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(54) Title: A METHOD FOR TREATING OBESITY

(57) Abstract: A method and pharmaceutical composition for treating a disease or condition associated with elevated hypothalamic T cell protein tyrosine phosphatase (TCPTP) such as obesity, weight gain, type 2 diabetes mellitus, insulin sensitivity, impaired glucose tolerance, and inflammation is disclosed. The method involves administering a subject with an agent which inhibits the activity or expression of TCPTP, wherein the agent is administered, adapted and/or formulated in a manner ensuring that an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP. Also disclosed is a method for alleviating or preventing leptin resistance in an obese or overweight subject, a method for sensitizing a subject to weight loss through dieting, and a dieting kit.



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A METHOD FOR TREATING OBESITY

5 FIELD OF THE INVENTION

The invention relates to a method and pharmaceutical composition for treating a disease or condition associated with elevated hypothalamic T cell protein tyrosine phosphatase (TCPTP) such as obesity, weight gain, type 2 diabetes mellitus, insulin sensitivity, impaired glucose tolerance, and inflammation.

10 INCORPORATION BY REFERENCE

This patent application claims priority from:

- Australian Provisional Patent Application No 2009905521 titled "A method for treating obesity" filed 11 November 2009, and

15 - Australian Provisional Patent Application No 2010903177 titled "A method for treating obesity" filed 16 July 2010.

The entire content of these applications is hereby incorporated by reference.

The following patent specifications and documents are referred to herein:

20 - United States Patent No 7504389 titled "Protein tyrosine phosphatase inhibitors and methods of use thereof",

- United States Patent No 7,381,736 titled "Thiazole and thiadiazole inhibitors of tyrosine phosphatases",

- United States Patent Publication No 2004/0121353 titled "Modulation of TCPTP signal transduction by RNA interference",

25 - International Patent Publication No WO 94/02178 titled "Targeting of liposomes to the blood-brain barrier",

- Zhang, S *et al.*, *J Am Chem Soc* 131:13072-13079 (2009),

- Silva, GA., *BMC Neuroscience* 9(suppl 3):S4 (2008), and

- Mathupala, SP., *Expert Opin Ther Pat* 19:137-140 (2009).

30 The entire content of these specifications and documents is also hereby incorporated by reference.

BACKGROUND TO THE INVENTION

Obesity is increasing at an alarming rate worldwide and is a major risk factor for type 2 diabetes mellitus, cardiovascular disease and the metabolic syndrome. It is now widely appreciated that obesity is
35 characterised by a state of chronic inflammation and that this, in turn, is causally linked to the development of insulin resistance, a major hallmark of type 2 diabetes. Also, it is known that adipose tissue-derived pro-inflammatory cytokines, or the activation of inflammatory pathways due to endoplasmic reticulum (ER) stress associated with overnutrition, suppresses insulin signalling downstream of the insulin receptor (IR) protein tyrosine kinase (PTK)¹⁻⁴. Further, although insulin

resistance in liver, muscle and fat (the peripheral insulin target tissues responsible for glucose homeostasis) is a key pathological feature of type 2 diabetes¹⁻³, recent evidence also points towards "central" insulin resistance being an important contributing factor to disease progression⁴⁻⁷

5 An added pathological feature linked to the inflammatory state of obesity is leptin resistance. Leptin is an adipocyte-derived hormone, which acts in the hypothalamus to activate the Janus kinase 2 (JAK2) PTK that signals via substrates such as STAT3 (signal transducer and activator of transcription 3) to suppress food intake, increase energy expenditure, decrease body weight and improve glucose tolerance^{8,9}. In principal, leptin should be an effective anti-obesity agent, however, inflammation/ER stress also
10 suppresses the leptin signal by promoting the expression of negative regulators such as SOCS3 (suppressor of cytokine signalling 3) and the prototypic protein tyrosine phosphatase, PTP1B^{4, 10-17}.

Approaches aimed at overcoming insulin and leptin resistance are attractive strategies for the treatment of
15 obesity and type 2 diabetes. One approach for enhancing insulin and/or leptin sensitivity and alleviating insulin and/or leptin resistance may involve the inhibition of protein tyrosine phosphatases (PTPs) that otherwise dephosphorylate and inactivate the IR or JAK2 PTKs, or their respective downstream target substrates such as IR substrate-1 (IRS-1) and STAT3. The ER-targeted PTP1B is a physiological negative regulator of the IR PTK in liver and muscle and JAK2 in the hypothalamus to regulate glucose
20 homeostasis, food intake and energy expenditure^{14, 18-24}. Accordingly, considerable attention has been focused on PTP1B as a target for the development of novel therapeutics for the treatment of type 2 diabetes and obesity²⁵⁻³².

In addition, another closely related phosphatase, TCPTP (T cell PTP), has been identified as a negative
25 regulator of insulin action, working coordinately with PTP1B to control the intensity and duration of IR phosphorylation and activation³³. It has also been found that TCPTP (also known as PTPN2) dephosphorylates and inactivates STAT3 in a cellular context³⁴. In work leading to the present invention, the applicant has found that TCPTP acts in the brain to control food intake, energy expenditure, body weight and insulin sensitivity, at least in part by controlling leptin sensitivity. Further, the present
30 applicant has now found that hypothalamic TCPTP expression is increased in obese mice indicating that elevated TCPTP expression is also a contributing factor to hypothalamic leptin and/or insulin resistance in obesity/type 2 diabetes.

SUMMARY OF THE INVENTION

The present invention provides a method for treating a disease or condition associated with elevated hypothalamic T cell protein tyrosine phosphatase (TCPTP) in a subject, said method comprising administering the subject with an agent which inhibits the activity or expression of TCPTP, wherein said agent is administered, adapted and/or formulated in a manner ensuring that an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

The method of the first aspect is suitable for a disease or condition associated with elevated hypothalamic TCPTP such as, for example, obesity (particularly, high fat diet-induced obesity (DIO)), weight gain, type 2 diabetes mellitus, insulin resistance, impaired glucose tolerance, and inflammation.

In a second aspect, the present invention provides a pharmaceutical composition comprising an agent which inhibits the activity or expression of TCPTP optionally in combination with a pharmaceutically-acceptable carrier, diluent or excipient, wherein said agent is adapted and/or formulated in a manner ensuring that, upon administration of the composition to a subject, an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

Preferably, said composition further comprises a PTP1B inhibitor.

In a third aspect, the present invention provides a method for alleviating or preventing leptin resistance in an obese or overweight subject, said method comprising administering the subject with an agent which inhibits the activity or expression of TCPTP, wherein said agent is administered, adapted and/or formulated in a manner ensuring that an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

In a fourth aspect, the present invention provides a method for sensitising a subject to weight loss through dieting, the method comprising administering the subject with an agent which inhibits the activity or expression of TCPTP, wherein said agent is administered, adapted and/or formulated in a manner ensuring that an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

In a fifth aspect, the present invention provides a dieting kit comprising foodstuffs and a pharmaceutical composition according to the second aspect.

BRIEF DESCRIPTION OF THE ACCOMPANYING FIGURES

Figure 1 provides a schematic diagram of the mechanism by which agents such as insulin and leptin suppress appetite; the diagram shows that in the arcuate nucleus (ARC), these agents inhibit orexigenic neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons while stimulating anorexigenic proopiomelanocortin (POMC) neurons;

Figure 2 shows the increase in hypothalamic TCPTP and SOCS3 levels following high fat fed (HFF) obese Mice (ie relative to low fat chow fed lean mice). Quantified results shown are means $\pm$ SE.

* $p < 0.05$;

Figure 3 provides a gel image showing that TCPTP expression in the brain was ablated in *TCPTPloxlox:Nestin-Cre* transgenic mice (hereinafter referred to as brain-specific TCPTP KO (BTKO) mice), whereas control (*loxlox*) mice showed unaltered TCPTP expression in the brain, including hypothalamus, and four other tissues. The experiment was conducted using tubulin expression as a control, because tubulin is not expected to be altered as a result of Cre recombinase activity;

Figure 4 provides graphical results showing the increased insulin sensitivity and glucose tolerance exhibited by BTKO mice. Results shown are means $\pm$ SE. * $p < 0.05$;

Figure 5 provides graphical results showing increased oxygen consumption, energy expenditure and decreased food intake in BTKO mice; monitored using a Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments International Inc, Columbus, OH, United States of America). Results shown are means $\pm$ SE. * $p < 0.05$, ** $p < 0.01$;

Figure 6 provides the results of experimentation designed to assess leptin sensitivity in BTKO mice. The results in A) indicated that by virtue of a decrease in body weight following intraperitoneal (*ip*) administration of leptin, and B) increased STAT3 phosphorylation following bolus *ip* leptin administration, leptin sensitivity in BTKO mice was increased relative to control (*loxlox*) mice. Results shown in A are means $\pm$ SE. * $p < 0.05$;

Figure 7 provides graphical results showing the blood glucose, plasma insulin and plasma leptin levels in fed (low fat chow diet) and fasted BTKO mice and control (*loxlox*) mice. Results shown are means $\pm$ SE. * $p < 0.05$;

Figure 8 provides a diagrammatic representation of the coordinated regulation of hypothalamic signalling by TCPTP and PTP1B. In particular, the figure shows that they act coordinately to attenuate JAK2/STAT3 signalling;

Figure 9 provides further graphical results demonstrating that, in BTKO mice on a high fat diet (23% fat), there was a decreased weight gain (relative to control (*lox/lox*) mice), and following the cessation of high fat feeding after about 20 weeks (replaced with low fat chow diet), BTKO showed an enhanced level of weight loss. Results shown are means $\pm$ SE. * $p < 0.05$;

Figure 10 provides a gel image of hypothalamic TCPTP (both 48 kDa and 45 kDa forms) and actin expression in the hypothalamus of low fat chow fed control mice. The results in A) show that the TC45 form is relatively enriched in the hypothalamus when compared to TCPTP expression in the whole brain, and in B) shows that TCPTP is expressed in the ARC and ventromedial nucleus (VMN) regions of the hypothalamus which are critical for leptin signalling;

Figure 11 provides graphical results demonstrating that TCPTP protein A) and B) and mRNA C) expression is induced by leptin A) and B) in Chinese Hamster Ovary cells overexpressing the leptin receptor (CHO-LRb) *in vitro* and C) *in vivo*. Results shown in B), and C) are means $\pm$ SE. * $p < 0.05$, *** $p < 0.05$;

Figure 12 provides a schematic diagram and graphical results showing the effect of TCPTP deficiency on leptin-induced expression of POMC (the precursor of α -melanocyte-stimulating hormone; α -MSH), NPY and AgRP. In these experiments, mice were injected with leptin and the mRNA levels of POMC, AgRP and NPY were assessed using real time PCR. The results indicate that TCPTP deficiency enhances the effects of leptin on the expression of POMC and AgRP which mediate leptin's effects on food intake, energy expenditure and glucose homeostasis. Results shown are means $\pm$ SE. * $p < 0.05$;

Figure 13 provides the results of experiments involving the administration of a TCPTP inhibitor ("Compound 8"; Zhang, S *et al. J Am Chem Soc* 131:13072-13079 (2009)) depicted at A). As shown in A), Compound 8 was injected into the lateral ventricle of mice by intracerebral injection (*icv*), along with *ip* administration of leptin. The results shown at A) suggest that Compound 8 enhances the effects of leptin on STAT3 phosphorylation. The graphical results shown at B) and C) were achieved following equivalent administration of Compound 8 and leptin administered IP for two consecutive days, wherein body weight and energy expenditure was regularly monitored. The results indicate that TCPTP inhibition with Compound 8 enhances the effects of leptin in suppressing body weight and increasing energy expenditure. The results shown at D) indicate that Compound 8 has no effect on the body weight of BTKO mice demonstrating that this compound specifically inhibits TCPTP (ie without inhibiting PTP1B). Quantified results shown are means $\pm$ SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; and

Figure 14 provides results obtained using 8-10 week old BTKO and *lox/lox* male littermates that were fed a high fat diet for 12 weeks: wherein body weights were determined weekly (A); (B) Leptin sensitivity on body weight measured; (C) insulin tolerance tests conducted following fasting for 4 h; and blood glucose

and plasma insulin levels measured following 6 h fasting. Results shown are means $\pm$ SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

DETAILED DESCRIPTION OF THE INVENTION

In addition to regulating carbohydrate/lipid/protein metabolism in the periphery, insulin acts in the hypothalamus via the phosphatidylinositol 3-kinase (PI3K) pathway in areas such as the arcuate nucleus (ARC) to reduce food intake and lower blood glucose levels^{6, 35-40}. The IR is expressed in the ARC in anorexigenic (appetite-suppressing) POMC, as well as orexigenic NPY and AgRP neuropeptide expressing neurons⁴¹⁻⁴⁴. Agents such as insulin and leptin that suppress appetite inhibit orexigenic NPY/AgRP neurons whilst stimulating anorexigenic POMC neurons (Figure 1)^{7, 8, 45, 46}. Further, recent studies have shown that IR expression in AgRP neurons is required for the suppression of hepatic glucose output, wherein hypothalamic IR signalling promotes interleukin-6 (IL-6) release from Kupfer cells in the liver which, in turn, acts on hepatocytes to promote STAT3 phosphorylation and thus suppress gluconeogenic gene expression^{36-38, 47}.

As mentioned above, TCPTP has been identified as a negative regulator of insulin action, working coordinately with PTP1B to control the intensity and duration of IR phosphorylation and activation³³. TCPTP is a ubiquitous tyrosine-specific phosphatase which exists as two splice variants: a 48 kDa form (TC48) that, like PTP1B, is targeted to the ER and a 45 kDa variant (TC45)³³ that is targeted to the nucleus by a nuclear localisation signal (NLS). Despite an apparent exclusive nuclear localisation in resting cells, TC45 can exit the nucleus in response to varied stimuli and, therefore, access substrates both in the nucleus and cytoplasm⁴⁹⁻⁵¹. Cytoplasmic substrates for TC45 include the IR³³ and JAK1/3⁵² PTKs and nuclear substrates include STAT family members^{53, 54}, such as STAT3³⁴. TC45's spatial isolation in the nucleus may be essential for the initiation of signal transduction at the cell surface and its nuclear exit may represent a negative feedback loop for the suppression of signalling.

In work leading to the present invention, the present applicant has found that high fat diet-induced obesity (DIO) increases TCPTP expression in the hypothalamus (but not in the liver, muscle or fat) by ~ 2 fold (Figure 2). This increase in TCPTP expression is similar to that seen for SOCS3 (Figure 2) and that previously reported for PTP1B¹⁶. Increases in hypothalamic SOCS3 and PTP1B have been implicated in the development of leptin resistance and, possibly, central insulin resistance in the obese state¹⁰⁻¹⁶ and, accordingly, given TCPTP's capacity to dephosphorylate and inactivate the IR and STAT3, the applicant considers that increases in TCPTP expression in the brain similarly contribute to disease progression. To address TCPTP's role in insulin and leptin signalling in the brain, TCPTP "floxed" mice (*lox* sites flanking exons 4 and 5 encoding the catalytic domain) on a C57BL/6 background were generated and then bred with *Nestin-Cre* (Cre recombinase expressed from the nestin promoter-enhancer) transgenic mice (C57BL/6) to delete TCPTP specifically in neuronal cells (*TCPTP^{loxlox};Nestin-Cre*: BTKO).

Having confirmed that TCPTP expression is unaltered in "floxed" mice (data not shown), observed that TCPTP is specifically ablated in the brain on the *Nestin-Cre* background (Figure 3), and found that the gross phenotype, behaviour and life expectancy of BTKO mice appears to be normal, studies involving the comparison of chow fed floxed control, heterozygous (*TCPTPlox+;Nestin-Cre*) and BTKO mice

5 resulted in BTKO mice that were about 10-20% smaller (data not shown). This difference in body weight coincided with a decrease in epididymal fat pad mass and decreased bone density (data not shown) that was consistent with that previously reported for brain-specific PTP1B KO mice¹⁴ and the established role for hypothalamic leptin signalling in promoting bone resorption via sympathetic control of osteoblasts⁶⁵⁻⁶⁷. The BTKO mice also had significantly improved insulin sensitivity and glucose tolerance as measured

10 by insulin tolerance tests (ITT) and glucose tolerance tests (GTT) (Figure 4). In addition, oxygen consumption and energy expenditure were increased and food consumption decreased in BTKO mice (Figure 5) and, finally, leptin sensitivity, as monitored by the suppression of food intake (data not shown) and body weight (Figure 6a) after intraperitoneal (*ip*) leptin administration as well as hypothalamic STAT3 phosphorylation in response to bolus *ip* leptin administration, were increased in BTKO mice

15 (Figure 6b). Further, the BTKO mice showed reduced levels of plasma leptin (Figure 7). Moreover, the BTKO mice showed reduced blood glucose and plasma insulin following fasting (Figure 7). Taken together, these results indicate that TCPTP is an integral regulator of neuronal signalling to affect body weight and peripheral insulin sensitivity. In particular, these results indicate that TCPTP is a key regulator of hypothalamic leptin sensitivity and, while not wishing to be bound by theory, the present applicant

20 therefore believes that TCPTP acts in concert with PTP1B to coordinately attenuate JAK2/STAT3 signalling; with PTP1B acting on JAK2 in the cytoplasm, and the TC45 form of TCPTP regulating STAT3 in the nucleus (Figure 8).

Accordingly, hypothalamic TCPTP represents a novel target for therapeutic agents for treating obesity,

25 weight gain and type 2 diabetes mellitus, since the inhibition of activity and/or expression of this enzyme promotes weight loss by increasing energy expenditure and suppressing food intake, enhances glucose homeostasis and insulin sensitivity and thus decreases fasting hyperglycaemia. With dieting for weight loss, the inhibition of hypothalamic TCPTP activity and/or expression, also offers an obese or overweight subject with the possibility of an increased rate of weight loss. Indeed, it has been found that in BTKO

30 mice on HFF, there was a decreased weight gain (relative to control (*lox/lox*) mice), and following the cessation of HFF after about 20 weeks (replaced with low fat chow diet), BTKO showed an enhanced level of weight loss (Figure 9).

Thus, in a first aspect, the present invention provides a method for treating a disease or condition

35 associated with elevated hypothalamic T cell protein tyrosine phosphatase (TCPTP) in a subject, said method comprising administering the subject with an agent which inhibits the activity or expression of TCPTP, wherein said agent is administered, adapted and/or formulated in a manner ensuring that an

effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

The method of the first aspect is suitable for a disease or condition associated with elevated hypothalamic TCPTP such as, for example, obesity (particularly DIO), weight gain, type 2 diabetes mellitus, insulin resistance, impaired glucose tolerance, and inflammation.

Preferably, the agent administered in the method of the first aspect specifically inhibits the activity or expression of TCPTP; by the term "specifically inhibits", it is to be understood that the agent acts so as to substantially exclusively inhibit the activity and/or expression of TCPTP with no or only a minimal effect on other mammalian proteins such as the closely related phosphatase, PTP1B. By way of an example, an agent that may be regarded as one which specifically inhibits TCPTP activity may show an IC_{50} for TCPTP inhibition of $< 10 \mu M$, preferably $\leq 1 \mu M$ and, more preferably, $\leq 50 nM$, and an IC_{50} for the inhibition of another PTP such as PTP1B of $> 100 \mu M$, preferably $> 200 \mu M$.

Suitable agents for inhibiting TCPTP include: small organic compounds which inhibit TCPTP activity such as certain phosphonic acid derivatives described in US Patent No 7,504,389 (the entire content of which is to be regarded as incorporated herein by reference), and aromatic amino acid derivatives and *o*- and *p*-substituted benzoic acid derivatives described in Zhang *et al.*¹¹ (the entire contents of which is to be regarded as incorporated herein by reference), or inhibit the formation of the TC45 form (ie "allosteric inhibitors") or otherwise "lock up" the TCPTP in an inactive form (nb. TCPTP is known to regulate its activity by an intramolecular mechanism that may be targeted by a suitable agent⁵⁸); antisense oligonucleotides targeting TCPTP (eg as available from Isis Pharmaceuticals, Inc, Carlsbad, CA, United States of America); and interference RNA (siRNA) targeting TCPTP such as that described in US Patent Publication No 2004/0121353 (the entire content of which is to be regarded as incorporated herein by reference).

Preferably, the agent administered in the method of the first aspect specifically inhibits the activity or expression of the TC45 form of TCPTP. One specific example of such an agent is Compound 8 described in Zhang *et al.*¹¹ (and depicted at Figure 13A). Compound 8 is a competitive TCPTP inhibitor with a K_i of $4.3 \pm 0.2 nM$ and IC_{50} of $8.7 \pm 1.4 nM$ for TCPTP and a K_i of $34 \pm 2.8 nM$ for PTP1B¹¹.

Since the hypothalamus is "behind" the blood-brain barrier (BBB), the agent must be either adapted and/or formulated in a manner such that at least some of said agent crosses the BBB so that an effective amount is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP, or otherwise the agent must be administered to the brain of the subject (eg by intracerebral or intracerebroventricular administration through intracerebral implantation or convection-enhanced delivery). An agent that is adapted to cross the BBB may be inherently adapted to do so by its lipophilic

nature or is conjugated or modified in a manner to enable the agent to cross the BBB (eg by conjugating the agent to carrier-mediated transporters such as glucose and amino acid carriers, or by conjugating to a lipophilic moiety to increase BBB permeability). Alternatively, or additionally, an agent may be formulated in a manner to enable the agent to cross the BBB (eg by formulating the agent into nanoparticles as described in Silva, GA. *BMC Neuroscience* 9(suppl 3):S4 (2008)¹⁵(the entire content of which is to be regarded as incorporated herein by reference) or liposomes as described in International Patent Publication No WO 94/02178 (the entire content of which is to be regarded as incorporated herein by reference). Suitable strategies for enabling antisense oligonucleotides and siRNA targeted to TCPTP to cross the BBB, including conjugation to a cholesterol moiety (or similar lipophilic molecule) or a cell penetrating peptide, are described in Mathupala, SP. *Expert Opin Ther Pat* 19:137-140 (2009) (the entire content of which is to be regarded as incorporated herein by reference).

The agent may be provided in any suitable pharmaceutical composition (optionally including a pharmaceutically-acceptable carrier, diluent or excipient) and dosage form (eg for oral, buccal, nasal, intramuscular and intravenous administration). Typically, such a composition will be administered to the subject in an amount which is effective to achieve a desired therapeutic effect, and may therefore provide between about 0.01 and about 100 µg/kg body weight per day of the agent, and more preferably, from 0.05 and 25 µg/kg body weight per day of the agent. A suitable composition may be intended for single daily administration, multiple daily administration, or controlled or sustained release, as needed to achieve the most effective results. However, notwithstanding the above, it will be understood by persons skilled in the art that the administered amount and frequency of administration for any particular subject may be varied and will depend upon a variety of factors including the activity of the particular agent, the metabolic stability and length of action of the agent, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion of the agent, and the severity of the disease or condition to be treated.

Since TCPTP acts coordinately with the hypothalamic PTP1B, the agent may be used in combination with a PTP1B inhibitor; that is, an inhibitor of PTP1B activity and/or expression. Suitable PTP1B inhibitors are well known to persons skilled in the art and include, for example, PTP1B inhibitors which are small organic molecules such as Trodusquemine (MSI-1436; Genaera Corporation, Southampton, PA, United States of America) which is highly selective for PTP1B and is capable of crossing the BBB, certain phosphonic acid derivatives described in US Patent No 7,504,389, thiazole and thiadiazole derivatives described in US Patent No 7,381,736 (the entire content of which is to be regarded as incorporated herein by reference), derivatives of 1,4-bis(3-hydroxycarbonyl-4-hydroxyl)styrylbenzene as described by Shrestha *et al.* (2008)⁶², and such compounds that inhibit so-called hypothalamic endoplasmic reticulum (ER) stress associated with obesity¹⁷. Other suitable PTP1B inhibitors include antisense oligonucleotides (ASOs) targeting PTP1B such as those presently being developed by Isis

Pharmaceuticals^{63, 64}. The PTP1B inhibitor may be administered shortly before, shortly after or concurrently with the TCPTP inhibitor so as to achieve a combination therapy.

Alternatively or additionally, the agent may be used in combination with leptin. In particular, it has been reported that in some subjects having undergone a Roux-en-Y gastric bypass (RYGBP), plasma leptin levels are significantly reduced (relative to BMI-matched controls) suggesting that these subjects are in a "leptin sensitive" state⁶⁵. It is therefore anticipated that such subjects would achieve further weight loss when administered leptin, and accordingly, leptin and a TCPTP inhibitor may represent a useful combination therapy. The leptin may therefore be administered shortly before, shortly after or concurrently with the TCPTP inhibitor so as to achieve a combination therapy.

In a second aspect, the present invention provides a pharmaceutical composition comprising an agent which inhibits the activity or expression of TCPTP optionally in combination with a pharmaceutically-acceptable carrier, diluent or excipient, wherein said agent is adapted and/or formulated in a manner ensuring that, upon administration of the composition to a subject, an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

Preferably, said composition further comprises a PTP1B inhibitor as described above.

For use with subjects who have RYGBP and are in a leptin sensitive state, said composition may preferably further comprise leptin.

The composition may also further comprise other useful compounds and/or substances such as an appetite suppressing agents such as amphetamine, norepinephrine, serotonin and dopamine uptake modulators.

In a third aspect, the present invention provides a method for alleviating or preventing leptin resistance in an obese or overweight subject, said method comprising administering the subject with an agent which inhibits the activity or expression of TCPTP, wherein said agent is administered, adapted and/or formulated in a manner ensuring that an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

Since leptin resistance in obese or overweight subjects promotes eating, conservation of energy and development of hyperglycaemia, it can be extremely difficult for such subjects to lose weight through dieting. The present invention therefore offers a means to "sensitise" obese or overweight subjects to weight loss through dieting.

Thus, in a fourth aspect, the present invention provides a method for sensitising a subject to weight loss through dieting. the method comprising administering the subject with an agent which inhibits the activity

or expression of TCPTP, wherein said agent is administered, adapted and/or formulated in a manner ensuring that an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

- 5 In a fifth aspect, the present invention provides a dieting kit comprising foodstuffs and a pharmaceutical composition according to the second aspect.

The foodstuffs may be low fat and/or low in kiloJoules and may be provided in the form of ready made meals for a dietary program.

10

The invention is hereinafter described by way of the following non-limiting examples.

EXAMPLES

15 **Example 1 TCPTP expression in the hypothalamus**

In a preliminary study it had been found that TCPTP is expressed in the hypothalamus. Further, the results of that study indicated that the TC45 variant is relatively enriched in the hypothalamus when compared to TCPTP expression in the whole brain (Figure 10A). It has also been found that TCPTP is strongly expressed in both the ARC and VMN regions of the hypothalamus (Figure 10B). The study of
20 this example is designed to assess whether TC45 is expressed abundantly in leptin receptor isoform b (LRb)- and IR-expressing neurons of the ARC and gain insight into the neurons and hypothalamic regions in which TCPTP regulates leptin and insulin signalling.

Materials and Methods

25 Determining the effect of TCPTP expression on obesity

Since hypothalamic TCPTP expression is increased in response to diet-induced obesity similarly to that seen for SOCS3 (Figure 2) and previously reported for PTP1B¹⁴⁻¹⁶, SOCS3 and PTP1B are implicated in the development of hypothalamic leptin and insulin resistance. Given TCPTP's capacity to negatively regulate STAT3 and the IR^{33, 34} and the enhanced leptin sensitivity and leptin-induced STAT3
30 phosphorylation in BTKO mice, the present applicant considers that increases in TCPTP expression in the obese state promotes leptin and insulin insensitivity, and thus contributes to disease progression. To confirm this, hypothalamic TCPTP expression will be assessed in other rodent models of obesity including Ob/Ob (leptin deficient) and Db/Db (LRb mutant) mice by immunoblot analysis.

35 Next, the molecular mechanism by which obesity induces TCPTP expression will be investigated. Since it has previously been shown that interleukin 4 (IL-4) can promote TCPTP expression in a negative feedback loop for the suppression of STAT6 signalling, it has been hypothesised that TCPTP functions in a negative feedback loop to inhibit leptin signalling. This possibility is consistent with finding that

TCPTP levels are not increased in the peripheral tissues of high fat fed mice (data not shown) and the primary target tissue for leptin being the hypothalamus.

Accordingly, TCPTP expression will be assessed by immunoblot analysis in a Chinese hamster ovary (CHO) cell line that stably overexpresses the LRB⁵⁷ after leptin stimulation (100 ng/ml) for 0-24 h. If TCPTP expression is altered, the contribution of individual pathways will be assessed using pharmacological inhibitors (2 μ M CMP6 to inhibit JAK2, 100 nM wortmannin to inhibit PI3K, or 50 μ M PD98059 to inhibit MAPK ERK1/2 activation) or by the specific stable knockdown of STAT3 by RNA interference (RNAi) using short-hairpin RNA (shRNA). The impact of leptin on TCPTP mRNA v/s protein stability will also be assessed by real-time PCR ($\Delta\Delta$ Ct) and 35S-methionine pulse-chase labelling respectively⁶¹. Subsequently, whether or not leptin enhances TCPTP expression *in vivo* will be investigated by administering leptin (1-2 μ g/g body weight/day) to wild type C57BL/6 mice over 3-5 days. If the hypothesis is correct, it will be expected that there will be no difference in TCPTP expression in Ob/Ob mice. However, if TCPTP expression is increased in Ob/Ob mice, then the impact of fatty acids (0.25 mM palmitate C16:0), hyperglycemia (25 mM glucose), hyperinsulinemia (1 μ M insulin) or inflammatory cytokines such as TNF (50 ng/ml TNF) on TCPTP expression (12-72 h) will be determined.

Determining TCPTPs role in insulin and leptin sensitivity using BTKO mice

It has been shown that TCPTP (TC48 and TC45) is expressed in the hypothalamus (Figures 3 and 10) and that STAT3 and the IR can serve as TCPTP substrates^{33, 34}. Further, the applicant's preliminary studies indicate that BTKO mice exhibit reduced body weight and food intake, increased energy expenditure and leptin sensitivity and enhanced leptin-induced hypothalamic STAT3 phosphorylation (Figures 4-6). Further, following HFF diet, BTKO mice showed an enhanced level of weight loss as compared to control (*loxlox*) mice. To confirm that TCPTP regulates hypothalamic IR and/or STAT3 signalling to control body weight and glucose homeostasis, the following experiments will be conducted:

1. Recognition of STAT3 as a substrate in response to leptin

TC45's capacity to dephosphorylate STAT3, as opposed to JAK2 or indeed the LRB in response to leptin, will be assessed initially by determining whether transiently overexpressed TC45 (Lipofectamine 2000; Invitrogen Corporation, San Diego, CA, United States of America) suppresses leptin induced LRB (pY1138; binds STAT3; Santa Cruz Biotechnology Inc, Santa cruz, CA, United States of America), JAK2 (pY1007/pY1008; BioSource International Inc, Camarillo, CA, United States of America) or STAT3 (pY705; Cell Signalling Technology, Inc, Danvers, MA, United States of America) phosphorylation in response to leptin (10ng/ml) in LRB-CHO or SH-SY5Y cells. Further, the ability of the ER-targeted TC48 to regulate leptin signalling will be assessed. In addition, a determination will be made of whether the corresponding "substrate trapping" mutant forms of TCPTP (ie D182A), which have the capacity to form stable complexes with tyrosine phosphorylated substrates in a cellular context and prevent dephosphorylation of substrates by endogenous PTPs^{57, 71}, enhance leptin-induced LRB, JAK2 or STAT3

phosphorylation in cell lysates by immunoblot analysis using phosphorylation-specific antibodies. Also, it will be determined as to whether the trapping mutants have the capacity to form stable complexes with LRb, JAK2 or STAT3 in TCPTP immunoprecipitates and whether or not these complexes occur in the nucleus or cytoplasm (eg by using immunofluorescence microscopy). Moreover, an assessment will be made to determine whether TCPTP stable knockdown by RNAi in SH-SY5Y cells using TC45 and TC48, or TC48-specific shRNA lentiviral particles results in enhanced leptin-induced LRb, JAK2 or STAT3 phosphorylation. Finally, it will be determined whether transient PTP1B knockdown by RNAi using small-interfering RNAs (Dharmacon, Inc, Lafayette, CO, United States of America) in control v/s TCPTP stably knocked-down cells results in additive increases leptin signalling as described previously^{59, 60}

2. Regulation of leptin-induced hypothalamic signalling

Using serum starved CHO-ObR cells left unstimulated or stimulated with recombinant murine leptin (10ng/ml) for 0-12 h, it has been found, following immunoblot analysis as quantified by densitometry, that TCPTP expression is induced by leptin *in vitro* (Figure 11 A and B). This has also been confirmed *in vivo* using C57BL/6 mice fasted for 18 h, left untreated or injected with leptin (5 µg/g BW, intraperitoneal (*ip*)), wherein the hypothalami were thereafter extracted and processed for real-time PCR ($\Delta\Delta Ct$) to measure *Ptpn2* expression (Figure 11C). Further, it has been found that leptin sensitivity and leptin-induced STAT3 phosphorylation are increased in BTKO mice. Further characterisation of these mice will allow for a more definitive/thorough assessment of the pathways modulated and the hypothalamic regions and neurons in which TCPTP may function to regulate leptin signalling. Accordingly, this experiment will assess TCPTP's potential to regulate leptin-induced LRb, JAK2 and STAT3 phosphorylation and signalling *in vivo* using BTKO v/s *TCPTPloxlox* control mice.

8-12 week old male BTKO and control mice will be fasted (18 h) and then injected *ip* with saline or murine leptin (1-2 µg/g; PeproTech Inc, Rocky Hill, NJ, United States of America) and hypothalami isolated at 15-60 min and processed in RIPA buffer for immunoblot analysis monitoring for LRb Y1138, JAK2 Y1007/Y1008 and STAT3 Y705 phosphorylation. Leptin-induced PI3K/Akt signalling (Akt S473 phosphorylation) and the suppression of AMPK activity (T172 phosphorylation) will also be monitored by immunoblot analysis since these pathways may occur downstream of JAK2 but in parallel to STAT3; it is expected that STAT3 but not PI3K/Akt or AMPK will be altered. STAT3 phosphorylation will also be examined by IHC using hypothalamic sections after intracerebroventricular (*icv*) administration of leptin (0.01-0.1 µg; 15-45 min; in 1 µl) or aCSF. To measure leptin *icv*-induced responses (0.01-0.1 µg; 45-60 min), the expression of the orexigenic *Npy* and *Agrp*, which are elevated after fasting and suppressed by leptin, and the anorexigenic neuropeptide precursor *Pomc1*, which is induced by leptin, will be assessed by quantitative ($\Delta\Delta Ct$) real time PCR (TaqMan™ Gene Expression; Applied Biosystems, Foster City, CA, United States of America). If necessary, hypothalamic sections could be incubated with leptin and neuropeptide secretion measured by radioimmunoassay (RIA). Leptin/STAT3 signalling promotes POMC and suppresses AgRP but other leptin pathways regulate NPY⁴⁸. Accordingly, if TCPTP

acts exclusively via STAT3, it will be expected that POMC/AgRP responses will be exacerbated, but NPY responses will be unaltered and, indeed, this was observed in preliminary experiments where mice were injected with leptin and the mRNA levels of POMC, AgRP and NPY assessed using PCR. The results shown in Figure 12 indicate that TCPTP deficiency enhances the effects of leptin on the expression of POMC and AgRP (but not NPY) which mediate leptin's effects on food intake, energy expenditure and glucose homeostasis.

3. *Regulation of insulin-induced hypothalamic signalling*

To assess TCPTP's potential to regulate hypothalamic insulin signalling, IR β Y1162/Y1163 phosphorylation (target site of TCPTP) and PI3K/Akt signalling (Akt pS473) will be monitored in isolated hypothalami from 8-12 week old chow fed BTKO and control mice (*TCPTP^{lox/lox}*) by immunoblot analysis after IP saline or insulin (0-120 min, 1mU/g) administration in 18 h fasted mice. Insulin-induced PI3K/Akt signalling will also be examined after *icv* administration of aCSF or insulin (10-50 nM; 15-45 min) by IHC staining for pAkt (pS473) in hypothalamic sections and by assessing hypothalamic *Npy*, *Agrp* and *Pomc1* expression by real time PCR (after 45-120 min of *ip* 1.0 mU/g insulin). Since insulin can act in AgRP neurons to induce hepatic interleukin 6 (IL-6) release (from Kupffer cells) and STAT3 phosphorylation in hepatocytes for the suppression of hepatic glucose output, hypothalamic IR β Y1162/Y1163 phosphorylation and PI3K/Akt signalling will be correlated with hepatic STAT3 (pY705) phosphorylation by immunoblot analysis and liver *IL-6* expression by quantitative ($\Delta\Delta C_t$) real time PCR (TaqMan™ Gene Expression) 2-3 h after *icv* administration of insulin (0-50 nM); hepatic *IL6* levels and STAT3 phosphorylation should be increased if hypothalamic insulin signalling is elevated³⁷. Increased hepatic STAT3 signalling results in reduced gluconeogenic gene expression [glucose-6 phosphatase (*G6pc*), PEPCCK (*Pck1*), fructose-1-6-bisphosphatase (*Fbp1*)]³⁶ and this will also be monitored by real-time PCR (TaqMan™ Gene Expression).

4. *The effect of neuronal TCPTP deficiency on DIO and insulin sensitivity*

Preliminary studies have indicated that hypothalamic TCPTP expression is increased by high fat diet-induced obesity, and that neuronal TCPTP deficiency enhances hypothalamic leptin sensitivity in chow fed mice. Accordingly, it is considered that increases in hypothalamic TCPTP expression contributes to the development of obesity and the associated central leptin/insulin resistance, peripheral glucose intolerance and insulin resistance. In particular, and while not wishing to be bound by theory, the present applicant believes that elevated hypothalamic TCPTP levels contribute to the development of obesity and type 2 diabetes. To confirm this, an investigation will be undertaken to determine whether TCPTP-deficiency protects from the development of DIO and insulin resistance. In particular, male v/s female BTKO and control (*TCPTP^{lox/lox}*) mice will be housed individually after weaning (3 weeks) and fed a high fat diet (23% fat; 45% energy from fat) for 6, 12 and 20 wks; body weights and food intake will be monitored and body composition (lean and fat mass) measured using a dual energy X-ray absorptiometry (DEXA) instrument. The results obtained will be correlated with liver and fat pad masses (epididymal,

infrarenal, subcutaneous, brown adipose tissue), liver and muscle triglycerides (GPO-Trinder, Sigma Aldrich Corporation, St Louis, MO, United States of America) and serum glucose, insulin (RIA; Linco Research, Inc, St Charles, MO, United States of America), FFAs (NEFA-Kit-U, Wako Chemicals USA Inc, Richmond, VA, United States of America) and adipokines (leptin, adiponectin, resistin (Linco), TNF (R&D Systems, Inc, Minneapolis, MN, United States of America) by ELISA) from fasted (18 h) and fed mice. The impact of TCPTP deficiency on glucose homeostasis will be measured using insulin (ITTs) and glucose (GTTs) tolerance tests and insulin sensitivity (glucose infusion rate) and glucose turnover determined in anaesthetised mice during fasting and hyperinsulinaemic euglycaemic clamps. Metabolic measurements for activity and energy expenditure will be performed using a 12 cage environmentally controlled CLAMS. The effect of neuronal TCPTP deficiency on obesity-induced central leptin resistance in mice fed a high fat diet for 6, 12 or 20 weeks will also be assessed by determining the effects of leptin on food intake and body weight; food intake and body weight baselines will be established and leptin (0.5-1.0 µg/g) administered *ip* twice daily for 3 days with food intake and body weights being monitored daily for up to 7 days as shown in Figure 6. These results will be correlated with an assessment of leptin-induced STAT3 signalling. Finally, recent studies have indicated that leptin sensitivity and glucose tolerance are restored after changing high fat fed mice to a standard chow diet, so accordingly, it will be assessed as to whether BTKO mice have any advantage in the loss of body weight/adiposity and the restoration of leptin and whole body insulin sensitivity when high fat fed (23% fat; 45% energy from fat) obese mice (20 weeks) are returned to a chow diet (4.6 % energy from fat). It is predicted that high fat fed BTKO will exhibit obesity resistance and maintain insulin/leptin sensitivity, or have delayed onset of obesity and insulin/leptin resistance as well as enhanced restoration of normal body weight and glucose homeostasis due to elevated hypothalamic insulin and leptin signalling.

Results from preliminary experiments using 8-10 week old BTKO and *lox/lox* male littermates that were fed a high fat diet for 12 weeks are shown at Figure 14: (A) body weights were determined weekly; (B) Leptin sensitivity on body weight measured (mice were injected *ip* with leptin (2µg/g body weight/day) during the indicated period and body weights determined; (C) Mice were fasted 4 h and insulin tolerance tests performed (0.5 mU insulin/g BW); and (D) Mice were fasted for 6 h and blood glucose and plasma insulin levels were measured. The results showed that TCPTP deficiency protects mice from the development of leptin resistance and obesity and the development of insulin resistance.

5. *Ascertaining the effect of a TCPTP inhibitor on body weight and energy expenditure*

Preliminary experiments were conducted using Compound 8 (described in Zhang *et al.*¹¹), a TCPTP inhibitor, to assess the effect of inhibiting TCPTP on the body weight and energy expenditure of mice.

Mice were administered with Compound 8 (1.5 µl, 0.2 nmol) or aCSF (1.5 µl) by injecting into the lateral ventricle of C57BL/6 or BTKO mice by intracerebral injection (*icv*), along with *ip* administration of leptin (1.0 µg/g) or PBS as a control. The results shown at Figure 13 indicate that Compound 8 enhances

the effects of leptin on STAT3 phosphorylation (Figure 13 A), and enhances the effects of leptin in suppressing body weight (Figure 13 B) and increasing energy expenditure (Figure 13 C). The results shown in Figure 13 D indicate that Compound 8 has no effect on the body weight of BTKO mice demonstrating that this compound specifically inhibits TCPTP (ie without inhibiting PTP1B).

5

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

10

All publications mentioned in this specification are herein incorporated by reference. Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia or elsewhere before the priority date of each claim of this application.

15

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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CLAIMS

1. A method for treating a disease or condition associated with elevated hypothalamic T cell protein tyrosine phosphatase (TCPTP) in a subject, said method comprising administering the subject
5 with an agent which inhibits the activity or expression of TCPTP, wherein said agent is administered, adapted and/or formulated in a manner ensuring that an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

2. The method of claim 1, wherein the agent specifically inhibits the activity or expression of
10 TCPTP variant TC45.

3. The method of claim 1 or 2, wherein the disease or condition is selected from the group consisting of obesity, weight gain, type 2 diabetes mellitus, insulin sensitivity, impaired glucose tolerance, and inflammation.

4. The method of claim 1 or 2, wherein the disease or condition is diet-induced obesity (DIO).

5. The method of any one of claims 1 to 4, wherein the method further comprises administering a
20 PTP1B inhibitor.

6. The method of claim 1 or 2, wherein the disease or condition is reduced plasma leptin levels associated with a Roux-en-Y gastric bypass (RYGBP).

7. The method of claim 6, wherein the method further comprises administering leptin.

8. The method of any one of claims 1 to 7, wherein the agent is adapted and/or formulated in a manner such that at least some of said agent, when administered to the subject, crosses the blood-brain barrier.

9. A pharmaceutical composition comprising an agent which inhibits the activity or expression of TCPTP optionally in combination with a pharmaceutically-acceptable carrier, diluent or excipient, wherein said agent is adapted and/or formulated in a manner ensuring that, upon administration of the composition to a subject, an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

10. The composition of claim 9, wherein the agent specifically inhibits the activity or expression of TCPTP variant TC45.

11. The composition of claim 9 or 10, wherein the composition further comprises a PTP1B inhibitor.

12. The composition of claim 11, wherein the composition further comprises leptin.

13. The composition of any one of claims 9 to 12, wherein the agent is adapted and/or formulated in a manner such that at least some of said agent, when administered to the subject, crosses the blood-brain barrier.

14. A method for alleviating or preventing leptin resistance in an obese or overweight subject, said method comprising administering the subject with an agent which inhibits the activity or expression of TCPTP, wherein said agent is administered, adapted and/or formulated in a manner ensuring that an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

15. A method for sensitising a subject to weight loss through dieting, the method comprising administering the subject with an agent which inhibits the activity or expression of TCPTP, wherein said agent is administered, adapted and/or formulated in a manner ensuring that an effective amount of said agent is delivered to the hypothalamus and inhibits the activity and/or expression of hypothalamic TCPTP.

16. The method of claim 14 or 15, wherein the agent specifically inhibits the activity or expression of TCPTP variant TC45.

17. The method of any one of claims 14 to 16, wherein the method further comprises administering a PTP1B inhibitor.

18. The method of any one of claims 14 to 17, wherein the agent is adapted and/or formulated in a manner such that at least some of said agent, when administered to the subject, crosses the blood-brain barrier.

19. A dieting kit comprising foodstuffs and a pharmaceutical composition according to any one of claims 9 to 13.

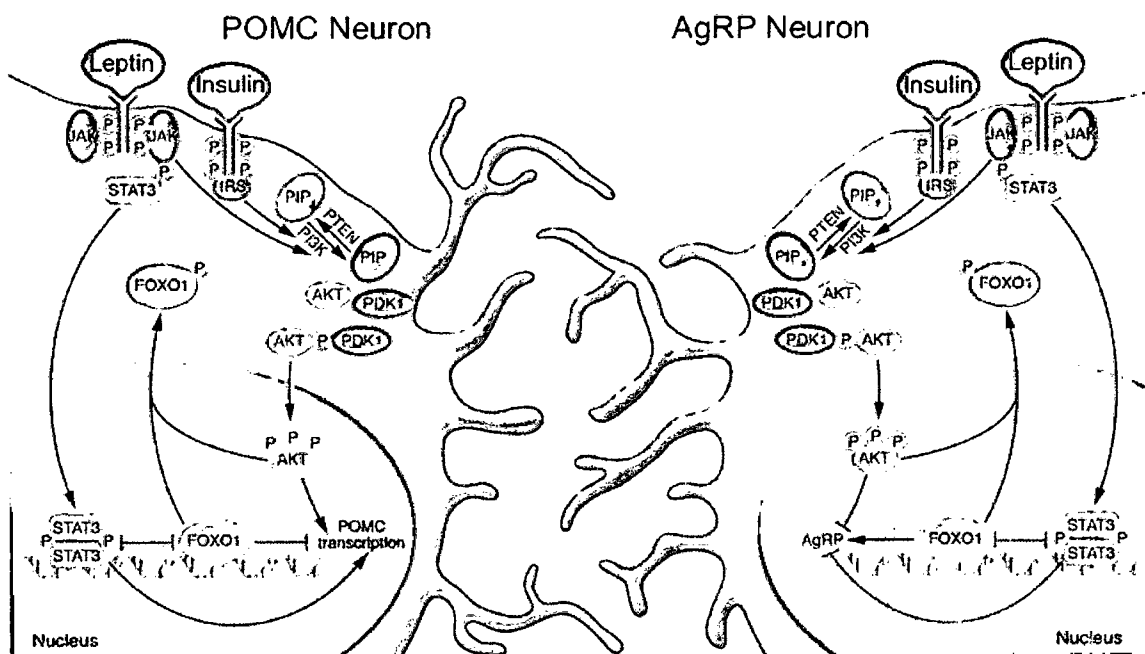


FIGURE 1

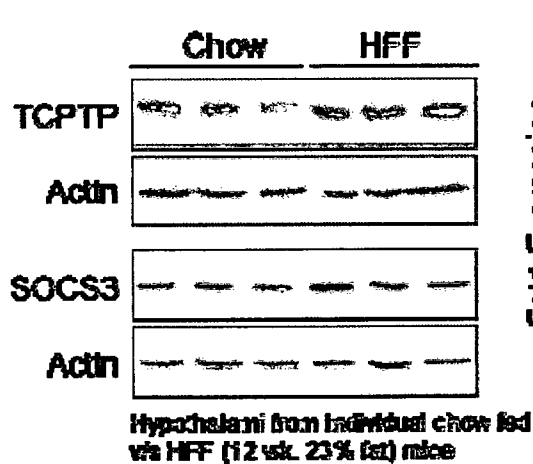


FIGURE 2A

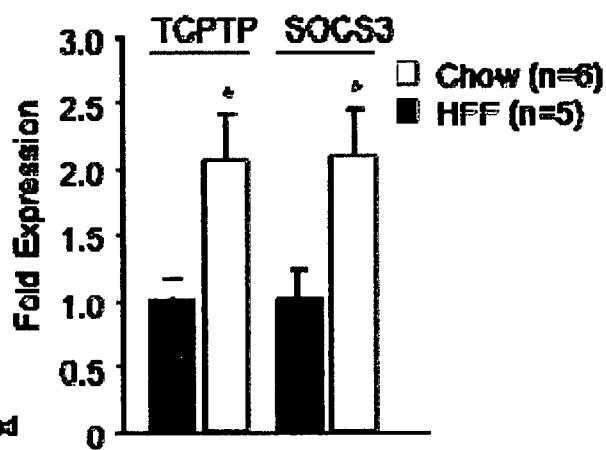


FIGURE 2B

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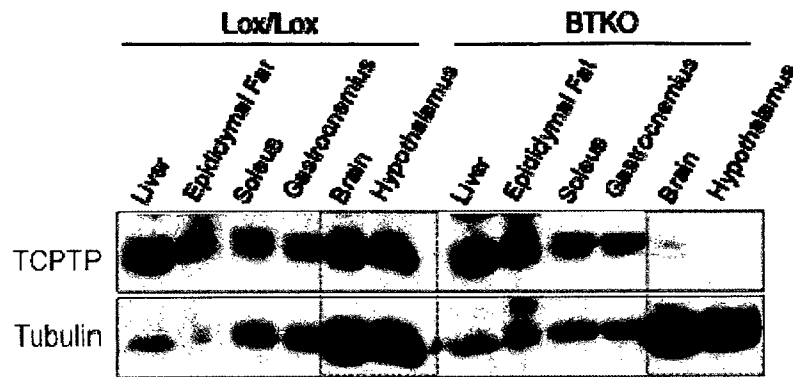


FIGURE 3

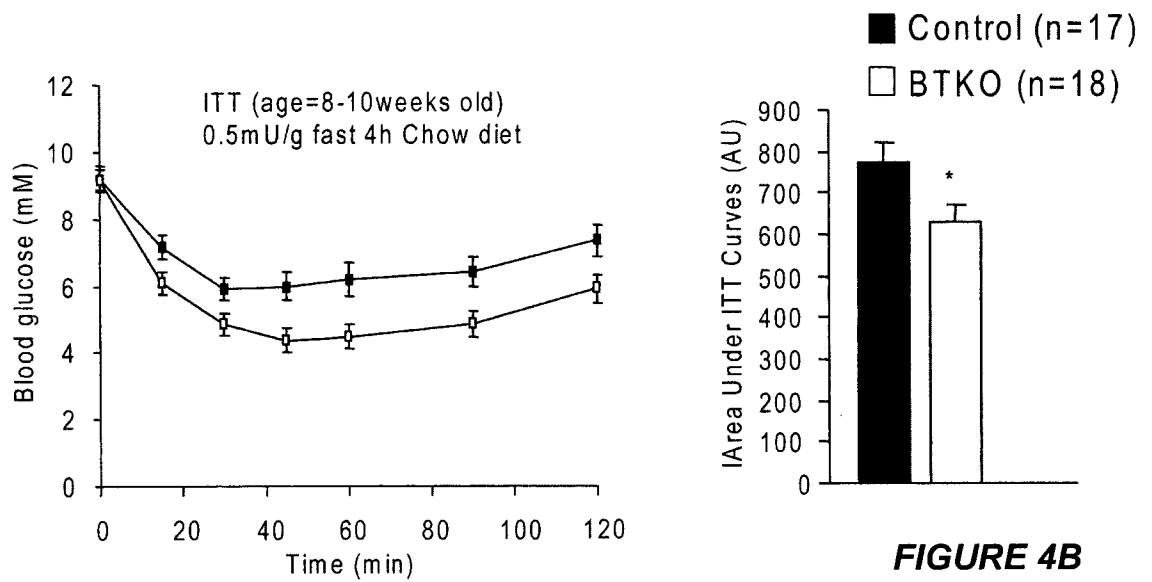


FIGURE 4B

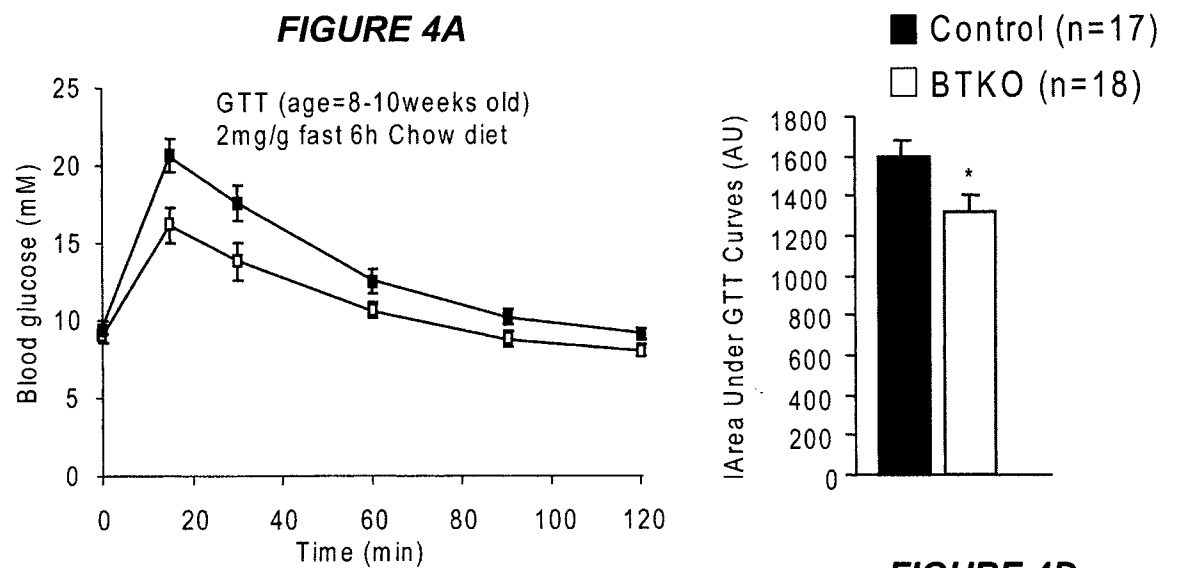
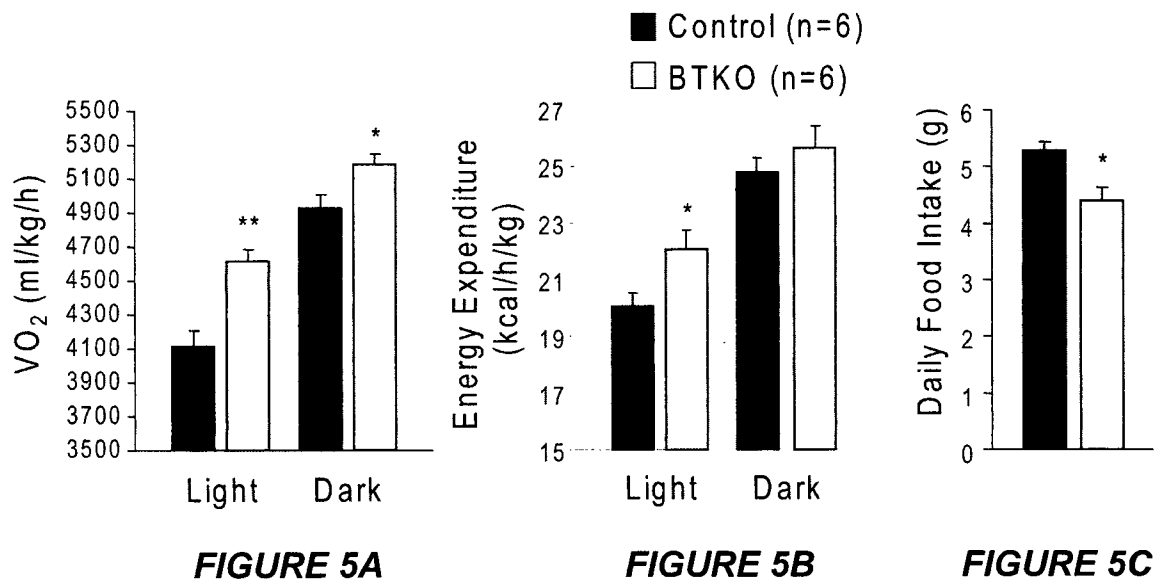
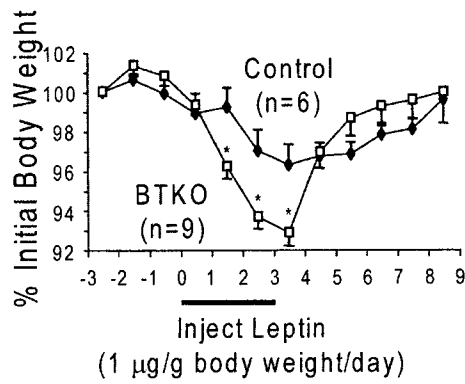


FIGURE 4D

FIGURE 4C



A) Effect of Leptin on Body Weight



B) Leptin Signalling

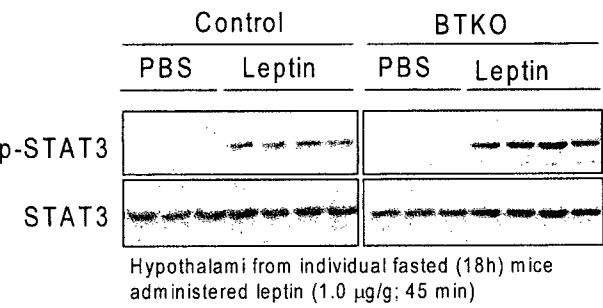


FIGURE 6

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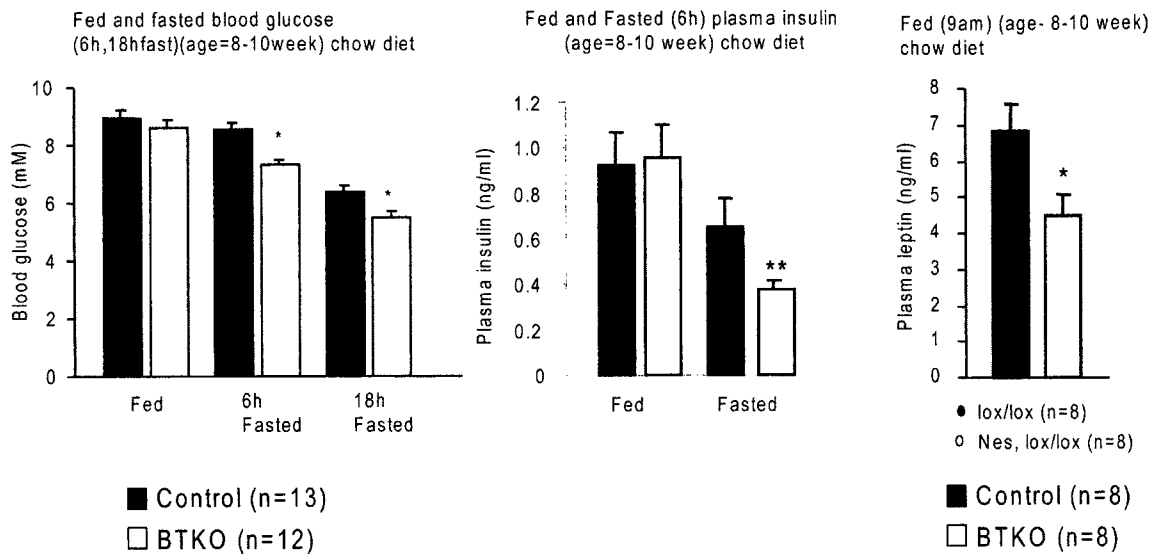


FIGURE 7A

FIGURE 7B

FIGURE 7C

Hypothalamic Signalling & Body weight Control

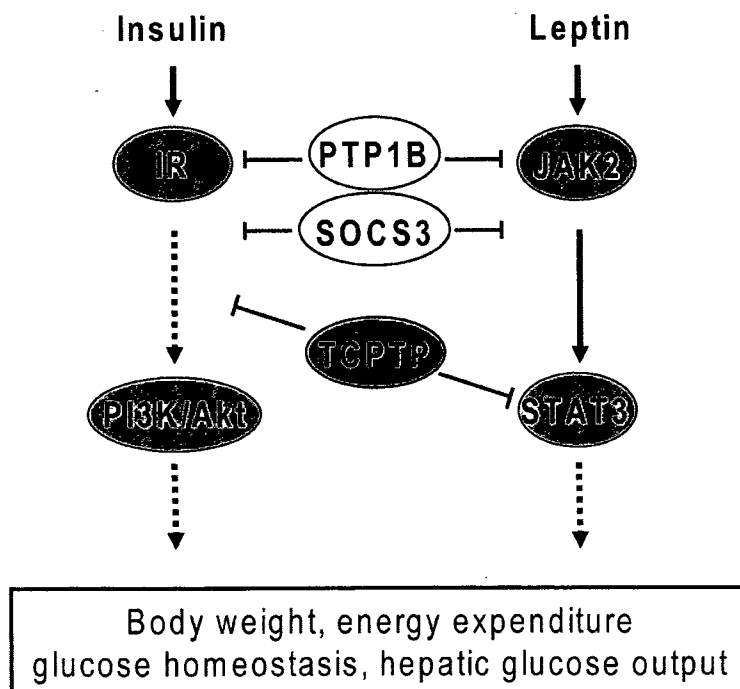


FIGURE 8

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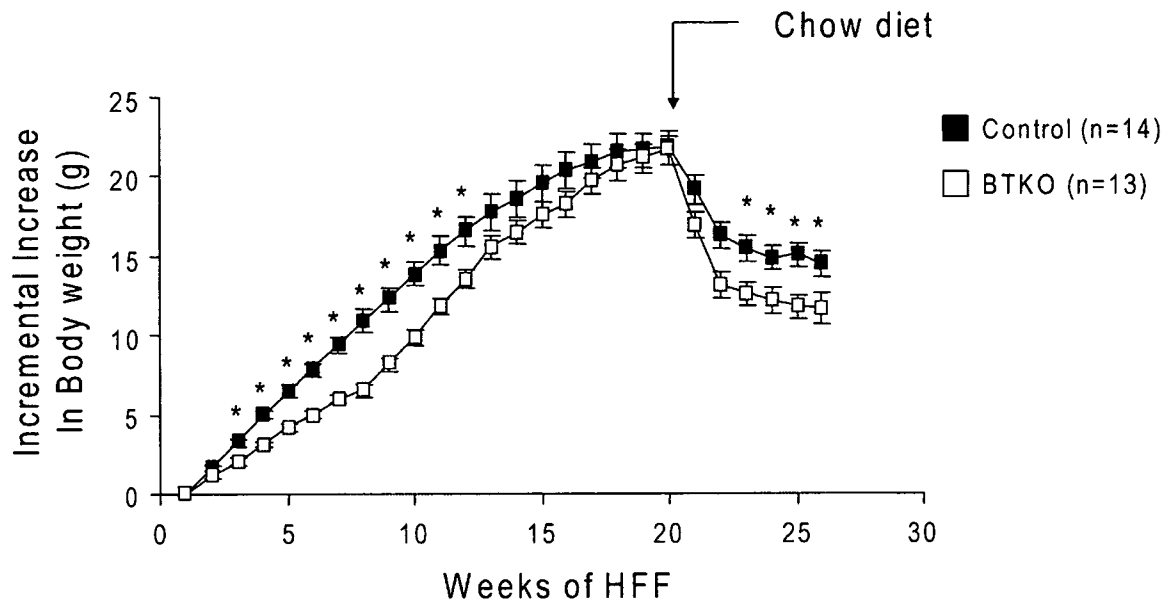


FIGURE 9

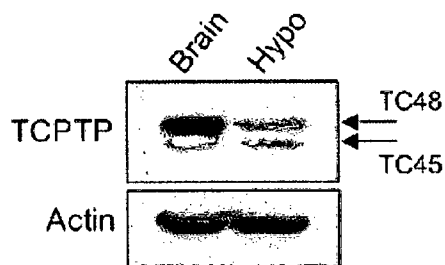


FIGURE 10A

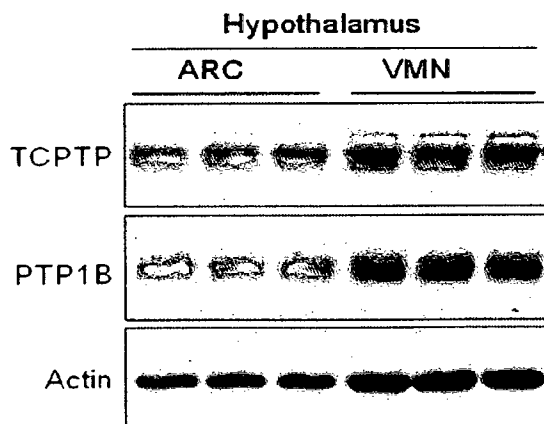
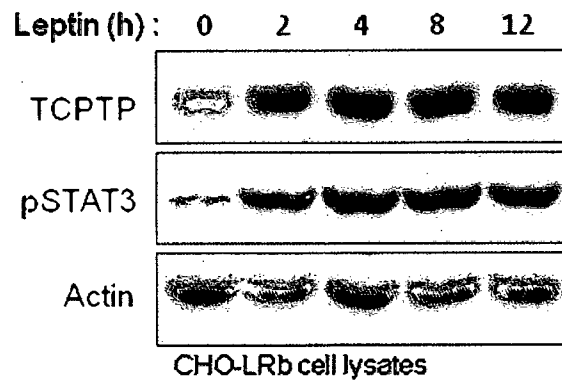
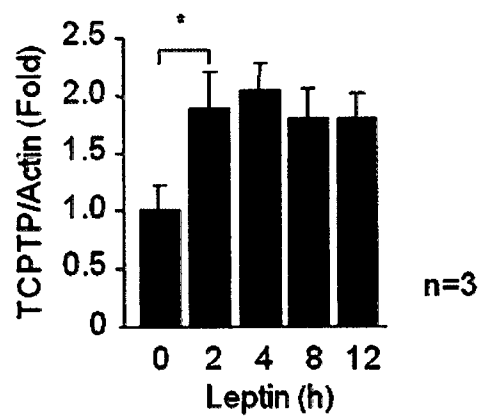
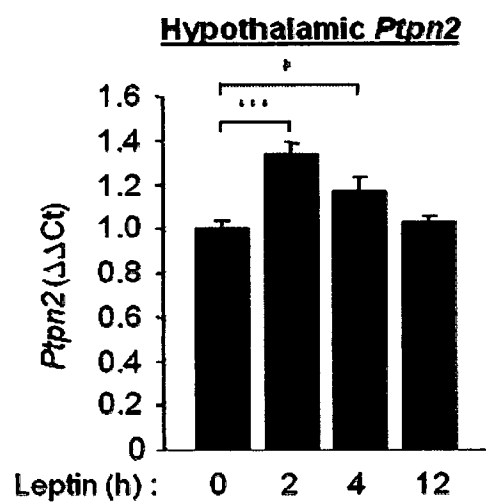


FIGURE 10B

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**FIGURE 11A****FIGURE 11B****FIGURE 11C**

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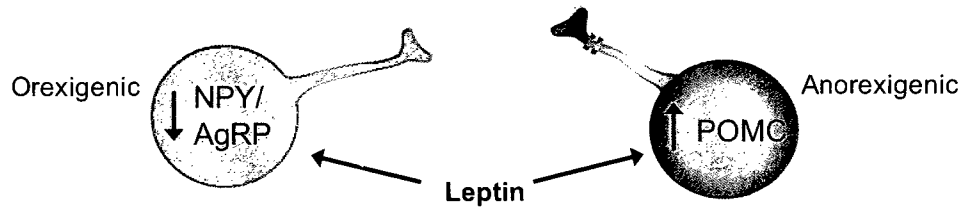


FIGURE 12A

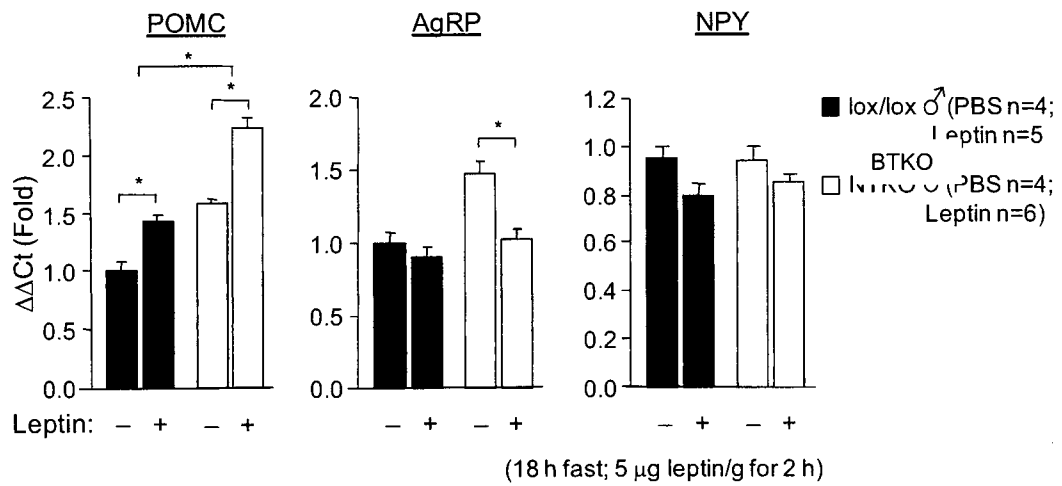
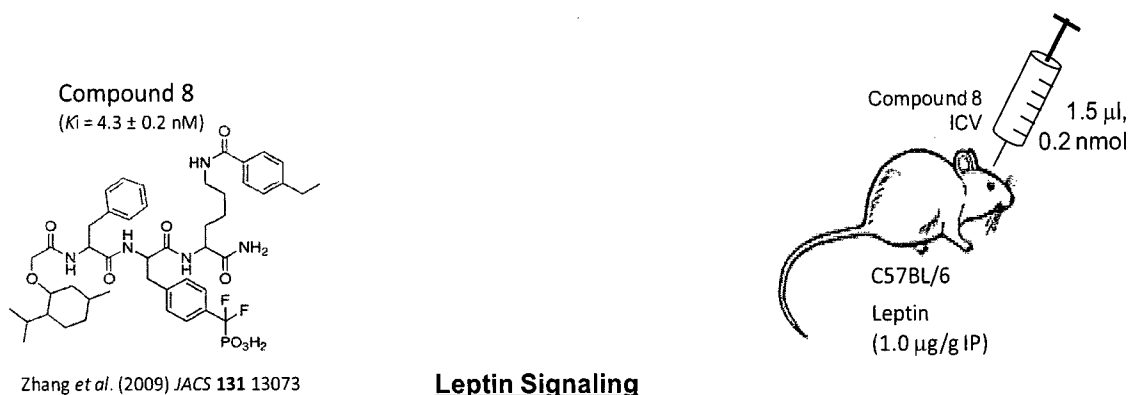
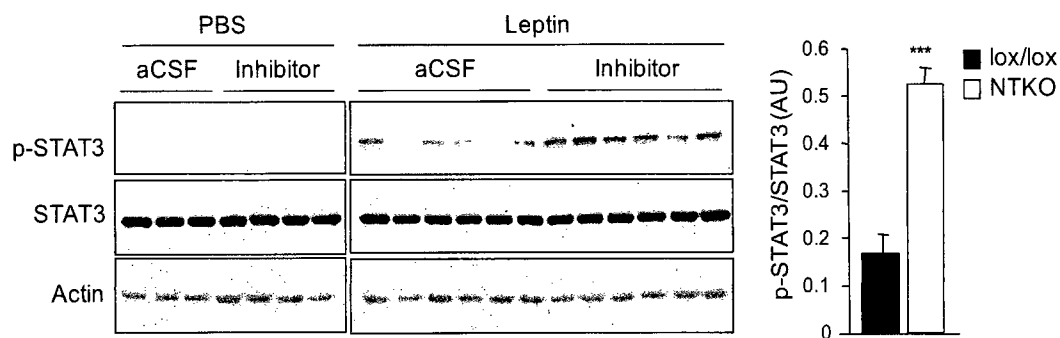
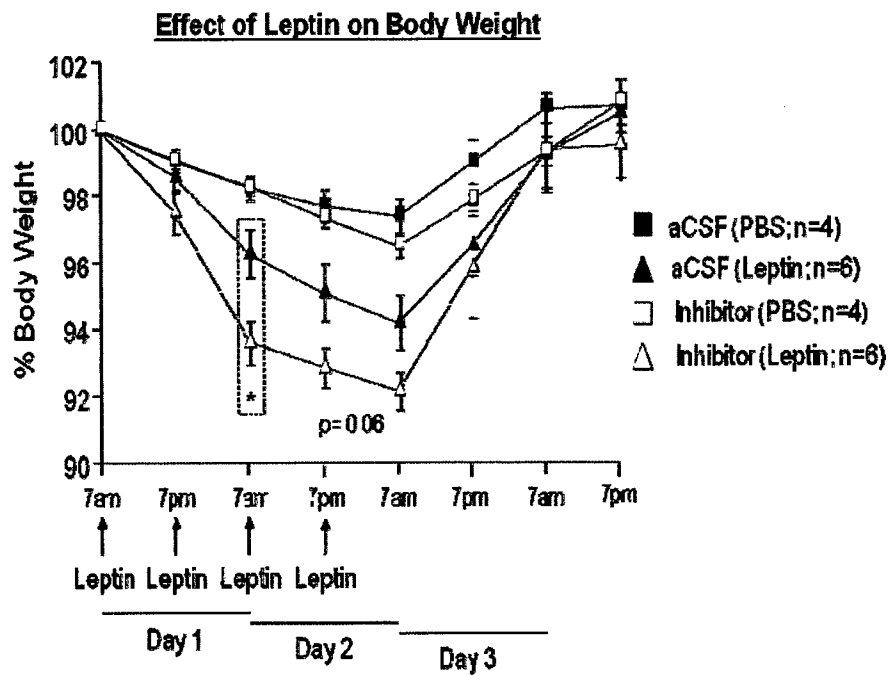


FIGURE 12B

FIGURE 12C

FIGURE 12D

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**Leptin Signaling****FIGURE 13A****FIGURE 13B**

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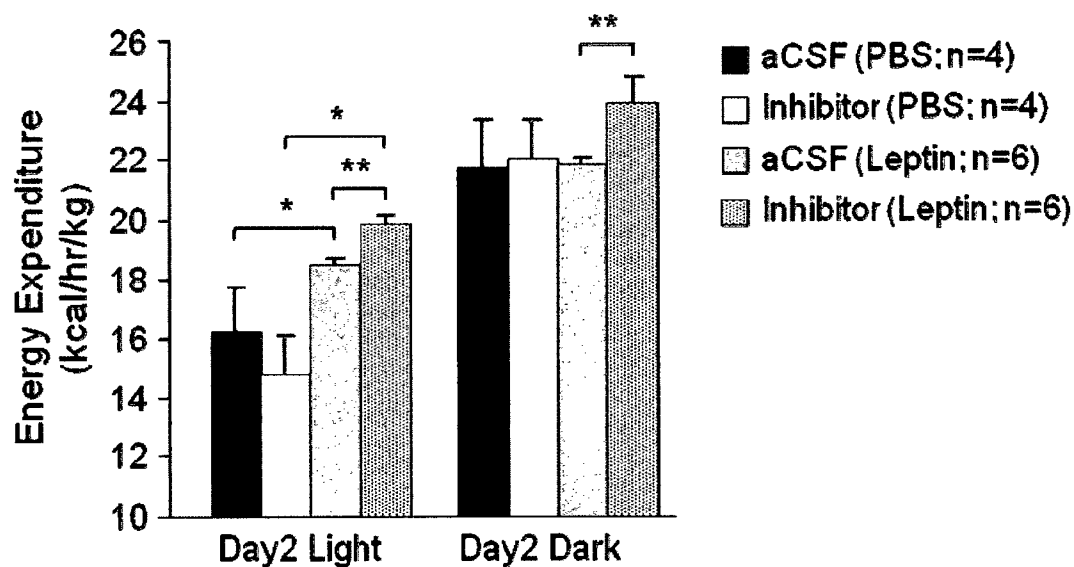


FIGURE 13C

Effect of Inhibitor on Leptin-Induced Changes in Body Weight in lox/lox control and BTKO mice

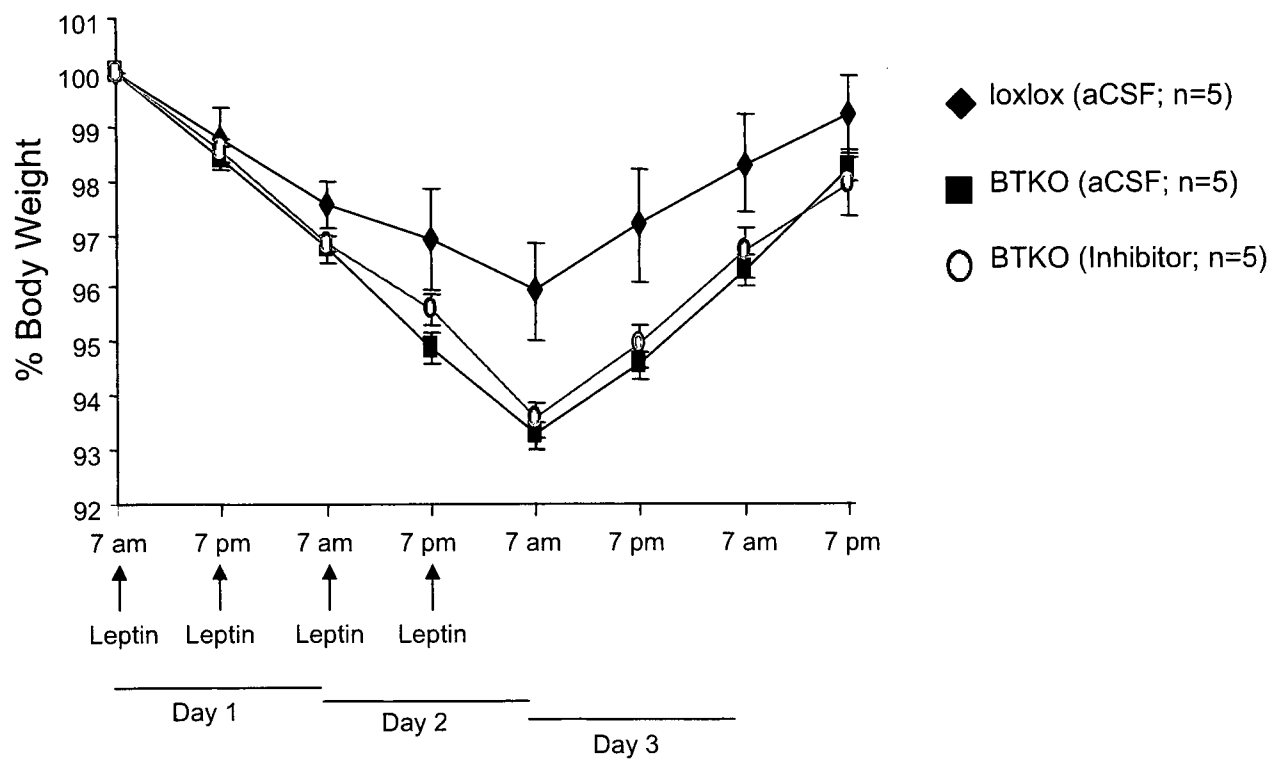


FIGURE 13D

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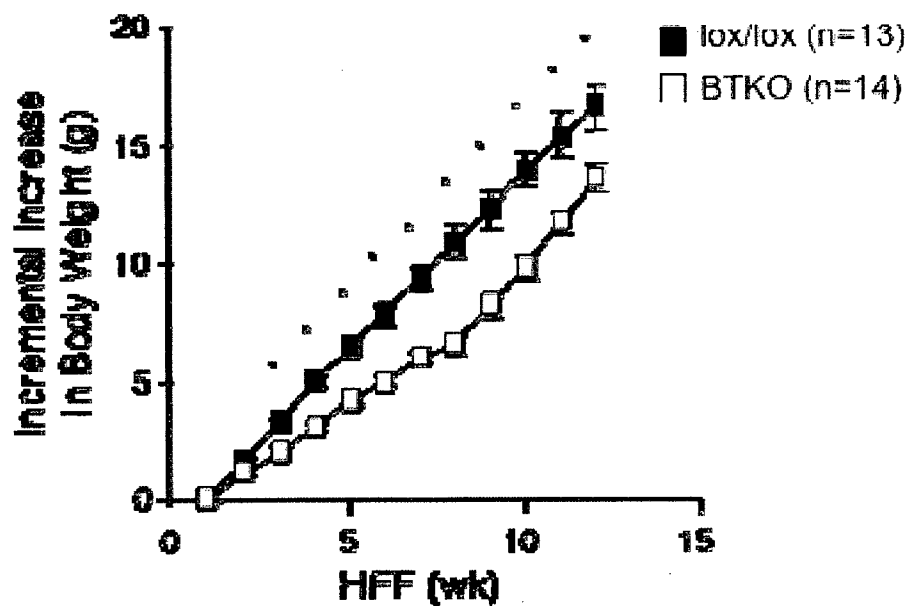


FIGURE 14A

Effect of Leptin on Body Weight

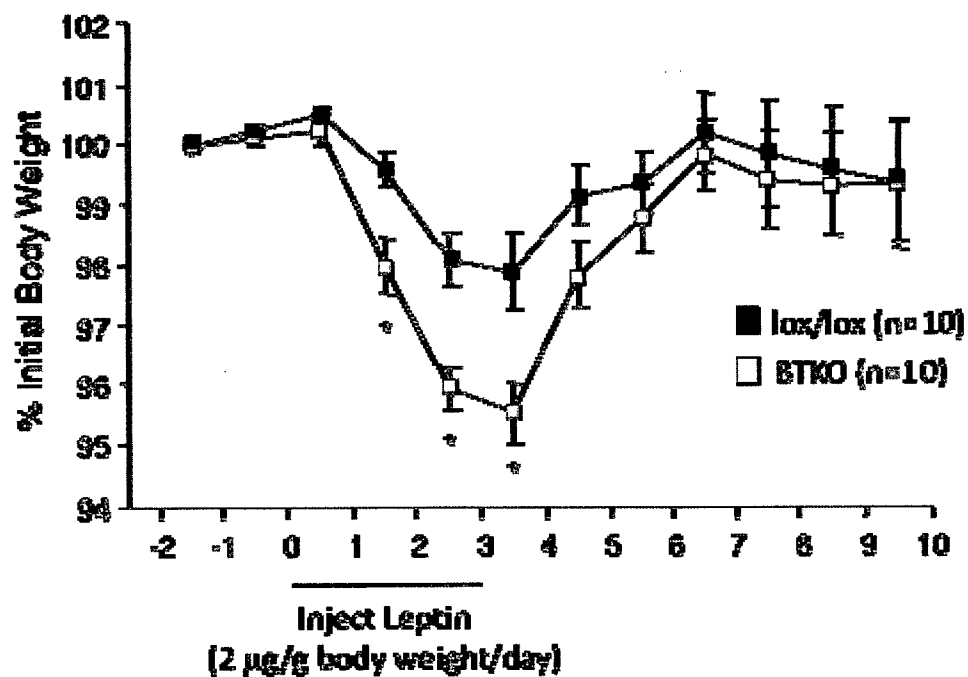


FIGURE 14B

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Insulin Tolerance Tests

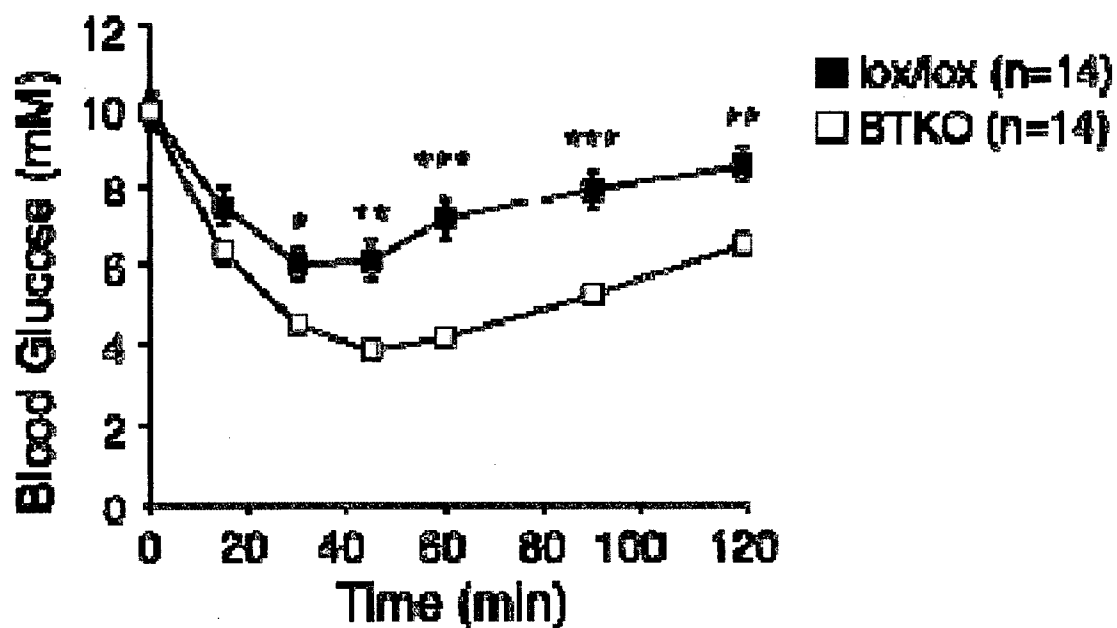


FIGURE 14C

Fed and Fasted Blood Glucose and Insulin

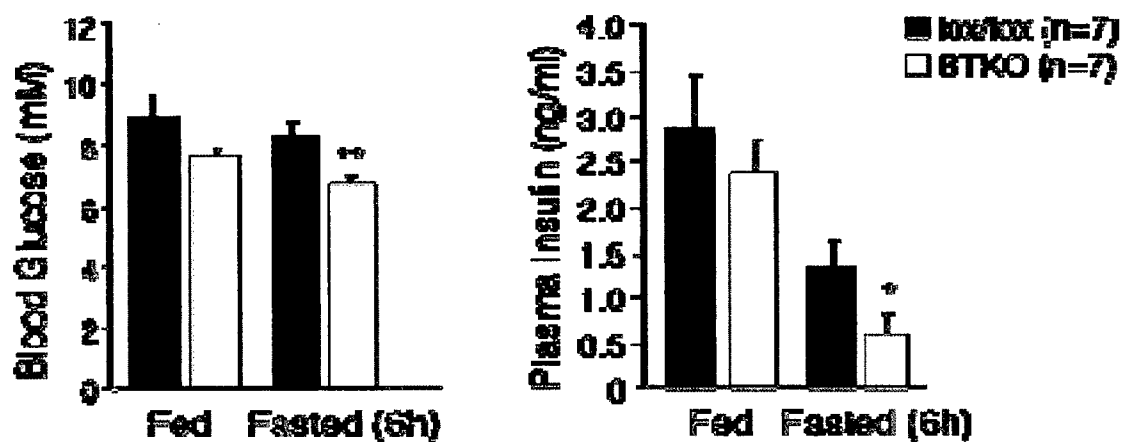


FIGURE 14D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2010/001501

A. CLASSIFICATION OF SUBJECT MATTER												
Int. Cl.												
<i>A61P 3/04</i> (2006.01) <i>A61K 31/66</i> (2006.01) <i>A61K 31/166</i> (2006.01) <i>A61P 3/10</i> (2006.01)												
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)												
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 practicable, search terms used)												
Medline, EPODOC and WPI. Keywords: protein tyrosine phosphatase, t cell, Tc-ptp, tc45, tc48, ptpn2, ptn2, Anti-body, inhibitor, antagonist, modulator, difluoromethyl phosphonic, obesity, diabetes, inflammation, insulin sensitivity, glucose tolerance, gastric bypass, plasma leptin and like terms												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
X	US 7,504,389 B2 (BLASKOVICH et al) 17 March 2009 See whole document, in particular abstract; col.4, lines 47-57; col.9, lines 14-21; col.21, lines 39-56; col.23, lines 4-33 and lines 42-47	1-19										
X	US 2004/0121353 A1 (LEWIS et al) 24 June 2004 See whole document, in particular [0017], [0027], [0058], [0099] and [0101]	1-4, 6-10, 13-16, 18 and 19										
Y	As above	5, 11, 12 and 17										
☒ Further documents are listed in the continuation of Box C ☒ See patent family annex												
* Special categories of cited documents: <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 09 December 2010		Date of mailing of the international search report 14 DEC 2010										
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. +61 2 6283 7999		Authorized officer EDWINA VANDINE AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6225 6113										

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RAO, G et al. "In Silico Structure-Based Design of a Potent and Selective Small Peptide Inhibitor of Protein Tyrosine Phosphatase 1B, A Novel Therapeutic Target for Obesity and Type 2 Diabetes Mellitus: A Computer Modeling Approach". JOURNAL OF BIOMOLECULAR STRUCTURE & DYNAMICS, 2006. Vol. 23, no.4, pages 377-384 See whole document, in particular Abstract and Conclusion	5, 11, 12 and 17
A	ZHANG, S et al. "Acquisition of a Potent and Selective TC-PTP Inhibitor via a Stepwise Fluorophore-Tagged Combinatorial Synthesis and Screening Strategy". JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, 2009, September. Vol.131, no.36, pages 13072-13079. See whole document, in particular Abstract; page 13072, right col. and page 1307, right col.	
A	SHI, L et al. "Corosolic Acid Stimulates Glucose Uptake via Enhancing Insulin Receptor Phosphorylation". EUROPEAN JOURNAL OF PHARMACOLOGY, 2008. Vol.584, no.1, pages 21-29. See whole document, in particular Abstract and page 28, last paragraph	
A	ZHANG, W et al. "Ursolic Acid and its Derivative Inhibit Protein Tyrosine Phosphatase 1B, Enhancing Insulin Receptor Phosphorylation and Stimulating Glucose Uptake". BIOCHIMICA ET BIOPHYSICA ACTA, 2006. Vol.1760, pages 1505-1512 See whole document, in particular page 1509: 3.3. Characterization of ursolic acid and its derivative	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2010/001501

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
US	7504389	AU	2005306612	BR	PI0518013	CA	2586363
		CN	101094677	EP	1812012	US	2006142250
		US	2008161592	US	7829737	US	2009131374
		WO	2006055525				
US	2004121353	AU	2003239897	AU	2003283951	CA	2525976
		EP	1546379	US	2004077574	US	7399586
		US	2004009946	WO	03099227	WO	2004016735
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							