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(54) Title: METHOD OF SCREENING FOR AN ANTAGONIST OF ADIPONECTIN RECEPTOR 2 AND USES THEREOF

(57) Abstract: This invention relates to methods of screening for an agent with therapeutic potential in treating or preventing a metabolic or neurological disease, comprising testing an agent for its ability to antagonise adiponectin receptor 2, wherein an agent which can antagonise adiponectin receptor 2 may have therapeutic potential in treating or preventing a metabolic or neurological disease. The invention also relates to methods of treating or preventing a metabolic or neurological disease in a patient by administering an agent capable of antagonising adiponectin receptor 2 and the use of adiponectin receptor 2 antagonists in treating such diseases.

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METHOD OF SCREENING FOR AN ANTAGONIST OF ADIPONECTIN RECEPTOR 2 AND USES THEREOF

This invention relates to methods of screening for an agent with therapeutic potential in treating or preventing a metabolic or neurological disease, comprising testing an agent for its ability to antagonise adiponectin receptor 2, wherein an agent which can antagonise adiponectin receptor 2 may have therapeutic potential in treating or preventing a metabolic or neurological disease. The invention also relates to methods of treating or preventing a metabolic or neurological disease in a patient by administering an agent capable of antagonising adiponectin receptor 2 and the use of adiponectin receptor 2 antagonists in treating such diseases.

Adiponectin is a hormone secreted by adipocytes (Scherer, 1995; Hu, 1996; Maeda 1996; Nakano 1996) that acts as an anti-diabetic and anti-atherogenic adipokine. Under conditions of obesity, insulin resistance and type 2 diabetes, levels of adiponectin in the blood are decreased (Yamauchi, 2003 Nature). Low adiponectin levels predict risk of developing type 2 diabetes (Lindsay, 2002; Spranger, 2003) and myocardial infarction (Pischon, 2004). Adiponectin-deficient mice are insulin resistant, diabetic and prone to develop atherosclerosis (Maeda, 2002; Kubota, 2002). Treatment with adiponectin causes glucose-lowering and has both anti-diabetic (Maeda, 2002; Fruebis, 2001; Yamauchi, 2001; Berg, 2001; Yamauchi, 2002; Tomas, 2002; Yamauchi, 2003 JBC) and anti-atherosclerotic (Yamauchi, 2003 JBC; Ouchi, 2001) effects in animals. The insulin-sensitising effect of adiponectin seems to be mediated by stimulation of fatty acid oxidation via AMP-activated protein kinase (AMPK) (Yamauchi, 2002; Tomas, 2002) and peroxisome proliferator-activated receptor (PPAR) α (Fruebis, 2001; Yamauchi, 2001; Yamauchi, 2003 JBC). In addition, adiponectin affects thermogenesis, weight-gain and serum lipid and glucose levels following intracerebroventricular injection (Qi, 2004).

Two putative adiponectin receptors located on different chromosomes and constituting a novel class of seven transmembrane domain receptors distinct from known G-protein-coupled receptors, have recently been cloned (Yamauchi, 2003 Nature). These receptors, termed adiponectin receptor 1 and adiponectin receptor 2, share 67% identity at amino acid level. Adiponectin receptor 1 is highly expressed in skeletal muscle, whereas adiponectin receptor 2 is predominantly expressed in the liver. Using overexpression and small interfering RNA (siRNA) gene knock-down, Yamauchi were able to show that adiponectin

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receptor 1 has preference for globular adiponectin while adiponectin receptor 2 preferentially binds full-length adiponectin (Yamauchi, 2003 Nature).

Studies involving treatment with either globular or full-length adiponectin increased PPAR α ligand activity and stimulated fatty-acid oxidation and glucose uptake (Yamauchi, 2003 Nature). Suppression of adiponectin receptor 1 expression using siRNA greatly reduced adiponectin-mediated increases in PPAR α ligand activity, fatty-acid oxidation and glucose uptake. Suppression of adiponectin receptor 2 expression using siRNA partially reduced adiponectin-mediated increases in PPAR α ligand activity and fatty-acid oxidation. These observations support the conclusion that the adiponectin receptors 1 and 2 serve as receptors for adiponectin, and that they mediate increased AMPK phosphorylation and PPAR α effects as well as fatty acid oxidation and glucose uptake by adiponectin (Yamauchi, 2003 Nature). Recently, adaptor protein contacting pleckstrin homology domain, phosphotyrosine binding (PTB) domain and leucine zipper motif (APPL1) was shown to bind to adiponectin receptors mediating adiponectin signaling and function (Mao, 2006).

Obesity has been shown to decrease expression levels of adiponectin receptor 1 and adiponectin receptor 2 which could influence adiponectin sensitivity. In addition, osmotin which is a ligand of the yeast homolog of adiponectin receptors, has recently been shown to activate AMPK via adiponectin receptor (Kadowaki, 2005).

These observations have led researchers to propose that adiponectin receptor agonists and adiponectin sensitisers would be important therapeutics for atherosclerosis, inflammatory diseases and obesity-linked diseases such as diabetes, dyslipidemia and metabolic syndrome (Kadowaki, 2005; International Patent Application Number WO2005/001061). The data indicate that agonism of adiponectin receptors and/or strategies to increase adiponectin receptor 1 and adiponectin receptor 2 expression may be a suitable approach to novel treatments for insulin resistance and type 2 diabetes (International Patent Publication Number WO2005/084697 which claims priority from US Patent Application Number US 60/549,561).

International Patent Publication Number WO2005/031345 describes the enumeration of the copy number of adiponectin receptor 2 nucleic acid and relative expression of adiponectin receptor 2 in particular tissues. Based on the result that adiponectin receptor 2 is expressed in various human tissues, the applicants speculate that regulating adiponectin receptor 2 would be applicable to treating an extensive range of distinct and disparate diseases, consisting of cardiovascular diseases, dermatological diseases, gastroenterological

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diseases, cancer, haematological diseases, respiratory diseases, inflammation, neurological diseases, or urological diseases. Further broadening the scope, the application states that a regulator of adiponectin receptor 2 may be

- an antagonist of adiponectin receptor 2 activity, or
- 5 a regulator which reduces adiponectin receptor 2 expression, or
- an agonist of adiponectin receptor 2 activity, or
- a regulator which increases adiponectin receptor 2 expression.

There is no indication of which regulator may be useful in the treatment of which of the listed diseases. Indeed the application makes contradictory statements that regulators which are

10 antagonists of adiponectin receptor 2 activity, or which reduce adiponectin receptor 2 expression could be used in the treatment of the listed diseases, and that regulators which are agonists of adiponectin receptor 2 activity, or which increase adiponectin receptor 2 expression could be used in the treatment of the listed diseases. It is therefore unclear to the skilled person which regulator, that is whether regulators which are agonists or regulators

15 which are antagonists, would be useful in the treatment of which of the listed diseases.

Although the scope of WO2005/031345 is large, exemplification is limited to expression profiling and suggested methods of screening for regulators of adiponectin receptor 2 for use in the treatment of the listed diseases.

In order to study the role of the adiponectin receptor 2, adiponectin receptor 2 knock-

20 out mice (*AdipoR2*^{-/-}) were created by exchanging exon 5 with a targeting vector containing the LacZ and Neomycin resistance genes. To their surprise, the inventors observed that both male and female *AdipoR2*^{-/-} were lean on regular chow and resistant to high-fat diet (HFD) induced weight-gain. Moreover resistance was not due to decreased food intake, as there was no change in food intake in relation to body weight or lean body mass. These results are

25 especially surprising given that adiponectin receptor 1 knock-out male mice (*AdipoR1*^{-/-}) had higher body weight and increased percentage of body fat compared to wild type (wt) mice. After 8 weeks of HFD, female *AdipoR2*^{-/-} had markedly decreased relative body fat mass and moderately decreased lean body mass compared to wt controls. After 14 weeks of HFD, both female and male *AdipoR2*^{-/-} showed markedly reduced ovarian and epididymal white adipose

30 tissue (WAT), perirenal WAT and brown adipose tissue weights compared to corresponding wt controls. Female *AdipoR2*^{-/-} on regular chow also displayed decreased ovarian WAT and perirenal WAT weights compared to wt control mice. Plasma levels of adiponectin tended to

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be elevated in *AdipoR2*^{-/-} and were significantly elevated in female *AdipoR2*^{-/-} on regular chow. The inventors also observed that both male and female *AdipoR2*^{-/-} had larger brains compared to wt controls. Staining of the whole *AdipoR2*^{-/-} brain with β -galactosidase, demonstrated that adiponectin receptor 2 is highly expressed in the brain. Thus, adiponectin receptor 2 may be important for brain development and function.

In summary, compared to wt littermate controls, *AdipoR2*^{-/-} are lean and markedly resistant to HFD induced body weight-gain and obesity associated with increased spontaneous locomotor activity and energy expenditure. Furthermore, *AdipoR2*^{-/-} showed improved oral glucose tolerance and reduced plasma cholesterol levels.

Contrary to the teaching of the prior art; that agonists of adiponectin receptor 2 could be beneficial in treating obesity, diabetes, atherosclerosis and inflammatory diseases, and the contradictory speculation of WO2005/031345 that both antagonists and agonists of adiponectin receptor 2 are useful for the treatment of cardiovascular diseases, dermatological diseases, gastroenterological diseases, cancer, haematological diseases, respiratory diseases, inflammation, neurological diseases, or urological diseases, our findings indicate that antagonising adiponectin receptor 2 represents a new approach to the prevention or treatment of metabolic diseases, such as obesity, hepatic steatosis, high cholesterol levels, high plasma lipids, insulin resistance, type 2 diabetes and cardiovascular disease, and neurological diseases, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, dementia, cognitive impairment, Huntington's disease, multiple sclerosis and other neurodegenerative diseases, or psychiatric disorders such as schizophrenia, psychoses, depression, anxiety and other neurotic diseases.

The present invention relates to methods of screening for an agent with therapeutic potential in treating or preventing a metabolic or neurological disease, comprising testing an agent for its ability to antagonise adiponectin receptor 2, wherein an agent which can antagonise adiponectin receptor 2 may have therapeutic potential in treating or preventing a metabolic or neurological disease. The invention also relates to methods of treating or preventing a metabolic or neurological disease in a patient by administering an agent capable of antagonising adiponectin receptor 2 and the use of adiponectin receptor 2 antagonists in treating such diseases.

According to one aspect of the invention there is provided a method of screening for an agent with therapeutic potential in treating or preventing a metabolic or neurological

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disease, comprising determining whether the agent antagonises adiponectin receptor 2, wherein an antagonist of adiponectin receptor 2 has therapeutic potential in treating or preventing a metabolic or neurological disease. In one embodiment there is provided a method of screening for an agent with therapeutic potential in treating or preventing a
5 metabolic or neurological disease, comprising determining whether the agent antagonises adiponectin receptor 2 comprising

contacting an adiponectin receptor 2 with a test agent, and

determining the ability of the test agent to antagonise adiponectin receptor 2.

According to another aspect of the invention there is provided use of adiponectin
10 receptor 2 in an assay to identify an agent with therapeutic potential in treating or preventing a metabolic or neurological disease, comprising determining whether the agent antagonises adiponectin receptor 2, wherein an antagonist of adiponectin receptor 2 has therapeutic potential in treating or preventing a metabolic or neurological disease. In one embodiment there is provided use of adiponectin receptor 2 in an assay to identify an agent with
15 therapeutic potential in treating or preventing a metabolic or neurological disease, comprising determining whether the agent antagonises adiponectin receptor 2 comprising

contacting an adiponectin receptor 2 with a test agent, and

determining the ability of the test agent to antagonise adiponectin receptor 2.

According to another aspect of the invention there is provided use of adiponectin
20 receptor 2 to screen for an agent with therapeutic potential in treating or preventing a metabolic or neurological disease. In one embodiment the use comprises determining whether the agent antagonises adiponectin receptor 2. In an alternative embodiment the use comprises

contacting an adiponectin receptor 2 with a test agent, and

25 determining the ability of the test agent to antagonise adiponectin receptor 2.

According to another aspect of the invention there is provided a method of identifying a lead compound in a drug discovery program for an agent with therapeutic potential in treating or preventing a metabolic or neurological disease comprising selecting as a lead compound in a drug discovery program an agent which antagonises adiponectin receptor 2,
30 wherein an antagonist of adiponectin receptor 2 has therapeutic potential in treating or preventing a metabolic or neurological disease.

In one embodiment the method comprises

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contacting an adiponectin receptor 2 with a test agent, and
determining the ability of the test agent to antagonise adiponectin receptor 2.

According to a another aspect of the invention there is provided a method of screening
for an agent with therapeutic potential in treating or preventing a metabolic or neurological
5 disease, comprising determining whether the agent antagonises adiponectin receptor 2
comprising

contacting an adiponectin receptor 2 with a test agent, and
determining the ability of the test agent to antagonise adiponectin receptor 2 (a
parent antagonist), and
10 optionally preparing a derivative of a parent antagonist, and
testing a derivative of a parent antagonist to determine whether the derivative
is an antagonist of adiponectin receptor 2, and
selecting a derivative, which is an antagonist of adiponectin receptor 2,
according to its properties,
15 wherein an antagonist of adiponectin receptor 2 has therapeutic potential in treating or
preventing a metabolic or neurological disease.

In one embodiment the antagonist of adiponectin receptor 2 may be selected according
to its pharmacokinetic, pharmacodynamic, toxicity, bioavailability, half life or other
properties.

20 The skilled person will appreciate that there are a number of methods that can be
employed to determine the ability of a test agent to antagonise adiponectin receptor 2. For
example the binding assay described by Yamauchi (Yamauchi, 2003 Nature). In this assay
cells expressing adiponectin receptor 2 are incubated with labelled adiponectin plus
unlabelled competitor substances. After washings, the cell bound radioactivity is determined
25 using a gamma-counter, and specific binding calculated by subtracting non-specific binding
from total binding. This assay could be adapted such that adiponectin is replaced by another
ligand known to be capable of binding to adiponectin receptor 2.

According to another aspect of the invention there is provided a method of screening
for an agent with therapeutic potential in treating or preventing a metabolic or neurological
30 disease, comprising determining whether the agent antagonises adiponectin receptor 2
comprising

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contacting a cell expressing adiponectin receptor 2, or a cell membrane preparation thereof, with radiolabelled adiponectin receptor 2 ligand plus unlabelled test agent, and
determining cell-bound radioactivity,

5 wherein cell-bound radioactivity is indicative of the ability of the test agent to antagonise adiponectin receptor 2.

In one embodiment the adiponectin receptor 2 ligand is adiponectin.

An alternative assay which may be employed comprises determining the ability of a test agent to compete away down-stream effects of adiponectin (either full-length, globular or
10 fragments of adiponectin) in cells expressing adiponectin receptor 2. Suitable down-stream effects for measurement include AMPK and acetyl-coenzyme A carboxylase (ACC) phosphorylation, AMPK activity, PPAR α activity, APPL1 association with the receptor, fatty acid oxidation or glucose uptake and conversion.

According to another aspect of the invention there is provided a method of screening
15 for an agent with therapeutic potential in treating or preventing a metabolic or neurological disease, comprising determining whether the agent antagonises adiponectin receptor 2 comprising

contacting a cell expressing adiponectin receptor 2, or a cell membrane preparation thereof, with adiponectin receptor 2 ligand plus test agent, and
20 determining the ability of the test agent to compete away the down-stream effect of adiponectin receptor 2 ligand.

In one embodiment the adiponectin receptor 2 ligand is adiponectin.

In one embodiment the down-stream effect for measurement is AMPK and acetyl-coenzyme A carboxylase (ACC) phosphorylation, AMPK activity, PPAR α activity, APPL1
25 association with the receptor, fatty acid oxidation or glucose uptake and conversion.

A further alternative assay which may be employed, comprises studying internalisation of adiponectin receptor 2 following receptor activation. Adiponectin receptor 2 is a membrane bound protein and analogous to other classes of cell surface receptors, adiponectin receptor 2 may be redistributed into intracellular compartments (internalised)
30 after activation of the receptor. Internalisation can be studied using fluorescence microscopy; either using a fluorescently labelled antibody recognising adiponectin receptor 2, or alternatively using a fluorescently labelled ligand that binds adiponectin receptor 2.

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According to another aspect of the invention there is provided a method of screening for an agent with therapeutic potential in treating or preventing a metabolic or neurological disease, comprising determining whether the agent antagonises adiponectin receptor 2 comprising

5 contacting a cell expressing adiponectin receptor 2, or a cell membrane preparation thereof, with adiponectin receptor 2 ligand plus test agent, and
 a fluorescently labelled adiponectin receptor 2 ligand, and
 measuring the internalisation of adiponectin receptor 2.

 In one embodiment the adiponectin receptor 2 ligand is adiponectin. In another
10 embodiment the fluorescently labelled adiponectin receptor 2 ligand is an antibody recognising adiponectin receptor 2. In a further embodiment internalisation is measured by fluorescence microscopy.

 The skilled person would realise that contacting an adiponectin receptor 2 with a test agent, or a cell expressing adiponectin receptor 2, or a cell membrane preparation thereof
15 means to bring adiponectin receptor 2 and the test agent together and includes, but is not limited to, incubating, combining and mixing together adiponectin receptor 2 and the test agent.

 Such assays can be employed to determine the ability of a test agent to antagonise adiponectin receptor 2 and therefore be used as a method of screening, or to identify, an agent
20 with therapeutic potential in treating or preventing a metabolic or neurological disease.

 According to another aspect of the invention there is provided an agent with therapeutic potential in treating or preventing metabolic or neurological disease, by virtue of the agents' ability to antagonise adiponectin receptor 2.

 According to another aspect of the invention there is provided an antagonist of
25 adiponectin receptor 2, or a pharmaceutically acceptable salt thereof, with therapeutic potential in treating or preventing a metabolic or neurological disease, identified by a method as described herein. In one embodiment there is provided an antagonist of adiponectin receptor 2, or a pharmaceutically acceptable salt thereof, for treating or preventing a metabolic or neurological disease, identified by a method as described herein.

30 According to another aspect of the invention the antagonist of adiponectin receptor 2 may bind to adiponectin receptor 2. The mechanism of action of the antagonist may include binding of the antagonist to adiponectin receptor 2 and inhibiting the binding of adiponectin

receptor 2 with a native ligand, or inhibition of signal transduction. Alternatively the antagonist may enhance clearance or internalisation of adiponectin receptor 2 therein reducing the effective concentration of adiponectin receptor 2 polypeptide. Alternatively the antagonist may reduce expression of adiponectin receptor 2 therein reducing the effective concentration
5 of adiponectin receptor 2 polypeptide.

Preferably the agent is able to antagonise adiponectin receptor 2 *in vitro* and *in vivo*.

In one embodiment of the invention in the methods as described hereinabove adiponectin receptor 2 may be in the form of a cell expressing adiponectin receptor 2, a cell membrane preparation thereof or a purified or recombinant preparation of adiponectin
10 receptor 2.

In one embodiment of the invention, the methods as described hereinabove may be employed to screen for an agent with therapeutic potential in promoting weight loss or reducing obesity in a patient for medical reasons, comprising determining whether the agent antagonises adiponectin receptor 2, wherein an antagonist of adiponectin receptor 2 has
15 therapeutic potential in promoting weight loss or reducing obesity in a patient for medical reasons.

According to another aspect of the invention there is provided a method of screening for an agent with therapeutic potential in treating or preventing a metabolic disease or in promoting weight loss or reducing obesity, comprising determining whether an agent
20 antagonises adiponectin receptor 2 and recording the result. In one embodiment the result may be recorded by electronic means, or on paper.

According to another aspect of the invention there is provided a pharmaceutical composition comprising an antagonist of adiponectin receptor 2 for treating or preventing a metabolic or neurological disease.

25 According to another aspect of the invention there is provided a method of preparing a pharmaceutical composition comprising

determining whether a test agent is an antagonist of adiponectin receptor 2, and incorporating the agent, or a derivative thereof, with a pharmaceutically acceptable carrier.

30 Incorporating the agent, or a derivative thereof, with a pharmaceutically acceptable carrier includes, but is not limited to, mixing, admixing and combining.

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A derivative is an agent which has been designed, synthesised and tested for antagonism of adiponectin receptor 2 based on the parent agent initially identified in the method as described herein. Such a derivative agent is generally identified using conventional structure activity relationship (SAR) studies. Furthermore, such derivative
5 compounds will generally possess shared structural features with the parent compound, but with one or more structural moieties altered. A derivative compound is likely to be one whose structure has been optimised to make the compound more suitable for therapeutic treatments, such as by removal of groups known to be associated with toxic effects; being more bioavailable; having a longer half-life *in vivo* etc.

10 A pharmaceutically acceptable carrier may be a carrier, diluent or excipient. The pharmaceutically acceptable carrier may serve as a filler or a binding agent, but has no pharmacological activity and is merely present as an inert component. For example, for parenteral administration, the antagonists of the invention may be formulated for injection in pharmaceutically acceptable carriers such as saline, dextrose solution, serum albumin and
15 Ringer's solution. Carriers can also be selected from starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the various oils, including those of petroleum, animal, vegetable or synthetic origin (peanut oil, soybean oil, mineral oil, sesame oil) saline, buffered saline, dextrose,
20 and/or water.

A pharmaceutically acceptable composition may be administered to a patient alone, or in combination with at least one other agent.

According to another aspect of the invention there is provided a method of antagonising adiponectin receptor 2 comprising administering an antagonist of adiponectin
25 receptor 2 with therapeutic potential in treating or preventing a metabolic or neurological disease.

According to another aspect of the invention there is provided a method of treating or preventing a metabolic or neurological disease in a patient comprising administering an antagonist of adiponectin receptor 2. In one embodiment the method comprises administering
30 a pharmaceutical composition comprising an antagonist of adiponectin receptor 2 as described herein. In another embodiment the method comprises selecting a patient in need of treatment for, or prevention of, a metabolic or neurological disease and administering to a patient a

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therapeutically effective dose of an antagonist of adiponectin receptor 2. In another embodiment the metabolic disease is selected from obesity, hepatic steatosis, high cholesterol, high plasma lipids, insulin resistance, type 2 diabetes or cardiovascular disease. In another embodiment the neurological disease is selected from any one of Alzheimer's disease,

5 Parkinson's disease, amyotrophic lateral sclerosis, dementia, cognitive impairment, Huntington's disease, multiple sclerosis and other neurodegenerative diseases, or psychiatric disorders such as schizophrenia, psychoses, depression, anxiety and other neurotic diseases.

According to another aspect of the invention there is provided a method of weight loss or reducing obesity in a patient for medical reasons. In one embodiment the method
10 comprises administering a pharmaceutical composition comprising an antagonist of adiponectin receptor 2 as described herein. In another embodiment the method comprises selecting a patient in need of treatment for obesity and administering to a patient a therapeutically effective dose of an antagonist of adiponectin receptor 2.

According to another aspect of the invention there is provided use of an antagonist of
15 adiponectin receptor 2 in the manufacture of a medicament.

According to another aspect of the invention there is provided use of an antagonist of adiponectin receptor 2 in the manufacture of a medicament for the treatment or prevention of a metabolic or neurological disease. In one embodiment the use comprises selecting a patient in need of treatment for, or prevention of, a metabolic or neurological disease. In another
20 embodiment the metabolic disease is selected from obesity, hepatic steatosis, high cholesterol, high plasma lipids, insulin resistance, type 2 diabetes or cardiovascular disease. In another embodiment the neurological disease is selected from any one of Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, dementia, cognitive impairment, Huntington's disease, multiple sclerosis and other neurodegenerative diseases, or psychiatric
25 disorders such as schizophrenia, psychoses, depression, anxiety and other neurotic diseases.

According to a another aspect of the present invention there is provided a kit comprising:

an antagonist of adiponectin receptor 2, incorporated with a pharmaceutically acceptable carrier forming a pharmaceutically acceptable composition, and

30 a container means for containing said pharmaceutically acceptable composition.

In one embodiment, the invention provides an article of manufacture including a container. The container includes a pharmaceutically acceptable composition of an antagonist

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of adiponectin receptor 2, and a package insert or label indicating that the composition can be used to treat or prevent a metabolic or neurological disease.

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

5 An agent or antagonist may be a polypeptide, nucleic acid, carbohydrate, lipid, small molecular weight compound (for example those having a molecular weight of less than 2000 Daltons), an oligonucleotide, an oligopeptide, RNA interference (RNAi), siRNA, antisense, a recombinant protein, an antibody, or conjugates or fusion proteins thereof. For a review of RNAi see Milhavet O, Gary DS, Mattson MP. (Pharmacol Rev. 2003 Dec;55(4):629-48.
10 Review.) and antisense see Opalinska JB, Gewirtz AM. (Sci STKE. 2003 Oct 28;2003(206):pe47.). An antagonist of adiponectin receptor 2 may be an inverse agonist.

Metabolic disease includes diseases affected by metabolism and/or dietary intake, including obesity, hepatic steatosis, high cholesterol, high plasma lipids, insulin resistance, type 2 diabetes or cardiovascular disease.

15 Neurological disease includes Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, dementia, cognitive impairment, Huntington's disease, multiple sclerosis and other neurodegenerative diseases, or psychiatric disorders such as schizophrenia, psychoses, depression, anxiety and other neurotic diseases

Use of the term 'adiponectin receptor 2' includes any sequence homologous to
20 mammalian adiponectin receptor 2, in particular wild type mammalian adiponectin receptor 2 and especially human, mouse or rat adiponectin receptor 2. Preferably adiponectin receptor 2 is human adiponectin receptor 2 comprising the sequence recorded in the SwissProt database under accession numbers Q86V24 or Q9H737 (SwissProt ID ADR2_HUMAN) and as described by Yamauchi (Yamauchi, 2003 Nature). Adiponectin receptor 2 also includes full-
25 length or truncated versions of the adiponectin receptor 2 sequence and any man made or natural allelic variants thereof.

The invention will now be illustrated by the following non-limiting examples, which are provided for illustrative purposes only and are not to be construed as limiting upon the teachings herein.

30

Example 1 Creation of AdipoR2 gene knock-out mice

Heterozygous adiponectin receptor 2 knock-out mice were obtained from Deltagen

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(Catalogue No.1962, Deltagen, San Carlos CA, USA). The heterozygous adiponectin receptor 2 knock-out mice were generated by homologous recombination in 129/OlaHsd ES cells using a targeting vector containing neomycin resistance and lacZ genes. The mouse model was generated by breeding chimeras carrying a disrupted adiponectin receptor 2 gene with C57BL/6 females resulting in F1 heterozygous offspring. F1 generation mice were backcrossed to C57BL/6 females for 6 generations before intercrossing to generate homozygous adiponectin receptor 2 knock-out mice. All experiments in this study were performed using littermate wt control mice. Genotyping was done by a PCR strategy. One primer was located upstream of the deleted region in the short arm

10 (5'GACGGAGTTTGTATGTGGTAGCGTC 3' (SEQ ID NO:1)), a second primer located in exon 5 (5' TCTCTGCCTTTCCTTTTCATGGCTC 3' (SEQ ID NO:2)) and the third located in the targeting cassette (5' GGGCCAGCTCATTCCTCCCACTCAT 3' (SEQ ID NO:3)).

Example 2 Diets, body weight, food intake, body composition and termination

15 The mice were either given standard laboratory chow containing (energy %) 12% fat, 62% carbohydrates, and 26% protein, with a total energy content of 12.6 kJ/g (R3, Lactamin AB, Kimstad, Sweden) or a high-fat diet containing 39.9% fat (mainly saturated), 42.3% carbohydrates and 17% protein with a total energy content of 21.4 kJ/g (R638, Lactamin AB). Mice were weighed weekly starting from 5 weeks of age. Food intake was analyzed for 24h in

20 food-deprived (12h) mice housed individually as described before (Bohlooly, 2005). Body composition analysis was performed on isoflurane (Forene, Abbot Scandinavia AB, Sweden) anesthetized mice by dual-energy X-ray absorptiometry (PIXImus Lunar, GE Medical Systems, Madison, WI). At termination (9-11 a.m.), plasma was isolated from isoflurane anesthetized mice and organs were collected, weighed and snap frozen in N2 and stored at -

25 80°C. Experimental procedures were approved by the Local Ethics Review Committee on Animal Experiments (Gothenburg region).

Example 3 Indirect calorimetry and activity

Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) were measured using an open

30 circuit calorimetry system (Oxymax, Columbus Instruments International Corp., Columbus, OH) as described before (Bohlooly, 2005). Energy expenditure (kcal/h) was calculated:

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(3.815 + 1.232RER)×vO₂, where RER is the respiratory exchange ratio [volume of CO₂ produced per volume of O₂ consumed (both ml/kg/min)] and vO₂ is the volume of O₂ consumed per h per kg mass of animal. The value of energy expenditure was correlated to individual body weights. Locomotor and rearing activity and corner time were measured in
5 activity boxes (Kungsbacka mät- och reglerteknik AB, Kungsbacka, Sweden) over 1 hour, 10-11am.

Example 4 Oral glucose tolerance test (OGTT).

OGTTs were performed ~9 am after a fast by oral administration of 2g glucose per kg body
10 weight. Blood (12μl) was sampled from the tail vein at 0, 15, 30, 60 and 120 minutes for determination of glucose (2μl, Accu-Chek, Roche Diagnostics, Mannheim, Germany) and insulin (2 x 5μl, Ultra-sensitive rat insulin ELISA kit, Crystal Chem Inc., Downers Grove, IL) levels.

15 Example 5 Blood biochemistry

Plasma adiponectin levels were measured using a mouse radioimmunoassay from Linco Research (Cat. # MADP-60HK, St. Charles, MO). The assay utilizes ¹²⁵I-labeled murine adiponectin and a multispecies adiponectin rabbit antiserum to determine the level of adiponectin in plasma by the double antibody/PEG technique. Plasma cholesterol was
20 measured with an enzymatic colorimetric assay (Roche Diagnostics). The cholesterol distribution profiles were measured on individual mice using a size exclusion high performance liquid chromatography system, SMART, with column Superose® 6 PC 3.2/30, (Amersham Pharmacia Biotech, Sweden) as described before (Linden, 2001). The lipoproteins in a 10μl sample were separated within 60 minutes and the area under the curves
25 represents the cholesterol content.

Example 6 Histology

Tissues were fixed in 4% buffered paraformaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

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Example 7 RNA preparation and quantitative real-time PCR

Total RNA was extracted using Trizol (Cat. # 15596-018, Invitrogen, Carlsbad, CA). First strand cDNA was synthesized from total RNA using High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Real time PCR analysis was performed with an ABI
5 Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using labeled fluorogenic probes. The expression data were normalized against mouse acidic ribosomal phosphoprotein P0 (M36B4). The relative expression levels were calculated according to the formula $2^{-\Delta CT}$, where ΔCT is the difference in cycle threshold (CT) values between the target and the M36B4 internal control.

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Example 8 Immunoblotting

Antibodies against adiponectin receptor 2 were generated by immunising rabbits with the following peptide: NRLGCSRTPEPDIRLRKGH (SEQ ID NO:4). Antibodies were affinity purified by peptide columns. Tissues were homogenized in H buffer (10 mM Hepes-KOH,
15 pH7.4, 2mM EDTA, 40 mM sucrose and 125 mM manitol) in the presence of protease inhibitors and centrifuged at 800 x g to remove the nuclei. The supernatant was collected and centrifuged at 100,000 x g to collect total membranes. Protein (25 μ g) was separated on 4-12% Bis-Tris gels (Novex, San Diego, CA) and transferred to immobilon membrane (Millipor, Bedford, MA). The blots were incubated with primary antibody (1 μ g/ml) in PBS-
20 T, 5% milk and then secondary antibody followed by development using ECL reagents (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Results

Adiponectin receptor 2 knock-out mice (*AdipoR2*^{-/-}) were created by exchanging
25 exon 5 with a targeting vector containing the LacZ and Neomycin resistance genes by Deltagen. To their surprise the inventors observed that both male and female *AdipoR2*^{-/-} were lean on regular chow and resistant to high-fat diet (HFD) induced body weight-gain. Resistance was not due to decreased food intake as there was no change in food intake in relation to body weight or lean body mass. After 8 weeks of HFD, female *AdipoR2*^{-/-} had
30 markedly lower relative body fat mass and moderately lower lean body mass compared to wild type (wt) controls. After 14 weeks of HFD, *AdipoR2*^{-/-} showed markedly reduced reproductive white adipose tissue (WAT) (males, -55% and females, -67%, $p < 0.01$), perirenal

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WAT (males, -53% and females, -69%, $p < 0.01$) and brown adipose tissue (males, -44% and females, -26%, $p < 0.01$) weights compared to corresponding littermate wt controls. In contrast to the males, female *AdipoR2*^{-/-} on regular chow also displayed decreased reproductive WAT (-50%, $p < 0.01$) and perirenal WAT (-57%, $p < 0.01$) weights compared to wt control mice.

5 Plasma levels of adiponectin tended to be elevated in *AdipoR2*^{-/-} and were significantly elevated in female *AdipoR2*^{-/-} on regular chow (wt = 493 ± 13 nM, *AdipoR2*^{-/-} = 569 ± 17 nM, $p < 0.01$, $n = 6$). Thus, *AdipoR2*^{-/-} are lean and markedly resistant to HFD induced body weight-gain and obesity and this may in part be due to higher circulating levels of adiponectin.

10 At 15 weeks of age, there was a reduction in testes weight in *AdipoR2*^{-/-} males compared to wt controls (wt = 0.219 ± 0.004 g and *AdipoR2*^{-/-} = 0.095 ± 0.001 g, $p < 0.01$, $n = 5-7$). This weight reduction was associated with an atrophy of the seminiferous tubules and numerous multinucleated giant cells in the tubular lumen. A moderate amount of intratubular cell debris was present in the epididymal tubules, which contained no spermatozoa
15 (aspermia). Thus, adiponectin receptor 2 may have a role in testicular development and maturity as well as fertility.

Interestingly, both male and female *AdipoR2*^{-/-} had larger brains compared to wt controls. Staining of the whole *AdipoR2*^{-/-} brain with β -galactosidase, demonstrated that adiponectin receptor 2 is highly expressed in the brain. Thus, adiponectin receptor 2 may be
20 important for brain development and function.

Female *AdipoR2*^{-/-} on HFD had 76% lower fasting insulin levels ($p < 0.01$) and markedly lower insulin response following an oral glucose tolerance test (OGTT). The area under the curve for the insulin response curve was 70% lower ($p < 0.01$) and for glucose 9% lower ($p < 0.05$) in *AdipoR2*^{-/-} compared to wt controls. Thus, *AdipoR2*^{-/-} had an improved
25 glucose tolerance compared to wt controls indicating improved insulin sensitivity.

AdipoR2^{-/-} had increased energy expenditure but unchanged respiratory exchange ratio (RER) compared to corresponding wt controls. The increased energy expenditure in *AdipoR2*^{-/-} could be explained by a markedly increased locomotor and rearing activity at the expense of decreased corner time in activity boxes in both male and female *AdipoR2*^{-/-}. Thus, *AdipoR2*^{-/-}
30 may be protected from HFD-induced obesity because of increased energy expenditure possibly due to increased spontaneous locomotor activity.

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On HFD, female *AdipoR2*^{-/-} showed 49% lower total plasma cholesterol level (wt = 3.5 ± 0.1 mM, *AdipoR2*^{-/-} = 1.8 ± 0.1 mM, $p < 0.01$) mainly due to decreased HDL cholesterol levels. In addition, female *AdipoR2*^{-/-} had lower liver triglyceride content compared to wt controls (wt = 2.9 ± 0.32 % g, *AdipoR2*^{-/-} = 1.7 ± 0.16 % g, $p < 0.05$) and tended to have lower
5 liver triglyceride content on HFD (wt = 5.3 ± 0.76 % g, *AdipoR2*^{-/-} = 4.5 ± 0.63 % g, non-significant).

Interestingly, the down-stream adiponectin target gene PPAR α and the down-stream PPAR α target gene carnitine palmitoyl transferase-I α (CPT-I α) were down regulated in *AdipoR2*^{-/-} livers compared to wt controls. Thus, *AdipoR2*^{-/-} are not lean because of increased
10 activity via the PPAR α pathway. This underscores the increased energy expenditure as the prime reason for the lean phenotype of *AdipoR2*^{-/-}.

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International Patent Application Number WO2005/001061

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International Patent Publication Number WO2005/031345

International Patent Publication Number WO2005/084697 which claims priority from US Patent Application Number US 60/549,561

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Claims:

1. A method of screening for an agent with therapeutic potential in treating or preventing a metabolic or neurological disease, comprising determining whether the agent
5 antagonises adiponectin receptor 2, wherein an antagonist of adiponectin receptor 2 has therapeutic potential in treating or preventing a metabolic or neurological disease.
2. A method of screening according to claim 1 comprising
contacting an adiponectin receptor 2 with a test agent, and
10 determining the ability of the test agent to antagonise adiponectin receptor 2.
3. A method of screening according to claim 1 comprising determining whether the agent antagonises adiponectin receptor 2 comprising
contacting an adiponectin receptor 2 with a test agent, and
15 determining the ability of the test agent to antagonise adiponectin receptor 2 (a parent antagonist), and
optionally preparing a derivative of a parent antagonist, and
testing a derivative of a parent antagonist to determine whether the derivative is
an antagonist of adiponectin receptor 2, and
20 selecting a derivative, which is an antagonist of adiponectin receptor 2,
according to its properties.
4. An antagonist of adiponectin receptor 2 for treating or preventing a metabolic or neurological disease identified by the method of any of the preceding claims.
- 25 5. A pharmaceutical composition comprising an antagonist of adiponectin receptor 2 according to claim 4 for treating or preventing a metabolic or neurological disease.
6. A method of preparing a pharmaceutical composition according to claim 5 comprising
30 determining whether a test agent is an antagonist of adiponectin receptor 2, and
incorporating the agent, or a derivative thereof, with a pharmaceutically acceptable carrier.

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7. A method of treating or preventing a metabolic or neurological disease in a patient comprising administering the pharmaceutical composition of claim 5.
- 5 8. A method of treating or preventing a metabolic disease according to claim 7, wherein the metabolic disease is obesity, hepatic steatosis, high cholesterol, high plasma lipids, insulin resistance, type 2 diabetes or cardiovascular disease.
9. A method of treating or preventing a neurological disease according to claim 7,
10 wherein the neurological disease is any one of Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, dementia, cognitive impairment, Huntington's disease, multiple sclerosis and other neurodegenerative diseases, or psychiatric disorders such as schizophrenia, psychoses, depression, anxiety and other neurotic diseases.
- 15 10. Use of an antagonist according to claim 4 in the manufacture of a medicament for the treatment or prevention of a metabolic or neurological disease.
11. Use of an antagonist according to claim 10, wherein the metabolic disease is obesity,
20 hepatic steatosis, high cholesterol, high plasma lipids, insulin resistance, type 2 diabetes or cardiovascular disease.
12. Use of an antagonist according to claim 10, wherein the neurological disease is any one of Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis,
25 dementia, cognitive impairment, Huntington's disease, multiple sclerosis and other neurodegenerative diseases, or psychiatric disorders such as schizophrenia, psychoses, depression, anxiety and other neurotic diseases.

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2007/001521

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/74

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 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, EMBASE, BIOSIS, 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	EP 1 589 106 A1 (CT FOR ADVANCED SCIENCE & TECH [JP]; NISSAN CHEMICAL IND LTD [JP]) 26 October 2005 (2005-10-26) paragraph [0072] - paragraph [0081]; claims 1-8	1-12
X	WO 2005/031345 A (BAYER HEALTHCARE AG [DE]; GOLZ STEFAN [DE]; BRUEGGEMEIER ULF [DE]; GEE) 7 April 2005 (2005-04-07) cited in the application page 38, line 25 - page 39, line 6 page 53, line 29 - page 54, line 11 page 115, line 12 - page 116, line 23 claims 19-26 ----- -/--	4-12



Further documents are listed in the continuation of Box C.



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Date of the actual completion of the international search

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International application No

PCT/GB2007/001521

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