Major involvement of mTOR in the PPARγ-induced stimulation of adipose tissue lipid uptake and fat accretion

Pierre-Gilles Blanchard,† William T. Festuccia,† Vanessa P. Houde, Philippe St-Pierre, Sophie Brûlé, Véronique Turcotte, Marie Côté, Kerstin Bellmann, André Marette, and Yves Deshaies

Department of Medicine, Faculty of Medicine, Quebec Heart & Lung Institute, Laval University, Quebec G1V 4G5, Canada; and Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo 05508-900, Brazil

Abstract. Evidence points to a role of the mammalian target of rapamycin (mTOR) signaling pathway as a regulator of adiposity, yet its involvement as a mediator of the positive actions of peroxisome proliferator-activated receptor (PPAR)γ agonism on lipemia, fat accretion, lipid uptake, and its major determinant lipoprotein lipase (LPL) remains to be elucidated. Here we evaluated the plasma lipid profile, triacylglycerol (TAG) secretion rates, and adipose tissue LPL-dependent lipid uptake, LPL expression/activity, and expression profile of other lipid metabolism genes in rats treated with the PPARγ agonist rosiglitazone (15 mg/kg/day) in combination or not with the mTOR inhibitor rapamycin (2 mg/kg/day) for 15 days. Rosiglitazone stimulated adipose tissue mTOR complex 1 and AMPK and induced TAG-derived lipid uptake (136%), LPL mRNA/activity (2–6-fold), and fat accretion in subcutaneous (but not visceral) white adipose tissue (WAT; 50%) and in brown adipose tissue (BAT; 266%). Chronic mTOR inhibition attenuated the upregulation of lipid uptake, LPL expression/activity, and fat accretion induced by PPARγ activation in both subcutaneous WAT and BAT, which resulted in hyperlipidemia. In contrast, rapamycin did not affect most of the other WAT lipogenic genes upregulated by rosiglitazone. Together these findings demonstrate that mTOR is a major regulator of adipose tissue LPL-mediated lipid uptake and a critical mediator of the hypolipidemic and lipogenic actions of PPARγ activation.—Blanchard, P-G., W. T. Festuccia, V. P. Houde, P. St-Pierre, S. Brûlé, V. Turcotte, M. Côté, K. Bellmann, A. Marette, and Y. Deshaies. Major involvement of mTOR in the PPARγ-induced stimulation of adipose tissue lipid uptake and fat accretion. J. Lipid Res. 2012. 53: 1117–1125.

Supplementary key words. mammalian target of rapamycin • dyslipidemia • lipoprotein lipase • triglycerides • adipose tissue • obesity • peroxisome proliferator-activated receptor

Activation of the nuclear receptor peroxisome proliferator-activated receptor (PPAR)γ, a master regulator of adipogenesis and adipocyte lipid metabolism (1, 2), is associated with marked fat accretion in subcutaneous white adipose (WAT) and brown adipose (BAT) tissues. We have shown in rodent models that such fat accretion is mainly attributable to the vastly enhanced uptake and storage of circulating lipids due to increased expression of genes involved in lipid uptake and esterification (2–6). This in turn is thought to be largely responsible for the marked postprandial hypolipidemic action of PPARγ activation in these models, with some contribution from reduced liver VLDL secretion (7). Therefore, when solicited, the PPARγ pathway brings about an integrated set of metabolic adaptations that leads to fat deposition in metabolically safe adipose compartments, a concomitant reduction in circulating lipids, and less exposure of nonadipose tissues to lipotoxicity. This process is thought to contribute to the powerful insulin-sensitizing action of PPARγ agonists, such as thiazolidinediones (8).

Although of definite importance, PPARγ is only one of many key modulators of adiposity. More specifically, robust...
evidence points to an important role of the mammalian target of rapamycin (mTOR) signaling pathway as a possible regulator of adipose tissue mass. mTOR is a conserved serine-threonine kinase that controls protein synthesis; cell size and proliferation according to the availability of amino acids; growth factors; nutrients; and cell energy status (9). mTOR is the catalytic core of two distinct multiprotein complexes, mTOR complex 1 (mTORC1) and 2 (mTORC2), that have different downstream targets, biological functions, and sensitivity to inhibition by the bacterial macrolide rapamycin. Whereas mTORC1 activity is broadly inhibited by rapamycin, mTORC2 is negatively affected by this molecule after prolonged treatment and in certain cell types only (10). With regard to adiposity, expansion of fat mass in obesity, for example, is associated with marked activation of mTOR in adipose tissue (11), whereas fat mass retraction due to caloric restriction and fasting is associated with adipose tissue mTOR inhibition. Accordingly, chronic pharmacological or genetic inhibition of the mTORC1 signaling pathway is associated with a reduction in adipose tissue mass due to both reduced adipocyte size and number (11–13).

Despite the direct association between mTOR activity and adiposity, little is known of the mechanisms by which mTOR modulates fat mass. Importantly, evidence suggests that mTOR may affect adiposity by modulating the activity of PPARγ. Pharmacological mTOR inhibition, for example, impairs in vitro preadipocyte differentiation into mature adipocytes through PPARγ inhibition (14), an effect completely reversed by the presence of a synthetic PPARγ ligand. Likewise, in vivo rapamycin treatment reduces adipose tissue expression of several PPARγ target genes (12). The above evidence points to a possible cross-talk between PPARγ and mTOR; however, to our knowledge such interaction has not yet been explored directly in the in vivo setting.

Considering that ligand-mediated PPARγ activation induces subcutaneous and brown fat accretion in vivo and that mTOR appears as an important regulator of adiposity, we tested in the present study the hypothesis that mTOR is a major mediator of the increased lipid uptake, fat accretion, and resulting reduction in lipemia induced by ligand-mediated PPARγ activation in vivo. To this end, rats treated with the PPARγ agonist rosiglitazone in combination or not with the mTORC1 inhibitor rapamycin were evaluated for plasma lipids; triacylglycerol (TAG) secretion; hydrolysis and uptake by adipose tissues; activity and expression of lipoprotein lipase (LPL), the major determinant of lipoprotein-derived fatty acid uptake; and expression of several other key genes involved in fatty acid uptake and deposition.

RESEARCH DESIGN AND METHODS

Animals

Animal handling and treatment were approved by the Animal Care and Handling Committee of Laval University. Male Sprague-Dawley rats (200 g) purchased from Charles River Laboratories (St-Constant, QC, Canada) were housed individually in a room kept at 23 ± 1°C with a 12:12 h light-dark cycle. After a 4-day adaptation period, rats were matched by weight and divided into four groups: control, vehicle; rosiglitazone, vehicle; control, rapamycin; and rosiglitazone, rapamycin. Vehicle (0.1% MeSO, 0.2% carboxymethylcellulose) or rapamycin (LC laboratories, Woburn, MA) (2 mg/kg/day) were injected intraperitoneally once daily. The dose of rapamycin was chosen based on previous studies showing its efficiency to completely block the mTOR pathway in rats and mice, a dose within the range of those used in human studies (15, 16). Rats were fed a nonpurified powdered rodent diet (Charles River Rodent Diet #5075, Woodstock, ON, Canada) alone (control) or supplemented with the PPARγ agonist rosiglitazone (AVANDIA) at a dose of 15 mg/kg/day for 15 days. This dose of rosiglitazone was found in previous studies to be associated with subcutaneous fat accretion and improvement in the plasma lipid profile (3). After 15 days of treatment, rats were euthanized by decapitation for tissue and blood harvesting after a 12 h fasting period followed or not by 3 h of ad libitum refeeding.

Plasma determinations

Plasma adiponectin was measured by ELISA following supplier’s recommendations (ALPCO Diagnostics, Salem, NH). Plasma TAG (Roche Diagnostics, Montreal, QC, Canada) and nonesterified fatty acids (NEFA) (Wako Chemicals, Richmond, VA) levels were measured by enzymatic methods according to the manufacturer’s instructions.

Immunoblotting of phosphorylated proteins

Tissue samples were homogenized in buffer, subjected to SDS-PAGE, and transferred to nitrocellulose membranes as previously described (12). Antibodies used for immunoblotting are listed in supplementary Table I. Densitometric analysis was performed with ImageQuant TL software (GE Healthcare, Little Chalfont, United Kingdom).

Triacylglycerol rate of appearance in the circulation

The following procedure was carried out to assess the contribution to triglyceridemia of TAG rate of entry into the circulation from the intestine and liver (chylomicron- VLDL-bound TAG). After a 12 h fast and 3 h of refeeding the habitual diet, an initial blood sample (0.15 ml) was withdrawn from the tail vein in an EDTA-containing syringe, and rats were injected through the tail vein with 1 ml/kg Triton WR-1339 (300 mg/ml saline; Sigma-Aldrich, St. Louis, MO), a detergent that prevents intravascular TAG hydrolysis (17). Blood samples (0.15 ml) were then taken 20, 40, and 60 min after the injection. Rats were then injected with a lethal dose of ketamine-xylazine. Blood samples were centrifuged and plasma was collected and stored at 20°C for later TAG quantification. The rate of appearance of TAG in the circulation was determined from regression analysis of TAG accumulation in plasma versus time corrected for plasma volume estimated from body weight and was expressed as micromoles per minute (7).

Hydrolysis, uptake, and storage of triacylglycerol by adipose tissue

Ex vivo measurement of [3H]TAG hydrolysis and incorporation of generated [3H]fatty acids into adipose tissue lipids was performed as previously described (18, 19). Adipose tissue explants were cut into small pieces (25–35 mg), weighed, and preincubated for 10 min with 1 ml of Krebs Ringer buffer [pH 7.2, composed of (in mM) 5 glucose, 0.51 MgCl2, 4.56 KCl, 119.8 NaCl, 0.7 Na2HPO4, 1.3 NaH2PO4, and 15.0 NaHCO3, and 1% fatty acid-free BSA]. Fat pieces were then incubated for 4 h in a well containing 1 ml of [3H]TAG-rich lipoprotein substrate prepared as previously described (20). Briefly, triolein (1.41 mM), phosphatidylcholine (0.08 mM), and [3H]triolein (2.5 μCi, specific activity 1.77 μCi/mM TAG) were emulsified by sonication in an aqueous buffer (0.54 M Tris-HCl, pH 7.2, 5.1% BSA, and 7.5%
RNA extraction and quantitative PCR analysis

RNA extraction and quantitative PCR analysis were performed as described previously (21). In addition to LPL, we quantified expression levels of key genes involved in NEFA uptake (including those generated by LPL-mediated TAG hydrolysis), intracellular trafficking and esterification [FAT/CD36, fatty acid-binding protein 1 (FATP1), fatty acid binding protein 4 (FABP4), also known as aP2, glycerol kinase (GyK), and phosphoenolpyruvate carboxykinase (PEPCK)], PPARγ1 itself, and two additional PPARγ target genes [fatty acid synthase (FAS) and the glucose transporter GLUT4]. The primers used are listed in supplementary Table II. Data are expressed as the ratio between the expression of the target gene and the housekeeping gene 36B4 (also known as ARBP), the expression of which was not significantly affected by either rosiglitazone or rapamycin treatments.

Statistical analysis

Results are expressed as means ± SE. Multifactorial ANOVA followed by Newman-Keuls multiple-range test was used for multiple comparisons. P < 0.05 was taken as the threshold of significance.

RESULTS

First, we evaluated treatment effects on the activation state of relevant signaling pathways, including mTORC1 and 2 and AMPK (activated by PPARγ and a known inhibitor of mTOR activity). As depicted in Fig. 1A,
rosiglitazone significantly activated inguinal adipose tissue mTORC1 as evidenced by the increased ratio of p-S6(Ser240/4)/S6, a downstream target protein in the mTORC1 signaling pathway. Rosiglitazone also markedly reduced the ratio of p-Akt(Thr308)/Akt (Fig. 1C) without affecting that of the mTORC2 substrate p-Akt(Ser473)/Akt (Fig. 1B). Concomitant with activation of inguinal adipose tissue mTORC1 by rosiglitazone, there was a marked stimulation of AMPK, as evidenced by the increased content of total and p-AMPK(Thr172) (Fig. 1D). As previously reported (12), rapamycin inhibited adipose tissue mTORC1 and 2 as seen through reduced p-S6/S6 and p-Akt(Ser473)/Akt ratios without affecting p-Akt(Thr308). No effect of rapamycin, however, was seen on inguinal adipose tissue total AMPK content. Simultaneous administration of rosiglitazone and rapamycin completely blocked p-S6 upregulation and attenuated the increased p-AMPK induced by rosiglitazone in inguinal adipose tissue. As in inguinal WAT, rapamycin attenuated mTORC1 and 2 signaling and abolished p-S6 upregulation associated with rosiglitazone treatment in retroperitoneal WAT and BAT (supplementary Fig. I). Interestingly, rosiglitazone alone inhibited mTORC2 in BAT as evidenced by p-Akt(Ser473)/Akt, but it had no effect on the activity of this protein complex in subcutaneous and visceral WAT. As previously reported (22), supplementary Fig. I confirms the differential action of rapamycin on S6 versus 4EBP. To further assess tissue specificity of treatment actions, the above pathways were also evaluated in the liver (supplementary Fig. II). As previously described (12), rapamycin treatment inhibited mTOR signaling in the liver without affecting Akt phosphorylation (Thr308 and Ser473) (supplementary Fig. II, A–C). This was associated with activation of AMPK as revealed by an increase in p-AMPK/AMPK (supplementary Fig. II, D). In contrast to adipose tissue, rosiglitazone treatment did not increase mTOR signaling in the liver. Surprisingly, the combination of both drugs abolished the activation of AMPK induced by rapamycin alone. The above findings indicate that some of the effects of PPARγ activation and mTOR inhibition appear to be specific to selected adipose tissue depots, rather than generalized actions.

Rats treated with rosiglitazone had higher body weight gain (13%) and showed a weak tendency to have higher food intake and efficiency than control vehicle-treated rats (Table 1), confirming previous studies (23). Rapamycin treatment, on the other hand, markedly reduced body weight gain (~87%) in control and rosiglitazone-treated rats, an effect due to a reduction in both food intake (~16%) and food efficiency (~82%).

Because rapamycin dramatically affects growth and energy balance in rats, fat depot masses were expressed relative to body weight to minimize the impact of these rapamycin effects on the interpretation of the changes in adiposity. The higher body weight gain of rosiglitazone-treated rats was associated with an increase in relative adiposity (Fig. 2A), which could be mainly attributed to an enhanced fat accretion in the subcutaneous inguinal WAT depot (50%) and interscapular BAT (266%) (Table 1). As expected (3, 6), no effect of rosiglitazone was seen on visceral retroperitoneal WAT mass (Table 1). Rapamycin, on the other hand, significantly reduced relative adiposity (~20%, Fig. 2A) and retroperitoneal mass (~40%), and it attenuated the upregulation induced by rosiglitazone in relative adiposity and weights of inguinal WAT and BAT. Intriguingly, retroperitoneal WAT mass was preserved to control values in rats treated with both drugs. The upregulation of subcutaneous fat accretion by rosiglitazone treatment was associated with an increase in plasma adiponectin levels (2.8-fold), which was partially attenuated by rapamycin (Table 1).

As depicted in Fig. 2, the actions of rosiglitazone and rapamycin on plasma lipids were strongly dependent upon the nutritional status. In fasting conditions (12 h overnight), there were no significant differences in plasma NEFA and TAG levels between the groups (Fig. 2B, C). At the end of 3 h of refeeding after the 12 h fast, however, rosiglitazone markedly reduced plasma levels of NEFA and TAG. Rapamycin, on the other hand, increased plasma NEFA and TAG levels, and it abrogated the hypotriglyceridemic action of rosiglitazone (Fig. 2B, C). In contrast to triglyceridemia, rapamycin did not affect the reduction in plasma NEFA induced by rosiglitazone (Fig. 2B).

To elucidate the mechanisms by which rapamycin and rosiglitazone modulate lipemia, we measured both determinants of triglyceridemia, i.e., the in vivo rates of liver and gut TAG secretion and ex vivo adipose tissue clearance of a lipid emulsion. Because rosiglitazone and rapamycin modulated lipemia only in fed animals, measurements of TAG secretion and adipose tissue clearance were performed only in the

---

**Table 1.** Body weight gain, food intake, and relative adipose depot masses corrected for body weight of rats treated with rapamycin (Rapa) and/or rosiglitazone (RSO) for 15 days

|                          | Control | Rapa | RSG | Rapa + RSG |
|--------------------------|---------|------|-----|------------|
| Body weight gain (g)     | 98.3 ± 2.7<sup>a</sup> | 12.6 ± 2.3<sup>a</sup> | 111.2 ± 2.7<sup>a</sup> | 9.61 ± 3.0<sup>a</sup> |
| Food intake (g)          | 214 ± 9.6<sup>a</sup> | 179 ± 10.1<sup>a</sup> | 242 ± 12.0<sup>a</sup> | 167 ± 8.6<sup>a</sup> |
| Food efficiency (%)      | 65.3 ± 1.2<sup>a</sup> | 11.6 ± 2.3<sup>a</sup> | 71.6 ± 2.1<sup>a</sup> | 13.6 ± 2.6<sup>a</sup> |
| Retroperitoneal fat (%)  | 0.87 ± 0.05<sup>a</sup> | 0.53 ± 0.04<sup>a</sup> | 0.84 ± 0.05<sup>a</sup> | 0.79 ± 0.04<sup>a</sup> |
| Inguinal fat (%)         | 1.06 ± 0.08<sup>a</sup> | 0.90 ± 0.04<sup>a</sup> | 1.58 ± 0.06<sup>a</sup> | 1.26 ± 0.04<sup>a</sup> |
| Brown fat (%)            | 0.09 ± 0.01<sup>a</sup> | 0.08 ± 0.01<sup>a</sup> | 0.33 ± 0.02<sup>a</sup> | 0.18 ± 0.02<sup>a</sup> |
| Adiponectin (µg/ml)      | 2.44 ± 0.25<sup>a</sup> | 3.17 ± 0.28<sup>a</sup> | 9.38 ± 1.01<sup>a</sup> | 6.66 ± 1.45<sup>a</sup> |

*Data are average ± SE, n = 12–20 rats. All groups had the same average body weight (230 g) at the onset of treatments.

<sup>a,b,c</sup>Means not sharing a common superscript are significantly different from each other, P < 0.05.

<sup>d</sup>Calculated as grams of body weight gain per 100 g of food ingested.
hand, significantly reduced fatty acid incorporation into lipids in retroperitoneal WAT explants and blocked their upregulation by rosiglitazone in inguinal WAT and BAT explants.

The robust stimulation of adipose tissue lipid clearance by rosiglitazone was associated with a marked increase in LPL activity and mRNA levels in inguinal (7-fold) and brown fat (2-fold) and to a lesser extent in retroperitoneal fat (35%) (Fig. 5A–D). Rapamycin treatment, on the other hand, significantly reduced LPL activity and mRNA levels in all WAT and BAT depots (except LPL mRNA in BAT), and it completely blocked their upregulation by rosiglitazone. Because of the suspected importance of LPL-dependent lipid uptake in the modulation of adiposity by rosiglitazone and rapamycin, correlative relationships between those variables were deemed of particular interest.

As depicted in Fig. 6, we found robust, significant correlations between inguinal mass, LPL activity, and lipid uptake, suggesting that LPL-mediated lipid clearance/uptake is a major factor in the modulation of adiposity upon rosiglitazone and/or rapamycin treatments. Noteworthy, similar robust correlations between these variables were found in BAT, but not in retroperitoneal WAT (data not shown), corroborating the depot specificity of rosiglitazone action promoting subcutaneous white and brown fat adiposity.

Because PPARγ targets several genes other than LPL that are involved in lipid trafficking and storage, and because rapamycin was unable to counteract the rosiglitazone-induced reduction in plasma NEFA (as opposed to TAG), we investigated whether mTOR plays a role therein by assessing in inguinal fat the mRNA levels of several PPARγ target proteins. As shown in Fig. 7, rosiglitazone significantly increased mRNA levels of its well-characterized target genes involved in the uptake (CD36 and FATP1), transport (FABP4), and storage into TAG (GyK, PEPCK, FAS) of NEFA, in the uptake of glucose (GLUT4), and in the control of lipogenesis (SREBP1). Rosiglitazone-mediated upregulation of GyK, FAS, and SREBP1 mRNA expression levels was abolished by rapamycin, whereas those of all the other lipogenic genes remained robustly stimulated under the dual treatment. Remarkably, the dual treatment greatly impaired rosiglitazone’s ability to induce the expression of its target genes in retroperitoneal WAT (supplementary Fig. III) and BAT (supplementary

![Fig. 2](image2.png)

**Fig. 2.** Relative adiposity [sum of inguinal, epididymal, retroperitoneal, and BAT masses corrected for body weight (bw), panel A], plasma nonesterified fatty acids (NEFA, panel B), and TAG (panel C) levels in rats treated with rapamycin (Rapa) or rosiglitazone (RSG) for 15 days. Plasma lipids were measured in rats 12 h fasted or 12 h fasted and 3 h refed. n = 12–20 for each group. Means not sharing a common superscript are significantly different from each other, *P* < 0.05.

![Fig. 3](image3.png)

**Fig. 3.** TAG secretion rate in refed rats treated with rapamycin (Rapa) or rosiglitazone (RSG) for 15 days. n = 4 for each group. Means not sharing a common superscript are significantly different from each other, *P* < 0.05.

![Fig. 4](image4.png)

**Fig. 4.** In vitro incorporation of 3H-fatty acids from triolein into explants of retroperitoneal (panel A), inguinal (panel B), and brown adipose (panel C) depots of rats treated with rapamycin (Rapa) or rosiglitazone (RSG) for 15 days. n = 6 for each group. Means not sharing a common superscript are significantly different from each other, *P* < 0.05.
between the nutrient sensors PPARγ and mTOR in the regulation of adiposity, adipose tissue lipid uptake, and triglyceridemia. Our main findings demonstrate that mTOR is an important regulator of adiposity, TAG-derived lipid uptake and LPL activity, and triglyceridemia under normal conditions, as well as its previously unrecognized role as a critical mediator of the positive actions of rosiglitazone toward these variables. More specifically, mTORC1 is activated in adipose tissue by ligand-mediated PPARγ stimulation and acts as a key mediator of the positive actions of rosiglitazone toward LPL activity, lipid clearance/uptake, and subcutaneous and brown fat accretion. Taken together, our results establish mTOR as a modulator of PPARγ activity, lipid homeostasis, and adiposity in vivo.

In line with the initial hypothesis of a possible implication of mTOR in the positive actions of PPARγ activation on adiposity, the study unveiled the as yet unsuspected ability of rosiglitazone to activate mTORC1 in adipose tissue, as evidenced by the increased content of p-S6 in all adipose depots examined, and that of p-4EBP in inguinal fat. The means by which rosiglitazone activates mTORC1 are unknown at the moment, but the reduction in p-Akt(Thr308) and absence of changes in p-Akt(Ser473) suggest that rosiglitazone activates mTORC1 by a mechanism that does not involve an increase in insulin sensitivity, one of the major phenotypes of PPARγ activation in vivo. Surprisingly, rosiglitazone activates mTORC1 despite overactivating AMPK, a kinase that inhibits mTORC1 activity by phosphorylating raptor (24) and TSC2 (25). These results are in agreement with our previous study showing a simultaneous activation of mTORC1 and AMPK in the heart upon rosiglitazone treatment (26). Such higher AMPK activity could prevent a possibly deleterious overactivation of mTORC1 by rosiglitazone by an unknown mechanism, which clearly deserves further investigation.

Confirming previous studies (12, 23), body weight gain was enhanced by rosiglitazone and drastically reduced by rapamycin alone or in combination with rosiglitazone. These findings suggest that mTOR inhibition overrides the positive actions of PPARγ activation on energy balance. At this point the mechanisms underlying the marked reduction in body weight gain by rapamycin are unknown, but beyond reduced food intake, the major decrease in food efficiency found in the present study suggests an increase in energy expenditure as its main driving component. Noteworthy, rapamycin affected neither intestinal

**DISCUSSION**

In the present study, by using a combination of rosiglitazone and rapamycin pharmacotherapy, we were able to elucidate several aspects of the complex interrelationship

---

**Fig. 5.** LPL activity and mRNA levels in retroperitoneal (panels A, B), inguinal (panels C, D), and brown adipose (panels E, F) depots of rats treated with rapamycin (Rapa) or rosiglitazone (RSG) for 15 days. n = 6 for each group. Means not sharing a common superscript are significantly different from each other, P < 0.05.

**Fig. 6.** Correlations (panels A–C) between inguinal adipose tissue mass, LPL activity, and TAG-derived lipid uptake in rats treated with rapamycin or rosiglitazone for 15 days. Each square represents an individual rat.
Adipose tissue mTORC2 activity by rictor deletion results in increased epididymal and parametrial fat masses (27, 28), which is the exact opposite of the phenotype seen with rapamycin treatment. On the other hand, as with rapamycin treatment, adipose tissue-specific inhibition of mTORC1 by raptor deletion (13) and whole body knock-out of the mTORC1 downstream target S6K (11) both result in reduced adiposity and protection against diet-induced obesity.

In contrast to adiposity, the adipose tissue mTOR activation status was inversely related to plasma TAG levels: mTOR inhibition by rapamycin and activation by rosiglitazone were associated with hypertriglyceridemia and hypotriglyceridemia, respectively. Further supporting an involvement of mTOR in the modulation of plasma TAG levels upon PPARγ activation, rapamycin treatment abolished the hypotriglyceridemic action of rosiglitazone. In the present study, we elucidated the as yet unknown mechanism underlying the hypertriglyceridemia associated with mTOR inhibition. Such dyslipidemia is one of the major side effects adversely affecting transplanted and cancer patients under rapamycin therapy and one of the most prevalent reasons for treatment discontinuation (29–34). Plasma TAG levels represent the balance between gut and liver TAG-rich lipoprotein secretion and LPL-mediated clearance in extrahepatic tissues, adipose depots being responsible for a large fraction of total body lipid clearance in the postprandial state. Our findings that pharmacological mTOR inhibition with rapamycin, even in the presence of vastly reduced TAG secretion, resulted in hypertriglyceridemia strongly suggest that an impairment in the ability of adipose tissue to hydrolyze, take up, and store circulating lipids is a major determinant of the phenotype. In accordance with this hypothesis, rapamycin treatment reduced the ability of adipose tissue explants to incorporate fatty acids from a lipid emulsion in vitro. Rapamycin also completely blocked the upregulation of this process induced by rosiglitazone, which underlines the major role of adipose lipid uptake in determining triglyceridemia in the present conditions. Thus, our findings suggest that the changes in adipose tissue lipid clearance/uptake induced by rapamycin and/or rosiglitazone accounts, at least in part, for the depot-specific changes in adiposity found in the present study. In agreement with this hypothesis we found a strong correlation between subcutaneous fat accretion and lipid uptake.

Although the alterations in energy balance elicited by rapamycin may constitute a potential confounding factor in establishing the involvement of PPARγ and mTOR in the modulation of lipid metabolism, it must be noted that PPARγ reduces triglyceridemia despite weight gain, whereas rapamycin favors hypertriglyceridemia despite a large reduction in weight gain. These observations are not supportive of indirect mechanisms related to energy balance but, instead, strongly suggest a direct involvement of PPARγ and mTOR in the modulation of plasma and adipose tissue lipid metabolism.

Adipose tissue clearance of circulating neutral lipids is mainly catalyzed by LPL, an endothelium-bound enzyme that hydrolyzes circulating TAG, releasing fatty acids and food absorption as estimated from fecal energy content, nor BAT UCP1 mRNA levels and core rectal temperature (data not shown), suggesting that nonshivering thermogenesis is not involved in the enhanced energy expenditure found upon rapamycin treatment. Further research is needed to elucidate the mechanisms underlying the increase in energy expenditure by pharmacological mTOR inhibition.

Although energy intake and expenditure obviously determine global energy balance, many modulatory systems affect energy partitioning among tissues, including fat accretion in various adipose depots. We found in the present study a direct relationship between adipose tissue mTORC1 activation status and relative adiposity, which was expressed per body weight to take into account the impact of the changes in energy balance and growth induced by rapamycin. Adipose tissue mTORC1 activation by rosiglitazone was associated with an increase in relative adiposity, which was mainly due to subcutaneous and brown fat accretion, whereas pharmacological mTOR inhibition with rapamycin was associated with reduced adiposity. Importantly, mTOR inhibition attenuated the increase in relative adiposity and subcutaneous and brown fat masses induced by rosiglitazone, establishing mTOR as a major modulator of adiposity under normal conditions and as a critical mediator of the fat accretion induced by pharmacological PPARγ activation. Despite the reduction in both adipose tissue mTORC1 and 2 signaling, the modulation of adiposity seen under rapamycin treatment seems to be due to mTORC1 rather than mTORC2 inhibition. Indeed, abrogation of adipose tissue mTORC2 activity by rictor deletion

Fig. 7. mRNA levels (panels A–H) of lipogenic proteins in inguinal adipose depot of rats treated with rapamycin (Rapa) or rosiglitazone (RS) for 15 days. n = 6–8 for each group. Means not sharing a common superscript are significantly different from each other, P < 0.05.
monoacylglycerol for tissue uptake. Corroborating the above-described mTORC1 actions regulating adipose tissue lipid clearance, activation of mTORC1 by rosiglitazone was associated with a significant increase in LPL mRNA levels and activity in inguinal and brown fat, whereas pharmacological mTOR inhibition not only reduced LPL expression and activity in vehicle-treated rats but also markedly attenuated their activation by rosiglitazone. In fact, we found a very strong correlation between subcutaneous fat accretion, lipid uptake, and LPL activity, suggesting that mTORC1 impacts the tissue-specific storage of energy substrates by modulating the ability of adipose tissue to take up and store circulating lipids at least partly via altering LPL.

Analysis of mRNA levels of several other PPARγ regulated genes revealed that, similar to LPL, the upregulation in GyK mRNA levels by rosiglitazone depended on mTOR activity. GyK is a cytosolic enzyme that catalyzes the phosphorylation of glycerol into glycerol 3-phosphate, supplying the carbon backbone for fatty acid esterification and TAG synthesis (35). Because LPL and GyK have in their promoter a functional PPAR response element (PPRE) and their transcription is increased by PPARγ ligands (35, 36), the attenuation of the rosiglitazone-mediated upregulation in their mRNA levels by rapamycin suggests that mTOR signaling is required for maximal PPARγ transcriptional activity toward a subset of genes. More importantly, the sensitivity of PPARγ DNA binding activity to mTOR blockade appears to be tissue-specific as rapamycin abolished the ability of rosiglitazone to upregulate lipogenic genes in retroperitoneal fat and BAT, in contrast to the inguinal depot where the mRNA levels of the glucose transporter GLUT4, the fatty acid transporters CD36, FATP1, and FABP4, and the key glyceroneogenic enzyme PEPCK were not negatively affected by the dual treatment. The lack of effect of mTOR inhibition on the rosiglitazone-mediated induction of key fatty acid uptake/transport genes in the inguinal depot may partially explain why the PPARγ agonist remains fully able to reduce circulating NEFA (as opposed to TAG). Although the precise mechanisms underlying this mTOR effect are unknown at the moment, the absence of change in PPARγ mRNA levels by rapamycin suggests that it involves covalent or allosteric modulation of PPARγ and/or its coregulators rather than changes in receptor availability. These findings not only exclude changes in receptor content as a possible mediator of rapamycin effects but also strongly suggest that some genes are more sensitive than others to mTOR-mediated regulation of PPARγ transcriptional activity.

Although the present findings suggest that mTOR signaling is required for maximal PPARγ transcriptional activity, a model of positive feed-forward regulation between the nutrient sensors PPARγ and mTOR, in which PPARγ activation stimulates adipose tissue mTORC1 activity that, in turn, further enhances PPARγ transcriptional action toward some specific genes, cannot be ruled out. Thus, in situations of nutrient abundance such as obesity, mTOR activation would enhance adipose tissue PPARγ transcriptional activity toward genes that enhance the ability and propensity to store the excess nutrients as TAG in a depot-specific manner.

A similar feed-forward loop of regulation was previously described to occur between PPARγ and adiponectin, in which constitutive increases in plasma adiponectin levels are associated with PPARγ activation that, in turn, further stimulates adiponectin synthesis and secretion (37). Interestingly, mice genetically modified to oversecrete adiponectin display hypotriglycerideremia and subcutaneous fat accretion similar to what is seen upon rosiglitazone treatment (37). Adiponectin exerts its actions in adipose tissue in part by activating AMPK (38, 39), which is a known inhibitor of mTORC1 activity. Our findings that rosiglitazone treatment was effective in increasing plasma adiponectin levels without concomitantly activating adipose tissue AMPK in rapamycin-treated rats or inducing hypotriglycerideremia and subcutaneous fat accretion together suggest that mTOR is somehow important in the modulation of these variables by the PPARγ-adiponectin feed-forward loop. The mechanisms underlying mTOR modulation of the interrelationships between adiponectin-AMPK and PPARγ are unknown, but they clearly deserve further investigation.

In conclusion, we have shown in the present study that mTOR is essential in the modulation of plasma lipid homeostasis and adiposity and is a key mediator of in vivo upregulation of adipose tissue LPL activity, lipid clearance/uptake, and fat accretion induced by rosiglitazone. As such, PPARγ agonists would offer little therapeutic value to hypertriglycerideremic transplanted and cancer patients under rapamycin treatment, although the impact of reduced postprandial NEFA levels remains to be assessed. Beyond lipidemia, the elucidation of the mechanisms by which mTOR modulates PPARγ might be of great interest in the development of future pharmacological strategies to more efficiently fine-tune PPARγ activity and adiposity.

The authors thank Yves Gélinas for invaluable technical assistance.

REFERENCES
1. Rosen, E. D., and O. A. MacDougald. 2006. Adipocyte differentiation from the inside out. Nat. Rev. Mol. Cell Biol. 7: 885–896.
2. Festuccia, W. T., and Y. Deshaies. 2009. Depot specificities of PPARγ ligand actions on lipid and glucose metabolism and their implication in PPARγ-mediated body fat redistribution. Clinical Lipidology. 4: 635–642.
3. Laplante, M., H. Sell, K. L. MacNaul, D. Richard, J. P. Berger, and Y. Deshaies. 2003. PPARγ activation mediates adipose depot-specific effects on gene expression and lipoprotein lipase activity: mechanisms for modulation of postprandial lipemia and differential adipose accretion. Diabetes. 52: 291–299.
4. Laplante, M., W. T. Festuccia, G. Soucy, Y. Gélinas, J. Lalonde, J. P. Berger, and Y. Deshaies. 2006. Mechanisms of the depot specificity of peroxisome proliferator-activated receptor γ action on adipose tissue metabolism. Diabetes. 55: 2771–2778.
5. Festuccia, W. T., P. G. Blanchard, V. Turcotte, M. Laplante, M. Sarlahmetoglu, D. N. Brindley, D. Richard, and Y. Deshaies, 2009. The PPAR γ agonist rosiglitazone enhances rat brown adipose tissue lipogenesis from glucose without altering glucose uptake. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296: R1327–R1335.
positive energy balance in the at is associated with reduced sympathetic drive to adipose tissues and thyroid status. Endocrinology. 149: 2121–2130.

24. Gwinn, D. M., D. B. Shackelford, D. F. Egan, M. M. Mihaylova, A. Merry, D. S. Vasquez, B. E. Turk, and R. J. Shaw. 2008. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol. Cell. 30: 214–226.

25. Inoki, K., Y. Li, T. Zhu, J. Wu, and K. L. Guan. 2002. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat. Cell Biol. 4: 648–657.

26. Festuccia, W. T., M. Laplante, S. Brule, V. P. Houde, A. Achourba, D. Lachance, M. L. Pedrosa, M. E. Silva, R. Guerra-Sa, J. Coutet, et al. 2009. Rosiglitazone-induced heart remodelling is associated with enhanced turnover of myofibrillar protein and mTOR activation. J. Mol. Cell. Cardiol. 47: 85–95.

27. Kumar, A., J. C. Lawrence, Jr., D. Y. Jung, H. J. Ko, S. R. Keller, J. K. Kim, M. A. Magnunson, and T. E. Harris. 2010. Fat cell-specific ablation of rictor in mice impairs insulin-regulated fat cell and whole-body glucose and lipid metabolism. Diabetes. 59: 1397–1406.

28. Cybulski, N., P. Polak, J. Anwerx, M. A. Ruegg, and M. N. Hall. 2009. mTOR complex 2 in adipose tissue negatively controls whole-body growth. Proc. Natl. Acad. Sci. USA. 106: 9902–9907.

29. Morisset, J. D., G. Abdel-Fattah, R. Hoogeveen, E. Mitchell, C. M. Ballantyne, H. J. Powall, A. R. Opekun, J. S. Jaffe, S. Oppermann, and B. D. Kahn. 2002. Effects of sirolimus on plasma lipids, lipoprotein levels, and fatty acid metabolism in renal transplant patients. J. Lipid Res. 43: 1170–1180.

30. Brattström, C., H. E. Wileczek, G. Tyden, Y. Bottiger, J. Sawe, and C. G. Groth. 1998. Hypertriglyceridemia in renal transplant recipients treated with sirolimus. Transplant. Proc. 30: 3950–3951.

31. Fernandez-Bussy, S., O. Akindipe, M. Baz, P. Gosain, A. Rosenberg, and M. Zumberg. 2010. Sirolimus-induced severe hypertriglyceridemia in a lung transplant recipient. Transplantation. 89: 481–482.

32. Malizzi, L. J., and A. Hsu. 2008. Temsirolimus, an mTOR inhibitor for treatment of patients with advanced renal cell carcinoma. Clin. J. Oncol. Nurs. 12: 639–646.

33. Fraenkel, M., M. Ketzelin-Gilad, Y. Ariav, O. Pappo, M. Karaca, J. Castel, M. F. Berthault, C. Magnan, E. Cerasi, N. Kaiser, et al. 2008. mTOR inhibition by rapamycin prevents beta-cell adaptation to hyperglycemia and exacerbates the metabolic state in type 2 diabetes. Diabetes. 57: 945–957.

34. Aggarwal, D. M., L. Fernandez, and G. A. Solomon. 2006. Rapamycin, an mTOR inhibitor, disrupts triglyceride metabolism in guinea pigs. Metabolism. 55: 794–802.

35. Guan, H. Y., Y. Li, M. V. Jensen, C. B. Newgard, C. M. Steppan, and M. A. Lazar. 2002. A futile metabolic cycle activated in adipocytes by antidiabetic agents. Nat. Mol. 8: 1122–1128.

36. Schoonjans, K., J. Pelinados-Onsurbe, A. M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb, B. Stach, and J. Anwerx. 1996. PPAR ald and PPAR activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J. 15: 5336–5348.

37. Kim, J. Y., E. van de Wall, M. Laplante, A. Azzara, M. E. Trujillo, S. M. Hofmann, T. Schraw, J. L. Durand, H. Li, G. Li, et al. 2007. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J. Clin. Invest. 117: 2621–2637.

38. Kadowaki, T., T. Yamauchi, N. Kubota, K. Harra, K. Ueki, and K. Tobe. 2006. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J. Clin. Invest. 116: 1784–1792.

39. Kadowaki, T., and T. Yamauchi. 2005. Adiponectin and adiponectin receptors. Endocr. Rev. 26: 430–451.