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Green Tea Polyphenol Extract Regulates the Expression of Genes Involved in Glucose Uptake and Insulin Signaling in Rats Fed a High Fructose Diet

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Green tea has antidiabetic, antiobesity, and anti-inflammatory activities in animal models, but the molecular mechanisms of these effects have not been fully understood. Quantitative real-time polymerase chain reaction (PCR) was used to investigate the relative expression levels and the effects of green tea (1 and 2 g solid extract/kg diet) on the expression of glucose transporter family genes (*Glut1*/*Slc2a1*, *Glut2*/*Slc2a2*, *Glut3*/*Slc2a3*, and *Glut4*/*Slc2a4*) and insulin signaling pathway genes (*Ins1*, *Ins2*, *Insr*, *Irs1*, *Irs2*, *Akt1*, *Grb2*, *Igf1*, *Igf2*, *Igf1r*, *Igf2r*, *Gsk3b*, *Gys1*, *Pik3cb*, *Pik3r1*, *Shc1*, and *Sos1*) in liver and muscle of rats fed a high-fructose diet known to induce insulin resistance and oxidative stress. Glut2 and Glut4 were the major Glut mRNAs in rat liver and muscle, respectively. Green tea extract (1 g) increased Glut1, Glut4, Gsk3b, and Irs2 mRNA levels by 110, 160, 30, and 60% in the liver, respectively, and increased Irs1 by 80% in the muscle. Green tea extract (2 g) increased Glut4, Gsk3b, and Pik3cb mRNA levels by 90, 30, and 30% but decreased Shc1 by 60% in the liver and increased Glut2, Glut4, Shc1, and Sos1 by 80, 40, 60, and 50% in the muscle. This study shows that green tea extract at 1 or 2 g/kg diet regulates gene expression in the glucose uptake and insulin signaling pathway in rats fed a fructose-rich diet.

KEYWORDS: Diabetes; fructose-rich diet; green tea; glucose transporter; insulin signaling pathway; obesity; polyphenol extract, rat

INTRODUCTION

Insulin resistance, diabetes, and obesity have been studied extensively, but the prevention and treatment of obesity and type 2 diabetes mellitus (type 2 DM) has not been resolved (*1*−*5*). Diet plays an important role in the development of these conditions, and the diets widely consumed in developed countries may increase the incidence of diabetes (*6*). The combination of a higher content of refined sugars and fats and a lower intake of traditional herbs, spices, and other plant products may contribute to the higher incidences of diabetes and obesity in the United States (*7*). Drug treatment for diabetes in the developing countries is not feasible for the majority of people, and alternative and inexpensive therapies need to be evaluated.

Medicinal plants have been used for the treatment of diabetes and related disorders for thousands of years (*8*). Herbal medicines are playing a role in the prevention and control of type 2 DM for people with elevated levels of blood glucose and glucose intolerance who have a greater risk of developing diabetes. Plant seeds, fruits, leaves, and bark contain polyphenolic compounds. These compounds are the end products of the plant flavonoid biosynthetic pathway and are used by plants for protection against predators (*9*). Plant polyphenols are also widely present in the diet (*10*) and are thought to be important for human health (*11*−*13*).

Tea (*Camellia sinensis*) is a popular beverage worldwide. Recent studies indicate that tea has a wide range of preventive effects on diabetes and obesity for animal and human health (*14*, *15*). A number of studies have suggested that green tea polyphenols mimic insulin action. First, rat epididymal adipocyte assays indicate that green tea extract has an insulin-potentiating activity on the utilization of glucose (*16*, *17*) and that the predominant active ingredient is epigallocatechin gallate (EGCG) (*16*, *17*), the major polyphenol in green tea. Second, green tea

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Table 1. Nucleotide Sequences of Real-Time PCR Primers and TaqMan Probes

mRNA	accession no.	amplicon	forward primer (5′ to 3′)	TaqMan probe (TET–BHQ1)	reverse primer (5′ to 3′)
Glut1/Slc2a1	M13979	123 bp	CGTGCTTATGGGTTTCTCCAAA	CAGGATCAGCATCTCAAAGGACTTGCCC	GACACCTCCCCCACATACATG
Glut2/Slc2a2	NM_012879	80 bp	TTTGCAGTAGGCGGAATGG	TGTCCCCAAGCCACCCACCAAA	GCCAACATGGCTTTGATCCTT
Glut3/Slc2a3	NM_017102	112 bp	TGAAGCCATGAGCTTTGTCTGT	CCCCATTCCCTGGTTTATTGTGGC	GCCCTGGCTGAAGAGTTCAG
Glut4/Slc2a4	NM_012751	87 bp	CAACTGGACCTGTAACTTCATCGT	TCCGCAACATACTGGAAACCCATGC	ACGGCAAATAGAAGGAAGACGTA
Akt1	NM_033230	90 bp	TGGACTACTTGCACTCCGAGAA	CCAGCTTGAGGTCCCGGTACACCAC	TTATCTTGATATGCCCGTCCTT
Grb2	NM_030846	119 bp	GGCTTCATCCCCAAGAATTACA	AGGCAGAAGAAATGCTTAGCAAACAGCG	ATCAGGAAGGCCCCGTCAT
Gsk3b	NM_032080	106 bp	TTAAGGAAGGAAAAGGTGAATCGA	AACCACCTCCTTTGCGGAGAGCTGCA	CCAAAAGCTGAAGGCTGCTG
Gys1	XM_229128	119 bp	TCCACTGTGCCTGTGTCTTCA	CTCAAGAGGAAACCAGATATTGTGACCCCCA	AGAGAACTTCTTCACATTCAGTCCATT
Ins1	NM_008386	89 bp	AGGTGGCCCGGCAGAAG	ATTGTGGATCAGTGCTGCACCAGCA	GCCTTAGTTGCAGTAGTTCTCCAGCT
Ins2	NM_008387	100 bp	TTGTGGTTCCCACTTGGTGGA	TTCTACACACCCATGTCCCGCCG	CACTTGTGGGTCCTCCACTTC
Insr	NM_017071	137 bp	CAAAAGCACAATCAGAGTGAGTATGAC	TCCTCCAGCTCCTTCAGGATCTGAGAGTC	ACCACGTTGTGCAGGTAATCC
Igf1	NM_184052	78 bp	GCTGTGCAATTTACTCATTG	TGGACAAAAGGCAGTTTACCCAGGCTC	TTGGACACCCAGGCAGGTAT
Igf1r	NM_010513	62 bp	CCGCTGCTGGACCACAA	TCGCTGCCAGAAAATGTGCCCA	AGGCTCGCTTCCCGCACA
Igf2	NM_010514	78 bp	TGTCTACCTCTCAGGCCGTACTT	CCCCAGATACCCCGTGGGCAA	TCCAGGTGTCATATTGGAAGAA
Igf2r	NM_010515	91 bp	GGATTCCACTCAAGTCAAAATAGCA	AAGACACCAGAACCAGACACTCCGGTAC	ACCTCCAGAATAGATGAGGGTGAGGTC
Irs1	NM_012969	68 bp	GCCTGGAGTATTATGAGAACGAGAA	CGCTCGACTTGTGCCGCCACTT	GGGGATCGAGCGTTTGG
Irs2	AF050159	69 bp	AAGATAGCGGGTACATGCGAAT	TGGTGTGGCTCCAAGCTGTCCATG	GCAGCTTAGGGTCTGGGTTCT
Pik3cb	NM_053481	134 bp	TCCAGACCAGTATGTCCGAGAGTA	CACTCATCTGTCGCAGGCAGCCTACA	ATCTGGAGAGGGCACAGTCAA
Pik3r1	NM_013005	118 bp	CCTCTCCTTATAAAGCTCCTGGAA	CAACTCTATACAGAACACAGAGCTCCAGCAACCC	GATCACAATCAAGAAGCTGTCGTAA
Shc1	XM_216176	85 bp	GCGACGACGAGGAGAAAGTC	CAGCTCTATCCTGGGGAGGAGTAACCTGAA	TGATCGGCACTCCAGCAAA
Sos1	D83014	104 bp	CAGAAGAAAGACAGTATCTACGGGAACT	TTCCGGGAAGCCTTTCTTTCAGACAAA	CTTTTCAATTTCAGAAGACTTGAACAA
Rpl32	NM_172086	66 bp	AACCGAAAAGCCATTGTAGAAA	AGCAGCACAGCTGGCCATCAGAGTC	CCTGGCGTTGGGATTGG

powder and its polyphenols decrease fasting plasma levels of glucose, insulin, triglyceride, and free fatty acid and increase the insulin-stimulated glucose uptake in rats fed green tea extract and polyphenols for 12 weeks (*18*). Third, EGCG inhibits BCH-stimulated insulin secretion and glutamate dehydrogenase (*19*). Fourth, EGCG induces forkhead transcription factor family O phosphorylation by a similar but not identical mechanism to insulin and insulin growth factor I (IGF-I) (*20*). Finally, both insulin and green tea polyphenol extract increase mRNA levels coding for the anti-inflammatory mRNA destabilizing protein tristetraprolin (TTP/ZFP36/TIS11) (*21*−*23*). However, a recent study suggests that green tea consumption did not improve blood glucose, lipid profiles, insulin resistance, or serum adiponectin levels in type 2 diabetes patients (*24*). Clearly, more studies are needed to demonstrate tea's insulin-like activity and the underlining molecular mechanisms.

A fructose-rich diet has been used as a model for the study of insulin resistance and oxidative stress in rats (*25*). Green tea (0.5 g powder/100 mL for 12 weeks) was shown to decrease fasting plasma levels of glucose, insulin, triglyceride, and free fatty acids and increase the insulin-stimulated glucose uptake and glucose transporter 4 (GLUT4) protein in rats fed a fructose-rich diet for 12 weeks (*26*). However, that study did not address more potential targets in the GLUT family and the insulin signal transduction pathway (*27*).

The aims of this study were to explore the effects of green tea extract on the expression of multiple genes coding for GLUT family (*28*) and components in the insulin signal transduction pathway (*27*, *29*). Quantitative real-time polymerase chain reaction (PCR) was used to investigate the profiles and the effects of green tea polyphenol extract on the expression levels of Glut family and insulin signal transduction pathway family genes. Our results suggest that green tea consumption regulates gene expression in glucose uptake and insulin signaling pathways in rats fed a fructose-rich diet. These results suggest that drinking adequate amounts of green tea may play a role in the prevention of diabetes and obesity.

MATERIALS AND METHODS

Green Tea Extract and Fructose-Rich Diet. The green tea polyphenolic extract was prepared by boiling leaves in hot water. The water-soluble extract was dried before adding it to the diet. The polyphenol extract contained 12.75% (w/w) EGCG, 9.21% epigallocatechin, 3.73% epicatechin gallate, 2.4% epicatechin, 5.94% caffeine, and 0.195% L-theanine, according to the manufacturer's analysis (Unilever France). The fructose-rich diet contained 60% (w/w) fructose, 20.7% casein, 5% corn oil, 8% alphacel, 1% mineral mix, 1% vitamin mix, and 0.3% casein (SAFE, 89290, Augis, France).

Animal Study. Male Wistar rats (6 weeks old, 150 ± 10 g, Charles River Laboratories, Les Oncins, France) were kept as previously described (*22*, *30*). All rats were fed a standard Purina chow for 1 week before being randomly divided into three groups with 10 rats per group. The first group of rats was given the high-fructose diet (control diet). The second group of rats was given the high-fructose diet plus 1 g of green tea solid extract/kg diet (1 g tea solid extract), and the third group of rats was given the high-fructose diet plus 2 g of green tea solid extract/kg diet (2 g tea solid extract). Previous studies have shown that a high fructose diet causes insulin resistance and increased oxidative stress (*25*). Therefore, a high fructose diet was selected as the base for the comparison of tea effects. Animals were sacrificed after 6 weeks on the diet. Rat tissues were frozen in liquid nitrogen and stored at −80 °C. The mean food intake was 20.7, 20.5, and 20.3 g/day for rats fed the control diet, 1 g of tea solid extract, and 2 g of tea solid extract, respectively. The mean body weight was 360, 350, and 353 g for rats fed the control diet, 1 g of tea solid extract, and 2 g of tea solid extract for 6 weeks, respectively. The mean body gain by week was about 35, 34, and 34.5 g for rats fed the control diet, 1 g of tea solid extract, and 2 g of tea solid extract, respectively. The insulin levels were 412 ± 100, 113 ± 28, and 197 ± 43 pmol/L for the control diet, 1 g of tea solid extract, and 2 g of tea solid extract treatments for 6 weeks, respectively (*30*). All procedures were in accord with guidelines of the National Institutes of Health and were approved by the French Army Ethical Committee.

RNA Isolation and cDNA Synthesis. The procedures for RNA isolation and cDNA synthesis have been described (*22*). Briefly, rat liver and muscle were homogenized in TRI_{ZOL} reagent (Invitrogen, Carlsbad, CA). RNA was isolated according to the manufacturer's instructions. RNA integrity and concentrations were determined using RNA 6000 Nano Assay Kit and the Bioanalyzer 2100 according to the manufacturer's instructions (Agilent Technologies, Palo Alto, CA) with RNA 6000 Ladder as the standards (Ambion, Inc., Austin, TX). Total cDNA synthesis was performed using the ImProm-II Reverse Transcription System (Promega, Madison, WI). The reaction mixture contained 5 μg of total RNA, 1 μg of oligo$(dT)_{15}$ primer (Invitrogen), 0.25 μg of random primers (Invitrogen), 500 μM dNTPs, 5 mM $MgCl_2$, 2.5 μL of RNasin ribonuclease inhibitor, and 5 μL of ImProm-II reverse transcriptase in 1X ImProm-II reaction buffer in a total volume of 20 μL.

PCR Primers and TaqMan Probes. The primers and probes were designed using Primer Express software (Applied Biosystems, Foster

City, CA) and synthesized by BioSource International, Invitrogen Corp. (Camarillo, CA) or Biosearch Technologies, Inc. (Navato, CA). The mRNA names, GenBank accession numbers, amplicon sizes, and the sequences (5′ to 3′) of the forward primers, TaqMan probes (TET-BHQ1), and reverse primers, respectively, are described in **Table 1**. The nomenclature of genes, mRNAs, and proteins is as described (*22*). For example, *Glut4*, Glut4, and GLUT4 represent Glut4 gene, mRNA, and protein, respectively.

Quantitative Real-Time PCR Assays. Real-time PCR assays were performed using an ABI Prism 7700 real time PCR instrument (Applied Biosystems) as described (*22*). Briefly, the TaqMan reaction mixture (25 μL) contained 25−50 ng of total RNA-derived cDNAs, 200−1000 nM each of the forward primer and reverse primer, 200−280 nM TaqMan probe, and 12.5 μL of 2× Absolute QPCR Mix (ABgene House, Epson, Surrey, United Kingdom). The thermal cycle conditions were as follows: 2 min at 50 °C and 10 min at 95 °C, followed by 40−60 cycles at 95 °C for 15 s and 60 °C for 60 s. Fluorescence signals measured during amplification were considered positive if the fluorescence intensity was more than 20-fold greater than the standard deviation of the baseline fluorescence (*31*). The $\Delta\Delta C_T$ method of relative quantification was used to determine the fold change in expression (*32*). This was done by first normalizing the resulting threshold cycle (C_T) values of the target mRNAs to the C_T values of the internal control ribosomal protein L32 (Rpl32) in the same samples ($\Delta C_T = C_{Ttarget} - C_{TRpl32}$). It was further normalized with the samples with only the high-fructose diet but without tea supplement ($\Delta\Delta C_T = \Delta C_{Ttea} - \Delta C_{Tdiet}$). The fold change in expression was then obtained ($2^{-\Delta\Delta C_T}$) as described (*22*).

Statistical Analyses. Real-time PCR data were analyzed by SigmaStat 3.1 software (Systat Software, Inc., Point Richmond, CA) using one-way analysis of variance (ANOVA). Multiple comparisons were performed with Duncan's multiple range test as described (*22*). Values with different lower case and upper case letters displayed above the columns of the figures are significantly different at $p < 0.05$ and $p < 0.01$, respectively.

RESULTS

Glut Family mRNAs in Rat Liver and Muscle. Multiple members of the Glut family exist in mammals (GLUT1/SLC2A1, GLUT2/SLC2A2, GLUT3/SLC2A3, GLUT4/SLC2A4, and GLUT5/SLC2A5) (*28*). We examined the expression levels of the first four forms of genes in the liver and skeletal muscle of rats fed a high-fructose diet known to induce insulin resistance and oxidative stress (*25*). The C_T values of the Glut family and the internal control Rpl32 mRNAs and the relative ratios of Glut family mRNAs in the liver and muscle are shown in **Table 2**. Glut2 was the major Glut mRNA in the liver, and Glut4 was the major one in the muscle. In liver, the relative mRNA levels of Glut1, Glut2, Glut3, and Glut4 were 1-, 32-, 0.02-, and 0.01-fold, respectively. In muscle, the relative mRNA levels of Glut1, Glut2, Glut3, and Glut4 were 1-, 0.003-, 1.6-, and 294.1-fold, respectively (**Table 2**, middle column). Glut1, Glut2, Glut3, and Glut4 mRNAs in the muscle were 0.1-, 0.00001-, 9.2-, and 4705.1-fold of those in the liver, respectively (**Table 2**, right column).

Insulin Signaling Pathway mRNA Levels in Rat Liver and Muscle. Numerous components exist in the insulin signal transduction pathway (*29*). We analyzed the mRNA levels of the following genes coding for insulin signaling pathway components [insulin I (*Ins1*), insulin II (*Ins2*), insulin receptor (*Insr*), insulin receptor substrate 1 (*Irs1*), insulin receptor substrate 2 (*Irs2*), thymoma viral proto-oncogene 1 (*Akt1*), growth factor receptor bound protein 2 (*Grb2*), insulin-like growth factor 1 (*Igf1*), insulin-like growth factor 2 (*Igf2*), insulin-like growth factor I receptor (*Igf1r*), insulin-like growth factor 2 receptor (*Igf2r*), glycogen synthase kinase 3 β (*Gsk3b*), glycogen synthase 1 (*Gys1*), phosphatidylinositol 3-kinase, catalytic, β (*Pik3cb*), phosphatidylinositol 3-kinase, regulatory subunit 1 (*Pik3r1*), Src homology 2 domain-containing transforming protein 1 (*Shc1*), and son of sevenless 1 (*Sos1*)] in liver and muscle of rats fed a high-fructose diet known to induce insulin resistance and oxidative stress (*25*). The C_T values of the insulin signaling pathway family mRNAs and the internal control Rpl32 mRNAs (**Table 2**, left column) and the relative ratios of the insulin signaling pathway family mRNAs in the liver and muscle are shown in **Table 2**. Akt1, Grb2, Gsk3b, Insr, Igf2r, Irs1, Irs2, Pik3cb, Pik3r1, and Shc1 mRNAs were more abundant in the liver than those in the muscle, whereas Gys1, Ins2, Igf2, and Igf1r mRNAs were more abundant in the muscle than those in the liver (**Table 2**, right column). Ins1, Ins2, Igf1, and Igf2 mRNAs were either undetectable or expressed at very low levels (**Table 2**, right column).

Table 2. mRNA Levels of Glut/Slc2a Family and Insulin Signaling Pathway Components in Rat Liver and Muscle[a]

			expression ratio	
tissue	mRNA	diet control cycle of threshold (CT ± SD)	relative to Glut1 (fold)	relative to liver (fold)
liver	Rpl32	18.8 ± 0.6	internal control	internal control
	Glut1/Slc2a1	25.8 ± 0.6	1.0	1.0
	Glut2/Slc2a2	20.8 ± 0.3	32.0	1.0
	Glut3/Slc2a3	31.7 ± 0.9	0.02	1.0
	Glut4/Slc2a4	33.2 ± 0.7	0.01	1.0
	Akt1	22.7 ± 0.9		1.0
	Grb2	22.0 ± 0.7		1.0
	Gsk3b	23.7 ± 0.7		1.0
	Gys1	28.9 ± 0.9		1.0
	Ins1	37.2 ± 1.0		1.0
	Ins2	38.4 ± 1.2		1.0
	Insr	23.3 ± 0.6		1.0
	Igf1	0		
	Igf2	38.6 ± 2.6		1.0
	Igf1r	26.6 ± 0.4		1.0
	Igf2r	24.0 ± 0.6		1.0
	Irs1	22.3 ± 0.6		1.0
	Irs2	23.6 ± 0.5		1.0
	Pik3cb	24.6 ± 0.5		1.0
	Pik3r1	23.6 ± 0.6		1.0
	Shc1	29.6 ± 0.5		1.0
	Sos1	25.7 ± 0.6		1.0
muscle	Rpl32	18.4 ± 0.4	internal control	internal control
	Glut1/Slc2a1	28.7 ± 0.6	1.0	0.1
	Glut2/Slc2a2	37.2 ± 1.4	0.003	0.00001
	Glut3/Slc2a3	28.0 ± 0.3	1.6	9.2
	Glut4/Slc2a4	20.5 ± 0.2	294.1	4705.1
	Akt1	23.6 ± 0.3		0.4
	Grb2	24.0 ± 0.5		0.2
	Gsk3b	24.2 ± 0.9		0.5
	Gys1	23.2 ± 0.3		36.8
	Ins1	36.6 ± 1.2		1.1
	Ins2	36.0 ± 0.4		3.7
	Insr	25.1 ± 0.7		0.2
	Igf1	0		
	Igf2	36.8 ± 1.2		2.5
	Igf1r	25.0 ± 0.6		2.1
	Igf2r	24.9 ± 0.5		0.4
	Irs1	23.4 ± 0.6		0.3
	Irs2	24.9 ± 0.5		0.3
	Pik3cb	29.3 ± 0.6		0.03
	Pik3r1	26.3 ± 0.6		0.1
	Shc1	32.8 ± 0.6		0.1
	Sos1	25.0 ± 0.6		1.1

[a] The relative ratios of mRNA levels were calculated using the $\Delta\Delta C_T$ method normalized with the Rpl32 C_T value as the internal control and the TTP C_T value or liver C_T value as the calibrator.

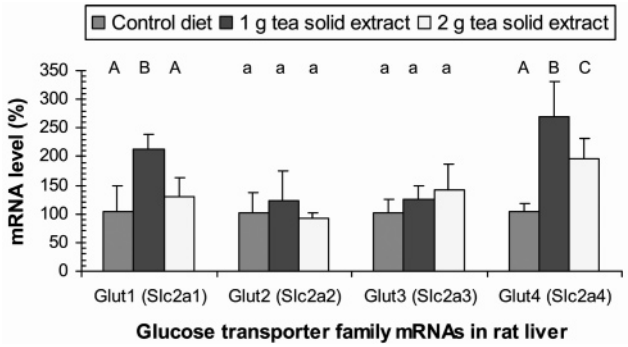

Figure 1. Green tea extract effects on Glut family mRNA levels in rat liver. Total RNAs were isolated from livers of rats with metabolic syndrome induced by a high-fructose diet and reverse transcribed into cDNAs. Twenty-five to 50 ng of RNA-derived cDNAs was used for quantitative real-time PCR assays. The $\Delta\Delta C_T$ method of relative quantification was used to determine the fold change in expression. The results represent the percentage means and the standard deviations from five to eight samples with 2–4 repetitions of each sample. Values with different upper case and lower case letters displayed above the columns of the figure are significantly different at $p < 0.01$ and $p < 0.05$, respectively.

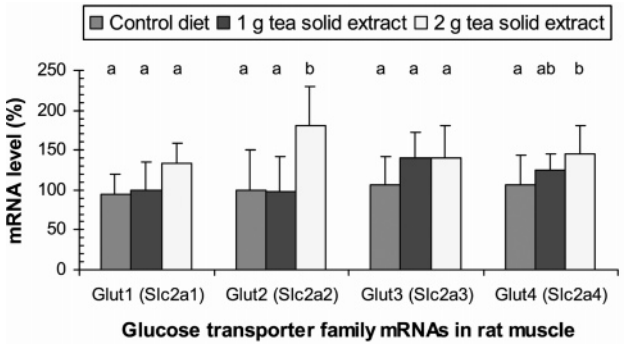

Figure 2. Green tea extract effects on Glut family mRNA levels in rat muscle. RNA isolation, cDNA synthesis, real-time PCR assays, and statistical analyses are described in the **Figure 1** legend.

Green Tea Effects on Glut Family mRNA Levels in Rat Liver. Green tea (1 g solid extract/kg diet) increased Glut1 and Glut4 mRNA levels by 111 and 165%, respectively but did not have statistically significant effects on Glut2 or Glut3 mRNA levels in the liver (**Figure 1**). Green tea (2 g solid extract/kg diet) significantly increased Glut4 mRNA levels by 92% but did not have statistically significant effects on Glut1, Glut2, or Glut3 mRNA levels in the liver (**Figure 1**).

Green Tea Effects on Glut Family mRNA Levels in Rat Muscle. Green tea (1 g solid extract/kg diet) did not significantly alter the mRNA levels of Glut1, Glut2, Glut3, or Glut4 (**Figure 2**). Green tea (2 g solid extract/kg diet) significantly increased Glut2 and Glut4 mRNA levels by 81 and 38% over the diet control, respectively, but did not have statistically significant effect on Glut1 or Glut3 mRNA levels in the muscle (**Figure 2**).

Green Tea Effects on Insulin Signaling Pathway Family mRNA Levels in Rat Liver. Green tea (1 g solid extract/kg diet) increased Gsk3b and Irs2 mRNA levels by 34 and 55%, respectively (**Figure 3**) but did not have statistically significant effects on Akt1, Grb2, Gys1, Ins1, Ins2, Insr, Igf1r, Igf2r, Irs1, Pik3r1, Shc1, or Sos1 mRNA levels in the liver (**Figure 3** and data not shown). Green tea (2 g solid extract/kg diet) significantly increased Gsk3b and Pik3cb mRNA levels by 30% (**Figure 3**) and decreased Shc1 mRNA levels by 63% but did not have statistically significant effects on Akt1, Grb2, Gys1, Ins1, Ins2, Insr, Igf1r, Igf2r, Irs1, Irs2, Pik3r1, or Sos1 mRNA levels in the liver (**Figure 3** and data not shown).

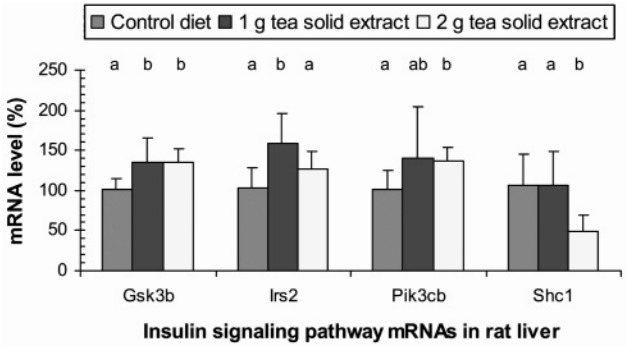

Figure 3. Green tea extract effects on insulin signaling pathway mRNA levels in rat liver. RNA isolation, cDNA synthesis, real-time PCR assays, and statistical analyses are described in the **Figure 1** legend.

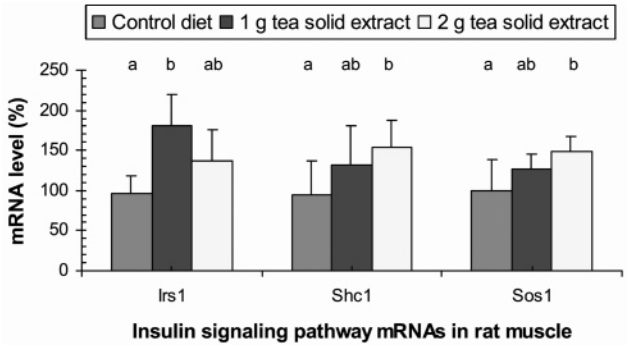

Figure 4. Green tea extract effects on insulin signaling pathway mRNA levels in rat muscle. RNA isolation, cDNA synthesis, real-time PCR assays, and statistical analyses are described in the **Figure 1** legend.

Green Tea Effects on mRNA Levels of Insulin Signal Pathway in Rat Muscle. Green tea (1 g solid extract/kg diet) increased Irs1 mRNA levels by 84% (**Figure 4**) but did not have statistically significant effects on Akt1, Grb2, Gsk3b, Gys1, Ins1, Ins2, Insr, Igf1r, Igf2r, Irs2, Pik3cb, Pik3r1, Shc1, or Sos1 mRNA levels in the liver (**Figure 4** and data not shown). Green tea (2 g solid extract/kg diet) significantly increased Shc1 and Sos1 mRNA levels by 60 and 49%, respectively (**Figure 4**), but did not have statistically significant effects on Akt1, Grb2, Gsk3b, Gys1, Ins1, Ins2, Insr, Igf1r, Igf2r, Irs1, Irs2, Pik3cb, or Pik3r1 mRNA levels in the liver (**Figure 4** and data not shown).

DISCUSSION

Plants have been used for the treatment of diabetes for thousands of years (*8*). In search for plant products for diabetic prevention, a number of studies have shown that tea and common spices (cinnamon, cloves, tumeric, and bay leaves) display insulin-like activity in vitro. We have demonstrated in one study that cinnamon improved glucose and lipid profiles of people with type 2 diabetes (*33*) and that cinnamon exhibited insulin-like activity in cells, animals, and in people with type 2 diabetes (*33*−*37*). We further showed that cinnamon extract and polyphenols with doubly linked procyanidin type-A polymers affect the expression of TTP/Zfp36/Tis11), Insr, and Glut4 in mouse 3T3-L1 adipocytes (*27*) (**Figure 5**). Several studies also indicate that tea extract and its major polyphenol EGCG have insulin-potentiating activity in in vitro and animal models (*16*, *17*, *26*, *30*, *38*, *39*); however, no investigation at the molecular level was substantially performed to support these observations.

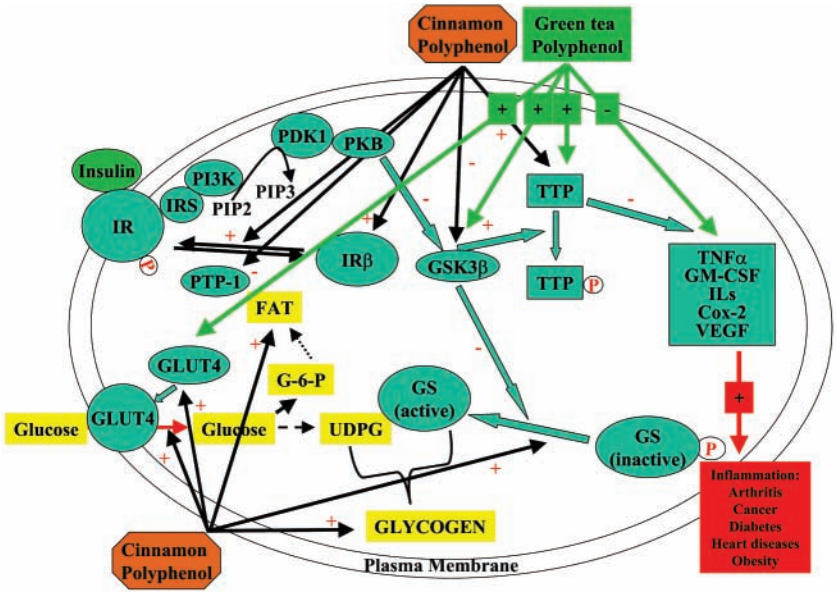

Figure 5. Model of actions by green tea and cinnamon polyphenols in the insulin signal transduction pathway leading to potential beneficial effects. This model was modified from a previous model for cinnamon polyphenol effects (*27*): The effects of green tea polyphenol extract on TTP and Tnf gene expression are described in a previous paper (*22*). The effects of green tea polyphenol extract on Glut4 and Gsk3b gene expression are described in this manuscript ("+" represents a positive effect, and "–" represents a negative effect).

In this study, we analyzed the mRNA profiles and the effects of a green tea polyphenolic extract on the mRNA levels of Glut family and insulin signal transduction pathway family in liver and muscle of rats fed a high-fructose diet known to induce insulin resistance and oxidative stress in rats (*25*).

The major finding reported in this study is that the solid green tea polyphenolic extract fed increased Glut4 mRNA levels by approximately 169 and 20% (1 g of tea solid extract) and 90 and 40% (2 g of tea solid extract) in rat liver and muscle, respectively (**Figure 5**). The increases of Glut4 mRNA level in the muscle may be physiologically significant for the following reasons: (i) Glut4 is the major Glut family mRNAs in the muscle (**Table 2**); (ii) GLUT4 protein is the insulin-responsive Glut in the muscle (*28*); (iii) the amount of GLUT4 protein is decreased in obese subjects (*40*); (iv) insulin increases Glut4 mRNA and protein levels in fetal rat brown adipocytes (*41*, *42*) and increases GLUT4 protein level in 3T3-F442A adipocytes (*43*); and (v) the amount of GLUT4 protein is increased in fructose-fed rats with green tea powder supplementation resulting in amelioration of fructose-induced insulin resistance (*26*). The increased Glut4 mRNA levels with tea extract reported here suggest a positive effect of these polyphenolic compounds on the long-term regulation of glucose transport, although GLUT4 protein levels need to be confirmed in future studies. The significance of the increased levels of Glut1 and Glut4 mRNAs in the liver and Glut2 mRNA in the muscle is not clear because these Glut mRNAs are the minor forms in their respective rat tissues (**Table 2**).

The other finding is that feeding solid green tea polyphenolic extract increased Gsk3b mRNA levels in the liver (**Figure 5**). GSK3B protein is a multifunctional protein kinase, and its activity is inhibited by AKT1/PKB-medicated phosphorylation at its serine residue, which is induced by insulin (*44*). Less GSK3 activity results in increases in activities of glycogen synthase and initiation factor 2B and results in stimulating glycogen and protein synthesis (*44*). It was also reported that GSK3B phosphorylated TTP in vitro (*21*), and phosphorylated TTP has less mRNA ARE-binding activity than the unphosphorylated form (*45*, *46*). TTP is a hyperphosphorylated protein (*47*) with anti-inflammatory function through the down-regulation of proinflammatory cytokines (*48*, *49*). TTP binds to and subsequently promotes the degradation of those mRNAs encoding proinflammatory cytokines such as TNF-α and GM-CSF (*45*, *50*, *51*). The mRNA and protein levels of TTP are dramatically induced by nanomolar concentrations of insulin in minutes (*23*). Therefore, the elevated Gsk3b mRNA levels appear to be in conflict with green tea's effect in rats of increasing insulin sensitivity (*30*) and anti-inflammatory activity (*22*). We did not pursue this further in the current study.

Green tea extract consumption also affected the expression of some other genes involved in the insulin signaling pathway. Specifically, green tea increased Irs2 and Pik3cb but decreased Shc1 mRNA levels in the liver, and green tea increased Irs1, Shc1, and Sos1 mRNA levels in the muscle. However, green tea consumption exhibited no or minimal effects on the other mRNA levels in the liver or muscle, including Ins1, Ins2, Insr, Akt1, Grb2, Igf1, Igf2, Igf1r, Igf2r, Gys1, and Pik3r1 mRNAs. The small effects of green tea consumption on mRNA levels in the insulin signaling pathway reported here are in agreement with previous observations that the effects of insulin on gene expression in the insulin signaling pathway are minimal in mouse 3T3-L1 adipocytes (*52*). The plausible reason for the small effects of green tea and insulin in mRNA levels in the insulin signal transduction pathway in rat liver and muscle, as well as mouse 3T3-L1 adipocytes, is probably due to the functional effects of insulin signal transduction mainly through protein phosphorylation. It is also possible that the effectiveness of green tea is dependent on factors such as the timing and dosages and the physiological status of the target cells, since green tea consumption did not have significant effects on inflammation and insulin resistance in type 2 diabetes patients (*24*). Additional studies are needed to determine the role of green tea or its components in the prevention and alleviation of glucose intolerance, insulin resistance, diabetes, and obesity.

The reason(s) why green tea extract at 2 g/kg high fructose diet did not produce more mRNA levels than 1 g of tea extract/kg diet in the liver is not obvious. Our earlier study also documented that green tea extract at 1 g/kg high fructose diet

is better than 2 g/kg diet in increasing TTP and decreasing Tnf mRNA levels in rat liver (*22*) (**Figure 5**). Furthermore, the effects of green tea extract on insulin levels in the plasma showed a similar pattern (*30*). It is possible that 2 g of tea extract contained more of the other compositions, which might reduce gene expression in the liver. Therefore, it is important to conduct more extensive analyses of the dosage effect on gene expression. Food intake for rats fed 1 g green tea extract/kg diet was 20.5 g/day. On a per kg basis, the rats consuming 1 g of tea solids per day consume approximately 60 mg/kg body weight per day. Humans consuming 1 cup of tea made from a tea bag containing 2 g of solids consume roughly 900 mg of tea solids or approximately 12.8 mg/kg. On a per kilogram basis, the lowest level used in this study is equivalent to humans drinking approximately 5 cups of tea per day. Clearly, the effects of varying levels of tea on parameters related to this study on humans are needed.

In conclusion, this study describes the mRNA profiles of the Glut family and the insulin signaling pathway family in the liver and muscle of rats with metabolic syndrome induced by a high-fructose diet. A green tea polyphenolic extract significantly increased the mRNA levels of Glut4 in the muscle and of Gsk3b in the liver (**Figure 5**). Green tea also increased Irs2 and Pik3cb but decreased Shc1 mRNA levels in the liver and increased Irs1, Shc1, and Sos1 mRNA levels in the muscle. However, green tea consumption exhibited no or minimal effect on Ins1, Ins2, Insr, Akt1, Grb2, Igf1, Igf2, Igf1r, Igf2r, Gys1, and Pik3r1 mRNAs in the liver or muscle. These results suggest that consumption of green tea or its extract could regulate gene expression in the glucose uptake and the insulin signal transduction pathways in rats fed a fructose-rich diet. These findings support a plausible mechanism for drinking adequate amounts of green tea to affect glucose intolerance, insulin resistance, diabetes, and obesity.

ABBREVIATIONS USED

ANOVA, one way analysis of variance; Akt1, thymoma viral proto-oncogene 1; EGCG, epigallocatechin gallate; Glut, glucose transporter; Grb2, growth factor receptor bound protein 2; Gsk3b, glycogen synthase kinase 3 β; Gys1, glycogen synthase 1; Igf1, insulin-like growth factor 1; Igf1r, insulin-like growth factor I receptor; Igf2, insulin-like growth factor 2; Igf2r, insulin-like growth factor 2 receptor; Ins1, insulin I; Ins2, insulin II; Insr, insulin receptor; Irs1, insulin receptor substrate 1; Irs2, insulin receptor substrate 2; Pik3cb, phosphatidylinositol 3-kinase, catalytic, β; Pik3r1, phosphatidylinositol 3-kinase, regulatory subunit 1; Shc1, Src homology 2 domain-containing transforming protein 1; Sos1, son of sevenless 1; Rpl32, ribosomal protein L32; TTP, tristetraprolin.

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