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Administration of tauroursodeoxycholic acid prevents endothelial dysfunction caused by an oral glucose load

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Abstract

Postprandial hyperglycemia leads to a transient impairment in endothelial function; however, the mechanisms remain largely unknown. Previous work in cell culture models demonstrate that high glucose results in endoplasmic reticulum (ER) stress and, in animal studies, ER stress has been implicated as a cause of endothelial dysfunction. Herein we tested the hypothesis that acute oral administration of tauroursodeoxycholic acid (TUDCA, 1500mg), a chemical chaperone known to alleviate ER stress, would prevent hyperglycemia-induced endothelial dysfunction. In 12 young healthy subjects (seven men, five women), brachial artery flow-mediated dilation (FMD) was assessed at baseline, 1 hour, and 2 hours post an oral glucose challenge. Subjects were tested on two separate visits in a single-blind randomized crossover design: after oral ingestion of TUDCA or placebo capsules. FMD was reduced from baseline during hyperglycemia under the placebo condition (−32% at 1 hr and −28% at 2 hr post oral glucose load; $p < 0.05$ from baseline) but not under the TUDCA condition (−4% at 1 hr and +0.3% at 2 hr post oral glucose load; $p > 0.05$ from baseline). Postprandial plasma glucose and insulin were not altered by TUDCA ingestion. Plasma oxidative stress markers 3-nitrotyrosine and TBARs remained unaltered throughout the oral glucose challenge in both conditions. These results suggest that hyperglycemia-induced endothelial dysfunction can be mitigated by oral administration of TUDCA, thus supporting the

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DECLARATIONS OF INTEREST

The authors have nothing to disclose.

AUTHOR CONTRIBUTION STATEMENT

LKW, RMR, and JP conceptualized and designed the study; LKW performed the experiments and analyzed the data; LKW, RMR, MN, CM, and JP interpreted the results; LKW and JP prepared the figures; LKW and JP drafted the manuscript; LKW, RMR, MN, CM, and JP edited and revised the manuscript; LKW, RMR, MN, CM, and JP approved final version of manuscript.

hypothesis that ER stress may contribute to endothelial dysfunction during postprandial hyperglycemia.

Keywords

Postprandial hyperglycemia; TUDCA; endothelial function

INTRODUCTION

Current evidence indicates that postprandial hyperglycemia is an independent predictor of cardiovascular disease and events (3, 9, 13, 19, 24, 51, 60). Indeed, reduction of postprandial hyperglycemia attenuates the risk of cardiovascular disease in subjects with impaired glucose tolerance and diabetes (12–14). The link between postprandial hyperglycemia and cardiovascular disease is likely due to the detrimental effects of glycemic excursions on the artery wall. In this regard, accumulating evidence demonstrates that postprandial hyperglycemia promotes endothelial dysfunction in both healthy subjects and patients with insulin resistance (4, 7, 12, 13, 15, 44, 45, 47, 51, 53). The mechanisms of endothelial dysfunction caused by high glucose ingestion and resulting hyperglycemia are not fully established; however, increased oxidative stress and consequent reduction in endothelial nitric oxide (NO) bioavailability have been implicated (7, 13, 15, 18, 24, 31, 34, 35, 37, 38, 39, 40, 44). In support of this, administration of antioxidant vitamins C and E have been shown to prevent endothelial dysfunction following an oral glucose challenge (46, 47, 61).

A potential mechanism by which hyperglycemia causes an increase in reactive oxygen species (ROS) is endoplasmic reticulum (ER) stress (5, 7, 48). Studies in cultured endothelial cells and other cell types demonstrate that glucotoxicity induces ER stress and formation of ROS (54, 55, 57, 58). Notably, high glucose-induced generation of ROS can be abrogated by treating cells with the chemical ER chaperone tauroursodeoxycholic acid (TUDCA), a bile acid known to inhibit ER stress (16). Given that TUDCA can be safely administered to humans (6, 8, 33, 36, 56), herein we tested the hypothesis that oral supplementation of TUDCA would prevent postprandial hyperglycemia-induced endothelial dysfunction. To test this hypothesis, in a randomized cross-over design, measurements of brachial artery endothelial function were performed before and following an oral glucose challenge under placebo versus TUDCA conditions. This is the first study to examine the vascular effects of TUDCA in humans.

MATERIALS AND METHODS

Twelve young healthy subjects were recruited to participate in this study. Subject characteristics are presented in Table 1. All experimental procedures and measurements conformed to the Declaration of Helsinki and were approved by the University of Missouri Health Sciences Institutional Review Board. Before participating in the study, each subject provided written informed consent. Subjects were recreationally active, nonsmokers, with no history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease as determined from a detailed medical health history questionnaire. No subjects were using prescribed or over-the-counter medications. Women were tested on days 1–7 of the

menstrual cycle following previously reported guidelines for flow-mediated dilation (FMD) (59).

Experimental Procedures

A schematic of the randomized, cross-over study design is presented in Figure 1. Height, weight, and body composition (dual energy X-ray absorptiometry, Hologic QDR 4500A) were measured prior to the two experimental visits. Both experimental visits were scheduled within ~30 days but at least 7 days apart. On each experimental visit, subjects arrived to the laboratory under fasted conditions eight hours after ingestion of either TUDCA (1500mg; Olympus Labs) or placebo (University of Missouri Hospital Pharmacy) capsules. TUDCA was administered 8 hours prior to baseline vascular measurements (Figure 1). This timing was based on pilot experiments performed to establish the pharmacokinetics of TUDCA in nonhuman primates (rhesus macaque; n=3). We determined that plasma levels of TUDCA peak 8 hours after ingestion and return to basal levels at ~24 hours (data not shown). Of note, the bile acid metabolism of nonhuman primates is very similar to that of humans and this dose of TUDCA has been shown to be well tolerated (6, 8, 36). The order of placebo versus TUDCA administration was randomized by study personnel and subjects were blinded to the experimental condition. Upon arrival, subjects were positioned supine in a quiet, climate controlled room (22–23°C) and instrumented with an automated sphygmomanometer (SphygmoCor XCEL, AtCor Medical, Itasca, IL) for assessment of blood pressure (BP). Thereafter, measurements of brachial artery FMD were performed at baseline as well as 60 and 120 minutes following ingestion of a glucose drink as illustrated in Figure 1 and described below.

Brachial artery FMD—Brachial artery FMD was assessed using Doppler ultrasound (Logiq P5; GE Medical Systems, Milwaukee, WI) as previously described (10, 52). Briefly, an 11-MHz linear array transducer was placed over the brachial artery and secured with a clamp. A rapid inflating cuff (Hokanson, Bellevue, WA) was placed on the forearm ~5cm distal to the antecubital fossa. Simultaneous diameter and velocity signals were obtained in duplex mode at a pulsed frequency of 5 MHz and corrected with an insonation angle of 60°. Sample volume was adjusted to encompass the entire lumen of the vessel without extending beyond the walls, and the cursor was set at mid-vessel. Two minutes of baseline hemodynamics were recorded and the cuff was then inflated to a pressure of 220 mmHg for 5 minutes. Continuous diameter and blood velocity measures were recorded for 3 minutes following cuff deflation. Recordings of all vascular variables were analyzed off-line using specialized edge-detection software (Cardiovascular Suite, Quipu srl, Pisa, Italy). Mean blood flow was calculated from continuous diameter and mean blood velocity recordings at each of the experimental time points using the following equation: $3.14 \cdot (\text{diameter}/2)^2 \cdot \text{mean blood velocity} \cdot 60$. Brachial artery FMD percent change was calculated using the following equation: $\% \text{FMD} = (\text{peak diameter} - \text{base diameter}) / (\text{base diameter}) \cdot 100$. Shear rate was defined as $8 \cdot \text{mean blood velocity} / \text{diameter}$ (50). Hyperemic shear rate area under the curve (AUC) up to peak diameter was calculated as stimulus for FMD, as previously described (10, 52).

Oral Glucose Challenge—An intravenous catheter was placed in the antecubital vein and a baseline blood sample was taken. The subjects then ingested a standard 75g glucose drink (Fisher Health Care, Houston, TX) within 3 minutes. Brachial artery FMD measures were repeated at 60 and 120 min post glucose ingestion and blood samples were collected at baseline and at 30 minute intervals for 120 minutes following glucose ingestion (Figure 1). Collected plasma was stored at -80°C for subsequent analysis.

Plasma Analysis—Analysis of plasma glucose, insulin, cholesterol, triglycerides, high- and low-density lipoproteins (HDL and LDL, respectively), as well as non-esterified fatty acids (NEFA) were performed by a third party laboratory (Comparative Clinical Pathology Services, Columbia, MO). Baseline plasma TUDCA and the taurine unconjugated form ursodeoxycholic acid (UDCA) were assessed using high performance liquid chromatography at the Bioanalytical Shared Resource/Pharmacokinetics Core, Oregon Health & Science University. In addition, plasma thiobarbituric acid reactive substances (TBARS) and 3-nitrotyrosine, markers of oxidative stress, were quantified in samples taken at baseline and 60 minutes during the glucose challenge using commercially available ELISA kits (TBARS: #10009055, Cayman Chemical, Ann Arbor, MI; 3-nitrotyrosine: # ab116691, Abcam, Cambridge, England).

Statistical analysis

A two-way (condition $\times$ time) repeated-measures ANOVA using Tukey HSD test for pairwise comparisons was performed on all dependent variables. FMD was also adjusted for hyperemic shear rate AUC via ANCOVA to statistically control for the influence of shear stimulus on the FMD response (2, 30, 50). Significance was accepted at $p < 0.05$. Data are expressed as means $\pm$ SE.

RESULTS

Plasma TUDCA and UDCA levels were both significantly elevated 8 hours after ingestion of the TUDCA versus placebo capsules (Figure 2A, $p < 0.05$). The increase in plasma glucose and insulin following ingestion of the glucose drink was similar between placebo and TUDCA visits (Figure 2B, $p > 0.05$). Notably, following the ingestion of the glucose drink, brachial artery FMD was significantly reduced below baseline under the placebo condition ($p < 0.05$), but not under the TUDCA condition ($p > 0.05$) (Figure 2C). Baseline brachial artery FMD was not different between visits ($p = 0.3$). FMD corrected for hyperemic shear rate AUC by ANCOVA did not affect the interpretation of these findings (Table 2). Additional brachial artery hemodynamic parameters, as well as plasma measurements of oxidative stress, are summarized in Table 2. Plasma TBARS and nitrotyrosine were unchanged at 60 min after glucose ingestion in both placebo and TUDCA conditions ($p > 0.05$; Table 2).

DISCUSSION

The major novel finding of the present study is that transient endothelial dysfunction caused by an oral glucose load was prevented by a single dose administration of TUDCA. Given that TUDCA is known to act as a chemical chaperone and alleviates ER stress, this study

supports the hypothesis that postprandial hyperglycemia-induced endothelial dysfunction may be in part attributable to ER stress.

It is well established that hyperglycemia is a major underlying contributor to cardiovascular disease (3, 12, 13, 14, 41, 51, 60, 63). Notably, consumption of excess added sugars, a common behavior in the Western world (32), leads to acute hyperglycemia which is considered a better predictor of future cardiovascular disease events than fasting glycemia in healthy and diabetic populations (13, 15, 42, 43, 62). It should be noted that the increased cardiovascular disease risk associated with postprandial hyperglycemia occurs with levels of glycemia below the cutoff for diabetes or impaired glucose tolerance (9, 13, 19, 28, 29, 42). That is, several prospective epidemiologic studies of populations without diabetes indicate that postprandial plasma glucose levels as low as 110 mg/dL are associated with an increased risk of eventual cardiovascular mortality (21, 24, 51). The precise mechanisms by which postprandial hyperglycemia leads to increased cardiovascular disease risk remain unknown, however, it is likely due to the direct detrimental effect of high glucose on the artery wall. In this regard, there is accumulating evidence that postprandial hyperglycemia following an oral glucose drink causes endothelial dysfunction (4, 7, 12, 13, 15, 44, 45, 47, 51, 53), a central feature and early event in the development of atherosclerosis (44, 45, 53). These detrimental endothelial effects of postprandial hyperglycemia have been documented in healthy adults, as well as in patients with glucose intolerance and type 2 diabetes; and have also been recently summarized in a meta-analysis study (43).

Although reducing the consumption of sugary foods and drinks would be the most logical approach at preventing the vascular burden associated with postprandial hyperglycemia, current trends indicate that this behavioral change is unlikely to occur (32). Because the vasculature is frequently exposed to postprandial hyperglycemia, an understanding of the mechanisms by which acute glucotoxicity suppresses endothelial cell function is of paramount importance for development of strategies that confer vascular protection. During acute hyperglycemia, increased oxidative stress has been proposed as a key trigger of endothelial dysfunction by reducing NO production or bioavailability (7, 13, 15, 38, 43, 44). In support of this idea, administration of antioxidant vitamins C and E prevent postprandial hyperglycemia-induced endothelial dysfunction in healthy subjects (47, 61). Current data from cell culture and rodents indicate that ER stress is implicated in hyperglycemia-induced oxidative stress (1, 17, 45, 48, 54, 57). Markers of ER stress are elevated with acute and chronic hyperglycemia (40, 48, 54) and ER stress is now considered to be the basis of a number of chronic metabolic and cardiovascular diseases (11, 18, 20, 22, 23, 25, 26). In endothelial cells, ER stress is associated with a pro-atherogenic phenotype characterized by a redox imbalance, inflammation, apoptosis, and endothelial dysfunction (5, 17, 18, 20, 26, 39). Importantly, alleviation of ER stress with TUDCA has been shown to reduce oxidative stress in cultured cells and *in vivo* in rodent models (1, 48). Indeed, evidence from rodent studies demonstrate that TUDCA protects against ER stress in several tissues including brain, liver, adipose tissue, pancreas, heart, skeletal muscle, and vasculature (1, 16, 33, 36, 48, 64, 65). For example, recent data indicate that inhibition of ER stress with TUDCA improves coronary endothelial function in hyperglycemic type 2 diabetic mice (17).

Given that TUDCA can be safely administered to humans (6, 8, 33, 36, 56), here we conducted the first human study to investigate the vascular effects of TUDCA. Consistent with the notion that hyperglycemia induced-ER stress causes endothelial dysfunction, we found that oral TUDCA administration prevented the impairment in brachial artery FMD following an oral glucose challenge. Of note, TUDCA did not alter the glycemic and insulinemic responses to the glucose drink suggesting that vascular protection afforded by TUDCA was not attributed to a lessened hyperglycemic insult to the vasculature. To examine whether TUDCA-mediated preservation of endothelial function following the oral glucose load was associated with reduced oxidative stress, we examined plasma levels of TBARS and nitrotyrosine, two common markers of oxidative stress. We found no evidence of increased plasma TBARS and nitrotyrosine in response to the glucose test under the placebo condition, nor an effect of TUDCA on these markers. However, the observation that postprandial hyperglycemia did not increase plasma markers of oxidative stress cannot be used as evidence that vascular oxidative stress was not manifested. That is, it is possible that hyperglycemia increases production of ROS in vascular endothelial cells but that common markers of oxidative stress assessed in plasma are not reflective of this effect. In this regard, previous studies have also reported a lack of an increase in plasma markers of oxidative stress following the same oral glucose challenge, despite a reduction in FMD that was prevented with oral antioxidants (46, 62).

Therefore, while it is possible that ER stress-mediated vascular oxidative stress is responsible for the impairment in endothelial function following an oral glucose load, other potential mechanisms should be considered. Previous work by Sage et al. (54) found that 60 min after the same oral glucose load of 75 g in healthy subjects, circulating leukocytes exhibited an increased expression of ER stress markers. This time point corresponds with our first measurement of postprandial FMD where a decline was noted under the placebo condition. With regards to this timing, we recently showed that 60 min of ER stress, induced pharmacologically via tunicamycin, was sufficient to impair endothelial function in isolated rat aortic rings and this effect was associated with a marked production of endothelin-1 (49). Importantly, we found that inhibition of endothelin-1 receptors abrogated endothelial dysfunction caused by ER stress, suggesting that ER stress-induced endothelial dysfunction may be attributed to increased endothelin-1 signaling (49). Taken together, it is possible that in the present study, impaired endothelial function following an oral glucose load is mediated by ER stress-induced production of ET-1 and not ROS; however, additional studies are needed to examine this interesting hypothesis. In addition, it is also possible that reduced endothelial function during the postprandial period may be related to ER stress-mediated changes in intracellular Ca²⁺ homeostasis and subsequent changes in eNOS activity (27).

Discussion of several considerations for the overall interpretation of the current findings is warranted. First, while previous *in vitro* and *in vivo* studies demonstrated that TUDCA alleviates ER stress, this study did not include measures of ER stress, in part because plasma markers of ER stress are unavailable. Future human studies using skeletal muscle or endothelial cell biopsies should determine whether the protective vascular effect of TUDCA against postprandial hyperglycemia is indeed associated with a reduction in vascular ER stress. In addition, this study included only healthy young subjects, thus the generalizability of the findings remain limited to this population. Future studies should examine if TUDCA

also prevents hyperglycemia-induced endothelial dysfunction in patients with glucose intolerance and diabetes as well as determine if chronic TUDCA administration improves basal vascular function in these patients as previously shown in rodents (17).

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ABBREVIATIONS LIST

AUC	area under the curve
BP	blood pressure
ER	endoplasmic reticulum
FMD	flow-mediated dilation
NO	nitric oxide
ROS	reactive oxygen species
TBARS	thiobarbituric acid reactive substances
TUDCA	tauroursodeoxycholic acid
UDCA	ursodeoxycholic acid

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SUMMARY STATEMENT

The key finding of this study is that vascular dysfunction caused by an oral glucose load can be prevented by a single dose oral administration of tauroursodeoxycholic acid, a compound known to alleviate endoplasmic reticulum stress.

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Clinical Perspectives

- We found for the first time that endothelial dysfunction caused by an oral glucose load was abrogated by oral administration of TUDCA in healthy subjects.
- TUDCA is known to act as a chemical chaperone and alleviates ER stress, this study supports the hypothesis that postprandial hyperglycemia-induced endothelial dysfunction may be in part attributable to ER stress.
- Future studies should now examine the therapeutic potential of TUDCA in patients with insulin resistance, diabetes and associated cardiovascular complications.

Figure 1. Experimental design and timeline

A: Schematic diagram of experimental protocol and measurements over the course of the study. Subjects were given either tauroursodeoxycholic acid (TUDCA) or placebo capsules 8 hours prior to arrival to the laboratory where experimental measurements were then taken at time points throughout the oral glucose load. FMD: flow-mediated dilation; BP: blood pressure; IV, intravenous.

Figure 2. Plasma analysis and flow-mediated dilation (FMD) in response to an oral glucose load
 A: Plasma tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA) levels in samples taken at baseline. B: Plasma insulin and glucose levels measured at baseline (BL) and 30, 60, 90, 120 min after the oral glucose load. C: Brachial artery FMD performed at BL, 60, and 120 min from oral glucose load after administration of either TUDCA or placebo capsules. Data are expressed at means $\pm$ SE. * $p < 0.05$ vs. baseline.

Table 1

Subject characteristics.

Age (years)	26 ± 2.5
Sex (M/F)	7/5
Body Mass Index (kg/m²)	23 ± 1.4
Total Body Fat (%)	28 ± 3
LDL (mg/dL)	87.42 ± 12.37
HDL (mg/dL)	48.50 ± 2.87
Total Cholesterol (mg/dL)	147.92 ± 5.94
Triglycerides (mg/dL)	87.44 ± 12.37
Systolic Blood Pressure (mmHg)	122 ± 2
Diastolic Blood Pressure (mmHg)	73 ± 2

Values are means ± SE. Blood lipids and blood pressure measures were obtained under the placebo condition prior to ingestion of the glucose drink.

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein

Table 2

Brachial artery hemodynamics as well as plasma measurements of oxidative stress before and after the oral glucose load.

		Time after oral glucose ingestion		
		0hr	1hr	2hr
Resting blood flow (ml/min)	Placebo	43.9 ± 14.2	27.1 ± 7.9	35.5 ± 11.4
	TUDCA	38.5 ± 10.9	29.4 ± 10.4	39.8 ± 24.7
Hyperemic Shear Rate AUC (A.U.)	Placebo	73568 ± 14880	61295 ± 14459	80310 ± 15511
	TUDCA	96613 ± 18926	62734 ± 14075	99957 ± 44649
Basal Diameter (cm)	Placebo	0.59 ± 0.02	0.58 ± 0.01	0.57 ± 0.03
	TUDCA	0.61 ± 0.03	0.59 ± 0.02	0.60 ± 0.02
ANCOVA- Corrected FMD (%)	Placebo	6.96 ± 0.74	4.72 ± 0.74 *	5.03 ± 0.78
	TUDCA	5.75 ± 0.74	5.50 ± 0.74	5.80 ± 0.74
Nitrotyrosine (μmol/L)	Placebo	63.83 ± 24.11	85.69 ± 38.85	
	TUDCA	67.05 ± 25.86	50.46 ± 8.36	
TBARs (μmol/L)	Placebo	2.03 ± 0.13	1.75 ± 0.16	
	TUDCA	2.16 ± 0.19	2.27 ± 0.21	

Values are means ± SE. ANCOVA- corrected flow-mediated dilation (FMD) data are adjusted for hyperemic shear rate area under the curve (AUC).

*
p<0.05 vs. Baseline (0hr).

Abbreviations: A.U., arbitrary units; TBARs, thiobarbituric acid reactive substances; TUDCA, tauroursodeoxycholic acid.