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Treating NIDDM with RXR agonists

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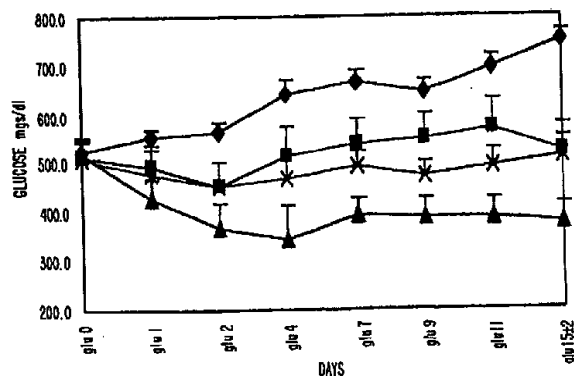
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(54) Title: TREATING NIDDM WITH RXR AGONISTS

(57) Abstract

This invention relates to methods and compositions for the treatment of non-insulin-dependent diabetes mellitus using an RXR agonist alone or in combination with a PPARγ agonist such as a thiazolidinedione compound.

TREATING NIDDM WITH RXR AGONISTS

FIELD OF THE INVENTION

This invention relates to methods and pharmaceutical

5   compounds for treating diabetes and related symptoms.

BACKGROUND OF THE INVENTION

Non-insulin-dependent diabetes mellitus (NIDDM, type
II diabetes) is characterized by abnormalities in insulin

10   secretion and insulin action. NIDDM constitutes 90-95% of the
approximately 6 million diagnosed diabetics in the United
States. NIDDM is characterized by hyperglycemia, the result
of insulin resistance in peripheral tissues (skeletal muscle
and adipose tissue), where insulin-stimulated

15   uptake/utilization of glucose is blunted, and in liver, where
insulin suppression of glucose output is insufficient. These
impairments in insulin action play an important role in the
development of elevated fasting blood glucose and glucose
intolerance.

20   Diet and exercise are first-line therapy for NIDDM
patients. NIDDM patients also take oral hypoglycemic drugs to
control blood glucose levels. The most widely used
hypoglycemic agents are various formulations of insulin and
sulfonylureas. A major drawback with these therapies is the

25   occurrence of potentially life-threatening hypoglycemia due to
hyperinsulinemia.

The hyperinsulinemia that can occur with these
therapies is also associated with an elevated risk of
cardiovascular disease, a major killer of diabetics.

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Therefore, a need exists for antidiabetic drugs which do not increase circulating insulin concentrations.

A new class of compounds, thiazolidinediones, have been documented to effect antihyperglycemic activity by

5 increasing insulin action rather than by promoting insulin secretion. Thiazolidinediones ameliorate insulin resistance and normalize plasma glucose and insulin (where elevated) without causing a hypoglycemic state, even at very high doses. The thiazolidinedione insulin sensitizers, e.g., ciglitazone,

10 englitazone, pioglitazone, BRL 49653 (5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]methyl]-2,4-thiazolidinedione), and troglitazone, enhance insulin-mediated suppression of hepatic glucose output and insulin-stimulated glucose uptake and utilization by adipose tissue. Thiazolidinediones also

15 change glucose transporter (e.g. Glut 4) expression to contribute to increased insulin responsiveness.

SUMMARY OF THE INVENTION

Applicant has found that RXR agonists mimic or

20 enhance the antidiabetic effects of thiazolidinedione compounds. RXR agonists activate the transcriptional activity of RXR/PPARγ heterodimers, increase insulin stimulated glucose uptake, lower the level of triglyceride, suppress the level of insulin, and increase the level of HDL cholesterol. Two RXR

25 agonists have been shown to lower glucose, triglycerides and insulin levels in two established animal models of NIDDM, i.e. the ob/ob and db/db mice. Therefore, RXR agonists may be used as insulin sensitizers or insulin mimetics in the treatment of NIDDM and related symptoms.

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In addition, the combination of an RXR agonist and a PPARγ agonist, such as a thiazolidinedione, achieves synergistic activation of the RXR/PPARγ heterodimers so as to enhance adipogenic and antidiabetic effects of PPARγ. In

5   db/db mice, the combination of an RXR agonist and a PPARγ agonist was shown to lower the level of glucose more than individual compounds did.

        Therefore, the present invention relates to methods and compositions for treating a host having NIDDM or insulin

10 resistant diabetes by administering to the host a composition containing a pharmaceutically effective amount of an activator of the RXR/PPARγ heterodimer, including, but not limited to, an RXR agonist. The host may be a human patient or an animal model of human NIDDM. The compositions of this invention are

15 adapted to cure, improve or prevent one or more symptoms of NIDDM in the host. A preferred drug is highly potent and selective with low toxicity. In this regard, those skilled in the art will recognize NIDDM as an example of a metabolic disease that can be treated with the RXR agonist-containing

20 compounds and compositions of the present invention. Other examples of metabolic diseases treatable with the compounds and compositions of the present invention include, but are not limited to, obesity and thyroid hormone abnormalties.

        By "pharmaceutically effective amount" is meant an

25 amount of a pharmaceutical compound or composition having a therapeutically relevant effect on NIDDM. A therapeutically relevant effect relieves to some extent one or more symptoms of NIDDM in a patient or returns to normal either partially or completely one or more physiological or biochemical parameters

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associated with or causative of NIDDM, e.g. increasing the
sensitivity of cellular response to circulating insulin,
curing, reducing, or preventing one or more clinical symptoms
of NIDDM, including, but not limited to, hyperglycemia,
5   hyperinsulinemia and hypertriglyceridemia. In a preferred
embodiment, a pharmaceutically effective amount of a compound
or composition means an amount that increases the uptake of
glucose by adipose tissue or muscle tissue. In another
preferred embodiment, a pharmaceutically effective amount of a
10   compound or composition means an amount that increases the
uptake of triglyceride by adipose tissue.

By "activator of the RXR/PPARγ heterodimer" is meant
a compound or composition which when combined with the
RXR/PPARγ heterodimer increases the transcriptional regulation
15   activity of the heterodimer, as measured by an assay known to
one skilled in the art, including, but not limited to, the
"co-transfection" or "cis-trans" assays described or disclosed
in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429,
5,506,102, WO89/05355, WO91/06677, WO92/05447, WO93/11235,
20   WO95/18380, PCT/US93/04399, PCT/US94/03795 and CA 2,034,220,
which are incorporated by reference herein. It includes, but
is not limited to, compounds that bind RXR, PPARγ, or both.

By "RXR agonist" is meant a compound or composition
which when combined with RXR homodimers or heterodimers
25   increases the transcriptional regulation activity of RXR, as
measured by an assay known to one skilled in the art,
including, but not limited to, the "co-transfection" or "cis-
trans" assays described or disclosed in U.S. Patent Nos.
4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355,

WO91/06677, WO92/05447, WO93/11235, WO95/18380, PCT/US93/04399, PCT/US94/03795 and CA 2,034,220, which are incorporated by reference herein. It includes, but is not limited to, compounds that preferentially activate RXR over RAR (i.e. RXR specific agonists), and compounds that activate both RXR and RAR (i.e. pan agonists). It also includes compounds that activate RXR in a certain cellular context but not others (i.e. partial agonists). Compounds disclosed or described in the following articles, patents and patent applications which have RXR agonist activity are incorporated by reference herein: U.S. Patents 5,399,586 and 5,466,861, WO96/05165, PCT/US95/16842, PCT/US95/16695, PCT/US93/10094, WO94/15901, PCT/US92/11214, WO93/11755, PCT/US93/10166, PCT/US93/10204, WO94/15902, PCT/US93/03944, WO93/21146, provisional applications 60,004,897 and 60,009,884, Boehm et al., J. Med. Chem. 38(16):3146-3155, 1994, Boehm, et al., J. Med. Chem. 37(18):2930-2941, 1994, Antras et al., J. Biol. Chem. 266:1157-1161 (1991), Salazar-Olivo et al., Biochem. Biophys. Res. Commun. 204:157-263 (1994) and Safanova, Mol. Cell. Endocrin. 104:201-211 (1994). RXR specific agonists include, but are not limited to, LG 100268 (i.e. 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-cyclopropyl]-pyridine-5-carboxylic acid) and LGD 1069 (i.e. 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethen-1-yl]-benzoic acid), and analogs, derivatives and pharmaceutically acceptable salts thereof. The structures and syntheses of LG 100268 and LGD 1069 are disclosed in Boehm, et al. J. Med. Chem. 38(16):3146-3155, 1994, incorporated by reference herein. Pan agonists include, but are not limited

to, ALRT 1057 (i.e. 9-cis retinoic acid), and analogs,
derivatives and pharmaceutically acceptable salts thereof.

In a preferred embodiment, the pharmaceutical
composition also contains a pharmaceutically effective amount
5   of a PPARγ agonist. Alternatively, a second composition
containing a pharmaceutically effective amount of a PPARγ
agonist is administered to the host separately. In a further
preferred embodiment, a compound having agonist activity for
both RXR and PPARγ is used.

10   By "PPARγ agonist" is meant a compound or
composition which when combined with PPARγ increases a
reaction typical for the receptor, e.g., transcriptional
regulation activity, as measured by an assay known to one
skilled in the art, including, but not limited to, the "co-
15   transfection" or "cis-trans" assays described or disclosed in
U.S. Patent Nos. 4,981,784 and 5,071,773 and Lehmann, et al.,
J. Biol. Chem. 270:12953-12956 (1995), which are incorporated
by reference herein. A preferred PPARγ agonist is a
thiazolidinedione compound, including, but not limited to, BRL
20   49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744,
englitazone, AD 5075, darglitazone, and analogs, derivatives
and pharmaceutically acceptable salts thereof. Compounds
disclosed in Tontonez et al., Genes & Develop. 8:1224-1234
(1994), Tontonez et al., Cell 79:1147-1156 (1994), Lehmann et
25   al., J. Biol. Chem. 270(22):1-4, 1995, Amri et al., J. Lipid
Res. 32:1449-1456 (1991), Amri et al., J. Lipid Res. 32:1457-
1463, (1991) and Grimaldi et al., Proc. Natl. Acad. Sci. USA
89:10930-10934 (1992) are incorporated by reference herein.

In a further preferred embodiment, the pharmaceutical composition also contains a pharmaceutically effective amount of insulin, insulin derivative. insulin secretagogue, insulin sensitizer. or insulin mimetic. Alternatively, a composition containing a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer, or insulin mimetic is administered to the host separately.

Accordingly there is disclosed herein a method for treating a host having NIDDM, the method comprising the step of administering to said host (i) a composition comprising a pharmaceutically effective amount of an RXR agonist or (ii) a composition comprising

(a)   a pharmaceutically effective amount of an RXR agonist; and

(b)   a pharmaceutically acceptable carrier

and a method for increasing glucose uptake in adipose or muscle tissue. comprising the step of administering to said tissue a composition comprising a pharmaceutically effective amount of an RXR agonist.

A composition containing a pharmaceutically effective amount of an active ingredient may be administered orally or systemically to a host. In a preferred embodiment, it is administered orally.

In another aspect, this invention features a pharmaceutical composition when used for treating NIDDM containing a pharmaceutically effective amount of an RXR agonist: and a pharmaceutically acceptable carrier adapted for a host having NIDDM. In a preferred embodiment. the pharmaceutical composition also includes a pharmaceutically effective amount of insulin. insulin derivative, insulin secretagogue, insulin sensitizer, insulin mimetic or PPARγ agonist.

In a preferred embodiment, the composition is held within a container which includes a label stating to the effect that the composition is approved by the FDA in the United States (or an equivalent regulatory agency in a foreign country) for treating NIDDM or for treating hyperglycemia. hyperinsulinemia or hypertriglyceridemia. Such a container provides a therapeutically effective amount of the active ingredient to be administered to a host.

In another aspect. the invention features a method for increasing glucose uptake in adipose or muscle tissue, comprising the step of administering to said tissue a composition comprising a pharmaceutically effective amount of an RXR agonist. In a preferred embodiment. the method further comprises the step of administering to the tissue a composition comprising a pharmaceutically effective amount of a PPARγ agonist. Alternatively the composition administered to the tissue may comprise both an RXR agonist and a PPARγ agonist.

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In another aspect, this invention features methods for screening for candidate compounds useful for treating NIDDM. These methods select compounds or compositions which when combined with the RXR/PPARγ heterodimer increase the

5   transcriptional regulation activity of the heterodimer, as measured by an assay known to one skilled in the art, including, but not limited to, the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355,

10   WO91/06677, WO92/05447, WO93/11235, WO95/18380, PCT/US93/04399, PCT/US94/03795 and CA 2,034,220, which are incorporated by reference herein. In one example, a candidate compound such as a potential RXR agonist is administered to an adipocyte or a preadipocyte. The level of lipid in the cell

15   is measured, and an increased accumulation of lipid after the treatment with the candidate compound indicates that the candidate compound is useful for treating NIDDM. In preferred embodiments, the level of lipid is measured by oil red O staining or detecting the level of triglyceride in the cell.

20   In another example, a candidate compound such as a potential RXR agonist is administered to an adipocyte or a preadipocyte and the transcription level of a adipocyte specific gene (e.g. lipoprotein lipase gene or PPARγ gene) is measured. An increased transcription of the adipocyte

25   specific gene after the treatment with the candidate compound indicates that the candidate compound is useful for treating NIDDM.

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In yet another example, a candidate compound such as
a potential RXR agonist is administered to an adipocyte or a
preadipocyte and the level of glucose uptake is measured. An
increased glucose uptake after the treatment with the
5   candidate compound indicates that the candidate compound is
useful for treating NIDDM. Alternatively, both the candidate
compound and insulin are administered to the cell and the
level of glucose uptake is compared to that in the same cell
treated with insulin alone. A higher level of glucose uptake
10   in the cell treated by the candidate compound and insulin
indicates that the candidate compound is an insulin sensitizer
and useful for treating NIDDM.

Other features and advantages of the invention will
be apparent from the following detailed description of the
15   invention, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a is a graph showing the extent of adipocyte
differentiation in 3T3-LI cells as measured by the levels of
20   triglyceride in 3T3-LI preadipocytes treated with various
combinations of an RXR agonist (LG 100268), a PPARγ agonist
(thiazolidinedione compound BRL 49653), and insulin. The
retinoid and BRL 49653 were used at 1 μM.

Figure 1b is a graph showing the extent of adipocyte
25   differentiation in 3T3-LI cells as measured by the level of
triglyceride in 3T3-LI preadipocytes treated with various
combinations of an RXR agonist (LGD 1069), a PPARγ agonist
(thiazolidinedione compound BRL 49653), and insulin. The
retinoid and BRL 49653 were used at 1 μM.

Figure 2a is a graph showing the level of LPL mRNA
in 3T3-LI cells treated with various combinations of an RXR
agonist (LG 100268), a PPARγ agonist (thiazolidinedione
compound BRL 49653), and insulin. The retinoid and BRL 49653

5   were used at 1 μM.

Figure 2b is a graph showing the level of PPARγ mRNA
in 3T3-LI cells treated with various combinations of an RXR
agonist (LG 100268), a PPARγ agonist (thiazolidinedione
compound BRL 49653), and insulin. The retinoid and BRL 49653

10   were used at 1 μM.

Figure 3 is a graph showing the level of glucose in
db/db mice treated with LG 100268, BRL 49653, and a
combination of LG 100268 and BRL 49653, respectively.

Figure 4 is a graph showing the level of

15   triglycerides in db/db mice treated with LG 100268, BRL 49653,
and a combination of LG 100268 and BRL 49653, respectively.

Figure 5 is a graph showing the level of HDL
cholesterol in db/db mice treated with LG 100268, BRL 49653,
and a combination of LG 100268 and BRL 49653, respectively.

20   Figure 6 is a graph showing the level of
triglycerides in ob/ob mice treated with LGD 1069, LG 100268,
and BRL 49653, respectively.

Figure 7 is a graph showing the level of glucose in
ob/ob mice treated with LGD 1069, LG 100268, and BRL 49653,

25   respectively.

Figure 8 is a graph showing the level of insulin in
ob/ob mice treated with LGD 1069, LG 100268, and BRL 49653,
respectively.

DETAILED DESCRIPTION OF THE INVENTION

TZD achieves antidiabetic and adipogenic effects through PPARγ

Thiazolidinediones are insulin sensitizers that significantly reduce glucose and lipid levels in animal models of NIDDM and obesity (Kees, et al., _J. Medicinal Chem._ 38(4):617-628, 1995; Willson, et al., _J. Medicinal Chem._ 39(3):665-668, 1996; Young, et al. _Diabetes_ 44:1087-1092, 1995). Thiazolidinediones improve glucose utilization without stimulating insulin release.

For example, repeated administration of BRL 49653 to obese mice improves glycemic control by increasing insulin responsiveness of target tissues. BRL 49653 potentiates insulin-stimulated glucose transport in adipocytes from insulin-resistant obese mice, both by increasing insulin receptor number and by facilitating translocation of GLUT4, from an expanded intracellular pool, to the cell surface.

Thiazolidinediones are also selective PPARγ agonists (Lehmann, et al. _J. Biol. Chem._ 270(22):1-4, 1995). Comparison of the EC_{50} for activation of PPARγ with the minimum effective dose (MED) for anti-hyperglycemic activity revealed a significant correlation. The correlation between _in vitro_ PPARγ activity and _in vivo_ antihyperglycemic activity of thiazolidinediones implicates PPARγ as the molecular target for the antidiabetic effects of thiazolidinediones.

PPARγ is a member of the nuclear receptor superfamily of ligand-activated transcription factors. It is expressed in an adipose-specific manner and its expression is induced early during the differentiation of several

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preadipocyte cell lines. Forced expression of PPARγ in
fibroblasts resulted in adipocyte differentiation.

In addition to insulin sensitizing activity,
thiazolidinediones have marked adipogenic effects on
5   preadipocyte and mesenchymal stem cells (Tontonoz, et al.,
Cell 79:1147-1156, 1994). Treatment of C3H/10T1/2 cells with
BRL 49653 resulted in efficient adipocyte differentiation,
showing that ligand-mediated activation of PPARγ is sufficient
to initiate the adipogenic signaling cascade in a mesenchymal
10   stem cell line. PPARγ is the molecular target for the
adipogenic effects of thiazolidinediones.

Adipogenesis plays a role in the development of
NIDDM, which is characterized by not only unbalanced glucose
homeostasis, but also elevated levels of circulating lipids.
15   Increases in lipid levels have been shown to interfere with
glucose disposal.

Adipocytes are highly specialized cells that play a
critical role in lipid metabolism and energy homeostasis.
Their primary role is to store triglycerides in times of
20   caloric excess and to mobilize this reserve during periods of
nutritional deprivation.

Adipocyte differentiation is characterized by a
coordinate increase in adipocyte-specific gene expression.
PPARγ is specifically expressed in adipocytes. Its expression
25   is induced early during the course of differentiation of
several preadipocyte cell lines. Forced expression of PPARγ
in fibroblasts resulted in adipocyte differentiation.

Synergistic effects of RXR and PPARγ on adipogenesis

Expression of PPARγ is induced early during the differentiation of cultured adipocyte cell lines and is expressed at very high levels specifically in adipose tissue. PPARγ regulates adipogenesis by modulating the transcription of other adipocyte-specific genes, e.g. adipocyte P2 gene (aP2 gene). aP2 gene encodes an intracellular lipid-binding protein and is expressed exclusively in adipose cells.

A 518-bp DNA fragment from the 5'-flanking region of the aP2 gene has been identified as an enhancer that directs high-level adipocyte-specific gene expression in both cultured cells and transgenic mice. A pair of elements in the aP2 enhancer, ARE6 and ARE7, bind a nuclear factor termed ARF6 that is detected only in nuclear extracts derived from adipocytes. The ARF6-binding sites are both necessary and sufficient for adipocyte-specific expression, suggesting that the trans-acting factor ARF6 functions as a differentiation-dependent and tissue-specific switch for the aP2 enhancer (Tontonoz, et al. Genes & Development, 8:1224-1234, 1994).

The ARF6 recognition sequence resembles a type of nuclear hormone receptor-binding site known as DR-1 (direct repeat with 1-nucleotide spacer). This motif has been shown to preferentially bind heterodimers of RXR and COUP-TF and heterodimers of RXR and the PPARs. DNA mobility retardation experiments using various HRE sequences as competitor demonstrated that ARF6 preferentially recognizes DR-1 sites.

ARF6 has been identified as a heterodimeric complex of RXRα and PPARγ. It has been shown that PPARγ and RXRα form heterodimers on ARF6-binding sites in vitro. Forced

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expression of these factors in transient transfections is
sufficient to activate the adipocyte-specific aP2 enhancer in
nonadipose cells such as fibroblasts. This activation is
potentiated by peroxisome proliferators, fatty acids, and 9-
5   *cis* retinoic acid. Antiserum to RXRα specifically inhibits
ARF6 activity in adipocyte nuclear extracts.

Cotransfection of the RXRα expression vector and the
PPARγ expression vector has a synergistic effect to activate
the aP2 enhancer in nonadipose cells. Maximal activation of
10   the aP2 enhancer is observed when both PPARγ, RXRα and their
agonists are present.

Without being bound by any theory, applicant
proposes that an RXR agonist affects glucose usage in tissues
through the synergistic effects of RXR and PPARγ heterodimers.
15   The RXR/PPARγ heterodimers, when activated by an RXR agonist
or a combination of an RXR agonist and a PPARγ agonist, induce
adipogenesis and modulates the levels of glucose and
triglyceride uptake. Alternatively or in addition, the
RXR/PPARγ heterodimers, when activated by an RXR agonist or a
20   combination of an RXR agonist and a PPARγ agonist, regulate
signaling molecules secreted by adipose tissue such as tumor
necrosis factor-α or leptin, which in turn modulates glucose
metabolism in other tissues.

25   Using RXR agonists to mimic or enhance the antidiabetic
effects of thiazolidinediones

PPARα, β and γ all form heterodimers with RXRs.
These RXR/PPAR heterodimers bind to DNA and regulate
transcription activity. RXR activators cooperate with PPARα

activators to activate the activity of PPARα protein (Kliewer,
et al. Nature 358:771-774 (1992) and Mukherjee, et al.
Steroid Biochem. Molec. Biol. 51:157-166 (1994)).

In this invention, a similar synergistic activation
5   was observed with a PPARγ activator and several RXR
activators.

According to this invention, RXR agonists, e.g. LGD
1069, ALRT 1057 and LG 100268, may be utilized in the
treatment of diabetes. We examined four independent
10 parameters for the effects of RXR agonists, i.e. morphological
changes, lipid accumulation, regulation of gene expression and
increased glucose uptake.

Two pre-adipocyte cell lines were used to test the
theory of RXR activation in the PPARγ/RXR heterodimer. 3T3-LI
15 and C3H/10T1/2 cells were obtained from ATCC and are derived
from mouse embryo. They are contact inhibited and can be
induced to differentiate into adipocyte cells containing large
lipid droplets within the cytoplasm. Adipocyte
differentiation can be observed by oil red O staining which
20 stains the lipid droplets within the cytoplasm red. The
extent of adipocyte differentiation can then be monitored by
microscope observation.

For a more quantitative assay and rapid screening of
compounds, a 96-well plate assay was developed to quantitate
25 the amount of triglyceride produced by the differentiating
adipocytes. In this assay, cells are grown as a monolayer to
confluence on a 96-well plate and treated with BRL 49653,
insulin, and retinoids alone or in various combinations.
These treatments induce differentiation to different extent in

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both 3T3-LI and C3H/10T1/2 cells. The level of triglyceride
accumulation can then be measured via an enzymatic color
reaction which can be read in a plate reader.

A third measure of adipocyte differentiation is to
5   examine regulation of gene expression. The mRNA expression
levels of both PPARγ and lipoprotein lipase (LPL) have been
shown to be modulated during adipocyte differentiation.
Northern blot analysis was used to dissect the molecular
aspects of how retinoids effect target genes of
10   differentiating adipocytes. PPARγ, lipoprotein lipase (LPL),
and β-actin (loading control) mRNA levels were monitored after
cells were treated with thiazolidinediones and retinoids.

A fourth indicator for the utility of a compound in
treating NIDDM or insulin-resistant diabetes is the compound's
15   ability to enhance insulin-stimulated glucose uptake. Labeled
2-deoxyglucose (2-DOG, a glucose analog) assay was performed
with a preadipocyte cell line in the presence of insulin and a
candidate compound to measure the level of 2-DOG
incorporation.

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A)   Retinoid Modulation of Lipid Accumulation in 3T3-LI
Cells Stained with Oil Red O

Table 1 shows the percent of 3T3-LI cells that had
differentiated into adipocytes as observed by oil red O
25   staining assay. BRL 49653 and LG 100268 were used at 1 μM,
insulin was used at 0.01 mg/ml. Wells treated with LG 100268
had bigger redder lipid droplets within the cytoplasm.

BRL 49653 and LG 100268 treatment alone induced 50%
of the cells to differentiate into adipocytes. This was
increased dramatically with the addition of insulin. Insulin
in combination with BRL induced 80% of the 3T3-LI cells into
5   adipocytes while the combination of insulin and LG 100268
induced 90% of the cells to differentiate. When BRL 49653 was
used in combination with LG 100268, an RXR agonist, the amount
of adipocytes differentiation was also increased dramatically.
Other RXR agonists mimic the activity of LG 100268. For
10   example, the addition of ALRT 1057 (pan agonist) or LGD 1069
(RXR specific agonist) in combination with BRL 49653 increased
the amount of differentiation, albeit to a lesser extent than
the strong RXR agonist LG 100268. The combination of insulin
with BRL 49653 and LGD 1069 had a strong differentiating
15   effect (95%) on the 3T3-LI cells.

        B) Retinoid Modulation of Triglyceride Content in
        Differentiated Adipocytes
        The retinoid modulation of lipid formation was
20   quantitated by monitoring triglyceride formation. Figures 1a
and 1b show triglyceride accumulation in 3T3-LI cells treated
with a retinoid (LG 100268 or LGD 1069) alone or in
combination with a thiazolidinedione and insulin. Retinoids
and BRL 49653 were used at 1 μM, insulin was used at 0.01
25   mg/ml for all experimental combinations.
        Insulin, BRL 49653 and retinoids all induced some
triglyceride accumulation when used alone, with LG 100268
giving the largest response. The addition of retinoids (LGD
1069, LG 100268) with the thiazolidinedione (BRL 49653) to the

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assay increased the amount of triglyceride accumulation in
3T3-LI differentiating adipocytes. This was also observed
when BRL 49653 or LG 100268 was used in combination with
insulin. The largest accumulation of triglyceride was seen in
5   the cells treated with LG 100268, BRL 49653 and insulin
together. Similar results were observed when LGD 1069
replaced LG 100268 in the study. These results concur with
those obtained in the oil red O staining assays.

10   C) PPARy and LPL mRNA modulation in differentiating 3T3-
        LI cells

        Adipocyte specific genes were monitored via northern
blot analysis. Figure 2a and 2b show the expression pattern
of LPL (lipoprotein lipase) mRNA and PPARy mRNA in cells that
15   were treated for 7 days with BRL 49653 (1 μM), LG 100268 (1
μM) and insulin (0.01 mg/ml), alone or in combination.

        Northern blot analysis shows an increase in the
relative signal normalized to β-actin of both LPL and PPARy
mRNA expression in cells treated with either compound alone.
20   There was a three to five fold increase in mRNA levels for
these adipocyte target genes demonstrating that
transcriptional regulation occurs with treatment by insulin,
BRL 49653 and LG 100268. Combination of insulin, BRL 49653
and LG 100268 did not further enhance the mRNA level.

25   These data demonstrate that, in 3T3-LI cells, RXR
agonists induce adipocyte differentiation by themselves or in
combination with thiazolidinediones or insulin. RXR agonists
enhance the activity of thiazolidinediones and insulin. Three
independent measurements support that RXR agonists contribute

to the modulation of the RXR/PPARγ heterodimer in regulating
adipocyte differentiation and useful in treating NIDDM.

    D) LG 100268 enhances insulin stimulated glucose
5   uptake in 3T3-L1 cells

        A murine preadipocyte cell line, 3T3-L1, is widely
used to study glucose uptake, adipogenesis, and has been used
in the characterization of thiazolidinediones and other PPARγ
activators. Insulin stimulated uptake of labeled 2-
10   deoxyglucose (2-DOG, a glucose analog) was observed in 3T3-L1
cells treated with BRL 49653 or LG 100268.

        3T3-L1 cells were treated with BRL 49653 (10 μM) or
LG 100268 (1 μM) for 10 days. Insulin was added to the cells
at a concentration of 0.01 mg/ml for 5 days, thereafter no
15   insulin was added. Labeled 2-deoxyglucose uptake assay was
performed (Szalkowski, et al., J. Endocrin. 136:1474-1481,
1995). The level of incorporated labeled 2-DOG was normalized
to the amount of total cellular protein. About 5-fold
increase in 2-DOG uptake was observed in 3T3-L1 cells treated
20   with BRL 49653 alone. About 2-fold increase in 2-DOG uptake
was observed in 3T3-L1 cells treated with LG 100268 alone.

        This experiment shows that an RXR agonist increases
insulin mediated glucose uptake in 3T3-L1 cells like a known
insulin sensitizer, a thiazolidinedione. It demonstrates
25   directly that RXR agonists can be used to treat a major
symptom of NIDDM, i.e. insulin resistance. Other RXR agonists
useful for the treatment of NIDDM can be confirmed using this
assay.

E) __Retinoid Modulation of Lipid Accumulation in__
__C3H/10T1/2 Cells Stained with Oil Red O__

To further assess the retinoid regulation of
adipocyte function we examined C3H/10T1/2 cells, which are
mouse embryo fibroblast/multipotent stem cells that can be
induced to differentiate into adipocytes or muscle cells.
Table 2 shows the percent of C3H/10T1/2 cells that have
differentiated into adipocytes as observed by oil red O
staining assay. Experiment A was conducted in the presence of
BRL 49653. Experiment B was conducted in the presence of both
BRL 49653 and insulin. ALRT 1057 and LGD 1069 were used at 1
µM and TTNPB (RAR selective compound) was used at 10 nM
concentration. Insulin was used at 0.01 mg/ml; and BRL 49653
was used at 0.1, 1 and 10 µM. C3H/10T1/2 cells were treated
for 7 days, cells were stained with oil red O and observed
under the microscope.

Morphological changes were observed when the cells
were treated with retinoids alone, even though the cells did
not fully differentiate into adipocytes. BRL 49653 alone
caused no more than 10 percent of the cells to undergo
adipocyte differentiation. However, when BRL 49653 was used
in combination with an RXR agonist, LGD 1069 or ALRT 1057,
there was a large increase in the amount of differentiation,
the same effect was seen when BRL 49653 was used in
combination with insulin.

The RAR agonist, TTNPB (which does not activate
RXR), did not have the same effect as RXR agonist LGD 1069 and
ALRT 1057. In fact, TTNPB inhibited the differentiation
induced by BRL 49653 or BRL/insulin.

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The above experiments show that a thiazolidinedione (BRL 49653) alone induces a minimal amount of differentiation in C3H/10T1/2 cells. However, when BRL 49653 is used in combination with a retinoid such as LG 100268, LGD 1069, or

5   ALRT 1057, which are RXR agonists, differentiation is dramatically increased. This is not seen with the pure RAR agonist, TTNPB. These data support that the PPARγ/RXR heterodimers, which drive the adipocyte differentiation process, can be activated and enhanced by the binding of an

10 RXR agonist. RXR agonists are useful for modulating the levels of glucose and triglyceride uptake.

Using RXR agonists to lower levels of glucose and triglycerides in animal models of NIDDM

15   (A) *In vivo experiment with db/db mice*
        Animals: Strain Diabetic C57BLKS/J - *m* +/+*db*, 82 mice
        Source: Jackson Lab
        Stock number 000642
        Genotype *m* +/+*db* x*m* +/+*db*

20   DOB 6/19/96±3d, DOA 7/23/96 - 34d old
        Date of study: 8/5/96 - 8/21/96, 44-63d old
        Mice were identified by an ear punch code (#1-82) and separated into 8 groups (A-H). Each group consisted of 10 mice separated into 4 cages with 2-3 mice/cage. Several mice

25 were lost during the course of the study from fluid injections into the lungs resulting in 2 groups with 9 mice/group. Control group C consisted of 12 mice. The mice were fed pelleted Purina Lab chow #5015 containing 3.83kcal/g with a caloric composition of 56% carbohydrate, 26% fat and 18%

30 protein. Food and water were provided ad libitum. Food

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intake/cage was measured over selected periods and expressed as g food consumed/100g mouse/day.

On days of study, food was removed from the cages at selected intervals of time between 6:15 and 7:00 AM. Animal weights were recorded 2h after start of fast and blood samples taken after a 3h fast. Blood was drawn from a cut at the tip of the tail and collected into a heparinized capillary tube (approx. 75μl volume). After centrifugation, the hematocrit was read in a microcapillary reader, recorded and the tube broken for recovery of plasma for analysis of glucose, triglyceride and insulin concentration.

Blood samples were collected on days 0.$_1$, 0, 3, 7, 10, and on the final day of study, days 13-15. After collection of blood samples on day 0, animals were refed their Purina chow diet and subsequently gavaged with control solution in group C and one of seven test solutions in groups designated A, B, D-H. The volume administered was equivalent in each group averaging .6ml/42g mouse (.01429 ml/g). The various solutions were gavaged daily to their respective groups based on the animals weight taken that morning or the weight of the animal taken on the preceding day of weighing. To assess alterations in plasma FFA, on day 10 an addition 75 μl blood sample was collected into an EDTA-coated capillary tube immediately after collecting the basal heparinized capillary tube sample.

On the final day of study, mice were not gavaged with test solution. The last gavage was administered on the day preceding the final day, i.e., day 12 for animals terminated on day 13, day 13 for animals terminated on day 14

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and day 14 for animals terminated on day 15. Terminal blood
was collected by decapitation to provide serum for assessment
of HDL.

5   Results:

Figure 3 shows that BRL49653 and LG100268 each
independently lowered the level of glucose in db/db mice. In
addition, the combination of BRL49653 and LG100268 lowered the
glucose level more than each compound did by itself. BRL

10   49653 (1 mg/kg) and LG 100268 (20 mg/kg) lowered the level of
glucose by 40% by day 15 in comparison to control. The
thiazolidinedione BRL 49653 at 1 mg/kg showed similar
efficacy. The combination of an RXR activator and a PPAR
activator showed greater efficacy, leading to almost 50% drop

15   in the level of glucose. The effect of the combination was
rapid, glucose levels were reduced by day 1 of the study and
reached a steady state by day 4. This indicates a rapid
resetting of the steady state levels of glucose homeostasis.
Therefore, RXR activators enhance the efficacy of PPARγ

20   activators, and vice vesa.

Figure 4 shows that RXR activators lowered the level
of triglycerides in db/db mice. LG 100268 (20 mg/kg) lowered
the level of triglycerides 40% by day 15 of the study. BRL
49653 (1 mg/kg) showed similar efficacy. The combination of

25   these two compounds worked even better.

Figure 5 shows that RXR activators modulators
increased the level of HDL cholesterol in db/db mice. LG
100268 (20 mg/kg) increased HDL-cholesterol levels (20%) in
comparison to controls in db/db mice. BRL 49653 caused an

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equivalent level of increase. The combination of these two
compounds showed a higher increase. HDL-C was measured by the
precipitation method using kits obtained from Bohringer-
Mannheim (catalog # 543004 and 427578).

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       (B) *In vivo experiment with ob/ob mice*

       Animals: Strain Obese C57 BL/6J-$Lep^{ob(4)}$, 121 mice

       Source: Jackson Lab Stock number 000632

       Genotype $Lep^{ob(4)}$/+ x $Lep^{ob(4)}$/+

10   DOB 5/22/96±3d, DOA 7/2/96 - 41d old

       Date of study: 7/21/96 - 8/2/96 - 49-70d old

       Mice were identified by an ear punch code (#1-121)
and separated into 12 groups. Each group consisting of 10
mice. (one mouse was terminated before start of the study
15 because of bad teeth causing initial weight loss. As in
preceding studies, mice in each group were housed in 4 cages
(2-3 mice/cage) and provided water and Purina Lab chow #505\15
ad libitum.

       On days of study, food was removed from the cages at
20 selected intervals of time between 6:15 and 7:00 AM. Animal
weights were recorded 2h after start of fast and blood samples
taken after a 3h fast. Blood was drawn from a cut at the tip
of the tail and collected into a heparinized capillary tube
(approx. 75µl volume). After centrifugation, the hematocrit
25 was read in a microcapillary reader, recorded and the tube
broken for recovery of plasma for analysis of glucose,
triglyceride and insulin concentration.

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        Blood samples were collected on days 0₋₃, 0, 3, 6, 8,
10, 14±1 and final collections were made on days 15, 16 or 17.
FFA samples were collected on day 10 by drawing a second 75μl
blood sample in a non-heparinized tube coated with EDTA. This
5   tube was collected immediately after the heparinized tube
collection.
        Animals in groups H were administered control gavage
solution (0.6 ml/42 g) daily commencing on day 0 and ending on
the day preceding the final day. 11 test solutions were
10   administered to animals in groups designated A-G, I-L. All
gavage solutions were administered after refeeding on days in
which mice were fasted for blood sampling.

Results:
15   Figure 6 shows that RXR modulators lowered the level
of triglycerides in ob/ob mice. RXR activators LGD 1069 (30
mg/kg) and LG 100268 (20 mg/kg) lowered the level of
triglycerides by 34% and 60% respectively in ob/ob mice by
day 14 of the study. BRL 49653 was also able to lower the
20   level of triglycerides, although not as efficacious as
LG100268.
        Figure 7 shows that RXR modulators lowered the level
of glucose in ob/ob mice. RXR activators LGD 1069 at 30 mg/kg
and LG 100268 at 20 mg/kg lowered the level of glucose nearly
25   50% in comparison to control. The level of glucose was
reduced to almost euglycemic levels by day 14 of the study.
BRL 49653 (0.4 mg/kg) showed similar efficacy.

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Figure 8 shows that RXR modulators, LGD 1069 (30 mg/kg) and LG 100268 (20 mg/kg), lowered the level of insulin in *ob/ob* mice. LG100268 lowered the level of insulin by 66% by day 14 of the study. LGD 1069 showed lower efficacy.

5   There was a very rapid effect of the compounds since the level of insulin started to drop in day 1.

Pharmaceutical Formulations and Modes of Administration

The particular compound that affects the disorders

10   or conditions of interest can be administered to a patient either by themselves, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of a agent or agents such as

15   these is administered. A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient.

The compounds also can be prepared as

20   pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include acid addition salts such as those containing hydrochloride, sulfate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-

25   toluenesulfonate, cyclohexylsulfamate and quinate. (*See e.g.*, PCT/US92/03736). Such salts can be derived using acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid,

benzenesulfonic acid, p-toluenesulfonic acid,
cyclohexylsulfamic acid, and quinic acid. These salts can be
prepared by standard techniques. For example, the free base
form of the compound is first dissolved in a suitable solvent
such as an aqueous or aqueous-alcohol solution, containing the
appropriate acid. The salt is then isolated by evaporating
the solution. In another example, the salt is prepared by
reacting the free base and acid in an organic solvent.

Carriers or excipients can be used to facilitate
administration of the compound, for example, to increase the
solubility of the compound. Examples of carriers and
excipients include calcium carbonate, calcium phosphate,
various sugars or types of starch, cellulose derivatives,
gelatin, vegetable oils, polyethylene glycols and
physiologically compatible solvents.

In addition, the molecules tested can be used to
determine the structural features that enable them to act on
the RXR/PPARγ heterodimer, and thus to select molecules useful
in this invention. Those skilled in the art will know how to
design drugs from lead molecules, using techniques such as
those disclosed in PCT publication WO 94/18959, incorporated
by reference herein.

Toxicity and therapeutic efficacy of such compounds
can be determined by standard pharmaceutical procedures in
cell cultures or experimental animals, e.g., for determining
the LD_{50} (the dose lethal to 50% of the population) and the ED_{50}
(the dose therapeutically effective in 50% of the population).
The dose ratio between toxic and therapeutic effects is the
therapeutic index and it can be expressed as the ratio

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LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies

5   preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Levels in plasma may be measured, for example, by HPLC.

10   The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl et al., in The Pharmacological Basis of Therapeutics, 1975, Ch. 1 p. 1). It should be noted that the attending physician would know how to

15   and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose

20   in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose

25   frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific conditions being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in *Remington's Pharmaceutical Sciences*, 18th ed., Mack

5   Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous,

10   intraperitoneal, intranasal, or intraocular injections, just to name a few.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's

15   solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to

20   formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular,

25   those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the

invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly
5   may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at
10   the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small
15   organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve
20   its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharma-
25   ceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceuti-cally. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or

solutions. The pharmaceutical compositions of the present
invention may be manufactured in a manner that is itself
known, e.g., by means of conventional mixing, dissolving,
granulating, dragee-making, levitating, emulsifying,
5   encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral
administration include aqueous solutions of the active
compounds in water-soluble form. Additionally, suspensions of
the active compounds may be prepared as appropriate oily
10   injection suspensions. Suitable lipophilic solvents or
vehicles include fatty oils such as sesame oil, or synthetic
fatty acid esters, such as ethyl oleate or triglycerides, or
liposomes. Aqueous injection suspensions may contain
substances which increase the viscosity of the suspension,
15   such as sodium carboxymethyl cellulose, sorbitol, or dextran.
Optionally, the suspension may also contain suitable stabiliz-
ers or agents which increase the solubility of the compounds
to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be
20   obtained by combining the active compounds with solid
excipient, optionally grinding a resulting mixture, and
processing the mixture of granules, after adding suitable
auxiliaries, if desired, to obtain tablets or dragee cores.
Suitable excipients are, in particular, fillers such as
25   sugars, including lactose, sucrose, mannitol, or sorbitol;
cellulose preparations such as, for example, maize starch,
wheat starch, rice starch, potato starch, gelatin, gum
tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose,
sodium carboxymethylcellulose, and/or polyvinylpyrrolidone

(PVP). If desired, disintegrating agents may be added, such
as the cross-linked polyvinyl pyrrolidone, agar, or alginic
acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings.
5   For this purpose, concentrated sugar solutions may be used,
which may optionally contain gum arabic, talc, polyvinyl
pyrrolidone, carbopol gel, polyethylene glycol, and/or
titanium dioxide, lacquer solutions, and suitable organic
solvents or solvent mixtures. Dyestuffs or pigments may be
10   added to the tablets or dragee coatings for identification or
to characterize different combinations of active compound
doses.

Pharmaceutical preparations which can be used orally
include push-fit capsules made of gelatin, as well as soft,
15   sealed capsules made of gelatin and a plasticizer, such as
glycerol or sorbitol. The push-fit capsules can contain the
active ingredients in admixture with filler such as lactose,
binders such as starches, and/or lubricants such as talc or
magnesium stearate and, optionally, stabilizers. In soft
20   capsules, the active compounds may be dissolved or suspended
in suitable liquids, such as fatty oils, liquid paraffin, or
liquid polyethylene glycols. In addition, stabilizers may be
added. Liposomes may be used for encapsulated delivery.

Pharmaceutical formulations disclosed or described
25   in Boehm, et al., WO94/15902 are incorporated by reference
herein.

All publications referenced are incorporated by
reference herein, including the nucleic acid sequences and
amino acid sequences listed in each publication. All the

compounds disclosed and referred to in the publications
mentioned above are incorporated by reference herein,
including those compounds disclosed and referred to in
articles cited by the publications mentioned above.

5   Other embodiments of this invention are disclosed in
the following claims.

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TABLE 1: Oil Red O Staining in 3T3-L1 Differentiating

Adipocytes

Treatment	Percent of Differentiated Cells
Control	0.1
BRL 49653	50
Insulin	20
LG 100268	50
BRL+Insulin	80
BRL+LG 100268	90
LG 100268+Insulin	90

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TABLE 2: Retinoid Modulation of Adipocyte differentiation in C3H/10T1/2 Cells

Treatment	Experiment A (+BRL) % differentiated cells	Experiment B (+BRL+Insulin) % differentiated cells
BRL (10μM)	10	80
BRL (1μM)	8	80
BRL (0.1μM)	5	60
BRL (10μM)	10	80
+ALRT 1057(1μM)	80	99
+LGD 1069(1μM)	80	99
+TTNPB(10nM)	5	50
BRL (1μM)	8	80
+ALRT 1057(1μM)	65	90
+LGD 1069(1μM)	65	90
+TTNPB(10nM)	2	30
BRL (0.1μM)	5	60
+ALRT 1057(1μM)	45	85
+LGD 1069(1μM)	45	90
+TTNPB (10nM)	0.2	

Undifferentiated controls 0% differentiation.

The claims defining the invention are as follows:

1.   Method for treating a host having NIDDM, the method comprising the step of administering to said host a composition comprising a pharmaceutically effective amount of an RXR agonist.

2.   The method of claim 1, wherein said RXR agonist is an RXR specific agonist.

3.   The method of claim 1 or claim 2, wherein said RXR agonist is LG 100268.

4.   The method of claim 1 or claim 2, wherein said RXR agonist is LGD 1069.

5.   The method of claim 1, wherein said RXR agonist also activates a retinoic acid receptor.

6.   The method of claim 5, wherein said RXR agonist is 9-cis retinoic acid.

7.   The method of any one of claims 1 to 6, further comprising the step of administering to said host a composition comprising a pharmaceutically effective amount of a PPARγ agonist.

8.   The method of any one of claims 1 o 6, wherein said composition further comprises a pharmaceutically effective amount of a PPARγ agonist.

9.   The method of claim 7 or claim 8, wherein said PPARγ agonist is a thiazolidinedione compound.

10.   The method of claim 9, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120, 744, englitazone, AD 5075 and darglitazone.

11.   The method of any one of claims 1 to 10, further comprising the step of administering to said host a composition comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

12.   The method of any one of claims 8 to 10, wherein said composition further contains a pharmaceutically effective amount of insulin, insulin mimetic, or insulin sensitizer.

13.   An RXR agonist when used for treating a host having NIDDM, wherein said use comprises administration to said host a composition comprising a pharmaceutically effective amount of an RXR agonist.

14.   An RXR agonist when used according to claim 13 wherein said RXR agonist is an RXR specific agonist.

15.   An RXR agonist when used according to claim 13 or claim 14 wherein said RXR agonist is LG 100268.

16.   An RXR agonist when used according to claim 13 or claim 14 wherein said RXR agonist is LGD 1069.

17.   An RXR agonist when used according to claim 13, wherein said RXR agonist also activates a retinoic acid receptor.

18.   An RXR agonist when used according to claim 17, wherein said RXR agonist is 9-cis retinoic acid.

19. An RXR agonist when used according to any one of claims 13 to 18 wherein said use further comprises administering a composition comprising a pharmaceutically effective amount of a PPARγ agonist.

20. An RXR agonist when used according to any one of claims 13 to 18 wherein said composition further comprises a pharmaceutically effective amount of a PPARγ agonist.

21. An RXR agonist when used according to claim 19 or claim 20, wherein said PPARγ agonist is a thiazolidinedione compound.

22. An RXR agonist when used according to claim 21, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120, 744, englitazone, AD 5075 and darglitazone.

23. An RXR agonist when used according to any one of claims 13 to 22, wherein said use further comprises administration to said host a composition comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

24. An RXR agonist when used according to any one of claims 20 to 22, wherein said composition further contains a pharmaceutically effective amount of insulin, insulin mimetic, or insulin sensitizer.

25. The use of an RXR agonist for the manufacture of a medicament for treating a host having NIDDM.

26. The use according to claim 25 wherein said RXR agonist is an RXR specific agonist.

27. The use according to claim 25 or claim 26 wherein said RXR agonist is LG 100268.

28. The use according to claim 25 or claim 26 wherein said RXR agonist is LGD 1069.

29. The use according to claim 25, wherein said RXR agonist also activates a retinoic acid receptor.

30. The use according to claim 29, wherein said RXR agonist is 9-cis retinoic acid.

31. The use according to any one of claims 25 to 30 wherein said medicament is for administration with a composition comprising a pharmaceutically effective amount of a PPARγ agonist.

32. The use according to any one of claims 25 to 30 wherein said medicament further comprises a pharmaceutically effective amount of a PPARγ agonist.

33. The use according to claim 31 or claim 32, wherein said PPARγ agonist is a thiazolidinedione compound.

34. The use according to claim 33, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120, 744, englitazone, AD 5075 and darglitazone.

35. The use according to any one of claims 25 to 34, wherein said medicament is for administration with a composition comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

36. The use according to any one of claims 32 to 34, wherein said medicament further contains a pharmaceutically effective amount of insulin, insulin mimetic, or insulin sensitizer.

37. Method for treating a host having NIDDM, the method comprising the step of administering to said host a pharmaceutical composition adapted for the treatment of NIDDM, the composition comprising

    (a)   a pharmaceutically effective amount of an RXR agonist; and

    (b)   a pharmaceutically acceptable carrier.

38. The method of claim 49, the composition further comprising a pharmaceutically effective amount of a PPARγ agonist.

39. The method of claim 37 or claim 38, wherein said RXR agonist is LG 100268.

40. The method of claim 37 or claim 38, wherein said RXR agonist is LGD 1069.

41. The method of claim 37 or claim 38, wherein said RXR agonist is 9-cis retinoic acid.

42. The method of any one of claims 37 to 41, wherein said PPARγ agonist is a thiazolidinedione compound.

43. The method of claim 42, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120, 744, englitazone, AD 5075 and darglitazone.

44. The method of any one of claims 37 to 43 wherein said composition further comprises a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

45. A composition defined in the method of any one of claims 37 to 44 when used for treating a host having NIDDM.

46. Use of a composition defined in the method of any one of claims 37 to 44 in the manufacture of a medicament for treating a host having NIDDM.

47. Method for increasing glucose uptake in adipose or muscle tissue, comprising the step of administering to said tissue a composition comprising a pharmaceutically effective amount of an RXR agonist.

48. The method of claim 47, wherein said RXR agonist is an RXR specific agonist.

49. The method of claim 47 or claim 48, wherein said RXR agonist is LG 100268.

50. The method of claim 47 or claim 48, wherein said RXR agonist is LGD 1069.

51. The method of claim 47, wherein said RXR agonist also activates a retinoic acid receptor.

52. The method of claim 51, wherein said RXR agonist is 9-cis retinoic acid.

53. The method of any one of claims 47 to 52, further comprising the step of administering to said tissue a composition comprising a pharmaceutically effective amount of a PPARγ agonist.

54. The method of any one of claims 47 to 52, wherein said composition further comprises a pharmaceutically effective amount of a PPARγ agonist.

55. The method of claim 53 or claim 54, wherein said PPARγ agonist is a thiazolidinedione compound.

56. The method of claim 55, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120, 744, englitazone, AD 5075 and darglitazone.

57. The method of any one of claims 47 to 56, further comprising the step of administering to said tissue a composition comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

58. The method of any one of claims 54 to 56, wherein said composition further contains a pharmaceutically effective amount of insulin, insulin mimetic, or insulin sensitizer.

59. An RXR agonist when used for increasing glucose uptake in adipose or muscle tissue, wherein said use comprises administering to said tissue a composition comprising a pharmaceutically effective amount of an RXR agonist.

60. An RXR agonist when used according to claim 59, wherein said RXR agonist is an RXR specific agonist.

61. An RXR agonist when used according to claim 59 or claim 60, wherein said RXR agonist is LG 100268.

62. An RXR agonist when used according to claim 59 or claim 60, wherein said RXR agonist is LGD 1069.

63. An RXR agonist when used according to claim 59, wherein said RXR agonist also activates a retinoic acid receptor.

64. An RXR agonist when used according to claim 63, wherein said RXR agonist is 9-cis retinoic acid.

65. An RXR agonist when used according to any one of claims 59 to 64, wherein said composition is administered to said tissue with a composition comprising a pharmaceutically effective amount of a PPARγ agonist.

66. An RXR agonist when used according to any one of claims 59 to 64, wherein said composition further comprises a pharmaceutically effective amount of a PPARγ agonist.

67. An RXR agonist when used according to claim 65 or claim 66, wherein said PPARγ agonist is a thiazolidinedione compound.

68. An RXR agonist when used according to claim 67, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120, 744, englitazone, AD 5075 and darglitazone.

69. An RXR agonist when used according to any one of claims 59 to 68, wherein said composition is administered to said tissue with a composition comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

70. An RXR agonist when used according to any one of claims 66 to 68, wherein said composition further contains a pharmaceutically effective amount of insulin, insulin mimetic, or insulin sensitizer.

71. The use of an RXR agonist for the manufacture of a medicament for increasing glucose uptake in adipose or muscle tissue.

72. The use according to claim 71, wherein said RXR agonist is an RXR specific agonist.

73. The use according to claim 71 or claim 72, wherein said RXR agonist is LG 100268.

74. The use according to claim 71 or claim 72, wherein said RXR agonist is LGD 1069.

75. The use according to claim 71, wherein said RXR agonist also activates a retinoic acid receptor.

76. The use according to claim 75, wherein said RXR agonist is 9-cis retinoic acid.

77. The use according to any one of claims 71 to 76, wherein said medicament is for administration with a composition comprising a pharmaceutically effective amount of a PPARγ agonist.

78. The use according to any one of claims 71 to 76, wherein said medicament further comprises a pharmaceutically effective amount of a PPARγ agonist.

79. The use according to claim 77 or claim 78, wherein said PPARγ agonist is a thiazolidinedione compound.

80. The use according to claim 79, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120, 744, englitazone, AD 5075 and darglitazone.

81. The use according to any one of claims 71 to 80, wherein said medicament is for administration with a composition comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

82. The use according to any one of claims 71 to 80, wherein said medicament further contains a pharmaceutically effective amount of insulin, insulin mimetic, or insulin sensitizer.
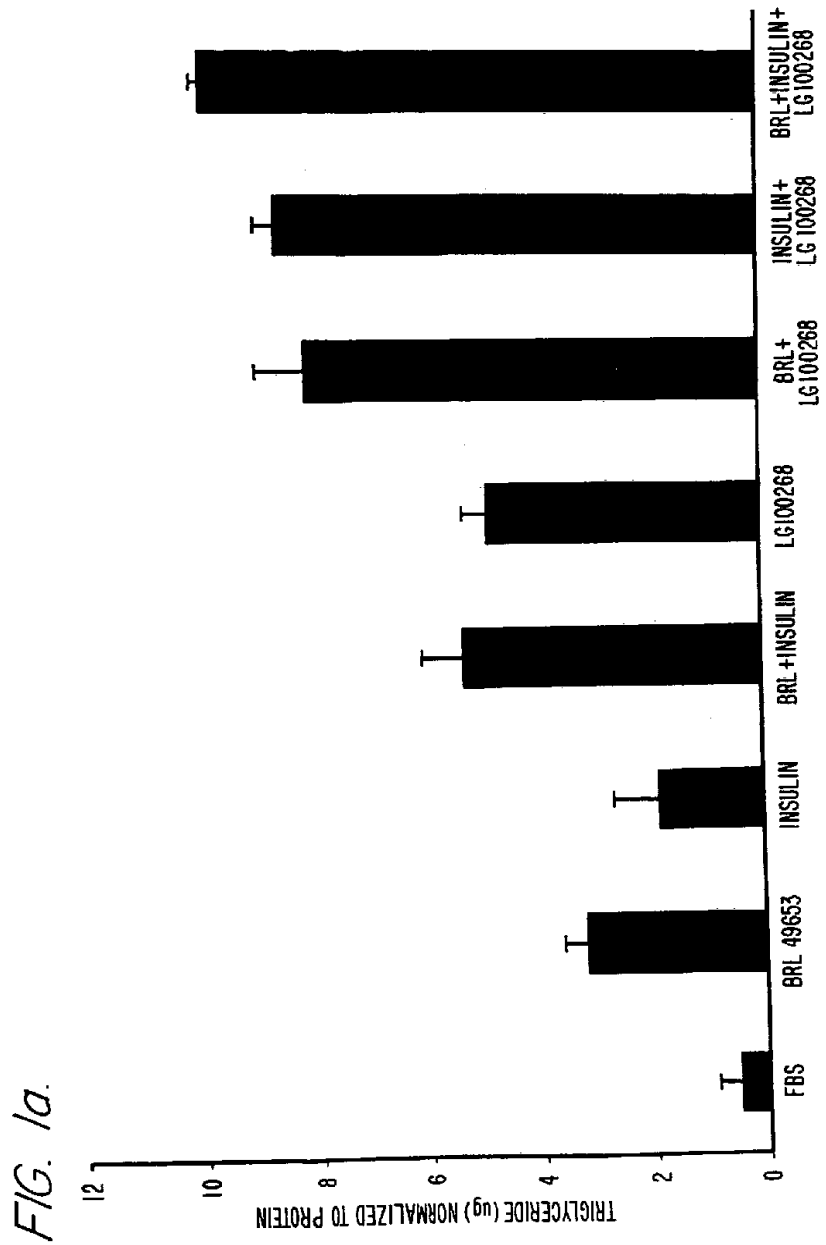
Dated 1 September, 2000

Ligand Pharmaceuticals Incorporated

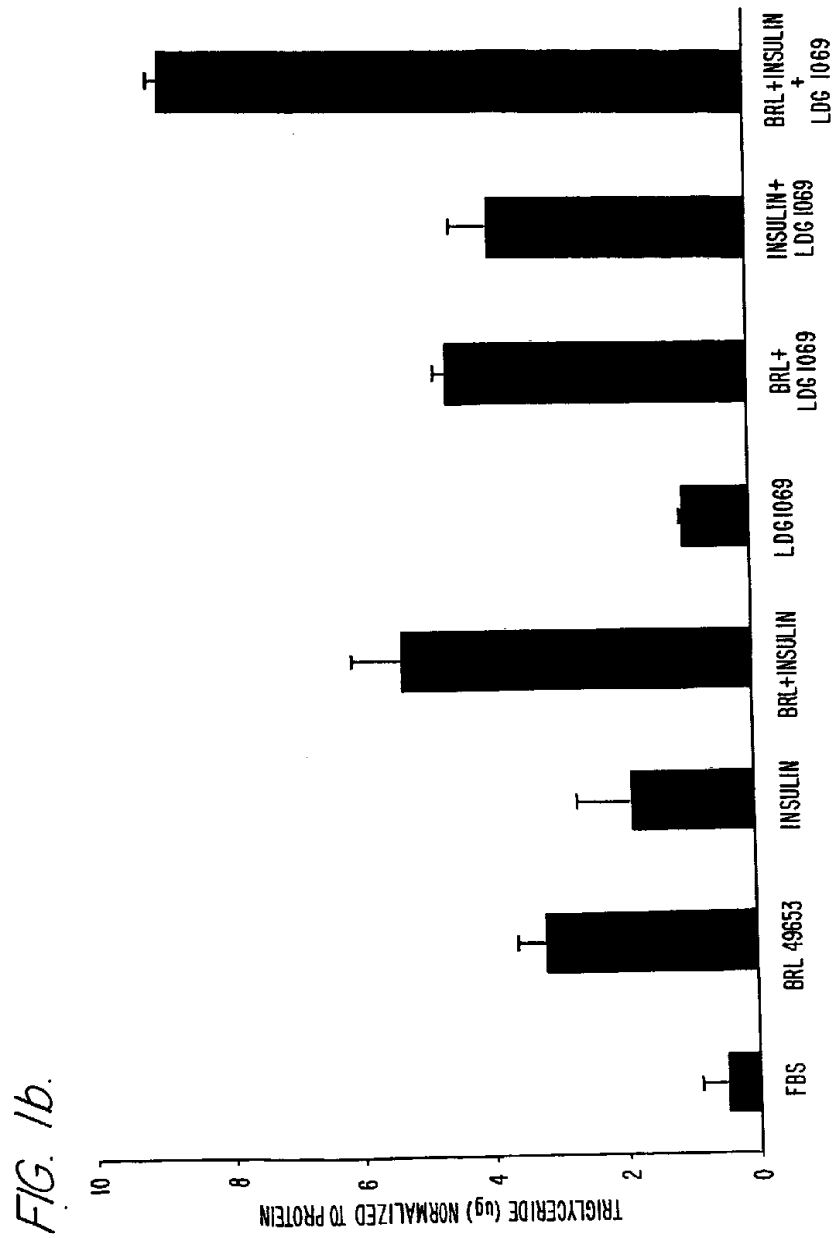Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

FIG. 1a.

FIG. 1b.

FIG. 2a.

FIG. 2b.

FIG. 3.

- ◆ — CONTROL
- ■ — BRL (1)
- ▲ — BRL (1)+LG 268
- ✕ — LG268 (20)

FIG. 4.

FIG. 5.

FIG. 6.

VEHICLE   LG 69 (30)   BRL(.4)   LG 268 (20)

TRIGLYCERIDES (mgs/dl)

140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0

RECTIFIED SHEET (RULE 91)
ISA/EP

FIG. 7.

VEHICLE
LG 69 (30)
BRL (.4)
LG 268 (20)

FIG. 8.