

# Effect of Rosiglitazone on Peroxisome Proliferator-Activated Receptor $\gamma$ Gene Expression in Human Adipose Tissue Is Limited by Antiretroviral Drug-Induced Mitochondrial Dysfunction

**Patrick W. G. Mallon,<sup>1,2,a</sup> Rebecca Sedwell,<sup>1</sup> Gary Rogers,<sup>4,a</sup> David Nolan,<sup>5</sup> Patrick Unemori,<sup>1,a</sup> Jennifer Hoy,<sup>6</sup> Katherine Samaras,<sup>3</sup> Anthony Kelleher,<sup>1,2</sup> Sean Emery,<sup>1</sup> David A. Cooper,<sup>1,2</sup> and Andrew Carr<sup>2</sup> for the Rosey Investigators<sup>b</sup>**

<sup>1</sup>National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, <sup>2</sup>St. Vincent's Hospital, and <sup>3</sup>Garvan Institute of Medical Research, Sydney, <sup>4</sup>Department of General Practice, University of Adelaide, <sup>5</sup>Royal Perth Hospital, Perth, and <sup>6</sup>Alfred Hospital and Monash University, Melbourne, Australia

(See the editorial commentary by Hadigan, on pages 1729–31.)

**Background.** Treatment of human immunodeficiency virus (HIV)-1 with thymidine-analogue nucleoside reverse-transcriptase inhibitors (tNRTIs) causes lipoatrophy, mitochondrial toxicity, and lower adipose tissue expression of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$  [PPARG gene]). Rosiglitazone (RSG), a PPAR $\gamma$  agonist, improves congenital lipoatrophy but not HIV lipoatrophy.

**Methods.** Serial fat biopsies were taken from HIV-infected, lipoatrophic men randomized to receive RSG or placebo for 48 weeks. Adipose tissue mitochondrial and nuclear gene expression and mitochondrial DNA content were quantified by real-time polymerase chain reaction. Nonparametric analyses were applied.

**Results.** Subjects receiving tNRTI-containing antiretroviral therapy had lower baseline mitochondrial RNA expression and DNA content. In subjects receiving tNRTIs, exposure to RSG did not affect PPARG expression at either week 2 or 48. At week 2, RSG increased PPARG expression only in subjects not treated with tNRTIs, whereas at week 48, increased PPARG expression was observed in subjects not treated with tNRTIs, regardless of RSG use. Similar findings were observed for the PPARG-responsive gene fatty acid-binding protein 4. Changes in PPARG expression were associated with increases in limb fat mass.

**Conclusions.** These data suggest that in HIV-infected, lipoatrophic men, adipose PPARG expression and function are dependent on intact mitochondrial function. These data support a direct link between mitochondrial toxicity and adipose tissue PPARG expression and help explain the poor clinical response to RSG observed in clinical trials.

Highly active antiretroviral therapies (HAART) used to treat HIV-1 infection are associated with metabolic

complications including peripheral lipoatrophy [1], disturbed lipid and glucose metabolism and increased risk of both diabetes [2, 3] and cardiovascular disease [4]. Nucleoside reverse-transcriptase inhibitors (NRTI), important components of HAART, include the thymidine

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<sup>a</sup> Present affiliations: School of Medicine and Medical Science, University College Dublin, Ireland (P.W.G.M.); Department of Internal Medicine, University of California, San Francisco (P.U.); School of Medicine, Griffith University, Queensland, Australia (G.R.).

<sup>b</sup> Members of the Rosey Investigators are listed at the end of the text.

Reprints or correspondence: Dr. Patrick W. G. Mallon, University College Dublin School of Medicine and Medical Science, Catherine McAuley Education and Research Centre, Mater Misericordiae University Hospital, Nelson Street, Dublin 7, Ireland (paddy.mallon@ucd.ie).

analogue (tNRTIs) stavudine (d4T) and zidovudine (AZT). Both d4T and AZT are commonly used to treat HIV, particularly in resource-poor countries. In adipose tissue, exposure to d4T or AZT causes mitochondrial toxicity, depletes mitochondrial DNA (mtDNA) [5], and affects expression of mitochondrial-encoded genes as well as genes encoding the adipocyte transcription factor peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$  [*PPARG* gene]) and its coactivator, the PPAR $\gamma$  coactivator 1 $\alpha$  (*PPARGC1A*) [6].

PPAR $\gamma$  is a ligand-activated transcription factor that binds as a heterodimer with the retinoid X receptor to PPAR-responsive regions of genes [7]. Target genes include those encoding adipocyte fatty acid-binding protein (*FABP4* gene or aP2), acyl-CoA synthase, and lipoprotein lipase [8, 9]. Activation of PPAR $\gamma$  modifies lipid-related cellular functions such as adipocyte differentiation, hepatic glucose and fatty acid uptake, and cholesterol transport in macrophages [9–11]. Synthetic ligands of PPAR $\gamma$  include drugs of the thiazolidinedione (TZD) class such as rosiglitazone and pioglitazone, which are licensed to treat type 2 diabetes mellitus. PPAR $\gamma$  activation increases murine subcutaneous adipose tissue and decreases murine visceral adipose tissue [8], and TZDs similarly affect adipose tissue in humans with congenital lipodystrophy, obesity, and type 2 diabetes mellitus [12–14].

In contrast, TZDs such as rosiglitazone and pioglitazone have shown mixed effects on limb fat mass in lipoatrophic, HIV-infected adults [15–20], despite improving insulin sensitivity and increasing concentrations of adiponectin, an adipose tissue-derived cytokine [15, 16, 19, 20]. Available data suggest that only a modest increase in limb fat with TZD therapy has been observed in patients who did not receive a tNRTI [15, 16]. In healthy volunteers, 2 weeks of NRTI exposure decreased adipose expression of both mitochondrial RNA (mtRNA) and *PPARG*; the changes in the expression of mtRNA and *PPARG* were significantly correlated [6], suggesting a link between tNRTI-induced mitochondrial dysfunction and *PPARG* expression.

We investigated the molecular mechanisms underlying the failure of RSG to increase limb fat mass in HIV-infected lipoatrophic adults to the same extent as that observed in subjects with congenital lipodystrophy. Our hypotheses were as follows: (1) ongoing mitochondrial dysfunction from continued tNRTI therapy would inhibit RSG-induced increases in *PPARG* expression, (2) RSG would have no impact on mitochondrial gene expression or mitochondrial DNA copy number in subjects with tNRTI-induced mitochondrial dysfunction, and (3) increases in *PPARG* expression would predict greater increases in limb fat.

## METHODS

**Population and study design.** This was a substudy of a randomized, placebo-controlled trial to examine the effect of 48 weeks of treatment with RSG, 4 mg administered twice daily, on

limb fat in lipoatrophic, HIV-infected adults [16]. From an original cohort of 108 subjects recruited through 17 Australian sites, 44 were recruited to this molecular substudy from 10 study sites in 4 Australian cities, with biopsies performed in 1 center in each city. Of these subjects 44, 41, and 28 underwent biopsies at baseline, week 2, and week 48, respectively. From these biopsy samples, suitable RNA was extracted from 38, 41, and 27, and suitable genomic DNA (gDNA) was extracted from 38, 37, and 28, respectively. The reduction in numbers at week 48 was largely the result of 1 study site declining to offer week-48 biopsies to enrolled subjects. All subjects recruited to the main study were eligible to enroll in this molecular substudy provided that their study site had access to the biopsy procedure. All subjects provided written, informed consent and the study protocol was approved by the relevant local human research ethics committees.

**Clinical assessments.** As part of the main clinical trial, fasting lipid and glycemic levels and body composition were measured at baseline and at regular intervals during the 48 weeks of follow-up [16]. In addition, subjects enrolled onto this substudy underwent subcutaneous fat biopsies at weeks 0, 2, and 48. We chose week 2 for the determination of earlier drug-induced effects in gene expression while also avoiding the potential for confounding effects of changes in diet or exercise arising over time. Biopsies were obtained under local anesthetic through an incision in the flank performed using aseptic technique, as described elsewhere [6]. Harvested fat samples were snap frozen in liquid nitrogen at the study site and transported for storage at a central site (St. Vincent's Hospital, Sydney, Australia), where batched extraction and testing was performed at the end of the study.

**Primer selection.** We used primers described elsewhere [6], targeting the following nuclear-encoded genes: *ACTB*, the gene encoding  $\beta$ -actin; *PPARG*; and *PPARGC1A*. Primers directed toward *FABP4* (also known as aP2; accession number NM\_001442) were designed using Primer3 Web-based software (<http://frodo.wi.mit.edu>; forward primer, 5'-TACTGGGCCAGGAAT TTGAC3'; reverse primer, 5'-GTGGAAGTGACGCCCTTCAT-3'). mtDNA content and mtRNA expression were measured by using primers directed against *MTCYB*, the gene encoding mitochondrial cytochrome b.

**Sample preparation and polymerase chain reaction (PCR).** RNA was extracted from homogenized frozen fat samples stored in TRIzol reagent (Invitrogen Life Technologies), as described elsewhere [6]. After RNA extraction, gDNA was extracted from the remaining homogenate by using the High Pure Viral Nucleic Acid Kit (Roche Applied Science), in accordance with the manufacturer's instructions.

We prepared first-strand complementary DNA (cDNA) from RNA samples (200 ng) by using SuperscriptII reverse transcriptase (RT) and oligo dT primers (Invitrogen) to a final volume of 20  $\mu$ L. To adjust for inter-RT variability, we performed 4 RT reactions per sample and pooled the cDNA as described else-

**Table 1.** Baseline characteristics of study subjects.

Characteristic	Main study cohort (n = 108)	Molecular substudy treatment group	
		Rosiglitazone (n = 21)	Placebo (n = 23)
Age, median (IQR), years	45 (10)	47 (8)	48 (10)
Male sex, no. (%)	106 (98)	21 (100)	21 (100)
Receipt of PI, no. (%)	66 (61)	16 (76)	14 (61)
PI therapy duration, median (IQR), months	NA	53 (41)	47 (45)
Receipt of tNRTIs, no. (%)	64 (59)	13 (62)	8 (35)
tNRTI therapy duration, median (IQR), months	NA	70 (41)	77 (45)
Last tNRTI exposure, median (IQR), months	NA	16 (11)	19 (16)
CD4 <sup>+</sup> T cell count, median (IQR), cells/mm <sup>3</sup>	542 (296)	525 (299)	532 (263)
HIV RNA, median (IQR), log copies/mL	1.7 (0.97)	1.7 (0.7)	1.7 (0.5)
AIDS diagnosis, no. (%)	20 (19)	5 (24)	3 (13)
BMI, median (IQR), kg/m <sup>2</sup>	23.3 (3.2)	23.3 (3.4)	23.3 (2.3)
Limb fat, median (IQR), kg	2.36 (1.9)	1.88 (1.1)	2.31 (1.24)
Adipose tissue gene expression			
ACTB, log ng/μL	NA	3.11 (0.4)	3.2 (0.36)
MTCYB vs. value for ACTB (IQR)	NA	4.5 (5.6)	6.2 (7.0)
PPARG vs. value for ACTB (IQR)	NA	0.13 (0.12)	0.12 (0.07)
PPARGC1A vs. value for ACTB (IQR)	NA	0.13 (0.10)	0.12 (0.12)
FABP4 vs. value for ACTB (IQR)	NA	6.4 (4.9)	6.6 (4.0)
mtDNA, copies/cell	NA	607 (801)	560 (601)

**NOTE.** Data on baseline antiretroviral regimens were available for all but 1 subject. Compared with the main study cohort [16], fewer subjects in the placebo group were prescribed thymidine-analogue nucleoside reverse-transcriptase inhibitor (tNRTI)-containing antiretroviral therapy. When the substudy groups were compared, subjects randomized to receive rosiglitazone (RSG) had lower trunk and limb fat and more visceral adipose tissue. *ACTB*, gene encoding β-actin; BMI, body mass index; *FABP4*, gene encoding fatty acid-binding protein 4; IQR, interquartile range; last tNRTI exposure, time since last exposure to tNRTIs in those not currently taking tNRTIs; *MTCYB*, gene encoding mitochondrial cytochrome b; NA, data not available; PI, protease inhibitor; *PPARG*, gene encoding peroxisome proliferator-activated receptor γ (PPARγ); *PPARGC1A*, gene encoding PPARγ coactivator 1α.

where [21]. We used 2 μL (20 ng) aliquots of cDNA or gDNA for real-time quantitative PCR (Lightcycler; Roche Applied Science). Samples were run in duplicate, with internal positive and negative controls, and gene product was quantified by comparing samples to known standard concentrations of pure gene product.

**Statistical analysis.** For mRNA expression, results were presented as a ratio relative to β-actin expression. There were no significant differences in *ACTB* (β-actin) expression between the randomized groups at baseline, week 2, or week 48 (all  $P > .4$ ). There were also no significant changes in *ACTB* expression between time points either for the cohort as a whole or within the randomized groups ( $P > .2$  for change from baseline to week 2 and  $P > .15$  for change from baseline to week 48, for all analyses).

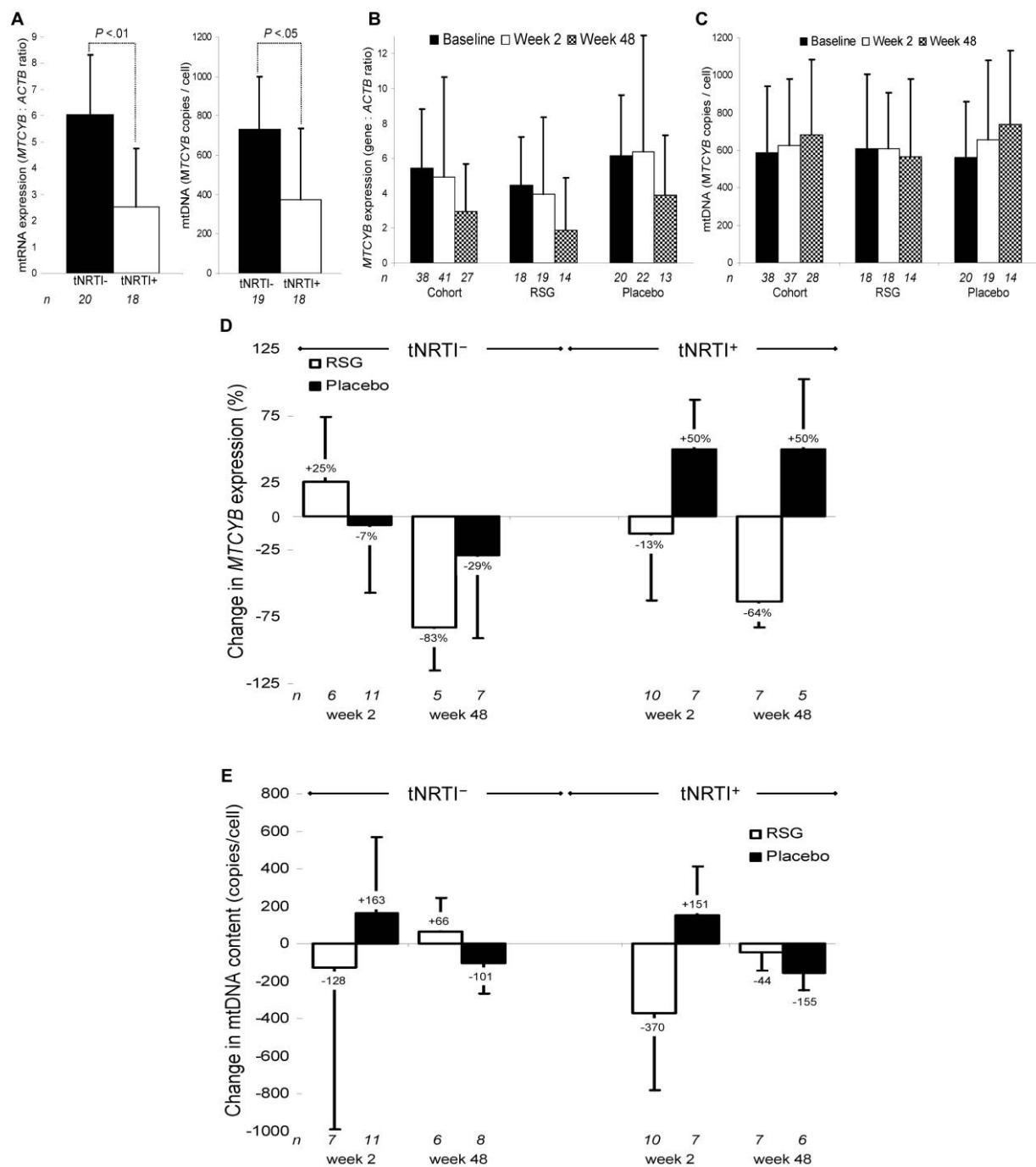
For mtDNA content, we used a nuclear gene (*PPARG*) measured in copy number (2 copies per cell) and a mitochondrial gene (*MTCYB*), with results expressed as copies per cell. Gene expression was calculated at individual time points, and the percentage change in the expression of genes of interest from baseline to week 2 and week 48 was determined. Unless stated otherwise, results are presented as medians and interquartile ranges (IQRs). Nonparametric analyses were applied to univariate and

bivariate comparisons, and regression analysis was used to control for potential confounders in adjusted bivariate analyses. Potential confounders were identified as baseline limb fat mass, trunk fat mass, BMI, lipid levels (total, low-density lipoprotein and high-density lipoprotein cholesterol and triglycerides), glucose level, insulin level, leptin level, adiponectin level, CD4<sup>+</sup> T cell count, age, HIV infection stage, randomization to RSG, exposure to tNRTI therapy, and exposure to PI therapy. As this was exploratory research, no power calculations were available to guide sample size. We did not adjust  $P$  values for multiple comparisons.

## RESULTS

**Baseline characteristics.** Subjects randomized to receive RSG and subjects randomized to receive placebo were similar with respect to all baseline clinical and demographic characteristics (table 1).

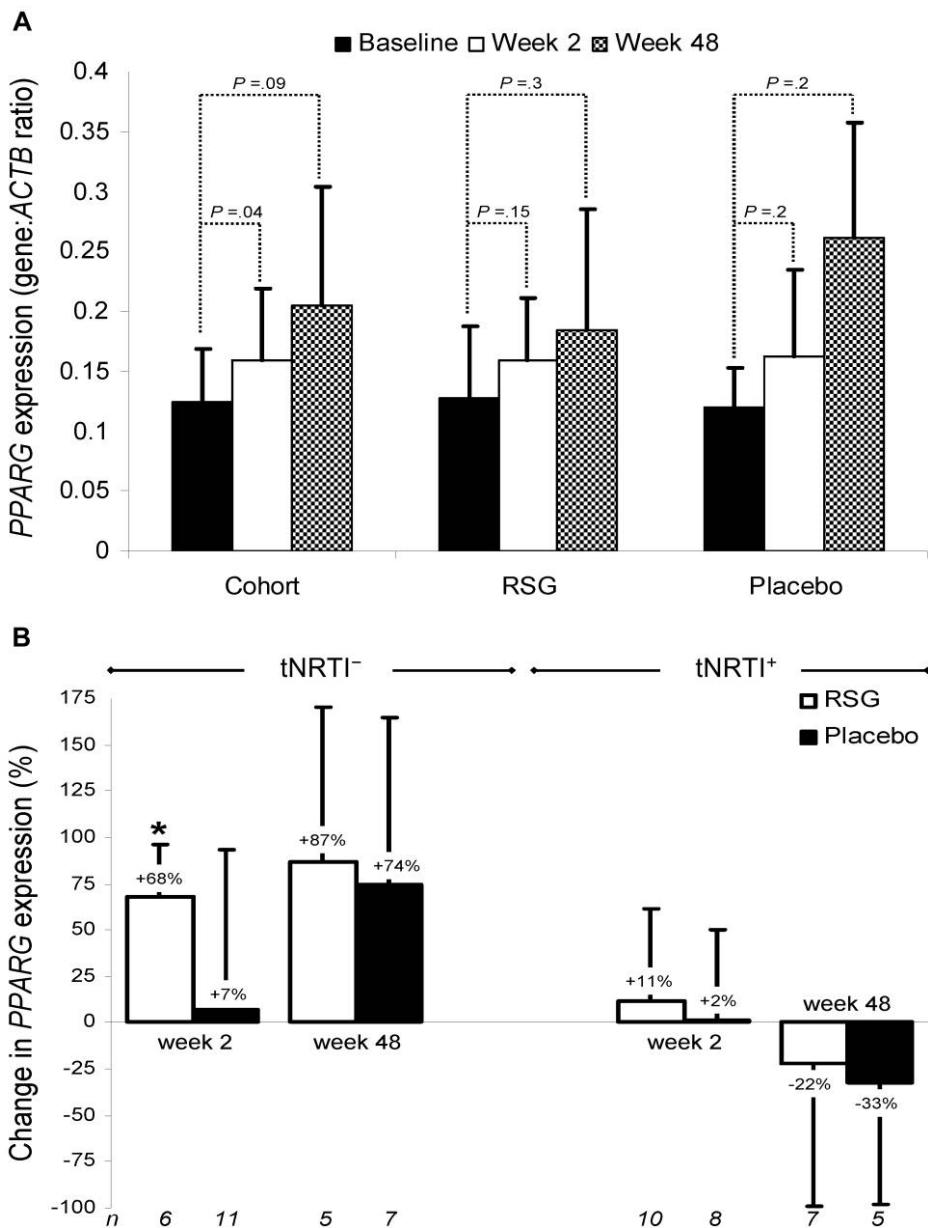
**mtRNA expression and mtDNA content.** At baseline, mtRNA expression and mtDNA content were significantly lower (58% and 53%, respectively) in subjects who received tNRTI-containing HAART, compared with those who received HAART that did not include tNRTIs (figure 1A). RSG did not signifi-



**Figure 1.** Baseline values and changes in adipose tissue mitochondrial RNA expression (mtRNA) and mitochondrial DNA content (mtDNA). At baseline those receiving continued therapy that contained thymidine-analogue nucleoside reverse-transcriptase inhibitors (tNRTI<sup>+</sup>) had lower levels of mtDNA and lower mtRNA expression, compared with those who received therapy that did not include tNRTIs (tNRTI<sup>-</sup>)/A). Therapy with rosiglitazone (RSG) had no effect on either mtDNA levels or mtRNA transcription (B and C). Expression of the gene encoding mitochondrial cytochrome b (*MTCYB*) and mtDNA content did not significantly change between the randomized groups, regardless of their exposure to tNRTIs (D and E). Numbers label median values, and whiskers indicate the interquartile range divided by 2. *ACTB*, gene encoding  $\beta$ -actin; cohort, main study cohort.

cantly alter adipose mtRNA expression at 2 or 48 weeks (figure 1B). Similarly, RSG did not affect adipocyte mtDNA at 2 and 48 weeks (figure 1C). Concurrent exposure to tNRTIs did not affect these results (figures 1D and 1E).

**PPAR $\gamma$ (PPARG) expression.** Higher baseline expression of adipose tissue PPARG was associated with lower trunk fat mass on dual energy x-ray absorptiometry ( $\rho = -0.37$ ;  $P = .02$ ), lower VAT on CT ( $\rho = -0.34$ ;  $P = .04$ ) and higher high-density lipoprotein

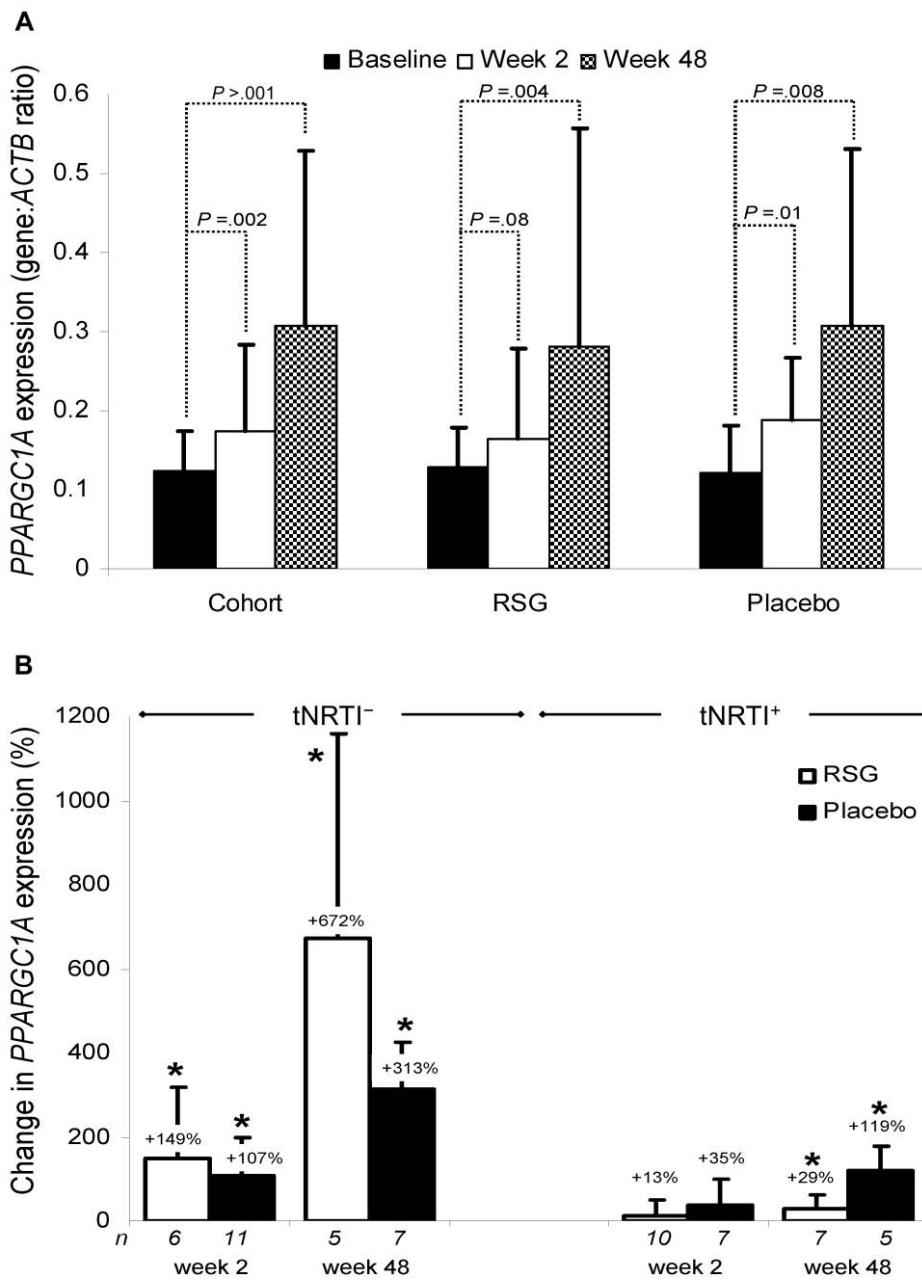


**Figure 2.** Changes in adipose tissue expression of the gene encoding peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$  [*PPARG*]). Comparison of the randomized groups did not show any differences in the changes in *PPARG* (A). At week 2, increased expression of *PPARG* was only noted in those randomized to receive rosiglitazone (RSG) who were not receiving ongoing thymidine-analogue nucleoside reverse-transcriptase inhibitor (tNRTI $^-$ ) therapy, although by week 48 an increase in *PPARG* was noted in the tNRTI $^-$  group regardless of their receipt of RSG therapy (B). There was no significant difference in baseline *PPARG* expression between the randomized groups (median [interquartile range {IQR}] for RSG vs. placebo, 0.13 [0.12] vs. 0.12 [0.07];  $P = .8$ ), between those on continued protease inhibitor therapy (PI) (median [IQR] for PI vs. no PI, 0.13 [0.11] vs. 0.12 [0.05];  $P > .7$ ), or between those on continued tNRTI therapy (tNRTI $^+$ ) and the tNRTI $^-$  group (median [IQR], 0.13 [0.11] vs. 0.12 [0.07];  $P > .2$ ). \* $P < .05$ , by the Wilcoxon signed rank test. Numbers label median values, and whiskers indicate the interquartile range divided by 2. *ACTB*, gene encoding  $\beta$  actin; cohort, main study cohort.

cholesterol ( $\rho = 0.33$ ;  $P = .047$ ). None of these factors, however, predicted change in *PPARG* expression (data not shown).

Overall, *PPARG* expression increased at both week 2 (median [IQR], 13% [103%]) and week 48 (median [IQR], 121% [298%]), although RSG did not significantly increase *PPARG* expression at either time point, compared with

placebo (figure 2A). However, *PPARG* expression increased in subjects not receiving tNRTIs, compared with those receiving tNRTIs (median [IQR] week 48 difference between subjects not receiving tNRTIs and those receiving tNRTIs, 81% [165%] vs. -25% [142%];  $P = .04$ ). In those subjects not receiving tNRTIs who were receiving RSG, *PPARG* expression increased at

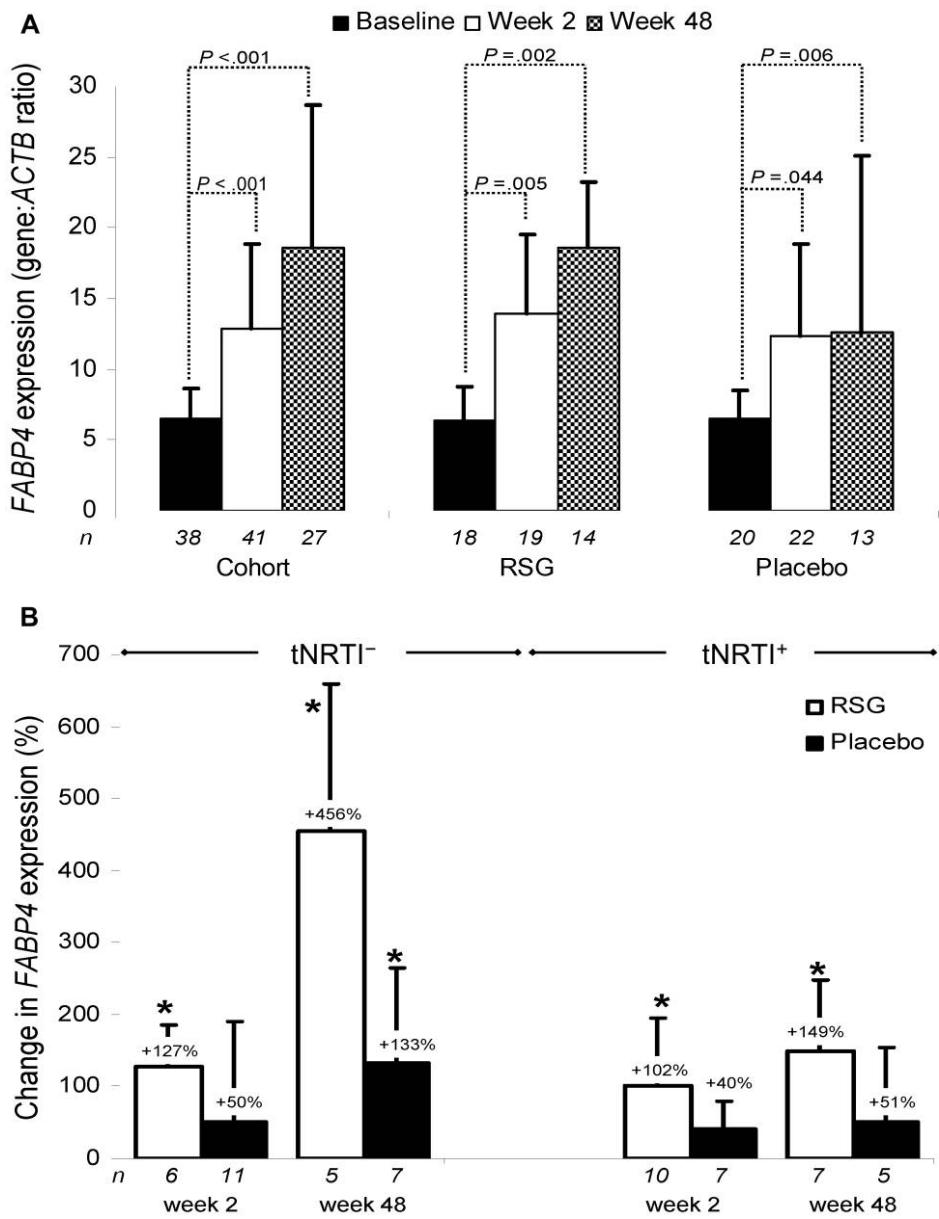


**Figure 3.** Changes in adipose tissue expression of the gene encoding peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) coactivator 1 $\alpha$  (PGC-1 $\alpha$  [PPARGC1A]). Similar to changes in expression of the gene encoding PPAR $\gamma$  (PPARG), there were no between-group differences with respect to change in PPARGC1A expression (A), and the largest changes in PPARGC1A expression occurred in subjects who were not receiving continued thymidine-analogue nucleoside reverse-transcriptase inhibitor therapy (tNRTI-) (B), an effect evident at both week 2 and week 48. \* $P < .05$ , by the Wilcoxon signed rank test. Numbers label median values, and whiskers indicate the interquartile range divided by 2. ACTB, gene encoding  $\beta$  actin; RSG, rosiglitazone; tNRTI+, subjects who were receiving tNRTI therapy.

week 2, but no difference was found at week 48, as PPARG expression also improved in the placebo group in those not receiving tNRTIs (figure 2B). In contrast, there was no increase in PPARG expression at 2 or 48 weeks in the subjects who were receiving tNRTI-based antiretroviral therapy, whether randomized to receive RSG or placebo (figure 2B).

**PGC-1 $\alpha$  (PPARGC1A) expression.** In common with changes in PPARG expression, expression of PPARGC1A in-

creased at week 2 (median [IQR], 41% [133%]) and week 48 (median [IQR], 121% [298%]) (figure 3A), with similar changes observed across randomized groups (between group difference  $P = .9$  for week 2 and  $P = .4$  for week 48). When the groups were analyzed on the basis of current tNRTI use, significantly greater increases in PPARGC1A were seen in subjects not receiving tNRTIs at week 2 (median [IQR] for subjects not receiving tNRTIs vs. those receiving tNRTIs, 107% [240%] vs. 21%



**Figure 4.** Changes in adipose tissue expression of the gene encoding fatty acid–binding protein 4 (*FABP4*). Although *FABP4* expression increased significantly in both randomized groups, there were no between-group differences in *FABP4* expression (A). The greatest changes in *FABP4* expression were seen in subjects randomized to receive rosiglitazone (RSG) who were not receiving thymidine-analogue nucleoside reverse-transcriptase inhibitor therapy (tNRTI<sup>-</sup>) (B), increases were seen in all patients treated with RSG, which suggests that RSG may have some biological activity even in the presence of continued tNRTI therapy. \* $P < .05$ , by the Wilcoxon signed rank test. Numbers label median values, and whiskers indicate the interquartile range divided by 2. *ACTB*, gene encoding  $\beta$  actin; tNRTI<sup>+</sup>, subjects who were receiving tNRTI therapy.

[81%];  $P = .02$ ) and week 48 (median [IQR] for subjects not receiving tNRTIs vs. those receiving tNRTIs, 315% [615%] vs. 71% [97%];  $P = .008$ ). As with *PPARG*, the greatest increases in *PPARGC1A* were observed in those randomized to RSG who were not receiving tNRTIs, an effect apparent at both week 2 and week 48 (figure 3B).

**Adipocyte fatty acid–binding protein (FABP4) expression.** To determine whether changes in *PPARG* and *PPARGC1A* expression were biologically relevant, we examined changes in the ex-

pression of *FABP4*, a PPAR $\gamma$ -responsive gene that encodes adipocyte fatty acid–binding protein (aP2). Changes in *PPARG* and *FABP4* were closely correlated at both week 2 ( $\rho = 0.7$ ;  $P < .001$ ) and week 48 ( $\rho = 0.79$ ;  $P < .001$ ). As with changes in other lipid metabolism genes, the expression of *FABP4* increased in both the RSG and placebo groups at weeks 2 and 48 (figure 4A). At week 2, the increases in *FABP4* expression were larger in those randomized to receive RSG, regardless of their exposure to tNRTIs, whereas at week 48, the greatest increases in

*FABP4* expression were observed in subjects randomized to receive RSG who were not receiving tNRTIs (figure 4B).

**Associations between changes in gene expression and changes in limb fat.** Subjects with greater increases in limb fat at week 48 had greater percentages of change in the expression of *PPARG* ( $\rho = 0.56$ ;  $P = .007$ ) and *FABP4* ( $\rho = 0.45$ ;  $P = .03$ ) in adipose tissue at week 48. For *PPARG*, this effect persisted in adjusted analyses, whereas the significance of the association between the change in limb fat and the change in *FABP4* expression was lost when adjusted for either receipt of tNRTI therapy or randomization to receive RSG. Changes in nuclear or mitochondrial gene expression or mtDNA content to week 2 did not predict the change in limb fat at either week 24 or week 48, and there were no other significant associations between the percentage change in limb fat at week 48 and baseline or the change from baseline in the expression of other nuclear or mitochondrial genes or mtDNA content (all  $P > .1$ ).

**Changes in gene expression and previous antiretroviral therapy exposure.** There were no significant associations between duration of antiretroviral exposure and changes in *PPARG* expression during the study. Greater total exposure to tNRTIs was associated with lower baseline expression of both *MTCYB* ( $\rho = -0.45$ ;  $P = .007$ ) and *PPARG* ( $\rho = -0.42$ ;  $P = .01$ ). In addition, a longer period of time since last exposure to tNRTIs was associated with greater *MTCYB* expression at baseline ( $\rho = 0.63$ ;  $P < .001$ ) and greater adipose tissue mtDNA content at all 3 time points (baseline:  $\rho = 0.46$ ,  $P = .009$ ; week 2:  $\rho = 0.68$ ,  $P < .001$ ; and week 48:  $\rho = 0.56$ ,  $P = .005$ ). Greater time since tNRTI exposure was also associated with greater changes in *PPARGC1A* expression at week 2 ( $\rho = 0.4$ ;  $P = .03$ ) and week 48 ( $\rho = 0.75$ ;  $P < .001$ ), effects that remained significant in adjusted analyses.

## DISCUSSION

The results from this study suggest that intact mitochondrial function is required for TZD-induced stimulation of *PPARG* expression in human adipose tissue and help explain why treatment with 4 mg of RSG twice daily for 48 weeks did not increase limb fat mass in a group of HIV-infected men with lipoatrophy. These data are consistent with the lack of effect of RSG in those receiving tNRTIs, both at a clinical and molecular level. Although both mtRNA expression and mtDNA content were unaffected by treatment with RSG, those receiving continued tNRTI therapy had significantly lower baseline adipose tissue mtDNA content and mtRNA expression, consistent with impaired mitochondrial function. In addition, the close associations between mtRNA and *PPARG* expression previously noted in our healthy volunteer study [6] were also noted in this study, indicating that any relationship between mitochondrial function and *PPARG* expression is not mediated by HIV infection.

The observed changes in adipose tissue *PPARG* expression are likely to be biologically and functionally relevant, since changes in *PPARG* expression, the PPAR $\gamma$ -responsive gene *FABP4*, and limb fat mass were significantly related. This underscores the importance of *PPARG* expression in the control of limb fat mass and its likely role in the development of lipoatrophy, as well as corroborating the clinical impression that cessation of tNRTIs is more useful than TZD therapy as an intervention for lipoatrophy in this population [16, 22].

In contrast to the changes in expression of *PPARG* and *PPARGC1A*, increases in *FABP4* expression were observed with RSG therapy at both weeks 2 and 48, regardless of ongoing tNRTI exposure. This suggests RSG has physiological activity even in the presence of ongoing tNRTI therapy. Corroborative clinical evidence of this was provided by the observation that rosiglitazone increased adiponectin levels at 48 weeks and improved insulin resistance, despite its lack of effect on fat mass [16]. These results suggest that either the inhibitory effects of tNRTIs on *PPARG* expression are specific to adipose tissue or that both the insulin-sensitizing effects of RSG and the RSG-induced increases in adiponectin levels may be at least partially independent of adipose PPAR $\gamma$  pathways, an effect not previously described.

The patterns of change in expression of *PPARGC1A* in response to RSG were similar to, although larger than, those observed for *PPARG* expression. These changes, and the association between changes in *PPARGC1A* expression and the interval since last exposure to tNRTIs, support a scenario in which recovery from tNRTI-induced mitochondrial toxicity and effects secondary to treatment with RSG both contribute to the observed changes in *PPARGC1A* expression. Whether or not the effects of RSG on *PPARGC1A* expression arise as a result of a direct ligand-binding effect of RSG or secondary to changes in RSG-stimulated *PPARG* expression requires further study.

The *PPARGC1A* gene encodes PGC-1 $\alpha$ , a transcriptional co-factor important in the regulation of both oxidative phosphorylation and mitochondrial biogenesis [23–27]. In rodents, lack of PGC-1 $\alpha$  results in decreased oxidative capacity, increased total body fat, and lower tissue mitochondrial number [28]. Its expression in humans is affected by several factors, including age, hereditary status (polymorphisms), and plasma insulin levels [29]. Although there were no correlations between change in *PPARGC1A* expression and mtRNA or mtDNA, the relationship between changes in *PPARGC1A* expression and time since last exposure to tNRTIs, with greater increases in *PPARGC1A* expression seen in those who had been off tNRTI therapy longer, is in keeping with what is known about relationships between PGC-1 $\alpha$  and mitochondrial biogenesis [23–26]. Although such interactions could help explain the effect of continued tNRTI exposure in limiting RSG-induced *PPARG* expression, results from healthy volunteers given tNRTIs showed NRTI-induced reductions in both *PPARG* and mtRNA expression coinciding

with compensatory increases, rather than decreases, in *PPARGC1A* expression [6]. This suggests an alternative, more direct, but as yet undetermined mechanism that controls the interaction between mRNA and *PPARG* expression, rather than one mediated through the action of *PPARGC1A*.

This study shares some similarities with a previous smaller study by Sutinen et al., which also found increased expression of *PPARG* and *PPARGC1A* but no change in *FABP4* expression in adipose tissue biopsies from a study in which lipoatrophic patients received 8 mg of RSG daily for 24 weeks [30]. It did not examine mitochondrial gene expression and reported no associations between changes in expression of *PPARG* and changes in limb fat. These differences may be the result of the shorter duration of therapy (24 versus 48 weeks), the needle biopsy procedure, the smaller number of subjects in the Sutinen et al. study, or the greater percentage of subjects for whom tNRTIs were prescribed in their study (93%, compared with 48% in the present study).

Our study has important limitations. No women or children were recruited, because of the demographics of HIV infection in Australia. Fewer subjects underwent biopsy at week 48 than at baseline and week 2. This reduced the statistical power of the observations at that time point. As the treatment of HIV is constantly changing, therapies also change, and although subjects largely received a single HAART combination during the study, 5 of 43 subjects had discontinued tNRTI therapy as part of their HAART regimen in the 12 months prior to study enrolment. Such variability makes it difficult to control for the effects of discontinuation of tNRTI therapy on adipose gene expression and limb fat mass. Despite these limitations, the study offers new insights into clinically relevant relationships between mitochondrial dysfunction and lipid metabolism. Although the data explain why RSG may be ineffective in subjects who receive tNRTI-containing HAART, further research is needed to determine whether TDZ may have a role to play in other clinical scenarios of lipoatrophy, such as in patients who switch from tNRTIs to other antiretrovirals. These data are not only relevant to our understanding of the pathogenesis of HIV-associated lipoatrophy but also reinforce and offer further insights into previously suggested roles for mitochondrial dysfunction in other related conditions, such as lipotoxicity and type 2 diabetes [31]. Given recent controversies surrounding the use of RSG and cardiovascular disease [32], dissecting the mechanism whereby mitochondrial function affects *PPARG* expression points to the possibility of manipulating this relationship through the identification of new therapeutic targets to help in the management of these common conditions.

## ROSEY INVESTIGATORS

Steering committee: Andrew Carr (St. Vincent's Hospital, Sydney; coprincipal investigator); David A. Cooper (coprincipal in-

vestigator), Dianne Carey, Sean Emery, Matthew Law, and Allison Martin (National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney); and Katherine Samaras (Garvan Institute of Medical Research, University of New South Wales, Sydney).

Other investigators (listed in order of the number of patients recruited): Jacinta Perram and Janice Forrester (Ground Zero Medical Centre, Sydney); Sarah Makinson and Michael Curry (Department of General Practice, University of Adelaide); Hugh MacLeod, Robert McFarlane, William Genn, and Robyn Vale (407 Doctors, Sydney); Robert Finlayson, John White, Ross Price, Neil Bodsworth, Cathy Pell, and Wilma Goodyear (Taylor Square Private Clinic, Sydney); Patrick Mallon and Sheena McLeod (St. Vincent's Hospital, Sydney); Norman Roth, Sven Strecker, Beng Eu, and Helen Wood (Prahran Market Clinic, Melbourne); David Austin, Mark Bloch, Dick Quan, Andrew Gowers, Rohan Holland, Cari Egan, and Samantha Miller (Holdsworth House General Practice, Sydney); Kit Fairley, Tim Read, Tina Schmidt, Catriona Bradshaw, Julie Silvers, and Helen Kent (Melbourne Sexual Health Centre); Jonathan Anderson and Julie Patching (Carlton Clinic, Melbourne); Roger Garsia and Marry Moussa (Royal Prince Alfred Hospital, Sydney); John Chuah, Stuart Aitken, Robyn James, and Fiona Clark (Gold Coast Sexual Health Centre, Miami); Mark Kelly, Don Smith, Harry Michelmore, and Tonia Rihs (Albion Street Centre, Sydney); Jenny Hoy, Olga Vujovic, Margaret Hellard, Anna Pierce, Lyn-li Lim, and Sally Algar (The Alfred Hospital, Melbourne); David Orth, Graham Lister, Kate Evans, and David Youds (Brunswick Street Medical Centre, Melbourne); Nicholas Medland, Claudio Villella, and Helen Wood (Centre Clinic, Melbourne); Martyn French and Esther Edward (Royal Perth Hospital); and Nicholas Doong and Jeff Hudson (Burwood Road Practice, Sydney).

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