DOI 10.1002/mnfr.200900306 *Mol. Nutr. Food Res.* 2010, *54*, S14–S23

RESEARCH ARTICLE

Green tea polyphenols improve cardiac muscle mRNA and protein levels of signal pathways related to insulin and lipid metabolism and inflammation in insulin-resistant rats

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Received: July 1, 2009
Revised: August 6, 2009
Accepted: August 20, 2009

Epidemiological studies indicate that the consumption of green tea polyphenols (GTP) may reduce the risk of coronary artery disease. To explore the underlying mechanisms of action at the molecular level, we examined the effects of GTP on the cardiac mRNA and protein levels of genes involved in insulin and lipid metabolism and inflammation. In rats fed a high-fructose diet, supplementation with GTP (200 mg/kg BW daily dissolved in distilled water) for 6 wk, reduced systemic blood glucose, plasma insulin, retinol-binding protein 4, soluble CD36, cholesterol, triglycerides, free fatty acids and LDL-C levels, as well as the pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and IL-6. GTP did not affect food intake, bodyweight and heart weight. In the myocardium, GTP also increased the insulin receptor (*Ir*), insulin receptor substrate 1 and 2 (*Irs1* and *Irs2*), phosphoinositide-3-kinase (*Pi3k*), v-akt murine thymoma viral oncogene homolog 1 (*Akt1*), glucose transporter 1 and 4 (*Glut1* and *Glut4*) and glycogen synthase 1 (*Gys1*) expression but inhibited phosphatase and tensin homolog deleted on chromosome ten (*Pten*) expression and decreased glycogen synthase kinase 3β (*Gsk3*β) mRNA expression. The sterol regulatory element-binding protein-1c (*Srebp1c*) mRNA, microsomal triglyceride transfer protein (*Mttp*) mRNA and protein, Cd36 mRNA and cluster of differentiation 36 protein levels were decreased and peroxisome proliferator-activated receptor (*Ppar*)γ mRNA levels were increased. GTP also decreased the inflammatory factors: *Tnf*, *Il1b* and *Il6* mRNA levels, and enhanced the anti-inflammatory protein, zinc-finger protein, protein and mRNA expression. In summary, consumption of GTP ameliorated the detrimental effects of high-fructose diet on insulin signaling, lipid metabolism and inflammation in the cardiac muscle of rats.

Keywords:
Cardiac insulin / Green tea polyphenols / Lipid and inflammatory signaling

1 Introduction

Despite remarkable therapeutic advances, cardiovascular disease (CVD) is responsible for more deaths worldwide than any other condition, and a large proportion of healthcare budgets are spent on its treatment [1]. Type 2 diabetes has reached epidemic proportions globally contributing to significant cardiovascular morbidity and mortality. The high prevalence of CVD can largely be attributed to the metabolic syndrome with its multiple cardiovascular

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Abbreviations: ***Akt,*** thymoma viral proto-oncogene; ***Cd36*/CD36,** cluster of differentiation 36; **FFA,** free fatty acids; ***Glut,*** glucose transporter; ***Gys,*** glycogen synthase; ***Gsk3β,*** glycogen synthesis kinase; **HFD,** high-fructose diet; ***Ir,*** insulin receptor; ***Irs,*** insulin receptor substrate; ***Il,*** interleukin; ***Mttp*/MTTP,** microsomal triglyceride transfer protein; ***Pi3k-r1,*** phosphatidylinositol 3-kinase, regulatory subunit 1; ***Pten,*** phosphatase and tensin homolog deleted on chromosome ten; ***Ppar,*** peroxisome proliferator-activated receptor; ***Ppia,*** peptidylprolyl isomerase A; ***Rbp4*/RBP4,** retinol-binding protein 4; ***Srebp,*** sterol regulatory element-binding protein; ***Tnf,*** tumor necrosis factor; ***Zfp36*/ZFP36,** zinc-finger protein 36

risk factors, including insulin resistance, chronic inflammation, dyslipidemia, obesity and hypertension. Heart diseases are the leading causes of death in diabetic patients [2], with coronary artery disease and atherosclerosis being the primary reasons for increased incidence of cardiovascular dysfunction [2, 3].

Epidemiological studies suggest that green tea consumption lowers the risk of CVDs and mortality [4]. Increasing experimental evidence [5] suggests that green tea consumption is associated with beneficial effects on cardiovascular risk factors, including improvement of dyslipidemia [6], hypertension [7], obesity [8, 9], and diabetes [10, 11]. Green tea is also effective in inhibiting atherosclerosis [12], ameliorating endothelial function [5, 13], and is anti-inflammatory [14]. Furthermore, recent evidence suggests that green tea also prevents myocardial hypertrophy [15, 16], and has beneficial effects with regard to myocardial ischemia–reperfusion injury [17, 18]. Li *et al.* [19] reported that green tea polyphenols (GTP) regulate myocardial contractility in cardiomyocytes *via* a protein kinase-Cε-dependent signaling pathway, without increasing intracellular Ca^{2+} in isolated rat hearts. Hsieh *et al.* [20] recently reported that one novel action of GTP in protecting against myocardial damage and enhancing cardiac contractility is by modulating myofilament Ca^{2+} sensitivity in post-myocardial infarction rats. However, little is known of the molecular effects of GTP on cardiac muscle itself. Cardiac muscle is also a target of insulin [21]; impairment of insulin-stimulated cardiac glucose uptake has been described in animal models of diabetes [22], obesity [23], hypertension [24] and high cholesterol/fructose feeding [25]. To our knowledge there are no direct studies of the link between GTP consumption and systemic metabolic environment and cardiac insulin signaling, lipid metabolism and inflammatory.

The aim of this study was to investigate whether supplementation with GTP improves biomarkers associated with insulin resistance, CVD, and inflammation, using a high-fructose diet (HFD)-induced insulin-resistant model. We investigated whether GTP consumption affects the patterns of gene expression related to proximal insulin signaling, lipid metabolism and glucose uptake pathways that are negatively affected by a HFD in cardiac tissue. We also evaluated two recently identified biomarkers of insulin action, plasma retinol-binding protein (RBP)4 [26] and soluble CD36 (sCD36) [27]. Finally, we determined the effects of GTP on the expression of genes and proteins associated with inflammation, and the anti-inflammatory zinc finger protein, Zfp36 [28], in cardiac tissue.

2 Materials and methods

2.1 Materials

Male Wistar rats weighing 100–130 g were housed in a temperature-controlled room ($22 \pm 2^{\circ}C$) on a 12 h light cycle (6:00 a.m. to 6:00 p.m.) according to the Guidelines for Animal Care of the Beltsville Area Animal Care and Use Committee. After a 1 wk acclimation period, rats were placed on either a normal chow diet or fructose-enriched diet (casein 207.0; DL-methionine 3.0; fructose 600.0; lard 50.0; cellulose 79.81; AIN mineral mix, 50.0; zinc carbonate 0.04; AIN vitamin mix 10.0; green food color 0.15 g/kg (89247-Teklad Animal Diets, Madison, WI)) for 10 wk. After the fifth week, fructose diet-fed rats were assigned randomly to receive either GTP or vehicle for an additional 6 wk. During the experimental period, the consumption of food and fluid were monitored every other day. GTP were dissolved in distilled water, based on fluid consumption and the bodyweight, the concentrations of GTP in each bottle were regulated to maintain the same dosage, 200 mg/kg body weight *per* day. This dosage has been shown to effectively inhibit insulin resistance [29] and dyslipidemia [30]. At the termination of the feeding experiment, following an overnight fast, the blood glucose levels were tested from blood collected from the tail vein. Rats were anesthetized and blood was collected from the portal vein in pre-cooled tubes containing EDTA and centrifuged at 5000 rpm for 15 min at 4°C. The hearts were carefully cleaned and weighed before being snap frozen in liquid nitrogen and stored at −80°C until analyses.

GTPs were obtained from Integrity Nutraceuticals International (Spring Hill, TN, USA). The extract was characterized using HPLC. The green tea extract contained 3.47% caffeine and 71.4% total catechins consisting of 66.8% EGCG, 7% epigallocatechin, 20% epicatechin gallate, and 5.1% epicatechin.

2.2 Immunoblotting

To determine plasma RBP4 (retinol-binding protein 4) and sCD36, the plasma samples were diluted (1:50) and samples were resolved by SDS-PAGE and subjected to immunoblotting using anti-rat RBP4 (ALPCO, Salem, NH, USA) and anti-rabbit cluster of differentiation 36 (CD36) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies as described previously [31]. For cardiac immunoanalysis, approximately 50 mg of cardiac tissue was homogenized (Polytron, Fisher Scientific Rockford, IL, USA) at 4°C for 30 s in lysis buffer containing 20 mM Tris (pH 7.4), 2 mM EDTA, 50 mM NaF, 200 μM Na_3VO_4, 250 μM PMSF, 1 μM leupeptin, 1 μM pepstatin, and 0.36 μM aprotinin. Protein concentrations were determined by a commercial assay (Bio-Rad D_c protein assay) using BSA as a standard. The procedures for detecting and quantifying the amounts of cardiac microsomal triglyceride transfer protein (MTTP), CD36 and zinc-finger protein (ZFP36) were carried out as described previously [28, 31, 32].

2.3 Cardiac gene expression

Gene expression was measured with real-time quantitative PCR. Total cardiac RNA was extracted with Trizol® reagent

Table 1. Real-time PCR primers and TaqMan probes

Gene	Sequence (5′–3′) forward	TaqMan probe (TET-BHQ1)	Sequence (5′ to 3′) reverse)
Ir	CAAAAGCACAATCAGAGTGAGTATGAC	TCCTCCAGCTCCTTCAGGATCTGAGAGTC	ACCACGTTGTGCAGGTAATCC
Irs1	GCCTGGAGTATTATGAGAACGAGAA	CGCTCGACTTGTGCCGCCACTT	GGGGATCGAGCGTTTGG
Irs2	AAGATAGCGGGTACATGCGAAT	TGGTGTGGCTCCAAGCTGTCCATG	GCAGCTTAGGGTCTGGGTTCT
Pi3k-r1	CCTCTCCTTATAAAGCTCCTGGAA	CAACTCTATACAGAACACAGAGCTCCAGCAACCC	GATCACAATCAAGAAGCTGTCGTAA
Akt1	TGGACTACTTGCACTCCGAGAA	CCAGCTTGAGGTCCCGGTACACCAC	TTATCTTGATATGCCCGTCCTT
Pten	AAGATGCTCAAAAAGGACAAAATGT	CACTTTTGGGTAAATACGTTCTTCATACCAGGACC	GATTTCCTGATCACAAAGACTTCCAT
Glut1	CGTGCTTATGGGTTTCTCCAAA	CAGGATCAGCATCTCAAAGGACTTGCCC	GACACCTCCCCCACATACATG
Glut4	CAACTGGACCTGTAACTTCATCGT	TCCGCAACATACTGGAAACCCATGC	ACGGCAAATAGAAGGAAGACGTA
Gys1	TCCACTGTGCCTGTGTCTTCA	CTCAAGAGGAAACCAGATATTGTGACCCCCA	AGAGAACTTCTTCACATTCAGTCCATT
Gsk3β	TTAAGGAAGGAAAAGGTGAATCGA	AACCACCTCCTTTGCGGAGAGCTGCA	CCAAAAGCTGAAGGCTGCTG
Srebp1c	GGCCCGGCTGTACACTTCT	AGACATCGCAAACAAGCTGACCTGGA	AATCTCCCCGGGCCAAA
Mttp	GATCGTAGGAAAGGACAACTTGGA	ACCTTCCCCCGGACCAGATGAAGAA	ACGGGCTCATTTTCATAGGAGTAG
Pparg	CTTGGCCATATTTATAGCTGTCATTATT	AGTGGAGACCGCCCAGGCTTGC	CCTCGATGGGCTTCACGTT
Cd36	AGGAATTTGTCCTATTGGGAAAGTT	ACATGATTAATGGCACAGATGCAGCCTC	CCGCAGTACCCGAGACTTCT
Tnf-α	GCTGTCGCTACATCACTGAACCT	TGCTCCCCACGGGAGCCG	TGACCCGTAGGGCAATTACA
Il1b	GGTTGAATCTATACCTGTCCTGTGTG	CACACCCACCCTGCAGCTGGAGAGT	TTTTCCATCTTCTTCTTTGGGTATT
Il6	ACACATGTTCTCTGGGAAATCGT	TGAGAAAAGAGTTGTGCAATGGCAATTCTG	AAGTGCATCATCGTTGTTCATACA
Zfp36	GGTACCCAGGCTGGCTTT	AACTCAATATAATCCTGCCTTAGCCTT	ACCTGTAACCCAGAACTTGGA
Ppia	TGTAATCTCTGCTCTCACTGAAGTTCT	TCCATATTTTCCTCATTCCCCTTCAAGTCTAGC	TATTCATAATCATAAACTTAACTTTGCAATCC

(Invitrogen, Carlsbad, CA, USA) following instructions of the manufacturer. RNA concentrations and integrity were determined using RNA 6000 Nano Assay Kit and the Bioanalyzer 2100 according to the manufacturer's instructions (Agilent, Santa Clara, CA, USA). The cDNAs were synthesized from total RNA using SuperScript II RT (Invitrogen). The primers, probes and reverse primers used are described in Table 1. PCR determinations were performed in an ABI Prism 7700 real-time PCR instrument (Applied Biosystems, Foster City, CA, USA). The expression of the housekeeping gene *Ppia* (peptidylprolyl isomerase A) was used to normalize the expression of target genes.

2.4 Plasma biochemistry

Glucose was determined on whole blood using a glucometer (One Touch, Milpitas, CA, USA). Serum insulin concentrations were determined by RIA using a rat insulin kit from Linco Research (St. Louis, MO, USA). Serum TNF-α (tumor necrosis factor-α) and IL-6 were determined with a rat ultrasensitive EIA (ALPCO). Triglycerides (TG) and cholesterol, LDL-cholesterol, and free fatty acids (FFA) were measured using a colorimetric assay (Wako, Richmond, VA, USA).

2.5 Statistical analyses

Data were analyzed by the one-way analysis of variance followed by LSD. p-Values < 0.05 were considered significant.

3 Results

3.1 General observation and plasma biochemistry

Food intake and water consumption, body and heart weight: Feeding the HFD for 10 wk did not affect body and heart weights (data not shown); 6 wk of GTP consumption did not affect food and water consumption and also did not affect body and heart weights compared with the HFD-fed group (data not shown).

Glucose and insulin concentrations and plasma RBP4 and sCD36 levels: Dietary fructose increased blood glucose ($p < 0.05$) and plasma insulin levels ($p < 0.01$) and GTP significantly attenuated these (Figs. 1A and B, $p < 0.05$, respectively). In rats receiving the HFD, plasma RBP4 levels were significantly (Fig. 1C, $p < 0.01$) higher than in chow feeding. GTP consumption lowered ($p < 0.05$) plasma RBP4 levels, compared with HFD. HFD significantly induced the overproduction of sCD36 in plasma, compared with chow diet ($p < 0.01$) and GTP consumption inhibited the increases in sCD36 levels due to fructose feeding (Fig. 1D, $p < 0.05$).

Plasma triglyceride, cholesterol, LDL-C and FFA concentrations: HFD feeding significantly elevated plasma

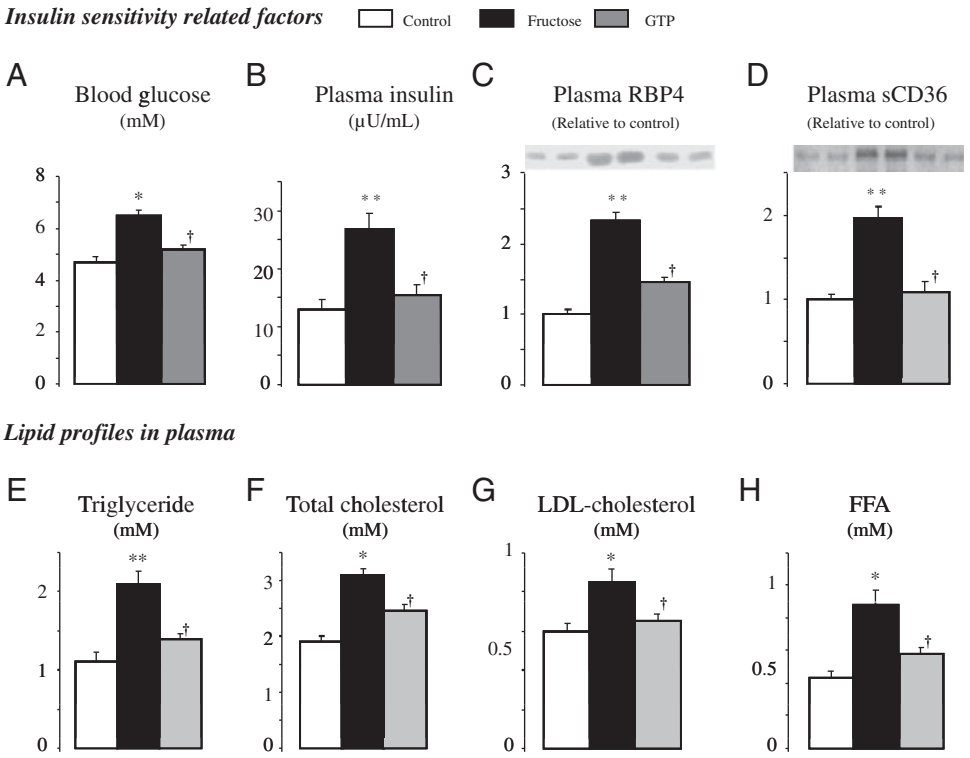

Figure 1. Systemic environment, insulin sensitivity-related factors and lipid profiles in plasma. (A–H): blood glucose (A); insulin (B); RBP4 (C) and sCD36 (D), representative experiments on RBP4 and sCD36, immunoblots were analyzed using densitometry; TG (E); cholesterol (F); LDL-cholesterol (G); and FFAs (H). Values given are means ± SE, ($n = 7$); $^{*}p < 0.05$ and $^{**}p < 0.01$ *versus* control; † $p < 0.05$ *versus* HFD.

TG, total cholesterol, LDL-C and FFA levels, compared with the controls and GTP improved the dyslipidemia for all these variables (Figs. 1E–H; $p < 0.05$, respectively).

Plasma TNF-α and IL-6 concentrations: Plasma TNF-α levels were higher in rats on HFD when compared with the chow diets (350 ± 20 and 30 ± 5 pg/mL, $p < 0.01$) and GTP significantly reversed this trend (about 120 pg/mL, $p < 0.05$). Dietary fructose also significantly induced plasma IL-6 production when compared with the chow diet (280 ± 18 and 45 ± 4 pg/mL, $p < 0.01$) and GTP consumption suppressed HFD-induced IL-6 expression (about 115 pg/mL, $p < 0.05$).

3.2 GTP altered the mRNA expression of the impaired cardiac insulin signaling pathway in rats fed the HFD

Rats on the HFD had impaired cardiac mRNA levels of the proximal insulin-signaling pathway, including decreases in mRNA levels of *Ir* by 15%, *Irs1* by 25%, *Irs2* by 21%, *Pi3k* by 14%, and *Akt1* by 19%. Because of high inter-animal variability, however, the differences were not statistically significant. In contrast, GTP significantly enhanced the mRNA levels of *Ir*, *Irs2*, *Pi3k* (Figs. 2A, C and D, $p < 0.05$, respectively). There was also a trend that GTP enhanced the lower *Irs1* and *Akt1* mRNA levels (Figs. 2B and E). HFD induced cardiac *Pten* mRNA overexpression and GTP inhibited the increased *Pten* expression (Fig. 2F, $p < 0.05$).

3.3 GTP regulated cardiac mRNA expression of glucose transporters and glycogen synthesis in rats fed the HFD

HFD feeding decreased cardiac mRNA expression of *Glut1* by 27% ($p < 0.05$) and *Glut4* by 24% ($p = 0.08$) relative to that of rats on the chow diet, while GTP consumption enhanced the impaired *Glut1* and *Glut4* mRNA expression (Fig. 2G and $p < 0.05$, respectively). HFD feeding also decreased the cardiac mRNA expression of *Gys1* by 43% ($p < 0.05$), but GTP enhanced the expression by 40% (Fig. 2I, $p < 0.05$). The HFD induced *Gsk3*β mRNA expression by 250% relative to control ($p < 0.01$), but GTP inhibited the *Gsk3*β overexpression (Fig. 2J, $p < 0.05$).

3.4 GTP modulated the expression of genes and the protein involved in cardiac lipid metabolism in rats fed the HFD

The HFD induced *Srebp1c* expression by 70% relative to control and GTP inhibited these increases (Fig. 3A, $p < 0.05$). The HFD also induced *Mttp* mRNA and protein expression by 80% and 40% relative to control and GTP also inhibited these increases in gene expression and protein levels (Figs. 3B and C, $p < 0.05$, respectively). Cardiac *Pparγ* mRNA levels were decreased by 30% relative to chow diet due to consumption of the HFD

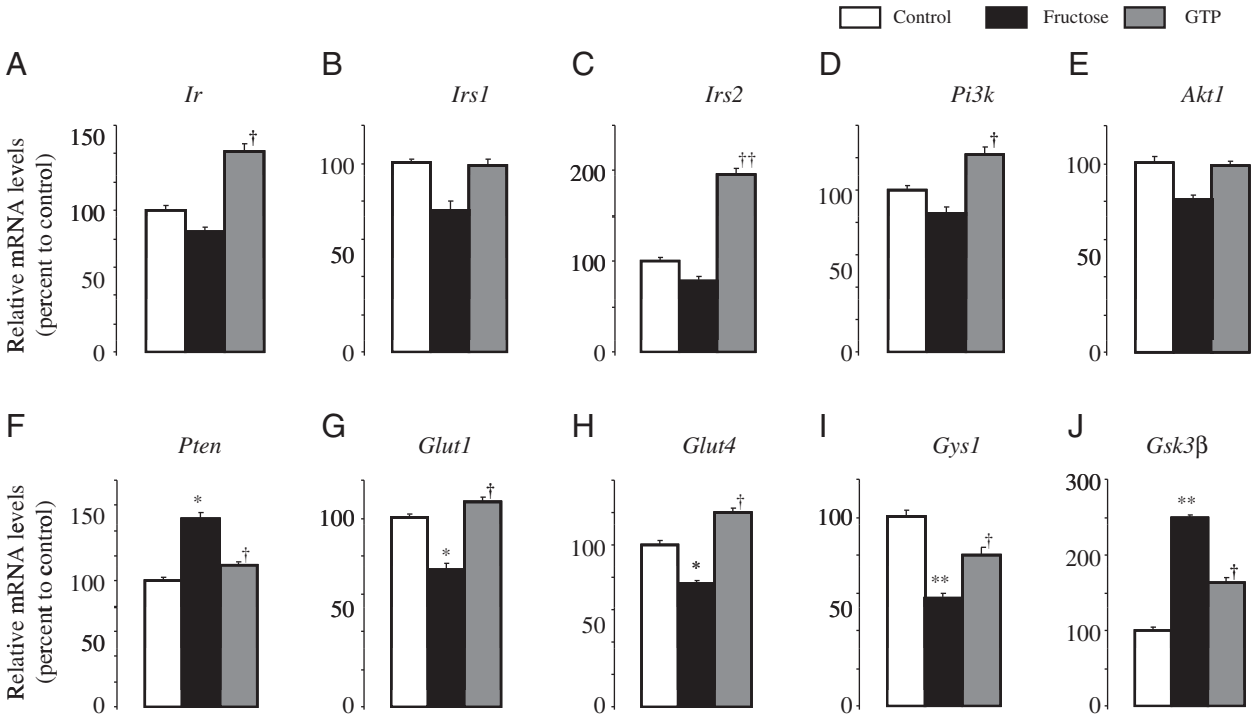

Figure 2. Effects of GTP on the cardiac mRNA levels related proximal insulin signaling, glucose transporters, and glycogen synthesis. (A–J): *Ir* (A), *Irs1* (B), *Irs2* (C), *Pi3k* (D), *Akt1* (E), *Pten* (F), *Glut1* (G), *Glut4* (H), *Gys1* (I) and *Gsk3*β (J). Values are mean ± SE and are presented as percent of control ($n = 5$–7). *$p < 0.05$ and **$p < 0.01$ *versus* control; † $p < 0.05$ and †† $p < 0.01$ *versus* HFD.

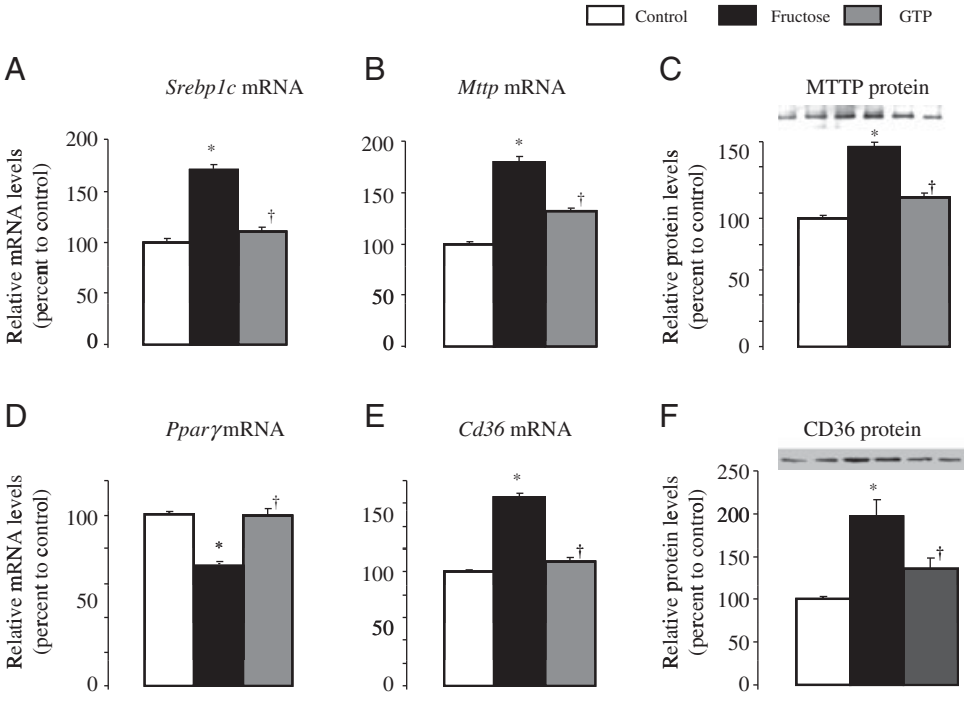

Figure 3. Effects of GTP on the expression of genes and protein involved in cardiac lipid metabolism in rats fed a HFD. (A–F): *Srebp1c* mRNA (A), *Mttp* mRNA (B), MTTP protein (C), *Pparγ* mRNA (D), *Cd36* mRNA (E), CD36 protein (F). (C) and (F): representative experiments on MTTP and CD36, immunoblots were analyzed using densitometry; values are mean ± SE and are presented as percent of control ($n = 5$–7). *$p < 0.01$ *versus* control; † $p < 0.05$ *versus* HFD.

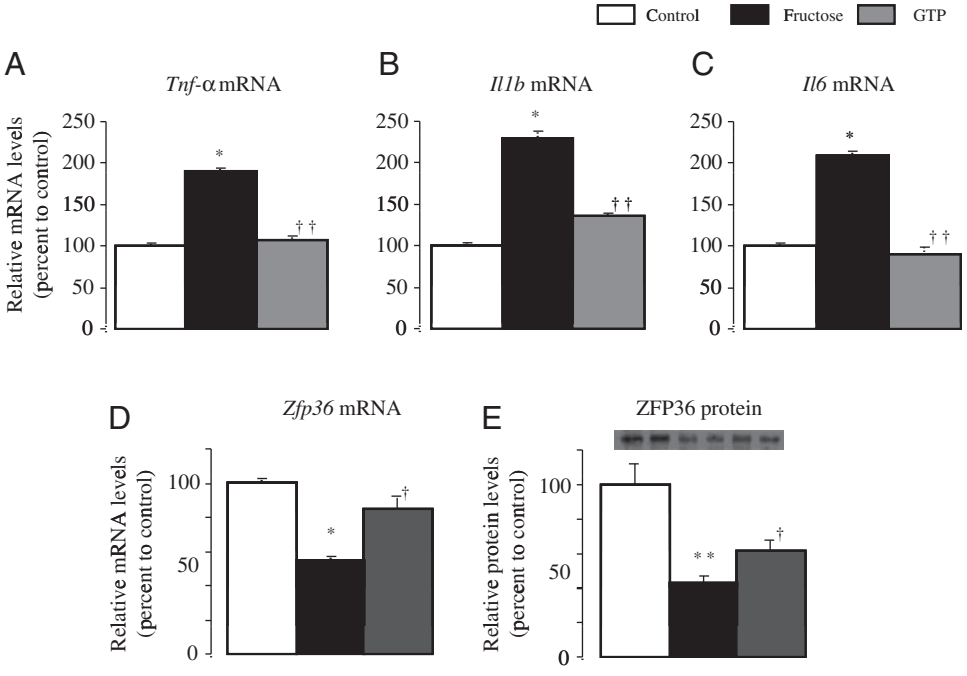

Figure 4. Effects of GTP on the expression of genes and protein involved in cardiac inflammation factors in rats fed a HFD. (A–F): *Tnf*-α mRNA (A), *Il1b* mRNA (B), *Il6* (C), *Zfp36* mRNA (D) and ZFP36 protein (E). (E): ZFP36 were immunoblotted using anti-mouse Zfp36 antibody. Immunoblots were analyzed using densitometry; values are mean ± SE and are presented as percent of control ($n = 5$–7). $^{*}p < 0.01$ *versus* control; † $p < 0.05$ and †† $p < 0.01$ *versus* HFD.

and GTP prevented these effects (Fig. 3D, $p < 0.05$). Cardiac *Cd36* mRNA and protein levels were significantly higher in HFD group than in control diet and supplementation with GTP attenuated the high of *Cd36* mRNA and protein levels (Figs 3E and F, $p < 0.05$, respectively).

3.5 GTP regulated inflammation-related gene expression and induces *Zfp36* expression

The HFD significantly induced the inflammation factors, *Tnf*-α, *Il1b* and *Il6* mRNA expression, by 90, 130 and 110 relative to the controls, respectively, and GTP inhibited mRNA expression of all three of these factors (Figs 4A–C, $p < 0.05$, respectively). Cardiac *Zfp36* mRNA and protein levels were significantly lower in the HFD group than in chow-fed group, and supplementation with GTP enhanced the levels of *Zfp36* expression (Figs 4D and E, $p < 0.05$).

4 Discussion

Using a HFD-induced insulin-resistant animal model, the present study provides evidence that GTP: (i) improved the systemic metabolic environment including glucose and lipid metabolism and related factors and especially lowered the biomarkers of insulin resistance, plasma RBP4 and sCD36 levels, and inhibited plasma inflammatory markers; (ii) improved the cardiac mRNA expression of proximal insulin signaling, and *Glut1* and *Glut4*, and inhibited *Gsk3*β expression; (iii) regulated the cardiac expression of lipid-related factors including *Srebp1c*, *Mttp*, *Pparγ* and *Cd36*; (iv) inhibited the cardiac mRNA expression of inflammation factors, and upregulated mRNA and protein expression of the anti-inflammation ZFP36 protein.

HFD has been implicated as a contributing factor to metabolic abnormalities such as insulin resistance and hypertriglyceridemia. In the current study, HFD feeding induced an abnormal metabolic environment including mild hyperglycemia, hyperinsulinemia, dyslipidemia and inflammation (Fig. 1). Our data provide strong evidence that GTP improved the systemic metabolic environment including plasma glucose, insulin, triglycerides, cholesterol, LDL, FFA and other metabolic biomarkers [29, 30, 33]. Koo and Noh [6] reported that green tea catechins are not readily absorbed and only small percentages of catechins appear in the blood. However, even at low levels, GTP might influence the uptake and intracellular processing of lipids and assembly and secretion of chylomicrons.

During the course of the development CVD, the heart is exposed to a dysmetabolic environment. Initially, the heart adapts to this environment and is able to maintain cardiac output. Long-term exposure of the heart to a dysmetabolic environment eventually results in myocardial dysfunction [34]. During insulin resistance, the heart rapidly modifies its energy metabolism, resulting in augmented lipids and decreased glucose consumption. Accumulating evidence suggests that the alteration of cardiac metabolism plays an important role in the development of cardiomyopathy [35]. Increasing evidence suggests that dietary fructose consumption induces systemic and peripheral insulin resistance [36]. At the molecular level, insulin resistance in hearts from animals fed a high-fat diet was reflected by

impaired activation of insulin receptor and Irs1/PI3k/Akt-mediated signaling [37]. Defects in insulin signaling also were reported in the hearts from *ob/ob* mice [38] and Zucker fatty rats [39]. Deng *et al.* [40] reported that high-cholesterol/fructose-feeding impaired cardiac insulin signaling and myocardial contractile performance. Our data suggest that GTP improved the impaired mRNA expression of the cardiac proximal insulin signaling pathway in rats fed a HFD, and inhibited HFD-induced mRNA expression of *Pten*. This gene encodes for an antagonist of the PI3k/Akt pathway [41, 42] and is a negative regulator of glucose metabolism in fructose-fed lean and obese Zucker rats [41].

RBP4 is a vitamin A transporter, synthesized by liver and adipose tissue. The close relationship between RBP4, obesity, and type 2 diabetes was first described by Yang *et al.* [43]. Plasma RBP4 levels and expression of RBP4 are elevated in insulin-resistant mice; elevated RBP4 levels can impair insulin signaling in muscle and increase hepatic glucose output. Plasma RBP4 levels are inversely correlated with the expression of GLUT4 in adipose tissue [44, 45]. In our study, GTP inhibited the overproduction of plasma RBP4 in HFD rats.

Glucose uptake by cardiomyocytes occurs primarily through the GLUT1 and GLUT4 [46]. GLUT1 mediates primarily basal glucose uptake, while GLUT4 mediates uptake in response to insulin stimulation [47]. GTP consumption enhanced the mRNA levels of *Glut1* and *Glut4*. Additionally, we observed that HFD feeding impaired glycogen synthase mRNA expression and increased *Gsk3*β mRNA levels. GSK3β is a key regulatory enzyme in glucose metabolism that, when activated, phosphorylates/inactivates target enzymes of the insulin-signaling pathway. Previous reports show that inhibition of GSK3β increases insulin action, resulting in augmented glucose uptake [48, 49].

In the insulin-resistant state, excessive lipid levels, in the presence of impaired glucose utilization, result in accumulation of lipids in the myocardium. Previous studies suggest that green tea extracts modulate cardiac lipid metabolism in hyperlipidemic rats fed a high-sucrose diet [50] and decrease cholesterol, TG, and FFA levels and lipoprotein lipase activity in the myocardium of diabetic rats [33]. Increased cardiac production of lipoproteins is associated with increased TG secretion from the heart and decreased stores of TG within the heart [51]. Microsomal triglyceride transfer protein is rate limiting for the production and secretion of apoB-containing lipoproteins. Microsomal triglyceride transfer protein gene expression and activity are increased in the *ob/ob* mouse hearts [52], which provides evidence that an upregulation of *Mttp* expression in the heart serves as a protective mechanism to unload excess lipids when the supply of fatty acids exceeds the utilization. MTTP might play an important role in controlling cardiac lipid homeostasis, and ultimately, cardiac function [51]. In the current study, HFD feeding induced *Mttp* mRNA and protein levels and GTP reversed this. Sterol-regulatory-element-binding protein-1c, an important regulator of lipogenesis has a crucial role in the regulation of TG accumulation in the liver [53] and preferentially enhances the transcription of the gene involved in fatty acid synthesis and triglyceride metabolism [54]. In the current study, GTP inhibited HFD-induced increased cardiac expression of *Srebp1c*. Recently, Handberg *et al.* [55]reported that sCD36 is highly related to risk factors of accelerated atherosclerosis in type 2 diabetes and insulin resistance. sCD36 is also reported as a novel marker of insulin resistance [55] and inflammation [56]. Higher levels are also found in other animal models [57]. The authors concluded that CD36 expression contributes to age-induced cardiomyopathy in mice [57]. In our study, GTP consumption reversed the overexpression of CD36 in plasma and cardiac muscle.

PPARγ is a lipid sensor that regulates lipid metabolism [58], and plays an important role in the regulation of CD36 expression [59]. Recent attention has focused on PPARγ ligands for their actions on the myocardium [60]. Several roles have been postulated for PPARγ including as an inhibitor of cardiac hypertrophy [61] that can decrease the nuclear factor kappa B-binding activity in the left ventricle of stroke-prone spontaneously hypertensive rats [62]. In the current study, GTP attenuated lower cardiac mRNA levels of *Pparγ* induced by HFD feeding. Taken together, these data suggest that GTP might play an important role in decreasing cardiac lipid accumulation and lipoprotein lipase activity in the myocardium of diabetic rats [33].

Proinflammatory cytokines such as TNF-α and IL-6 may contribute to the development of CVD by promoting insulin resistance, dyslipidemia, and endothelial dysfunction [63]. In this study, fructose feeding not only induced plasma TNF-α and IL-6 overproduction, but also stimulated cardiac *Tnf*, *Il1b* and *Il6* mRNA expression. GTP consumption inhibited expression of these biomarkers of inflammation in plasma and in cardiac muscle. Additionally, we also found that HFD feeding impaired ZFP36 protein and mRNA expression. Importantly, GTP attenuated this. ZFP36 is an anti-inflammatory protein and the potential therapeutic target for the treatment of inflammation-related diseases [28]. Moreover, recent study reported that ZFP36 in omental adipose tissue may offer partial protection against the development of insulin resistance and diabetes [64]. Our data suggest that GTP inhibits the systemic and cardiac inflammation factors induced by feeding a HFD.

In summary, we provide evidence that feeding GTP ameliorated multiple pathways in cardiac muscle related to insulin signaling, lipid metabolism and inflammation that were negatively influenced by feeding a HFD. These findings contribute to a better understanding of the molecular mechanisms by which green tea consumption lowers the risk of CVD [4].

This work was supported by a USDA Cooperative Research and Development Agreement (CRADA No. 58-3K95-7-1184) with Integrity, Spring Hill, TN, to R. A. Dr. Ji-Young Lee, Dr.

Joseph Urban and Dr. Allison Yates are thanked for support and advice. The authors also thank Dr. Heping Cao for his assistance and the antibody for Zfp36.

Conflict of interest: B. Qin is a visiting scientist, working at the USDA/ARS/BHNRC and also employed by Integrity Nutraceuticals International.

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