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Cinnamon Extract Prevents the Insulin Resistance Induced by a High-fructose Diet

Abstract

The aim of this study was to determine whether cinnamon extract (CE) would improve the glucose utilization in normal male Wistar rats fed a high-fructose diet (HFD) for three weeks with or without CE added to the drinking water (300 mg/kg/day). *In vivo* glucose utilization was measured by the euglycemic clamp technique. Further analyses on the possible changes in insulin signaling occurring in skeletal muscle were performed afterwards by Western blotting. At 3 mU/kg/min insulin infusions, the decreased glucose infusion rate (GIR) in HFD-fed rats (60% of controls, $p < 0.01$) was improved by CE administration to the same level of controls (normal chow diet) and the improving effect of CE on the GIR of HFD-fed rats was blocked by approximately 50% by N-monometyl-L-arginine. The same tendency was found during the 30 mU/kg/min insulin infusions. There were no differences in skeletal muscle insulin receptor (IR)-β, IR substrate (IRS)-1, or phosphatidylinositol (PI) 3-kinase protein content in any groups. However, the muscular insulin-stimulated IR-β and IRS-1 tyrosine phosphorylation levels and IRS-1 associated with PI 3-kinase in HFD-fed rats were only 70 ± 9%, 76 ± 5%, and 72 ± 6% of controls ($p < 0.05$), respectively, and these decreases were significantly improved by CE treatment. These results suggest that early CE administration to HFD-fed rats would prevent the development of insulin resistance at least in part by enhancing insulin signaling and possibly via the NO pathway in skeletal muscle.

Key words
Traditional herbal medicine · Insulin action · Nitric oxide · Insulin signaling

Introduction

The consumption of fructose has increased worldwide in the past two decades. This increase is largely because of an augmentation in the consumption of soft drinks and many other beverages high in fructose, and the consumption of foods such as breakfast cereals, baked goods, condiments, and desserts sweetened with sucrose and high-fructose corn syrup [1,2]. Furthermore, fructose consumption makes up a significant proportion of energy intake in the Japanese [1] and American [2] diet. In humans, insulin resistance may result from inherited factors, or it may develop through lifestyle and environmental effectors [3,4]. Non-genetic factors such as increased consumption of dietary fructose might be one of the environmental factors contributing to the develop-

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ment of obesity and the accompanying abnormalities in insulin-resistance syndrome [2]. Several clinical reports have shown that a seven-day high-fructose feeding period was accompanied by a reduction in insulin binding and insulin sensitivity [5], and that diets containing 15% of energy from fructose gave rise to detrimental changes in the glucose metabolism in both normal and hyperinsulinemic males [6]. Animal studies have also suggested that a high-fructose diet (HFD) induces insulin resistance in both skeletal muscle and liver [7–10], and also leads to hypertriglyceridemia [11,12] and hypertension [7]. Furthermore, decreased changes in the early steps of insulin signal transduction may play an important role in the insulin resistance induced by HFD feeding [8,9].

Cinnamon, also known as Cassia, Sweet Wood, and Gui Zhi, is an ancient herbal medicine mentioned in Chinese texts as long ago as 4,000 years [13]. It has a broad range of historical uses as a medicine [13], and is often used as a flavoring agent in different cultures. Several studies have reported that cinnamon has vasodilative, anti-thrombotic, anti-spastic, anti-ulcerous, and anti-allergic action [13]. *In vitro* studies have revealed that at least one of the effective components extracted from cinnamon – methylhydroxychalcone polymer (MHCP) – potentiates insulin action in isolated adipocytes [14–17]. Some of these studies have suggested that MHCP may be useful in the treatment of insulin resistance by increasing glucose utilization and improving the insulin receptor function in adipocytes [16,17]. Additionally, our previous study has shown that the oral treatment with CE would improve *in vivo* insulin-regulated glucose utilization in a dose-dependent fashion in rats, at least in part by enhancing insulin signaling in skeletal muscle [18]. The mixtures of crude extracts found in Chinese medicine prescriptions containing cinnamon have been reported to improve insulin resistance induced by streptozotocin in rats [19,20]. These findings prompted us to study whether CE alone is capable of preventing the insulin resistance and other abnormalities induced by HFD in rats. Therefore, the present study was firstly undertaken to determine whether CE could prevent the development of insulin resistance induced by HFD feeding in normal rats. *In vivo* insulin action was measured using the euglycemic clamp technique in chronically catheterized, conscious animals.

Recent evidences suggest that endothelial nitric oxide (NO) production could be decreased in HFD-fed rats [21,22], and that chronic fructose feeding seems to induce the development of an impaired response to insulin dependent on nitric oxide [23]. In addition, continuous infusion of sodium nitroprusside (SNP, an NO donor) could improve the insulin resistance induced by HFD [24]. Based on our preliminary results and on the above-mentioned findings, our second objective was to investigate whether the expected effect produced by CE treatment on the insulin action of HFD-fed rats was mediated by the NO pathway. Additionally, we further studied the effect of CE on the insulin-signaling pathway in skeletal muscle, the main site of post-absorptive glucose disposal and a major insulin target [25,26].

Materials and Methods

Animals and materials

Male Wistar rats aged 6 weeks were purchased form CLEA (Japan). N^G-monomethyl-L-arginine (L-NMMA), a nitric oxide synthase (NOS) inhibitor, was obtained from Calbiochem (USA). Anti-IR-β (insulin receptor β-subunit), anti-IRS-1 (insulin receptor substrate-1), anti-PI 3-k (phosphatidylinositol 3-kinase), and anti-phosphotyrosine antibodies were purchased from Santa Cruz Biotechnology (USA). All other reagents were of biochemical grade. The cinnamon extract (stored at 4 °C) was kindly provided by Tsumura Co. (Tokyo, Japan).

Experimental protocols

All experimental procedures complied with the Nagoya University *Guide for the Care and Use of Laboratory Animals*. Rats weighing between 145 and 160 g were used for the study. The animals were housed in individual cages in a room with controlled temperature (23 ± 1 °C) and light (12-hour light/dark cycle; lighting between 8:00 a. m. and 8:00 p. m.) and had free access to a standard diet and water. After a one-week acclimation period, the rats were divided into two batches, and were fed one of the following diets for 3 weeks: normal chow diet (n = 6; MF; Oriental Yeast, Chiba, Japan), and high-fructose diet (n = 18; HFD; AIN-93M, Oriental Yeast, Chiba, Japan). Twelve HFD-fed rats received drinking water with added CE (300 mg/kg/day). The CE dosage complied with the findings of our preliminary study [18]. The control diet was standard chow containing 59% carbohydrate, 29% protein, and 12% fat (in % calories). The fructose content provided 60% of the total calories in the high-fructose diet, which also contained 28% of the total calories from protein, and 12% from fat.

Surgery procedures

At the end of the second week, all rats were submitted to the surgery procedures. The animals were anesthetized with an intraperitoneal injection of 50 mg/kg BW sodium pentobarbital. After that, a middle ventral incision was made in the neck, and the right jugular vein and left carotid artery were cannulated with Silascon SH tubing. The catheters were tunneled subcutaneously to the dorsal region of the neck and flushed with 300 µl of saline containing heparin (40 U/ml) and 500 µl of sodium penicillin G (10,000 U/ml). The catheters were then filled with a viscous solution of 50% polyvinylpyrrolidone (PVP) and capped with a piece of polyethylene tubing melted and sealed at one end. After surgery, the rats were kept in the same preoperative conditions. Normal food intake was recovered 3–4 days after surgery.

Euglycemic clamp procedures

One week after surgery, each rat was submitted to a two-step hyperinsulinemic euglycemic clamp procedure [24] performed after a 16 h overnight fast to assess whole-body insulin action. The rat was placed in a restraining cage, and extension tubing was attached to the jugular catheter by an adapter for the continuous infusion of insulin and glucose. The carotid catheter was used for blood sampling. A primed infusion was delivered at a rate of 3 mU/kg/min (low-dose) for 90 minutes and then at an increased rate of 30 mU/kg/min (maximal stimulation, high-dose) for an additional 90-minute period. Based on the blood glucose concentration measured every 10 minutes, the glycemia was kept constant at the basal level with a variable infusion of a 20%

(w/v) glucose solution. The glucose infusion rate (GIR, ml/kg/min) was calculated every 10 minutes during the clamp study. The means of GIR values from 60 to 90 min and from 150 to 180 min for the two-step sequential euglycemic clamp procedure were regarded as an index of whole body insulin activity since a plateau in the GIR was achieved during these periods of time. During the clamp period, L-NMMA was infused with a microinjection pump (Terumo, Japan) in one half of the CE treated HFD-fed rats (n = 6), and the remaining animals were given saline only, according to the same protocol described above. The dosage for an intravenous administration of L-NMMA was 1 mg/kg/min, which we expected to be enough to effectively inhibit NOS [27]. Additional blood samples were collected in heparinized tubes at 20 min before the starting of the clamp study (basal), 90 min, and 180 min for the determination of plasma insulin and basal free fatty acids (FFA) concentrations. At the end of the experiment, skeletal muscle was excised and immediately frozen at liquid nitrogen temperature, and stored at – 80 °C until analysis.

Blood assays

The blood glucose concentration was determined using a glucose analyzer (model 2300; Yellow Springs Instrument, OH). Plasma insulin was assayed with a radioimmunoassay kit (Phadesepa Insulin RIA, Pharmacia AB, Sweden). Plasma FFA was determined with a commercial kit (Wako pure Chemical Industries, Osaka, Japan).

Preparation of tissue extracts

Muscle biopsies were homogenized in ice-cold homogenizing buffer (20 mM Tris-Cl, pH 7.6, 150 mM NaCl, 1% Nonidet P-40, 10 mM NaF, 10 mM Na_3VO_4, 10 mM EDTA, 1 mM PMSF, 1 µg/ml Leupeptin, and 1 µg/ml Aprotinin) using a Polytron homogenizer as described elsewhere [28]. The homogenates were kept on ice for 1 h and then centrifuged at 38,000 rpm at 4 °C for 1 h using a Hitachi RP55 rotor (Hitachi, Tokyo, Japan). The protein concentration of the supernatants was determined using a commercial kit (Bio-Rad, Richmond, CA). Supernatants were stored at – 80 °C until analysis.

IR-β, IRS-1 and PI 3-k protein content analysis

For the assays of the protein amounts of the IR-β, IRS-1, and PI 3-k, an aliquot (40 µg) of the supernatant was resuspended in treatment buffer containing β-mercaptoethanol and boiled for 5 min. The supernatant proteins were size-fractionated by SDS-PAGE (6% or 10% acrylamide gels). After electroblotting, the PVDF membranes were incubated overnight with anti-IR-β, anti-IRS-1, and anti-PI 3-k antibodies each at 4 °C. The membranes were then incubated with Goat Anti-Rabbit IgG (1:2,000) in Tris-buffer (pH 7.4) containing 3% BSA for 1 h at room temperature. After washing, blotted proteins were visualized using a Western blotting detection system (ECL Plus, Amersham). Quantification of the band intensity was performed using the public domain NIH image software.

Determination of IR-β and IRS-1 tyrosine phosphorylation and IRS-1 associated with PI 3-k

The supernatants containing equal amounts of protein (1 mg/ml each tube) were incubated overnight with anti-IR-β (5 mg/ml) or anti-IRS-1 (5 mg/ml) at 4 °C, and then with 20 µl of protein A agarose beads at 4 °C for 4 h. The immunoprecipitated complexes were washed as described previously [29]. Samples were resuspended in treatment buffer containing β-mercaptoethanol and boiled for 5 min. Phosphorylated proteins were separated by SDS-PAGE as above. After electroblotting, the PVDF membranes were incubated with phosphotyrosine antibody or PI 3-k antibody. Bound antibodies were visualized and quantified by the same method described above.

Statistical analysis

All data are expressed as means ± S.E. Changes within each group over time were assessed by the two-way ANOVA, and a comparison of all groups was performed by the one-way ANOVA. When a significant difference was found ($p < 0.05$), the results were further compared with the Fisher's PLSD test. The StatView 5.0 software (SAS Institute Inc., Cary, NC) was used for the statistical analysis.

Results

Food intake, body weight, plasma FFA, blood glucose, and plasma insulin levels

The food intake, body weight, plasma FFA, blood glucose, and plasma insulin levels before, during and immediately after the euglycemic clamp are shown in Table **1**. Daily food consumption and body weights of all rats were comparable throughout the experimental period. After surgery for catheter placement, food consumption and body weight transiently decreased in all rats, but returned to normal levels after 3 – 4 days. CE treatment for three weeks did not affect the higher levels of basal plasma FFA in the HFD-fed rats compared with controls. The fasting blood glucose (FBG) concentrations and steady state plasma insulin concentrations during the low-dose and the high-dose insulin clamp procedures were not significantly different among all groups. In summary, 3-week CE administration did not affect plasma insulin, FBG, and plasma FFA levels in HFD-fed rats.

Table **1** Diet intake, body weight, plasma FFA, and the concentrations of blood glucose and plasma insulin before and during euglycemic clamp procedures at 3.0 and 30.0 mU/kg/min insulin infusions

	Control (Chow-fed)	*HFD (High-fructose-fed)*	*CE*	*CE + L-NMMA*
Diet intake (g/day)	17 ± 3	16 ± 3	15 ± 2	15 ± 2
Body wt (g)	271 ± 3	275 ± 4	272 ± 5	276 ± 7
Basal FFA (µmol/l)	640 ± 40	1100 ± 30*	1260 ± 13*	1090 ± 40*
Glucose (mmol/l)				
Basal	4.1 ± 0.1	4.0 ± 0.2	3.9 ± 0.2	4.2 ± 0.2
Low-dose	4.1 ± 0.2	4.0 ± 0.2	4.1 ± 0.1	4.2 ± 0.3
High-dose	4.0 ± 0.2	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.2
Insulin (pmol/l)				
Basal	55 ± 2	60 ± 4	58 ± 5	61 ± 4
Low-dose	211 ± 21	224 ± 14	220 ± 15	230 ± 20
High-dose	4060 ± 105	4270 ± 140	4138 ± 125	4310 ± 163

Values are mean ± S.E. * Different from controls ($p < 0.001$).

Effect of CE administration on the in vivo GIR

Glucose utilization was measured using the euglycemic clamp technique. A plateau GIR was achieved during the last 30 min of the low- and high-dose clamp and the average GIR was calculated (Fig. **1**). At the low-dose clamp, HFD feeding induced a marked decrease in GIR compared with the controls (5.2 ± 0.3 vs. 10.2 ± 0.4 ml/kg/min, $p < 0.001$). CE treatment produced a significant increase in GIR (9.4 ± 0.6 ml/kg/min), which reached similar levels as controls. However, the increased GIR in CE-treated HFD-fed rats was approximately 50% blocked by L-NMMA infusion. The same tendency was found during the high-dose clamp, namely high-fructose feeding also reduced GIR compared with the controls (34.3 ± 0.9 *vs.* 41.5 ± 1.2 ml/kg/min, $p < 0.05$) and CE treatment improved it (39.5 ± 1.0 ml/kg/min, $p < 0.05$). Improved GIR by CE treatment was just about disappeared by L-NMMA infusion.

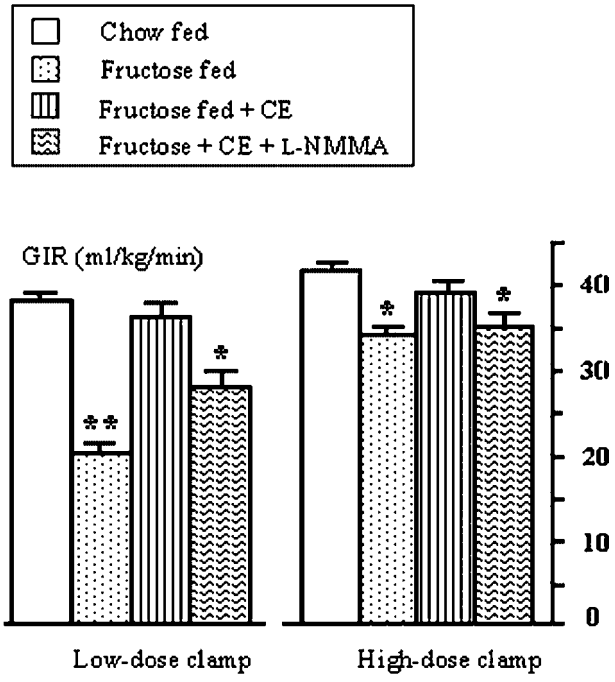

Fig. **1** 3-week CE administration effect: GIRs during the euglycemic clamp procedure in chow fed and fructose fed, fructose fed + CE and fructose fed + CE + L-NMMA groups (n = 6, respectively) at the low-dose and high-dose insulin infusion. Data are expressed as the means ± S.E. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. controls and CE treated group.

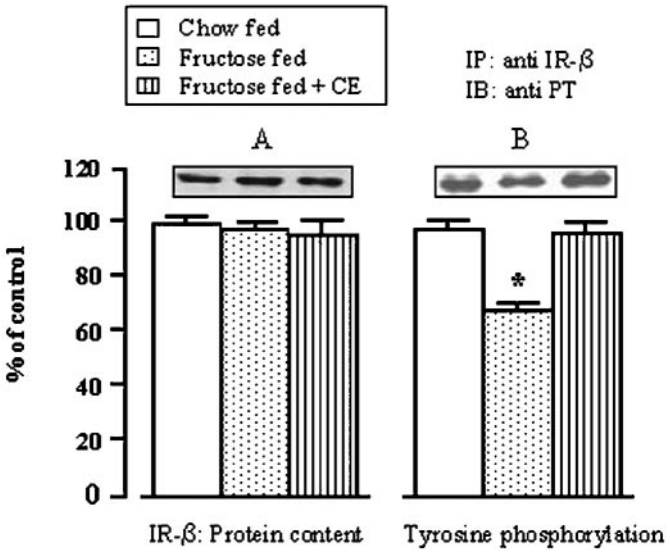

Fig. **2** Effect of 3-week administration of CE on IR-β protein content (A) and tyrosine phosphorylaton (B) in rat gastrocnemius muscle. Data are expressed as the means ± S.E. for 5 rats, respectively. $^{*}p < 0.05$ vs. controls and CE treated group. Immunoprecipitation: IP; Immunoblotting: IB; Phosphotyrosine: PT.

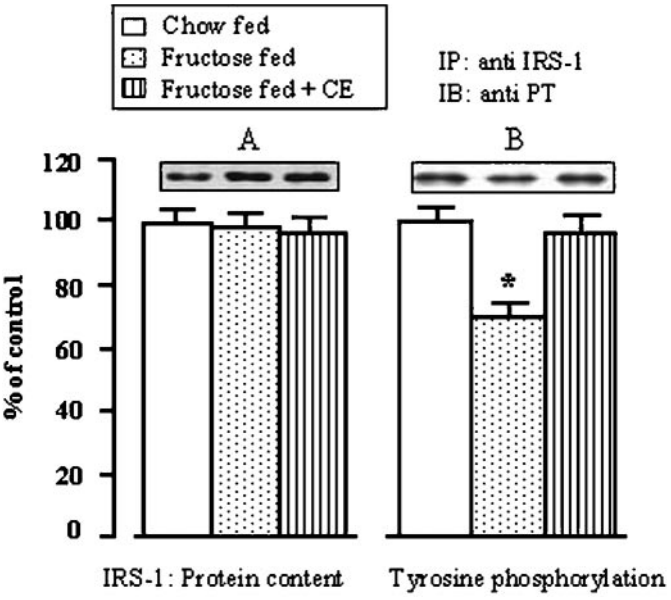

Fig. **3** Effect of 3-week administration of CE on IRS-1 protein content (A) and tyrosine phosphorylation (B) in rat gastrocnemius muscle. Data are expressed as the means ± S.E. for 5 rats, respectively. $^{*}p < 0.05$ vs. controls and CE treated group. Immunoprecipitation: IP; Immunoblotting: IB; Phosphotyrosine: PT.

Effect of CE administration on IR-β, IRS-1, and PI 3-k protein contents

The total protein contents of skeletal muscle IR-β, IRS-1, and PI 3-k were not significantly different between HFD-fed rats and controls and were not affected by CE administration (Fig. **2A**, Fig. **3A**, and Fig. **4A**, respectively).

Effect of CE administration on the tyrosine-phosphorylation levels of IR-β and IRS-1, and the IRS-1 association with PI 3-k

The tyrosine phosphorylation level of IR-β was determined by immunoblotting the phosphotyrosine antibody immunoprecipitates with the IR-β antibody. The tyrosine phosphorylation levels of IR-β in skeletal muscle of HFD-fed rats were significantly decreased when compared to the controls (70 ± 9% of control, $p < 0.05$). In CE-treated HFD-fed rats, the levels of IR-β tyrosine phosphorylation were not different from those of controls (Fig. **2B**). The same tendency was found for the decreased IRS-1 tyrosine phosphorylation in HFD-fed rats (76 ± 5% of controls, $p < 0.05$), which was also improved to the same level of the controls by CE treatment (Fig. **3B**). The IRS-1/PI 3-k association level was determined by immunoblotting the PI 3-k antibody immunoprecipitates with the IRS-1 antibody. The association of PI 3-k

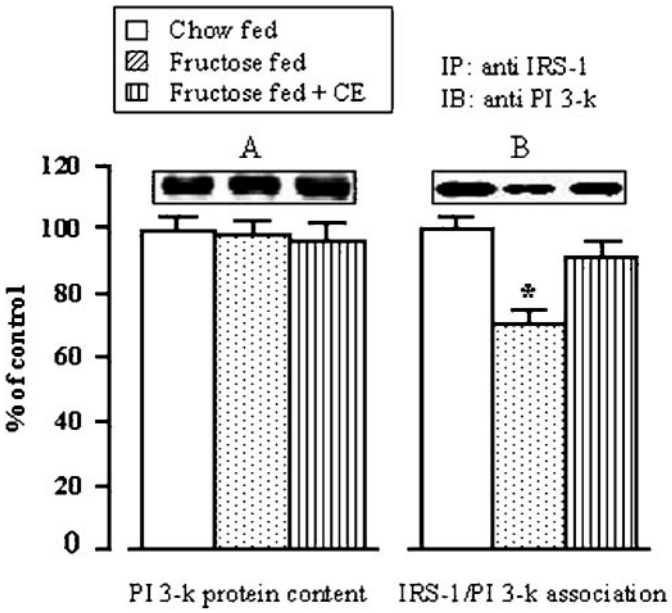

Fig. **4** Effect of 3-week administration of CE on PI 3-k protein content (A) and the association of PI 3-k with IRS-1 (B) in rat gastrocnemius muscle. Data are expressed as the means ± S.E. for 5 rats, respectively. *$p < 0.05$ vs. controls and CE treated group. Immunoprecipitation: IP; Immunoblotting: IB.

with IRS-1 was also reduced in HFD-fed rats (72 ± 6% of controls, $p < 0.05$), and the impaired association was ameliorated by CE treatment (Fig. **4B**). These results indicated that CE administration was correspondingly effective in correcting decreased tyrosine phosphorylation in the skeletal muscle of HFD-fed rats. In addition, the tyrosine-phosphorylation of IR-β, IRS-1, and the IRS-1/PI 3-k association was also compared between the CE-treated group and the CE plus L-NMMA-treated group, and no difference was detected (data not shown).

Discussion

In vitro studies have suggested that CE treatment enhances glucose uptake [14–17] and improves the insulin receptor kinase activity and its autophosphorylation level [17] in adipocytes. The present study was undertaken to assess the preventive effect of CE administration on the glucose utilization in the HFD-fed rats using the euglycemic clamp technique. The GIR at low-dose insulin infusion primarily reflects insulin sensitivity in peripheral tissue, and these changes are thought to be caused mainly by changes in the insulin receptor binding. High-dose insulin infusion leads to a maximal insulin action and responsiveness, predominantly indicating the capacity of post-receptor binding mechanisms [4,30]. The results of the euglycemic clamp study suggested that firstly, the traditional herb cinnamon extract administration may improve the decreased insulin sensitivity and insulin responsiveness induced by environmental factors (that is, HFD feeding) and, secondly, the CE preventive effect is probably mediated by the NO pathway.

Studies indicated that NO can contribute to the insulin-mediated muscle glucose uptake independent of the blood flow in muscle [31], and glucose uptake in peripheral tissues *in vivo* is NO-dependent [32]. Exogenously administered NO, which is generated from NO donors such as SNP, stimulates glucose transport in isolated skeletal muscle [33] by increasing GLUT4 levels at the cell surface [34]. Furthermore, SNP administration resulted in significantly increased *in vivo* glucose utilization in HFD-fed rats [24]. In addition, recent evidences suggest that endothelial NO production could be decreased in HFD-fed rats [21,22], and chronic fructose feeding seems to develop an impaired response to insulin, dependent on NO [23]. On the other hand, administration of L-NMMA results in the development of marked insulin resistance [35] and hyperglycemia [36], suggesting an important role for NO in the skeletal muscle glucose metabolism. In this study, an intravenous administration of L-NMMA to CE-treated HFD-fed rats showed to significantly decrease the previously improved GIR (Fig. **1**). In addition, the same dose of L-NMMA infused in healthy controls did not significantly affect GIR in agreement with previous reports that used similar experimental conditions [19,37]. These results suggested that a consequent increase in NO production after CE administration might be an important factor to the effect of CE on improving insulin resistance on HFD-fed rats. Thus, we speculate that CE and SNP probably improve insulin action in HFD-fed rats by the same metabolic pathway, by increasing NO production; further research on this NO-mediated mechanism is needed.

Insulin resistance induced by HFD feeding results from the impairment of the insulin action on the peripheral glucose uptake along with an impairment in the suppressing effect on hepatic glucose production (HGP) [7–10]. In the present study, high physiological insulin levels of about 210 pmol/l achieved during the low-dose clamp reflects insulin sensitivity in peripheral tissues (such as skeletal muscle) and liver in the postprandial state. These levels of insulin are enough to inhibit the HGP in normal rats [38] but not in insulin-resistant rats fed on a high-fructose diet [10]. Therefore, we speculated that an inhibitory effect of CE on HGP in HFD rats cannot be ruled out. The high-dose insulin infusion resulted in the pharmacological insulin range, which sufficiently suppresses HGP [38], therefore we are able to conclude that the impaired insulin responsiveness in peripheral tissues of HFD-fed rats may be improved by CE administration.

Although the mechanisms on the relationship between insulin signaling and NO are still not exactly known, some evidence suggest that IRS-1 plays a necessary role in coupling signaling from the insulin receptor to PI 3-k and the subsequent activation of the endothelial NOS (eNOS) [39]. PI 3-k was reported to play a significant role in the insulin-signaling pathway related to NO production [40]. Additionally, studies have provided direct evidence for a complete biochemical pathway involving the IR-β, IRS-1, PI 3-k, and eNOS that may account for important physiological actions of insulin in stimulating the production of NO [39–43]. Moreover, in a previous study carried out *in vitro*, Wortmannin, a potent PI 3-k inhibitor, decreased the biological response to insulin and bioactive compounds from cinnamon similarly, indicating that cinnamon is affecting elements on the upstream of PI 3-k [16]. Based on these reports, the impaired glucose disposal during the euglycemic clamp is mainly due to the

reduced glucose uptake in the skeletal muscle of type 2 diabetic subjects [26,44,45]. Therefore, we further clarified the effects of CE on the molecular mechanisms of the *in vivo* insulin signaling in skeletal muscle obtained from the experimental rats.

Several defects in insulin signaling have been reported in type 2 diabetes, including a modest reduction in the insulin receptor phosphorylation and tyrosine kinase activity [46], a decrease in insulin-stimulated IRS-1 tyrosine phosphorylation [47,48], and reduced PI 3-k activity [47–49]. However, the IRS-1 protein expression appears to be unchanged in these studies [47–49]. Our data showed that HFD feeding does not affect the IRS-1 protein amount (Fig. **4A**) in good agreement with the results reported by Ueno et al. [8] and Bezerra et al. [9]. However, the significantly decreased tyrosine phosphorylation level of IRS-1 in the HFD-fed rats agreed with previous studies [8,9], and this decrease was significantly improved by CE administration (Fig. **3B**). Additionally, the same trend was found in the IR-β, reflecting the metabolic upstream effects of IRS-1 (Fig. **2B**). The activation of PI 3-kinase is required for insulin to stimulate glucose transport [50,51]. Although we have not directly assessed the PI 3-k activation level, it was previously described that there is an increase in insulin-stimulated IRS-1 tyrosine phosphorylation and IRS-1-associated PI 3-k activity in parallel with an increase in IRS-1/PI 3-k association in skeletal muscle of streptozotocin-diabetes [52]. To be precise, PI 3-k activation closely correlates with IRS-1 phosphorylation [52]. Our results revealed that CE administration increases IRS-1 tyrosine phosphorylation levels and the IRS-1/PI 3-kinase association (Fig. **4B**); therefore, these changes are thought to result in improved PI 3-k activation.

The present study demonstrated for the first time that firstly, cinnamon extract administration prevents insulin resistance in HFD-fed rats, and secondly, cinnamon extract improves insulin-stimulated decreased tyrosine phosphorylation of IR-β and IRS-1 while increasing the association between IRS-1 and PI 3-k in HFD-fed rats compared to non-treated HFD-fed animals, and these effects might be related to improved NO production. Thus, modulation of the early steps of insulin signaling in the skeletal muscle of CE treated HFD-fed rats for 3-wk may play a central role in improving glucose metabolism *in vivo*.

In conclusion, the current study suggests that early CE administration would prevent the development of insulin resistance in HFD-fed rats due at least in part to enhancement in decreased insulin signaling. Overall, based on our results and others reports [14–17,19,20], CE may be a useful food additive agent or a component herb of synthetic traditional Chinese medicine prescription for preventing insulin resistance induced by everyday life. However, further investigation on the molecular details about the effect of CE from natural sources on the insulin resistance induced by HFD and type 2 diabetes mellitus is needed.

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