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(54) Title: METHOD FOR MONITORING THE EFFECT OF ACE INHIBITOR THERAPY ON PERIPHERAL INSULIN RESISTANCE

(57) Abstract: The present invention relates to the field of diagnostics. Particularly, it relates to the use of adiponectin, C-reactive protein and retinol binding protein 4 for the monitoring of a therapy with ACE-inhibitors for the treatment of peripheral insulin resistance.



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Method for monitoring the effect of ACE inhibitor therapy on peripheral insulin resistance

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10 The present invention relates to the field of diagnostics. Particularly, it relates to the use of adiponectin, C-reactive protein and retinol binding protein 4 for the monitoring of a therapy with ACE-inhibitors for the treatment of peripheral insulin resistance.

There are two main categories of diabetes mellitus, type 1 and type 2, which can be distinguished by a combination of features known to the person skilled in the art. Type 1 diabetes is caused by the loss of the beta cells of the islets of Langerhans in the pancreas. Endogenous insulin production is, thus, effectively abolished in these patients. In type 2 diabetes (previously called adult-onset or non-insulin-dependent), insulin secretion is not diminished, at least not in the early stages of the disease. Often insulin levels are very high, especially early in the disease, but peripheral insulin resistance and increased hepatic production of glucose make insulin levels inadequate to normalize plasma glucose levels. Insulin production then falls, further exacerbating hyperglycemia. The disease generally develops in adults and becomes more common with age. Plasma glucose levels reach higher levels after eating in older than in younger adults, especially after high carbohydrate loads, and take longer to return to normal, in part because of increased accumulation of visceral and abdominal fat and decreased muscle mass.

25 The metabolic syndrome is associated with an imbalance between energy intake and the capacity for energy storage and results in the ectopic deposition of lipids in visceral fat, liver, skeletal muscle, pancreatic beta cells and vessel walls (Smith, 2006, Obesity Vol. 14 Suppl. 128S-134S). It is a constellation of interrelated risk factors of metabolic origin that is considered to directly promote the development of atherosclerotic cardiovascular disease and diabetes type 2 (Grundy et al., 2005, Circulation 112, 2735-2752).

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The predominant underlying risk factors for the syndrome are, presumably, abdominal obesity and insulin resistance. Other conditions that are associated with the metabolic syndrome can be physical inactivity and hormonal imbalances. The metabolic syndrome
5 can occur both in obese and in non-obese patients. In non-obese patients, lipids are stored in visceral reservoirs. Once these reservoirs are filled, lipids are also stored in other tissues and organs. Especially, in the presence of visceral obesity, there is a significantly increased risk of progressing to diabetes type 2 and cardiovascular disease.

10 Type 2 diabetes can be treated by dietary and lifestyle modification, oral antidiabetics and insulin substitution. Dietary and life style modifications aim at weight reduction in obese patients and at increased physical exercise. There are several groups of oral antidiabetics: sulfonyureas, meglitinides and biguanides increase the secretion of insulin by the beta cells in the pancreas. Thiazolidinediones reduce the peripheral insulin resistance. If life style
15 modification and oral antidiabetics fail to lower blood glucose levels sufficiently, insulin substitution therapy becomes necessary.

In the context of the present invention it is important to note, that medicaments that influence the renin-angiotensin system (RAS), i.e. ACE-inhibitors and AT₁-receptor
20 inhibitors, have in many cases been shown to protect against or to slow down the onset of type 2 diabetes (McGuire et al., 2008, Diabetes Vasc Dis Res, 5: 59-66; Karagiannis et al., 2007, Expert Opin Ther Targets, 11: 191-205). They increase the plasma level of adiponectin in hypertensive patients with insulin resistance (Perkins and Davis, 2008, Current Opinion in Endocrinology, Diabetes & Obesity, 15: 147-152).

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ACE-inhibitors inhibit the angiotensin converting enzyme which converts angiotensin I into angiotensin II. They reduce cardiac myocyte growth, myocyte apoptosis, aldosterone release, norepinephrine release, fibroblast proliferation, smooth muscle proliferation and vasoconstriction. ACE-inhibitors are frequently used for the treatment or prevention of
30 heart failure.

However, therapy of insulin resistance with ACE-inhibitors is not in all patients successful. Consequently, the problem underlying the present invention can be viewed as providing means and methods for monitoring the therapy against peripheral insulin resistance with ACE-inhibitors.

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The problem is solved by the embodiments characterized in the claims and below.

The present invention relates to a method for monitoring the therapy with at least one ACE-inhibitor in a patient comprising the steps of

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- a) measuring the level of RBP4 in a first sample from a patient;
- b) measuring the level of RBP4 in at least one further sample from a patient; and
- c) comparing the level of RBP4 in the at least one further sample to the level of the respective marker in the previous sample or samples.

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As set forth elsewhere herein, the method of the present invention, preferably, may further comprise the steps of

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- a1) measuring the level of CRP (C-reactive binding protein) and/or Adiponectin in a first sample from a patient;
- b1) measuring the level of CRP and/or Adiponectin in at least one further sample from the patient; and
- c1) comparing the level of Adiponectin and/or CRP in the at least one further sample

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to the level of the respective marker in the previous sample or samples.

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In the course of the study underlying the present invention it was surprisingly discovered that ACE-inhibitors influence not only the level of adiponectin. Additionally, they increase the levels of retinol-binding protein 4 (RBP4). This effect of ACE-inhibitors is undesirable because RBP4 induces insulin resistance and is increased in type 2 diabetes patients (Lin et al., 2008, Translational Research, 151: 309-314). Thus, for the therapy of peripheral insulin resistance or type 2 diabetes with an ACE-inhibitor the dosage has to be sufficiently high

to increase the insulin sensitivity of the patient. On the other hand, the undesired effects of high dosages of ACE-inhibitors on the levels of RBP4 have to be avoided. In patients who receive ACE-inhibitors for the treatment of other disorders than peripheral insulin resistance or type 2 diabetes undesired effects of ACE-inhibitors on peripheral insulin resistance have to be monitored as well.

The surprising finding that ACE-inhibitors have positive (increase of adiponectin levels, decrease of CRP levels) as well as negative (increase of RBP4 levels) effects on insulin resistance demonstrates that the proper treatment of peripheral insulin resistance with ACE-inhibitors is more complex than previously thought.

In the context of the method of the present invention, the level of RBP4 (and, optionally, CRP and/or adiponectin) in the at least one further sample shall be compared to the level of the respective marker in the previous sample or samples. Preferably, the level in the at least one further sample shall be compared to the level in the first sample. More preferably, the at least one further sample is the second sample.

In principle, the first sample and the second sample as well as any further sample can be taken at any time during the treatment with the ACE-inhibitor(s) provided that the first sample is taken before the second sample. A first sample from a patient is, preferably, taken before the start therapy against peripheral insulin resistance with at least one ACE-inhibitor. At least one further sample is taken, preferably, after the onset of therapy against peripheral insulin resistance with at least one ACE-inhibitor. However, monitoring can, in principle, be started at any time during treatment.

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The Person skilled in the art knows that different sampling intervals are possible. If the therapy with ACE-inhibitors is started, if the therapy with ACE-inhibitors is altered in any way or if there are changes in the physical conditions or the life style of the patient, sampling is performed, preferably, 6 weeks after the event in question allowing the RBP4 level to adapt to the new conditions. If there are no changes in the treatment or the

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condition of the patient, samples are, preferably, taken in intervals of 3 or 12 months, most preferably 6 months.

Thus, the first sample is, preferably, obtained at the start of the therapy with the at least one
5 ACE inhibitor, shortly before the start of said therapy or during said therapy. "Shortly before the start of the therapy" as used herein, preferably, means 1, 2, 3, 4, 6 or 7 days before the therapy is initiated. If the first sample is obtained during therapy, it is particularly envisaged to obtain said sample if the therapy with ACE-inhibitors is altered in any way or if there are changes in the physical conditions or the life style of the patient.

10 The second sample is, preferably, obtained 6 weeks, 3 months or 12 months after the first sample.

The monitoring of the levels of the markers of the present invention in a patient over a longer time allows the early identification of the need for adjusting the therapy.

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Changes in the life style or the general physical condition of a patient are, preferably, weight loss, weight gain, increased or decreased physical activity, additional illnesses or pregnancy. All these changes potentially change the peripheral insulin resistance of a patient.

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An increase of the level of RBP4 in the second sample (or in further samples) from a patient as compared to the first sample is indicative of an increase of peripheral insulin resistance.

25 Thus, an increase, a significant increase, or, more preferably, a statistically significant increase of the level of RBP4 in the second sample as compared to the level in the first sample, preferably, indicates an increase in the peripheral insulin resistance. Preferably, said increase of said level indicates that the therapy with the at least one ACE inhibitor has

to be adjusted (e.g., by lowering the dosage of said ACE inhibitor, the term "adjusting the therapy with at least one ACE inhibitor" is explained elsewhere herein).

Thus, an unchanged level or a decrease, or a statistically significant decrease of the level of
5 RBP4 in the second sample as compared to the level in the first sample, preferably, indicates an unchanged or decreased peripheral insulin resistance. Preferably, said unchanged or decreased level indicates that the therapy with the ACE can either be continued without changing the dosage of said ACE inhibitor or can be continued with an increased dosage of said ACE inhibitor.

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Particularly, a significant increase (or decrease) is an increase (or decrease) of a size which is considered to be significant for diagnosis, particularly statistically significant. The terms "significant" and "statistically significant" are known to the person skilled in the art. Whether an increase (or a decrease) is statistically significant can be determined without
15 further ado by the person skilled in the art using various well known statistic evaluation tools including those referred to herein.

Preferred significant increases of the levels of RBP4 in samples (preferably in plasma samples within an interval of 6 weeks) which have been found in the course of the
20 invention to be indicative for an increase in the peripheral insulin resistance.

Preferably, an increase of the level of RBP4 in the second sample compared to the level in the first sample, preferably, of at least 7 %, more preferably of at least 10%, and even, more preferably, of at least 15 % or of at least 20 %, and most preferably of at least 30% is,
25 considered to be significant and, thus, to be associated with a change with respect to the peripheral insulin resistance of a patient, and, thus, to be indicative for an adjustment of the therapy with the ACE inhibitor.

With respect to the absolute level: Preferably, an increase of the level of RBP4 in the second sample compared to the level in the first sample, preferably, of at least 4 mg/l, more preferably of at least 7 mg/l, and even, more preferably, of at least 10 mg/l or of at least 15 mg/l and most preferably of at least 20 mg/l is, considered to be significant and, thus, to is
5 indicative for an increase in the peripheral insulin resistance (and, thus, to be indicative for an adjustment of the therapy with the ACE inhibitor).

Preferred significant decreases of the levels of RBP4 in samples (preferably in plasma samples within an interval of 6 weeks) which have been found in the course of the
10 invention to be indicative for a decrease in the peripheral insulin resistance.

Preferably, a decrease of the level of RBP4 in the second sample compared to the level in the first sample, preferably, at least 10 %; at least 20 %; at least 30 %; at least 40 %; at least 50 %; at least 60 %; at least 70 %; at least 80 % or at least 100 % is, considered to be
15 significant and, thus, to be associated with a change with respect to the peripheral insulin resistance of a patient. Said decrease indicates that the therapy may be continued (with an unchanged dosage or an increased dosage).

With respect to the absolute level: Preferably, a decrease of the level of RBP4 in the
20 second sample compared to the level in the first sample, preferably, of at least 4 mg/l, more preferably of at least 5 mg/l, and even, more preferably, of at least 7 mg/l or of at least 10 mg/l, 20 and most preferably of at least 25 mg/l is, considered to be significant and, thus, to is indicative for a decrease in the peripheral insulin resistance (and, thus, to be indicative for an adjustment of the therapy with the ACE inhibitor). Said decrease indicates that the
25 therapy may be continued (with an unchanged dosage or an increased dosage).

The term "ACE-inhibitor", preferably refers to benazepril, captopril, cilazapril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril, and trandolapril. The most preferred ACE-inhibitor in the context of the method of the present invention is
30 lisinopril.

Retinol binding protein 4 (RBP4) is a protein of the lipocalin family. It is the specific carrier of retinol in blood and transports retinol from the storages in the liver to the peripheral tissues. It is an adipokine, i.e. it is a cell-to-cell signalling molecule secreted by adipocytes. As described above it has recently been associated with the occurrence of insulin resistance. Preferably, RBP-4 has the same amino acid sequence as the protein with the GenBank accession number CAH72329.1. Increased RBP4-levels are associated with type 2 diabetes and the metabolic syndrome (see EP 08 168 164)

CRP, RBP4 and adiponectin used herein also encompass variants of the aforementioned specific CRP, RBP4 or adiponectin polypeptides. Such variants have at least the same essential biological and immunological properties as the specific CRP, RBP4 or adiponectin polypeptides. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA assays using polyclonal or monoclonal antibodies specifically recognizing the said CRP, RBP4 or adiponectin polypeptides. Moreover, it is to be understood that a variant as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino sequence of the specific CRP, RBP4 or adiponectin polypeptide (preferably over the entire length of the CRP, RBP4 or adiponectin polypeptide). The degree of identity between two amino acid sequences can be determined by algorithms well known in the art. Preferably, the degree of identity is to be determined by comparing two optimally aligned sequences over a comparison window, where the fragment of amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the

percentage of sequence identity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Add. APL. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (USA)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, PASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by visual inspection. Given that two sequences have been identified for comparison, GAP and BESTFIT are preferably employed to determine their optimal alignment and, thus, the degree of identity. Preferably, the default values of 5.00 for gap weight and 0.30 for gap weight length are used. Variants referred to above may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments or subunits of the specific CRP, RBP4 or adiponectin polypeptides or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above. Such fragments may be, e.g., degradation products of the CRP, RBP4 or adiponectin peptides. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation.

Preferably, a variant shall have the biological properties of the corresponding polypeptide. E.g. a variant of RBP4, preferably, shall have the ability to bind to retinoids (preferably retinol) and/or the ability to bind to transthyretin. Whether a variant of RBP4 binds to retinoids or to transthyretin can be determined by well known methods. A particularly preferred method for determining whether a variant binds to retinoids or to transthyretin is described by Sharif et al. (2009, Time-resolved fluorescence resonance energy transfer and surface plasmon resonance-based assays for retinoid and transthyretin binding to retinol-binding protein 4, 2009, *Analytical Biochemistry*, Volume 392, Issue 2, Pages 162-168) which hereby is incorporated by reference with respect to its entire disclosure content.

Determining the amount (level) of CRP, RBP4, adiponectin or any other peptide or polypeptide referred to in this specification relates to measuring the amount or

concentration, preferably semi-quantitatively or quantitatively. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of the peptide or polypeptide based on a signal which is obtained from the peptide or polypeptide itself and the intensity of which directly correlates with the number of molecules of the peptide present in the sample. Such a signal - sometimes referred to herein as intensity signal - may be obtained, e.g., by measuring an intensity value of a specific physical or chemical property of the peptide or polypeptide. Indirect measuring includes measuring of a signal obtained from a secondary component (i.e. a component not being the peptide or polypeptide itself) or a biological read out system, e.g., measurable cellular responses, ligands, labels, or enzymatic reaction products.

In accordance with the present invention, determining the amount of a peptide or polypeptide can be achieved by all known means for determining the amount of a peptide in a sample. Said means comprise immunoassay devices and methods which may utilize labelled molecules in various sandwich, competition, or other assay formats. Said assays will develop a signal which is indicative for the presence or absence of the peptide or polypeptide. Moreover, the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse- proportional) to the amount of polypeptide present in a sample. Further suitable methods comprise measuring a physical or chemical property specific for the peptide or polypeptide such as its precise molecular mass or NMR spectrum. Said methods comprise, preferably, biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass- spectrometers, NMR- analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoassays (available for example on ElecsysTM analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-HitachiTM analyzers), and latex agglutination assays (available for example on Roche-HitachiTM analyzers).

Preferably, determining the amount of a peptide or polypeptide comprises the steps of (a) contacting a cell capable of eliciting a cellular response the intensity of which is indicative of the amount of the peptide or polypeptide with the said peptide or polypeptide for an

adequate period of time, (b) measuring the cellular response. For measuring cellular responses, the sample or processed sample is, preferably, added to a cell culture and an internal or external cellular response is measured. The cellular response may include the measurable expression of a reporter gene or the secretion of a substance, e.g. a peptide, polypeptide, or a small molecule. The expression or substance shall generate an intensity signal which correlates to the amount of the peptide or polypeptide.

Also preferably, determining the amount of a peptide or polypeptide comprises the step of measuring a specific intensity signal obtainable from the peptide or polypeptide in the sample. As described above, such a signal may be the signal intensity observed at an m/z variable specific for the peptide or polypeptide observed in mass spectra or a NMR spectrum specific for the peptide or polypeptide.

Determining the amount of a peptide or polypeptide, preferably, comprises the steps of (a) contacting the peptide with a specific ligand, (b) (optionally) removing non-bound ligand, (c) measuring the amount of bound ligand. The bound ligand will generate an intensity signal. Binding according to the present invention includes both covalent and non-covalent binding. A ligand according to the present invention can be any compound, e.g., a peptide, polypeptide, nucleic acid, or small molecule, binding to the peptide or polypeptide described herein. Preferred ligands include antibodies, nucleic acids, peptides or polypeptides such as receptors or binding partners for the peptide or polypeptide and fragments thereof comprising the binding domains for the peptides, and aptamers, e.g. nucleic acid or peptide aptamers. Methods to prepare such ligands are well-known in the art. For example, identification and production of suitable antibodies or aptamers is also offered by commercial suppliers. The person skilled in the art is familiar with methods to develop derivatives of such ligands with higher affinity or specificity. For example, random mutations can be introduced into the nucleic acids, peptides or polypeptides. These derivatives can then be tested for binding according to screening procedures known in the art, e.g. phage display. Antibodies as referred to herein include both polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(ab)₂ fragments that are capable of binding antigen or hapten. The present invention also includes single

chain antibodies and humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Preferably, the ligand or agent binds specifically to the peptide or polypeptide. Specific binding according to the present invention means that the ligand or agent should not bind substantially to ("cross-react" with) another peptide, polypeptide or substance present in the sample to be analyzed. Preferably, the specifically bound peptide or polypeptide should be bound with at least 3 times higher, more preferably at least 10 times higher and even more preferably at least 50 times higher affinity than any other relevant peptide or polypeptide. Non-specific binding may be tolerable, if it can still be distinguished and measured unequivocally, e.g. according to its size on a Western Blot, or by its relatively higher abundance in the sample. Binding of the ligand can be measured by any method known in the art. Preferably, said method is semi-quantitative or quantitative. Suitable methods are described in the following.

First, binding of a ligand may be measured directly, e.g. by NMR or surface plasmon resonance.

Second, if the ligand also serves as a substrate of an enzymatic activity of the peptide or polypeptide of interest, an enzymatic reaction product may be measured (e.g. the amount of a protease can be measured by measuring the amount of cleaved substrate, e.g. on a Western Blot). Alternatively, the ligand may exhibit enzymatic properties itself and the "ligand/peptide or polypeptide" complex or the ligand which was bound by the peptide or polypeptide, respectively, may be contacted with a suitable substrate allowing detection by the generation of an intensity signal. For measurement of enzymatic reaction products, preferably the amount of substrate is saturating. The substrate may also be labeled with a detectable label prior to the reaction. Preferably, the sample is contacted with the substrate for an adequate period of time. An adequate period of time refers to the time necessary for

an detectable, preferably measurable, amount of product to be produced. Instead of measuring the amount of product, the time necessary for appearance of a given (e.g. detectable) amount of product can be measured.

- 5 Third, the ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand. Labelling may be done by direct or indirect methods. Direct labeling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labeling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand.
- 10 Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often used to increase the signal. Suitable secondary and higher order ligands may include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.). The ligand or substrate may also be
- 15 "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin, digoxigenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, influenza A virus haemagglutinin (HA), maltose binding protein, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus. Suitable labels are any labels detectable by an
- 20 appropriate detection method. Typical labels include gold particles, latex beads, acridan ester, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels ("e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels. Enzymatically active labels include e.g. horseradish peroxidase, alkaline phosphatase, beta-Galactosidase, Luciferase, and derivatives thereof. Suitable substrates
- 25 for detection include di-amino-benzidine (DAB), 3,3'-5,5'-tetramethylbenzidine, NBT-BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, available as ready-made stock solution from Roche Diagnostics), CDP-Star™ (Amersham Biosciences), ECF™ (Amersham Biosciences). A suitable enzyme-substrate combination may result in a colored reaction product, fluorescence or chemoluminescence, which can
- 30 be measured according to methods known in the art (e.g. using a light-sensitive film or a suitable camera system). As for measuring the enzymatic reaction, the criteria given above

apply analogously. Typical fluorescent labels include fluorescent proteins (such as GFP and its derivatives), Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568). Further fluorescent labels are available e.g. from Molecular Probes (Oregon). Also the use of quantum dots as fluorescent labels is contemplated. Typical radioactive labels
5 include ^{35}S , ^{125}I , ^{32}P , ^{33}P and the like. A radioactive label can be detected by any method known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according to the present invention also include precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent
10 assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFI), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid
15 gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can be used alone or in combination with labeling or other detection methods as described above.

The amount of a peptide or polypeptide may be, also preferably, determined as follows: (a) contacting a solid support comprising a ligand for the peptide or polypeptide as specified
20 above with a sample comprising the peptide or polypeptide and (b) measuring the amount peptide or polypeptide which is bound to the support. The ligand, preferably chosen from the group consisting of nucleic acids, peptides, polypeptides, antibodies and aptamers, is preferably present on a solid support in immobilized form. Materials for manufacturing solid supports are well known in the art and include, inter alia, commercially available
25 column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracytes, wells and walls of reaction trays, plastic tubes etc. The ligand or agent may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses,
30 natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Suitable

methods for fixing/immobilizing said ligand are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like. It is also contemplated to use "suspension arrays" as arrays according to the present invention (Nolan 2002, Trends Biotechnol. 20(1):9-12). In such suspension arrays, the carrier, e.g. a microbead or
5 microsphere, is present in suspension. The array consists of different microbeads or microspheres, possibly labeled, carrying different ligands. Methods of producing such arrays, for example based on solid-phase chemistry and photo-labile protective groups, are generally known (US 5,744,305).

10 The term "amount" as used herein encompasses the absolute amount (level) of a polypeptide or peptide, the relative amount (level) or concentration of the said polypeptide or peptide as well as any value or parameter which correlates thereto or can be derived therefrom. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the said peptides by direct measurements,
15 e.g., intensity values in mass spectra or NMR spectra. Moreover, encompassed are all values or parameters which are obtained by indirect measurements specified elsewhere in this description, e.g., response levels determined from biological read out systems in response to the peptides or intensity signals obtained from specifically bound ligands. It is to be understood that values correlating to the aforementioned amounts or parameters can
20 also be obtained by all standard mathematical operations.

The term "sample" refers to a sample of a body fluid, to a sample of separated cells or to a sample from a tissue or an organ. Samples of body fluids can be obtained by well known techniques and include, preferably, samples of blood, plasma, serum, or urine, more
25 preferably, samples of blood, plasma or serum. Tissue or organ samples may be obtained from any tissue or organ by, e.g., biopsy. Separated cells may be obtained from the body fluids or the tissues or organs by separating techniques such as centrifugation or cell sorting. Preferably, cell-, tissue- or organ samples are obtained from those cells, tissues or organs which express or produce the peptides referred to herein.

The term “patient”, preferably, refers to a human. More preferably, the patient suffers from high blood pressure, heart failure, diabetes or peripheral insulin resistance. More preferably, the patient suffers from type 2 diabetes or the metabolic syndrome. Most preferably, the patient suffers from type 1 diabetes.

5

The term “diabetes”, preferably, refers to type 1 or type 2 diabetes. In type 1 diabetes (previously called juvenile-onset or insulin-dependent diabetes), insulin production is absent because of autoimmune pancreatic beta-cell destruction, possibly triggered by environmental exposure in genetically susceptible people. Destruction progresses subclinically over months or years until beta-cell mass decreases to the point that insulin concentrations are no longer adequate to control plasma glucose levels. The type 1 diabetes generally develops in childhood or adolescence and until recently was the most common form diagnosed before age 30; however, it can also develop in adults.

10

In type 2 diabetes (previously called adult-onset or non-insulin-dependent diabetes), insulin secretion is inadequate. Often insulin levels are very high, especially early in the disease, but peripheral insulin resistance and increased hepatic production of glucose makes insulin levels inadequate to normalized plasma glucose levels. Insulin production then falls, further exacerbating hyperglycemia. The disease generally develops in adults and becomes more common with age. Plasma glucose levels reach higher levels after eating in older than in younger adults, especially after high carbohydrate loads, and take longer to return to normal, in part because of increased accumulation of visceral and abdominal fat and decreased muscle mass.

20

The term “metabolic syndrome” refers to combination of peripheral insulin resistance, increased blood pressure, obesity and other factors as outlined below. The criteria according to the WHO (1998) require, for the diagnosis of a metabolic syndrome in a subject, the presence of insulin resistance identified by one of the following: diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or for those with normal fasting glucose levels (< 110 mg/dL) glucose uptake below the lowest quartile for

30

background population under investigation under hyperinsulinemic, euglycemic conditions. In addition to insulin resistance, the presence at least two of the following is required:

- 5 a) blood pressure: ≥ 140 mmHg systolic or ≥ 90 mmHg diastolic (or on treatment for high blood pressure)
- b) plasma triglycerides (TG): ≥ 1.695 mmol/L and high-density lipoprotein cholesterol (HDL C) ≤ 0.9 mmol/L (for male subjects), ≤ 1.0 mmol/L (for female subjects)
- c) waist to hip ratio > 0.90 (for male subjects); > 0.85 (for female subjects), and/or body mass index (BMI) > 30 kg/m²
- 10 d) urinary albumin excretion ratio ≥ 20 μ g/min or albumin to creatinine ratio ≥ 30 mg/g.

The US National Cholesterol Education Program Adult Treatment Panel III (ATP-III/NCEP, 2001) requires, for the presence/diagnosis of a metabolic syndrome, at least three of the following:

- 15 a) abdominal obesity given as waist circumference ≥ 102 cm (for male subjects), ≥ 88 cm (for female subjects)
- b) plasma triglycerides ≥ 1.695 mmol/L (150 mg/dl)
- c) HDL-C < 40 mg/dL (for male subjects), < 50 mg/dL (for female subjects)
- d) blood pressure $\geq 130/ \geq 85$ mmHg
- 20 e) fasting plasma glucose ≥ 6.1 mmol/L (110 mg/dl)

The European Group for the Study of Insulin Resistance (EGIR) requires, for the diagnosis/ presence of a metabolic syndrome, insulin resistance defined as the top 25% of the fasting insulin values among non-diabetic individuals, and two or more of the
25 following:

- a) central obesity: waist circumference ≥ 94 cm (male), ≥ 80 cm (female) and/or body mass index (BMI) $> 30 \text{ kg/m}^2$
- b) plasma triglycerides $\geq 150 \text{ mg/dL}$ and/or HDL-C $< 39 \text{ mg/dL}$ (or treated for dyslipidaemia)
- 5 c) hypertension: blood pressure $\geq 140/90 \text{ mm Hg}$ (or antihypertensive medication)
- d) fasting plasma glucose $\geq 6.1 \text{ mmol/L}$

The AHA/NHLBI criteria American Heart Association/Updated NCEP require, for the presence/diagnosis of a metabolic syndrome, at least three of the following: elevated waist
10 circumference: (Men: Equal to or greater than 102 cm, women equal to or greater than 88 cm); elevated triglycerides (equal to or greater than 150 mg/dl); reduced HDL cholesterol (men: less than 40 mg/dL; women: less than 50 mg/dL); elevated blood pressure (equal to or greater than 130/85 mm Hg or use of medication for hypertension) elevated fasting glucose (equal to or greater than 100 mg/dL (5.6 mmol/L) or use of medication for
15 hyperglycemia).

The term "comparing" as used herein encompasses comparing the amount of the peptide or polypeptide comprised by the sample to be analyzed with an amount of a suitable reference source specified elsewhere in this description. Preferably, the amount (level) of
20 polypeptide as referred to herein in the first sample shall be compared with the amount (level) in the second sample. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount is compared to an absolute reference amount while a concentration is compared to a reference concentration or an intensity signal obtained from a test sample is compared to the same
25 type of intensity signal of a reference sample. The comparison referred to in step (c) of the method of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount may be compared to values corresponding to suitable references which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison, i.e.
30 automatically provide the desired assessment in a suitable output format. Based on the

comparison of the amount determined in step a) and the reference amount, it is possible to determine the effective dosage of at least one ACE-inhibitor that has to be administered to a patient. Furthermore, during the therapy with at least one ACE-inhibitor the comparison of the measured amount of CRP, RBP4 and/or adiponectin with the desired amounts
5 enables the adjustment of the therapy against peripheral insulin resistance to the patient's needs.

Preferably, the method of the present invention is an in-vitro method. That means that the determination of the respective peptide (CRP, RBP4 and/or adiponectin, in particular
10 RBP4) to be determined is carried out in vitro.

Advantageously, the present invention provides a method for the monitoring of the therapy of peripheral insulin resistance with at least one ACE-inhibitor. Based on the results the therapy can be adjusted according to the needs of the individual. As every person differs
15 regarding body weight, peripheral insulin resistance and the response to ACE-inhibitors, the required dosage has to be determined individually. The effect of ACE-inhibitors on blood glucose levels can be easily monitored. However, the fact that control of blood glucose levels in a patient who takes ACE-inhibitors is insufficient does not indicate the required cause of action. The poor glucose control may be caused by a dosage of the ACE-
20 inhibitor(s) which is too low to significantly reduce peripheral insulin resistance. Alternatively, the ACE-inhibitors may increase RBP4 levels, thus leading to increased peripheral insulin resistance and poor glucose control. Depending on the cause underlying poor control of blood glucose levels different courses of action are required. In the former case, if the dosage of the ACE-inhibitor is insufficient as indicated by low adiponectin
25 levels and high CRP levels, an increased dosage is required to counter peripheral insulin resistance. In the latter case if the ACE-inhibitor causes an increase of RBP4 levels in the patient, the dosage of the ACE-inhibitor(s) has to be reduced or substituted and/or additional medication against peripheral insulin resistance has to be administered.

30 In a preferred embodiment of the method of the present invention, the level of Troponin T and/or NT-proBNP is measured instead of the level of RBP4.

The definitions given herein above, preferably, apply *mutatis mutandis* to the following.

In a further embodiment of the present invention, the results of a direct measurement of the glucose metabolism of the patient are combined with the above described method. It is preferred to measure the glucose metabolism in those patients directly who display increased RBP4 amounts in the at least one further sample. Thus, it is possible to select those patients for closer monitoring whose increasing RBP4 levels indicate that they respond to the treatment with at least one ACE-inhibitor with increased peripheral insulin resistance.

Methods for directly measuring the glucose metabolism in a patient are well known to the person skilled in the art. Preferably, they comprise glucose tolerance testing, determination of fasting glucose and determination of HbA_{1C}.

A fasting glucose level above 126 mg/dL (7.0 mmol/l) is generally considered a sign of diabetes.

Determination of HbA_{1C} indicates the long term control of blood glucose levels. During its life time the haemoglobin within the red blood cells becomes progressively glycosylated. This process is irreversible. Higher blood glucose levels lead to a higher rate of glycosylation. Thus, the glycosylation state of haemoglobin reflects the blood glucose levels over its life cycle.

Glucose tolerance testing, preferably, is performed as oral glucose tolerance testing. After a predetermined time of fasting the patient is given a certain amount of glucose dissolved in water. Blood glucose levels are determined directly before drinking the glucose solution and two hours thereafter. Individuals with increased glucose tolerance display higher blood glucose levels after two hours than healthy individuals.

After measuring the glucose metabolism in the patient a therapeutic decision is made. A combination of at least one sign of an impaired metabolism of glucose in combination with

increased RBP-levels, preferably, indicates that an adjustment of the therapy with ACE-inhibitors is necessary.

5 In those patients, where the therapy with at least one ACE-inhibitor is directed against peripheral insulin resistance or type 2 diabetes a preferred embodiment of the present invention includes determining the amount of at least one marker selected from the group consisting of CRP and adiponectin in the first sample from the patient and in each subsequent sample (e.g. in the second sample). It further includes comparing the amounts of the aforementioned markers determined in the first sample and the at least one
10 subsequent sample.

Adiponectin is a polypeptide (one of several known adipocytokines) secreted by the adipocyte. In the art, adiponectin is frequently also referred to as Acrp30 and apM1. Adiponectin has recently been shown to have various activities such as anti-inflammatory,
15 antiatherogenic, preventive for metabolic syndrome, and insulin sensitizing activities. Adiponectin is encoded by a single gene, and has 244 amino acids, the molecular weight is approximately 30 kilodaltons. The mature human adiponectin protein encompasses amino acids 19 to 244 of full-length adiponectin. A globular domain is thought to encompass amino acids 107 – 244 of full-length adiponectin. The sequence of the adiponectin
20 polypeptide is well known in the art, and, e.g., disclosed in WO/2008/084003.

Adiponectin associates itself into larger structures. Three adiponectin polypeptides bind together and form a homotrimer. These trimers bind together to form hexamers or dodecamers. Adiponectin is known to exist in a wide range of multimer complexes in
25 plasma and combines via its collagen domain to create 3 major oligomeric forms: a low-molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer, and high-molecular weight (HMW) 12- to 18-mer adiponectin (Kadowaki et al. (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 116(7): 1784–1792; Rexford S. Ahima, Obesity 2006;14:242S-249S).
30 Adiponectin has been reported to have several physiological actions, such as protective

activities against atherosclerosis, improvement of insulin sensitivity, and prevention of hepatic fibrosis.

Adiponectin as used herein, preferably, relates to low molecular weight adiponectin, mid
5 molecular weight adiponectin, more preferably, to total adiponectin, and, most preferably, to high molecular weight adiponectin. The terms high molecular weight adiponectin (12- to 18-mer adiponectin, preferably, 18-mer adiponectin), low and mid molecular weight adiponectin and total adiponectin are understood by the skilled person. Preferably, said adiponectin is human adiponectin. Methods for the determination of adiponectin are, e.g.,
10 disclosed in US 2007/0042424 A1 as well as in WO/2008/084003. Preferably, the amount of adiponectin is determined in a serum sample.

CRP, herein also referred to as C-reactive protein, is an acute phase protein that was discovered more than 75 years ago to be a blood protein that binds to the C-polysaccharide
15 of pneumococci. CRP consists of five single subunits, which are non covalently linked and assembled as a cyclic pentamer with a molecular weight of approximately 110-140 kDa. Preferably, CRP as used herein relates to human CRP. The sequence of human CRP is well known and disclosed, e.g., by Woo et al. (J. Biol. Chem. 1985. 260 (24), 13384-13388). The level of CRP is usually low in normal individuals but can rise 100- to 200-fold or
20 higher due to inflammation, infection or injury (Yeh (2004) Circulation. 2004; 109:II-11–II-14). It is known that CRP is an independent factor for the prediction of a cardiovascular risk. Particularly, it has been shown that CRP is suitable as a predictor for myocardial infarction, stroke, peripheral arterial disease and sudden cardiac death. Moreover, elevated CRP amounts may also predict recurrent ischemia and death in subjects with acute
25 coronary syndrome (ACS) and those undergoing coronary intervention. Determination of CRP is recommended by expert panels (e.g. by the American Heart Association) in patients with a risk of coronary heart disease (see also Pearson et al. (2003) Markers of Inflammation and Cardiovascular Disease. Circulation, 107: 499-511).

30 Preferably, the amount of CRP in a sample of a subject is determined by using CRP assays with a high sensitivity. The CRP determined by such assays is frequently also referred to as

high sensitivity CRP (hsCRP). hsCRP assays are nowadays used to predict the risk of heart disease. Suitable hsCRP assays are known in the art. A particularly preferred hsCRP assay in the context of the present invention is the Roche/Hitachi CRP (Latex) HS test with a detection limit of 0.1 mg/l.

5

By determining the amount of RBP4 it is possible to detect an increasing peripheral glucose resistance in a patient caused by ACE-inhibitors. CRP and adiponectin, in contrast, are markers for the beneficial role of ACE-inhibitors in patients with peripheral insulin resistance or type 2 diabetes.

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Thus, the success of a therapy against peripheral insulin resistance is advantageously monitored with a combination of a marker for the negative effects and at least one marker for the positive effects of ACE-inhibitors on peripheral insulin resistance.

15 A further embodiment of the present invention relates to a method for deciding about adjusting the therapy with at least one ACE-inhibitor in a patient comprising the steps of

- a) measuring the level of RBP4 in a first sample from a patient;
- b) measuring the level of RBP4 in at least one further sample from a patient;
- 20 c) comparing the level of RBP4 in the at least one further sample to the level of the respective marker in the previous sample or samples (preferably in the first sample); and
- d) deciding about adjusting the therapy with the at least one ACE-inhibitor.

25 Preferred, time intervals between the first sample and the at least one further sample (preferably, the second sample) are given elsewhere herein.

Preferably, an increase, a significant increase, or, more preferably, a statistically significant increase of the level of RBP4 in the second sample (or at least one further sample) as
30 compared to the level in the first sample that the therapy with the at least one ACE inhibitor has to be adjusted (e.g., by lowering the dosage of said ACE inhibitor; the term “adjusting the therapy with at least one ACE inhibitor” is explained elsewhere herein).

Preferably, an unchanged level or a decrease, or a statistically significant decrease of the level of RBP4 in the second sample (or at least one further sample) as compared to the level in the first sample, preferably, indicates that the therapy with the ACE inhibitor can
5 be continued (either without changing the dosage of said ACE inhibitor or with an increased dosage of said ACE inhibitor).

The term “adjusting the therapy with at least one ACE-inhibitor”, preferably, refers to decreasing the dosage of the one or more ACE-inhibitors, to switching from ACE-
10 inhibitors to AT₁-receptor inhibitors, aldosterone antagonists, renin antagonists and/or to the supplementing administration of thiazolidinediones.

Preferred aldosterone receptor antagonists are spironolactone and eplerenone. Preferred AT₁-receptor inhibitors are telmisartan, losartan, lalsartan, cadesartan and irbesartan. A
15 preferred renin antagonist is aliskiren.

AT₁-receptor inhibitors interfere with the renin-angiotensin system at a different level. They do not inhibit the conversion of angiotensin I to angiotensin II, instead they block the binding of angiotensin II to its receptor.
20

Renin inhibitors are a new class of drugs which interfere with the renin-angiotensin system (RAS). They block the action of renin which transforms angiotensinogen to angiotensin I. Renin inhibitors, thus, interfere with the RAS at the beginning of the activation cascade.

25 If the at least one ACE-inhibitor is to be substituted or augmented by an AT₁-receptor inhibitor, telmisartan, irbesartan and losartan are preferred, because they have been shown to activate PPAR γ (Schupp et al., 2004, Circulation 109: 2054-2057; Schupp et al., 2006, Hypertension 47: 586-589). Telmisartan is especially preferred. Said adjustment of the therapy is, preferably, initiated if the RBP4 levels in a patient increase.

The increase of RBP4 is, preferably, statistically significant. More preferably, the level of RBP4 increases by at least 10 %; at least 20 %; at least 30 %; at least 40 %; at least 50 %; at least 60 %; at least 70 %; at least 80 % or at least 100 %. Most preferably, an increase of RBP-4 to levels above 42.0 mg/l is indicative of an undesired increase of peripheral insulin resistance. Whether an increase or decrease is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001.

In a further preferred embodiment of the present invention the decision about adjusting the therapy is additionally based on the results of a direct measurement of glucose metabolism as described above.

In another preferred embodiment of the present invention the level of at least one of the markers selected from the group consisting of CRP and adiponectin is determined in the first and the at least one further sample of the patient. Preferably, the level of the said at least one marker in the at least one further sample is compared to the level of said at least one marker in a previous sample (preferably, the first sample).

Preferably, the determination of the level of CRP and adiponectin is carried out, if the RBP4 level is decreased in the at least one further sample as compared with the first sample. This combination of markers gives valuable information for the therapy of peripheral insulin resistance or type 2 diabetes with ACE-inhibitors. Thus, the further determination of adiponectin and/or CRP is, particularly, contemplated in patients with type 2 diabetes.

If the amount of RBP4 is decreased in the at least one further sample as compared to the amount in the first sample, the following applies:

If the level of adiponectin fails to increase in the at least one further sample and/or if the level of C-reactive protein (CRP) fails to decrease in the at least one further sample, the dosage of the ACE inhibitor, is preferably raised. The increase of adiponectin and the decrease of CRP is, preferably, statistically significant.

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Thus, an increase (preferably, a statistically significant increase) of the level of adiponectin in the at least one further sample as compared to the level of adiponectin in the first sample and/or (preferably "and") a decrease (preferably, a statistically significant decrease) of the level of CRP in the at least one further sample as compared to the level of adiponectin in the first sample, preferably, indicates that the therapy with at least one ACE inhibitor can be continued (without changing the dosage).

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A decrease (preferably, a statistically significant decrease) of the level of adiponectin in the at least one further sample as compared to the level of adiponectin in the first sample and/or (preferably "and") an increase (preferably, a statistically significant an increase) of the level of CRP in the at least one further sample as compared to the level of adiponectin in the first sample, preferably, indicates that the therapy can be continued with an increased dosage of the ACE inhibitor. An increased dosage of the ACE inhibitor is possible, since the ACE inhibitor did not increase or change the peripheral insulin resistance (as indicated by the RBP4 level).

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Preferred increases/decreases are given herein below:

More preferably, the level of adiponectin increases by at least 10 %; at least 20 %; at least 30 %; at least 40 %; at least 50 %; at least 60 %; at least 70 %; at least 80 % or at least 100 %. Adiponectin is, preferably, determined as high molecular weight adiponectin. The decrease in the level of CRP is, preferably, statistically significant. Preferably, a decrease of the level of CRP of at least 10 %; at least 20 %; at least 30 %; at least 40 % or at least 50 % is considered to be statistically significant. More preferably, the level of CRP drops by at least 10 %; at least 20 %; at least 30 %; at least 40 % or at least 50 %. CRP is, preferably, determined with a highly sensitive test. Whether an increase is statistically significant can be determined by the person skilled in the art as described above.

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Moreover, the present invention relates to a method for deciding whether therapy with at least one ACE-inhibitor is suitable for a patient comprising the steps of

- 5 a) measuring the level of RBP4 in a first sample from a patient;
 b) comparing the measured RBP4 level with a suitable reference level; and
 c) deciding about the suitability of therapy with at least one ACE-inhibitor for the patient, wherein an RBP4 level lower than the reference amount indicates that the therapy with at least one ACE-inhibitor is suitable for the patient.

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Accordingly, the term "reference amount" as used herein, preferably, refers to an amount which allows deciding whether therapy with at least one ACE inhibitor is suitable for a patient. Accordingly, the reference may e.g. be derived from (i) a subject known to susceptible to said therapy or (ii) a subject known to be not susceptible to said therapy.

15 Moreover, the reference amount may define a threshold amount or range, whereby dependent on the type of reference a change in the determined amount with respect to the threshold is either indicative for a patient being susceptible or not being susceptible to said therapy. The reference amount applicable for an individual subject may vary depending on various physiological parameters such as age, gender, or subpopulation, as well as on the
20 means used for the determination of the polypeptide or peptide referred to herein. A suitable reference amount may be determined from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the test sample.

Reference amounts can be calculated for a cohort of subjects (i.e. (i) subjects which are
25 known to be susceptible to a therapy with an ACE inhibitor or (ii) subjects known not to be susceptible to a therapy with an ACE inhibitor among the cohort of subjects suffering from acute inflammation) based on the average or mean values for a given biomarker by applying standard statistically methods. In particular, accuracy of a test such as a method aiming to diagnose an event, or not, is best described by its receiver-operating
30 characteristics (ROC) (see especially Zweig 1993, Clin. Chem. 39:561-577). The ROC graph is a plot of all of the sensitivity/specificity pairs resulting from continuously varying the decision threshold over the entire range of data observed. The clinical performance of a

diagnostic method depends on its accuracy, i.e. its ability to correctly allocate subjects to a certain prognosis or diagnosis. The ROC plot indicates the overlap between the two distributions by plotting the sensitivity versus 1-specificity for the complete range of thresholds suitable for making a distinction. On the y-axis is sensitivity, or the true-positive fraction which is defined as the ratio of number of true-positive test results to the product of number of true-positive and number of false-negative test results. This has also been referred to as positivity in the presence of a disease or condition. It is calculated solely from the affected subgroup. On the x-axis is the false-positive fraction, or 1-specificity which is defined as the ratio of number of false-positive results to the product of number of true-negative and number of false-positive results. It is an index of specificity and is calculated entirely from the unaffected subgroup. Because the true- and false-positive fractions are calculated entirely separately, by using the test results from two different subgroups, the ROC plot is independent of the prevalence of the event in the cohort. Each point on the ROC plot represents a sensitivity/-specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions of results) has an ROC plot that passes through the upper left corner, where the true-positive fraction is 1.0, or 100% (perfect sensitivity), and the false-positive fraction is 0 (perfect specificity). The theoretical plot for a test with no discrimination (identical distributions of results for the two groups) is a 45° diagonal line from the lower left corner to the upper right corner. Most plots fall in between these two extremes. (If the ROC plot falls completely below the 45° diagonal, this is easily remedied by reversing the criterion for "positivity" from "greater than" to "less than" or vice versa.) Qualitatively, the closer the plot is to the upper left corner, the higher the overall accuracy of the test. Dependent on a desired confidence interval, a threshold can be derived from the ROC curve allowing for the diagnosis or prediction for a given event with a proper balance of sensitivity and specificity, respectively. Accordingly, the reference to be used for the aforementioned method of the present invention, i.e. a threshold which allows to discriminate between subjects being susceptible to a therapy with ACE inhibitors or those which are not susceptible to a therapy with ACE inhibitors can be generated, preferably, by establishing a ROC for said cohort as described above and deriving a threshold amount therefrom.

The reference amount is, preferably, about 43 mg/l, about 50 mg/l, about 55 mg/l or about 60 mg/l. More preferably it is about 60 mg/l.

5 About in the context of the present invention means +/- 20%, +/- 10%, +/- 5%, +/- 2 % or +/- 1% +/- 0% from the said values. This also takes into account usual deviations caused by measurement techniques and the like.

10 Amounts higher than that are indicative for patients suffering from the metabolic syndrome or type 2 diabetes (see European patent application 08 168 164). Both conditions are characterized by increased insulin resistance. Because therapy with ACE-inhibitors potentially increases the RBP4 level further, patients who have already elevated RBP4 levels are especially prone to developing insulin resistance as a consequence of treatment with ACE-inhibitors. Therefore, therapy with ACE-inhibitors has to be considered carefully for patients with RBP4 levels higher than the reference amount. Preferably, these
15 patients are treated with other drugs, preferably renin antagonists, AT1-receptor inhibitors or aldosterone antagonists instead of ACE-inhibitors. Also preferably, the treatment with at least one ACE-inhibitor in these patients is closely monitored according to the method of the present invention. More preferably, if a treatment with at least one ACE-inhibitor is initiated, it is combined with administering a thiazolidinedione and is monitored closely
20 according to the method of the present invention.

Thus, an amount of RBP4 in a sample from a patient lower than the reference amount, preferably, indicates that the therapy with at least one ACE inhibitor is suitable for the patient.
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Furthermore, the present invention relates to a device for monitoring the therapy with at least one ACE-inhibitor against insulin resistance comprising

- 30 a) means for measuring the level of RBP4 and at least one marker selected from the group comprising adiponectin and CRP in a patient;
b) means for comparing the measured levels of the markers to reference levels.

The term "device" as used herein relates to a system of means comprising at least the aforementioned means operatively linked to each other as to allow the prediction. Preferred means for determining the amount of CRP, RBP4 and/or adiponectin, and means for carrying out the comparison are disclosed above in connection with the method of the invention. How to link the means in an operating manner will depend on the type of means included into the device. For example, where means for automatically determining the amount of the peptides are applied, the data obtained by said automatically operating means can be processed by, e.g., a computer program in order to obtain the desired results. Preferably, the means are comprised by a single device in such a case. Said device may accordingly include an analyzing unit for the measurement of the amount of the peptides or polypeptides in an applied sample and a computer unit for processing the resulting data for the evaluation. Alternatively, where means such as test stripes are used for determining the amount of the peptides or polypeptides, the means for comparison may comprise control stripes or tables allocating the determined amount to a reference amount. The test stripes are, preferably, coupled to a ligand which specifically binds to the peptides or polypeptides referred to herein. The strip or device, preferably, comprises means for detection of the binding of said peptides or polypeptides to the said ligand. Preferred means for detection are disclosed in connection with embodiments relating to the method of the invention above. In such a case, the means are operatively linked in that the user of the system brings together the result of the determination of the amount and the diagnostic or prognostic value thereof due to the instructions and interpretations given in a manual. The means may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further ado. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of raw data which need interpretation by the clinician. Preferably, the output of the device is, however, processed, i.e. evaluated, raw data the interpretation of which does not require a clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the peptide, Plasmon surface resonance devices, NMR spectrometers, mass-spectrometers etc.) or evaluation units/devices referred to above in accordance with the method of the invention.

Moreover, the present invention relates to a kit for monitoring the therapy with at least one ACE-inhibitor against insulin resistance comprising

- 5 a) means for measuring the level of RBP4 and at least one marker selected from the group comprising adiponectin and CRP in a patient;
- b) means for comparing the measured levels of the markers to reference levels.

The term “kit” as used herein refers to a collection of the aforementioned compounds,
10 means or reagents of the present invention which may or may not be packaged together. The components of the kit may be comprised by separate vials (i.e. as a kit of separate parts) or provided in a single vial. Moreover, it is to be understood that the kit of the present invention is to be used for practising the methods referred to herein above. It is, preferably, envisaged that all components are provided in a ready-to-use manner for
15 practising the methods referred to above. Further, the kit preferably contains instructions for carrying out the said methods. The instructions can be provided by a users manual in paper- or electronic form. For example, the manual may comprise instructions for interpreting the results obtained when carrying out the aforementioned methods using the kit of the present invention.

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Moreover, the present invention relates to the use of RBP4, or of a ligand thereof, for monitoring a therapy with at least one ACE inhibitor in a patient.

The present invention also contemplates the use of RPB, or of a ligand thereof, and at least
25 one further marker selected from the group consisting of CRP and adiponectin, or of a ligand of said at least one further marker for monitoring a therapy with at least one ACE inhibitor in a patient.

The present invention also relates to the in vitro use of RBP4, or of a ligand thereof, for
30 deciding whether a therapy with at least one ACE inhibitor in a patient shall be adjusted.

The present invention also contemplates the use of RBP, or of a ligand thereof, and at least one further marker selected from the group consisting of CRP and adiponectin, or of a ligand of said at least one further marker, for deciding whether a therapy with at least one ACE inhibitor in a patient shall be adjusted.

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The present invention also relates to the use of RBP4 or of a ligand thereof for deciding whether therapy with at least one ACE-inhibitor is suitable for a patient.

Moreover, the present invention relates to RBP4, or a ligand thereof, for monitoring a therapy with at least one ACE inhibitor in a patient.

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The present invention also contemplates RBP4, or a ligand thereof, and at least one further marker selected from the group consisting of CRP and adiponectin, or a ligand of said at least one further marker for monitoring a therapy with at least one ACE inhibitor in a patient.

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The present invention also relates to RBP4 or a ligand thereof for deciding whether a therapy with at least one ACE inhibitor in a patient shall be adjusted.

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The present invention also contemplates RBP4, or a ligand thereof, and at least one further marker selected from the group consisting of CRP and adiponectin, or a ligand of said at least one further marker, for deciding whether a therapy with at least one ACE inhibitor in a patient shall be adjusted.

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The present invention also relates to the use of RBP4 or a ligand thereof for deciding whether therapy with at least one ACE-inhibitor is suitable for a patient.

Preferred ligands are described elsewhere herein. Preferably, the ligand specifically binds to RBP4, CRP and adiponectin, respectively. The most preferred ligands are antibodies which specifically bind CRP and adiponectin, respectively.

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The literature cited in this application is incorporated by reference with respect to its whole disclosure content.

The following examples are only intended to illustrate the invention. They shall not limit
5 the scope of the claims in any way.

Example 1:

The study was performed in a collective of 49 patients with type 1 diabetes patients and
10 mostly elevated RBP-4 levels at baseline. The characteristics of the collective were as shown in table 1:

Table 1: Characteristics of the patient collective

Sex (male/female):	16/33
Age ¹ (years):	49 ($\pm$ 10)
Diabetes duration ¹ (years):	33 ($\pm$ 10)
24-hour ambulatory blood pressure ¹ (mm Hg):	142 ($\pm$ 14)/ 74 ($\pm$ 8)
UAER ² (mg/24 hours):	362 (240-545)
GFR ¹ (mL min ⁻¹ 1.73 m ⁻²):	81 (30)

¹data given with standard deviation

²geometric mean (95 % CI)

15 In an initial wash-out period all antihypertensive medication except from slow-release furosemide was withdrawn. After the wash-out period patients were given 20 mg lisinopril daily. After 6 weeks the dosage was escalated to 40 mg and after another 6 weeks it was escalated to 60 mg. Before each escalation RBP-4, adiponectin and hsCRP were determined. RBP-4 was determined with the retinol binding protein (RBP)/RBP4 ELISA
20 kit from Immundiagnostik AF, Bensheim. Adiponectin was determined with the Adiponectin (Multimeric) EIA produced by ALPCO Diagnostics, Salem, New Hampshire. hsCRP was determined with the Tina-quant C-reactive protein (latex) high sensitive assay from Roche/Hitachi.

Table 2: Influence of lisinopril on RBP-4, adiponectin and hsCRP

	Lisinopril			
	Baseline	20 mg	40 mg	60 mg
RBP-4 ¹ (mg/l)	43.55 (34.49/58.41)	56.41 (41.62/74.16)	53.84 (38.13/70.12)	50.84 (39.11/66.06)
Adiponectin ¹ (µg/l)	4.88 (3.27/7.35)	6.31 (3.68/10.29)	5.39 (3.11/10.22)	5.98 (4.07/10.16)
hsCRP ¹ (mg/l)	1.41 (0.82/2.47)	148 (0.79/2.28)	1.34 (0.69/2.12)	1.37 (0.83/2.20)

¹Data are given as median (25th perc./75th perc)

14 patients had a decrease of the RBP4 level indicating that the therapy with the ACE inhibitor did not negatively effect peripheral insulin resistance. Of these 14 patients, 5 showed a decrease of the adiponectin level and a decrease of the CRP level indicating that the dosage of the ACE inhibitor may be increased (without significantly effecting peripheral insulin resistance). In patients with increased adiponectin and decreased CRP levels, the therapy may be continued without changing the dosage of the ACE inhibitor.

35 patients showed an increase of the RBP4 level indicating increased peripheral insulin resistance. Administration of insulin sensitizers such as thiozolidinedione is indicated.

Example 2

A 56-year old patient presents a clinical manifestation of heart failure (NYHA class 1) and signs of the metabolic syndrome. It is intended to treat the heart failure with ACE-inhibitors. Before the onset of therapy RBP4 is 42.9 mg/l, CRP is 1.6 md/dl and adiponectin is 4.2 µg/l. Therapy with lisinopril is initiated at 20 mg/d. After 6 weeks RBP4 increases to 52.6 mg/l, the other markers remain constant. Additionally, there are signs of pathological glucose tolerance test. Therefore therapy with piogliazone is initiated.

After 6 further weeks RBP4 drops to 42.5 mg/l, levels of the other markers remain constant. The dosage of lisinopril is increased to 40 mg/d. After 6 further weeks levels of all markers remain constant, a further increase of the dosage of lisinopril is not required.

Example 3

A patient suffering from type 2 diabetes is to receive an ACE-inhibitor. Before the onset of therapy RBP4 is 56.2 mg/l, adiponectin is 4.6 µg/l and CRP is 1.6 mg/dl. Due to the high RBP4 level therapy is initiated with an AT₁-receptor inhibitor instead of the ACE-inhibitor. Furthermore treatment with metformin is replaced by treatment with pioglitazone. After 6 weeks RBP4 has decreased to 41.9 mg/l, CRP is 1.4 mg/l and adiponectin has not changed. It is considered to administer an ACE-inhibitor instead of the AT₁-receptor inhibitor. However, the treatment is not changed because the AT₁-receptor inhibitor is well tolerated.

Example 4

A patient suffering from type 2 diabetes has a RBP4 level of 40.9 mg/l before treatment. The adiponectin level is 4.9 µg/l and CRP is 1.2 mg/l. Therapy is initiated with 20 mg/d lisinopril. The levels of the markers have not changed after 6 weeks and the dosage is raised to 40 mg/d. After further 6 weeks not change of the marker levels can be observed and the dosage is increased to 60 mg/d. After 6 further weeks CRP and RBP4 remain constant, adiponectin increases to 5.4 µg/l. The treatment is now considered to be well adjusted and controls are scheduled every 6 months.

Example 5

A patient with heart failure and no signs of the metabolic syndrome is to be treated with an ACE-inhibitor. The level of RBP4 is 41.0 mg/l, CRP is 1.12 mg/l and adiponectin is 6.1 µg/l. Therapy is initiated and RBP4 increases after 6 weeks to 44.0 mg/l, CRP decreases to 0.9 mg/l and adiponectin remains constant. The dosage of the ACE-inhibitor is increased first to 40 mg/d and then to 60 mg/d at normal CRP and Adiponectin levels, the glucose tolerance test is within normal range.

Claims

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1. A method for monitoring the therapy with at least one ACE-inhibitor in a patient comprising the steps of

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- a) measuring the level of RBP4 in a first sample from a patient;
- b) measuring the level of RBP4 in at least one further sample from a patient; and
- c) comparing the level of each marker in the at least one further sample to the level of the respective marker in the previous sample or samples.

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2. The method of claim 1, wherein the patient suffers from high blood pressure, heart failure, type 1 diabetes, type 2 diabetes and/or the metabolic syndrome.

3. The method of claim 2, wherein the patient suffers from type 2 diabetes and/or the metabolic syndrome.

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4. The method of any of claims 1, 2 or 3, wherein additionally the results of a direct measurement of the glucose metabolism of the patient are used for the monitoring.

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5. The method of any of claims 1 to 4, wherein the therapy with ACE-inhibitors is directed against insulin resistance.

6. The method of claim 5, wherein at least one marker selected from the group consisting of adiponectin and CRP is determined in steps a) and b) additionally to RBP4.

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7. The method of claim 6, wherein adiponectin is high molecular weight adiponectin.

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8. A method for deciding about adjusting the therapy against insulin resistance with at least one ACE-inhibitor comprising

- a) measuring the level of RBP4 in a first sample from a patient;
- b) measuring the level of RBP4 in at least one further sample from a patient; and

- c) comparing the level of RBP4 in the at least one further sample to the level of the respective marker in the previous sample or samples, and
- d) deciding about adjusting the therapy based on the results of step c).

5 9. The method of claim 8, wherein an unchanged level of RBP4 or a decrease of RBP4 in the further sample as compared to the first sample indicates that the therapy can be continued.

10 10. The method of claim 8, wherein an increase of the RBP4 level is indicative of the need to administer a thiazolidinedione, and/or wherein an increase of the RBP4 level is indicative of the need to decrease the dosage of the at least one ACE-inhibitor or to replace the ACE-inhibitor(s) by an aldosterone antagonist, a renin antagonist or an AT₁-receptor inhibitor.

15 11. The method of claim 9, further comprising determining the level of CRP and adiponectin in said first and said at least one further sample.

20 12. The method of claim 11, wherein the failure of the adiponectin level to increase and/or the CRP level to decrease is indicative of the need to increase the dosage of the at least one ACE inhibitor.

13. A device for monitoring the therapy with at least one ACE-inhibitor against insulin resistance comprising

- 25 a) means for measuring the level of RBP4 and at least one marker selected from the group comprising adiponectin and CRP in a patient; and
- b) means for comparing comparing the level of each marker in the at least one further sample to the level of the respective marker in the previous sample or samples.

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14. A kit for monitoring the therapy with at least one ACE-inhibitor against insulin resistance comprising

- 35 a) means for measuring the level of RBP4 and at least one marker selected from the group comprising adiponectin and CRP in a patient; and

- b) means for comparing comparing the level of each marker in the at least one further sample to the level of the respective marker in the previous sample or samples.
- 5 15. The device of claim 13 or the kit of claim 14, comprising means for determining high molecular weight adiponectin.
16. The method of claim 1, wherein the level of Troponin T is measured instead of the level of RBP4.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/058723

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/66 G01N33/68

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)

G01N

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, 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	GALLAGHER EMILY JANE; LEROITH DEREK; KARNIELI EDDY: "The metabolic syndrome--from insulin resistance to obesity and diabetes" ENDOCRINOLOGY AND METABOLISM CLINICS OF NORTH AMERICA, W.B. SAUNDERS COMPANY, PHILADELPHIA, vol. 37, no. 3, 1 September 2008 (2008-09-01), pages 559-579, VII, XP009121934 ISSN: 0889-8529	1-8, 11-15
Y	p.567 last two par., p.569 par.1, p.571 par.1, p.573 ----- -/--	9,10



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

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

"&" document member of the same patent family

Date of the actual completion of the international search

13 October 2010

Date of mailing of the international search report

29/11/2010

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/058723

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOHLSTEDT KARIN; GERSHOME CYNTHIA; FICHTLSCHERER STEPHAN; BUSSE RUDI; FLEMING INGRID: "Angiotensin converting enzyme (ACE) inhibitors modulate gene expression in human endothelial cells and adipocytes via the ACE-Dependent signaling" CIRCULATION, LIPPINCOTT WILLIAMS & WILKINS, US, vol. 114, no. 18, Suppl. S, 1 October 2006 (2006-10-01), page 119, XP009121933 ISSN: 0009-7322	1-3,5,6, 13
Y	abstract	9,10
X	----- WO 2008/131224 A2 (TETHYS BIOSCIENCE INC [US]; URDEA MICHAEL S [US]; MCKENNA MICHAEL P []) 30 October 2008 (2008-10-30) par.6, 16-19, 41, 53, 55, 122, 177-178, 187, 264, 269; Table 1: biomarkers 5, 65, 202; par.285-288; par.162-173 -----	1-6,8, 13,14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2010/058723

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

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

13-15(completely); 1-12(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 13-15(completely); 1-12(partially)

Method of using RBP4 as biomarker for therapy with at least one ACE-inhibitor in a patient. Kit and device related thereto.

2. claims: 16(completely); 1-12(partially)

Method of using Troponin T as biomarker for therapy with at least one ACE-inhibitor in a patient.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2010/058723

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008131224 A2	30-10-2008	AU 2008242764 A1	30-10-2008
		CA 2684308 A1	30-10-2008
		EP 2147315 A2	27-01-2010
		JP 2010525332 T	22-07-2010