PPAR γ agonism increases rat adipose tissue lipolysis, expression of glyceride lipases, and the response of lipolysis to hormonal control

W. T. Festuccia · M. Laplante · M. Berthiaume ·
Y. Glinas · Y. Deshaies

Received: 10 March 2006 / Accepted: 2 May 2006 / Published online: 14 August 2006
 Springer-Verlag 2006

Abstract

Aims/hypothesis The aim of this study was to investigate the effect and mechanisms of action of *in vivo* peroxisome proliferator-activated receptor γ (PPAR γ) activation on white adipose tissue (WAT) lipolysis and NEFA metabolism.

Materials and methods Study rats were treated for 7 days with 15 mg/kg of rosiglitazone per day; control rats were not treated. After a 6-h fast, lipolysis and levels of mRNA for lipases were assessed in explants from various adipose depots.

Results Rosiglitazone markedly increased basal and noradrenaline (norepinephrine)-stimulated glycerol and NEFA release from WAT explants, and amplified their inhibition by insulin. Primary adipocytes isolated from PPAR γ

agonist-treated rats were also more responsive to noradrenaline stimulation expressed per cell, ruling out a contribution of an altered number of mature adipocytes in explants. Rosiglitazone concomitantly increased levels of mRNA transcripts for adipose triglyceride lipase (ATGL) and monoglyceride lipase (MGL) in subcutaneous and visceral WAT, and mRNA for hormone-sensitive lipase (HSL) in subcutaneous WAT. Lipase expression increased within 12 h of *in vitro* exposure of nave explants to rosiglitazone, suggesting direct transcriptional activation. In parallel, chronic *in vivo* treatment with rosiglitazone lowered plasma NEFAs and exerted in WAT its expected stimulatory action on glycerol and NEFA recycling, and on the expression of genes involved in NEFA uptake and retention by WAT, such processes counteracting net NEFA export.

Conclusions/interpretation These findings demonstrate that, in the face of its plasma NEFA-lowering action, PPAR γ agonism stimulates WAT lipolysis, an effect that is compensated by lipid-retaining pathways. The results further suggest that PPAR γ agonism stimulates lipolysis by increasing the lipolytic potential, including the expression levels of the genes encoding adipose triglyceride lipase and monoglyceride lipase.

Electronic supplementary material Supplementary material is available for this article at <http://dx.doi.org/10.1007/s00125-006-0336-y> and is accessible for authorized users.

The first two authors contributed equally to the work reported in this manuscript.

W. T. Festuccia · M. Laplante · M. Berthiaume · Y. Glinas ·
Y. Deshaies
Laval Hospital Research Center, Faculty of Medicine,
Laval University,
Quebec G1V 4G5, Canada

W. T. Festuccia · M. Laplante · M. Berthiaume · Y. Glinas ·
Y. Deshaies
Department of Anatomy and Physiology, Faculty of Medicine,
Laval University,
Quebec G1V 4G5, Canada

Y. Deshaies ()
Laval Hospital Research Centre, Laval Hospital,
d'Youville Y3110, 2725 Ch Sainte-Foy,
Quebec G1V 4G5, Canada
e-mail: yves.deshaies@phs.ulaval.ca

Keywords Adipose triglyceride lipase · Desnutrin ·
Hormone-sensitive lipase · Insulin · Lipolysis ·
Monoglyceride lipase · NEFA · Noradrenaline ·
PPAR γ agonism · Rosiglitazone

Abbreviations

ATGL	adipose triglyceride lipase
DBcAMP	dibutyl cyclic AMP
EPI	epididymal adipose depot
ESM	Electronic supplementary material
FABP4	adipose fatty acid binding protein 4

FATP1	fatty acid transporter protein 1
GYK	glycerol kinase
HSL	hormone-sensitive lipase
ING	inguinal adipose depot
MES	mesenteric adipose depot
MGL	monoglyceride lipase
PEPCK	phosphoenolpyruvate carboxykinase
PPAR γ	peroxisome proliferator-activated receptor γ
RETRO	retroperitoneal adipose depot
WAT	white adipose tissue

Introduction

Thiazolidinediones are used in the treatment of insulin-resistant states. The compounds exert their potent insulin-sensitising action by interacting with, and activating, peroxisome proliferator-activated receptor γ (PPAR γ), a nuclear receptor that is mainly expressed in adipose tissue. Along with their effects on white adipose tissue (WAT) adipokine production and extra-adipose actions [1, 2], several lines of evidence suggest that one of the insulin-sensitising mechanisms of thiazolidinediones is a reduction in circulating NEFAs, which protects non-adipose tissues against lipid overload and consequent insulin resistance (reviewed in [2]).

Plasma concentrations of NEFAs represent the balance between their release from WAT and uptake by oxidative tissues, with a contribution from adipose reuptake. All of these processes are affected by thiazolidinediones. Indeed, rosiglitazone has been shown to reduce NEFA uptake by the liver and muscle [3], and to increase NEFA uptake, intracellular re-esterification, and storage into triglycerides in WAT [4]. Because of its effect on plasma NEFAs, PPAR γ agonism might also be expected to reduce lipolysis; however, previous studies investigating this issue have produced conflicting results. Whereas some studies have reported lower in vitro rates of glycerol and NEFA release from WAT [5, 6], others that include in vivo NEFA kinetic approaches [4, 7] suggest that PPAR γ agonism stimulates lipolysis, an effect that would be counteracted by its concomitant action on NEFA reuptake and re-esterification.

Until recently, the hydrolysis of triglycerides in WAT was thought to be catalysed exclusively by the enzyme hormone-sensitive lipase (HSL). Lipolytic hormone-stimulated, protein kinase A-mediated phosphorylation of both HSL and perilipin results in hydrolysis of triglyceride into NEFAs and monoglyceride, which is further converted to NEFA and glycerol by monoglyceride lipase (MGL) [8]. Recent studies showing that adipocytes from *HSL* knockout mice retain a marked basal and adrenergically stimulated lipolysis and accumulate diglycerides after stimulation of

lipolysis demonstrated that HSL is not the sole lipase involved in triglyceride catabolism [9–11]. Three independent groups have discovered a protein—alternatively termed adipose triglyceride lipase (ATGL) [12], desnutrin [13] or calcium-independent phospholipase A₂/lipase ζ (iPLA₂ ζ) [14]—that fulfils all the predicted characteristics of this new lipase: a preference for the hydrolysis of the first triglyceride ester bond, drastically reduced lipolysis following antibody or antisense ATGL inhibition, and enhanced basal and isoproterenol-stimulated lipolysis following *ATGL* overexpression [15]. ATGL therefore plays a particularly important role in the control of basal lipolysis, but also appears to participate in the lipolytic response to adrenergic stimulation [16].

The characterisation of ATGL prompted us to consider the possibility that, if PPAR γ agonism indeed enhances lipolysis, it may do so partly via altering lipase expression. The present study therefore tested the hypothesis that thiazolidinediones increase WAT lipolysis, through assessment of the impact of chronic rosiglitazone treatment of rats on NEFA and glycerol release by WAT explants. Because thiazolidinediones exert depot-specific actions [17] and their putative modulation of *ATGL* and *MGL* expression has not yet been addressed, four white adipose depots representing visceral and subcutaneous fat were investigated. Noradrenaline (norepinephrine)-induced stimulation and insulin-induced inhibition of lipolysis were assessed to establish the impact of PPAR γ agonism on the response of lipolysis to neuroendocrine/metabolic control. The depot-specific levels of *ATGL*, *HSL* and *MGL* mRNAs were determined to verify the second hypothesis: that rosiglitazone-induced changes in lipolysis are associated with the expression levels of its obligate lipases.

Materials and methods

Animals and treatment Male Sprague–Dawley rats (200–225 g, Charles River Laboratories, St Constant, QC, Canada) were housed individually in stainless steel cages (23±1°C, 12 h/12 h light/dark cycle, lights on at 12.00 h). The *Principles of Laboratory Animal Care* (NIH publication no. 85–23, revised 1985) were followed, and animal care and handling were performed in accordance with the *Canadian Guide for the Care and Use of Laboratory Animals*. All experimental procedures received prior approval by Laval University's animal protection committee. Rats were divided into a control group and a treated group that was given the PPAR γ agonist rosiglitazone (purchased as Avandia GlaxoSmithKline, Mississauga, ON, Canada at a local pharmacy) at a daily dose of 15 mg/kg body weight as an admixture to the food (Charles River Rodent Diet

#5075; Ralston Products, Woodstock, ON, Canada) for 1 week. This dose is slightly above that which elicits the maximal response of glucose and lipid metabolism to rosiglitazone in rats, as determined in pilot studies. For all experimental procedures described below, food was removed at 07.00 h, and rats were killed by decapitation after 6 h of fasting.

In vitro basal, NA-stimulated, and insulin-inhibited lipolysis Adipose explants (20–25 mg) of inguinal (ING), retroperitoneal (RETRO), epididymal (EPI) and mesenteric (MES) WAT were incubated in 1 ml of Krebs Ringer bicarbonate buffer of the following composition (in mmol/l): 118 NaCl, 4.8 KCl, 1.25 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 5 glucose, supplemented with 2.5% NEFA-free BSA (Sigma, Oakville, ON, Canada), pH 7.4. Fat explants were incubated for 2 h in a humidified atmosphere of 5% CO₂ and 95% O₂ at 37°C. Insulin (100 and 500 pmol/l), noradrenaline (1 µmol/l) and dibutyl cAMP (DBcAMP, 1 mmol/l) were added to the incubation buffer to inhibit or stimulate lipolysis. At the end of the incubation, WAT explants were removed and media were frozen until the measurement, using reagent kits, of NEFA (Wako Pure Chemical Industries, Richmond, VA, USA) and glycerol (Sigma). Data for NEFA and glycerol release are expressed as nmol µg DNA⁻¹ h⁻¹ (DNeasy Tissue Kit; Qiagen, Mississauga, ON, Canada) to correct for cell number. DNA content was determined according to the manufacturer's instructions. Additional experimental details are provided in the Electronic supplementary material (ESM text 1).

In vitro exposure of explants to rosiglitazone Minced pieces (40 mg/well) of each of the four depots described above, obtained from four control, untreated, fasted rats, were incubated in 1 ml of DMEM (Invitrogen, Burlington, ON, Canada) supplemented with pure rosiglitazone (Cayman Chemical Company, Ann Arbor, MI, USA) to a final concentration of 10 µmol/l, or carrier DMSO for 12 h. Treatments were performed in duplicate for each rat. Fat explants were then removed and frozen in liquid nitrogen until RNA isolation and analysis.

Triglyceride/NEFA cycling, PEPCK and GYK activities These variables were quantified according to established methods, as detailed in ESM text 2.

RNA isolation and analysis RNA was isolated from adipose depots using Qiazol and the RNeasy Lipid Tissue Kit (Qiagen). For cDNA synthesis, Expand reverse transcriptase (Invitrogen) was used following the manufacturer's instructions and cDNA was diluted in DNase-free water (1:25) before quantification by real-time PCR. The mRNA

transcript levels were measured in duplicate samples through chemical detection of the PCR products with SYBR Green I (Molecular Probes, Willamette Valley, OR, USA) using a Rotor Gene 3000 system (Montreal Biotech, Montreal, QC, Canada). Primers are listed in ESM Table 1, and additional methodological details are presented in ESM text 3.

Serum determinations Serum glucose concentrations were measured by the glucose oxidase method with the YSI 2300 STAT Plus glucose analyser (YSI, Yellow Springs, OH, USA). Insulin levels were determined by RIA (Linco Research, St Charles, MO, USA) with rat insulin as standard. Serum triglyceride levels were measured by an enzymatic method (Roche Diagnostics, Montreal, QC, Canada), and serum NEFAs and glycerol were determined enzymatically as described above.

Statistical analysis Data are expressed as means±SEM. Simple effects of rosiglitazone treatment were analysed by Student's unpaired *t*-test. Where appropriate, factorial ANOVA followed by Newman–Keuls multiple range test were used to compare the effects of rosiglitazone in various adipose depots. A *p* value<0.05 was used as the threshold of significance.

Results

Final body weight and body weight gain were significantly increased (6% and 50%, respectively) after 7 days of rosiglitazone treatment (Table 1), higher body weight being associated with an increase in both food intake (18%) and food efficiency (23%), and in the weight of all WAT depots (30–35%) and interscapular brown adipose tissue (116%). Rosiglitazone significantly reduced plasma levels of insulin (–35%), NEFAs (–57%), and triglycerides (–55%), but did not alter serum glucose or glycerol levels. Rosiglitazone therefore exerted its expected metabolic actions on energy balance, WAT mass, indices of insulin sensitivity, and lipidaemia.

The effects of rosiglitazone on rates of basal and noradrenaline/DBcAMP-stimulated glycerol and NEFA release normalised per DNA are illustrated in Fig. 1. All three visceral depots behaved similarly on a qualitative basis, and only data for the RETRO and ING (subcutaneous) depots are presented. In control rats, with the exception of basal NEFA release, rates of glycerol and NEFA release were, as expected, lower in the ING (Fig. 1a,b) than in the RETRO (Fig. 1c,d) depot. Noradrenaline and DBcAMP increased glycerol and NEFA release to the same extent in both adipose depots. Also, in both depots, chronic

Table 1 Measurements in rosiglitazone-treated and control rats

Parameter	Control rats	Rosiglitazone-treated rats
Body weight (g)	330±4	348±3 ^a
Body weight gain (g)	47±3	67±3 ^a
Food intake (g)	164±3	194±4 ^a
Food efficiency (%) ^b	28±1	35±1 ^a
ING (g)	3.2±0.2	4.3±0.2 ^a
RETRO (g)	2.0±0.2	2.7±0.1 ^a
EPI (g)	2.7±0.2	3.5±0.2 ^a
MES (g)	2.0±0.1	2.7±0.2 ^a
Brown adipose tissue (g)	0.31±0.01	0.67±0.02 ^a
Insulin (pmol/l)	186±22	122±18 ^a
Glucose (mmol/l)	7.9±0.3	7.8±0.1
NEFAs (mmol/l)	0.47±0.05	0.20±0.07 ^a
Glycerol (mmol/l)	0.128±0.011	0.121±0.003
Triglycerides (mmol/l)	2.0±0.2	1.1±0.1 ^a

Data are means±SEM of 6–12 rats. Treated rats received rosiglitazone for 7 days

^a $p<0.05$ vs controls (Student's unpaired *t*-test)

^bCalculated as body weight gain (g) per 100 g food ingested

rosiglitazone treatment markedly increased basal lipolysis, as demonstrated by the much larger release of glycerol (eight- to ten-fold) and NEFAs (five-fold) from explants of treated compared with untreated rats. Rosiglitazone retained its ability to stimulate the release of lipolytic products in the presence of noradrenaline and DBcAMP such that the lipolytic response to combined adrenergic or DBcAMP stimulation and chronic rosiglitazone treatment were additive. The possible contribution of rosiglitazone-induced changes in the number of lipolytically competent cells was excluded using isolated adipocytes, as discussed in ESM text 4 and illustrated in ESM Fig. 1.

Because PPAR γ agonism affects adipose lipid metabolism largely via modulation of gene expression, we next sought to determine whether the rosiglitazone-induced stimulation of lipolysis was associated with changes in mRNA levels of the major intracellular glyceride lipases. In control rats, levels of *ATGL* (Fig. 2a), *HSL* (Fig. 2b) and *MGL* mRNAs (Fig. 2c) were lower in ING than in all visceral depots, with the exception of *MGL* mRNA in MES. Concomitant with basal lipolysis, rosiglitazone increased levels of both *ATGL* (Fig. 2a; ING, six-fold; all visceral

Fig. 1 In vitro basal, noradrenaline (NA)- and DBcAMP-stimulated rates of glycerol (a, c) and NEFA (b, d) release by explants of ING (a, b) and RETRO (c, d) adipose depots of control (empty columns) and rosiglitazone-treated rats (filled columns). Each column represents

the mean±SEM for six rats. * $p<0.05$ vs untreated control under the same incubation conditions; † $p<0.05$ vs basal unstimulated conditions for same treatment group

Fig. 2 Levels of *ATGL* (a), *HSL* (b) and *MGL* (c) mRNAs in ING, RETRO, EPI and MES adipose depots of control (empty columns) and rosiglitazone-treated rats (filled columns). Each column represents the

mean±SEM for six rats. * $p < 0.05$ vs control in the same depot; † $p < 0.05$ vs ING depot of control rats

depots, two-fold) and *MGL* (Fig. 2c; ING, seven-fold; all visceral depots, three- to four-fold) mRNAs. Rosiglitazone increased *HSL* mRNA levels (2.5-fold) only in the ING depot (Fig. 2b). With data of the four adipose depots pooled, rates of glycerol release correlated with mRNA levels of both *ATGL* (Fig. 3a) and *MGL* (Fig. 3c), but not with those of *HSL* (Fig. 3b). Similar correlation coefficients were found between lipase mRNAs and NEFA release ($r = 0.59$ and 0.77 , $p < 0.0001$, for *ATGL* and *MGL*, respectively, and 0.07 , NS, for *HSL*).

To address the question of whether rosiglitazone directly stimulates lipase expression, adipose tissue explants from untreated rats were exposed for 12 h to the agonist, and lipase mRNA levels were compared with levels of *FABP4* mRNA, which codes for adipose fatty acid binding protein 4, a direct PPAR response element-containing target of PPAR γ agonism. As depicted in Fig. 4, within 12 h, rosiglitazone increased the mRNA levels of all lipases to approximately the same extent as that of *FABP4* in both ING and RETRO depots, without altering the mRNA level of the reference *L27* gene.

As rosiglitazone proved to increase key elements of the lipolytic machinery, we next sought to determine whether

the agonist altered the response of WAT to the antilipolytic action of physiological amounts of insulin. In control ING explants, insulin failed to inhibit basal lipolysis, represented by absolute glycerol release (Fig. 5a) or per cent of relative to basal (Fig. 5b), but did reduce NEFA release (Fig. 5c,d) by virtue of its stimulatory action on NEFA recycling. In ING explants of rosiglitazone-treated rats, insulin inhibited the release of both glycerol and NEFAs, and to a much greater extent than in control explants (Fig. 5a–d). In the RETRO depot, insulin reduced both glycerol (Fig. 5e,f) and NEFA release (Fig. 5g,h), regardless of treatment, and the magnitude of the inhibition was again greater in explants from rosiglitazone-treated than control rats. Notably, despite the greater magnitude of the relative antilipolytic effect of insulin, fat explants of rosiglitazone-treated rats continued to release more absolute amounts of NEFAs than control ING (Fig. 5c) or RETRO explants (Fig. 5g).

Quantification of major determinants of glycerol and NEFA re-esterification (*GYK* and *PEPCK* mRNA levels and activity of the respective proteins) and NEFA reuptake (*FABP4* and *FATP* fatty acid transporter protein mRNA levels) indicated that lipolysis was stimulated by rosiglitazone, despite large increases in these processes, which

Fig. 3 Correlations between glycerol release and levels of *ATGL* mRNA ($n = 46$, a), *HSL* mRNA ($n = 45$, b), and *MGL* mRNA ($n = 46$, c). The four adipose depots harvested from six control and six rosiglitazone-treated rats were included

Fig. 4 Levels of *ATGL*, *HSL* and *MGL* mRNAs, expressed as percent of control, in ING (**a**) and RETRO (**b**) explants from untreated rats incubated in vitro with or without (horizontal line) rosiglitazone for 12

h. *FABP4* and *L27* mRNA served as positive and negative controls, respectively. Each column represents the mean±SEM of explants from four rats. * $p < 0.05$ vs control explants

counteract the net release of lipolytic products, as further discussed in ESM text 5 and presented in ESM Fig. 2.

Discussion

This study investigated the impact and mechanisms of action of the thiazolidinedione rosiglitazone on WAT lipolysis and NEFA metabolism. Chronic rosiglitazone markedly induced WAT lipolysis, both under basal and stimulated conditions, and amplified the antilipolytic action of insulin under basal conditions. The study further revealed that rosiglitazone increases the levels of *ATGL* and *MGL* mRNAs in subcutaneous and visceral fat, and those of *HSL* mRNA in subcutaneous fat, suggesting that upregulation of lipase expression is part of the mechanisms of PPAR γ action on adipose lipolysis.

The study confirmed the well-established actions of rosiglitazone on morphometric variables, indices of insulin sensitivity and lipidaemia [18–21]. Several lines of evidence suggest that thiazolidinediones exert their beneficial effects on whole-body insulin sensitivity partly by reducing circulating NEFAs and the consequent exposure of non-adipose tissues to the deleterious effects of NEFA metabolites on insulin signal transduction [2]. The present study demonstrates that, paradoxically to its effect on plasma NEFA, rosiglitazone markedly stimulates WAT lipolysis. Of note, the stimulatory action of rosiglitazone on lipolysis assessed in WAT explants and expressed per μg of DNA, thus correcting for cellularity, was maintained in isolated mature adipocytes. This excludes the possibility of the effect being due to the presence of a larger number of mature, lipolytically competent adipocytes having arisen through PPAR γ -induced adipocyte differentiation [22]. Thiazolidinediones exert depot-specific effects on fat accretion in humans [17], which is why several depots were investigated in this study. The findings show that the

lipolysis-stimulating action of rosiglitazone extends to both subcutaneous and visceral adipose depots.

Studies that have addressed the effect of PPAR γ agonism on adipose lipolysis have reported mixed results. Two studies found a reduction in the release of lipolytic products from rodent WAT explants and 3T3-L1 adipocytes [5, 6]. These were, however, primarily designed to examine NEFA re-esterification under conditions that may have favoured the latter process. In contrast, studies that have specifically focused on in vivo NEFA kinetics in rats have shown that, in the fasting state, PPAR γ agonists increase the capacity of WAT to release NEFAs [4, 7]. In addition, human adipocytes stimulated to differentiate in the presence of rosiglitazone displayed increased basal and noradrenaline-stimulated lipolysis [23]. In humans, the impact of PPAR γ agonism on lipidaemia is more modest than in rodent models. Although some human in vivo turnover studies did not report any effect of PPAR γ agonism on the kinetics of lipolytic products [24, 25], a net transcapillary release of NEFA from subcutaneous WAT was recently demonstrated in fasted type 2 diabetic subjects treated with rosiglitazone [26]. Taken together, these findings suggest that PPAR γ agonism does stimulate the lipolytic process in the presence of low insulin concentrations.

Concomitant with lipolysis, rosiglitazone markedly increased mRNA levels of the lipases *ATGL* and *MGL* in all WAT depots, and those of *HSL* in the ING depot. A fairly robust correlation was found between basal rates of release of lipolytic products and mRNA levels of *ATGL*. This correlation obviously does not establish a cause and effect relationship; however, the importance of the expression level of *ATGL* in WAT lipolysis is highlighted by recent studies in which *ATGL* overexpression in 3T3-L1 adipocytes enhanced both fasting basal and isoproterenol-stimulated lipolysis, whereas the opposite effects were found with inhibition of *ATGL* through antisense technology [12, 16, 27]. The functional importance of lipase

Fig. 5 Antilipolytic effect of insulin, expressed as absolute levels of glycerol and NEFA release (**a**, **c**, **e**, **g**) and per cent of basal (without insulin, **b**, **d**, **f**, **h**), on basal (unstimulated) glycerol and NEFA release, by explants of ING (**a–d**) and RETRO (**e–h**) adipose depots of control (open squares and columns) and rosiglitazone-treated rats (full squares

and columns). Each symbol or column represents the mean $\pm$ SEM of six rats. * $p < 0.05$ vs untreated control under the same incubation conditions; † $p < 0.05$ vs without insulin in same treatment group; # $p < 0.05$ between control and rosiglitazone for dose–response curve analysed as a whole by ANOVA

expression levels is further supported by the depot-specific behaviour of *ATGL* mRNA levels and basal lipolysis in the present study: both were lower in ING than in visceral depots in control rats, but tended to respond more robustly to rosiglitazone, in relative terms, in the former than in the latter. This suggests that upregulation of *ATGL* expression constitutes one mechanism by which PPAR γ agonism enhances WAT basal lipolysis.

The mRNA levels of *MGL* were also well correlated with the rosiglitazone-induced changes in lipolytic rates. Although recognised as an important enzyme for the complete hydrolysis of triglycerides in WAT [28], the modulation of *MGL* has not received much attention. The finding that rosiglitazone increases *MGL* mRNA levels in congruence with WAT basal lipolysis indicates that this enzyme may also be one of its regulated steps, as well as one additional level of action of PPAR γ agonism thereupon.

With regard to *HSL*, other studies have reported a strong PPAR γ -induced increment in expression in human subcutaneous mature adipocytes [29] and in rodent brown adipocytes [30] incubated short-term with rosiglitazone. Also, *HSL* was recently shown to be a transcriptional target of PPAR γ in differentiating pre-adipocytes and other tissues, but not in visceral WAT in vivo [31]. The present study revealed a short-term (12 h) induction of *HSL* expression in vitro by rosiglitazone in both ING and RETRO depots, which persisted after a 7-day in vivo treatment in the ING but not in the RETRO depot. Taken together, these findings suggest depot and time dependence of the impact of PPAR γ agonism on *HSL* expression. The fact that *HSL* mRNA levels did not correlate with lipolysis, as did the other lipases, and that the absolute PPAR γ -mediated increase in lipolysis was quite similar in both basal and noradrenaline- or DBcAMP-stimulated conditions, leaves some uncertainty as to the contribution of *HSL* to the PPAR γ effect on lipolysis. Clearly, additional studies are needed to clarify this issue.

Under the present conditions, a direct action of rosiglitazone on expression of the lipases is supported by the observation that lipase mRNAs were increased within 12 h of in vitro exposure of naïve explants, as was the case for the PPAR response element-containing *FABP4* gene. Such stimulation of the expression of intracellular lipases would efficiently complement the PPAR γ -mediated upregulation of perilipin expression [32, 33], which facilitates lipid–lipase interactions and is a key player in the hormonal modulation of lipolysis. PPAR γ agonism therefore appears to exert a multilevel, concerted action on the production of several key proteins, which results in an increase in the lipolytic capacity of adipose tissue.

To gain further insight into the consequences of PPAR γ agonism on the physiological modulation of lipolysis, the

effects of rosiglitazone on the response to lipolytic and antilipolytic hormones were investigated. Noradrenaline released by sympathetic nerve terminals in WAT is a major positive modulator of WAT lipolysis [34]. Glycerol and NEFA release reached under noradrenaline and DBcAMP stimulation were much higher in explants from rosiglitazone-treated than control rats. That DBcAMP was approximately as efficient as noradrenaline in stimulating lipolysis excludes the possibility of increased β -adrenergic receptor functionality and signal transduction as a mechanism of action of rosiglitazone on lipolysis. Such a conclusion is further supported by the mild downregulatory action of PPAR γ agonism on β_3 -receptor expression [35]. Instead, it appears that rosiglitazone increases levels of components of the lipolytic machinery (e.g. expression of lipases and other lipolysis-related proteins), thus allowing it to respond more robustly to adrenergic stimulation. Such amplification of the lipolytic potential by PPAR γ agonism strikingly resembles that of the thermogenic potential reported in our previous study [35].

Insulin constitutes another major modulator of WAT lipolysis. The present study confirmed that rosiglitazone increases the response of WAT lipolysis to the inhibitory action of insulin [4]. It is worth noting that the inhibitory effect of insulin on NEFA was stronger than that on glycerol release, because of its additive effect on NEFA re-esterification to triglycerides [36, 37]. Despite the stronger antilipolytic effect of insulin on WAT lipolysis, explants of rosiglitazone-treated rats exposed to insulin concentrations approximating those found in vivo under fasting and postprandial conditions maintained a higher rate of NEFA release than controls. The apparent contradiction between this observation and the lower in vivo serum NEFA levels can be explained by the fact that, while reducing liver and muscle NEFA uptake, thiazolidinediones augment the ability of WAT to clear systemic NEFA through stimulation of protein-mediated NEFA uptake [3, 38]. The rosiglitazone-induced increase in *FABP4* and *FATP* expression observed in the present study confirms previous studies [39, 40] and supports this notion. In vivo, the rosiglitazone-induced increase in WAT NEFA uptake and retention obviously overwhelmed that of net NEFA output, thus reducing plasma NEFAs. Additional potential in vivo contributors to NEFA lowering include the prevailing levels of antagonistic lipolytic modulators, regional blood flow, and NEFA uptake by brown adipose tissue (M. Laplante, Y. Deshaies, unpublished data). It should be noted that the robust stimulation of lipolysis by rosiglitazone occurred in the face of a large increase in the recycling of lipolytic products. Such futile cycling between lipolysis and re-esterification may have contributed to the enhanced response of lipolysis to hormonal modulation (see further discussion in ESM text 6).

In conclusion, the results of this study establish that PPAR γ activation increases basal lipolysis in WAT and amplifies its response to both adrenergic stimulation and insulin inhibition. The study further revealed that PPAR γ agonism increases mRNA levels of *ATGL* and *MGL* in subcutaneous and visceral WAT, and that of *HSL* in subcutaneous WAT, apparently via a direct action on gene expression. Given the importance of lipase expression levels on lipolytic activity, the findings suggest that, at least in the case of *ATGL* and *MGL*, increased expression contributes to the positive action of PPAR γ agonism on adipose lipolysis. In the face of increased lipolysis, the net release of NEFAs into the bloodstream is counteracted by increased reuptake and cycling. PPAR γ agonism therefore reduces net NEFA release into the circulation while greatly increasing lipid substrate turnover, an action likely contributing to improve the hormonal fine tuning of adipose lipid metabolism.

Acknowledgements This work was supported by a grant from the Canadian Institutes of Health Research (CIHR) to Y. Deshaies. W. T. Festuccia was the recipient of a postdoctoral fellowship from a CIHR Training in Obesity Program grant. M. Laplante was the recipient of a studentship from the Natural Sciences and Engineering Research Council of Canada. The authors are very grateful for the invaluable professional assistance of J. Lalonde, M. Alain and S. Poulin. They also thank K. Cianflone and P. Mauriège for their helpful review of the manuscript.

Duality of interest The authors declare that there is no duality of interest associated with this study.

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