X₁ and X₂; (b) at positions X₁ and X₃; (c) at positions X₁ and X₄; (d) at positions X₁ and X₅; (e) at positions X₂ and X₃; (f) at positions X₂ and X₄; (g) at positions X₂ and X₅; (h) at positions X₃ and X₄; (i) at positions X₃ and X₅; and/or (j) at positions X₄ and X₅.

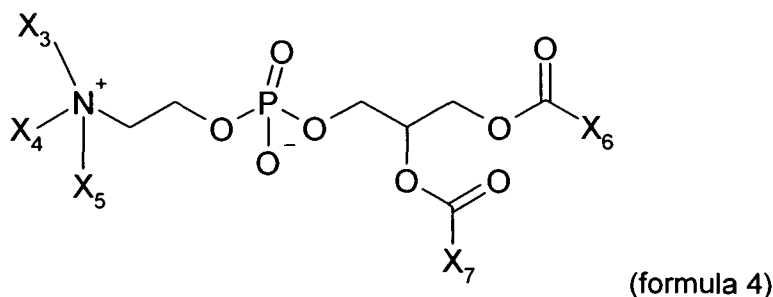
In another embodiment, PAF derivatives according to formula 3 may be identical to PAF at only one position selected from X₁, X₂, X₃, X₄ and X₅ (and, therefore, differ from PAF at four positions selected from X₁, X₂, X₃, X₄ and X₅). Thus, PAF derivatives according to formula 3 may be identical to PAF (a) only at position X₁; (b) only at position X₂; (c) only at position X₃; (d) only at position X₄; or (e) only at position X₅.

In another embodiment, PAF derivatives according to formula 3 may be identical to PAF in the PC moiety, and therefore differ from PAF in substituted glycerol-based moiety at one or both of positions X₁ or X₂ only.

In another embodiment, PAF derivatives according to formula 3 may be identical to PAF in substituted glycerol-based moiety, and therefore differ from PAF in the PC moiety at one, two or all three of positions X₃, X₄ and/or X₅ only.

In another embodiment, PAF derivatives according to formula 3 may differ from PAF at each of positions X₁, X₂, X₃, X₄ and X₅.

In formula 3, as given above, it may be that X₁ and X₂ are not moieties that both result in the formation of ester linkages to their adjacent carbons in the substituted glycerol-based moiety. Thus, for example, the term PAF derivative may optionally exclude molecules having the general formula 4

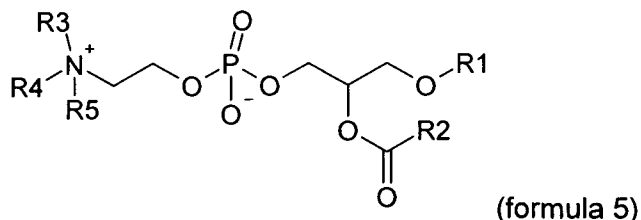


wherein X_3 , X_4 and X_5 are as defined above formula 3 (and in one embodiment are each methyl) and wherein X_6 and X_7 can be any substituent. For example, X_6 and X_7 may each independently be a C_2 to C_{25} (such as C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 , C_9 , C_{10} , C_{11} , C_{12} , C_{13} , C_{14} , C_{15} , C_{16} , C_{17} , C_{18} , C_{19} , C_{20} , C_{21} , C_{22} , C_{23} , C_{24} or C_{25}) alkylene, alkenylene, or alkynylene group.

Optionally, the term PAF derivative may, or may not, be construed to exclude 1,2-dimyristoyl-sn-glycero-3-phosphocholine and/or an analogue thereof .

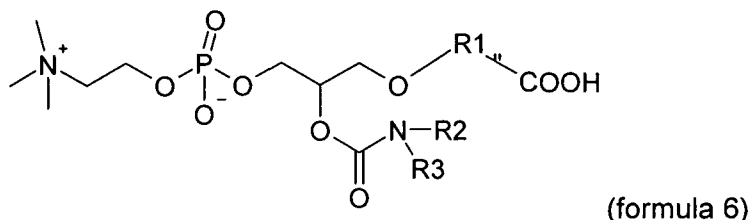
Further examples of derivatives of PAF according to the present invention include one or more compounds selected from the group consisting of –

(a) compounds according to the formula 5



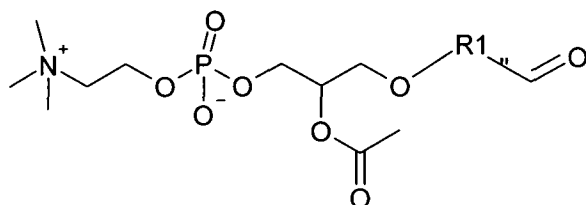
where R_1 is C_2 to C_{25} (such as C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 , C_9 , C_{10} , C_{11} , C_{12} , C_{13} , C_{14} , C_{15} , C_{16} , C_{17} , C_{18} , C_{19} , C_{20} , C_{21} , C_{22} , C_{23} , C_{24} or C_{25}) alkylene, alkenylene, or alkynylene linking group, and R_2 to R_5 are independently selected from C_1 to C_6 (such as C_1 , C_2 , C_3 , C_4 , C_5 , or C_6) alkyl, for example as described in WO 87/05904 and US 5,061,626,

(b) compounds according to the formula 6



where R1 is C₂ to C₁₈ (such as C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₅, C₁₆, C₁₇, or C₁₈) alkyl, preferably pentadecyl, R2 is H, methyl, ethyl, propyl, or isopropyl; R3 is methyl, ethyl, propyl or isopropyl, such as described by Karasawa K et al. J Biochem (Tokyo) 1991, 110:683-687,

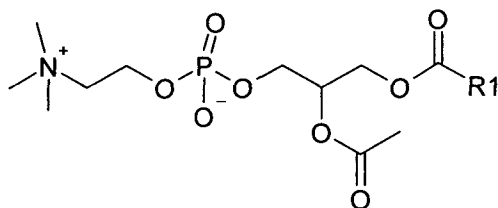
- 5 (c) compounds according to the formula 7



(formula 7)

where R1 is C₆ to C₁₈ (such as C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₅, C₁₆, C₁₇, or C₁₈) alkyl, preferably hexyl or dodecyl, such as described by Smal MA et al. Lipids 1991, 26:1130-5, or

- 10 (d) compounds according to the formula 8



(formula 8)

where R1 is C₆ to C₁₈ (such as C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₅, C₁₆, C₁₇, or C₁₈) alkyl, preferably hexyl or dodecyl (1-acyl-PAF), such as described by Muzya GI et al. Immunologiya (Moscow) 1997, 6, 9-11.

- 15 Optionally, the term "PAF derivative" may be construed to exclude compounds including any one or more compounds selected from the group consisting of 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine, 1-hexadecyl-2-azelaoyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphocholine (PGPC), 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC), 1-palmitoyl-2-(9-oxononanoyl)-sn-glycero-3-phosphocholine, 1-hexadecyl-2-acetoyle-sn-glycero-3-phosphocholine, 1-octadecyl-2-acetoyle-sn-glycero-3-phosphocholine, 1-hexadecyl-2-butyroyl-sn-glycero-3-phosphocholine, 1-octadecyl-2-butyroyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-acetoyle-sn-glycero-3-phosphocholine, 1-octadecenyl-2-acetoyle-sn-glycero-3-phosphocholine, 1-hexadecyl-2-(homogammalinolenoyl)-sn-glycero-3-phosphocholine, 1-hexadecyl-2-arachidonoyl-sn-glycero-3-phosphocholine, 1-
- 20
- 25

hexadecyl-2-eicosapentaenoyl-sn-glycero-3-phosphocholine, 1-hexadecyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine, 1-octadecyl-2-methyl-sn-glycero-3-phosphocholine, 1-hexadecyl-2-butenoyl-sn-glycero-3-phosphocholine, Lyso PAF C16, Lyso PAF C18, 1-O-1'-(Z)-hexadecenyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-sn-glycero-3-phosphocholine, 1-O-1'-(Z)-hexadecenyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-O-1'-(Z)-hexadecenyl-2-arachidonoyl-sn-glycero-3-phosphocholine, 1-O-1'-(Z)-hexadecenyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine.

Optionally, the term "PAF derivative" may be construed to exclude or include compounds including any one or more compounds selected from the group consisting of the products formed from the oxidation of low density lipoprotein (oxLDL), lysoPAF and/or lysophosphatidylcholine.

By a PAF conjugate is meant a PAF moiety linked to a carrier, optionally via a spacer. By conjugate of PAF derivative is meant a PAF derivative linked to a carrier, optionally via a spacer. The PAF moiety or PAF derivative can be covalently or non-covalently linked to the carrier. Preferably the PAF moiety or PAF derivative is linked to the carrier via the alkyl group (or corresponding portion thereof in the derivative, as generally identified as substituent R1 in the foregoing formulae describing certain derivatives).

Any suitable carrier can be used. For example, the carrier may be selected from the group consisting of a protein, a carbohydrate, a polymer, latex beads, or colloid metal. Typically, a carrier will have a mass of about, or at least, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more Daltons, such as about, or at least, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more kDa. For example, the protein carrier bovine serum albumin (BSA) has a molecular mass of just over 66 kDa.

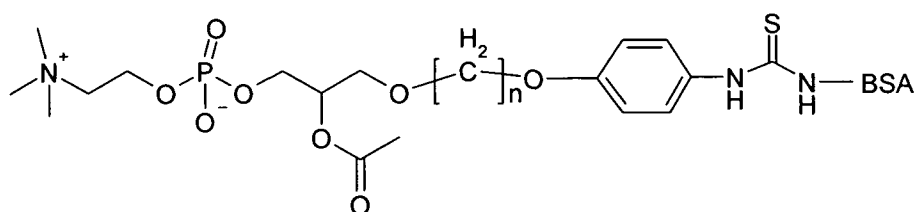
The PAF conjugate, or conjugate of a PAF derivative, may for example be a protein-PAF conjugate, such as a human serum albumin (HSA)-PC conjugate, a transferrin-PAF conjugate, a keyhole limpet hemocyanin (KLH)-PAF conjugate or a BSA-PAF conjugate, a conjugate of a protein and a PAF derivative, such as an HSA-PAF derivative conjugate, a transferrin-PAF derivative conjugate, a KLH-PAF derivative conjugate or a BSA-PAF derivative conjugate.

Optionally, the PAF derivative, PAF conjugate and/or conjugate of a PAF derivative is a molecule that does not include a moiety that has a separate,

independent, activity, such as a therapeutic, pharmacological or pharmaceutical activity, or is a pro-drug that can be activated to provide a separate, independent, activity, such as a therapeutic, pharmacological or pharmaceutical activity. Thus, for example, the terms PAF conjugate, PAF derivative, and conjugate of a PAF derivative
 5 may be optionally construed to exclude PAF, or a PAF derivative, that is conjugated to diclofenec of other non-steroidal anti-inflammatory drugs (NSAIDs).

In one option, it may be preferred that PAF is the sole therapeutically, prophylactically, pharmacologically and/or pharmaceutically active moiety present in a PAF derivative, PAF conjugate and/or conjugate of a PAF derivative for use in the
 10 present invention.

The following PAF-BSA conjugate was synthesized and used in methods according to the invention



n = 16

15 Examples of PAF conjugates and generation of anti-PAF antibodies are described in WO 87/05904 and by Karasawa K et al. (*Antibodies to synthetic platelet-activating factor (1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphocholine) analogues with substituents at the sn-2 position*. J Biochem (Tokyo). 1991, 110:683-7), Macpherson JL et al. (*Production and characterization of antibodies to platelet-activating factor*. J Lipid
 20 Mediat. 1992, 5:49-59), Smal MA et al. (*Synthesis of a PAF immunogen and production of PAF-specific antibodies*. Lipids. 1991, 26:1130-5), Tomii A and Masugi F. (*Production of anti-platelet-activating factor antibodies by the use of colloidal gold as carrier*. Jpn J Med Sci Biol. 1991, 44:75-80), and Wang C and Tai HH. (*A sensitive and specific radioimmunoassay for platelet-activating factor*. Lipids. 1992, 27:206-8), WO
 25 2009/056826, and US 5,061,626 the content of which is hereby incorporated by reference.

Metabolic diseases that can be treated, prevented and/or diagnosed according to the first or second aspects of the present invention are exemplified, but not limited to, metabolic syndrome, insulin resistance (IRS), glucose intolerance, hyperglycemia,
 30 type I diabetes also referred to as insulin-dependent diabetes mellitus or IDDM, type II

diabetes also referred to as noninsulin-dependent diabetes mellitus or NIDDM, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

Thus, an individual for treatment according to the method or use of the present invention may be an individual that is identified as suffering from, or being at risk of suffering from, a metabolic disease, such as, but not limited to, metabolic syndrome, insulin resistance (IRS), glucose intolerance, hyperglycemia, type I diabetes also referred to as insulin-dependent diabetes mellitus or IDDM, type II diabetes also referred to as noninsulin-dependent diabetes mellitus or NIDDM, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

In one embodiment, the individual for treatment is an individual that is not suffering from a disease or condition as discussed in WO 2009/056826.

Optionally, the individual to be treated may be an individual who is not suffering from, and/or has not been diagnosed as suffering from or being at risk of the progression or development of, conditions selected from one or more of cardiovascular disease, such as ischemic cardiovascular disease, atherosclerosis, an atherosclerotic related disease, acute myocardial infarction, stable and unstable angina, stroke, restenosis following artery grafting, artery stenting, and balloon angioplasty, especially restenosis following coronary artery bypass grafting, coronary artery stenting and coronary angioplasty. In another option, the individual to be treated may be an individual who is suffering from, and/or has been diagnosed as suffering from or being at risk of the progression or development of, conditions selected from one or more of cardiovascular disease, such as ischemic cardiovascular disease, atherosclerosis, an atherosclerotic related disease, acute myocardial infarction, stable and unstable angina, stroke, restenosis following artery grafting, artery stenting, and balloon angioplasty, especially restenosis following coronary artery bypass grafting, coronary artery stenting and coronary angioplasty, and who is receiving therapy or prophylaxis for that condition by administration of a therapeutic or prophylactic agent that does not comprise a composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.

Preferably, in the use according to the first aspect of the invention, or the method according to the second aspect of the invention, the medicament is for

administration by injection. However, in practice it can be administered by any suitable means that allows the PAF conjugate, a PAF derivative, or conjugate of a PAF derivative to provoke an immune response in, or allows efficient delivery of the antibody preparation to, the subject to which it is administered.

5 A clinician can determine the most appropriate administrative regimen for an individual based on factors such as the individual's weight, age, gender, diagnosis or prognosis, and the half-life of the administered therapeutic molecule. However, in general it may be suitable to treat an individual with a single dose, or multiple doses, of the composition comprising at least one PAF conjugate, a PAF derivative, or conjugate
10 of a PAF derivative and/or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative. Where multiple administrations are made, these may be made at a rate of, for example, once, twice, three times, four times or more often per day, week or month, and may be continued for a period of time necessary and effective to increase the levels of antibodies with
15 reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative in the individual and thereby obtain a therapeutically or prophylactically-beneficial effect in respect of metabolic disease. For example treatment may continue for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more days, weeks, months or years, or even for the rest of the life of the subject. In the case of the use of antibodies with reactivity to PAF, a PAF
20 conjugate, a PAF derivative, or conjugate of a PAF derivative, then administration would most typically be made weekly, or once or twice per month, and continue for as long as is clinically beneficial. In the case of the administration with a composition comprising at least one PAF conjugate, a PAF derivative, or conjugate of a PAF derivative then, in one embodiment, the treatment may involve an initial immunisation,
25 followed by a further administration as a booster (for example, within about one month of the initial immunisation), and optionally followed by yearly further administrations, continued for as long as is clinically beneficial.

Accordingly, the first aspect of the invention provides active (where the composition comprises at least one PAF conjugate, PAF derivative, or conjugate of a
30 PAF derivative), or passive (where the composition comprises the defined antibody), immunization having immunogenic or therapeutic properties against metabolic diseases.

In other words, the invention provides at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation (for example a
35 monoclonal antibody) with reactivity to PAF, a PAF conjugate, a PAF derivative, or

conjugate of a PAF derivative, for use in the prophylaxis, prevention and/or treatment of metabolic diseases, and provides a method for immunization and treatment against metabolic diseases. The method may comprise the step of administering to a subject a pharmaceutical composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation (for example a monoclonal antibody) with reactivity PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative. The pharmaceutical composition is intended to provide active or passive immunization having immunogenic or therapeutic properties against metabolic diseases.

10 Active immunization:

One embodiment of the present invention is thus to use a PAF conjugate, PAF derivative, or conjugate of a PAF derivative, for the preparation of a pharmaceutical composition to be used in the treatment, prophylaxis and/or prevention of metabolic diseases, such as metabolic syndrome, insulin resistance (IRS), glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

The conjugate can, for example, be PAF or a PAF derivative linked to a pharmaceutically acceptable carrier such as a protein, carbohydrate, or polymer.

The pharmaceutical composition is preferably given by injection, but can in practice be administered by any suitable means that allows the PAF conjugate, PAF derivative, or conjugate of a PAF derivative to provoke an immune response in the subject to which it is administered.

For the purposes of active immunisation of a patient, one or more PAF conjugate molecules, PAF derivative molecules, and/or conjugate of a PAF derivative molecules are prepared in an immunogenic formulation, optionally containing suitable adjuvants and carriers and administered to the patient in known ways. Suitable adjuvants include Freund's complete or incomplete adjuvant, muramyl dipeptide, the "Iscoms" of EP 109 942, EP 180 564 and EP 231 039, aluminium hydroxide, saponin, DEAE-dextran, neutral oils (such as miglyol), vegetable oils (such as arachis oil), liposomes, Pluronic polyols or the Ribic adjuvant system (see, for example GB-A-2 189 141). "Pluronic" is a Registered Trade Mark.

The proposed method of active immunization will modulate (preferably, increase) the antibody titre which in turn will have a positive effect on the development of metabolic diseases (that is, the development of metabolic disease will be reduced).

Passive immunization.

5 Another embodiment of the invention is to use an antibody preparation, for example a monoclonal antibody, recognizing PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, for the preparation of a pharmaceutical composition to be used in the treatment, prophylaxis and/or prevention of metabolic diseases, such as metabolic syndrome, insulin resistance (IRS), glucose intolerance, hyperglycemia,
10 type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

Monoclonal antibodies can be produced using methods known in the art and/or as discussed further below. Other antibody preparations may be used, such as preparations obtained from Intravenous immunoglobulin preparations that are enriched
15 for antibodies recognizing PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, recombinantly produced antibodies recognizing PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, and/or other artificially created antibody derivatives recognizing PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, as discussed above.

20 Antibodies against a PAF conjugate or a conjugate of a PAF derivative can be produced as described above. See, e.g., WO 87/05904, Macpherson JL et al. (*Production and characterization of antibodies to platelet-activating factor. J Lipid Mediat.* 1992, 5:49-59), and Smal MA et al. (*Synthesis of a PAF immunogen and production of PAF-specific antibodies. Lipids.* 1991, 26:1130-5).

25 Monoclonal antibodies reactive against PAF, PAF conjugates, PAF derivatives, or conjugates of a PAF derivative can be produced using any standard method known in the art. See for example Tomii A and Masugi F. (*Production of anti-platelet-activating factor antibodies by the use of colloidal gold as carrier. Jpn J Med Sci Biol.* 1991, 44:75-80).

30 Other antibodies reactive against PAF, PAF conjugates, PAF derivatives, or conjugates of a PAF derivative can be prepared using methods well known to those skilled in the art. For example, a sub-fraction of a human immunoglobulin preparation reactive with PAF, PAF conjugates, PAF derivatives, or conjugates of a PAF derivative

can be prepared, for example as described below, for example by affinity purification using PAF, a PAF conjugate, a PAF derivative, or a conjugate of a PAF derivative.

Intravenous immunoglobulin preparations (e.g., IGIV; Baxter and others) is a highly purified preparation of IgG commercially available and is used in the treatment of patients who have no, or very low levels of antibody production. Immunoglobulin preparations include those available from the following manufacturers: Baxter (US), e.g., Gammagard[®], Isiven (Antimo Naples, Italy), Omrix (Tel-Hashomer, Israel), Miles (Biological Products Division, West Haven, CT), Sclavo (Lucca, Italy), Sandoz (Novartis, Basel, Switzerland), e.g., Sandoglobulin[®], Biotest Diagnostic Corporation (Deville, NJ). Examples of immunoglobulin preparations are GammagardS/D[®], GammarIV[®], Gaimnar-PIV[®], Gammimune N[®], Iveegam[®], Panglobulin[®], Polygam S/D[®], Sandoglobulin[®], Venoglobulin[®]. Immunoglobulin preparations typically contain some IgM as well as IgG. Trace amounts of IgM are present in Gammagard[®]. Pentaglobin (Biotest) is an enriched IgM preparation which has been used for treatment of SARS. The subfraction with reactivity to PAF, PAF conjugates, PAF derivatives, or conjugates of a PAF derivative, may comprise both IgG and IgM, or may be selected to comprise mainly IgG (for example by starting with an IgG-rich preparation such as Gammagard[®] and/or by selecting for IgG); or mainly IgM (for example by starting with an IgM-rich preparation such as Pentaglobin and/or by selecting for IgM).

Additionally, the present invention contemplates the use of recombinantly produced antibodies with reactivity to PAF, PAF conjugates, PAF derivatives, or conjugates of a PAF derivative, and/or other artificially created antibody derivatives, such as CDR-grafted and/or humanised antibodies, scFv, dAb, Fab, or Fv or other molecules which comprise or consists of fragments of an antibody with binding activity to PAF, PAF conjugates, PAF derivatives, or conjugates of a PAF derivative.

Thus, passive immunisation may be used to increase the titre of antibodies recognizing PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, in an individual.

A further embodiment of the invention is to provide a method of diagnosing the absence, presence and/or levels of antibodies, for example IgA, IgM or IgG antibodies, with reactivity towards PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, which factor is related to an increased or decreased risk of developing metabolic diseases, such as metabolic syndrome, insulin resistance (IRS), glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia,

hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS), using PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative. A preferred method is an immunoassay. The method may be used in assessing the patient's risk of developing or progression of metabolic diseases.

5 Accordingly, a second aspect of the present invention provides a method for diagnosing metabolic disease, or assessing a patient's risk of developing or progression of metabolic disease, the method comprising the steps of –

- (a) assessing the patient's level of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative; and
- 10 (b) diagnosing metabolic disease or determining the patient's level of risk of developing or progression of metabolic disease based on the assessed levels of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.

Typically, the method of the second aspect of the invention comprises exposing
15 PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, to a sample (for example, an *ex vivo* sample) from an individual and detecting antibodies which have bound to PAF, the PAF conjugate, the PAF derivative, or the conjugate of a PAF derivative.

Preferably, in the second aspect of the invention, the individual is a human.

20 Preferably, in the method of the second aspect of the invention, the sample is blood, serum or plasma. Serum may be preferred in one embodiment.

Optionally, in the second aspect of the invention, in the case of a conjugate of PAF or a PAF derivative, the PAF or PAF derivative is linked to a carrier via a spacer. In this embodiment, typically the carrier may, for example, be a protein, such as KLH
25 (keyhole limpet hemocyanin), transferrin, human serum albumin (HSA) or bovine serum albumin (BSA). In an alternative embodiment, the carrier may be latex beads.

Typically, according to the second aspect of the invention, antibodies which have bound to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, are determined by an assay, preferably an immunoassay.

30 The patient's levels of antibodies, e.g., of all or a particular isotype such as IgM, IgG or IgA antibodies, with reactivity to PAF, a PAF conjugate, a PAF derivative, or

conjugate of a PAF derivative, may be assessed using an immunoassay. Examples of suitable immunoassays are described below and will in any case be apparent to those skilled in the art.

Typically, in the second aspect of the invention, low levels of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, are indicative of the presence of metabolic disease and/or an increased risk of developing metabolic disease. Conversely, high levels of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, are indicative of the absence of metabolic disease and/or a reduced risk of developing metabolic disease. Typically, although not necessarily, antibodies are determined in a sample of patient blood, plasma or serum.

In any given population, levels of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, are likely to vary. The level of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, determined for any given individual may be categorised as high or low by reference to the range observed in the wider population. For example, a level of such antibodies below a particular percentile value determined with reference to the wider population may be categorised as a low level. Suitably, a low level may correspond to a value below the 25th percentile, or below the 20th, 10th or 5th percentile. A high level may correspond to a value of above the 5th, 10th, 20th, or 25th percentile, for example.

Where an individual is characterised as possessing low levels of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, this information may assist in the diagnosis or prognosis of the presence of, or the increased risk of development or progression of, metabolic disease. A clinician may take other factors into account in arriving at a diagnosis or prognosis.

It may be desirable to measure antibodies reactive with phosphorylcholine (PC) or a PC conjugate as well as measuring antibodies, e.g., IgM, IgG and/or IgA antibodies, with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative,. It may further be desirable to measure antibodies reactive with oxidised low density lipoprotein (oxLDL) or malondialdehyde modified LDL (MD-LDL) as well as measuring antibodies, e.g., IgM, IgG and/or IgA antibodies, with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative,. It may alternatively or in addition be desirable to measure levels of lipoprotein associated

phospholipase A₂ (LpPLA₂), homocystein, C-reactive protein (CRP), HSP70, high density lipoprotein (HDL), TNF, in particular TNF α , and/or HSP60 as well as measuring antibodies, e.g., IgM, IgG and/or IgA antibodies, with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative,. Assaying for such factors may assist in the diagnosis or prognosis of increased risk of development or progression of metabolic disease.

Other factors presented by a patient may also be taken into account clinician in arriving at a diagnosis or prognosis.

For example, where the individual is assessed for the presence of, or the increased risk of development or progression of, metabolic syndrome then a clinician may take into account any one, two, three or more of the presence of obesity, insulin resistance, diabetes, hypertension and hyperlipidemia in the patient. Thus, for example, the clinician may also take into account whether the patient presents at least one, two, three or more of the following features (i) blood pressure >130/85 mmHg or antihypertensive treatment, (ii) fasting plasma glucose > 6.1 mmol/l, (iii) serum triglycerides >1.7 mmol/l, (iv) waist circumference > 102 cm in men and >88 cm in women, and (v) HDL-cholesterol < 1.0 mmol/l in men and <1.3 in women.

Where the individual is assessed for the presence of, or the increased risk of development or progression of, polycystic ovary syndrome (PCOS) the clinician may also take into account whether the female patient presents at least one, two or more of the following features: (i) oligoovulation or anovulation, (ii) excess androgen activity or (iii) the presence of polycystic ovaries.

Where the individual is assessed for the presence of, or the increased risk of development or progression of, diabetes mellitus, the clinician may also take into account whether the individual (such as the male or female) patient presents and one, two or more of the following features: (i) random plasma glucose concentration above 11.1 mmol/L [200mg/dl], or (ii) fasting plasma glucose above 7.0 mmol/L [126mg/dl], or (iii) 2-h plasma glucose concentration after 75 g anhydrous glucose in an oral glucose, tolerance test above 11.1 mmol/L [200mg/dl].

Where the individual is considered to have metabolic disease, treatments (including treatment in accordance with the first aspect of the present invention) and/or life-style changes may be recommended. Where the individual is considered to have an increased risk of developing metabolic disease, prophylactic treatments (including prophylactic treatment in accordance with the first aspect of the present invention)

and/or life-style changes may be recommended. Where the individual is diagnosed as having a progressive metabolic disease, his or her clinician may recommend treatments and/or life-style changes tailored to the individual.

5 In the second aspect of the invention, levels of antibodies may be characterised by assaying for all antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, or for only antibodies of a particular isotype, such as IgM, IgG or IgA, or for a combination of two or more antibody isotypes. Preferably, the level of IgM is determined.

10 Immunoassays can be competitive or noncompetitive. In a typical competitive immunoassay, the antibody in the sample competes with labeled antibody to bind with PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative. The amount of labeled antibody bound to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, is then measured. There is an inverse relationship between concentration of antibody in the sample and the quantity of labeled antibody
15 detected. In noncompetitive immunoassays, antibody in the sample is bound to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, then a labeled detection reagent, typically an anti-immunoglobulin antibody, is bound to the antibody. The amount of labeled detection reagent bound to the antibody is then measured. Unlike the competitive method, the results of the noncompetitive method will be directly
20 proportional to the concentration of the antibody.

In a noncompetitive immunoassay or western blot, a labeled detection reagent, typically an anti-immunoglobulin antibody, is used to detect antibody bound to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative. A suitable anti-immunoglobulin antibody must bind specifically to immunoglobulin of the species from
25 which the sample is obtained. It may bind to all immunoglobulin isotypes of that species, or only a subset of isotypes. For example, it may bind only to IgA, IgD, IgE, IgG or IgM, or combinations of two or more of these isotypes. The anti-immunoglobulin antibody may bind specifically only to certain subtypes of any given isotype. Subtypes of human IgA are IgA1 and IgA2. The anti-immunoglobulin antibody may bind to one
30 or both of these subtypes. Subtypes of human IgG are IgG1, IgG2, IgG3 and IgG4. The anti-immunoglobulin may bind to one or more of these human IgG subtypes. It will be appreciated that there are different isotypes and subtypes in different vertebrate species.

In radioimmunoassay, the antibody or detection reagent is labeled with a radioisotope, such as ^{131}I or ^{125}I . In enzyme immunoassays, the antibody or detection reagent is labeled with an enzyme. Suitable enzymes are capable of being detected with the use of a chromogenic substrate. A chromogenic substrate is a substance
5 which, as a result of the reaction with the enzyme, gives rise to a coloured product which can thus be detected spectrophotometrically. Enzymes such as horse radish peroxidase, alkaline phosphatase, beta-galactosidase, and pyrophosphatase from *E.coli* have been widely employed. Chemi-luminescent systems based on enzymes such as luciferase can also be used. Other labels include fluorescent labels such as
10 fluorophores of the Alexa series.

Conjugation of the antibody or detection reagent with the vitamin biotin is frequently used since this can readily be detected by its reaction with enzyme- or fluorophore-linked avidin or streptavidin to which it binds with great specificity and affinity.

15 In a typical noncompetitive enzyme immunoassay, the sample to be analyzed is placed in contact and incubated with PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, adsorbed on a solid substrate. Any antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, that are possibly present in the sample are thus specifically bound by the PAF, PAF
20 conjugate, PAF derivative, or conjugate of a PAF derivative, adsorbed on the solid substrate, producing a first complex between the PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative and the reactive antibody thereto in the sample. The sample is then separated from the solid substrate so as to eliminate non-bound materials, for example, by washing. In the next step of the method, an indicator
25 antibody capable of binding any antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative that are present on the substrate in the form of the first complex is added to the solid substrate, thus producing a second complex between PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, the reactive antibody thereto, and the indicator antibody. The indicator
30 antibody may, for example, be an anti-human IgG immunoglobulin raised in a non-human animal species. Finally, the presence of the second complex on the solid substrate is detected, the presence of said second complex on the solid substrate being indicative of the presence of antibodies reactive with PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative in the sample from the individual.

Typically, the solid substrate is a micro-titration plate, for example, of the type commonly used for performing ELISA immunological assays. The micro-titration plate is preferably a polystyrene plate. Other suitable solid substrates are latex particles, beads and coated red blood cells. Conveniently, the PAF, PAF conjugate, PAF derivative, or conjugate of a PAF derivative, is adsorbed to the solid substrate by incubating it in a buffer with the solid substrate. Suitable buffers include carbonate buffer or phosphate buffered saline. Alternatively, the PAF, PAF conjugate, PAF derivative, or conjugate of a PAF derivative, may be covalently linked to the solid substrate. Typically, after adsorption or covalent linkage of PAF, PAF conjugate, PAF derivative, or conjugate of a PAF derivative, to the solid substrate, the solid substrate is incubated with a blocking agent to reduce non-specific binding of matter from the sample to the solid substrate. Suitable blocking agents are known in the art and include bovine serum albumin.

It is preferred that a quantitative estimate of antibody which can bind to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, is obtained by one or more of the above techniques. In typical non-competitive assays, a linear relationship between the measured variable, whether it be optical density or some other read-out, and antibody concentration, is assumed. For example, if sample A has double the optical density of sample B in the assay (background having been subtracted from both), it is assumed that the concentration of antibody is double in A compared to B. However, it is preferable to construct a standard curve of serial dilutions of a pool of positive serum samples. Preferably, such dilutions are assayed at the same time as the test samples. By doing this, any variation from the linear relationship may be taken into account in determining the quantity of antibody in the samples.

In the following example, levels of antibodies reactive to a PAF-BSA conjugate (as defined above) were determined by the following protocol. A microtitre plate was coated with 5 $\mu\text{g/ml}$ PAF-BSA conjugate (described above), in phosphate buffered saline (PBS). After washings with PBS, the plates were blocked with a 1% BSA solution. Serum samples were diluted (1:101) in sample diluent and added to the plates. The plates were incubated for 30 minutes at room temperature and washed. Horse radish peroxidase conjugated rabbit anti- human IgG diluted 1:1000 was added and incubated at room temperature for 30 minutes. After washings, colour was developed by adding 3,3',5,5', tetramethyl benzidine (TMB) substrate and the plates were incubated for 10 minutes at room temperature in the dark. The absorbance was

read in a spectrophotometer at 450 nm. The levels of antibodies reactive with PAF-BSA conjugate was calculated as the ratio between the absorbance obtained from the tested sample and the absorbance obtained from a positive control included in each assay.

5 As discussed above, the level of antibodies with reactivity to PAF, PAF-conjugate, PAF derivative, or conjugate of a PAF derivative, determined for any given individual may be categorised as high or low by reference to the range observed in the wider population or test cohort. It may be appropriate to assess the level of antibodies with reactivity to PAF, PAF-conjugate, PAF derivative, or conjugate of a PAF derivative,
10 in blood samples taken from individuals in a cohort *before* the onset of metabolic disease (incident cases) compared to three unrelated age- and sex-matched controls at blood draw (+/- 1 year), and/or to assess the level of antibodies with reactivity to PAF, PAF-conjugate, PAF derivative, or conjugate of a PAF derivative, in blood samples taken from individuals in a cohort *after* the onset of disease (prevalent cases)
15 compared to three unrelated age- and sex-matched controls at blood draw (+/- 1 year). It may be possible to match the controls to more than one test case and so the effective number of controls may therefore be less than 3 x number of cases. The total number of test and controls individuals in a suitable cohort may be greater than 100, such as about 200, 300, 400, 500, 600, 700, 800, 900 or 1000. Where a test case
20 shows a level of anti-PAF antibodies below the mean average, or below a particular percentile value determined with reference to the wider population or cohort, it may be categorised as a low level. Suitably, a low level may correspond to a value below the 25th percentile, or below the 20th, 10th or 5th percentile. A high level may for example, correspond to a value of above the 5th, 10th, 20th, or 25th percentile, or above the mean
25 average level.

In practice, the skilled person will appreciate that any percentile value cut-off point can be used to indicate a low level of antibodies with reactivity to PAF, PAF-conjugate, PAF derivative, or conjugate of a PAF derivative, that is associated with increased risk of developing a metabolic disease, so long as, when conditional logistic regression analysis is performed on the measured antibody levels generated from a
30 test cohort:-

- the calculated odds ratio for all individuals within that percentile group is greater than 1 (indicating that a person having a level of antibodies with reactivity to PAF, PAF-conjugate, PAF derivative, or conjugate of a PAF derivative, within

the levels associated with that percentile is more likely to develop a metabolic disease than a person having a level above that percentile); and

- the p-value calculated from the measured antibody values for individuals within that percentile group is less than 0.05 and the 95% odds ratio confidence interval for that group provides a range in which the lower limit is above 1 (wherein such p-values and CI values indicate that the odds ratio value ascribed to individuals with levels of antibodies with reactivity to PAF, PAF-conjugate, PAF derivative, or conjugate of a PAF derivative, falling within that percentile group is statistically significant).

10 In practice, therefore, the skilled person can readily determine by statistical analysis of the data from a cohort, the highest percentile value for which levels of antibodies with reactivity to PAF, PAF-conjugate, PAF derivative, or conjugate of a PAF derivative, indicate a statistically significant risk of developing a metabolic disease, and can also calculate associated (and incrementally higher) hazard ratios for individuals
15 with levels of antibodies with reactivity to PAF, PAF-conjugate, PAF derivative, or conjugate of a PAF derivative, falling within lower percentile values.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. Similarly, any embodiment discussed with respect to one aspect of the invention may
20 be used in the context of any other aspect of the invention.

Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. Alternatively, it may be used to signify a value that is \pm 20, 10, 5, 4, 3, 2, 1 or less than 1% of the stated value.

25 The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

The use of the term "or" in the claims is used to mean "and/or" unless explicitly
30 indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

Other objects, features and advantages of the present invention will be apparent from the detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and
5 modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

The examples disclosed below are provided only for the purpose of illustrating the present invention and should not be considered as any limitation of the scope as outlined in the appended claims. Document referred to herein are hereby incorporated
10 by reference.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a graph showing (a) the levels of IgM anti-PAF, (b) IgG anti-PAF, and (c) IgM anti BSA in apoE^(-/-) mice following immunization with a PAF-conjugate. - ◆ - control, - ■- PAF-conjugate, - ▲ - sham.

- 5 Figure 2 is a graph showing (a) fasting glucose, (b) insulin, and (c) HOMA-IR in apoE^(-/-) mice following immunization with a PAF-conjugate. - ◆ - control, - ■- PAF-conjugate, - ▲ - sham.

EXAMPLE

To investigate the effects of PAF antibodies on the development of diabetes,
10 mice were immunized with PAF conjugated to BSA, as the PAF molecule itself is too small to trigger an antibody reaction. As adjuvant we used aluminium hydroxide (Alum, Aluminium hydroxide hydrate, batch 034K3685, Sigma, St Louis, MO).

Thirty three male apoE^(-/-) mice (Taconics, Denmark) were used in the current study. At age 8 weeks, they were switched from a standard mouse chow diet to a
15 Western type diet containing 21.2% and 0.15% (wt/wt) fat and cholesterol, respectively (Lantmännen R368). The mice were divided into three groups, receiving 200 µL subcutaneous injections containing 25 µg/mouse of the PAF-BSA conjugate and one mg alum (PAF group), 25 µg BSA and Alum (sham) or saline (n = 11 per group). For active immunisation, one µg of PAF-BSA was added to 8 µl of alum (5 mg/ml in NaCl),
20 the mixture was gently shaken for an hour before administration. 200 µL was injected subcutaneously in the neck at each occasion. For the sham group, PAF-BSA was replaced by BSA, and in the controls, only NaCl was administered. Starting when the mice were placed on the Western diet, they received injections every second week until termination of the experiment after 16 weeks on the diet. Fasting blood samples (4
25 hours fasting) were taken from the tail vein or v saphena before the experiment started and before each immunisation.

Fasting insulin, F_{insulin}, and glucose were measured every 2nd week throughout the study. Tail vein blood samples were obtained after a 4 hour fasting period. Fasting blood glucose levels, FPG, were measured using an Accu-Chek Compact glucometer
30 (Roche Diagnostics Corp., Indianapolis; Indiana, USA), and insulin was determined by a ELISA kit specific for mouse insulin (Ultra Sensitive Mouse Insulin ELISA kit #90080, Crystal Chem Inc.).

Insulin sensitivity was estimated as HOMA-IR, obtained as the product of FPG and F_{insulin} .

The levels of antibodies against PAF was measured using the ELISA method as described above.

5 Results.

The active immunisation resulted in markedly elevated levels of IgM antibodies against PAF, while levels in the other groups were low (Figure 1a). Antibody levels began to rise after approximately two weeks, and reached maximal levels after 6-8 weeks. After this they remained high, although a dip was found at 10 weeks. In control and sham treated mice IgM anti-PAF levels were low compared to actively immunized, although at 2 weeks there was a transient increase in the control group. IgG antibodies against PAF appeared after 8 to 10 weeks in the actively immunized mice (Figure 1b), but remained low in the other groups. Although the methods used to measure the IgM and IgG antibodies are not quantitative, the levels of IgG anti-PAF were lower than levels of the IgM anti-PAF. As the PAF was linked to BSA, we also measured if antibodies towards BSA was formed. Similar amounts of BSA was given to actively and sham immunized mice, and in both groups low levels of IgM anti-BSA was found, but not in the controls (Figure 1c). The levels were very low compared to the IgM anti-PAF levels found. The treatments did not induce formation of IgG anti-BSA.

It is well known that apoE^(-/-) mice develop insulin resistance or prediabetes when administered an atherogenic diet (Zhang SH, Reddick RL, Burkey B, Maeda N. *Diet-induced atherosclerosis in mice heterozygous and homozygous for apolipoprotein E gene disruption*. J Clin Invest. 1994 **94**(3):937-45). This was the case also in the current study. Throughout the study period of 16 weeks, there was a non-significant increase in fasting glucose levels in all three study groups, after 16 weeks the levels were still within what can be considered as normal levels in mice (Figure 2a). Insulin resistance can be measured as increased fasting insulin levels in serum, or as HOMA-IR. Both fasting insulin levels and HOMA-IR increased significantly, indicating peripheral insulin resistance (Figure 2b and c). This process started after approximately 8 weeks on the atherogenic diet, and insulin resistance then increased steadily.

Compared to controls, mice actively immunized with PAF-BSA had better peripheral insulin sensitivity as shown by significantly lower increases in fasting insulin and HOMA-IR, without major differences in fasting glucose levels. Mice receiving BSA and adjuvant did not show any improvement in fasting insulin or glucose levels.

Conclusions.

As expected, immunisation of mice with PAF-BSA resulted in markedly elevated levels of antibodies against PAF, especially of the IgM isotype. A surprising finding was that this effect led to improved peripheral insulin sensitivity in this model of peripheral
5 insulin resistance. This finding shows that inhibiting PAF action in general represent a new way to treat patients with metabolic disease, such as peripheral insulin resistance, and in particular that PAF vaccination is one such treatment.

CLAIMS

1. A composition comprising at least one platelet activating factor (PAF) conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, for use in the immunization or prophylaxis against, or the prevention or treatment of, metabolic diseases in mammals.
2. Use of a composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, in the manufacture of a medicament for the immunization or prophylaxis against, or the prevention or treatment of, metabolic diseases in mammals.
3. A method for the immunization or prophylaxis against, or the treatment of, metabolic diseases in a mammal, the method comprising the step of administering to the mammal a pharmaceutical composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.
4. The composition of claim 1, the use of claim 2, or the method of claim 3, wherein the mammal is a human.
5. The composition of claim 1 or 4, the use of claim 2 or 4, or the method of claim 3 or 4, wherein metabolic disease is a condition selected from the group consisting of metabolic syndrome, insulin resistance, glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).
6. The composition of claim 1, 4 or 5, the use of claim 2, 4 or 5, or the method of claim 3, 4 or 5, wherein the composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, is a pharmaceutical composition comprising at least one PPAF conjugate, PAF derivative, or conjugate of a PAF derivative, optionally in combination with an adjuvant.
7. The composition of claim 1, 4 or 5, the use of claim 2, 4 or 5, or the method of claim 3, 4 or 5, wherein the antibody preparation with reactivity to PAF, a PAF

conjugate, a PAF derivative, or conjugate of a PAF derivative, comprises a monoclonal antibody with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.

- 5 8. The composition of any of claims 1 or 4 to 7, the use of any of claims 2 or 4 to 7, or the method of any of claims 3 to 7, for the therapeutic treatment of a mammal suffering from metabolic disease, or for the prophylactic treatment of a mammal facing the risk of developing metabolic disease.
- 10 9. The method of any of claims 3 to 8, wherein a therapeutically effective amount of a composition comprising at least one PAF conjugate, PAF derivative, or conjugate of a PAF derivative, or an antibody preparation with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, is administered to the mammal.
- 15 10. A method for diagnosing metabolic disease, or assessing a patient's risk of developing or progression of metabolic disease, the method comprising the steps of –
- (a) assessing the patient's level of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative; and
- (b) diagnosing metabolic disease or determining the patient's level of risk of developing or progression of metabolic disease based on the assessed levels of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.
- 20 11. The method of claim 10 comprising the step of assessing the patient's level of IgM, IgG or IgA antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative.
- 25 12. The method of claim 10 or 11, wherein the patient's level of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, are assessed by analysis of an *ex vivo* sample taken from the patient.
13. The method of any of claims 10 to 12, wherein the patient is human.
- 30 14. The method of any of claims 10 to 13, wherein the patient's level of antibodies with reactivity to PAF, a PAF conjugate, a PAF derivative, or conjugate of a

PAF derivative, correlates negatively with the patient's risk of developing or progression of the metabolic disease.

15. The method of any of claims 1- to 14 wherein metabolic disease is a condition selected from the group consisting of metabolic syndrome, insulin resistance, glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).
- 5
16. Use of PAF, a PAF conjugate, a PAF derivative, or conjugate of a PAF derivative, in a method of diagnosing metabolic disease and/or for assessing a patient's risk of developing or progression of metabolic disease as defined by any of claims 10 to 15.
- 10

Figure 1

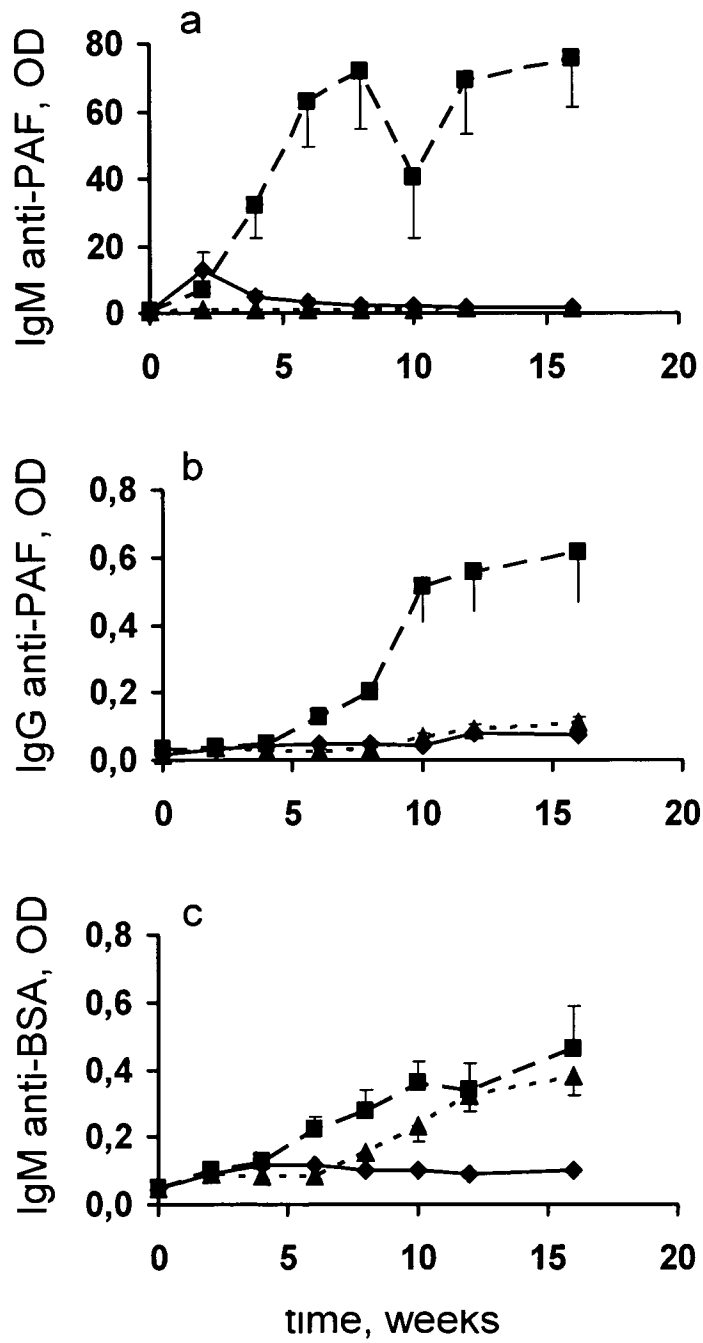
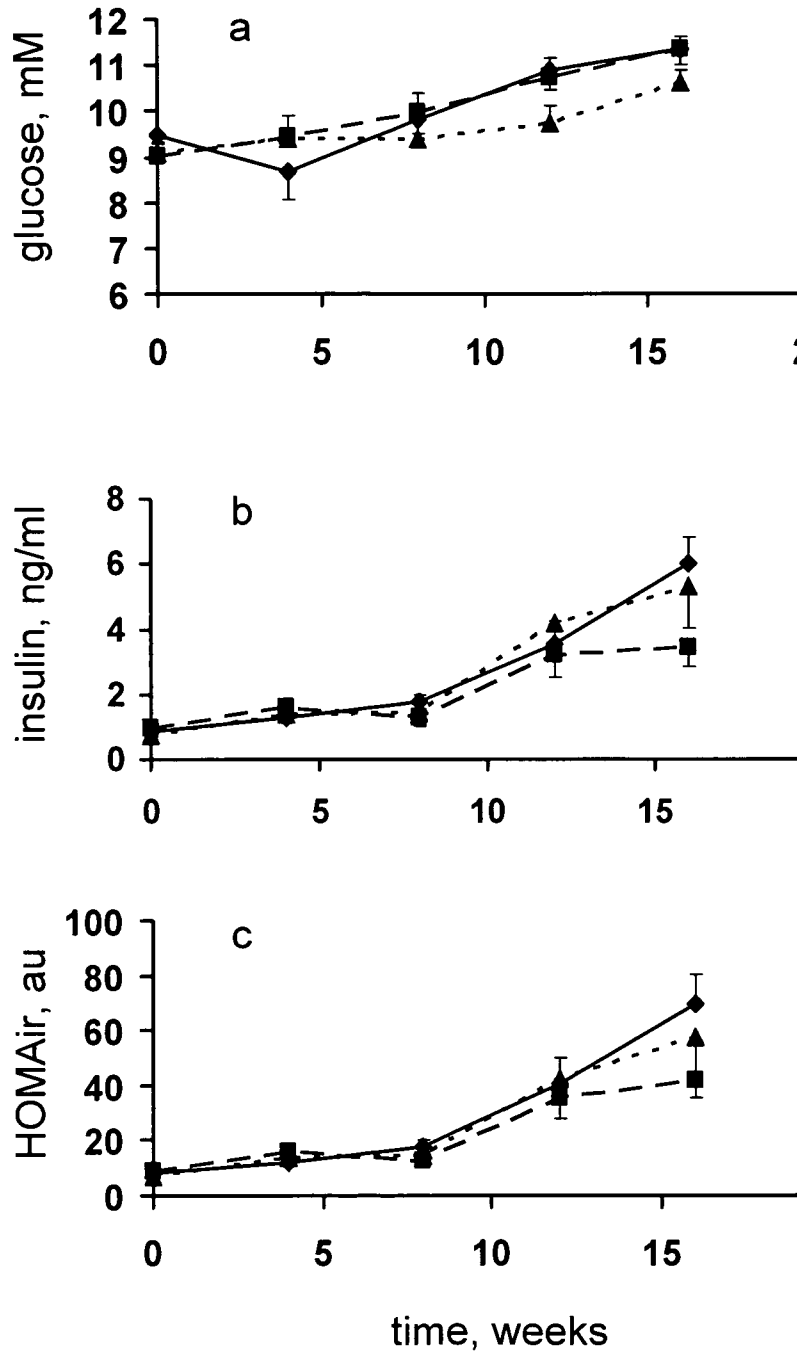


Figure 2



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2011/003603

A. CLASSIFICATION OF SUBJECT MATTER
 INV. A61K39/00 A61K39/395 A61K47/48 A61P3/08
 ADD.

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)
 A61K

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, BIOSIS, CHEM ABS Data, EMBASE, SCISEARCH, WPI 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 2009/056826 A1 (ATHERA BIOTECHNOLOGIES AB [SE]; WRIGHT ANDREW [GB]; GROENLUND HANS [SE]) 7 May 2009 (2009-05-07) pages 5,6,9; claims 1-3,8,17; example 1 -----	1-4, 6-14,16
Y	KUDOLO GEORGE B ET AL: "Chronic hyperinsulinemia inhibits platelet-activating factor (PAF) biosynthesis in the rat kidney", JOURNAL OF LIPID MEDIATORS AND CELL SIGNALLING, vol. 16, no. 1, 1997, pages 23-37, XP002664192, ISSN: 0929-7855 the whole document ----- -/--	1-16



Further documents are listed in the continuation of Box C.



See patent family 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

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"&" document member of the same patent family

Date of the actual completion of the international search

23 November 2011

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2011/003603

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WU R ET AL: "Antibodies to platelet-activating factor are associated with borderline hypertension, early atherosclerosis and the metabolic syndrome", JOURNAL OF INTERNAL MEDICINE, vol. 246, no. 4, October 1999 (1999-10), pages 389-397, XP002664193, ISSN: 0954-6820 the whole document</p>	1-16
A	<p>TEKTONIDOU M G ET AL: "Clinical importance of antibodies against platelet activating factor in antiphospholipid syndrome manifestations", EUROPEAN JOURNAL OF CLINICAL INVESTIGATION, vol. 30, no. 7, July 2000 (2000-07), pages 646-652, XP002664194, ISSN: 0014-2972 the whole document</p>	1-16
A	<p>RIZOS E ET AL: "Lipoprotein-associated PAF-acetylhydrolase activity in subjects with the metabolic syndrome", PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS, CHURCHILL LIVINGSTONE, EDINBURGH, vol. 72, no. 3, 1 March 2005 (2005-03-01), pages 203-209, XP004721686, ISSN: 0952-3278, DOI: 10.1016/J.PLEFA.2004.10.021 the whole document</p>	1-16
Y	<p>WO 87/05904 A1 (UNIV SYDNEY [AU]; UNIV MACQUARIE [AU]; ROYAL NORTH SHORE HOSPITAL [AU]) 8 October 1987 (1987-10-08) the whole document</p>	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2011/003603

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		EP 2217280	A1 18-08-2010
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		US 2010278836	A1 04-11-2010
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WO 8705904	A1	08-10-1987	
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		JP H01502584	A 07-09-1989
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