

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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Running title: Lipid metabolism in subcutaneous human fat.

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ABSTRACT

OBJECTIVE – The sympathetic nervous system and thiazolidinediones control lipid metabolism and have been implicated in body weight regulation. The study was undertaken 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 non-diabetic subjects.

RESEARCH DESIGN AND METHODS – Fifty seven women and men were randomized into 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); 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 % in ECP and 4.6 % in ECPio groups, whereas remained unchanged in the PPio or PP groups. Triglycerides levels decreased by -7.7, -24, -15.2, and -41mg/dL after 16 weeks treatment in the PP, PPio, ECP and ECPio groups respectively; indicating that pio with or without EC decreased triglycerides and EC with or without Pio decreased body weight. The mRNA for sirtuin 1 and CD36 increased only in ECPio group. Carnitine palmitoyltransferase-1, medium-chain acyl coenzyme A dehydrogenase and malonyl-CoA decarboxylase increased with PPio and ECPio. SteroylCoA desaturase decreased with ECP.

CONCLUSIONS – Combined activation of PPAR α and beta-adrenergic receptors has beneficial effects on body weight, plasma triglycerides and lipid metabolism in subcutaneous fat by increasing the expression of genes required for fatty acid catabolism.

Clinical Trial Number: NCT00377975

INTRODUCTION

In mammals, mature fat cells are characterized by a high degree of plasticity and the ability to transdifferentiate between the white (WAT) and brown (BAT) 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 antidiabetic maneuver in rodents (3, 4). Furthermore, beta-adrenergic stimulation remodels white type adipocytes into 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 BAT or WAT (9-11). Peroxisome proliferator activated receptor (PPAR) coactivator 1 α (PGC-1 α) (12, 13) and PPAR α are key factors driving these two processes (14). Uncoupling protein-1 (UCP-1) is considered as a marker of the brown fat thermogenic phenotype. Beta-adrenergic stimulation increases PGC-1 α 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 MAPK activation by peroxisome proliferators-activated receptor gamma (PPAR γ), controls lipid metabolism and body weight. PPAR α 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 rodents (16). This was accompanied by an increase in the number of small, insulin sensitive adipocytes, mitochondrial biogenesis and increased expression of UCP-1. Above observations suggest the existence of cross-talk between two distinct signal transduction pathways (cAMP and PPAR α in adipose

tissue (16). Indeed, both systems drive the expression of important genes necessary for lipid uptake and oxidation. For example, lipoprotein lipase was upregulated after cAMP (17) and PPAR α (18, 19) activation. Combined therapy with ephedrine/caffeine plus pioglitazone significantly reduced plasma triglycerides, VLDL and LDL level while increased HDL total mass (20). Our and several previous results demonstrated that PPAR α 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, above results suggests that activation of two signaling systems described previously might synergize to exert beneficial effects on body weight, body fat, and blood lipids via the regulation of key genes expression involved in lipid metabolism and mitochondrial biogenesis in subcutaneous fat.

RESEARCH DESIGN AND METHODS

Study population. Non-diabetic patients were enrolled in a clinical trial performed in Baton Rouge, LA at the Pennington Biomedical Research Center (14 men and 43 women between 18 and 50 years of age with a BMI of 30 to 37 kg/m²). The characteristics the study population were previously reported (20). Subjects were healthy and were not taking thiazolidinediones, beta-blockers, orlistat, sibutramine, ephedrine, phenylpropanolamine (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 group); 2) ephedrine HCl plus caffeine/placebo (ECP); 3) placebo/pioglitazone (PPio); 4) ephedrine HCl plus caffeine/pioglitazone (ECPio). The placebo, the pharmaceutical ephedrine HCl (25mg) ("Breathe Easy", Contract Pharmacal Corporation, Hauppauge, NY)

and caffeine (200mg) (Contract Pharmacal Corporation) were dosed as follows: one of each pill per day for seven days at breakfast, then increased to one each at breakfast and lunch for the next seven days, then increased to one each at breakfast, lunch, and dinner for the remainder of the 16 week protocol. Subjects that could not tolerate three doses of E+C per day were allowed to continue in the protocol 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 hours 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 endpoint was the % fat measured with DEXA after 16 weeks 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 fasting. Fat samples were cleaned of visible connective tissue and blood vessels, immediately frozen and stored at -70°C until assays.

Body composition. Body fat mass and lean mass were measured on a Hologic Dual Energy X-ray Absorptiometer (QDR 4500A, Hologic, Inc, Waltham, MA). Visceral adiposity was determined by multi-slice CT scanning using a GE High Speed™ CT scanner under an established protocol (21).

RNA and DNA Extraction. Total RNA from 50-100 mg of adipose tissue was isolated with Trizol reagent (Invitrogen, Carlsbad, CA) and purified with RNeasy columns

(QIAGEN, Valencia, CA) according to manufacturer's 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 Complete DNA and RNA Purification Kit (EPICENTRE, Madison, WI). The total amount of 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 described previously (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: one cycle of 48°C for 30 min, then 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. A standard curve was prepared by serial dilution of pooled total RNA and each gene / sample was compared to 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 ± SE unless otherwise noted as SD. Significant differences were assumed for P<0.05. Gene expression data was analyzed by paired t-test within treatment group using GraphPad Prism version 4.0 (GraphPad Software Inc., San Diego, CA). Serial lipid data (Table 2) was analyzed using a mixed model (PROC MIXED) in SAS (Cary, NC). Pearson correlations were performed in JMP v 3.0 (SAS; Cary, NC).

RESULTS

Subjects characteristics.

The characteristics of the overweight / obese study population are presented in Table 1. Generally, the treatment groups were well balanced except for the baseline triglycerides which were by chance higher in the PioP patients. One subject from each PPio and ECP groups and three subjects in the ECPio were able to tolerate only two E+C 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 on Figure 1. We observed a 6.6 % weight loss in the ephedrine/caffeine group (ECP) and 5.2 % weight decrease after combined intervention with E/C plus pioglitazone (ECPio) (Fig. 1A). The 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 and 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 ECP group and 4.6 % in ECPio group, whereas body fat remained unchanged in the PPio or PP groups (Fig. 1B). Visceral and subcutaneous fat measured by CT decreased in proportion to the reduction in weight and body fat (Fig. 1C, D).

After combination therapy triglycerides fell by 41 mg/dL in the ECPio group, while treatment with ephedrine/caffeine alone or pioglitazone alone decreased the levels by 15.2 mg/dL and 24 mg/dL respectively (Table 2). HDL cholesterol increased by 7.8 in the ECPio group, 3.7 in the ECP group and 5.1mg/dL in the PPio group. Thus the reduction in TG levels can be largely attributed to the Pio treatment, with no statistically significant impact from adding EC.

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

Pioglitazone treatment (PPio) markedly increased mRNA expression for phosphoenolpyruvate carboxykinase

(PEPCK1, $P=0.01$, Fig. 2C), but did not affect lipoprotein lipase (LPL; Fig.2A), fatty acid transporter (FATP/CD36; Fig. 2B), fatty acid synthase (data not shown) or stearoyl-CoA desaturase-1 (SCD1; Fig. 2D) mRNA levels when compared to the placebo treated subjects. Pioglitazone treatment also increased carnitine palmitoyltransferase-1 (CPT1; $P<0.01$; Fig. 3A), medium-chain acyl coenzyme A dehydrogenase (MCAD; $P<0.05$; Fig. 3B) and malonyl-CoA decarboxylase (MLYCD; $P<0.01$; Fig. 3C), but did not change PPAR α (Fig. 3D) or cytochrome C (data not shown) mRNA levels. The treatment with pioglitazone alone did not change the expression of genes involved in mitochondrial biogenesis including PGC-1 α , mtTFA, NRF-1, ERR α , AMPK α , or SIRT1-3 (data not shown). The upregulation of these three lipid oxidation genes (CPT1, MCAD, and MYLCD) 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 ephedrine/caffeine (ECP) decreased only SCD1 mRNA expression ($P<0.001$; Fig. 2D), but did not change expression of the 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, 2E). SIRT1 has been implicated in energy homeostasis and recently described as a regulator of fat oxidation in adipocytes (23). The administration of pioglitazone or ephedrine/caffeine alone did not change SIRT1 mRNA expression (Fig. 2E). Importantly, the effect of pioglitazone to increase CPT1, MCAD, and MYLCD was maintained in the combination [CPT1 ($P<0.05$; Fig.3A), MCAD ($P<0.05$; Fig. 3 B) and MLYCD ($P<0.05$; Fig 3C)]. This suggests a potential for synergy between EC and pioglitazone; reprogramming of

adipocytes towards oxidation by Pioglitazone and activation of oxidation by EC. The decrease SCD1 produced by EC was slightly blunted by the combination ($p = 0.0004$ for EC and $p = 0.07$ 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. The baseline mRNA for SIRT1 was positively correlated with PPAR α ($r=0.65$; $P<0.001$; 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), TFA ($r=0.77$; $P<0.001$; Fig. 4E), PGC-1 α ($r=0.36$; $P<0.001$; Fig. 4F), ERR α ($r=0.35$; $P<0.01$; data not shown) but not with CytC ($r=0.22$, data not shown), CPT1 ($r=0.21$; data not shown) or CAP ($r=0.25$; data not shown). The mRNA expression for the mitochondrial SIRTs (SIRT3 and SIRT5) showed a much lower correlations with TFA ($r=0.32$ and 0.54 respectively, $P<0.01$; data not shown), LPL ($r=0.31$ and $r=0.48$ respectively; $P<0.01$; data not shown), PPAR α ($r=0.51$ and 0.58 respectively; $P<0.01$; data not shown), MCAD ($r=0.43$ and 0.44 respectively; $P<0.01$; data not shown).

DISCUSSION

Preclinical data suggests that combined PPAR α and β -adrenergic therapy synergize to increase oxidative capacity in adipose tissue. This study was performed to determine whether the combined pharmacological intervention with pioglitazone (PPAR α and ephedrine / caffeine (β -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 the existence of cross-talk between these two distinct pathways in the white adipose tissue in rodents produced weight loss beyond that seen with either agent alone (16). In the present study, the addition of pioglitazone to ephedrine / caffeine (ECPio group) had no additional effect on the loss of body weight. It should be also pointed out 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 α system will cause obligatory fat gain (21). In obese hypertensives without diabetes, pioglitazone did not increase body weight (24).

Several previous studies have observed a decrease in VAT with no changes in SAT in pioglitazone treated patients consistent with our results in the Pioglitazone Placebo treated patients. That total fat was not decreased 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 ~4kg represents only a 500g change. One possibility is that DEXA 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 subcutaneous adipose tissue. 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.

It has been proposed that the beneficial effect of TZDs on plasma lipids and glucose concentration in type 2 diabetic subjects is due to several mechanisms within white adipocytes and involves a) higher LPL leading to increased fatty acid transport into adipocyte, b) increased fat catabolism in the cell, c) increased lipogenesis and d) mitochondrial biogenesis (18, 22, 25). The

unexpected finding of unchanged body weight after PPAR γ activation in the present study could be partly explained by the fact that our subjects were healthy, without diabetes. Activation of PPAR γ 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/CD 36. The increased expression of several genes involved in beta oxidation (CPT1, MCAD, MLYCD) is consistent with our previous experiments *in vivo* and *in vitro* (18, 22) and suggests that increased lipid oxidation, not sequestration / storage might be important for the improved triglycerides. Taken together, these results suggest that the benefit of ECP combination treatment is that the weight loss effects of EC treatment are retained, along with the hypotriglyceridemic actions of Pioglitazone and a suggestion of a somewhat greater effect of the combination on triglycerides and HDL-cholesterol. Pioglitazone increased CPT-I, 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 towards oxidation by Pioglitazone and activation of lipolysis by EC providing fatty acids substrate towards 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 ability of pioglitazone to activate the transcription of lipid oxidizing genes which [we have previously shown are increased in diabetics *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 ephedrine plus caffeine 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 up regulated by the combination of PPAR γ activation and cAMP activation. In contrast to the pre-clinical 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 catabolism or mitochondrial biogenesis, as previously demonstrated in numerous rodent or *in vitro* studies (11, 12, 15). Activation of cAMP/PKA pathway increases LPL expression during the differentiation of adipocytes, but may decrease LPL activity through post-transcriptional 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 complicated than simply changes in gene expression as post-translational mechanisms such as allosteric regulation and translocation to the cell surface play a critical role in determining final activities (26, 27) and so this result must be interpreted with caution. Ephedrine / caffeine (ECP) reduced mRNA expression of stearoyl-CoA deaturase 1 (SCD1), whereas pioglitazone did not. Combined therapy with pioglitazone and beta-adrenergic stimulation (ECPio group) tended to decrease SCD1 mRNA expression.

This effect was predominant with ephedrine/caffeine treatment but was not seen with pioglitazone treatment. SCD1 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 SCD1 and fatty acid synthase (FAS) at baseline ($r=0.51$; $P<0.001$; data not shown). Reduced SCD1 activates pathways promoting fatty acid oxidation and decreases triglycerides synthesis in fat and muscle (28).

Our study showed that combination therapy with pioglitazone and ephedrine/caffeine 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 α , MCAD) and mitochondrial biogenesis (TFA, PGC-1 α).

To summarize, these results indicate that combined therapy with PPAR α 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 down-regulation of genes triggering fat accumulation (SCD1) and up-regulating genes required for fatty acid catabolism (SIRT1, CPT1, MCAD, MLYCD).

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Table 1. Baseline Characteristics of the Four Treatment Groups

Variable	PI/PI	PI/Pi	E + C/Pi	E + C/PI	p-Value
Number of subjects	21	20	20	20	
Gender (Female/Male)	17/4	16/4	16/4	17/3	
Age (y)	35.80+7.6	38.85+9.39	37.95+8.02	39.30+8.14	0.52
Anthropometry					
Height (cm)	168.16+8.26	166.24+9.43	167.67+6.86	167.27+10.36	0.90
Weight (kg)	94.94+11.67	93.74+12.0	95.37+11.14	93.16+11.12	0.91
Waist	101.20+10.21	99.88+9.26	95.37+11.14	97.53+8.12	0.58
BMI (kg/m ²)	33.52+2.92	33.82+2.31	33.99+2.34	33.23+1.78	0.74
Body composition					
Body fat (kg)	37.02+7.56	37.05+6.59	37.12+6.71	36.21+5.64	0.96
Body fat (%)	39.16+6.18	39.83+7.13	39.24+6.24	39.29+5.59	0.98
Whole Body Lean (kg)	57.44+9.41	56.93+1.24	57.75+10.22	56.46+10.12	0.98
Computed Tomography					
VAT Mass (kg)	4.08+1.50	3.60+1.33	4.16+1.91	3.37+1.20	0.26
SAT Mass (kg)	12.23+3.50	12.84+2.20	12.34+3.31	11.79+2.06	0.69
Blood Lipids					
Total Cholesterol (mg/dL)	206.57+33.70	205.30+43.50	184.00 + 29.61	192.25 + 20.97	0.08
HDL cholesterol (mg/dL)	52.32 +17.15	53.66 +14.19	45.17 + 8.54	50.36 +10.35	0.17
LDL cholesterol (mg/dL)	121.94+35.50	128.34+35.98	116.25 + 24.51	120.98 + 24.19	0.64
Triglycerides (mg/dL)	176.00 +121.70	116.50+42.82	112.85 + 51.28	104.55 + 54.85	0.01

Data are Mean + SD. P value is for ANOVA

Table 2. Baseline Values and Change from Baseline for Blood Lipids.

Placebo/Placebo					
	Baseline	Week 4	Week 8	Week 12	Week 16
CHOL	199.40 + 30.79	-6.66 + 18.48	-13.53 + 21.64	-9.80 + 19.60	-9.46 + 19.94
HDL	46.31 + 12.46	-0.94 + 3.84	-0.60 + 3.49 <i>a</i>	0.39 + 4.64 <i>a</i>	-0.22 + 6.63 <i>a</i>
LDL	115.75 + 35.25	-3.45 + 20.84	-7.67 + 21.95	-5.64 + 19.46	-5.01 + 18.66
TRIG	194.60 + 121.33 #	-3.78 + 37.89 <i>a</i>	-30.13 + 69.16	-27.33 + 53.85	-7.75 + 39.99 <i>a</i>

Placebo / Pioglitazone					
	Baseline	Week 4	Week 8	Week 12	Week 16
CHOL	201.84 + 41.87	-12.92 + 20.62	-6.92 + 21.48	-5.92 + 10.75	-6.23 + 14.90
HDL	54.33 + 16.64	0.34 + 3.51	3.22 + 4.49 <i>ab</i>	4.10 + 4.66 <i>ab</i>	5.09 + 6.26 <i>ab</i>
LDL	123.50 + 30.49	-7.56 + 17.67	-6.70 + 22.16	-3.33 + 13.66	-4.84 + 12.56
TRIG	111.61 + 42.52	-20.15 + 34.45 <i>ab</i>	-8.84 + 39.86	-25.07 + 36.42	-24.00 + 40.86 <i>ab</i>

Ephedrine + Caffeine / Placebo					
	Baseline	Week 4	Week 8	Week 12	Week 16
CHOL	194.17 + 22.20	-8.70 + 17.64	-11.58 + 17.01	-11.58 + 16.14	-8.05 + 19.54
HDL	52.00 + 10.35	4.21 + 4.64	2.85 + 3.45 <i>ab</i>	3.57 + 4.47 <i>ab</i>	3.66 + 4.81 <i>ab</i>
LDL	122.59 + 25.21	-10.97 + 16.49	-10.84 + 16.70	-10.95 + 14.68	-8.40 + 15.34
TRIG	96.61 + 41.41	-8.44 + 28.43 <i>ab</i>	-16.73 + 23.46	-19.79 + 33.90	-15.20 + 24.24 <i>ab</i>

Ephedrine + Caffeine / Pioglitazone					
	Baseline	Week 4	Week 8	Week 12	Week 16
CHOL	184.38 + 31.89	-14.69 + 30.86	-9.00 + 21.82	-16.61 + 23.12	-10.07 + 22.87
HDL	43.33 + 7.75	4.41 + 6.44	6.16 + 5.86 <i>b</i>	6.75 + 3.02 <i>b</i>	7.78 + 5.09 <i>b</i>
LDL	118.76 + 26.51	-17.10 + 20.04	-11.04 + 14.30	-16.76 + 21.88	-11.06 + 19.81
TRIG	112.30 + 42.56	-43.00 + 21.56 <i>b</i>	-22.07 + 46.81	-34.46 + 50.28	-41.00 + 21.56 <i>b</i>

Data are presented as raw mean + 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 analysis of the ANOVA; cells with different letters are significantly different within time point with a Tukey