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Decaffeinated Green Tea and Voluntary Exercise Induce Gene Changes Related to Beige Adipocyte Formation in High Fat-Fed Obese Mice*

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Abstract

We have previously reported that decaffeinated green tea extract (GTE) in combination with voluntary exercise (Ex) reduces metabolic syndrome in high fat-fed C57BL/6J mice. Here, we examined for the first time the effect of treatment with 77 mg/g GTE, Ex, or both (GTE + Ex) on genes related to the conversion of white adipose tissue (WAT) to brown fat-like adipose tissue (BLAT) in this model. GTE+Ex induced genes related to lipolysis (hormone sensitive lipase [3.0-fold] and patatin-like phospholipase domain-containing protein 2 [2-fold]), mitochondrial β-oxidation (NADH dehydrogenase 5 [2.3-fold], cytochrome B [2.0-fold], and cytochrome C oxidase III [1.9-fold increase]), and adipose tissue browning (peroxisome proliferator-activated receptor-γ coactivator-1α [1.8-fold], bone morphogenetic protein 4 [2.6-fold], and phosphatase and tensin homolog [2.6-fold]) in visceral WAT compared to HF-fed mice. These results suggest that GTE+Ex function in part by inducing the conversion of WAT to BLAT and provides novel mechanistic insight into this combination.

*Abbreviations: Bmp4, bone morphogenetic protein 4; EGCG, (−)-epigallocatechin-3-gallate; Ex, voluntary exercise; GTE, decaffeinated green tea extract; HF, high fat diet; Lipe, hormone sensitive lipase; LF, low fat; mt-Co3, cytochrome C oxidase subunit III; mt-Cytb, cytochrome B; mt-Nd5, NADH dehydrogenase 5; PGC1α, proliferator-activated receptor-γ coactivator-1α; Pnpla2, patatin-like phospholipase domain-containing protein 2; PPARγ, peroxisome proliferator-activated receptorγ; PRDM16, PR domain containing 16; Pten, phosphatase and tensin homolog; Srebf1, sterol regulatory element-binding protein-1c; Ucp1, uncoupling protein-1

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Keywords

green tea; *Camellia sinensis*; voluntary exercise; obesity; beige adipose tissue

1. Introduction

Obesity is a risk factor for chronic disease (Flegal, Carroll, Ogden, & Curtin, 2010). We and others have previously reported that green tea (*Camellia sinensis*, Theaceae) and its major polyphenol (−)-epigallocatechin-3-gallate (EGCG) have obesity preventive effects (Huang et al., 2009; Zhang et al., 2012). Many mechanisms have been proposed to explain the anti-obesity effects of green tea including modulation of pancreatic lipase, inhibition of *de novo* lipogenesis, and enhanced fatty acid oxidation (Grove, Sae-tan, Kennett, & Lambert, 2012; Lee, Kim, Kim, & Kim, 2009; Sae-Tan, Grove, Kennett, & Lambert, 2011).

More recently, it has been shown that combination treatment with green tea and exercise results in greater obesity preventive activity in high fat-fed mice (Murase et al., 2005; Sae-Tan, Rogers, & Lambert, 2014). Gene expression analysis showed that combination treatment increased expression of genes related to lipid oxidation and decreased expression of genes related to *de novo* lipogenesis in both skeletal muscle and the liver. To date, there has been no investigation of the effect of this combination on markers of lipolysis, *de novo* lipogenesis, and energy utilization in adipose tissue.

It was previously believed that there were two types of adipose tissue: white (WAT) and brown adipose tissue (BAT). WAT stores energy as TAGs; whereas BAT uses stored triacylglyceride (TAG)s to generate energy mostly in form of heat (Qian et al., 2013). Recently, a new form of adipose tissue, known as brown fat-like adipose tissue (BLAT), has been discovered and identified as a potential target for obesity treatment (Wu, Cohen, & Spiegelman, 2013). WAT and BLAT adipocytes are derived from two distinct populations of $Pax7^{-}$ and $Myf5^{-}$ precursor cells, whereas BAT adipocytes originate from $Pax7^{+}$ and $Myf5^{+}$ precursors (Wu, Cohen, & Spiegelman, 2013). BLAT adipocytes have more mitochondria than white adipocytes and respond to cyclic AMP stimulation in a manner similar to BAT.

Peroxisome proliferator activated receptor (PPARγ, gene: *Pparg*) in concert with PPARγ coactivator-1(PGC1α, gene: *Ppargc1a*) controls mitochondrial biogenesis and oxidative metabolism (Bostrom et al., 2012). PR domain containing 16 (PRDM16), a master regulator of adipocyte differentiation and involved in both white and brown adipogenesis (Koppen & Kalkhoven, 2010). Transcriptional activation of PPARγ in conjunction with PGC1α and PRDM16 results in increased expression of genes related to mitochondrial biogenesis and adaptive thermogenesis, including: uncoupling protein 1 (UCP1), NADH dehydrogenase 5 (mtND5), cytochrome B (mtCYTB), and cytochrome C oxidase subunit III (mtCO3) (Bostrom et al., 2012; Wu et al., 2012).

Here, we used samples derived from a larger study to investigate for the first time the impact of the combination of decaffeinated green tea extract (GTE) and voluntary exercise on the

expression of genes related to conversion of WAT to BLAT in the high fat-fed C57BL/6J mice.

2. Materials and Methods

2.1 Chemicals and diets

GTE (per g: 312 mg EGCG, 174 mg (−)-epigallocatechin, 177 mg (−)-epicatechin, and 174 mg (−)-epicatechin-3-gallate) was donated by Nature's Sunshine Products, Inc (Spanish Fork, UT, USA). Experimental diets were prepared by Research Diets, Inc (New Brunswick, NJ, USA) as previously described (Sae-Tan, Rogers, & Lambert, 2014).

2.2 Animals and treatment

Samples used here were generated as part of a larger study to examine the effect of green tea and exercise on high fat diet-induced obesity (Sae-Tan, Rogers, & Lambert, 2014). This previous report examined changes in the liver and skeletal muscle. The animal experiment was approved by the Institutional Animal Care and Use Committee (IACUC#37115). Male C57BL/6J mice (4 wks, Jackson Laboratories, Bar Harbor, ME, USA) had access to food and water *ad libitum*. Mice were randomized to low fat diet (LF, 10% energy from fat, n=12), high fat diet (HF, 60% energy from fat, n=22), HF supplemented with decaffeinated green tea extract (7.7 g GTE/kg diet, n=22), HF plus access to running wheel (Ex, n=22, Techniplast, Exton, PA, USA), or HF plus GTE and running wheel (GTE+Ex, n=22) (Sae-Tan, Rogers, & Lambert, 2014). Mice were treated for 16 wk, food deprived for 7 h (7:00–14:00 h), anesthetized, and killed by exsanguination. Epididymal AT was harvested, rinsed with saline, and frozen at −80°C.

2.3 Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Gene expression analysis in the epididymal AT from treated mice was performed by qRT-PCR as previously described (Sae-Tan, Rogers, & Lambert, 2014). Total RNA was isolated from epididymal AT by using the RNeasy Lipid Tissue Mini Kit (Qiagen Inc., Alameda, CA, USA), quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and cDNA was synthesized using reverse transcriptase. PCR was performed using SYBR Green PCR Master Mix (Thermo Scientific) according to the manufacturer's protocol and amplified on an ABI Prism 7000 sequence detection system (Thermo Scientific). mRNA levels were normalized to GAPDH and fold-changes were calculated compared to HF-fed mice. The sequences for the primers used are listed in Supplemental Table 1.

2.4 Statistical Analysis

All plots show the mean ± standard error of the mean. Data were analyzed by One-way ANOVA with Tukey's post-test. $P < 0.05$ indicates statistical significance.

3. Results and Discussion

We and others have previously examined the impact of green tea alone or in combination with exercise on obesity (Sae-Tan, Rogers, & Lambert, 2014; Zhang et al., 2012). In the

present study, we examined, for the first time, the impact of treatment with GTE, Ex, or the combination on the expression of genes related to the conversion of WAT to BLAT in high fat-fed mice.

Analysis of gene expression in the epididymal AT depot showed that GTE+Ex treatment significantly increased hormone sensitive lipase (*Lipe*, 3.0-fold) and patatin-like phospholipase domain containing protein 2 (*Pnpla2*, 2.0- fold) compared to HF-fed mice (Fig. 1A and B). These genes encode enzymes that play a critical role in lipolysis and fatty acid mobilization. Although, a previous study reported that supplementation with EGCG increased the expression of these genes in AT from high fat-fed mice, the impact of addition of Ex has not previously been assessed (Lee, Kim, & Kim, 2009). Consistent with these studies, we observed that GTE alone tended to increase the expression of both genes. We also found that GTE+Ex increased the expression of sterol regulatory element binding transcription factor 1 (*Srebf1*) by 1.8-fold in GTE+Ex-treated mice compared to HF-fed mice (Fig. 1C). This transcription factor plays a key role in *de novo* lipogenesis, and its increase by GTE+Ex was counterintuitive (Griffin & Sul, 2004). The ratio of *Lipe*/*Srebf1* mRNA, however, was increased by 65.9% by GTE+Ex (Fig. 1D) but no significant change was observed in GTE- and Ex-treated mice. Our results suggest that the combination favors lipolysis over lipogenesis in WAT.

An number of studies have focused on the conversion of WAT to BLAT as a treatment strategy for obesity: exposure to cold or treatment with PPARγ agonists have been shown to induce this conversion (Fisher et al., 2012; Ohno, Shinoda, Spiegelman, & Kajimura, 2012; Qian et al., 2013; Wu et al., 2012; Wu, Cohen, & Spiegelman, 2013). To date, there have been no studies on the effect of green tea and exercise on the expression of BLAT-related genes. BLAT has a larger number of mitochondria than WAT. The expression of *mt-Nd5*and *mt-Cytb*, genes involved in oxidative phosphorylation, was increased in GTE+Ex treated mice by 2.3- and 2.0-fold, respectively (Fig. 2A and B). The expression of *mt-Co3* was significantly increased in GTE+Ex treated mice (1.9-fold, Fig. 2C). UCP1 is highly expressed in BAT and is a marker of the WAT to BLAT conversion (Wu et al., 2012). *Ucp1* expression tended to increase in Ex- and GTE+Ex-treated mice, but the effect was not significant (Fig. 2D). Our results suggest that the effects of the combination of GTE and Ex on these genes may be due primarily to the Ex.

BMP4 has been reported to play an important role in the conversion of WAT to BLAT in mice (Qian et al., 2013). BMP4 causes dissociation of the the cytosolic complex between WNT1 inducible signaling pathway protein 2 and PPARγ transcriptional activator zinc finger protein 423 (ZFP423). ZFP423 can then translocate into the nucleus and induce PPARγ transcription (Hammarstedt et al., 2013). The activation of PPARγ has been reported to induce the conversion to BLAT (Ohno, Shinoda, Spiegelman, & Kajimura, 2012). Here, we report for the first time that expression of *Bmp4* was significantly increased by GTE+Ex treatment (2.6- fold, Fig. 3A). The expression of *Pparg* was increased by 1.8- and 2.1-fold by Ex and GTE+Ex, respectively, compared to HF-fed mice (Fig. 3B). *Ppargc1a* expression tended to increase in both Ex- (1.3-fold) and GTE+Ex-treated mice (1.8-fold) although this did not reach statistical significance (Fig. 3C). PGC1α, a transcriptional coactivator of PPARγ has been shown to increase UCP1 expression, mitochondrial biogenesis, and

oxidative metabolism (Bostrom et al., 2012). Phosphatase and tensin homolog (PTEN) has been reported to dephosphorylate intermediate molecules in insulin signaling by counteracting the activity of phosphatidylinositol 3-kinase type I/Akt pathway. Akt-mediated phosphorylation has been shown to inhibit the transcriptional co-activation activity of PGC1a (Li, Monks, Ge, & Birnbaum, 2007). *Pten* was increased by 2.0-fold in GTE+Ex treated mice (Fig. 3D).

4. Conclusions

We report for the first time that the combination of GTE and Ex alters gene expression in a manner suggesting conversion of WAT to BLAT. These results in combination with previous studies demonstrating the ability of green tea to reduce energy absorption and increase thermogenesis, provide a more complete understanding of the obesity preventive effects of GTE alone and in combination with exercise.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

1. We report green tea plus exercise induced changes in adipose genes in obese mice.
2. The results suggest a new anti-obesity mechanism of action for the combination.
3. The combination appears to convert white visceral adipocytes to beige adipocytes.
4. The expression of lipolytic genes in the visceral adipose tissue was increased.
5. The combination increased the expression of mitochondrial complex genes.

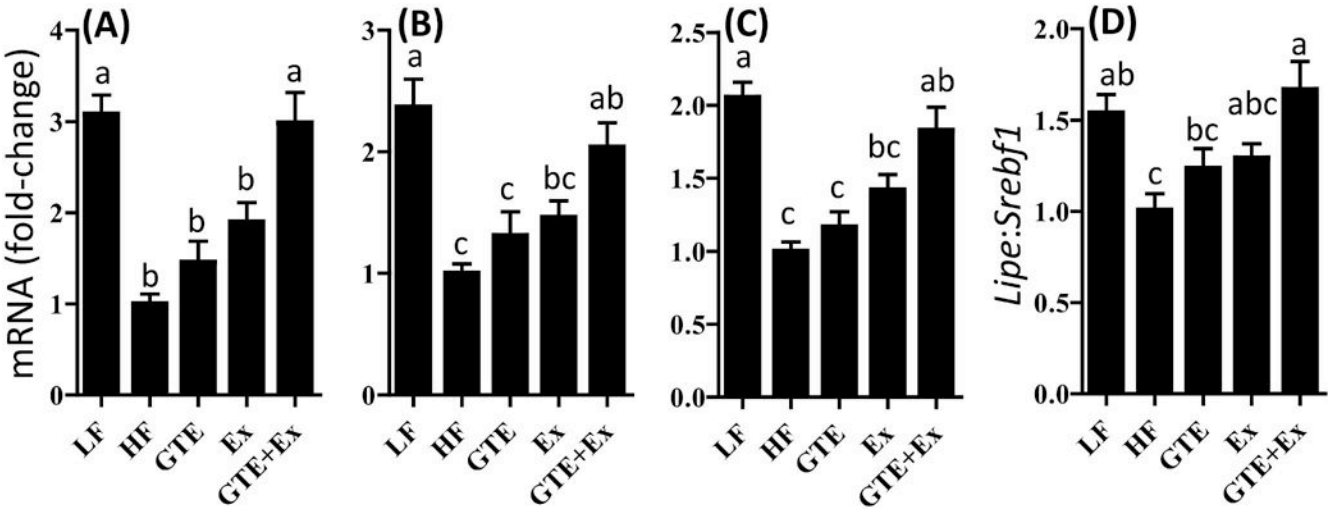

FIGURE 1.

The effect of GTE, Ex or GTE+Ex on genes related to lipolysis and lipogenesis in the adipose tissue from high fat-fed mice. Expression of (A) *Lipe*, (B) *Pnpla2*, and (C) *Srebf1* was determined in the epididymal adipose depot by qRT-PCR. Expression was normalized to *Gapdh*. The ratio of *Lipe* to *Srebf1* (D) was determined as an indication of lipolysis to lipogenesis. N = 12 for LF and 22 for other groups. The error bars represent the SEM. Different superscript letters indicate $P < 0.05$ by one-way ANOVA with Tukey's post-test.

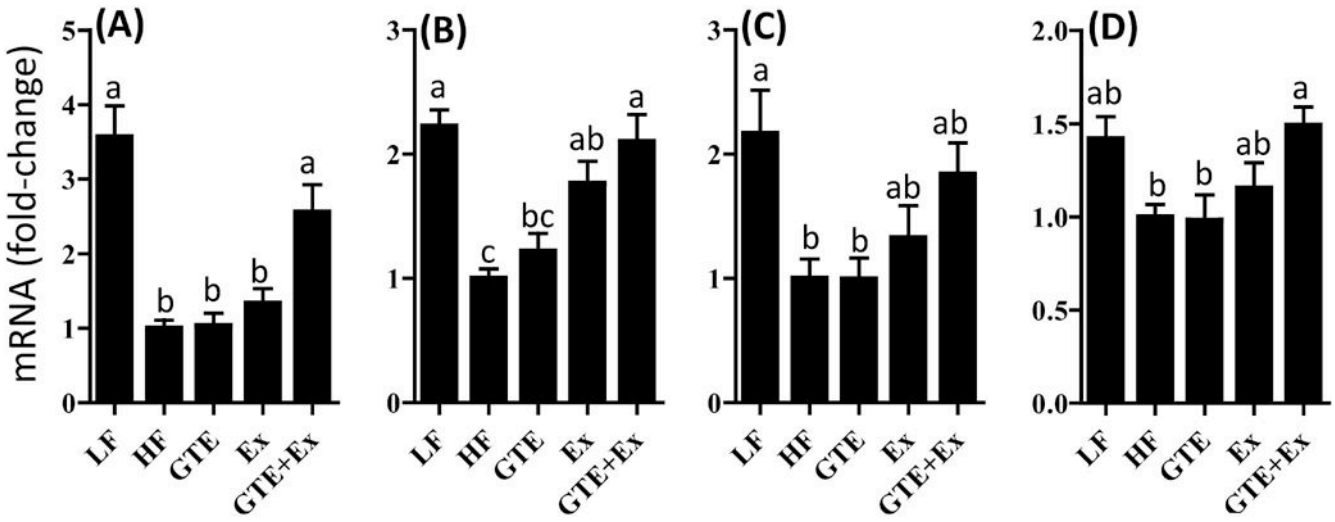

FIGURE 2.

The effect of GTE, Ex or GTE+Ex on genes related to thermogenesis, mitochondrial function in the adipose tissue of high fat-fed mice. Expression of (A) *mt-Nd5*,(B) *mt-Cytb*, (C) *mt-Co3*, and (D) *Ucp1* was determined in the epididymal adipose depot by qRT-PCR. Expression was normalized to *Gapdh*. N = 12 for LF and 22 for other groups. The error bars represent the SEM. Different superscript letters indicate $P < 0.05$ by one-way ANOVA with Tukey's post-test.

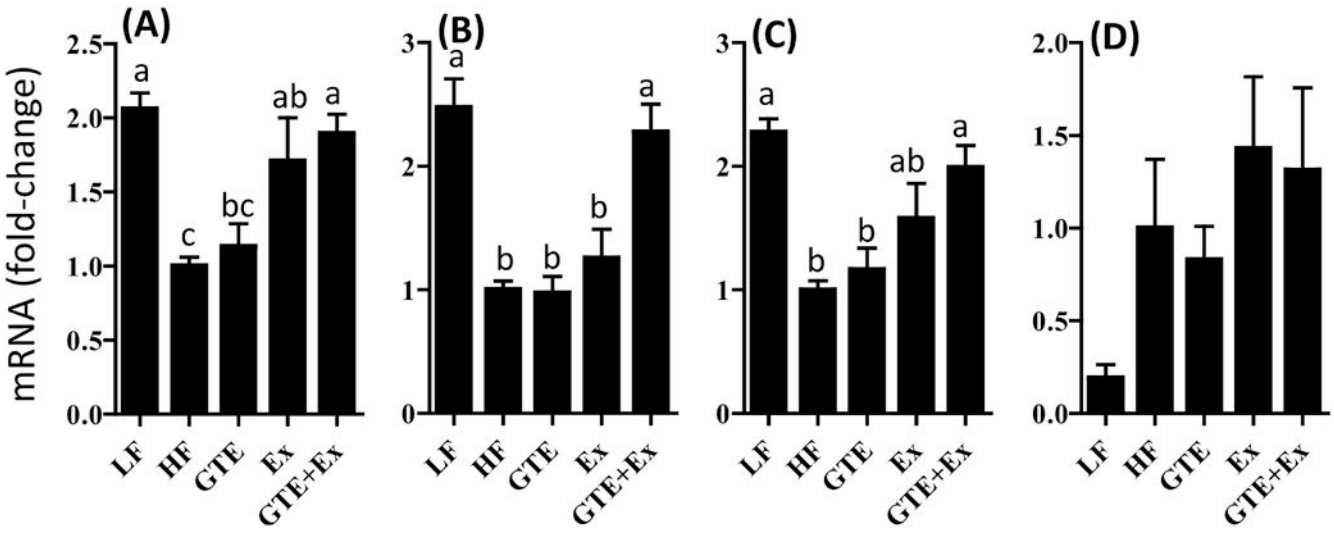

FIGURE 3.

The effect of GTE, Ex or GTE+Ex on genes related to PPARγ signaling in the adipose tissue of high fat-fed mice. Expression of (A) *Bmp4*, (B) *Pparg*, (C) *Ppargc1a*, and (D) *Pten* was determined in the epididymal adipose depot by qRT-PCR. Expression was normalized to *Gapdh*. N = 12 for LF and 22 for other groups. The error bars represent the SEM. Different superscript letters indicate $P < 0.05$ by one-way ANOVA with Tukey's post-test.