Title: CXCR2 RECEPTOR ANTAGONISTS FOR THE TREATMENT OR THE PREVENTION OF INSULIN RESISTANCE

Abstract: The present invention relates to CXCR2 receptor antagonists or inhibitors of CXCR2 receptor gene expression for the treatment or the prevention of insulin resistance. The present invention also relates to a method for diagnosing and/or prognosing insulin resistance in a subject comprising detecting the presence and/or quantifying CXCL5 in a biological sample obtained from said subject.
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CXCR2 RECEPTOR ANTAGONISTS
FOR THE TREATMENT OR THE PREVENTION OF INSULIN RESISTANCE

FIELD OF THE INVENTION:

The present invention relates to CXCR2 receptor antagonists or inhibitors of CXCR2 receptor gene expression for the treatment or the prevention of insulin resistance.

BACKGROUND OF THE INVENTION:

Insulin resistance (IR) is the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from adipose, muscle and liver cells. Insulin resistance in fat cells reduces the effects of insulin and results in elevated hydrolysis of stored triglycerides in the absence of measures which either increase insulin sensitivity or which provide additional insulin. Increased mobilization of stored lipids in these cells elevates free fatty acids in the blood plasma. Insulin resistance in muscle cells reduces glucose uptake (and so local storage of glucose as glycogen), whereas insulin resistance in liver cells results in impaired glycogen synthesis and a failure to suppress glucose production. Elevated blood fatty acid levels (associated with insulin resistance and diabetes mellitus Type 2), reduced muscle glucose uptake, and increased liver glucose production all contribute to elevated blood glucose levels. High plasma levels of insulin and glucose due to insulin resistance are believed to be the origin of metabolic syndrome and type 2 diabetes, including its complications.

White adipose tissue (WAT) is primarily involved in energy storage in the form of triglycerides, and energy release in the form of free fatty acids. However, WAT is no longer considered only as a fat storage organ. Instead, WAT secretes numerous factors that play a role in several physiological and pathological processes including insulin resistance (reviewed in (Ahima and Flier, 2000; Trujillo and Scherer, 2006)). WAT can therefore be considered as a sensory organ. External factors such as nutritional status, stress, physical exercise, infection or trauma, will affect WAT biology through various signaling pathways including growth factors and fatty acids.
In turn, WAT will then signal other organs in an endocrine manner, thereby transmitting a metabolic, proliferative, growth, or differentiation response. Adipokines are factors secreted by the different cell compartments of WAT, such as adipocytes or macrophages, and were initially characterized as regulators of metabolic processes, such as regulation of food intake, energy homeostasis, adipocyte differentiation, or insulin sensitivity. Subsequently, it was found that adipokines could modulate inflammatory processes. These adipokines include WAT-specific factors, such as leptin, adiponectin, and well-known cytokines secreted by several cell types, such as TNFα, IL-6, IL-8, IL-1, or monocyte chemoattractant protein-1 (MCP-1).

Increased WAT mass, such as observed in obese subjects, is correlated with chronic systemic inflammatory response, demonstrated by increased infiltration of macrophages in WAT (Weisberg et al., 2003; Xu et al., 2003). This inflammatory state results in the development of obesity-associated pathologies including atherosclerosis, hypertension, non-alcoholic steatohepatitis, and insulin resistance. These obesity-associated pathologies are the result, at least in part, of changes in the secretion of adipokines, and illustrate the link between inflammatory state and metabolic response (Hotamisligil, 2006).

Chemokines are proinflammatory cytokines that stimulate leukocyte chemotaxis and are produced in response to infectious and other inflammatory stimuli by a number of different cell types. To date more than 50 proinflammatory cytokines or chemokines have been identified and have been classified into four groups according to the location of the conserved cysteine residues: CXCL, CCL, CL, and CX3CL (Zlotnik et al., 2006). These chemokines play differential roles in specifically recruiting different cell types to an inflammatory site. CXCL5 or epithelial neutrophil activating peptide (ENA-78) is a cytokine belonging to the family of chemokines that is mainly implicated in the chemotaxis of inflammatory cells through the generation of local concentration gradients (Walz et al., 1991; Walz et al., 1997). It has been shown, to be a recruiter of neutrophils and involved in their activation, through interaction with the CXCR2 receptor. This C-X-C chemokine has been implicated in pulmonary disease, lung cancer, arthritis, and other pathological states (Walz et al., 1991; Walz et al., 1993; Wislez et al., 2004).

However, no investigation on CXCR2 receptor pathway has been made on insulin resistance.
SUMMARY OF THE INVENTION:

The present invention relates to a CXCR2 receptor antagonist for the treatment or the prevention of insulin resistance.

The present invention also relates to an inhibitor of CXCR2 receptor gene expression for the treatment or the prevention of insulin resistance.

The present invention further relates to a method for diagnosing and/or prognosing insulin resistance in a patient comprising detecting the presence and/or quantifying CXCL5 in a biological sample obtained from said patient.

DETAILED DESCRIPTION OF THE INVENTION:

Definitions:

The terms "CXCR2" or "CXCR2 receptor" are used interchangeably and have their general meaning in the art. The CXCR2 receptor can be from any source, but typically is a mammalian (e.g., human and non-human primate) CXCR2 receptor, particularly a human CXCR2 receptor.

The terms "CXCL5", "LIX" or "ENA-78" are used interchangeably and have their general meaning in the art. They refer to chemokine (C-X-C motif) ligand 5 which is a natural ligand of the CXCR2 receptor. CXCL5 can be from any source, but typically is a mammalian (e.g., human and non-human primate) CXCL5, particularly a human CXCL5.

An "inhibitor of gene expression" refers to a natural or synthetic compound that has a biological effect to inhibit or significantly reduce the expression of a gene. Consequently an "inhibitor of CXCR2 receptor gene expression" refers to a natural or synthetic compound that has a biological effect to inhibit or significantly reduce the expression of the gene encoding for the CXCR2 receptor.

By "receptor antagonist" is meant a natural or synthetic compound that has a biological effect opposite to that of a receptor agonist. The term is used indifferently to denote a "true" antagonist and an inverse agonist of a receptor. A "true" receptor antagonist is a compound which binds the receptor and blocks the biological activation of the receptor, and thereby the action of the receptor agonist, for example, by competing with the agonist for said receptor. An inverse agonist is a compound
which binds to the same receptor as the agonist but exerts the opposite effect. Inverse agonists have the ability to decrease the constitutive level of receptor activation in the absence of an agonist.

The terms "CXCR2 receptor antagonist" includes any entity that, upon administration to a patient, results in inhibition or down-regulation of a biological activity associated with activation of the CXCR2 receptor by CXCL5 in the patient, including any of the downstream biological effects otherwise resulting from the binding to CXCR2 receptor with CXCL5. Such CXCR2 receptor antagonists include any agent that can block CXCR2 receptor activation or any of the downstream biological effects of CXCR2 receptor activation. For example, such a CXCR2 receptor antagonist (e.g. a small organic molecule, an antibody directed against CXCR2) can act by occupying the ligand binding site or a portion thereof of the CXCR2 receptor, thereby making CXCR2 receptor inaccessible to its natural ligand, CXCL5, so that its normal biological activity is prevented or reduced. The term CXCR2 receptor antagonist includes also any agent able to interact with the natural ligand of CXCR2, namely CXCL5. Said agent may be an antibody directed against CXCL5 which can block the interaction between CXCL5 and CXCR2 or which can block the activity of CXCL5 ("neutralizing antibody").

In the context of the present invention, when CXCR2 receptor antagonists are small organic molecules, said antagonists are preferably selective for the CXCR2 receptor as compared with the other CXC receptors, such as CXCR1 receptor. By "selective" it is meant that the affinity of the antagonist for the CXCR2 receptor is at least 10-fold, preferably 25-fold, more preferably 500-fold, still preferably 150-fold higher than the affinity for the other CXC receptor (CXCR1).

The affinity of an antagonist for CXCR2 receptor may be quantified by measuring the activity of CXCR2 receptor in the presence a range of concentrations of said antagonist in order to establish a dose-response curve. From that dose response curve, an IC₅₀ value may be deduced which represents the concentration of antagonist necessary to inhibit 50% of the response to an agonist in defined concentration. The IC₅₀ values may be readily determined by the one skilled in the art by fitting the dose-response plots with a dose-response equation as described by De Lean et al. (1979). IC₅₀ values can be converted into affinity constant (Ki) using the assumptions of Cheng and Prusoff (1973).
The term "small organic molecule" refers to a molecule of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e.g., proteins, nucleic acids, etc.). Preferred small organic molecules range in size up to about 5000 Da, more preferably up to 2000 Da, and most preferably up to about 1000 Da.

As used herein, the term "subject" denotes a mammal, such as a rodent, a feline, a canine, and a primate. Preferably, a subject according to the invention is a human.

The term "insulin resistance" refers to a condition where a normal amount of insulin is unable to produce a normal physiological or molecular response and preferably to obesity-induced insulin resistance.

In its broadest meaning, the term "treating" or "treatment" refers to reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition.

In particular, "prevention" of insulin resistance may refer to the administration of the compounds of the present invention prevent the symptoms of insulin resistance.

As used herein, "detecting" means determining if CXCL5 is present or not in a biological sample and "quantifying" means determining the amount of CXCL5 in a biological sample.

"Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

**Therapeutic methods and uses:**

The present invention provides methods and compositions (such as pharmaceutical compositions) for treating or preventing insulin resistance.

According to a first aspect, the invention relates to a CXCR2 receptor antagonist for treating or preventing insulin resistance.
In one embodiment, the CXCR2 receptor antagonist may be a low molecular weight antagonist, e.g., a small organic molecule.


In a particular embodiment, the CXCR2 receptor antagonists described in the international Patent Application WO96/25157 have the following formula:

\[
\begin{align*}
\text{n(Y)} & \text{X} \text{R} \text{(R1)m} \\
\text{N} & \text{H} \text{N} \text{H} \\
\end{align*}
\]

wherein

- X is oxygen or sulfur;
- R is any functional moiety having an ionizable hydrogen and a pKa of 10 or less;
- Ri is independently selected from hydrogen; halogen; nitro; cyano; halosubstituted CMO alkyl; CMO alkyl; C2,10 alkenyl; CMO alkoxy; halosubstituted C1,10 alkoxy; azide; S(O)JtR4; hydroxy; hydroxy C1,4 alkyl; aryl C1,4 alkyl; aryloxy; ary1 C1,4 alkoxy; heteroaryl; heteroarylalkyl; heterocyclic; heterocyclic C1,4 alkyl; heteroaryl C1,4 alkoxy; aryl C2,10 alkyl; heteroaryl C2,10 alkyl; heterocyclic C2,10 alkyl; heterocyclic C2,10 alkyl; N, R4, R5, C2,10 alkyl; C(O)NR4 R5; C(O)NR4 R5; C(O)NR4, R5; S(O)3 H; S(O)3 R8; CMO alkyl C(O)Rn; C2,10 alkyl C(O)Rn; C2,10 alkyl C(O)ORn; C(O)Rn; C(O)ORn; C(O)ORi2.
OC(O) R_i; NR_{4}C(O)R_{n}; or two R_i moieties together may form O-(CH_{2})_{s}O or
a 5 to 6 membered unsaturated ring;
s is 0 or an integer having a value of 1 or 2;
R_{4} and R_{5} are independently hydrogen, optionally substituted C_{i-4} alkyl,
optionally substituted aryl, optionally substituted aryl C_{i-4} alkyl, optionally
substituted heteroaryl, optionally substituted heteroaryl C_{i-4} alkyl, heterocyclic,
heterocyclic C_{i-4} alkyl, or R_{4} and R_{5} together with the nitrogen to which they
are attached form a 5 to 7 member ring which may optionally comprise an
additional heteroatom selected from O/N/S;
Y is independently selected from hydrogen; halogen; nitro; cyano;
halosubstituted C_{1-10} alkyl; C_{1-10} alkyl; C_{2-10} alkenyl; C_{M_0} alkoxy;
halosubstituted C_{M_0} alkoxy; azide; S(O)R_{4}; hydroxy; hydroxy C_{i-4} alkyl; aryl;
aryl C_{i-4} alkyl; aryloxy; aryl C_{i-4} alkyloxy; heteroaryl; heteroarylalkyl;
heteroaryl C_{i-4} alkyloxy; heterocyclic; heterocyclic C_{i-4} alkyl; aryl C_{2-10} alkenyl;
heteroaryl C_{2-10} alkenyl; heterocyclic C_{2-10} alkenyl; NR_{4}R_{5}; C_{2-10} alkynyl
C(O)NR_{4}R_{5}; C(O)NR_{4}R_{5}; C(O)NR_{4}R_{1}; S(O)_{3}H; S(O)_{3}R_{5}; C_{M_0} alkyl C(O)R_{n};
C_{2-10} alkenyl C(O)R_{n}; C_{2}-10 alkenyl C(O)OR_{n}; C(O)R_{n}; C(O)OR_{n}; C(O)OR_{1};
OC(O) R_{1}; NR_{4}C(O)R_{n}; or two R_i moieties together may form O-(CH_{2})_{s}O or
n is an integer having a value of 1 to 3;
m is an integer having a value of 1 to 3;
R_{8} is hydrogen or C_{i-4} alkyl;
R_{10} is C-i-O alkyl C(O)_{2}R_{8};
Rn is hydrogen, C_{i-4} alkyl, optionally substituted aryl, optionally substituted
aryl C_{i-4} alkyl, optionally substituted heteroaryl, optionally substituted
heteroaryl C_{i-4} alkyl, optionally substituted heterocyclic, or optionally
substituted heterocyclic C_{i-4} alkyl;
R_{i_2} is hydrogen, C_{M_0} alkyl, optionally substituted aryl or optionally substituted
arylalkyl;
or a pharmaceutically acceptably salt thereof.
According to this particular embodiment, the receptor CXCR2 antagonists are selected from those described in the international Patent Application WO96/251577 and that are listed below:

N-(2-Hydroxy-4-nitrophenyl)-N'-(2-methoxyphenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-phenylphenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-methylthiophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2,3-dichlorophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-chlorophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2,3-methylenedioxyphenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-methoxy-3-chlorophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-phenyloxyphenyl)urea,
N-(3-Chloro-2-hydroxyphenyl)-N'-(bromophenyl)urea,
N-(2-Hydroxy-3-glycememethylestercarbonylphenyl)-N'-(2-bromophenyl)urea,
N-(3-Nitro-2-hydroxyphenyl)-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2-methoxyphenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2-phenylphenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2,3-dichlorophenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2-methylphenyl)urea,
N-(2-Hydroxy-3-cyano-4-methylphenyl)-N'-(2-bromophenyl)urea,
N-(4-Cyano-2-hydroxyphenyl)-N'-(2-thfluoromethylphenyl)urea,
N-(3-Trifluoromethyl-2-hydroxyphenyl)-N'-(2-bromophenyl)urea,
N-(3-Phenylaminocarbonyl-2-hydroxyphenyl)-N'-(2-bromophenyl)urea,
N-(2-Hydroxy 4-nitro phenyl) N'-(2-iodo phenyl) urea,
N-(2-Hydroxy 4-nitro phenyl) N'(2-bromo phenyl)thiourea,
N-(2-Phenylsulfonamido)-4-cyanophenyl-N'-(2-bromo phenyl)urea,
(E)-N-[3-[(2-Aminocarbonyl)ethenyl]-2-hydroxyphenyl]-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-3,4-dichlorophenyl)-N'-(2,3-dichlorophenyl)urea,
N-(2-Hydroxy-5-nitrophenyl)-N'-(2,3-dichlorophenyl)urea;  and
N-(2-Hydroxy-3-cyanophenyl)-N'-(2,3-dichlorophenyl)urea.

In a particular embodiment, the CXCR2 receptor antagonist is N-(2-Hydroxy-4-
nitrophenyl)-N'-(2-bromophenyl)urea (also named SB 225002) described by White
J.R. et al. (1998). This selective antagonist binds with high affinity the human CXCR2
receptor (Ki = 22 nM). This affinity is 150-fold more selective over the CXCR2
receptor (White J.R. et al. 1998).

In another embodiment the CXCR2 receptor antagonist may consist in an
antibody (the term including antibody fragment) that can block CXCR2 receptor
activation.

In particular, the CXCR2 receptor antagonist may consist in an antibody
directed against the CXCR2 receptor or CXCL5, in such a way that said antibody
impairs the binding of a CXCL5 to CXCR2.

In a particular embodiment, the CXCR2 receptor antagonist may be an anti-
CXCL5 neutralizing antibody (Arenberg et al., 1998; Halloran et al., 1999).

Antibodies directed against the CXCR2 receptor or CXCL5 can be raised
according to known methods by administering the appropriate antigen or epitope to a
host animal selected, e.g., from pigs, cows, horses, rabbits, goats, sheep, and mice,
among others. Various adjuvants known in the art can be used to enhance antibody
production. Although antibodies useful in practicing the invention can be polyclonal,
monoclonal antibodies are preferred. Monoclonal antibodies against CXCR2 receptor
or CXCL5 can be prepared and isolated using any technique that provides for the
production of antibody molecules by continuous cell lines in culture. Techniques for
production and isolation include but are not limited to the hybridoma technique
originally described by Kohler and Milstein (1975); the human B-cell hybridoma
technique (Cote et al., 1983); and the EBV-hybridoma technique (Cole et al. 1985).
Alternatively, techniques described for the production of single chain antibodies (see,
e.g., U.S. Pat. No. 4,946,778) can be adapted to produce anti-CXCR2, or anti-
CXCL5 single chain antibodies. CXCR2 receptor antagonists useful in practicing the
present invention also include anti- CXCR2, or anti-CXCL5 antibody fragments
including but not limited to F(ab')2 fragments, which can be generated by pepsin
digestion of an intact antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab and/or scFv expression libraries can be constructed to allow rapid identification of fragments having the desired specificity to CXCR2 receptor.

Humanized anti-CXCR2 receptor or anti-CXCL5 antibodies and antibody fragments therefrom can also be prepared according to known techniques. "Humanized antibodies" are forms of non-human (e.g., rodent) chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (CDRs) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Methods for making humanized antibodies are described, for example, by Winter (U.S. Pat. No. 5,225,539) and Boss (Celltech, U.S. Pat. No. 4,816,397).

Then after raising antibodies directed against the CXCR2 receptor or CXCL5 as above described, the skilled man in the art can easily select those blocking CXCR2 receptor activation.

In another embodiment the CXCR2 receptor antagonist is an aptamer directed against CXCR2 or CXCL5. Aptamers are a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide or oligopeptide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Such ligands may be isolated through Systematic Evolution of Ligands by Exponential enrichment
(SELEX) of a random sequence library, as described in Tuerk C. and Gold L., 1990. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., 1999. Peptide aptamers consists of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods (Colas et al., 1996).

Then after raising aptamers directed against the CXCR2 receptor or CXCL5 as above described, the skilled man in the art can easily select those blocking CXCR2 receptor activation.

Another aspect of the invention relates to an inhibitor of CXCR2 receptor gene expression for treating or preventing insulin resistance.

Inhibitors of CXCR2 receptor gene expression for use in the present invention may be based on anti-sense oligonucleotide constructs. Anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, would act to directly block the translation of CXCR2 receptor mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of CXCR2 receptors, and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding CXCR2 receptor can be synthesized, e.g., by conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically inhibiting gene expression of genes whose sequence is known are well known in the art (e.g. see U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

Small inhibitory RNAs (siRNAs) can also function as inhibitors of CXCR2 receptor gene expression for use in the present invention. CXCR2 receptor gene expression can be reduced by contacting a subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that CXCR2 receptor gene expression is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose
sequence is known (e.g. see Tuschl, T. et al. (1999); Elbashir, S. M. et al. (2001); Hannon, GJ. (2002); McManus, MT. et al. (2002); Brummelkamp, TR. et al. (2002); U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836).

Ribozymes can also function as inhibitors of CXCR2 receptor gene expression for use in the present invention. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of CXCR2 receptor mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

Both antisense oligonucleotides and ribozymes useful as inhibitors of CXCR2 receptor gene expression can be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramidite chemical synthesis. Alternatively, anti-sense RNA molecules can be generated by in vitro or in vivo transcription of DNA sequences encoding the RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the invention can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.
Antisense oligonucleotides siRNAs and ribozymes of the invention may be delivered in vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the antisense oligonucleotide siRNA or ribozyme nucleic acid to the cells and preferably cells expressing CXCR2 receptor. Preferably, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the the antisense oligonucleotide siRNA or ribozyme nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rouse sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses (e.g., lentivirus), the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Khegler, 1990 and in Murry, 1991).

Preferred viruses for certain applications are the adeno-viruses and adeno-associated viruses, which are double-stranded DNA viruses that have already been
approved for human use in gene therapy. The adeno-associated virus can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as, heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well known to those of skill in the art. See e.g. Sambrook et al., 1989. In the last few years, plasmid vectors have been used as DNA vaccines for delivering antigen-encoding genes to cells in vivo. They are particularly advantageous for this because they do not have the same safety concerns as with many of the viral vectors. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUC19, pRC/CMV, SV40, and pBlueScript. Other plasmids are well known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA. Plasmids may be delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA plasmid can be injected by intramuscular, intradermal, subcutaneous, or other routes. It may also be administered by intranasal sprays or drops, rectal suppository and orally. It may also be administered into the epidermis or a mucosal surface using a gene-gun. The plasmids may be given in an aqueous solution, dried onto gold particles or in association with another DNA delivery system including but not limited to liposomes, dendhmers, cochleate and microencapsulation.
Another object of the invention relates to a method for treating or preventing insulin resistance comprising administering a subject in need thereof with a CXCR2 receptor antagonist or an inhibitor of CXCR2 receptor gene expression.

CXCR2 receptor antagonists or inhibitors of CXCR2 receptor gene expression may be administered in the form of a pharmaceutical composition, as defined below. Preferably, said antagonist or inhibitor is administered in a therapeutically effective amount.

By a "therapeutically effective amount" is meant a sufficient amount of the CXCR2 receptor antagonist or inhibitor of CXCR2 receptor gene expression to treat or to prevent insulin resistance at a reasonable benefit/risk ratio applicable to any medical treatment.

It will be understood that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Preferably, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.
**Screening methods:**

Inhibitors of the invention can be further identified by screening methods described in the state of the art. The screening methods of the invention can be carried out according to known methods.

The screening method may measure the binding of a candidate compound to the receptor, or to cells or membranes bearing the receptor, or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound. Alternatively, a screening method may involve measuring or, qualitatively or quantitatively, detecting the competition of binding of a candidate compound to the receptor with a labelled competitor (e.g., antagonist or agonist). Further, screening methods may test whether the candidate compound results in a signal generated by an antagonist of the receptor, using detection systems appropriate to cells bearing the receptor. Antagonists can be assayed in the presence of a known agonist (e.g. CXCL5) and an effect on activation by the agonist by the presence of the candidate compound is observed. Competitive binding using known agonist such as CXCL5 is also suitable.

**Pharmaceutical compositions:**

The CXCR2 receptor antagonist or inhibitor of CXCR2 receptor gene expression may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

In the pharmaceutical compositions of the present invention, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.
Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The CXCR2 receptor antagonist or inhibitor of CXCR2 receptor gene expression of the invention can be formulated into a composition in a neutral or salt form. Pharmacologically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and
by the use of surfactants. The prevention of the action of microorganisms can be
brought about by various antibacterial and antifungal agents, for example, parabens,
chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be
preferable to include isotonic agents, for example, sugars or sodium chloride.
Prolonged absorption of the injectable compositions can be brought about by the use
in the compositions of agents delaying absorption, for example, aluminium
monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active
colloidal protein or polypeptides in the required amount in the appropriate solvent with various of the
other ingredients enumerated above, as required, followed by filtered sterilization.
Generally, dispersions are prepared by incorporating the various sterilized active
ingredients into a sterile vehicle which contains the basic dispersion medium and the
required other ingredients from those enumerated above. In the case of sterile
powders for the preparation of sterile injectable solutions, the preferred methods of
preparation are vacuum-drying and freeze-drying techniques which yield a powder of
the active ingredient plus any additional desired ingredient from a previously sterile-
filtered solution thereof.

Upon formulation, solutions will be administered in a manner compatible with
the dosage formulation and in such amount as is therapeutically effective. The
formulations are easily administered in a variety of dosage forms, such as the type of
injectable solutions described above, but drug release capsules and the like can also
be employed.

For parenteral administration in an aqueous solution, for example, the solution
should be suitably buffered if necessary and the liquid diluent first rendered isotonic
with sufficient saline or glucose. These particular aqueous solutions are especially
suitable for intravenous, intramuscular, subcutaneous and intraperitoneal
administration. In this connection, sterile aqueous media which can be employed will
be known to those of skill in the art in light of the present disclosure. For example,
one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to
1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some
variation in dosage will necessarily occur depending on the condition of the subject
being treated. The person responsible for administration will, in any event, determine
the appropriate dose for the individual subject.
The CXCR2 receptor antagonist or inhibitor of CXCR2 receptor gene expression of the invention may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses can also be administered.

In addition to the compounds of the invention formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; liposomal formulations; time release capsules; and any other form currently used.

**Diagnostic methods:**

Another aspect of the invention is a method for diagnosing and/or prognosing insulin resistance in a subject in need thereof, said method comprising detecting the presence and/or quantifying CXCL5 in a biological sample obtained from said subject.

According to the invention, the biological sample susceptible to contain CXCL5 is a biological sample, such as cell lysates (WAT, liver and muscle) or culture medium, or is a body fluid such as serum, plasma.

In one embodiment, said sample is WAT.

In another embodiment, said sample is a serum sample or a plasma sample.

In a particular embodiment of the invention, the detection and/or the quantification of CXCL5 is carried out by immunological detection.

In one embodiment, the immunological detection or quantification of CXCL5 is achieved by any methods known in the art using at least one antibody that binds specifically to CXCL5.

Examples of said methods include, but are not limited to, standard electrophoretic and immunodiagnostic techniques such as western blots, immunoprecipitation assay, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassay, gel diffusion precipitation reaction, immunodiffusion assay, precipitation reaction, agglutination assay (such as gel
agglutination assay, hemagglutination assay, etc.), complement fixation assay, protein A assay, immunoelectrophoresis assay, high performance liquid chromatography, size exclusion chromatography, solid-phase affinity, etc.

According to the invention, an antibody that binds specifically to CXCL5 is an antibody that does not cross react with others C-X-C chemokines. Examples of said antibody include, but are not limited to, the antibody described in Arenberg et al., 1998; Halloran et al., 1999.

According to the invention, the antibody that binds to CXCL5 may be labelled with a detectable molecule or substance. Examples of suitable labels for this purpose include a chemiluminescent agent, a colormetric agent, an energy transfer agent, an enzyme, a substrate of an enzymatic reaction, a fluorescent agent, or a radioisotope. The label may be coupled directly or indirectly by any known method in the art.

In one embodiment of the invention, the detection or quantification of CXCL5 in a biological sample may be achieved by a competitive immunoassay. Example of competitive immunoassays include enzyme immunoassay or enzyme-linked immunoassay (EIA or ELISA), fluorescent immunoassay, radiometric or radioimmunoassay (RIA), magnetic separation assay (MSA), lateral flow assay, diffusion immunoassay, immunoprecipitation immunoassay.

In another embodiment of the invention, the quantification of CXCL5 in a biological sample may be achieved by a non-competitive immunoassay referred as immunomethodc, "two-site" or "sandwich" immunoassays, wherein the CXCL5 may be bound to or sandwiched between two antibodies that bind to CXCL5.

Examples of non-competitive immunoassays include enzyme immunoassay or enzyme-linked immunoassay (EIA or ELISA), fluorescent immunoassay, radiometric or radioimmunoassay (RIA), magnetic separation assay (MSA), lateral flow assay, diffusion immunoassay, immunoprecipitation immunoassay, immnosorbent or "antigen-down" assay using antibodies that bind to CXCL5 bound to a solid support, or agglutination assay.

Examples of said assays are the human CXCL5 /ENA-78 DuoSet kits DY254 and DY443 purchased by R1D Systems.
In this embodiment, the quantification of CXCL5 in a biological sample is achieved by
- contacting said biological sample with two antibodies that bind to CXCL5,
- measuring the amount of bound CXCL5 antibody and
- calculating the amount of CXCL5 in the sample.

In one embodiment of the invention, the first antibody that binds to CXCL5 is an antibody that binds specifically to a first domain of CXCL5 and the second antibody is an antibody directed to an epitope in a second domain of CXCL5.

In one embodiment, a one-step assay (simultaneous incubation of the two antibodies that bind to CXCL5) is useful. In another embodiment, a two-step assay (sequential incubation of the two antibodies that bind to CXCL5) is useful. A two-step assay is preferred in the case where other molecules could compete for binding to the antibodies that bind to CXCL5.

Antibodies useful in the various embodiments of the invention encompass commercially available antibodies and antibody fragments, as well as any novel antibodies generated to bind to a suitable epitope on CXCL5. The antibodies used in various embodiments exemplified herein are monoclonal or polyclonal in nature. Other antibodies and antibody fragments, such as recombinant antibodies, chimeric antibodies, humanised antibodies, Fab or Fv fragments are also useful.

The amount of CXCL5 quantified may thus be compared with the corresponding amount detected in the samples of control subjects, in previous samples obtained from the subject or with normal reference values.

While the method of the invention is intended for the diagnosis and/or the prognosing of insulin resistance, control subjects are for example subjects that have not been diagnosed for said insulin resistance. Normal reference values refer to the amount of CXCL5 that can be determined by the method of the invention in a subject that has not been diagnosed for insulin resistance.

In one embodiment of the invention, said control value or reference value is determined by using the average values obtained from at least 10, preferably from at least 100 control subjects.
According to the invention, said method for diagnosing and/or prognosing an insulin resistance in a subject in need thereof, comprises the steps of:

- providing a biological sample obtained from a subject,
- measuring CXCL5 in said biological sample by immunological detection,
- quantifying the amount of CXCL5 present in said biological sample,
- correlating said amount of CXCL5 with the diagnosis and/or the prognosis of insulin resistance in said subject.

The invention will further be illustrated in view of the following figures and examples.

**FIGURES:**

**Figure 1: CXCL5 is expressed by WAT resident macrophages:** A. CXCL5 level was measured by ELISA assay in total protein extract of WAT, liver and muscle from 10-weeks-old C57Bl/6 mice (n=4 mice). B. WAT from 10-weeks-old C57Bl/6 mice (n=4 mice) were incubated in KRBH buffer for 1h, 3h, or 22h. At each time point, secreted CXCL5 in medium was analyzed by ELISA and normalized by DNA content in tissue. C. CXCL5 mRNA quantification by QPCR in different fractions of isolated human subcutaneous WAT as indicated. SVF: stromal vascular fraction. Quantification of mRNA was normalized by the expression level of rS9, here and in the following figures. D-E. Quantification of relative CXCL5 mRNA levels at the indicated times of 3T3-L1 (D) or human preadipocyte (E) differentiation. F. aP2 mRNA expression was analyzed by quantitative RT-PCR on 3T3L1 adipocytes differentiated with or without recombinant mouse CXCL5 (50ng/ml).

**Figure 2: Obesity increases CXCL5 release from WAT and CXCL5 systemic level:** A. Quantification by ELISA methods of CXCL5 secretion by WAT explants from C57Bl/6 and db/db mice after 6h in incubation media. Results were normalized by DNA content in tissue. B. Circulating CXCL5 levels were measured by ELISA methods in serum from control C57Bl/6, db/db, and ob/ob male mice. C. Circulating CXCL5 levels were measured by ELISA methods in serum from control C57Bl/6 mice and mice fed with a high fat diet (HFD) for 12 weeks D. Quantification of CXCL5 concentration in serum, as measured by ELISA between normal lean
(n=41) and obese patients (n=82). E. Quantification of CXCL5 concentration in serum, as measured by ELISA in response to body weight loss during a 4-week very low calorie diet (VLCD) in 24 obese patients.

**Figure 3: CXCL5 is involved in insulin resistance:** A. Comparative expression of CXCR2 mRNA in WAT, liver and muscle from 6-month-old C57Bl/6 mice. B. 2-deoxy-glucose uptake in isolated soleus muscles from mice. Muscle strips were incubated with or without recombinant CXCL5 (100ng/ml) for 1h before stimulation with insulin (10^{-7}M). C. Protein expression of phospho-Akt, and total Akt in whole cells lysates from isolated muscles (upper panel) described in (B) and from primary MEFs (lower panel) as analyzed by Western blotting with anti-phospho-Ser473-Akt and anti-Akt antibodies. D. Relative mRNA level of SOCS2 in primary MEFs treated or not with CXCL5 (300ng/ml) and cotreated with or without Jak2 inhibitor (AG490 50µM) or CXCR2 inhibitor (SB225002 10-6M). E. Protein expression of phospho-STAT5a/b and total STAT5 in whole cells lysates from primary MEFs treated with or without insulin (10-7M) and/ or CXCL5 (300ng/ml) as analyzed by Western blotting with anti-phospho-Y694/Y699-STAT5a/b and anti-STAT5 antibodies. F. Relative quantification of circulating CXCL5 concentration (fold) in mice after 12 days treatment with 20mg/Kg rosiglitazone (rosi) compared to non-treated mice (control). G. CXCL5 serum concentration is increased in obese patients with insulin resistance (n=28), compared to obese non-insulin resistant subjects (n=24). Insulin resistance status was assessed by the HOMA index (non IR: HOMA<2.6; IR: HOMA>2.6). H. Quantification of CXCL5 concentration in serum, as measured by ELISA between normal lean (n=9), lipodystrophic patients (n=12) and obese patients (n=1 1).

**Figure 4: Inhibition of CXCL5 action in obese mice improves insulin sensitivity:** A-D. Fasting glucose (A-B) and insulin (C-D) in 18-week-old obese HFD fed mice (A-C) and 9-weeks-old db/db mice (B-D) after 7 days of treatment with neutralizing antibody against CXCL5 (anti-CXCL5) or control IgG (cont IgG) (n=1 0 mice per group). E-H. Glucose clearance after intraperitoneal injection of insulin (0.75U/kg) (E-F)) or glucose (G-H) in animals treated with anti-CXCL5 or control IgG. For the graph E, F and G, glucose values are relative to initial glucose levels. Area under the curve was analyzed (AUC).
Figure 5: Inhibition of CXCR2 signaling improves insulin sensitivity: A-B. Fasting glucose (A) and insulin (B) in 18-week-old HFD fed mice after 9 days of treatment with CXCR2 antagonist, SB225002 (100mg/kg) or vehicle (n=10 mice per group). C-D. Glucose clearance after intraperitoneal injection of insulin (0.75U/kg) (C) or glucose (D) in animals treated with SB225002 or vehicle. Area under the curve was analyzed (AUC).

Figure 6: CXCR2 -/- mice improves insulin sensitivity: A-B. Fasting glucose (A) and insulin (B) in 10-weeks-old CXCR2 -/- and control mice before (chow) and after 7 weeks of high fat diet (HFD) (n=10 mice per group). C. Body weight in control and CXCR2 -/- mice in chow and high fat diet. D. Food intake in control and CXCR2 -/- mice. E-F. Glucose clearance after intraperitoneal injection of insulin (E) or glucose (F) in control and CXCR2 -/- mice under chow or high fat diet. Area under the curve was analyzed (AUC).

Figure 7: CXCL5 expression is regulated by TNFα and rosiglitazone: A. CXCL5 level in conditioned medium of human subcutaneous adipose tissue explants after treatment with vehicle (control) or TNFα (10ng/ml) for 24h (n=3 per group). B. Quantification of relative CXCL5 mRNA levels in WAT from obese ob/ob TNFα receptor -/- mice compared to the control obese mice (n=3 per group) . C-D. CXCL5 levels in WAT (A) from C57Bl/6 mice or THP1 macrophages (B) after treatment with vehicle (control), TNFα (10ng/ml), rosiglitazone (10⁻⁸M) or co-treated with TNFα/rosiglitazone for 6h. Secreted CXCL5 in medium was analyzed by ELISA and normalized by DNA tissue content. E. Q-PCR analysis of CXCL5 mRNA in THP-1 macrophages treated as in (C). F. Luciferase activity expressed as relative luciferase units of a 1422 bp DNA fragment of the CXCL5 promoter coupled to the luciferase gene in the absence or in the presence of a PPARY expression vector in 293T cells treated with vehicle, TNFα (10ng/ml), rosiglitazone (10⁻⁸M) or both TNFα/rosiglitazone. Results were normalized to β galactosidase activity. G. ChIP analysis of the NFkB binding site of the CXCL5 promoter in immunoprecipitated chromatin from THP-1 macrophages either non-treated (cont.) or treated with TNFα, rosi, or a combination of both (upper panel). Chromatin was immunoprecipitated with
mock antibody (IgG) or anti p65 antibody as indicated. Quantification of the ChIP analysis in lower panel. Image J software was used to measure the optical density of the bands. Results are corrected by the signal of the corresponding input lanes.

**Figure 8:** Comparison of mRNA levels in adipocytes, stromavascular fraction (SVF), and macrophages from human subcutaneous adipose tissue. Data are means ± SEM. Relative mRNA levels are expressed as arbitrary units obtained after normalization by 18S ribosomal RNA subunit.

**Figure 9:** Quantification of relative PPARY mRNA levels at the indicated times of 3T3-L1 preadipocyte differentiation.

**Figure 10:** Obesity increases CXCL5 systemic level: Quantification of CXCL5 concentration in serum, as measured by ELISA between normal lean (n=9) and obese patients (n=11) from an English cohort.

**Figure 11:** Rosiglitazone effect on diabetes: Fasting glucose level in db/db mice after 12 days treatment with 20mg/Kg rosiglitazone (rosi) compared to non-treated mice (control).

**Figure 12:** Neutralizing anti-CXCL5 antibody and CXCR2 antagonist effects on body weight and food intake: Body weight in 18-week-old obese high fat diet fed mice (A) and 9-weeks-old db/db mice (B) after 7 days of treatment with neutralizing antibody against CXCL5 (anti-CXCL5) or control IgG (cont IgG) and in 18-week-old mice after 9 days of treatment with CXCR2 antagonist SB225002 (100mg/kg) or vehicle (D) (n=10 mice per group). Food intake in neutralizing antibody (C) and in CXCR2 antagonist-treated mice (E).

**Figure 13:** CXCR2 antagonist treatment in mice: (A) Fasting glucose in 18-week-old obese mice before and after 9 and 14 days of treatment with CXCR2 antagonist, SB225002 (100mg/kg) (SB225002) or vehicle (control) (n=10 mice per group). (B) Glucose clearance after intraperitoneal injection of glucose in 12-week-old lean animals treated with anti-CXCL5, with CXCR2 antagonist (100mg/kg) or vehicle. Area under the curve was analyzed (AUC).
EXAMPLE:

Material & Methods

Materials: All chemicals, except if stated otherwise, were purchased from Sigma (St-Louis, Missouri). Recombinant human TNFα and recombinant mouse CXCL5 were purchased from AbCys s.a. (France) and R&D systems (France), respectively. Rosiglitazone was purchased from Molekula (France).

Animals and Experimental Design: Male ob/ob mice, male db/db mice and male C57BL/6 mice were purchased at 8-9 weeks of age from Charles River laboratory. Male CXCR2 -/- mice were purchased at 9 weeks of age from Jackson Laboratory. Animals were maintained according to European Union guidelines for use of laboratory animals. In vivo experiments were performed in compliance with the French guidelines for experimental animal studies (Agreement No. B-34-1 72-27). For the diet-induced obesity model, 6-weeks-old C57BL/6 mice were fed ad libitum with a diet including 45% from fat (TestDiet) for 12 weeks. For antibody treatment, the neutralizing CXCL5 antibody we purchased from Dr. Strieter and injected every other day as described previously (Arenberg et al., 1998). The neutralizing CXCL5 antibody has been shown to inhibit rat peritoneal neutrophil recruitment to recombinant CXCL5 in vivo (Halloran et al., 1999). For antagonist treatment, SB225002 was synthetized as previously described (White et al., 1998) and injected in intraperitoneal everyday for 9 days (100mg/kg). For rosiglitazone treatment, 11 weeks-old db/db mice were gaved with 20mg/kg everyday for 12 days. Body weights and glycemia was measured every 3 days and blood samples were collected. Food intake was measured every second day for 6 consecutive days. For serum preparation, blood samples were collected. The blood let to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. For glucose tolerance test, 18h-fasted mice were injected i.p. with glucose (1.7g/kg) and glycemia were measured at time 0, 30, 60 and 90 min after injection. For insulin tolerance test, 8h-fasted mice were injected i.p. with insulin (0.75U/kg) and glucose level was measured at time 0, 20 and 40 min after injection.
**Human studies:** Serum samples and subcutaneous abdominal WAT were obtained from subjects in agreement with French, Czech and English laws on biomedical research. Human adipocytes in primary culture were differentiated as described (Tiraby et al., 2003). The different cell types of human subcutaneous WAT were isolated as previously described (Curat et al., 2006). Human white adipose tissue was isolated as described (Tiraby et al., 2003). Results are expressed in pg/ml/g of tissue.

**WAT explants culture:** Fat pads from male C57BL/6 mice and/or db/db mice were dissected under sterile conditions, washed in Krebs Ringer Bicarbonate Hepes buffer (KRBH), minced finely, and incubated in 12-well tissue culture plates containing KRBH supplemented with 1% bovine serum albumin (BSA). The fat tissue samples were left unstimulated at 37°C under 5% CO2 for indicated time or were stimulated with vehicle (DMSO), TNFα (10ng/ml), rosiglitazone (10-6M) or co-treated with TNFα / rosiglitazone. At different time point, conditioned medium was removed and CXCL5 was measured by ELISA assay. At the end of experiment, fat tissue was removed and DNA extraction was done to normalize. Results are expressed in pg/ml/cells.

**Protein extraction:** WAT, liver and muscle were removed from C57BL/6 mice and were homogenized by Polytron in TEG buffer (10mM Ths-Hcl, pH 7.4, 1.5mM EDTA and 10% glycerol) containing protease inhibitors cocktail (Roche). The mixture was then sonicated and the cellular debris were pelleted by centrifugation at 13,000g for 10 minutes at 4°C. CXCL5 level was analyzed by ELISA assay.

**ELISA and Bio-plex assay:** CXCL5 concentration in cellular extracts, conditioned medium and serum was determined by ELISA with Duoset kit (DY254 and DY443, R&D Systems) as recommended by the manufacturer. CXCL1 and CXCL2 concentrations in serum were evaluated by Bio-Plex Cytokine Assay (Bio-Rad) as recommended by the manufacturer.

**RNA Extraction and Reverse Transcriptase, Quantitative PCR:** Total RNA from cells and tissues were isolated with TRlzol reagent (Invitrogen) as described by the manufacturer. Reverse transcription was performed using 5 µg total RNA,
random primers and MMLV enzyme (Invitrogen). Quantitative PCR was performed with FastStart DNA Master SYBR Green I kit (Roche, Manheim, Germany) on a Light Cycler instrument (Roche) as specified by the manufacturer. Ribosomal protein S9 (rS9) was used as an internal control. The sequence of the primers used is available upon request.

**Cell culture, transient transfection, and glucose transport:** Preadipocytes 3T3-L1 cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 100U/ml penicillin and 100 g/ml streptomycin. 3T3-L1 cells were differentiated as previously described (Sarruf et al., 2005). To analyze CXCL5 effect on adipocyte differentiation, 3T3-L1 preadipocytes were differentiated with mix supplemented with recombinant mouse CXCL5 (50ng/ml). Glucose transport experiments were performed as previously described (Abella et al., 2005). Human monocytic cell line Thp-1 were grown in RPMI 1640 supplemented with 10% FBS, 100U/ml penicillin and 100 g/ml streptomycin. Macrophage differentiation was induced by stimulating cells with TPA 10OnM for 48h. Then, macrophages Thp-1 were maintained in DMEM 10% FBS and stimulated or not with TNFα (10ng/ml), rosiglitazone (10-6M) or co-treated with TNFα / rosiglitazone for 18h. CXCL5 mRNA level was analyzed by Q-PCR. Secreted CXCL5 was measured by ELISA assay and normalized by DNA content. Fibroblastic 293T cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, 100U/ml penicillin and 100 g/ml streptomycin. Transient transfections were performed as described previously (Annicotte et al., 2005) and luciferase activity measurements were normalized for β-galactosidase activity to correct for differences in transfection efficiency. Graph values represent the mean of three independent experiments.

Primary MEFs were obtained from embryos at day 13.5 as previously described (Abella et al., 2005) and maintained in DMEM supplemented with 25mM glucose and 10% FBS. MEFs were starved 1h in DMEM containing 0.2% BSA and then treated 30min with Jak2 inhibitor AG490 (50µM) or CXCR2 antagonist SB225002 (10-6M) before to stimulate 1h with CXCL5 (300ng/ml). For insulin signaling, cells were stimulated with insulin (10-9M) for 10min.

**Chromatin immunoprecipitation (ChIP):** ChIP assays were performed as described previously (Annicotte et al., 2006). Briefly, proteins from Thp-1 cells were
formaldehyde cross-linked to DNA. After homogenization, lysis and DNA sonication, proteins were then immunoprecipitated using anti-p65 antibody. After washing, DNA-protein-complexes were subsequently eluted and cross-linking was reversed by heating the samples at 65°C for 16 h. DNA was then purified using Qiagen PCR purification kit (Qiagen, Courtabceuf, France) and PCR amplification was performed using cxc5 promoter-specific oligonucleotide primers.

**Plasmids:** Human CXCL5 promoter corresponding to -1359/+63 was cloned from breast cancer cell line MCF-7 using 5'-TCA GAA CCA GCC AGA AGA GGA-3' and 5'-AGC GGA GAT TGG AGG AGC GAA GAT-3' and subcloned in xp-2 luciferase reporter vector. CMV-GAL corresponds to the β-galactosidase gene under the control of the cytomegalovirus (CMV) promoter.

**Statistical analysis:** Data are presented as means ± SEM. Group means were compared by factorial analysis of variance (ANOVA). Upon significant interactions, differences between individual group means were analyzed by Fisher’s protected least squares difference (PLSD) test. Differences were considered statistically significant at p < 0.05 (‘), 0.01 ≤ p ≤ 0.001 (’’), p ≤ 0.001 (’’’).

In human studies, statistical comparisons were performed using non-parametric Wilcoxon-Mann Whitney test (p values < 0.05 were considered to be statistically significant).

**Results**

**CXCL5 is expressed by WAT resident macrophages:** Global analysis of cytokine expression in insulin-sensitive tissues highlighted the presence of high levels of CXCL5 protein expression in WAT in mice, compared to liver or muscle (Figure 1A), suggesting that CXCL5 could be a new adipocytokine. Consistent with this hypothesis, CXCL5 was found to be secreted by WAT explants in culture (Figure 1B). Furthermore, fractionation of human subcutaneous WAT by collagenase digestion showed that the stromal vascular fraction (SVF) expressed higher levels of CXCL5 than did mature adipocytes, as assessed by real-time PCR analysis (Figure 1C). A more detailed analysis by immunoselective isolation demonstrated that the major contribution to CXCL5 concentration was from macrophages from the SVF of human WAT (Figure 1C). Isolation of specific cell types was validated with specific
markers of each fraction (Figure 8). The observed high levels of expression in WAT could point to the participation of CXCL5 in adipogenesis. To address the role of CXCL5 in adipocyte differentiation, we first analyzed CXCL5 mRNA expression during this process. CXCL5 mRNA was highly expressed in mouse and human preadipocytes whereas its expression progressively decreased during adipocyte differentiation (Figures 1D and 1E). PPARγ mRNA level is showed as specific marker of adipocyte differentiation (Figure 9). This result suggested that CXCL5 was not critical for adipogenesis. To demonstrate this hypothesis the effects of recombinant CXCL5 on 3T3-L1 preadipocyte differentiation were evaluated. 3T3-L1 cells differentiated into adipocytes, as measured by the expression of the adipocyte marker aP2, regardless of the absence or the presence of CXCL5 (Figure 1F). Altogether these results suggested that CXCL5 is a new adipokine secreted by WAT macrophages.

**CXCL5 expression and secretion is increased in obesity:** The inflammatory state associated with obesity includes macrophage infiltration in WAT, and increased cytokine secretion by these macrophages. Since it is expressed in WAT macrophages (Figure 1C), we hypothesized that CXCL5 expression would be increased in obesity. Consistent with this hypothesis CXCL5 secretion in WAT explants from obese db/db mice was markedly increased compared to non-obese control mice (2.5-fold, Figure 2A). Most interestingly, circulating serum CXCL5 concentration was increased 2-fold in obese ob/ob and db/db and high fat diet-fed mice compared to lean mice (Figures 2B and 2C). These results prompted us to analyze CXCL5 concentration in human obese subjects. Similar to the results observed in mice, CXCL5 serum concentration was significantly increased in an obese French population, compared to lean control subjects (median: 1545 [295-25900] versus 970 [395-2790] pg/ml, p=0.025) (Figure 2D). Similar results were observed when an English cohort was analyzed (Figure 10). To further demonstrate a positive correlation between fat mass and CXCL5 levels, calorie restriction experiments were performed. Obese women were subjected to very low calorie diet (VLCD, 800kcal/d) for a period of 4 weeks (Vitkova et al., 2007). An average decrease of 7% in body weight was observed during VLCD. Strikingly, the weight loss is associated with a decrease of 10% in serum CXCL5 concentration (median: 2230 versus 2040 pg/ml, p=0.006), suggesting that CXCL5 circulating concentration
is controlled by WAT mass (Figure 2E).

**CXCL5 mediates insulin resistance:** Some of the insulin desensitizing effects observed during obesity development are mediated by adipokines. The positive association of CXCL5 concentration with obesity suggested that this adipokine could be a mediator of insulin resistance. The biological effects of CXCL5 are mediated through interaction with the chemokine receptor CXCR2. To investigate the role of CXCL5 in insulin resistance, we first aimed to identify tissues that are potentially responsive to CXCL5. Expression analysis in insulin-sensitive tissues showed that CXCR2 mRNA was expressed at higher levels in muscle than in liver or WAT (Figure 3A). The physiological effects of CXCL5 in muscle were next analyzed. Incubation of isolated soleus muscles with recombinant CXCL5 resulted in a diminution of insulin-stimulated glucose transport in this tissue (Figure 3B). This suggested a role of CXCL5 in mediating insulin resistance. Strikingly, CXCL5 treatment inhibited insulin-induced Akt phosphorylation (Figure 3C), which was consistent with the observed decrease in glucose transport in CXCL5-treated muscles (Figure 3B). Similarly, CXCL5 inhibited Akt phosphorylation also in primary cultures of mouse embryonic fibroblasts (MEFs) (Figure 3C). A more in detail analysis showed that CXCL5 was inhibiting, insulin signaling through activation of the JAK/STAT pathway, and consequently through increasing the expression of SOCS2, which is a known inhibitor of the insulin receptor, and increasing the activity of Stat5 signaling (Figures 3 D and E). Strikingly, SOCS 2 regulation by CXCL5 is blunted by a Jak2 inhibitor, AG490 and by CXCR2 antagonist treatments (Figure 3D). From these results we concluded that CXCL5 blocks insulin signaling in muscle through activation of the Jak/STAT/SOCS pathway through the CXCR2 receptor. We attempted next to demonstrate a positive correlation between insulin resistance and serum CXCL5 concentration in mice. Obese db/db mice were treated with the insulin-sensitizing drug rosiglitazone. This resulted, as expected in a decrease in glycemia (Figure 11). Decreased circulating glucose concentration was significantly associated with a 30% decrease in serum CXCL5 concentration in rosiglitazone-treated mice (Figure 3F).

We next examined the relationship between CXCL5 and insulin sensitivity in a population of obese subjects. The subjects (n=52) were stratified into two groups according to HOMA values, as a measure of insulin resistance index. We found that
serum CXCL5 concentration was higher in insulin-resistant compared to non insulin-resistant obese subjects, suggesting that CXCL5 levels are causative of insulin resistance in these subjects (Figure 3G; IR: 3960pg/ml versus non IR: 3110pg/ml, P<0.021). Furthermore, we have analyzed CXCL5 levels in a human model of non-obese insulin resistance. We show that lipodystrophic patients, who are insulin resistant do not have increased levels of CXCL5 (Figure 3H). We can conclude from this data that it is CXCL5 that induces insulin resistance, and not the opposite. Taken together these results suggested that CXCL5 is involved in the development of insulin resistance during obesity.

**Inhibition of CXCL5 signaling in obese and insulin resistant mice improves insulin sensitivity:** To unequivocally demonstrate the participation of CXCL5 in obesity-induced insulin resistance we analyzed the effects of CXCL5 inhibition in two mice model of insulin resistance. C57BL/6 mice rendered obese by feeding high fat diet for 12 weeks, and obese db/db mice were treated every other day with intraperitoneal injection of neutralizing anti-CXCL5 antibody or control IgG. After one week of treatment, a significant decrease in fasting glucose was observed in mice injected with anti-CXCL5 compared to mice injected with control IgG (Figures 4A and 4B). Anti-CXCL5-treated obese mice were more sensitive to insulin as measured by fasted insulin level (Figures 4C and 4D) and by insulin tolerance test (Figures 4E and 4F). Moreover, treatment with anti-CXCL5 improved glucose tolerance during an intraperitoneal glucose tolerance test in both mouse models of obesity (Figure 4G and 4H).

Since CXCL5 signaling is mediated by the CXCR2 receptor, we next studied the impact of a selective antagonist of CXCR2, SB225002, on insulin sensitivity. Obese C57BL/6 mice were treated daily with intraperitoneal injection of SB225002 (100mg/kg) for 10 days. Similar to the effect of neutralizing antibody, SB225002 treatment also decreased fasting glucose and insulin level (Figures 5A and 5B), and increased insulin sensitivity and glucose homeostasis as demonstrated by insulin and glucose tolerance tests (Figures 5C and 5D). No differences in body weight or food intake were observed as a result of either SB225002 or anti-CXCL5 antibody treatments, suggesting a direct rather than a secondary effect (Figures 12A, 12B and 12C). Since CXCL5 is secreted in normal mice we tested the effect of the treatments...
in these mice. Even in these SB225002- and anti CXCL5 treated lean mice a significant increase in glucose clearance was observed (Figure 13B).

To unequivocally demonstrate the participation of the CXCL5-CXCR2 signaling axis in insulin sensitivity we next analyzed the phenotype of CXCR2 -/- mice. The most concluding observation was the protection of CXCR2/- mice to obesity-induced insulin resistance (Figure 6). Fasting glucose concentration remained normal in CXCR2/- mice fed high fat diet, compared to CXCR2+/+ mice (Figure 6A). Surprisingly, an increase in fasted insulin levels was observed suggesting that the genetic deletion of CXCR2 could also affect insulin secretion in pancreas (Figure 6B). Moreover, no differences in body weight or food intake were observed (Figures 6C and 6D) whereas improved insulin sensitivity and glucose tolerance in both chow and high fat diet CXCR2/-, compared to +/- mice were observed (Figures 6E and 6F). Interestingly, genetic CXCR2 ablation or CXCR2 antagonist treatment are more effective than CXCL5 blocking, suggesting that other CXCR2 ligands could be involved in this pathway. Strikingly, circulating levels of CXCL1 and CXCL2, which are also CXCR2 ligands, are increased in mice models of obesity, although to a lesser extent than CXCL5. Taken together, these results show that CXCL5 plays a major role in insulin resistance.

**CXCL5 is regulated by TNFα:** We finally analyzed the molecular mechanisms implicated in the observed increase in CXCL5 expression in the macrophage fraction of WAT. WAT secretes large amounts of TNFα in obese states, suggesting that this cytokine could regulate the expression of CXCL5. This was shown by the observation that CXCL5 secretion by human WAT explants was increased upon treatment with TNFα for 24h (Figure 7A). TNFα is a key factor regulating CXCL5 level in WAT since we demonstrated that CXL5 mRNA expression is strongly decreased in WAT from obese ob/ob TNFα receptor -/- mice compared to the ob/ob mice (Figure 7B). Furthermore, we showed in WAT explants from mice that TNFα-induced CXCL5 secretion was reduced by cotreatment with the PPARY agonist rosiglitazone, which is known to antagonize the effects of TNFα (Figure 7C). Similar results were observed when THP-1 macrophages, instead of WAT explants were treated with TNFα (Figure 7D). Moreover, increased CXCL5 secretion in response to TNFα correlated to increased CXCL5 mRNA expression in these cells
Rosiglitazone cotreatment of the cells also resulted in a reduction of TNFα pro-secretory (Figure 7D) and mRNA stimulation effects (Figure 7E). This suggested that TNFα regulation of CXCL5 expression is at the transcriptional level. Computational analysis of the human CXCL5 promoter sequence revealed the presence of several NFkB binding sites located upstream of the transcription start site, which could mediate the effects of TNFα. Transient transfection experiments using the CXCL5 promoter driving the expression of the luciferase gene demonstrated a 6.5-fold increase in CXCL5 promoter activity upon stimulation of the transfected cells with TNFα (Figure 7F). Similar to that observed when mRNA levels were analyzed (Figure 7E) the induction of CXCL5 promoter activity by TNFα was reduced by rosiglitazone co-treatment in cells cotransfected with PPARY (Figure 7F). To demonstrate that the regulation of the CXCL5 promoter in response to TNFα is mediated by NFkB, chromatin immunoprecipitation (ChIP) studies were performed using a specific anti-p65 antibody or preimmune serum in THP-1 cells. No amplification of the CXCL5 promoter was observed when non-specific antibodies were used to immunoprecipitate the chromatin (IgG, Figure 7G), whereas a specific fragment corresponding to the NFkB binding site of the CXCL5 promoter was amplified when chromatin of TNFα-treated cells was immunoprecipitated with an anti-NFkB antibody (Figure 7G). Furthermore, rosiglitazone treatment resulted in decreased binding of p65 to this site, which was consistent with the transfection data (Figure 7F). Altogether, these results suggest that TNFα triggers the expression of CXCL5 in WAT through the NFkB pathway.

**Conclusion:**

The most important finding in this study is the insulin sensitizing effects of both anti-CXCL5 neutralizing antibodies, and CXCR2 antagonists. This finding underscores a role of WAT-secreted CXCL5 in the development of insulin resistance in obese subjects, and validates an anti-CXCL5 therapy for the treatment or prevention of obesity-induced insulin resistance.
REFERENCES:


reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity. J Clin Endocrinol Metab 92, 2330-2335.


CLAIMS:

1. A CXCR2 receptor antagonist for the treatment or the prevention of insulin resistance.

2. The CXCR2 receptor antagonist according to claim 1, wherein said CXCR2 receptor antagonist is a small organic molecule.

3. The CXCR2 antagonist according to claim 1 wherein said antagonist is an antibody and or an aptamer directed against CXCR2 or CXCL5.

4. The CXCR2 receptor antagonist according to claim 2, wherein said CXCR2 receptor antagonist has the following formula:

   ![Chemical Structure](image)

   wherein
   
   X is oxygen or sulfur;
   R is any functional moiety having an ionizable hydrogen and a pKa of 10 or less;
   Ri is independently selected from hydrogen; halogen; nitro; cyano; halosubstituted CMO alkyl; C2-10 alkenyl; CMO alkoxy; halosubstituted C1-10 alkoxy; azide; S(O)R; hydroxy; hydroxy C1-4 alkyl; aryl C1-4 alkyl; aryloxy; aryl C1-4 alkoxy; heteroaryl; heteroarylalkyl; heterocyclic; heterocyclic C1-4 alkyl; heteroaryl C1-4 alkyl; aryloxy C2-10 alkenyl; heteroaryl C2-10 alkenyl; heterocyclic C2-10 alkyl; heterocyclic C2-10 alkyl; NR4R6; C2-10 alkenyl; C(O)NR4R6; C(O)NR4R5; C(O)NR4R5; S(O)3H; S(O)3R8; CMO alkoxy C(O)Rn; C2-10 alkenyl C(O)Rn; C2-10 alkenyl C(O)ORn; C(O)Rn; C(O)ORn; C(O)ORi; OC(O)Ri; NR4C(O)Rn; or two Ri moieties together may form O-(CH2)S or or a 5 to 6 membered unsaturated ring;
   t is O or an integer having a value of 1 or 2;
   s is an integer having a value of 1 to 3;
R₄ and R₅ are independently hydrogen, optionally substituted Cᵢ-₄ alkyl, optionally substituted aryl, optionally substituted aryl Cᵢ-₄ alkyl, optionally substituted heteroaryl, optionally substituted heteroaryl Cᵢ-₄ alkyl, heterocyclic, heterocyclic Cᵢ-₄ alkyl, or R₄ and R₅ together with the nitrogen to which they are attached form a 5 to 7 member ring which may optionally comprise an additional heteroatom selected from O/N/S;

Y is independently selected from hydrogen; halogen; nitro; cyano; halosubstituted C₁-₁₀ alkyl; C₀ M₀ alkyl; C₂₋₁₀ alkenyl; halosubstituted C₂₋₁₀ alkenyl; azide; S(O)₄R₄; hydroxy; hydroxy C₁-₄ alkyl; aryl; aryl C₁₋₄ alkyl; aryl C₁₋₄ alkyl; heterocyclic; heterocyclic Cᵢ₋₄ alkyl; aryl C₂₋₁₀ alkenyl; heteroaryl C₂₋₁₀ alkenyl; heteroaryl C₂₋₁₀ alkenyl; N₄R₅; C₂₋₁₀ alkenyl C(O)NR₄R₅; C(O)NR₄R₅; C(O)NR₄Rᵢ₀; S(O)₃H; S(O)₃R₈; C M₀ alkyl C(O)R₈; C₂₋₁₀ alkenyl C(O)R₈; C₂₋₁₀ alkenyl C(O)OR₈; C(O)R₈; C(O)OR₈; C(O)ORᵢ₂; OC(O) R₁₁; NR₄C(O)R₈; or two Rᵢ moieties together may form O-(CH₂)₅O or a 5 to 6 membered unsaturated ring;
n is an integer having a value of 1 to 3;
m is an integer having a value of 1 to 3;
R₅ is hydrogen or Cᵢ₋₄ alkyl;
R₁₀ is C₁₋₁₀ alkyl C(O)₂R₈;
R₁₁ is hydrogen, Cᵢ₋₄ alkyl, optionally substituted aryl, optionally substituted aryl Cᵢ₋₄ alkyl, optionally substituted heteroaryl, optionally substituted heteroaryl Cᵢ₋₄ alkyl, optionally substituted heterocyclic, or optionally substituted heterocyclic Cᵢ₋₄ alkyl;
Rᵢ₂ is hydrogen, Cₐ₀ alkyl, optionally substituted aryl or optionally substituted arylalkyl;
or a pharmaceutically acceptably salt thereof.

5. The CXCR2 receptor antagonist according to claim 4, wherein said CXCR2 receptor antagonist is selected in the group consisting of:

N-(2-Hydroxy-4-nitrophenyl)-N'-(2-methoxyphenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-phenylphenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-methylthiophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2,3-dichlorophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl) N'-(2-chlorophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2,3-methylenedioxyphenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-methoxy-3-chlorophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl)-N'-(2-phenyloxyphenyl)urea,
N-(3-Chloro-2-hydroxyphenyl)-N'-(bromophenyl)urea,
N-(2-Hydroxy-3-glycylmethylenelestercarbonylphenyl)-N'-(2-bromophenyl)urea,
N-(3-Nitro-2-hydroxyphenyl)-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-3,4-dichlorophenyl)-N'-(2-bromophenyl)urea,
N-(3-Cyano-2-hydroxyphenyl)-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2-methoxyphenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2-phenylphenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2,3-dichlorophenyl)urea,
N-(2-Hydroxy-4-cyanophenyl)-N'-(2-methylphenyl)urea,
N-(2-Hydroxy-3-cyano-4-methylphenyl)-N'-(2-bromophenyl)urea,
N-(4-Cyano-2-hydroxyphenyl)-N'-(2-trifluoromethylphenyl)urea,
N-(3-Trifluoromethyl-2-hydroxyphenyl)-N'-(2-bromophenyl)urea,
N-(3-Phenylaminocarbonyl-2-hydroxyphenyl)-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-4-nitrophenyl) N'-(2-iodo phenyl) urea,
N-(2-Hydroxy-4-nitrophenyl) N'(2-bromo phenyl)thiourea,
N-(2-Phenylsulfonamido)-4-cyanophenyl-N'(2-bromo phenyl)urea,
(E)-N-[3-[(2-Aminocarbonyl)ethenyl)]-2-hydroxyphenyl]-N'-(2-bromophenyl)urea,
N-(2-Hydroxy-3,4-dichlorophenyl)-N'-(2-methoxyphenyl)urea,
N-(2-Hydroxy-3,4-dichlorophenyl)-N'-(2-phenylphenyl)urea,
N-(2-Hydroxy-3,4-dichlorophenyl)-N'-(2,3-dichlorophenyl)urea,
N-(2-Hydroxy-5-nitrophenyl)-N'-(2,3-dichlorophenyl)urea; or
N-(2-Hydroxy-3-cyanophenyl)-N'-(2,3-dichlorophenyl)urea.

6. The CXCR2 receptor antagonist according to claim 3, wherein said CXCR2 receptor antagonist is an anti-CXCL5 neutralizing antibody or aptamer.
7. An inhibitor of CXCR2 receptor gene expression for the treatment or the prevention of insulin resistance.

8. The inhibitor of CXCR2 receptor gene expression according to claim 7, wherein said inhibitor is selected from the group consisting of antisense RNA or DNA molecules, small inhibitory RNAs (siRNAs), short hairpin RNA and ribozymes.

9. A pharmaceutical composition for the treatment or the prevention of an insulin resistance which comprises a CXCR2 receptor antagonist as defined in any of claims 1 to 6 or an inhibitor of CXCR2 receptor gene expression as defined in claims 7 and 8.

10. The CXCR2 receptor antagonist according to any one claims 1 to 6, the inhibitor of CXCR2 receptor gene expression according to claims 7 to 8 or the pharmaceutical composition according to claim 9 for the treatment or the prevention of obesity-induced insulin resistance.

11. A method of treating or preventing of insulin resistance in a subject in need thereof comprising a step of administrating to said subject an therapeutically effective amount of an inhibitor of CXCR2 receptor antagonist as defined in any of claims 1 to 6, or a CXCR2 receptor gene expression according to claims 7 to 8.

12. A method for diagnosing and/or prognosing an insulin resistance in a subject comprising detecting the presence and/or quantifying CXCL5 in a biological sample obtained from said subject.

13. The method according to claim 12, wherein the detection of the presence and/or the quantification of CXCL5 is carried out by immunological detection.

14. The method according to claims 12 or 13, wherein the biological sample obtained from said subject is a serum sample or a plasma sample.
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Figure 8
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K31/17
ADD. A61P5/48 A61P5/50

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

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

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

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

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>WO 2006/052722 A (SMITHKLINE BEECHAM CORP [US]; EVANS KAREN [US]; CICHY-KNIGHT MARIA [US] 18 May 2006 (2006-05-18) page 39, last par.; compounds 165, 166; 167-204; 206-238; 263; 317-325; 361-381; 388; 394; 475-483; 503-507; 510; 515; 521-530;</td>
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</tr>
<tr>
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<td>WO 97/29743 A (SMITHKLINE BEECHAM CORP [US]; WIDDOWSON KATHERINE LOUISA [US]; VEGER B) 21 August 1997 (1997-08-21) the whole document</td>
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| X        | See patent family annex |

**Date of the actual completion of the international search**

9 November 2009

**Date of mailing of the international search report**

18/11/2009

**Name and mailing address of the ISA/Authority**

European Patent Office  P B 5818 Patentlaan 2 NL-2280 HV Rijswijk
Tel (+31-70) 340-2040,  Fax (+31-70) 340-3016

**Authorized officer**

Hörtner, Michael
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<th>Relevant to claim No</th>
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<tr>
<td>Y</td>
<td>wo 2005/023238 A (POSEIDON PHARMACEUTICALS AS [DK]; OLESEN SOEREN PETER [DK] ; GRUNNET) 17 March 2005 (2005-03-17) page 4, line 14-31 ; page 6, line 23 - page 7, line 6; page 8, line 11 - page 9, line 5; page 10-12, compounds ; page 16, line 15 - page 21;</td>
<td>1,2,4,5 , 9-11</td>
</tr>
<tr>
<td>Y</td>
<td>EP 1 676 573 A (ESTEVE LABOR DR [ES]) 5 July 2006 (2006-07-05) paragraph [0039] ; compounds NS-1608</td>
<td>1,2, 4, 5, 9-11</td>
</tr>
<tr>
<td>A</td>
<td>wo 2004/058683 A (MITOKOR INC [US] ; GHOSH SOUMITRA S [US] ; PEI YAZH0N6 [US] ; TANG XIAO-Q) 15 July 2004 (2004-07-15) page 9, line 13 ; page 10, line 5 - page 11, line 7; page 15, formul a (V) ; page 16, formul a (VI );</td>
<td>1-14</td>
</tr>
<tr>
<td>A</td>
<td>EP 1 088 819 A (PFIZER PROD INC [US]) 4 April l 2001 (2001-04-04) page 18, scheme B2 ; formul a B-10, B-II ; page 19, scheme B3, formul a B-14, B15 ; par . 145 ; ex. 10-1</td>
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<td>T</td>
<td>CHAVEY C ET AL: &quot;CXC Ligand 5 Is an Adipose-Tissue Derived Factor that Links Obesity to Insulin Resistance&quot; CELL METABOLISM, CELL PRESS, CAMBRIDGE, MA, US, vol . 9 , no . 4 , 8 April l 2009 (2009-04-08) , pages 339-349 , XP007909843 ISSN : 1550-4131 the whole document</td>
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<tr>
<td>wo 2005019220</td>
<td>03-03-2005</td>
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</tr>
<tr>
<td>wo 2006052722</td>
<td>18-05-2006</td>
<td>AU 2005304962 Al</td>
<td>18-05-2006</td>
</tr>
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<td></td>
<td>BR PI0517567 A</td>
<td>17-06-2006</td>
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<td></td>
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<td>02-01-2008</td>
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<tr>
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<td>EP 1812383 Al</td>
<td>01-08-2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2008519761 T</td>
<td>12-06-2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 20070086044 A</td>
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<tr>
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<td></td>
<td>SG 155229 Al</td>
<td>30-09-2009</td>
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<td></td>
<td></td>
<td>US 2007249670 Al</td>
<td>25-10-2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 200703713 A</td>
<td>29-10-2008</td>
</tr>
<tr>
<td>wo 9729743</td>
<td>21-08-1997</td>
<td>AU 725456 B2</td>
<td>12-10-2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 6900796 A</td>
<td>02-09-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BG 102690 A</td>
<td>30-04-1999</td>
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<td>BR 9612779 A</td>
<td>24-10-2000</td>
</tr>
<tr>
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<td>CA 2245927 Al</td>
<td>21-08-1997</td>
</tr>
<tr>
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<td>CN 1215990 A</td>
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</tr>
<tr>
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<td>CN 1539816 A</td>
<td>27-10-2004</td>
</tr>
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<td></td>
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<td>EP 0809492 Al</td>
<td>03-12-1997</td>
</tr>
<tr>
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<td>EP 0896531 Al</td>
<td>17-02-1999</td>
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</tr>
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