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Cinnamon increases liver glycogen in an animal model of insulin resistance

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ABSTRACT

The objective of this study was to determine the effects of cinnamon on glycogen synthesis, related gene expression, and protein levels in the muscle and liver using an animal model of insulin resistance, the high-fat/high-fructose (HF/HFr) diet–fed rat. Four groups of 22 male Wistar rats were fed for 12 weeks with (1) HF/HFr diet to induce insulin resistance, (2) HF/HFr diet containing 20 g cinnamon per kilogram of diet, (3) control diet, and (4) control diet containing 20 g cinnamon per kilogram of diet. In the liver, cinnamon added to the HF/HFr diet led to highly significant increases of liver glycogen. There were no significant changes in animals consuming the control diet plus cinnamon. In the liver, cinnamon also counteracted the decreases of the gene expressions due to the consumption of the HF/HFr diet for the insulin receptor, insulin receptor substrates 1 and 2, glucose transporters 1 and 2, and glycogen synthase 1. In muscle, the decreased expressions of these genes by the HF/HFr diet and glucose transporter 4 were also reversed by cinnamon. In addition, the overexpression of glycogen synthase 3β messenger RNA levels and protein observed in the muscle of HF/HFr fed rats was decreased in animals consuming cinnamon. These data demonstrate that, in insulin-resistant rats, cinnamon improves insulin sensitivity and enhances liver glycogen via regulating insulin signaling and glycogen synthesis. Changes due to cinnamon in control animals with normal insulin sensitivity were not significant.

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1. Introduction

In Western countries, nutritional imbalances due to an overload in fat and/or refined carbohydrates are the leading causes of insulin resistance and associated increases in obesity, metabolic syndrome, diabetes, and cardiovascular diseases [1]. Although the pathogenesis of diabetes and its long-term consequences are well known, optimal treatment remains elusive; and despite conventional treatment, only half of the patients are achieving the recommended hemoglobin A1c target [2]. Clearly, additional treatments are needed. Some nutritional factors, such as polyphenols,

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counteract insulin resistance and therefore may be beneficial for patients with type 2 diabetes mellitus through their insulin-potentiating, antioxidant, anti-inflammatory, and related properties. Not only do common cinnamon (CN) (*Cinnamomum verum* and *C zeylanicum*) and cassia (*C aromaticum* and *C burmannii*) have a long history of uses as spices, flavoring agents, preservatives, and pharmacological agents; but CN is also a source of polyphenols that have been shown to have beneficial effects on insulin function. Several studies [3-6] demonstrate that, in animals and in humans, CN and aqueous extracts of cinnamon (CEs) improve blood glucose, lipids, insulin, and related variables and may be beneficial to counteract the features of insulin resistance, the metabolic syndrome, and ultimately the onset of type 2 diabetes mellitus and related diseases.

Glycogen synthesis represents a major pathway of glucose disposal after insulin stimulation. It is well known that glycogen is made primarily by the liver and the muscle as the secondary long-term form of energy storage. Previous studies have suggested that glycogen in the liver and skeletal muscle is important to maintain physical performance during prolonged exercise [7,8]; and the activity of glycogen synthase, the rate-limiting enzyme for glycogen synthesis, is reduced in type 2 diabetes mellitus [9]. Although CN has been shown to have beneficial effects on insulin function, the in vivo effects of CN consumption on liver and muscle glycogen metabolism have not been investigated.

The objective of this study was to determine the effects of CN consumption on glycogen synthesis, related gene expressions, and protein concentrations in muscle and liver using an animal model of dietary-induced insulin resistance and metabolic syndrome, the high-fat/high-fructose diet (HF/HFr)–fed rat.

2. Materials and methods

2.1. CN powder

The CN powder (*C burmannii*) was obtained from McCormick Spice, Baltimore, MD. A water extract of the CN contained more than 5% type A polyphenols with a tetramer with a molecular weight of 1152 and 2 trimers with a molecular weight of 864 [10,11]. The insulin-related bioactivity of the type A polyphenols has been documented [10-14].

2.2. Animals and diets

Eighty-eight male Wistar rats, 5 weeks old (Charles River, L'Arbresle, France), were individually housed in a temperature-controlled room (22°C) with a 12-hour light/12-hour dark cycle. All experimental procedures were reviewed and approved by the Institutional Ethic Committee for Animal Care (Center Research Military Health Service, protocol no. 2008/02.1 accepted December 2008). The rats were maintained and handled in accord with the Guide for the Care and Use of Laboratory Rats (National Institutes of Health, 1985). The diets were purchased from SAFE (89290, Augis, France). The control diet contained 5% cellulose, 20% casein, 25% corn starch, 25% potato starch, 16% maltodextrin, 4% soybean oil, 3.5% AIN mineral mix, 1% AIN vitamin mix, 0.3% DL-methionine, and 0.2% choline bitartrate. The HF/HFr diet was similar except that the corn starch, potato starch, and maltodextrin were replaced by 46% fructose and 20% lard. The rats were adapted and fed Purina chow (SAFE) for 3 weeks. They were then randomly divided into 4 groups of 22 rats and fed ad libitum for 12 weeks one of the following 4 diets: Purina chow as the control diet (C), the HF/HFr diet (described above) to induce insulin resistance, or the respective diets containing 20 g CN per kilogram of diet (C + CN or HF/HFr + CN). The amount of CN used was based upon our previous study showing a definite effect of 20 g of CN per kilogram of diet on spontaneously hypertensive rats. Similar effects were observed at 1%, 2%, and 4% added CN [15]. One, 3, and 6 g of CN per day were all shown to have beneficial effects in people with type 2 diabetes mellitus [16]. In this study, the measurements were performed after 12 weeks of consumption of the diets. In 10 animals of each group, the effects of the diets on insulin resistance were determined using the hyperinsulinemic-euglycemic clamp technique [17]. In the other rats (12 by group), glycogen levels and related gene expressions and protein levels in muscle and liver were evaluated.

2.3. Tissue sampling

After overnight fasting, 48 rats (12/group) were killed with fluoroethane anesthesia. The experiments were conducted between 8:00 AM and 10:30 AM. The median lobe of the liver and plantaris muscle were used for glycogen, gene expressions, and protein determinations. The organs were weighed and immediately frozen in liquid nitrogen and stored at –80°C until analyses.

2.4. Glycogen determinations

Thirty to 50 mg of liver or muscle was hydrolyzed in KOH for measurements of glycogen by a method derived from Keppler and Decker [18]. Glucose obtained from hydrolysis was determined routinely with the Glucose Assay Kit (Sigma Diagnostics, Isle d'Abeau, France).

2.5. Messenger RNA and protein determinations

Total RNA was isolated from liver and muscle tissues using Trizol reagent (Invitrogen, Carlsbad, CA). RNA concentrations and integrity were determined using RNA 6000 Nano Assay Kit and the Bioanalyzer 2100 according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA). The primers used for polymerase chain reaction (PCR) were as follows: Ir primers, 5'-CAAAAGCACAATCAGAGTGAGTATGAC-3' and 5'-ACCACGTTGTGCAGGTAATCC-3'; Irs1 primers, 5'GCCTGGAGTATTATGAGA ACGAGAA-3' and 5'-GGGGATCGAGCGTTTGG-3'; Irs2 primers, 5'-AAGATAGCGGGTACATGCGAAT -3' and 5'-GCAGCTTAGGGTCTGGGTTCT -3'; *Glut1* primers, 5'-GTGCTTATGGGTTTCTCCAAA-3' and 5'-GACACCTCCCCCACATACATG -3'; Glut2 primers, 5'-TTTGCAGTAGGCGGAATGG-3' and 5'-GCCAACATGGCTTTGATCCTT-3'; Glut4 primers, 5'-CAACTGGACCTGTAACTTCATCGT-3' and 5'-ACGGCAAATAGAAGGAAGACGTA-3'; *Gys1* primers, 5'-TCCACTGTGCCTGTGTCTTCA-3' and 5'-

AGAGAACTTCTTCACATTCAGTCCATT-3'; Gsk3b primers, 5'-TTAAGGAAGGAAAAGGTGAATCGA-3' and 5'-CCAAAAGCT-GAAGGCTGCTG-3'; and 18S primers, 5'-TAAGTCCCTGCCCTTTG-TACACA-3' and 5'-ATCCGAGGGCCTCACTAAAC-3'. Messenger RNA (mRNA) levels were assessed by real-time quantitative reverse transcriptase (RT)–PCR. All PCRs were performed in a total volume of 25 μL and included the following components: complementary DNA (cDNA) derived from 25 ng of total RNA, 400 nmol/L of each primer, RNase-free water, and 12.5 μL of SYBR Green PCR Master Mix (ABI), an optimized buffer system containing AmpliTaq Gold DNA polymerase (Applied Biosystems Inc., Carlsbad, CA, USA) and dNTPs. All PCRs were performed in duplicate, and cycling parameters were as follows: after an initial denaturation step for 10 minutes at 95°C, 40 subsequent cycles were performed in which samples were denatured for 15 seconds at 95°C followed by primer annealing and elongation at 60°C for 1 minute. Relative quantities of mRNA were normalized by 18S ribosomal RNA content.

Protein concentrations were determined by a commercial assay (Bio-Rad DC protein assay; BioRad, Hercules, CA, USA) using bovine serum albumin as a standard. The detection and quantification of the amounts of glycogen synthase and glycogen synthase kinase 3β (GSK3β) were determined with Western blotting.

2.6. *Statistical analysis*

Data are expressed as means ± SE. Two-way analysis of variance (ANOVA) was used to determine the significance of the effects of diet and CN treatment on the measured parameters. When significant intergroup differences were found ($P < .05$), the Tukey test was carried out. Different superscripts indicate significant differences among groups ($P < .05$).

3. Results

The initial body weight of the control group was 188 ± 3.8 and 410 ± 11.4 g after 12 weeks of the study. Initial body weight of the HF/HFr group was 188 ± 2.8 and 401 ± 6.6 after 12 weeks of consuming the diet. Addition of CN to the diets did not change the body weights of the animals [17]. Caloric intake of the animals consuming the HF/HFr diet was significantly higher than that of the animals on the control diet (Fig. 1). Caloric intake of the animals consuming HF/HFr + CN was significantly lower than that of the HF/HFr group and similar to that of the C and C + CN groups (Fig. 1).

In muscle, increases in glycogen due to CN were not significant either in the control animals or in the animals consuming the HF/HFr diet (Fig. 2A). However, muscle glycogen values of the animals consuming the HF/HFr diet plus CN were higher than those of the control animals (2-way ANOVA, $F_{diet} = 7.9$, $P < .01$; $F_{CN} = 3.4$, $P = .07$); there was not a significant interaction ($F = 0.03$, $P = .85$) . In the liver (Fig. 2B), increases in liver glycogen in animals consuming the control diet were also not significant due to CN. However, HF/HFr + CN rats exhibited a 2.5-fold increase in liver glycogen levels compared with the HF/HFr rats as well as those consuming the control diets (2-way ANOVA, $F_{diet} = 22$, $P < .001$; $F_{CN} = 14$, $P < .001$); there was also a significant interaction ($F = 7.1$, $P < .05$).

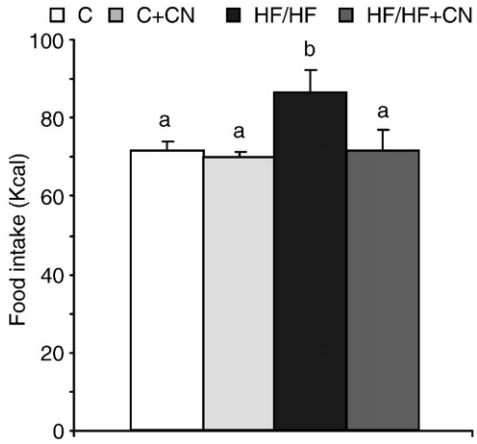

Fig. 1 – Effects of diet and CN on caloric intake. Values are means ± SE of n = 8 to 10 rats per group. Different letters indicate significant differences among groups ($P < .05$).

As shown in Fig. 3, HF/HFr diet decreased muscle Ir, Irs1, Irs2, Glut1, and Glut4 gene expression by 20%, 30%, 32%, 45%, and 25% of the control, respectively. These impaired gene expressions were ameliorated by CN, and values of the HF/HFr + CN animals were not significantly different from those of the control animals (Ir: $F_{diet} = 11$, $P < .01$; $F_{CN} = 15$, $P < .001$; Irs1: $F_{diet} = 25$, $P < .001$; $F_{CN} = 21$, $P < .001$; Irs2: $F_{diet} = 17$, $P < .001$; $F_{CN} = 14.5$, $P <$

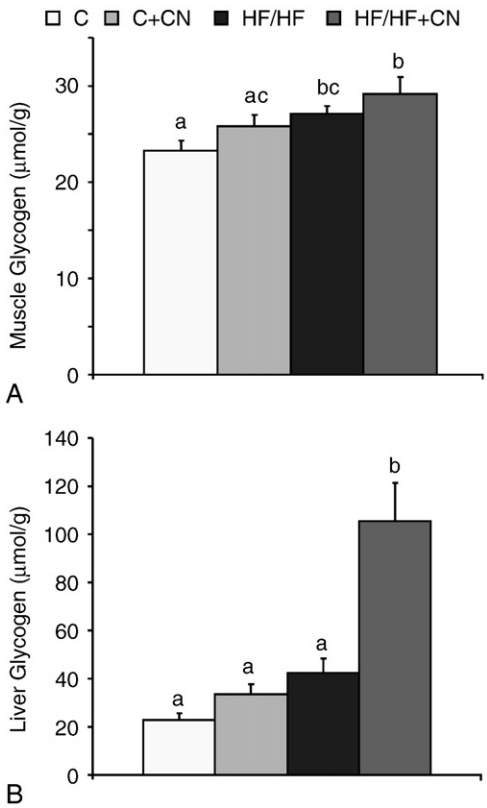

Fig. 2 – Effects of diet and CN on muscle (A) and liver (B) glycogen concentrations. Values are means ± SE of n = 9 to 12 rats per group. Different letters indicate significant differences among groups ($P < .05$).

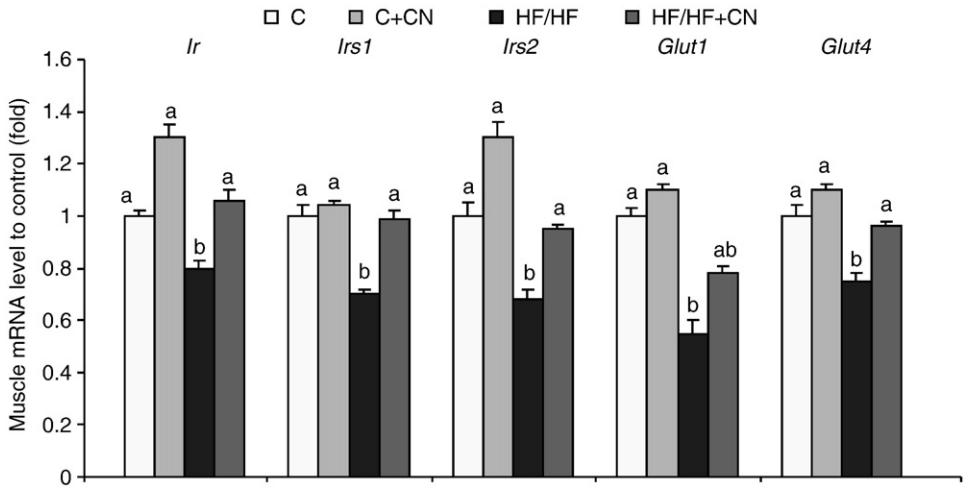

Fig. 3 – Effects of diet and CN on the muscle mRNA expression of Ir, Irs1, Irs2, Glut1, and Glut4 mRNA were determined with RT-PCR using cDNA made from 25 ng total RNA as template. Pairwise differences were analyzed by Tukey test after ANOVA. Values are mean ± SE (n =8 for each). Different superscripts indicate significant differences among groups ($P < .05$).

.001; Glut1: $F_{diet} = 24$, $P < .001$, $F_{CN} = 3.8$, $P = .06$; Glut4: $F_{diet} = 29$, $P < .001$; $F_{CN} = 15$, $P < .001$); there was a significant interaction between the HF/HFr diet and CN supplement in Irs1 mRNA ($F = 13.7$, $P < .001$), but not for Ir, Irs2, Glut1, and Glut4.

As shown in Fig. 4A, the mRNA level of muscle GS1 was decreased in HF/HFr-fed rats; and HF/HFr feeding plus CN values were similar to those of the control ($F_{diet} = 36$, $P < .001$; $F_{CN} = 14$, $P < .001$); the interaction was also significant ($F = 19$, $P < .001$). Neither diet nor CN affected the GS1 protein levels (Fig. 4C). In contrast, HF/HFr feeding significantly enhanced muscle Gsk3b mRNA and protein levels, with increases of 180% (Fig 4B) and 190% (Fig. 4D) of controls. There were significant effects of diet ($F_{diet} = 60$, $P < .0001$) and CN ($F_{CN} = 8$, $P < .01$) on Gsk3b mRNA. There was no significant interaction. As shown in Fig. 4D, the elevated GSK3β protein levels were improved by CN addition to the level of the control animals. There were significant effects of diet ($F_{diet} = 30$, $P < .0001$) and CN ($F_{CN} = 5$, $P < .050$) on GSK3b protein; the interaction was also significant ($F = 9.6$, $P < .05$).

As shown in Fig. 5, HF/HFr diet decreased liver Ir, Irs1, Irs2, Glut1, and Glut2 gene expression by 44%, 10% (not significant), 48%, 60%, and 52% of the control, respectively. These impaired gene expressions were ameliorated by the CN supplement to 78%, 140%, 70%, 60%, and 73% of the control, respectively. Cinnamon addition did not significantly regulate these gene expressions in chow-fed rats. The diet and CN effects were as follows: Ir: $F_{diet} = 44$, $P < .001$; $F_{CN} = 3.6$, $P = .068$; Irs1: $F_{diet} = 15$, $P < .001$; $F_{CN} = 20$, $P < .001$; Irs2: $F_{diet} = 21$, $P < .001$; Glut1: $F_{CN} = 1.4$, $P > 0.2$; Glut1: $F_{diet} = 326$, $P < .0001$; $F_{CN} = 23$, $P < .001$; Glut2: $F_{diet} = 25$, $P < .001$; $F_{CN} = 1.7$, $P > 0.2$. There was a significant interaction between HF/HFr diet and CN supplement for Ir, Irs1, Irs2, and Glut2 mRNA ($F = 9.8$, $P < .01$; $F = 31$, $P < .001$; $F = 4.9$, $P < .05$; and $F = 10$, $P < .01$, respectively), but not for Glut1.

The HF/HFr feeding significantly impaired liver Gsk3b mRNA and protein levels, with decreases of 62% and 31% of controls (Fig. 6A and C, respectively); the impaired GSK3β protein levels were increased by CN addition to those of the control animals. As shown in Fig. 6B, the protein level of liver glycogen synthase 2 was decreased in HF/HFr-fed rats (69% of control). Cinnamon supplement improved the decreased protein expression of GS2 to 80% of the control. For Gsk3b mRNA, there was a significant effect of diet ($F_{diet} = 14$, $P < .001$) but not CN ($F_{CN} = 0.98$, $P = .34$) or for the interaction ($F = 3.57$, $P = .06$). For GSK3b protein, there were significant effects of diet ($F_{diet} = 9$, $P < .01$) and CN ($F_{CN} = 5.8$, $P < .05$) but not for the interaction ($F = 3.9$, $P = .057$). For GS2 protein, there were significant effects of diet ($F_{diet} = 42$, $P < .001$) but not CN ($F_{CN} = 3$, $P = .09$); and the interaction was also not significant ($F = 1$, $P = .3$) by 2-way ANOVA.

4. Discussion

Glycogen synthesis and insulin sensitivity are closely related. Decreased insulin-stimulated glycogen synthesis and glucose transport activity are observed in insulin resistance [19]. The significant decrease of the glucose infusion rate, assessed by hyperinsulinemic-euglycemic clamps in HF/HFr rats, confirmed that consumption of a diet rich in fructose and fat leads to insulin resistance and alterations in glucose utilization [17]. Insulin sensitivity was significantly improved when CN was added to the HF/HFr diet [17]. These data are consistent with several other studies in vitro and in vivo reporting that polyphenols from CN improve insulin sensitivity (see review by Qin et al [6]). The decreased glucose infusion rate in HF/HFr rats (60% of normal controls) is in agreement with our previous data in high-fructose diet rats in which glucose infusion rate was improved by CE to the level of controls [20]. The beneficial effects of CN on insulin sensitivity have been also reported in healthy humans [21-24], in prediabetic men [25], in women with polycystic ovary syndrome [26], and in people with type 2 diabetes mellitus [16,27-29].

The increased caloric intake of the animals consuming the HF/HFr diet did not lead to increased body weight but did lead to changes in body composition. The fat pad weights per gram of body weight were higher in the animals consuming the HF/

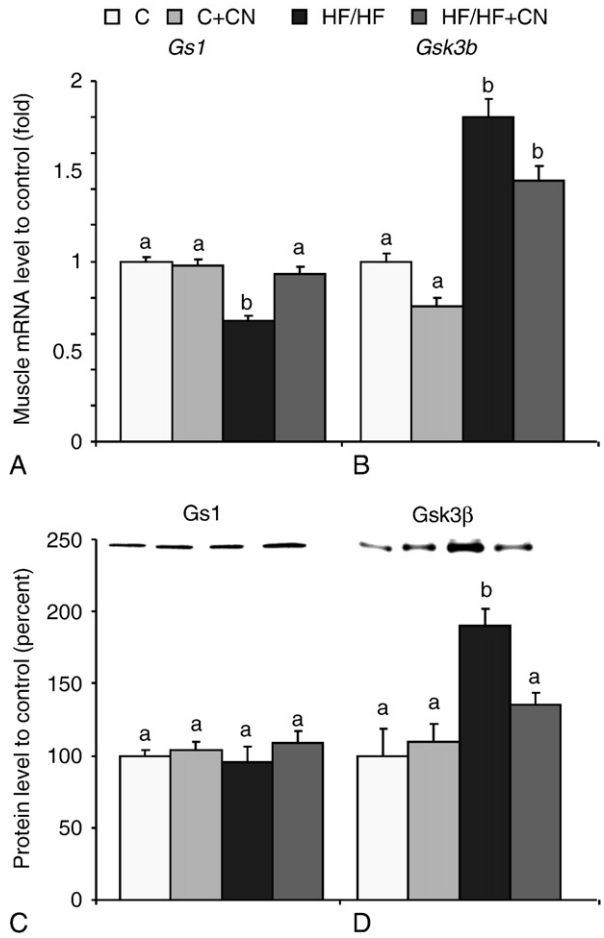

Fig. 4 – Effects of diet and CN on the muscle mRNA and protein expression of Gs1 and Gsk3b. A and B, Messenger RNA was determined with RT-PCR using cDNA made from 25 ng total RNA as template. C and D, Representative experiments on GS1 and glycogen synthase kinase-3b. Immunoblots were analyzed using densitometry. Pairwise differences were analyzed by Tukey test after 2-way ANOVA. Values are mean ± SE (n =8 for each). Different superscripts indicate significant differences among groups ($P < .05$).

HFr diet and the muscle weights were lower compared with the animals on the control diet [17]. Additional long-term studies are needed to evaluate the effects of CN on body weight and body composition of animals consuming diets that lead to insulin resistance.

Hepatic glycogen synthesis and breakdown play important roles in modulating blood glucose metabolism [30]. Conversion of glucose into glycogen is a major pathway for the removal of blood glucose by liver during the postprandial state [31]. In this study, we observed that intake of the HF/HFr diet tended to increase hepatic glycogen content, which is in agreement with a previous study reporting that long-term intake of a fructose-rich diet augmented glycogen content in liver and muscle tissues [32]. Cinnamon addition, like exercise training [32], further enhanced the hepatic glycogen content in HF/HFr-fed rats. Increases in liver glycogen due to CN in the animals consuming the control diet were not significant. There were also no changes in insulin sensitivity in animals consuming the control diet plus CN [17]. Increases in liver glycogen appear to be related to increases in insulin sensitivity. Similarly, an increase in glycogen levels in the liver in mice with type 2 diabetes mellitus and the increased hepatic glycogen synthesis and glycogen content involve enhanced insulin sensitivity [33].

Our data demonstrate that HF/HFr feeding decreases hepatic mRNA expression of proximal insulin signaling. Cinnamon addition enhances impaired gene expressions of Glut1 and Glut2, which are the main liver isoforms for the facilitated diffusion of glucose across the hepatocyte plasma membrane [9]. Consistent with our results, the hepatic mRNA and protein levels of glycogen synthase also were less in diabetic rats compared with the normal rats [34].

In mice with high-fat diet–induced diabetes, the activity of GSK3β was increased in epididymal fat, slightly decreased in liver, and not changed in skeletal muscle, demonstrating that regulation of GSK3 in diabetic conditions can differ dramatically among tissues [35]. Liver-specific GSK3β knockout mice display normal metabolic characteristics and insulin signaling, whereas mice lacking skeletal muscle GSK3β display improved glucose tolerance that is coupled with enhanced insulin-stimulated glycogen synthase regulation and glycogen deposition, indicating organ-specific functions [36]. Dysregulation of the protein kinase GSK3β has been implicated in the development of type 2 diabetes mellitus. The GSK3β protein expression and kinase activity are elevated in diabetes, whereas selective factors decreasing GSK3β have shown promise as modulators of glucose metabolism and insulin sensitivity. As proposed by Patel et al [36], decreased overexpression of GSK3β in skeletal muscle might lead to improved glucose tolerance that is coupled with enhanced insulin-stimulated glycogen synthase regulation and glycogen deposition [36]. The downregulation of GSK3β by CN in HF/HFr rats could therefore be the key step explaining the trend for increased glycogen synthesis in muscle. We also reported that, in vitro, a purified type A CN polyphenol stimulated glucose uptake and glycogen synthesis to a similar level as insulin in 3T3-L1 adipocytes [37]. In that study, CN, in agreement with our data in muscle, activated glycogen synthase–stimulated glucose uptake and inhibited GSK3β [12].

In the present study, HF/HFr feeding decreased the hepatic mRNA and protein expression of GSK3β; and CN reversed the impaired expression to levels of the control animals. In contrast, in the muscle, HF/HFr feeding increased Gsk3b mRNA and protein; and CN decreased protein levels to those of the control, with a similar trend in the mRNA. These apparently conflicting results are consistent with the organ-specific effects cited above in which liver-specific GSK3β knockout mice display normal metabolic characteristics and insulin signaling, whereas mice lacking skeletal muscle GSK3β display improved glucose tolerance that is coupled with enhanced insulin-stimulated glycogen synthase regulation and glycogen deposition [36].

Skeletal muscle is the major site of glucose uptake in the postprandial state. Under euglycemic-hyperinsulinemic conditions, roughly 80% of glucose uptake occurs in skeletal muscle [38]. Cinnamon addition to the HF/HFr diet enhanced the glucose uptake by euglycemic clamp and also tended to increase muscle glycogen. In muscle, decreased expressions

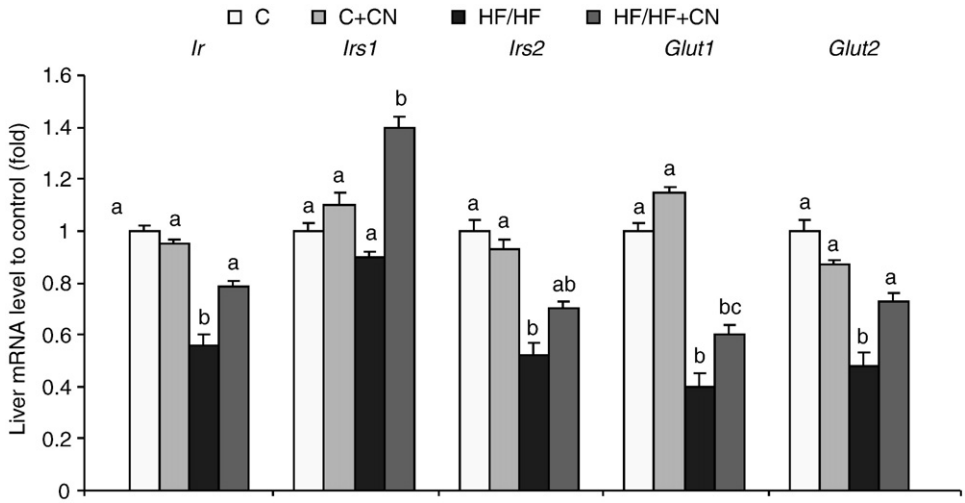

Fig. 5 – Effects of diet and CN on the liver mRNA expression of Ir, Irs1, Irs2, Glut1, and Glut2 in the liver. Messenger RNA was determined with RT-PCR using cDNA made from 25 ng total RNA as template. Pairwise differences were analyzed by Tukey test after 2-way ANOVA. Values are mean ± SE (n =8 for each). Different superscripts indicate significant differences among groups (P < .05).

of Ir, Irs1, Irs2, Glut1, and Glut4, induced by the HF/HFr diet, were also reversed by CN. These data suggest that CN prevents the development of insulin resistance, at least in part by enhancing insulin signaling. Cinnamon could be implicated in the regulation of insulin signaling via increased phosphorylation of signaling proteins as reported by Imparl-Radosevitch et al [39]. In agreement with these data, in previous animal studies in chow diet–fed rats [40] or high-fructose diet–fed rats [20], insulin receptor β and the IRS1 tyrosine phosphorylation levels and IRS1/PI3 kinase in skeletal muscle are enhanced by consumption of CEs. In the present work, the gene expression of the glucose transporter GLUT4, which facilitates the transport of glucose across plasma membranes in skeletal muscle, was increased when CN was added to the diet. These effects have been also observed for GLUT4 expression in 3T3-L1 adipose cells. More, recently Qin et al [41] observed that a water extract of CN (Cinnulin PF; Integrity, Spring Hill, TN, USA) could modulate multiple genes including Glut1, Glut4, and glycogen synthase 1 expression in the adipose tissue of fructose-fed rats.

The effects of CN on liver glycogen and insulin signaling are significant in animals consuming the HF/HFr diet, and there are some trends in the animals consuming the control diet. Similarly, in humans, the effects of CN are more pronounced in people with insulin resistance such as those with type 2 diabetes mellitus [27,28], polycystic ovary syndrome [26], and metabolic syndrome [42]; but there are also significant effects of CN on healthy normal subjects [21,24]. Insulin resistance is a continuum, and even healthy normal humans or animals consuming control diets would have

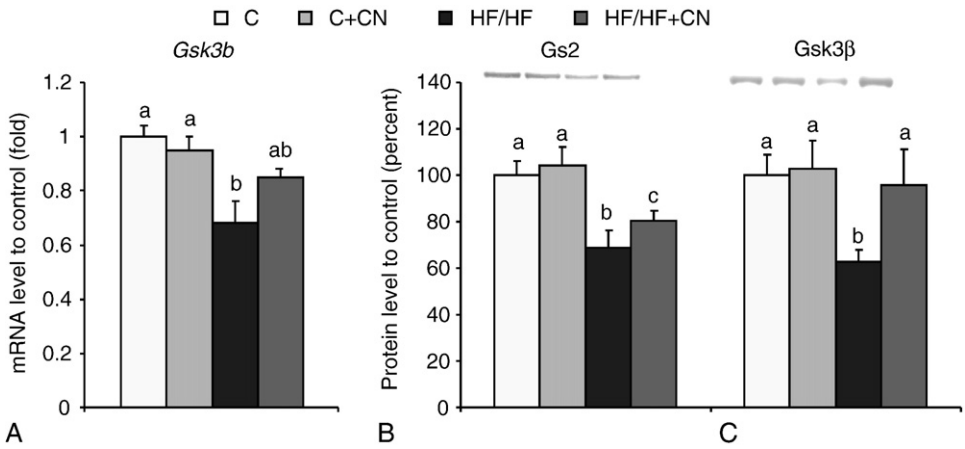

Fig. 6 – Effects of diet and CN on the liver Gsk3b mRNA and protein levels of GS2 and GSK3ß. A, Messenger RNA was determined with RT-PCR using cDNA made from 25 ng total RNA as template. B and C, Representative experiments on glycogen synthase 2 and glycogen synthase kinase-3b. Immunoblots were analyzed using densitometry. Pairwise differences were analyzed by Tukey test after 2-way ANOVA. Values are mean ± SE (n =8 for each). Different letters indicate significant differences among groups (P < .05).

varying degrees of insulin resistance. There also are factors, other than insulin resistance, that influence the response to CN [6].

Several bioactive components of CN could be involved in the observed effects. The insulin-like biological activity is likely related to polyphenol type A polymers present in CN [10]. Purified components of CN, procyanidin type A trimers (molecular weight, 864) and a tetramer (molecular weight, 1152), were found to enhance insulin activity [10], glucose uptake and glycogen synthesis [12], and mRNAs for the insulin receptor and glucose transporter 4 [37]. Purified type A CN polyphenols have also been shown to inhibit kinase activity of purified vascular endothelial growth factor receptor and related signaling pathways [11] and to have significant activity in the inhibition of protein tau polymerization [14]. Cinnamaldehyde, the major component of CN, reduced blood glucose and lipids in rats made chemically diabetic, increased circulating insulin, decreased glycated hemoglobin, and restored the activities of plasma enzymes including aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and alkaline and acid phosphatases [43]. However, cinnamaldehyde has no in vitro insulin-enhancing activity in epididymal cells [10] and does not alter vascular endothelial growth factor receptor activity like the purified type A trimers and tetramer [11]. Cinnamaldehyde also has a dual action of activating GLUT1 under basal conditions but inhibiting GLUT1 activation at low glucose and thus is not thought to be the active component in CN involved in increased insulin sensitivity [44]. Other possible components have been discussed [6].

The amount of CN shown to have beneficial effects on glucose and lipids in humans is 1 g or less per day [16]. In our initial study in humans, we tested 1, 3, and 6 g/d; and the benefits were similar at all 3 levels of CN tested. Beneficial effects of similar amounts of CN have been reported by others [3-6].

The main strength of his study is the demonstration that liver glycogen more than doubles in animals consuming the HF/HFr diet plus CN. Doubling of normal liver glycogen values is near the maximum for athletes preparing for a marathon [45]. The effects of CN on liver glycogen are consistent with improvements in insulin sensitivity. An apparent weakness of the study regarding possible effects on performance is that the increases in liver glycogen due to CN were not significant in animals consuming the control diet. Improvements in insulin sensitivity in animals consuming the control diet plus CN were also not significant. The combined effects of carbohydrate loading and CN on performance need to be determined. Additional studies are also needed to ascertain the effects of CN on long distance runners and cyclers trying to maximize performance.

In summary, the present work shows that addition of CN to an HF/HFr diet reversed insulin resistance and enhanced liver glycogen, in association with the regulation of the expression of multiple genes and proteins involved in insulin sensitivity and glycogen metabolism. These data suggest that CN intakes could play an important role in enhancing liver glycogen synthesis under conditions of reduced insulin sensitivity. Therefore, CN consumption could be of interest to help to maintain the appropriate metabolic balance for the partitioning of fuel substrates between glycogen and lipids in nutritional imbalances due to an overload in dietary fat and/or refined carbohydrates, as often encountered in Western countries. Through its important role in enhancing liver glycogen synthesis, CN should be considered of interest for athletes and lay public for increased energy output and endurance. Additional studies are needed.

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