

# The Effect of $\beta$ -Adrenergic and Peroxisome Proliferator-Activated Receptor- $\gamma$ Stimulation on Target Genes Related to Lipid Metabolism in Human Subcutaneous Adipose Tissue

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metabolism in subcutaneous fat by increasing the expression of genes required for fatty acid catabolism.

*Diabetes Care* 30:1179–1186, 2007

**OBJECTIVE** — The sympathetic nervous system and thiazolidinediones control lipid metabolism and have been implicated in body weight regulation. This study was conducted to determine whether the simultaneous activation of these two signaling systems might synergize to exert beneficial effects on the expression of key genes involved in lipid metabolism and mitochondrial biogenesis in subcutaneous fat in nondiabetic subjects.

**RESEARCH DESIGN AND METHODS** — A total of 57 women and men were randomized into four groups: 1) placebo/placebo (PP), 2) ephedrine HCl (25 mg, 3 times daily) plus caffeine (200 mg, 3 times daily)/placebo (ECP), 3) placebo/pioglitazone (45 mg) (PPio), and 4) ephedrine plus caffeine/pioglitazone (ECPio) for 16 weeks. Adipose tissue samples were obtained after 12 weeks of treatment to determine gene expression.

**RESULTS** — Body fat decreased by 6.0 and 4.6% in the ECP and ECPio groups, respectively, while remaining unchanged in the PPio and PP groups. Triglyceride levels decreased by  $-7.7$ ,  $-24$ ,  $-15.2$ , and  $-41$  mg/dl after 16 weeks treatment in the PP, PPio, ECP, and ECPio groups, respectively. This indicates that pioglitazone groups with or without EC (ephedrine HCl plus caffeine) decreased triglycerides, and EC groups with or without pioglitazone decreased body weight. The mRNA for sirtuin 1 and CD36 increased only in the ECPio group. Carnitine palmitoyltransferase-1, medium-chain acyl CoA dehydrogenase, and malonyl-CoA decarboxylase increased with PPio and ECPio. Stearoyl-CoA desaturase decreased with ECP.

**CONCLUSIONS** — Combined activation of peroxisome proliferator-activated receptor- $\gamma$  and  $\beta$ -adrenergic receptors has beneficial effects on body weight, plasma triglycerides, and lipid

In mammals, mature fat cells are characterized by a high degree of plasticity and the ability to transdifferentiate between the white adipose tissue (WAT) and brown adipose tissue phenotype (1). WAT is also important as a determinant of lifespan, and it has been proposed that a reduction in body fat will extend life (2). The exact mechanisms responsible for adipocyte remodeling are still not completely understood, but several signaling pathways have been implicated. Chronic stimulation of  $\beta$ -adrenergic receptors activates thermogenic systems, and it is an effective antiobesity and antidiabetes maneuver in rodents (3,4). Furthermore,  $\beta$ -adrenergic stimulation remodels white-type adipocytes into a brown, more oxidative phenotype in rodent models (5–8). A number of in vivo and in vitro experiments indicate that  $\beta$ -adrenergic activation increases the expression of several genes controlling mitochondrial biogenesis and oxidative metabolism in brown adipose tissue or WAT (9–11). Peroxisome proliferator-activated receptor (PPAR) coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) (12,13) and PPAR- $\alpha$  are key factors driving these two processes (14). Uncoupling protein-1 (UCP-1) is considered a marker of the brown fat thermogenic phenotype.  $\beta$ -Adrenergic stimulation increases PGC-1 $\alpha$  as well as UCP-1 mRNA and protein levels in WAT (11,15).

The sympathetic nervous system, via the intracellular messenger cAMP and/or the mitogen-activated protein kinase activation by PPAR- $\gamma$ , controls lipid metabolism and body weight. PPAR- $\gamma$  activation results in the remodeling of adipocytes, and combined therapy with  $\beta$ -adrenergic stimulators exerts a synergistic effect to produce a negative energy balance in ro-

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Received for publication 20 September 2006 and accepted in revised form 13 February 2007.

Published ahead of print at <http://care.diabetesjournals.org> on 10 March 2007. DOI: 10.2337/dc06-1962. Clinical trial reg. no. NCT00377975, clinicaltrials.gov.

S.R.S. has served as a member of an advisory board for and received grant funding from Takeda Pharmaceuticals.

Additional information for this article can be found in an online appendix at <http://dx.doi.org/10.2337/dc06-1962>.

**Abbreviations:** CPT-1, carnitine palmitoyltransferase-1; CT, computed tomography; EC, ephedrine HCl plus caffeine; FATP/CD36, fatty acid transporter; LPL, lipoprotein lipase; MCAD, medium-chain acyl CoA dehydrogenase; MLYCD, malonyl-CoA decarboxylase; PGC-1 $\alpha$ , peroxisome proliferator-activated coactivator 1 $\alpha$ ; PPAR, peroxisome proliferator-activated receptor; SAT, subcutaneous adipose tissue; SCD-1, stearoyl-CoA desaturase-1; UCP-1, uncoupling protein-1; VAT, visceral adipose tissue; WAT, white adipose tissue.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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dents (16). This was accompanied by an increase in the number of small, insulin-sensitive adipocytes, mitochondrial biogenesis, and increased expression of UCP-1. The above observations suggest the existence of cross-talk between two distinct signal transduction pathways (cAMP and PPAR- $\gamma$ ) in adipose tissue (16). Indeed, both systems drive the expression of important genes necessary for lipid uptake and oxidation. For example, lipoprotein lipase (LPL) was upregulated after cAMP (17) and PPAR- $\gamma$  (18,19) activation. Combined therapy with ephedrine plus caffeine/pioglitazone (ECPio) significantly reduced plasma triglycerides (triglycerides), VLDL, and LDL levels while it increased HDL total mass (20). Our and several previous results demonstrated that PPAR- $\gamma$  activation increases body weight and the expression of several genes involved in mitochondrial biogenesis and lipid metabolism in subcutaneous fat obtained from subjects with type 2 diabetes (18,20–22).

Taken together, the above results suggest that activation of the two described signaling systems might synergize to exert beneficial effects on body weight, body fat, and blood lipids via the regulation of key genes involved in lipid metabolism and mitochondrial biogenesis in subcutaneous fat.

## RESEARCH DESIGN AND METHODS

— Nondiabetic patients were enrolled in a clinical trial performed in Baton Rouge, Louisiana, at the Pennington Biomedical Research Center (14 men and 43 women between 18 and 50 years of age with BMI 30–37 kg/m<sup>2</sup>). The characteristics of the study population were previously reported (20). Subjects were healthy and not taking thiazolidinediones,  $\beta$ -blockers, orlistat, sibutramine, ephedrine, phenylpropranolamine (Dexatrim), corticosteroids, statins, fibrates, cholesterol-binding drugs, or herbal supplements containing ephedrine and/or caffeine; abusing alcohol; or using other illicit drugs. Subjects were randomized into four groups: 1) placebo/placebo (PP), 2) ephedrine HCl plus caffeine/placebo (ECP), 3) placebo/pioglitazone (PPio), and 4) ephedrine HCl plus caffeine/pioglitazone (ECPio). The placebo, the pharmaceutical ephedrine HCl (25 mg) (Breathe Easy; Contract Pharmacal Corporation, Hauppauge, NY) and caffeine (200 mg) (Contract Pharmacal Corporation) were dosed as follows: one of

each pill per day for 7 days at breakfast, increased to one of each pill at breakfast and lunch for the next 7 days, and then increased to one of each pill at breakfast, lunch, and dinner for the remainder of the 16-week protocol. Subjects who could not tolerate three doses of EC (ephedrine HCl plus caffeine) per day were allowed to continue on two doses per day. If subjects could not tolerate two doses per day they were dropped from the protocol. Medications were taken at least 4 h apart to reduce side effects (tremor and tachycardia). Pioglitazone was initiated at 15 mg/day and increased by 15 mg each week until the maximum dose of 45 mg was achieved. All subjects signed a consent form approved by the Pennington Biomedical Research Center Ethical Review Board after potential risks and procedures had been explained. At treatment initiation, all subjects were given a short standardized instruction on diet and behavior modification as well as instructions on healthy activity levels (walking). The primary end point was the percentage of fat measured with dual-energy X-ray absorptiometry after 16 weeks of treatment using a Hologic QDR 4500 DEXA.

Adipose tissue was obtained by Bergstrom needle biopsies from subcutaneous depots 6–10 cm lateral to the umbilicus at baseline (0 week) and after 12 weeks of treatment following an overnight fast. Fat samples were cleaned of visible connective tissue and blood vessels, immediately frozen, and stored at  $-70^{\circ}\text{C}$  until assays.

## Body composition

Body fat mass and lean mass were measured on a Hologic Dual Energy X-ray Absorptiometer (QDR 4500A; Hologic, Waltham, MA). Visceral adiposity was determined by multislice computed tomography (CT) scanning using a General Electric High-Speed Computed Tomography scanner under an established protocol (21).

## RNA and DNA extraction

Total RNA from 50 to 100 mg adipose tissue was isolated with Trizol reagent (Invitrogen, Carlsbad, CA) and purified with RNeasy columns (QIAGEN, Valencia, CA) according to manufacturers' procedure. The quantity and quality of the RNA were confirmed by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). TNA (total nucleic acid) was extracted from a separate sample of adipose tissue (20 mg) by isopropanol precipitation using MasterPure Com-

plete DNA and RNA Purification kit (EPICENTRE, Madison, WI). The total DNA recovered was determined by spectrophotometry.

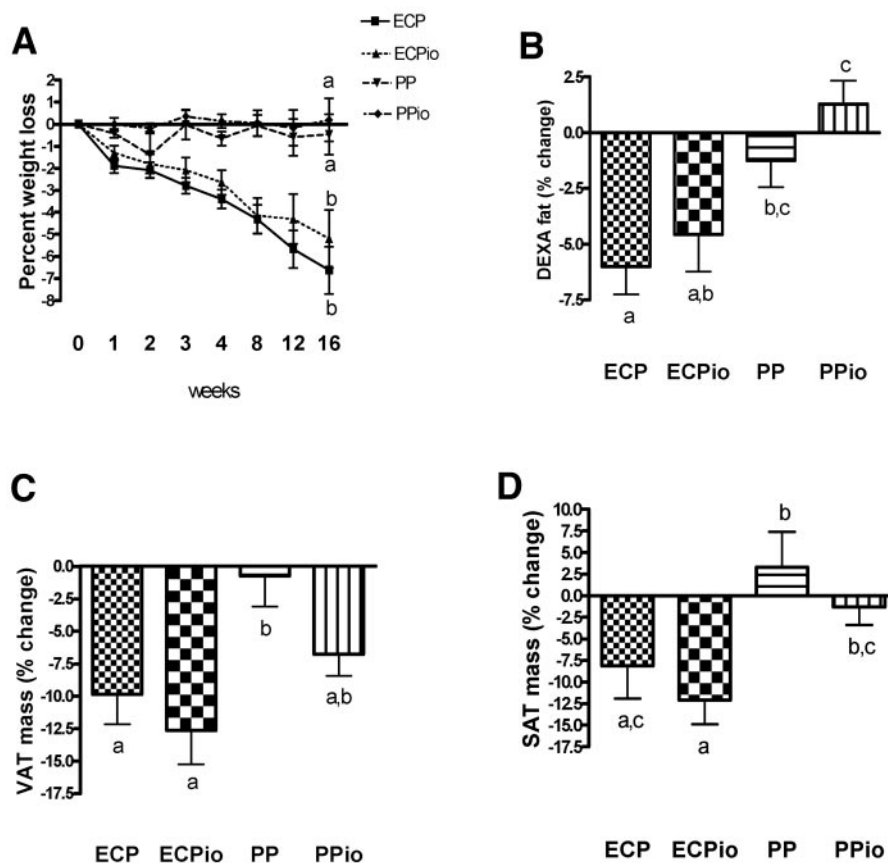
## Real-time RT-PCR for RNA

Real-time RT-PCR was performed using a Taqman 100Rxn PCR Core Reagent Kit (Applied Biosystems, Roche, Branchburg, NJ) as previously described (15,18,22). Real-time RT-PCR was carried out in an ABI PRISM 7900 sequence detector (Applied Biosystems, Branchburg, NJ) using the following parameters: 1 cycle of  $48^{\circ}\text{C}$  for 30 min and  $95^{\circ}\text{C}$  for 10 min followed by 40 cycles at  $95^{\circ}\text{C}$  for 15 s and  $60^{\circ}\text{C}$  for 1 min. A standard curve was prepared by serial dilution of pooled total RNA and each gene/sample was compared with this standard curve. All expression data were normalized by dividing the amount of target gene by the amount of cyclophilin B applied as an internal control. To determine the tissue content of mitochondria per cell, real-time PCR for mitochondrial DNA was applied as described previously (22).

## Statistical analysis

All values are presented in figures and tables as raw means  $\pm$  SE unless otherwise noted as SD. Significant differences were assumed for  $P < 0.05$ . Gene expression data were analyzed by paired *t* test within treatment group using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA). Serial lipid data was analyzed using a mixed model (PROC MIXED) in SAS (Cary, NC). Pearson's correlations were performed in JMP version 3.0 (SAS, Cary, NC).

**RESULTS**— The characteristics of the overweight/obese study population are presented in supplemental Table 1 (found in an online-only appendix at <http://dx.doi.org/10.2337/dc06-1962>). Generally, the treatment groups were well balanced except for the baseline triglycerides, which were by chance higher in the PPio patients. One subject in the PPio and ECP groups and three subjects in the ECPio group were able to tolerate only two EC doses per day. The clinical parameters of the study population expressed as percent of change after 16 weeks of treatment with ephedrine, caffeine, and/or pioglitazone are presented in Fig. 1. We observed a 6.6% weight loss in the ECP group and 5.2% weight decrease after combined intervention with ECPio (Fig. 1A). The



**Figure 1**—Effect of the 16 weeks of treatment with placebo (PP), pioglitazone (PPio), ephedrine/caffeine (ECP), and pioglitazone plus ephedrine/caffeine (ECPio) on weight loss (A), dual-energy X-ray absorptiometry (DEXA) fat (B), VAT (C), and SAT (D). Groups with the different letters are significantly different at  $P < 0.05$ .

body weight of volunteers treated with pioglitazone alone or placebo did not change (Fig. 1A). Thus, the major treatment effects on body weight and adiposity were due to the EC treatment; the addition of pioglitazone did not increase body weight loss as seen in the preclinical rodent models. The pattern of change in percent fat paralleled the observed changes in body weight (Fig. 1B). Body fat decreased by 6.0% in the ECP group and 4.6% in the ECPio group, while body fat remained unchanged in the PPio and PP groups (Fig. 1B). Visceral and subcutaneous fat measured by CT decreased in proportion to the reduction in weight and body fat (Fig. 1C and D).

After combination therapy, triglycerides decreased by 41 mg/dl in the ECPio group, whereas treatment with EC alone or pioglitazone alone decreased the levels by 15.2 and 24 mg/dl, respectively (Table 1). HDL cholesterol increased by 7.8 mg/dl in the ECPio group, 3.7 mg/dl in the ECP group, and 5.1 mg/dl in the PPio group. Thus, the reduction in triglyceride levels can be largely attributed to the pio-

glitazone treatment, with no statistically significant impact from adding EC.

#### Treatment effects on the expression of genes involved in lipid metabolism

Pioglitazone treatment markedly increased mRNA expression for PEPCK1 ( $P = 0.01$ ) (Fig. 2) but did not affect LPL (Fig. 2A), fatty acid transporter (FATP/CD36) (Fig. 2B), fatty acid synthase (data not shown), or stearoyl-CoA desaturase-1 (SCD-1) (Fig. 2D) mRNA levels when compared with the placebo-treated subjects. Pioglitazone treatment also increased carnitine palmitoyltransferase-1 (CPT-1,  $P < 0.01$ ) (Fig. 3A), medium-chain acyl CoA dehydrogenase (MCAD,  $P < 0.05$ ) (Fig. 3B), and malonyl-CoA decarboxylase (MLYCD) ( $P < 0.01$ ) (Fig. 3C) but did not change PPAR- $\alpha$  (Fig. 3D) or cytochrome C (data not shown) mRNA levels. Treatment with pioglitazone alone did not change the expression of genes involved in mitochondrial biogenesis including PGC-1 $\alpha$ , mtTFA, NRF-1, ERR $\alpha$ , AMPK $\alpha$ , or SIRT1s 1–3 (data not shown).

The upregulation of these three lipid oxidation genes (CPT01, MCAD, and MLYCD) suggests that the adipocytes might be reprogrammed for greater fat oxidation when stimulated by  $\beta$ -agonists.

The treatment of healthy subjects with the  $\beta$ -agonist ECP decreased only SCD-1 mRNA expression ( $P < 0.001$ ) (Fig. 2D) but did not change the expression of other genes involved in lipid metabolism (Fig. 2).

The combined administration of these three drugs (ECPio) resulted in a higher mRNA expression for FATP/CD36 ( $P = 0.03$ ) (Fig. 2B). Interestingly, only the combined treatments (ECPio) increased expression of SIRT1 and CD36 (both  $P = 0.03$ ) (Fig. 2B and E). SIRT1 has been implicated in energy homeostasis and was recently described as a regulator of fat oxidation in adipocytes (23). The administration of pioglitazone or EC alone did not change SIRT1 mRNA expression (Fig. 2E). Importantly, the effect of pioglitazone to increase CPT-1, MCAD, and MLYCD was maintained in the combination (CPT-1 [ $P < 0.05$ , Fig. 3A],

Table 1—Baseline values and change from baseline for blood lipids

	Baseline	Week 4	Week 8	Week 12	Week 16
PP (n = 15)					
Total cholesterol	199.40 ± 30.79	−6.66 ± 18.48	<b>−13.53 ± 21.64</b>	<b>−9.80 ± 19.60</b>	−9.46 ± 19.94
HDL cholesterol	46.31 ± 12.46	−0.94 ± 3.84	−0.60 ± 3.49*	0.39 ± 4.64*	−0.22 ± 6.63*
LDL cholesterol	115.75 ± 35.25	−3.45 ± 20.84	−7.67 ± 21.95	−5.64 ± 19.46	−5.01 ± 18.66
TG	194.60 ± 121.33†	−3.78 ± 37.89*	−30.13 ± 69.16	−27.33 ± 53.85	−7.75 ± 39.99*
PPio (n = 13)					
Total cholesterol	201.84 ± 41.87	<b>−12.92 ± 20.62</b>	−6.92 ± 21.48	−5.92 ± 10.75	−6.23 ± 14.90
HDL cholesterol	54.33 ± 16.64	0.34 ± 3.51	<b>3.22 ± 4.49*‡</b>	<b>4.10 ± 4.66*‡</b>	<b>5.09 ± 6.26*‡</b>
LDL cholesterol	123.50 ± 30.49	<b>−7.56 ± 17.67</b>	−6.70 ± 22.16	−3.33 ± 13.66	−4.84 ± 12.56
TG	111.61 ± 42.52	<b>−20.15 ± 34.45*‡</b>	−8.84 ± 39.86	<b>−25.07 ± 36.42</b>	<b>−24.00 ± 40.86*‡</b>
ECP (n = 13)					
Total cholesterol	194.17 ± 22.20	<b>−8.70 ± 17.64</b>	<b>−11.58 ± 17.01</b>	<b>−11.58 ± 16.14</b>	<b>−8.05 ± 19.54</b>
HDL cholesterol	52.00 ± 10.35	<b>4.21 ± 4.64</b>	<b>2.85 ± 3.45*‡</b>	<b>3.57 ± 4.47*‡</b>	<b>3.66 ± 4.81*‡</b>
LDL cholesterol	122.59 ± 25.21	<b>−10.97 ± 16.49</b>	<b>−10.84 ± 16.70</b>	<b>−10.95 ± 14.68</b>	<b>−8.40 ± 15.34</b>
TG	96.61 ± 41.41	−8.44 ± 28.43*‡	<b>−16.73 ± 23.46</b>	<b>−19.79 ± 33.90</b>	<b>−15.20 ± 24.24*‡</b>
ECPio (n = 17)					
Total cholesterol	184.38 ± 31.89	<b>−14.69 ± 30.86</b>	<b>−9.00 ± 21.82</b>	<b>−16.61 ± 23.12</b>	<b>−10.07 ± 22.87</b>
HDL cholesterol	43.33 ± 7.75	<b>4.41 ± 6.44</b>	<b>6.16 ± 5.86‡</b>	<b>6.75 ± 3.02‡</b>	<b>7.78 ± 5.09‡</b>
LDL cholesterol	118.76 ± 26.51	<b>−17.10 ± 20.04</b>	<b>−11.04 ± 14.30</b>	<b>−16.76 ± 21.88</b>	<b>−11.06 ± 19.81</b>
TG	112.30 ± 42.56	<b>−43.00 ± 21.56‡</b>	<b>−22.07 ± 46.81</b>	<b>−34.46 ± 50.28</b>	<b>−41.00 ± 21.56‡</b>

Data are raw means ± SD for completers. Bold indicates that the within-treatment group change from baseline is significant at  $P < 0.05$ . At each time point, the treatment effect across groups (slices) was tested by post-hoc ANOVA. Cells with different symbols are significantly different within time point with a Tukey adjusted  $P = 0.05$ .  $n = 15, 13, 13,$  and  $17$  completers for PPP, PPio, ECPio, and ECP groups, respectively. Post hoc analysis of the mixed model was performed to test for significance within time, i.e., slices. Values with different symbols are significantly different with  $P < 0.05$ .

MCAD [ $P < 0.05$ , Fig. 3B], and MLYCD [ $P < 0.05$ , Fig. 3C]). The decrease of SCD-1 produced by EC was slightly blunted by the combination ( $P = 0.0004$  for EC,  $P = 0.07$  for ECP), suggesting one potential opposing action of the combination. Additionally, we did not observe any changes in mitochondrial copy number (mtDNA) between groups (Fig. 3D). There was no significant relationship between baseline mRNA for SIRT and fasting triglycerides. Similarly, the correlation between the change in SIRT1 and the change in triglycerides was not significant.

### Correlations with the expression of SIRTs

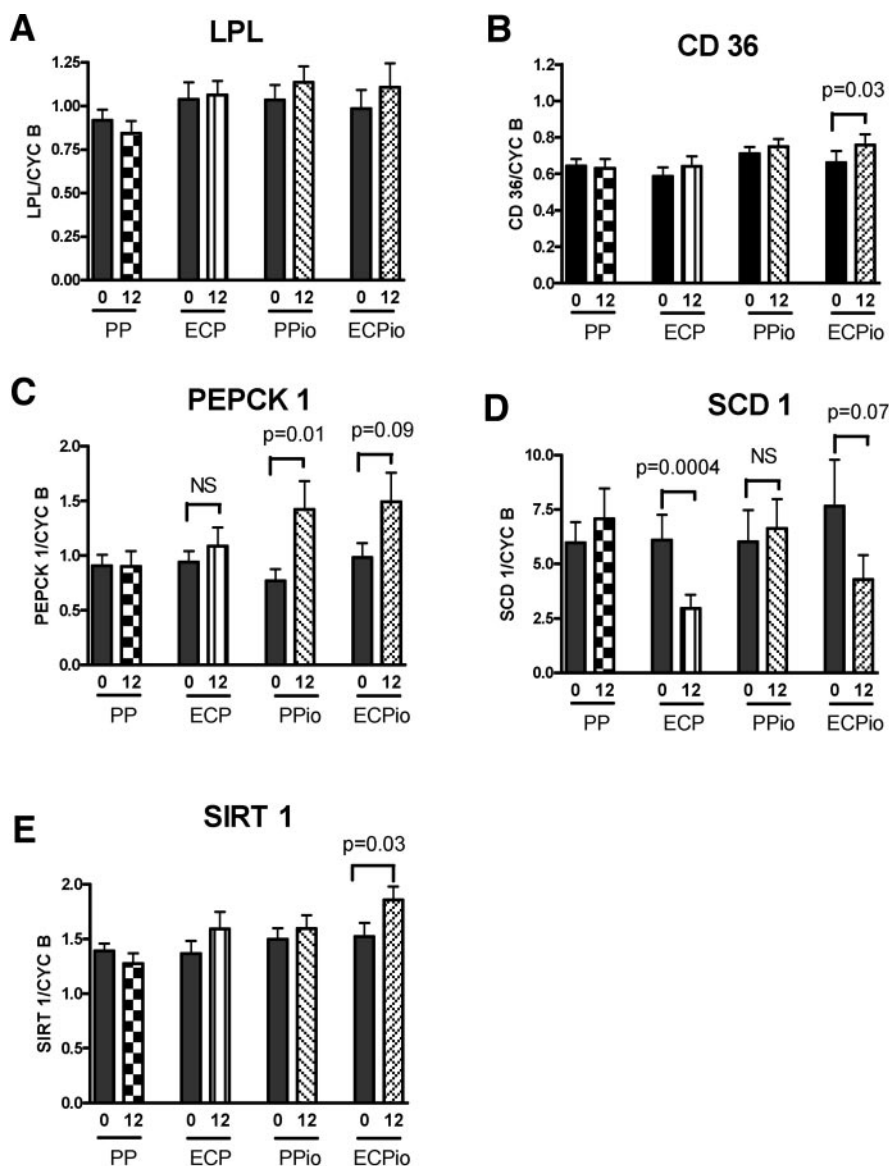
Given that the SIRT gene family has been implicated in the regulation of genes involved in fat oxidation, we explored the relationships between the expression of genes in the fat oxidation pathway and SIRT1 expression. Baseline mRNA for SIRT1 was positively correlated with PPAR- $\alpha$  ( $r = 0.65$ ,  $P < 0.001$ ) (Fig. 4A), MCAD ( $r = 0.61$ ,  $P < 0.001$ ) (Fig. 4B), LPL ( $r = 0.69$ ,  $P < 0.001$ ) (Fig. 4C), CD36 ( $r = 0.58$ ,  $P < 0.001$ ) (Fig. 4D), TFAM ( $r = 0.77$ ,  $P < 0.001$ ) (Fig. 4E), PGC-1 $\alpha$  ( $r = 0.36$ ,  $P < 0.001$ ) (Fig. 4F), and ERR $\alpha$  ( $r = 0.35$ ,  $P < 0.01$ ) (data not shown) but not with cytochrome C ( $r =$

$0.22$ , data not shown), CPT-1 ( $r = 0.21$ , data not shown), or CAP ( $r = 0.25$ , data not shown). The mRNA expression for the mitochondrial SIRTs (SIRT3 and SIRT5) showed much lower correlations with TFAM ( $r = 0.32$  and  $0.54$ , respectively,  $P < 0.01$ ; data not shown), LPL ( $r = 0.31$  and  $0.48$ ,  $P < 0.01$ ; data not shown), PPAR- $\alpha$  ( $r = 0.51$  and  $0.58$ ,  $P < 0.01$ ; data not shown), and MCAD ( $r = 0.43$  and  $0.44$ ,  $P < 0.01$ ; data not shown) (supplemental Table 2 of the online-only appendix).

**CONCLUSIONS**— Preclinical data suggest that combined PPAR- $\gamma$  and  $\beta$ -adrenergic therapy synergize to increase oxidative capacity in adipose tissue. This study was performed to determine whether the combined pharmacological intervention with pioglitazone (PPAR- $\gamma$ ) and EC ( $\beta$ -adrenergic) would have a beneficial effect on body weight, lipids, and the expression of genes involved in lipolysis/lipogenesis, mitochondrial biogenesis, and oxidative metabolism in subcutaneous fat. It has been previously suggested that the existence of cross-talk between these two distinct pathways in the WAT in rodents produced weight loss beyond that seen with either agent alone (16). In the present study, the addition of pioglitazone to EC (ECPio group) had no

additional effect on the loss of body weight. It should be noted that treatment with pioglitazone alone did not change body weight. This is slightly surprising and contrasts with the commonly held view that activation of the PPAR- $\gamma$  system will cause obligatory fat gain (21). In obese hypertensive subjects without diabetes, pioglitazone did not increase body weight (24).

Several previous studies have observed a decrease in visceral adipose tissue (VAT) with no changes in subcutaneous adipose tissue (SAT) in pioglitazone-treated patients, consistent with our results in the PP-treated patients. The reason total fat did not decrease could be due to one of two explanations. First, VAT could change without a change in whole-body SAT. A change in VAT of 13% for a depot that is only ~4 kg represents only a 500-g change. One possibility is that dual-energy X-ray absorptiometry is not sensitive enough to detect this change. Second, VAT could decrease and SAT could increase by an equivalent amount. Note that the SAT measure by CT represents only abdominal SAT and VAT. An increase in gluteal femoral SAT or arm/leg SAT that matched the decrease in VAT would result in a “no change” result for SAT.

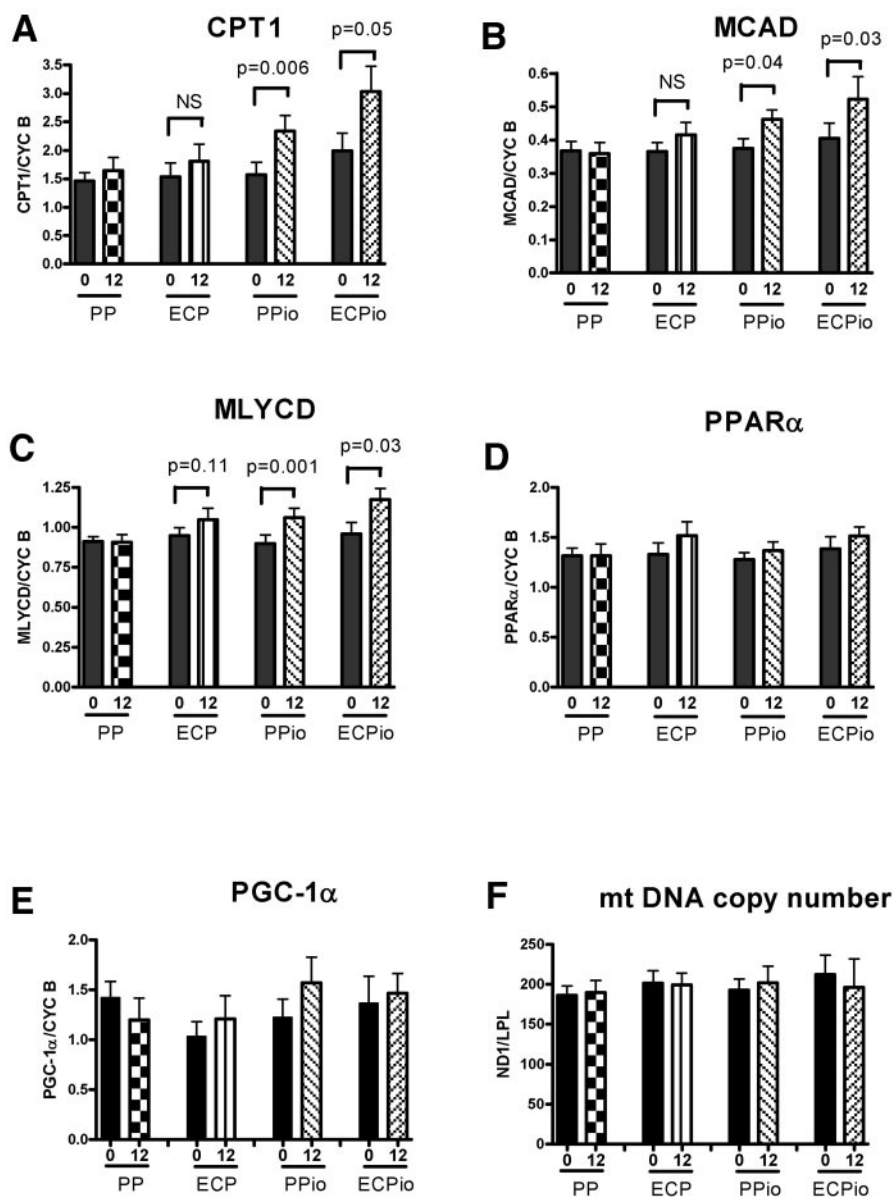


**Figure 2**—Effect of 12 weeks of treatment with placebo (PP), pioglitazone (PPio), ephedrine/caffeine (ECP), and pioglitazone plus ephedrine/caffeine (ECPio) on the expression of genes involved in lipid metabolism: LPL (A), fatty acid transport protein (FATP/CD36) (B), phosphoenolpyruvate carboxykinase 1 (PEPCK1) (C), SCD-1 (D), and sirtuin 1 (SIRT1) (E).

It has been proposed that the beneficial effect of thiazolidinediones on plasma lipids and glucose concentration in type 2 diabetic subjects is due to several mechanisms within white adipocytes and involves higher LPL leading to increased fatty acid transport into the adipocyte, increased fat catabolism in the cell, increased lipogenesis, and mitochondrial biogenesis (18,22,25). The unexpected finding of unchanged body weight after PPAR- $\gamma$  activation in the present study could be partly explained by the fact that our subjects were healthy and did not have diabetes. Activation of PPAR- $\gamma$  receptors may exert different effects on lipid metabolism in subcutaneous fat depots,

depending on the diabetes status. Our subjects did not change body weight after pioglitazone treatment but significantly reduced their blood triglyceride levels without an increase in mRNA for LPL or FATP/CD36. The increased expression of several genes involved in  $\beta$ -oxidation (CPT-1, MCAD, and MLYCD) is consistent with our previous experiments *in vivo* and *in vitro* (18,22) and suggests that increased lipid oxidation and not sequestration/storage might be important for the improved triglycerides. Taken together, these results suggest that the benefit of ECP combination treatment is that weight loss effects of EC treatment are retained along with the hypotriglyceridemic ac-

tions of pioglitazone and a suggestion of a somewhat greater effect of the combination on triglycerides and HDL cholesterol. Pioglitazone increased CPT-1, MCAD, and MYLCD, whereas EC, a  $\beta$ -agonist, is a known activator of lipolysis in adipocytes. This suggests a potential for synergy between EC and pioglitazone for lipid oxidation: reprogramming of adipocytes toward oxidation by pioglitazone and activation of lipolysis by EC providing fatty acids substrate toward oxidation. One unexpected finding was the increase in SIRT1. Furthermore, since this was unique to the ECPio group, there may be signaling or transcriptional synergy underlying this result. We speculate that the



**Figure 3**—Effect of 12 weeks of treatment with placebo (PP), pioglitazone (PPiO), ephedrine/caffeine (ECP), and pioglitazone plus ephedrine/caffeine (ECPIo) on expression of genes involved in oxidative metabolism and mitochondrial biogenesis: CPT-1 (A), MCAD (B), MLYCD (C), PPAR- $\alpha$  (D), PGC-1 $\alpha$  (E), and mitochondrial DNA (mt DNA) (F).

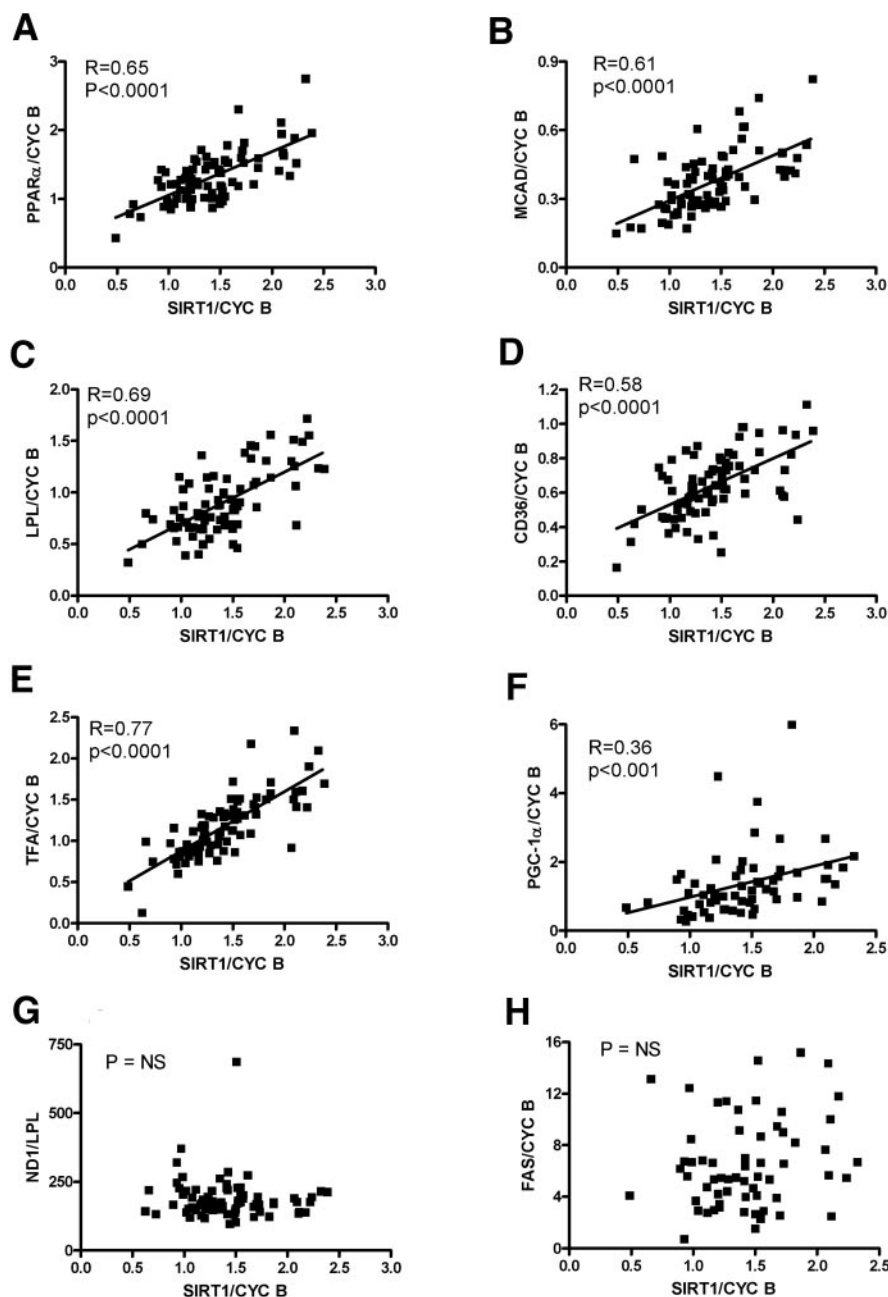
ability of pioglitazone to activate the transcription of lipid-oxidizing genes, which we have previously shown are increased in diabetic subjects in vivo and confirmed in vitro, combined with the known effects of the  $\beta$ -adrenergic system to activate fat oxidation might work together to lower triglycerides. Larger studies of this combination in subjects with elevated triglycerides and low HDL are clearly warranted.

One unique feature of the combination treatment is the increase in CD36 and SIRT1 mRNA. This is in contrast to ECP alone or pioglitazone alone, which had no effect on the expression of these two

genes. It is possible that these genes represent a larger set of genes that are specifically upregulated by the combination of PPAR- $\gamma$  activation and cAMP activation. In contrast to the preclinical rodent models, this synergistic effect on gene expression did not translate into differences in weight loss. No other candidate genes measured in this study were uniquely regulated by the combination treatment.

In the present study, the activation of  $\beta$ -adrenergic receptors reduced body weight and blood triglycerides, but surprisingly did not change mRNA expression of several tested genes involved in lipid catab-

olism or mitochondrial biogenesis, as previously demonstrated in numerous rodent or in vitro studies (11,12,15). Activation of cAMP-dependent protein kinase pathway increases LPL expression during the differentiation of adipocytes but may decrease LPL activity through posttranscriptional modifications. Therefore, the lack of changes in LPL mRNA levels in our study may suggest that LPL activity was not changed; however, LPL activity was not measured. Furthermore, in the present study we did not note alterations in CPT-1 and CD36 mRNA expression. Regulation of lipid oxidation is certainly more compli-



**Figure 4**—Correlations between *SIRT1* mRNAs at baseline and selected genes involved in lipid metabolism/mitochondrial biogenesis in human subcutaneous adipose tissue.

cated than simply changes in gene expression, as posttranslational mechanisms, such as allosteric regulation and translocation to the cell surface, play a critical role in determining final activities (26,27); thus, this result must be interpreted with caution. EC (ECP) reduced mRNA expression of SCD-1, whereas pioglitazone did not. Combined therapy with pioglitazone and  $\beta$ -adrenergic stimulation (ECPio group) tended to decrease SCD-1 mRNA expression. This effect was predominant with EC treatment but was not seen with pioglitazone treatment.

SCD-1 is the rate-limiting enzyme in the desaturation of saturated fatty acids to monounsaturated fatty acids and targets lipids to TAG synthesis. We found a significant correlation between mRNAs for SCD-1 and fatty acid synthase at baseline ( $r = 0.51$ ;  $P < 0.001$ ; data not shown). Reduced SCD-1 activates pathways promoting fatty acid oxidation and decreases triglyceride synthesis in fat and muscle (28).

Our study showed that combination therapy with pioglitazone and EC significantly increased the expression of *SIRT1*

transcript level, whereas the administration of these treatments separately did not affect gene expression. *SIRT1* is the closest mammalian ortholog of SIR 2 (silencing information regulator 2) that augments lifespan in lower organisms and mammalian cells (29–31) in response to caloric restriction. *SIRT1* regulates fat mobilization in white adipocytes (23). We noted a strong relationship between baseline mRNAs for *SIRT1* and genes involved in lipid uptake/oxidation (LPL, FATP/CD36, PPAR- $\alpha$ , and MCAD) and

mitochondrial biogenesis (TFA and PGC-1 $\alpha$ ).

To summarize, these results indicate that combined therapy with PPAR- $\gamma$  and  $\beta$ -adrenergic stimulators, representing two distinct intracellular signaling pathways, has a beneficial effect on body weight, plasma triglycerides, and lipid metabolism in subcutaneous fat depots through the downregulation of genes triggering fat accumulation (SCD-1) and up-regulating genes required for fatty acid catabolism (SIRT1, CPT-1, MCAD, and MLYCD).

**Acknowledgments**—This work was supported by an unrestricted grant from Takeda Pharmaceuticals (to S.R.S.) and National Institutes of Health Grant DK074772 (to T.W.G.).

The authors acknowledge Matt Hulver, Michele McNeil, David Hymel, Heather Loggins, and Leisel Hurder for advice and technical assistance and the volunteers who participated in this study.

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