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# Cinnamon extract inhibits the postprandial overproduction of apolipoprotein B48-containing lipoproteins in fructose-fed animals☆

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**Abstract**

We have reported previously that a cinnamon extract (CE), high in type A polyphenols, prevents fructose feeding-induced decreases in insulin sensitivity and suggested that improvements of insulin sensitivity by CE were attributable, in part, to enhanced insulin signaling. In this study, we examined the effects of CE on postprandial apolipoprotein (apo) B-48 increase in fructose-fed rats, and the secretion of apoB48 in freshly isolated intestinal enterocytes of fructose-fed hamsters. In an olive oil loading study, a water-soluble CE (Cinnulin PF, 50 mg/kg body weight, orally) decreased serum triglyceride (TG) levels and the over production of total- and TG-rich lipoprotein-apoB48. In ex vivo $^{35}$S labeling study, significant decreases were also observed in apoB48 secretion into the media in enterocytes isolated from fructose-fed hamsters. We also investigated the molecular mechanisms of the effects of CE on the expression of genes of the insulin signaling pathway [insulin receptor (IR), IR substrate (IRS)1, IRS2 and Akt1], and lipoprotein metabolism [microsomal TG transfer protein (MTP), sterol regulatory element-binding protein (SREBP1c) in isolated primary enterocytes of fructose-fed hamsters, using quantitative real-time polymerase chain reaction. The CE reversed the expression of the impaired IR, IRS1, IRS2 and Akt1 mRNA levels and inhibited the overexpression of MTP and SREBP1c mRNA levels of enterocytes. Taken together, our data suggest that the postprandial hypertriglycerides and the overproduction of apoB48 can be acutely inhibited by a CE by a mechanism involving improvements of insulin sensitivity of intestinal enterocytes and regulation of MTP and SREBP1c levels. We present both in vivo and ex vivo evidence that a CE improves the postprandial overproduction of intestinal apoB48-containing lipoproteins by ameliorating intestinal insulin resistance and may be beneficial in the control of lipid metabolism.
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*Keywords:* Cinnamon Extract; Postprandial apob-48; Intestinal Insulin Signaling

## 1. Introduction

Growing evidence suggests that postprandial hyperlipidemia contributes to the development of atherosclerosis and coronary artery disease[1–3]. Substantial evidence suggests that elevated levels of intestinal lipoproteins are associated with increased cardiovascular disease risk [4] and are particularly atherogenic [5,6]. Although the physiological basis of metabolic dyslipidaemia appears to be hepatic overproduction of apolipoprotein (apo)B-containing very low-density lipoprotein (VLDL) particles [7,8], emerging evidence suggests that intestinal overproduction of apoB-containing lipoproteins, in insulin-

*Abbreviations:* apoB48, apolipoprotein B48; CE, cinnamon extract; IR, insulin receptor; IRS-1, IR substrate-1; MTP, microsomal triglyceride transfer protein; TRL, triglyceride rich lipoprotein; VLDL, very low-density lipoprotein.

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resistant states, may be an important contributor to the elevation of circulating triglyceride (TG)-rich lipoproteins (TRLs) [9–11]. apoB-48 is an essential structural component of intestinally derived chylomicrons and its remnants, and a marker of TRLs of intestinal origin. Recent studies [12,13] suggest that the accumulation of postprandial apoB48-TRLs not only leads to the enhanced delivery of exogenous lipids to liver, which will result in an increased production of hepatic lipoproteins, but also delays in the clearance of hepatic-derived TRLs. Consequently, apoB-48 TRL and remnants may play a disproportionally more important role in postprandial lipoprotein metabolism, even if the absolute plasma concentrations of chylomicrons are low compared with levels of hepatic-derived VLDL particles [12,13].

Cinnamon is among the world's oldest and most frequently consumed spices, having been mentioned in Chinese texts as long as 4000 years ago and is often used as an herbal remedy [14,15]. Cinnamon has been shown also to have insulin-like biological activity [16]. Increasing evidence suggests that aqueous cinnamon extracts (CEs) mimic some of the effects of insulin and improve insulin action [17–19]. We have reported previously that a CE prevents fructose feeding-induced decreases in insulin sensitivity, which appears to be mediated via enhancing the insulin signaling pathway [15]. A water-soluble CE (Cinnulin PF) has been shown to reduce fasting blood sugar levels in prediabetic men and women with the metabolic syndrome [20], improve antioxidant status [21] and improve insulin sensitivity in women with polycystic ovary syndrome [22]. Mang et al. [23] also have reported that a CE improves blood glucose and lipids of patients with Type 2 diabetes [24]. Furthermore, evidence suggests that cinnamon, or its components, has potential lipid lowering properties in people with Type 2 diabetes [24], in fructose [25] and cholesterol-fed animals [26] and in streptozotocin (STZ)-induced diabetic rats [27]. However, whether CE effects the postprandial overproduction of apoB48-containing lipoproteins in insulin resistance is currently unknown.

The aim of this study was to investigate whether acute CE treatment inhibits the postprandial production of apoB48-containing lipoproteins in fructose-fed rats. Ex vivo pulse-chase labeling studies were performed using the freshly isolated enterocytes from Syrian golden hamsters, which have attracted increasing attention as a model for studies of lipoprotein metabolism, because hamster lipid metabolism more closely resembles that of humans [28,29]. Additionally, we also investigated the effects of CE on mRNA expression of the intestinal insulin signaling and lipoprotein metabolism. Both in vivo and ex vivo evidence that CE inhibits apoB-48 containing lipoprotein production are presented. We further demonstrate that the intestinal effects of CE are partially mediated by regulating the abnormal mRNA expression of intestinal insulin signaling and the key proteins involved in intestinal lipoprotein metabolism.

## 2. Materials and methods

### 2.1. Cinnamon extract

Water-soluble CE (Cinnulin PF) was obtained from Integrity Nutraceuticals International (Spring Hill, TN, USA); 50 mg of Cinnulin PF is equivalent to approximately 1 g of whole cinnamon powder (i.e., 20:1 extract) and contains at least 3% doubly-linked polyphenol Type-A polymers (considered to be the bioactive components) [17] The extract was dissolved in 50 mg per 5 ml saline before using in animal study. The purified extract from CE was prepared as described previously [17] for use in ex vivo study; the composition of Cinnulin PF and the purified extract are similar according high-performance liquid chromatography analysis. The dried extract was reconstituted at 100 mg/ml in dimethylsulfoxide and diluted with deionized water before being added to the culture medium and kept at −20°C.

### 2.2. Animals

Male Wistar rats, 5 weeks old ($n$=24) and 6-week old male Syrian golden hamsters ($n$=10) (both from Harlan, Indianapolis, IN, USA) were given free access to food and water. All animals were fed a normal chow diet for 7 days to allow acclimatization to the new environment and recovery from the stress of shipping. Animals were then placed on one of two feeding protocols: (1) normal chow or (2) high-fructose diet (diet with 60% fructose, Harlan) both for 3 weeks. At the end of 3 weeks, the animals underwent either the in vivo protocol described below or were sacrificed for isolation of enterocytes for the ex vivo protocols. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of the US Department of Agriculture (USDA).

### 2.3. Oral fat-loading test

To assess the postprandial increase in TG-rich lipoproteins, oral fat-loading tests were performed. Rats were fasted for 16 h and then orally administered 2 ml/kg body weight (BW) of olive oil emulsion (containing 50% olive oil, 5% Tween-20, and mixed with saline), with and without Cinnulin PF (10 or 50 mg/kg BW, 50 mg/5 ml solution). Blood samples were drawn from the tail vein at the baseline (fasting state) and 2 h after oral fat loading in tubes containing EDTA. Centrifugation at 2500×$g$ for 15 min at 4°C was performed to separate serum layer.

### 2.4. Preparation of TRL-apoB48 and Western blotting analysis

To isolate the TRL fraction, 200 μL of serum sample was centrifuged at 122,000×$g$ for 70 min at 15°C using a SW 55 Ti rotor (Beckman Coulter, Fullterton, CA, USA) with the sample overlayed with 4 ml KBr solution (density=1.006 g/ml). The top layer (300 μl), containing the TRL was removed. Samples were resolved in 5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and

subjected to immunoblotting using hamster apoB antiserum, apoB bands were visualized and quantified using an imaging densitometer as described in our previous studies [30,31]. To determine the total serum apoB48, the serum samples were diluted (1:200) and then treated as the described above.

### 2.5. Metabolic labeling of intact primary enterocytes

Primary enterocytes were isolated from hamsters and were used for pulse-chase experiments as described [9]. Enterocytes were first treated with CE (10 or 50 μg/ml) in methionine-free minimal essential medium at 37°C for 30 min and then pulsed with [$^{35}$S] methionine and chased for 60 min. The media samples were collected. apoB48 was visualized by autoradiography and quantified by liquid scintillation counting as described [9,30].

### 2.6. IR, IRS1, IRS2, Akt1, MTP and SREBP1c mRNA abundance

Primary enterocytes were isolated from chow and fructose-fed hamsters and incubated with or without CE (10 or 50 μg/ml) at 37°C for 4 h. Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA). 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, USA).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′IRS-2 primers, 5′-AAGATAGCGGGTACATGCGAAT-3′ and 5′-GCAG-CTTAGGGTCTGGGTTCT-3′; Akt1 primers, 5′-TGGAC-TACTTGCACTCCGAGAA-3′ and 5′-TTATCTTGATATGCCCGTCCTT-3′; MTP primers: 5′-T-CAGGAAGCTGTGTCAGAATG-3′ and 5′-CTCCTTTTTCTCTGGCTTTTCA-3′; SREBP1c primers: 5′GCGGACGCAGTCTGGG-3′ and 5′-AGCTG-GAGCATGTCTTCAAA-3′; 18S primers, 5′-TAAGTCCCTGCCCTTTGTACACA-3′ and 5′-GATCC-GAGGGCCTCACTAAAC-3′. mRNA levels were assessed

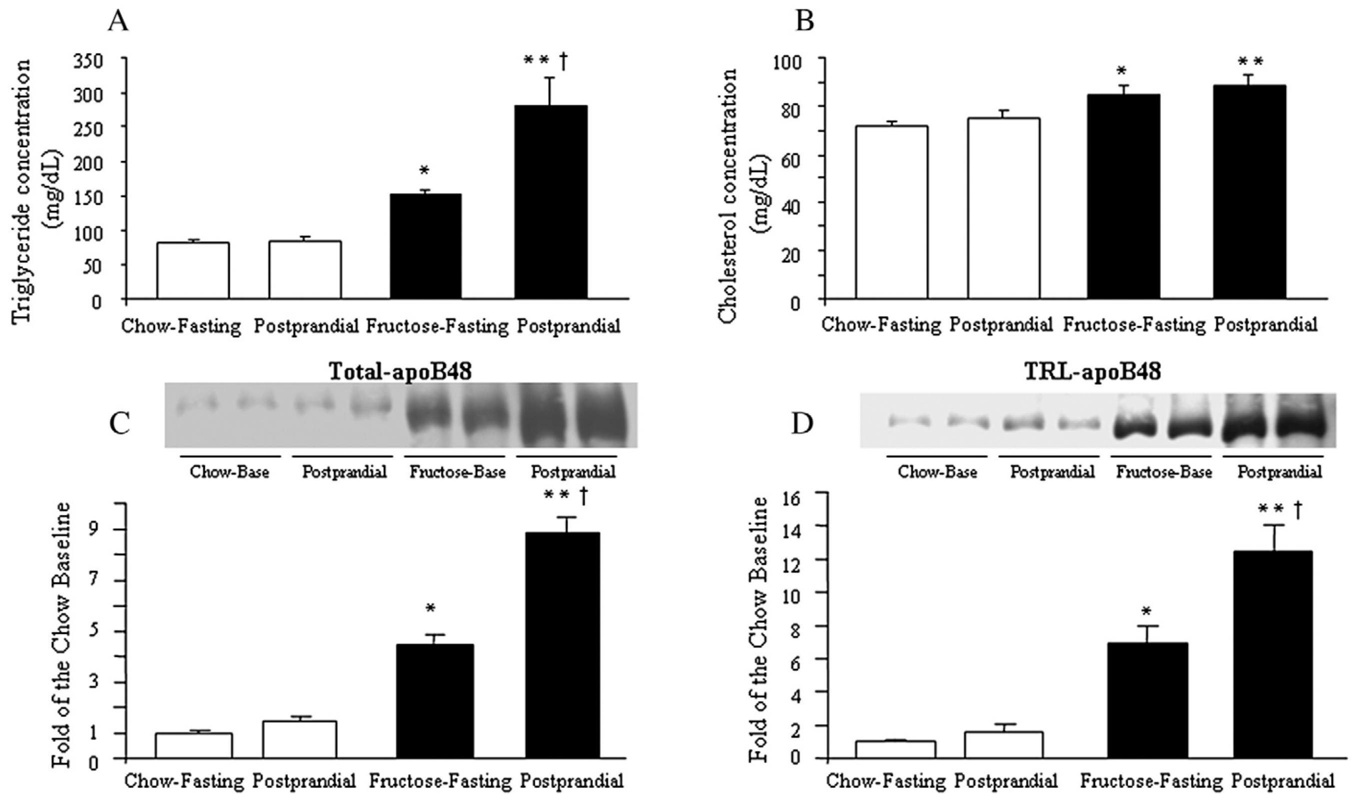


Fig. 1. Fructose feeding induces elevated triglycerides and overproduction of apoB48-containing lipoproteins in the fasting and postprandial state in rats. The concentrations of serum triglycerides (A) and cholesterol (B) were determined by enzymatic colorimetric assays in rats after overnight fast and after 2-h fat loading. The serum- (C) and TRL-apoB48 (D) were immunoblotted using an anti-hamster apoB primary antibody followed by antirabbit horseradish peroxidase secondary antibody. Immunoblots were analyzed using densitometry and expressed as a percentage of chow baseline. Values are represented as mean±S.E. from six animals of each group. *$P$<.05 vs. chow-baseline, respectively; **$P$<.05 vs. fructose-postprandial; †$P$<.05 vs. chow-postprandial.

by real-time quantitative reverse transcriptase-PCR (RT-PCR). All PCR reactions were performed in a total volume of 25 μl and included the following components: cDNA derived from 25 ng of total RNA, 400 nM of each primer, RNase-free water, and 12.5 μl of SYBR Green PCR Master Mix (Applied Biosystems, Foster, CA, USA), an optimized buffer system containing AmpliTaq Gold DNA polymerase and dNTPs. All PCR reactions were performed in duplicate and cycling parameters were as follows: after an initial denaturation step for 10 min at 95°C, 40 subsequent cycles were performed in which samples were denatured for 15 s at 95°C followed by primer annealing and elongation at 60°C for 1 min. Relative quantities of mRNA were normalized by 18S rRNA content.

### 2.7. Other laboratory methods

Serum insulin concentrations were determined by RIA using a rat insulin kit from Linco Research (St. Louis, MO, USA). TG and cholesterol were measured using a colorimetric assay (Wako, Richmond, VA, USA).

### 2.8. Statistical analyses

Statistical significance was calculated using a 2-tailed paired Student's $t$ test analysis or one-way analysis of variance. $P$ values less than .05 were considered significant.

## 3. Results

### 3.1. Effects of CE (Cinnulin PF) treatment on serum triglycerides and cholesterol levels and the production of total- and TRL-apoB48 in fructose-fed rats

Rats were fed a fructose-enriched diet for 3 weeks; the BWs did not significantly change between chow-, 314 ± 7 g, and fructose-fed animals, 318 ± 9 g. There was a significant increase in fasting serum TG levels by approximately 83% (Fig. 1A: $P < .001$ vs. chow-fed) and cholesterol levels by 18% (Fig. 1B: $P < .05$ vs. chow-fed). Fructose feeding also

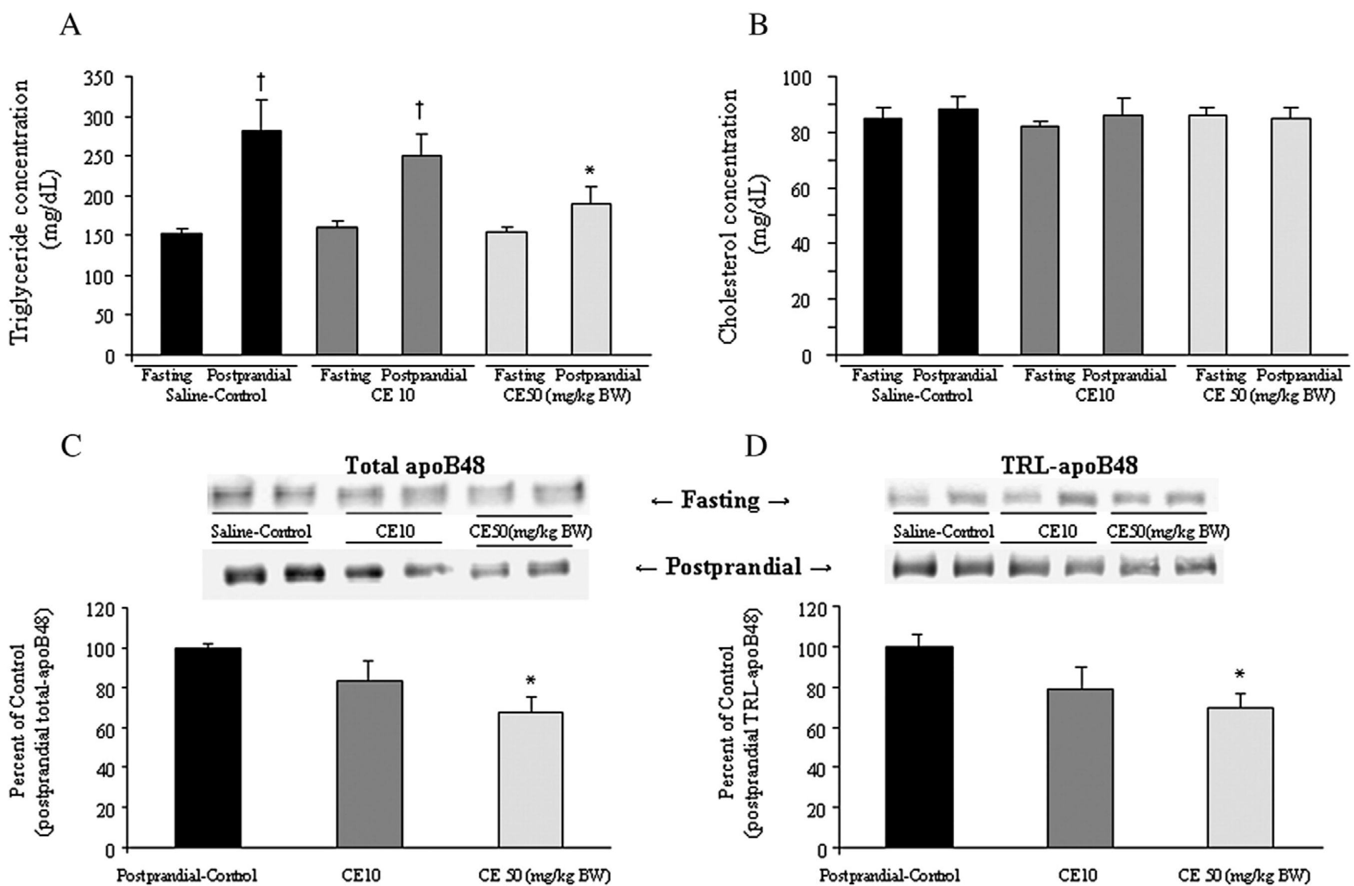


Fig. 2. In vivo acute effects of CE oral treatment on TG, cholesterol, total- and TRL-apoB48 production in fructose-fed rats. The concentrations of serum TG (A) and cholesterol (B) were determined by enzymatic colorimetric assays in fructose-fed rats after an overnight fast and after 2 h of fat loading. The upper representation of Panels C and D show total-and TRL-apoB48 in the fasting state; the apoB48 were immunoblotted using an anti-hamster apoB primary antibody followed by anti-rabbit horseradish peroxidase secondary antibody. Immunoblots were analyzed using densitometry and expressed as a percentage of the saline-baseline. Graphical representation of apoB48 in serum (C) and the TRL fraction (D) is in the postprandial state. Values are mean±S.E. from six animals of each group. †$P$<.05 vs. the baseline, respectively; *$P$<.05 vs. saline-postprandial.

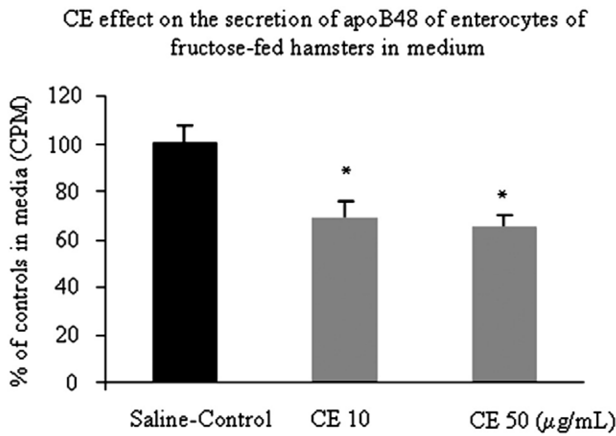


Fig. 3. *Ex vivo* effect of CE on apoB48 secretion in primary enterocytes in fructose-fed hamsters. Primary enterocytes, isolated from fasting fructose-fed hamsters, were pretreated with saline or CE (10 or 50 μg/ml) for 30 min at 37°C, then pulsed with [$^{35}$S] methionine and chased for 60 min. The media samples were collected and subjected to immunoprecipitation and then analyzed by SDS-PAGE and fluorography. apoB48 was visualized by autoradiography and quantified by liquid scintillation counting. Data are the means±SE ($n$=3 for each group); *$P$<.05 vs. saline treated control.

induced total-apoB and TRL-apoB48 overproduction in the fasting state (Fig. 1C: 4.4±0.4-; Fig. 1D: 6.9±1.0-fold of chow-fed; $P$<.01, respectively). The fasting serum insulin levels were also increased in the fructose-fed rats (0.67±0.04 vs. chow: 0.37 ± 0.03 ng/ml; $P$<.01).

In an olive oil loading test, postprandial serum TG, cholesterol levels as well as serum-and TRL-apoB48 did not significantly increase above baseline in the chow-fed rats (Fig. 1 A). However, in the fructose-fed group, the postprandial serum TG levels were significantly higher than the baseline with an approximate increase of 2.3-fold compared to the chow-fed ($P$ < .001). Fat loading did not affect the postprandial cholesterol levels, as the olive oil is cholesterol free, but values were higher than those of the chow-fed animals($P$ < .05) (Fig. 1B). The serum- and TRL-apoB48 were both significantly higher — increases of 5.2- and 7-fold to the chow-fed (Fig. 1C and D: $P$<.001, respectively); 2 h fat loading did not affect the insulin levels, compared with the baseline (data not shown).

After 2 h fat loading and CE treatment, there was a trend that the low-dose CE treatment decreased the postprandial serum TG levels, but the differences were not significant; 50 mg/kg BW CE treatment inhibited the increase of postprandial TG levels

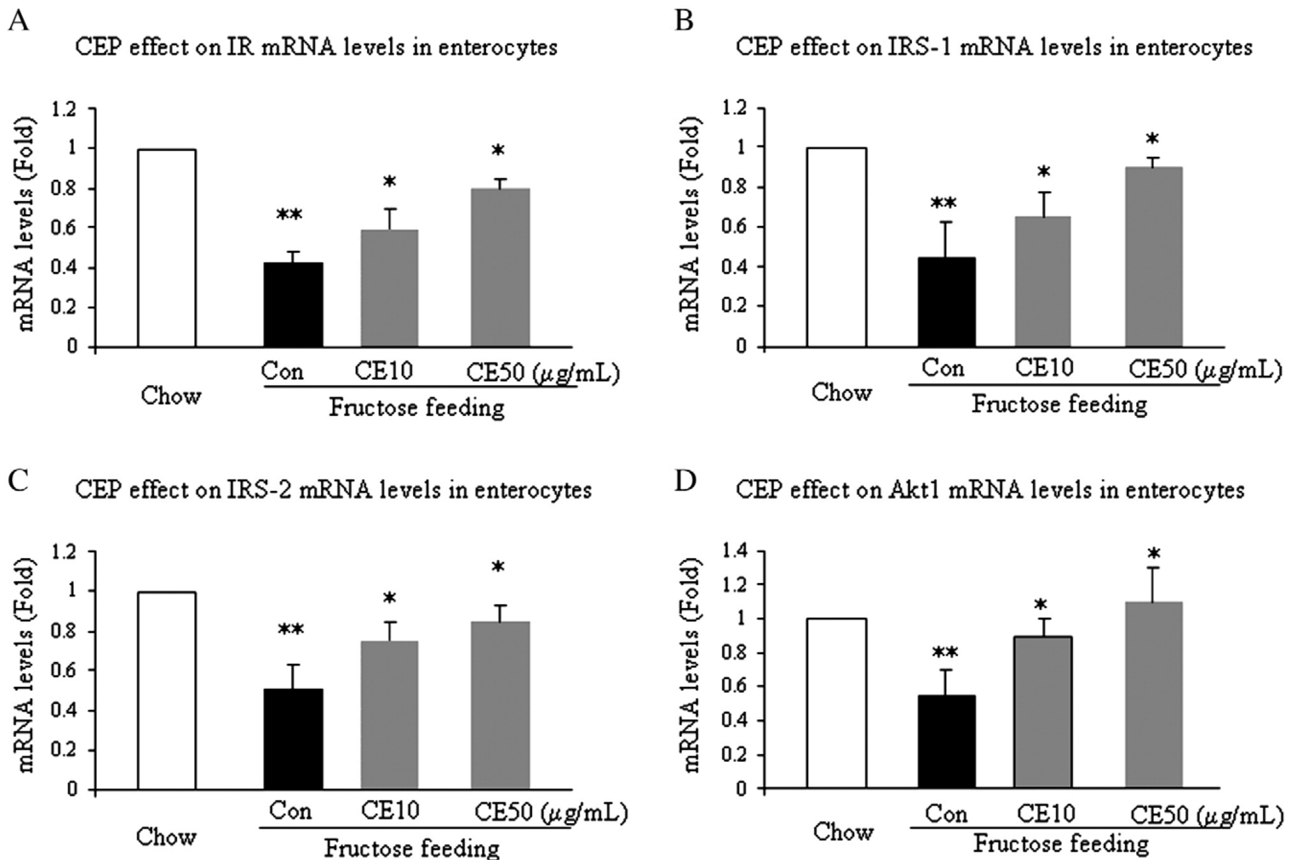


Fig. 4. Effect of CE on the mRNA levels of IR, IRS1, IRS2 and Akt1 in primary hamster enterocytes. The IR, IRS1, IRS2 and Akt1 mRNA levels are 2 h after CE (10 or 50 μ/ml) at 37°C, determined with RT-PCR using cDNA made from 25 ng total RNA as template. The mRNA levels were normalized using the 18s rRNA levels in each samples. Data are mean±S.E. ($n$=3 or 4 for each group). *$P$<.05 vs. fructose feeding control; **$P$<.001 vs. chow control.

about 33% of the control (Fig. 2A, $P<.05$). CE treatment and fasting and postprandial state did not affect the cholesterol values (Fig. 2B). The postprandial overproduction of serum-and TRL-apoB48 were both inhibited by high-dose CE treatment (Fig. 2C and D, $P<.05$, respectively). There was a trend for low-dose CE-treated rats to have lower total- and TRL-apoB48 production, but the differences were not significant. CE treatment did not affect the insulin levels, compared to the controls (data not shown).

### *3.2 Ex vivo CE treatment decreases apoB48 secretion from enterocytes from the fructose-fed hamsters*

We also examined the secretion of apoB48 in enterocytes isolated from fasted fructose-fed hamsters radiolabeled with [$^{35}$S] methionine for 60 min. Both CE levels tested significantly inhibited the amount of apoB48 secreted into the media (Fig. 3).

### *3.3. CE enhances the mRNA expression of the insulin receptor, IRS1, IRS2 and Akt1 in fructose-fed hamster enterocytes*

To assess whether intestinal enterocytes were responsive to CE, enterocytes were treated with CE (10 and 50 μg per ml) for 2 h at 37°C, using RT-PCR to measure the mRNA expression. Fructose-feeding significantly impaired the mRNA expression of IR, IRS1, IRS2 and Akt1 in hamster enterocytes (Fig. 4: 0.43 ± 0.06; B: 0.45 ± 0.18; C: 0.51± 0.12; D: 0.55 ± 0.15 fold of chow-fed, $P < .05$, respectively). Both CE treated groups showed significant increases in the mRNA expression of IR, IRS1, IRS2 and Akt1, compared with the enterocytes of fructose-fed hamsters (increases of about 39% and 86%, 44% and 100%, 47% and 66%, 63 and 100%; $P<.05$, respectively).

### *3.4. Effect of CE treatment on mRNA expression of MTP in the primary enterocytes of fructose-fed hamsters*

MTP is an important component for secretion of chylomicrons by the intestine [32]. MTP mRNA was markedly increased in enterocytes from fructose-fed hamsters, compared with chow control (Fig. 5A, 1.9±0.2 fold of chow-fed, $P<.05$). The overexpression of MTP mRNA was inhibited in both CE treated enterocyte groups.

### *3.5. CE treatment inhibits mRNA expression of SREBP1c in primary enterocytes of fructose-fed hamsters*

SREBP1c, an important regulator of lipogenesis, has an important role in the regulation of TG accumulation [33]. Enterocytes isolated from the fructose-fed hamsters had significantly increased SREBP1c mRNA levels compared with chow-fed hamsters (2.75±0.4-fold, $P<.05$). The over-expression of SREBP1c mRNA in the fructose-fed hamsters was inhibited in both CE treated groups by about 20% and 46%, respectively (Fig. 5A and B).

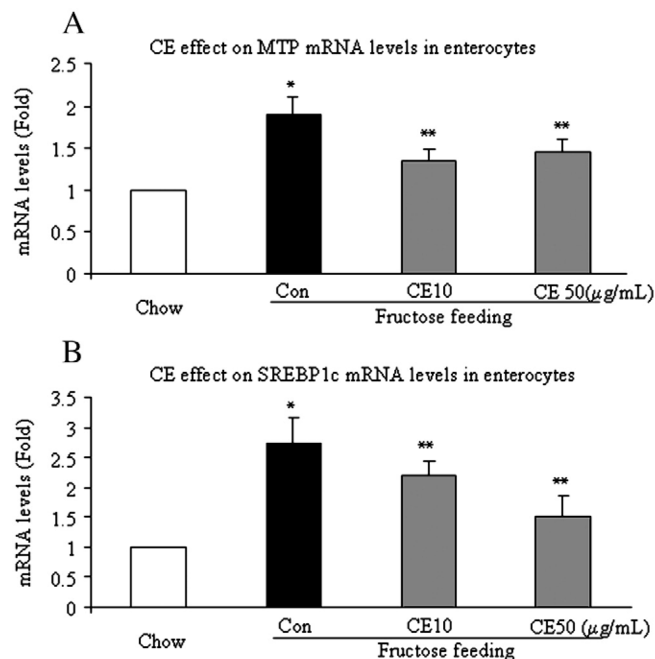


Fig. 5. Effect of CE on the mRNA levels of MTP and SREBP1c in primary hamster enterocytes. The MTP and SREBP1c mRNA levels of 2 h CE (10 or 50 μ/ml) at 37°C were determined with RT-PCR using cDNA made from 25 ng total RNA as template. The mRNA levels were normalized using the 18s rRNA levels in each sample. Data are mean±S.E. ($n$=3 or 4 for each group); *$P<.05$ vs. fructose feeding control; **$P<.001$ vs. chow control.

## 4. Discussion

Cinnamon has potential lipid lowering properties in animal and human studies [24–27]. In the present study, we examined the acute effects of oral treatment with a water-soluble CE on the postprandial apoB48 production in fructose-fed rats and the secretion of apoB48 in enterocytes from fructose-fed hamsters. We also investigated the potential molecular mechanism in intestinal enterocytes. The water-soluble CE (Cinnulin PF) markedly inhibited the postprandial hypertriglycerides and serum- and TRL-apoB48 overproduction in fructose-fed rats. In rats, apoB-48 is synthesized both in the liver and the intestine; however, in humans, apoB48-containing lipoproteins are almost exclusively secreted by the intestine. To focus on whether CE treatment affects intestinal-derived apoB48 secretion, the Syrian golden hamster model was used since the tissue-specific expression of apoB48 (only in the intestine) is a distinct advantage over other rodent models [34]. In the ex vivo labeling study, the data are consistent with in vivo results, CE significantly decreased the secretion of apoB48 in the enterocytes of fructose-fed hamsters.

Insulin has been shown to have acute inhibitory effects on apoB48 production in enterocytes [30]. Lewis et al. [35] reported that treatment of fructose-fed, insulin-resistant hamsters treated with an insulin sensitizer, improved whole-body and also decreased the over secretion of intestinal apoB48-containing particles. Moreover, Federico et al. [30] provided solid evidence that insulin insensitivity at the level of the intestine and aberrant insulin signaling are important underlying factors in intestinal overproduction of highly atherogenic apoB48

in the insulin-resistant state. We also reported that the inflammatory factor tumor necrosis factor-α induces whole-body and intestinal insulin resistance and also stimulates the overproduction of intestinal apoB48-containing lipoproteins [31]. We demonstrated previously that treatment of fructose-fed, insulin-resistant rats with CE improved whole-body insulin sensitivity and also enhanced insulin signaling in muscle [15]. In the current study, fructose-feeding markedly decreased the mRNA expression of the insulin receptor, and CE prevented the decreases of IRS1, IRS2 as well as Akt1 of enterocytes isolated from fructose-fed hamsters. Acute CE treatment inhibited the postprandial overproduction of apoB48-containing lipoproteins without affecting the insulin levels. Together, these data suggest that CE treatment increases the insulin sensitivity of enterocytes.

MTP is an important enabler for secretion of chylomicrons by the intestine [32]. Intestinal MTP mRNA is elevated in animal models of both insulin resistance and diabetes [36–38]. The increased MTP associated with oversecretion of intestinal apoB48 lipoproteins [10] and the increased chylomicron particle number may play a crucial role in the development of atherosclerosis [36]. Insulin sensitization treatment is associated with a reduction in intestinal MTP over expression and apoB48-containing particle hypersecretion [35]. The promoter region of the MTP gene contains a negative insulin-response element [39]. In the current study, we found that the over expression of MTP mRNA levels in the enterocytes of fructose-fed hamsters was significantly inhibited by CE. Therefore, it is possible that the reduction in MTP levels induced by CE treatment was the result of improved insulin signaling at the level of the enterocyte.

SREBP1c, which is an important regulator of lipogenesis, has a role in the regulation of TG accumulation in the liver [33]. A significant increase in the mature form of SREBP1c in intestinal enterocytes from the fructose-fed hamsters has been reported [30]. Here, we observed that fructose feeding induced the overexpression of SREBP1c mRNA in enterocytes, which was inhibited by CE treatment. Since SREBP1c expression has been shown to increase apoB secretion [40] and be associated with insulin resistance [41], CE effects on the SREBP1c mRNA expression might be involved in the improved intestinal enterocyte insulin signaling.

In summary, our results suggest that acute oral CE treatment inhibits the increase in postprandial triglycerides and the overproduction of apoB48-containing lipoproteins in fructose-fed, insulin-resistant rats. CE inhibits the secretion of apoB48 in the enterocytes isolated from fructose-fed hamsters and enhances the impaired mRNA expression of intestinal insulin signaling and down-regulates the over expression of MTP and SREBP1c mRNA levels. Dietary measures that effectively improve whole-body insulin resistance and down-regulate MTP and SREBP1c over expression in insulin-resistant state could be part of the control to correct the overproduction of intestinal lipoproteins associated with insulin resistance. Collectively, our findings suggest that Cinnulin PF attenuates the elevated postprandial lipoprotein production and secretion in insulin resistant states. Additional studies investigating the effect of Cinnulin PF on intestinal lipoprotein overproduction is needed to determine whether this process is relevant to humans associated with Type 2 diabetes and insulin resistance.

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