

The University of Akron  
IdeaExchange@UAkron

---

Chemical and Biomolecular Engineering Faculty  
Research

Chemical and Biomolecular Engineering  
Department

---

Fall 10-6-2014

# Hepatic Carboxylesterase 1 Is Induced by Glucose and Regulates Postprandial Glucose Levels

Jiesi Xu

Liya Yin

Yang Xu


Yuanyuan Li

Munaf Zalzal

*See next page for additional authors*

Please take a moment to share how this work helps you [through this survey](#). Your feedback will be important as we plan further development of our repository.

Follow this and additional works at: [http://ideaexchange.uakron.edu/chemengin\\_ideas](http://ideaexchange.uakron.edu/chemengin_ideas)

 Part of the [Biomedical Engineering and Bioengineering Commons](#), [Chemicals and Drugs Commons](#), and the [Pharmacy and Pharmaceutical Sciences Commons](#)

---

## Recommended Citation

Xu, Jiesi; Yin, Liya; Xu, Yang; Li, Yuanyuan; Zalzal, Munaf; Cheng, Gang; and Zhang, Yanqiao, "Hepatic Carboxylesterase 1 Is Induced by Glucose and Regulates Postprandial Glucose Levels" (2014). *Chemical and Biomolecular Engineering Faculty Research*. 583.

[http://ideaexchange.uakron.edu/chemengin\\_ideas/583](http://ideaexchange.uakron.edu/chemengin_ideas/583)

This Article is brought to you for free and open access by Chemical and Biomolecular Engineering Department at IdeaExchange@UAkron, the institutional repository of The University of Akron in Akron, Ohio, USA. It has been accepted for inclusion in Chemical and Biomolecular Engineering Faculty Research by an authorized administrator of IdeaExchange@UAkron. For more information, please contact [mjon@uakron.edu](mailto:mjon@uakron.edu), [uapress@uakron.edu](mailto:uapress@uakron.edu).

---

**Authors**

Jiesi Xu, Liya Yin, Yang Xu, Yuanyuan Li, Munaf Zalzal, Gang Cheng, and Yanqiao Zhang

# Hepatic Carboxylesterase 1 Is Induced by Glucose and Regulates Postprandial Glucose Levels

Jiesi Xu, Liya Yin, Yang Xu, Yuanyuan Li, Munaf Zalzal, Gang Cheng, Yanqiao Zhang

Published: October 6, 2014 • DOI: 10.1371/journal.pone.0109663

## Abstract

Metabolic syndrome, characterized by obesity, hyperglycemia, dyslipidemia and hypertension, increases the risks for cardiovascular disease, diabetes and stroke. Carboxylesterase 1 (CES1) is an enzyme that hydrolyzes triglycerides and cholesterol esters, and is important for lipid metabolism. Our previous data show that over-expression of mouse hepatic CES1 lowers plasma glucose levels and improves insulin sensitivity in diabetic *ob/ob* mice. In the present study, we determined the physiological role of hepatic CES1 in glucose homeostasis. Hepatic CES1 expression was reduced by fasting but increased in diabetic mice. Treatment of mice with glucose induced hepatic CES1 expression. Consistent with the *in vivo* study, glucose stimulated CES1 promoter activity and increased acetylation of histone 3 and histone 4 in the CES1 chromatin. Knockdown of ATP-citrate lyase (ACL), an enzyme that regulates histone acetylation, abolished glucose-mediated histone acetylation in the CES1 chromatin and glucose-induced hepatic CES1 expression. Finally, knockdown of hepatic CES1 significantly increased postprandial blood glucose levels. In conclusion, the present study uncovers a novel glucose-CES1-glucose pathway which may play an important role in regulating postprandial blood glucose levels.

**Citation:** Xu J, Yin L, Xu Y, Li Y, Zalzal M, et al. (2014) Hepatic Carboxylesterase 1 Is Induced by Glucose and Regulates Postprandial Glucose Levels. PLoS ONE 9(10): e109663. doi:10.1371/journal.pone.0109663

**Editor:** Tianru Jin, University of Toronto, Canada

**Received:** July 6, 2014; **Accepted:** September 12, 2014; **Published:** October 6, 2014

**Copyright:** © 2014 Xu et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability:** The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

**Funding:** This work was supported by National Institutes of Health (NIH) grants R01HL103227 and R01DK095895 to Y.Z. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** Yanqiao Zhang is a PLOS ONE Editorial Board member. However, this does not alter the authors' adherence to PLOS ONE Editorial policies and criteria.

## Introduction

Metabolic syndrome refers to a group of metabolic disturbances, including obesity, hyperglycemia, dyslipidemia and hypertension, which increase the risks for cardiovascular disease, diabetes and stroke. The prevalence of metabolic syndrome is estimated to be 34% of the U.S. adult population and the prevalence has risen since people adopt the unhealthy dietary and inactive lifestyle. Lipid and glucose metabolism is tightly regulated in the body by modulating their dietary intake, transport, synthesis, storage and elimination. Any disturbances of these metabolic processes may increase the risks for metabolic diseases.

Carboxylesterase 1 (CES1) is a drug-metabolizing enzyme that is highly expressed in the liver but also to a lesser extent in the intestine, macrophages and other tissues. CES1 catalyzes the hydrolytic reaction with the release of alcohol substituent and acyl group-containing molecule from the substrate [1]. CES1 possesses triglyceride (TG) and cholesterol ester (CE) hydrolase activity [2], [3], [4], [5], and is shown to play an important role in regulating lipid metabolism [4], [5], [6], [7], [8]. *Ces1<sup>-/-</sup>* mice developed obesity, fatty liver, hyperinsulinemia, and insulin insensitivity, thus highlighting the importance of CES1 in lipid metabolism [6]. CES1 has been shown to prevent TG accumulation in rat McArdle-RH7777 hepatocytes [4]. The intestinal CES1 regulates chylomicron assembly, secretion and clearance [9]. Our recent data show that adenovirus-mediated over-expression of mouse CES1 (Ad-CES1) lowers hepatic TG and plasma glucose levels in both wild-type and diabetic mice and improves glucose tolerance in diabetic mice [5]. These latter data indicate that CES1 plays an important role in glucose metabolism.

Glucose is a major energy source for the body to cope with nutrient deprivation [10]. Blood glucose level is tightly controlled to maintain systemic glucose homeostasis [10]. When blood glucose level rises, a number of events, including glucose transport, glycolysis, glycogenesis, lipogenesis and gluconeogenesis, are coordinately regulated to keep blood glucose level within a normal range [11], [12].

Glucose has been shown to directly regulate the transcription of genes involved in the conversion of carbohydrates to lipids in the liver [13], [14]. Carbohydrate response element-binding protein (ChREBP) mediates glucose-induced gene expression in the liver [15], [16], [17]. In response to glucose stimulation, ChREBP is activated and binds to carbohydrate response element (ChoRE) in the promoter of liver-type pyruvate kinase (*L-PK*), a glycolytic gene, and lipogenic genes, including acetyl-CoA carboxylase (*ACC*) and fatty acid synthase (*FAS*). In contrast, ChREBP is inactivated under starvation conditions, suggesting that ChREBP can sense blood glucose levels [18]. Liver X receptor (LXR) is reported to be another glucose sensor, which integrates hepatic glucose metabolism and fatty acid synthesis [19]. Recent data have shown that glucose induction of gene transcription is associated with epigenetic modifications of histone [20], [21]. ATP-citrate lyase (*ACL*) converts glucose-derived citrate to acetyl-CoA, a substrate for histone acetyltransferases which acetylate the lysine residue (K) in the histone 3 (H3) and H4 tails [21]. Therefore, nuclear ACL is required for glucose-mediated histone acetylation and gene activation [21], [22].

Modulation of postprandial glucose levels plays an important role in glycemic control. In humans, postprandial glucose levels peak at ~ 1 h after the start of the meal and then return to preprandial levels within 2–3 h [23], [24]. A link between high postprandial glycemic levels and the development of cardiovascular diseases (CVD) and diabetes underscores the significance of controlling postprandial glucose [25]. Our previous data [5] and data from Quiroga *et al.* [6] suggest that CES1 may play a role in the control of blood glucose levels. Whether CES1 regulates postprandial glucose levels remains unknown. In the present study, we determined the reciprocal regulation between glucose and the expression of mouse CES1 (also called *Ces1g*, *Es-x*) as well as the role of hepatic CES1 in postprandial glucose control. Our data suggest a novel glucose-CES1-glucose pathway that may play an important role in regulating postprandial glucose levels.

## Material and Methods

### Mice, diets and gavage

C57BL/6 mice, *ob/ob* mice and *db/db* mice were purchased from the Jackson Laboratories (Bar Harbor, ME). High fat/high cholesterol (HFHC) diet (21% kcal from fat, 1.5% cholesterol) was purchased from Research Diets (cat #D12108, New Brunswick, NJ). For glucose treatment, C57BL/6 mice were fasted 16 h, and 40% glucose (8 g/kg) was administered twice with 3 hours interval through oral gavage. Unless otherwise stated, male mice were used and all mice were fasted for 5–6 hours prior to euthanization using Isoflurane (Henry Schein, NY). All the animal studies have been approved by the Institutional Animal Care and Use Committee at Northeast Ohio Medical University.

### RNA isolation and quantitative real-time PCR

Total RNA was isolated using TRIzol Reagent (Life Technologies, NY). Reverse transcription and qPCR were performed as described previously [26]. Relative mRNA levels were calculated using the comparative cycle threshold ( $C_T$ ) method with experimental values normalized to the values of 36B4 (ribosomal protein, large, P0; Rplp0) [27].

### Primary hepatocyte isolation

Mouse primary hepatocytes were isolated as described previously [28], [29]. In brief, mice were anaesthetized by intraperitoneal injection of 50 mg/kg pentobarbital. The portal vein was cannulated with a 23-gauge plastic cannula. Mouse livers were perfused with perfusion buffer I containing 10 mM HEPES, 0.15 M NaCl, 0.42 g/L KCl, 0.99 g/L glucose, 2.1 g/L NaHCO<sub>3</sub>, and 0.19 g/L EDTA. Simultaneously, the inferior vena cava was cut open. Subsequently, livers were perfused with collagenase buffer containing CaCl<sub>2</sub> and collagenase (Sigma; St. Louis, MO). Primary hepatocytes were released from the Glisson capsule and collected in 50 mL centrifuge tubes. After serial centrifugations and washings, cells were cultured in 6-well plate pre-coated with collagen in 2 mL of DMEM supplemented with 10% FBS.

### Chromatin immunoprecipitation (ChIP) assay

200 mg liver tissues were used for ChIP assays as described previously [5], [30], [31] and following the manufacturer's instructions (cat# 17-295, Millipore, MA). Antibodies against acetyl-H3 (Lys9) and acetyl-H4 (Lys16) (Cell Signaling Technology, MA) were used to immuno-precipitate chromatin. Normal IgG was used as a measure of nonspecific background in immunoprecipitation. Chromatin purified from 10% sonicated tissue lysate was used as "input". Real-time PCR was performed to test the chromatin enrichment in the CES1 promoter region. Chromatin enrichment was determined based on the fold change between critical threshold ( $C_T$ ) of specific antibody and  $C_T$  of normal IgG [ $\Delta C_T = C_T(\text{specific antibody}) - C_T(\text{normal IgG})$ ]. The derived  $\Delta C_T$  was then normalized to  $C_T$  of the input. The primer sequences are AACTCTAGGTCGGTGTGAC (forward) and TGCCCCACAGCTATAAACTC (reverse), which amplified a fragment between -148 bp and -14 bp in the *Ces1* gene promoter.

### Western blotting

Tissues were homogenized in ice-cold modified RIPA buffer and protein concentrations were determined using a Pierce BCA Protein Assay Kit (Thermo Scientific, IL). Western blotting was performed as described previously [30]. An antibody against mouse CES1 (*Ces1g*) was purchased from Abcam (Cambridge, MA). Antibodies against P-AKT, and AKT were purchased from Cell Signaling Technology.

### Recombinant adenovirus

Adenoviruses expressing ChREBP [32], *Ces1* (*Ces1g*, *Es-x*) [5], sh*Ces1* (*Ces1g*, *Es-x*) [5] and sh*AcI* [33] have been described previously. Adenoviruses were grown in 293A cells, followed by subsequent purification by cesium chloride gradient centrifugation. About  $1-2 \times 10^9$  plaque formation units (pfu) of adenoviruses were injected into each mouse intravenously.

### Transient transfection assays

Transient transfections were performed in triplicate as described [34]. Briefly, pGL3-Ces1 luciferase reporter constructs were transfected into HepG2 cells, followed by treatment with either 5.5 mM glucose or 27.5 mM glucose. After 36 h, luciferase activities were determined and normalized to  $\beta$ -galactosidase activity.

## Plasma glucose analysis

Plasma glucose levels were measured using Infinity reagent from Thermo Scientific (Waltham, MA) or a glucometer (Onetouch).

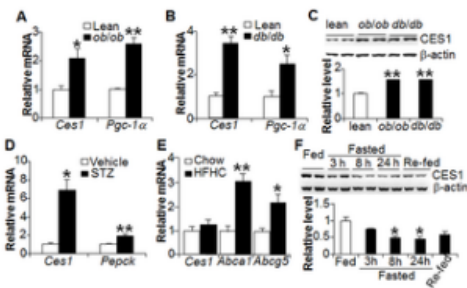
## Statistical Method

The data were analyzed statistically using unpaired Student's *t*-test (two-tailed) and ANOVA (for more than two groups), followed by a post hoc Newman-Keuls test. The data were expressed as mean $\pm$ SE. Only  $p < 0.05$  was considered statistically significant.

## Results

### Hepatic CES1 is regulated by nutritional status

To determine whether nutritional status affects CES1 expression, we first determined hepatic CES1 expression in diabetic mice. Our data indicated that hepatic *Ces1* mRNA (Figure 1A, B) and protein (Figure 1C) levels were significantly induced in both diabetic *ob/ob* mice and *db/db* mice. *ob/ob* mice and *db/db* mice are type 2 diabetes mouse models. Therefore, we also investigated the expression of CES1 in a type 1 diabetes mouse model. In streptozotocin (STZ)-treated mice, hepatic *Ces1* mRNA levels were induced by >7 fold (Figure 1D). Peroxisome proliferator-activated receptor gamma coactivator-1  $\alpha$  (PGC-1 $\alpha$ ) and phosphoenolpyruvate carboxykinase (PEPCK) served as positive controls. To investigate whether hepatic CES1 expression is affected by a Western diet, we fed C57BL/6 mice a high fat/high cholesterol (HFHC) diet for 3 weeks; the data show that treatment with an HFHC diet for a short time did not regulate hepatic *Ces1* expression (Figure 1E). ATP-binding cassette (ABC) transporter A1 (ABCA1) and ABC transporter G5 (ABCG5), which are known to be induced by dietary cholesterol [35], served as positive controls. Finally, we investigated the effect of fasting on hepatic CES1 expression. The data of Figure 1F show that fasting for 8 or 24 hours caused a reduction in hepatic CES1 protein levels. These data indicated that hepatic CES1 is regulated by nutritional status and glucose may induce hepatic CES1 expression.



**Figure 1. Hepatic CES1 is regulated by nutritional status.**

(A–C) Hepatic mRNA levels in *ob/ob* (A) and *db/db* (B) mice were determined by qRT-PCR and protein levels determined by Western blot assays (C, top) ( $n = 4–6$  mice per group). In (C, bottom), protein levels were also quantified by Image J. (D) C57BL/6 mice were treated with either vehicle (0.1 M sodium citrate, pH 4.5) or streptozotocin (STZ) (50 mg/kg/d) for 5 days. Seven days after STZ treatment, mice were euthanized and hepatic mRNA levels were quantified ( $n = 5$  mice per group). (E) Wild-type mice were fed a chow or high fat/high cholesterol (HFHC) diet (21% fat, 1.5% cholesterol) for 3 weeks and hepatic mRNA levels were determined ( $n = 8$  mice per group). (F) C57BL/6 mice were fed a chow diet, or fasted for 3, 8, 24 h, or fasted for 24 h followed by re-fed for 24 h ( $n = 5$  mice per group). Hepatic protein levels were determined by Western blot assays (top) and then quantified (bottom). *Pgc-1 $\alpha$* , peroxisome proliferator-activated receptor gamma coactivator-1 $\alpha$ . *Abca1*, ATP-binding cassette (ABC) transporter A1. *Abcg5*, ABC transporter G5. *Pepck*, phosphoenolpyruvate carboxykinase. \* $p < 0.05$ , \*\* $p < 0.01$ .  
doi:10.1371/journal.pone.0109663.g001

### Hepatic CES1 is regulated by glucose but not insulin

To test our hypothesis that glucose induces hepatic CES1 expression, saline or glucose (8 g/kg) were administered to C57BL/6 mice twice with 3 hours interval via oral gavage. Mice were sacrificed 3 hours after second oral gavage and hepatic CES1 expression was determined. Hepatic *Ces1* mRNA levels (Figure 2A) and protein levels (Figure 2B, C) were induced by 2 fold in response to glucose stimulation. In contrast, insulin did not induce hepatic CES1 expression (Figure 2D).

- [View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
24. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, et al. (1988) Abnormal Patterns of Insulin-Secretion in Non-Insulin-Dependent Diabetes-Mellitus. *New England Journal of Medicine* 318: 1231–1239. doi: 10.1056/nejm198805123181903  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
25. Bell DS (2001) Importance of postprandial glucose control. *South Med J* 94: 804–809. doi: 10.1097/00007611-200194080-00011  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
26. Zhang YQ, Ge XM, Heemstra LA, Chen WD, Xu JS, et al. (2012) Loss of FXR Protects against Diet-Induced Obesity and Accelerates Liver Carcinogenesis in ob/ob Mice. *Molecular Endocrinology* 26: 272–280. doi: 10.1210/me.2011-1157  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
27. Thompson GM, Trainor D, Biswas C, LaCerte C, Berger JP, et al. (2004) A high-capacity assay for PPARgamma ligand regulation of endogenous aP2 expression in 3T3-L1 cells. *Analytical Biochemistry* 330: 21–28. doi: 10.1016/j.ab.2004.03.061  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
28. Mastrodonato M, Calamita G, Rossi R, Mentino D, Bonfrate L, et al. (2011) Altered distribution of caveolin-1 in early liver steatosis. *European Journal of Clinical Investigation* 41: 642–651. doi: 10.1111/j.1365-2362.2010.02459.x  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
29. Edwards M, Houseman L, Phillips IR, Shephard EA (2013) Isolation of mouse hepatocytes. *Methods Mol Biol* 987: 283–293. doi: 10.1007/978-1-62703-321-3\_24  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
30. Yin L, Ma H, Ge X, Edwards PA, Zhang Y (2011) Hepatic hepatocyte nuclear factor 4alpha is essential for maintaining triglyceride and cholesterol homeostasis. *Arterioscler Thromb Vasc Biol* 31: 328–336. doi: 10.1161/atvbaha.110.217828  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
31. Ge X, Yin L, Ma H, Li T, Chiang JY, et al. (2011) Aldo-keto reductase 1B7 is a target gene of FXR and regulates lipid and glucose homeostasis. *J Lipid Res* 52: 1561–1568. doi: 10.1194/jlr.m015859  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
32. Ma L, Tsatsos NG, Towle HC (2005) Direct role of ChREBP.Mlx in regulating hepatic glucose-responsive genes. *J Biol Chem* 280: 12019–12027. doi: 10.1074/jbc.m413063200  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
33. Wang Q, Li S, Jiang L, Zhou Y, Li Z, et al. (2010) Deficiency in hepatic ATP-citrate lyase affects VLDL-triglyceride mobilization and liver fatty acid composition in mice. *J Lipid Res* 51: 2516–2526. doi: 10.1194/jlr.m003335  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
34. Ge XM, Yin LY, Ma HY, Li TG, Chiang JYL, et al. (2011) Aldo-keto reductase 1B7 is a target gene of FXR and regulates lipid and glucose homeostasis. *Journal of Lipid Research* 52: 1561–1568. doi: 10.1194/jlr.m015859  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
35. Oram JF, Vaughan AM (2006) ATP-Binding cassette cholesterol transporters and cardiovascular disease. *Circ Res* 99: 1031–1043. doi: 10.1161/01.res.0000250171.54048.5c  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
36. Dentin R, Pegorier JP, Benhamed F, Foufelle F, Ferre P, et al. (2004) Hepatic glucokinase is required for the synergistic action of ChREBP and SREBP-1c on glycolytic and lipogenic gene expression. *Journal of Biological Chemistry* 279: 20314–20326. doi: 10.1074/jbc.m312475200  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
37. Brasacchio D, Okabe J, Tikellis C, Balcerczyk A, George P, et al. (2009) Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that coexist on the lysine tail. *Diabetes* 58: 1229–1236. doi: 10.2337/db08-1666  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
38. Villeneuve LM, Reddy MA, Lanting LL, Wang M, Meng L, et al. (2008) Epigenetic histone H3 lysine 9 methylation in metabolic memory and inflammatory phenotype of vascular smooth muscle cells in diabetes. *Proceedings of the National Academy of Sciences of the United States of America* 105: 9047–9052. doi: 10.1073/pnas.0803623105  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
39. Zhang T, Zhao S, Li W, Ma L, Ding M, et al. (2014) High-fat diet from perilla oil induces insulin resistance despite lower serum lipids and increases hepatic fatty acid oxidation in rats. *Lipids in Health and Disease* 13: 15. doi: 10.1186/1476-511x-13-15  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
40. Rachek LI (2014) Free fatty acids and skeletal muscle insulin resistance. *Prog Mol Biol Transl Sci* 121: 267–292. doi: 10.1016/b978-0-12-800101-1.00008-9  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)

41. Xiong J, Shang W, Wu L, Chen R, Liu W, et al. (2014) Glucose dominates the regulation of carboxylesterases induced by lipopolysaccharide or interleukin-6 in primary mouse hepatocytes. *Life Sci* 112: 41–48. doi: [10.1016/j.lfs.2014.07.019](https://doi.org/10.1016/j.lfs.2014.07.019)  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)

of hepatic CES1 increases hepatic TG levels through increasing lipogenesis [5]. Lipid homeostasis has a profound impact on insulin sensitivity and glucose metabolism. Plasma free fatty acid (FFA) levels correlate negatively to the degree of insulin sensitivity [39], [40]. Due to the regulatory role of CES1 in TG hydrolysis and FAO, it is not surprising to see that increased hepatic CES1 expression lowers plasma glucose levels and improves insulin sensitivity. In the present study, we investigated the regulation of hepatic CES1 by glucose and the physiological role of such regulation. Our data reveal a novel role for hepatic CES1 in postprandial glucose control.

Poor control of postprandial glucose is a significant contributor to type 2 diabetes mellitus. Persistent, moderate increase in postprandial glucose levels is a significant risk factor for macrovascular complications, and is more indicative of atherosclerosis than fasting glucose [23], [24]. In light of the risks of postprandial hyperglycemia for vascular events, tight control of postprandial glucose levels is important for long-term indices of diabetes control. After the start of a meal, blood glucose levels are increased. The increased blood glucose levels induce hepatic CES1 expression, which in turn helps lower blood glucose levels likely by increasing peripheral insulin sensitivity (Figure 5). This conclusion is supported by the findings that increased hepatic CES1 expression lowers plasma glucose levels whereas loss of hepatic CES1 results in increased postprandial blood glucose levels.

We have previously shown that farnesoid X receptor regulates CES1 expression [5]. Our present study shows that hepatic CES1 is also regulated under physiological and pathological conditions. Under these latter conditions, hepatic CES1 expression is altered likely due to the change in plasma glucose levels. Indeed, our data show that glucose induces CES1 expression both *in vitro* and *in vivo*. Consistent with our finding, very recent data by Xiong *et al.* also show that glucose induces the expression of carboxylesterases in mouse primary hepatocytes [41].

Several lines of evidence suggest that glucose regulates gene expression through epigenetic modifications [20] and that nuclear ACL is important for glucose-mediated histone acetylation [21]. In this study, we show that glucose induces CES1 expression by epigenetic modifications of the acetylation status (H3 and H4) of the CES1 chromatin in an ACL-dependent manner. The histone tails interact with a region about 150 bp upstream of the transcription start site of CES1. Consistent with this finding, the data from the luciferase-promoter assays show that this region is required for glucose to induce CES1 promoter activity. Thus, glucose induces CES1 expression via acetylation of H3 and H4 in the CES1 chromatin.

Although our data show that hepatic CES1 is required for regulating postprandial glucose levels, the underlying mechanism remains to be fully determined. Global *Ces1*<sup>-/-</sup> mice have elevated plasma levels of triglycerides, free cholesterol, FFAs and insulin [6]. These mice also display insulin resistance, which results from reduced insulin sensitivity in both skeletal muscle and white adipose tissue [6]. However, CES1 is not expressed in skeletal muscle and its expression level in white adipose tissue is low (data not shown). Thus, insulin resistance observed in global *Ces1*<sup>-/-</sup> mice likely results from a deficiency in hepatic *Ces1*. Consistent with this speculation, our data show that hepatic *Ces1* deficiency results in impaired postprandial glucose clearance. Although hepatic CES1 deficiency may cause hepatic insulin resistance, skeletal muscle and white adipose tissues are the major organs responsible for plasma glucose clearance. Therefore, hepatic *Ces1* deficiency may affect peripheral insulin sensitivity. To precisely understand how hepatic CES1 deficiency regulates glucose homeostasis or insulin sensitivity, hyperinsulinemic-euglycemic clamp studies will be needed to help characterize the underlying mechanism.

In summary, the present study suggests a glucose-CES1-glucose cascade, in which glucose induces hepatic CES1 expression, which in turn lowers blood glucose levels. This cascade plays an important role in regulating postprandial glucose levels. Since high levels of postprandial blood glucose contribute to macrovascular complications, CES1 may be targeted for prevention of vascular diseases associated with hyperglycemia.

## Author Contributions

Contributed reagents/materials/analysis tools: None. None. Conceived and designed the experiments: JX LY GC YZ. Performed the experiments: JX LY YX YL MZ YZ. Analyzed the data: JX YZ. Wrote the paper: JX YZ.

## References

1. Satoh T, Hosokawa M (2006) Structure, function and regulation of carboxylesterases. *Chem Biol Interact* 162: 195–211. doi: 10.1016/j.cbi.2006.07.001  
View Article • PubMed/NCBI • Google Scholar
2. Shimada A, Tamai T, Oida K, Takahashi S, Suzuki J, et al. (1994) Increase in Neutral Cholesteryl Ester Hydrolase Activity Produced by Extralysosomal Hydrolysis of High-Density-Lipoprotein Cholesteryl Esters in Rat Hepatoma-Cells (H-35). *Biochimica Et Biophysica Acta-Lipids and Lipid Metabolism* 1215: 126–132. doi: 10.1016/0005-2760(94)90101-5  
View Article • PubMed/NCBI • Google Scholar
3. Zhao B, Song JM, Chow WN, Clair RWS, Rudel LL, et al. (2007) Macrophage-specific transgenic expression of cholesteryl ester hydrolase significantly reduces atherosclerosis and lesion necrosis in *Ldlr*(-/-) mice. *Journal of Clinical Investigation* 117: 2983–2992. doi: 10.1172/jci30485  
View Article • PubMed/NCBI • Google Scholar
4. Ko KWS, Erickson B, Lehner R (2009) *Es-x/Ces1* prevents triacylglycerol accumulation in McArdle-RH7777 hepatocytes. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids* 1791: 1133–1143. doi: 10.1016/j.bbali.2009.07.006  
View Article • PubMed/NCBI • Google Scholar
5. Xu J, Li Y, Chen W, Xu Y, Yin L, et al. (2013) Hepatic carboxylesterase 1 is essential for both normal and farnesoid X-receptor-controlled lipid homeostasis. *Hepatology* 59: 1761–1771. doi: 10.1002/hep.26714  
View Article • PubMed/NCBI • Google Scholar



6. Quiroga AD, Li L, Trotsmuller M, Nelson R, Proctor SD, et al. (2012) Deficiency of carboxylesterase 1/esterase-x results in obesity, hepatic steatosis, and hyperlipidemia. *Hepatology* 56: 2188–2198. doi: 10.1002/hep.25961  
View Article • PubMed/NCBI • Google Scholar
7. Jemas M, Olsson B, Arner P, Jacobson P, Sjostrom L, et al. (2009) Regulation of carboxylesterase 1 (CES1) in human adipose tissue. *Biochem Biophys Res Commun* 383: 63–67. doi: 10.1016/j.bbrc.2009.03.120  
View Article • PubMed/NCBI • Google Scholar
8. Bie J, Wang J, Yuan Q, Kakiyama G, Ghosh SS, et al. (2014) Liver-specific transgenic expression of cholesteryl ester hydrolase reduces atherosclerosis in Ldlr<sup>-/-</sup> mice. *Journal of Lipid Research* 55: 729–738. doi: 10.1194/jlr.m046524  
View Article • PubMed/NCBI • Google Scholar
9. Quiroga AD, Lian JH, Lehner R (2012) Carboxylesterase1/Esterase-x Regulates Chylomicron Production in Mice. *Plos One* 7.
10. Rodgers JT, Puigserver P (2007) Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. *Proceedings of the National Academy of Sciences of the United States of America* 104: 12861–12866. doi: 10.1073/pnas.0702509104  
View Article • PubMed/NCBI • Google Scholar
11. Vaulont S, Daines S, Evans M (1995) Disruption of the Adenosine-Deaminase (Ada) Gene Using a Dicistronic Promoterless Construct - Production of an Ada-Deficient Homozygote Es Cell-Line. *Transgenic Research* 4: 247–255. doi: 10.1007/bf01969118  
View Article • PubMed/NCBI • Google Scholar
12. Kannu PD, M JT (1999) Lightning induced voltages on conductors interlinking the towers of a satellite launch pad. *Proceeding of the International Conference on Electromagnetic Interference and Compatibility '99*: 479–484.  
View Article • PubMed/NCBI • Google Scholar
13. Girard J, Ferre P, Fougelle F (1997) Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. *Annual Review of Nutrition* 17: 325–352. doi: 10.1146/annurev.nutr.17.1.325  
View Article • PubMed/NCBI • Google Scholar
14. Towle HC, Kaytor EN, Shih HM (1997) Regulation of the expression of lipogenic enzyme genes by carbohydrate. *Annual Review of Nutrition* 17: 405–433. doi: 10.1146/annurev.nutr.17.1.405  
View Article • PubMed/NCBI • Google Scholar
15. Ma L, Robinson LN, Towle HC (2006) ChREBP center dot Mlx is the principal mediator of glucose-induced gene expression in the liver. *Journal of Biological Chemistry* 281: 28721–28730. doi: 10.1074/jbc.m601576200  
View Article • PubMed/NCBI • Google Scholar
16. Uyeda K, Repa JJ (2006) Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metabolism* 4: 107–110. doi: 10.1016/j.cmet.2006.06.008  
View Article • PubMed/NCBI • Google Scholar
17. Postic C, Dentin R, Denechaud PD, Girard J (2007) ChREBP, a transcriptional regulator of glucose and lipid metabolism. *Annual Review of Nutrition* 27: 179–192. doi: 10.1146/annurev.nutr.27.061406.093618  
View Article • PubMed/NCBI • Google Scholar
18. Kabashima T, Kawaguchi T, Wadzinski BE, Uyeda K (2003) Xylulose 5-phosphate mediates glucose-induced lipogenesis by xylulose 5-phosphate-activated protein phosphatase in rat liver. *Proceedings of the National Academy of Sciences of the United States of America* 100: 5107–5112. doi: 10.1073/pnas.0730817100  
View Article • PubMed/NCBI • Google Scholar
19. Mitro N, Mak PA, Vargas L, Godio C, Hampton E, et al. (2007) The nuclear receptor LXR is a glucose sensor. *Nature* 445: 219–223. doi: 10.1038/nature05449  
View Article • PubMed/NCBI • Google Scholar
20. El-Osta A, Brasacchio D, Yao D, Poci A, Jones PL, et al. (2008) Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia (vol 205, pg 2409, 2008). *Journal of Experimental Medicine* 205: 2683–2683. doi: 10.1084/jem.20081188092608c  
View Article • PubMed/NCBI • Google Scholar
21. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, et al. (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* 324: 1076–1080. doi: 10.1126/science.1164097  
View Article • PubMed/NCBI • Google Scholar
22. Li T, Chanda D, Zhang Y, Choi HS, Chiang JY (2010) Glucose stimulates cholesterol 7 $\alpha$ -hydroxylase gene transcription in human hepatocytes. *Journal of Lipid Research* 51: 832–842. doi: 10.1194/jlr.m002782  
View Article • PubMed/NCBI • Google Scholar
23. Kahn R, Assoc AD (2001) Postprandial blood glucose. *Diabetes Care* 24: 775–778. doi: 10.2337/diacare.24.4.775

- [View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
24. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, et al. (1988) Abnormal Patterns of Insulin-Secretion in Non-Insulin-Dependent Diabetes-Mellitus. *New England Journal of Medicine* 318: 1231–1239. doi: 10.1056/nejm198805123181903  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
25. Bell DS (2001) Importance of postprandial glucose control. *South Med J* 94: 804–809. doi: 10.1097/00007611-200194080-00011  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
26. Zhang YQ, Ge XM, Heemstra LA, Chen WD, Xu JS, et al. (2012) Loss of FXR Protects against Diet-Induced Obesity and Accelerates Liver Carcinogenesis in ob/ob Mice. *Molecular Endocrinology* 26: 272–280. doi: 10.1210/me.2011-1157  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
27. Thompson GM, Trainor D, Biswas C, LaCerte C, Berger JP, et al. (2004) A high-capacity assay for PPARgamma ligand regulation of endogenous aP2 expression in 3T3-L1 cells. *Analytical Biochemistry* 330: 21–28. doi: 10.1016/j.ab.2004.03.061  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
28. Mastrodonato M, Calamita G, Rossi R, Mentino D, Bonfrate L, et al. (2011) Altered distribution of caveolin-1 in early liver steatosis. *European Journal of Clinical Investigation* 41: 642–651. doi: 10.1111/j.1365-2362.2010.02459.x  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
29. Edwards M, Houseman L, Phillips IR, Shephard EA (2013) Isolation of mouse hepatocytes. *Methods Mol Biol* 987: 283–293. doi: 10.1007/978-1-62703-321-3\_24  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
30. Yin L, Ma H, Ge X, Edwards PA, Zhang Y (2011) Hepatic hepatocyte nuclear factor 4alpha is essential for maintaining triglyceride and cholesterol homeostasis. *Arterioscler Thromb Vasc Biol* 31: 328–336. doi: 10.1161/atvbaha.110.217828  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
31. Ge X, Yin L, Ma H, Li T, Chiang JY, et al. (2011) Aldo-keto reductase 1B7 is a target gene of FXR and regulates lipid and glucose homeostasis. *J Lipid Res* 52: 1561–1568. doi: 10.1194/jlr.m015859  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
32. Ma L, Tsatsos NG, Towle HC (2005) Direct role of ChREBP.Mlx in regulating hepatic glucose-responsive genes. *J Biol Chem* 280: 12019–12027. doi: 10.1074/jbc.m413063200  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
33. Wang Q, Li S, Jiang L, Zhou Y, Li Z, et al. (2010) Deficiency in hepatic ATP-citrate lyase affects VLDL-triglyceride mobilization and liver fatty acid composition in mice. *J Lipid Res* 51: 2516–2526. doi: 10.1194/jlr.m003335  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
34. Ge XM, Yin LY, Ma HY, Li TG, Chiang JYL, et al. (2011) Aldo-keto reductase 1B7 is a target gene of FXR and regulates lipid and glucose homeostasis. *Journal of Lipid Research* 52: 1561–1568. doi: 10.1194/jlr.m015859  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
35. Oram JF, Vaughan AM (2006) ATP-Binding cassette cholesterol transporters and cardiovascular disease. *Circ Res* 99: 1031–1043. doi: 10.1161/01.res.0000250171.54048.5c  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
36. Dentin R, Pegorier JP, Benhamed F, Foufelle F, Ferre P, et al. (2004) Hepatic glucokinase is required for the synergistic action of ChREBP and SREBP-1c on glycolytic and lipogenic gene expression. *Journal of Biological Chemistry* 279: 20314–20326. doi: 10.1074/jbc.m312475200  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
37. Brasacchio D, Okabe J, Tikellis C, Balcerzyk A, George P, et al. (2009) Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that coexist on the lysine tail. *Diabetes* 58: 1229–1236. doi: 10.2337/db08-1666  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
38. Villeneuve LM, Reddy MA, Lanting LL, Wang M, Meng L, et al. (2008) Epigenetic histone H3 lysine 9 methylation in metabolic memory and inflammatory phenotype of vascular smooth muscle cells in diabetes. *Proceedings of the National Academy of Sciences of the United States of America* 105: 9047–9052. doi: 10.1073/pnas.0803623105  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
39. Zhang T, Zhao S, Li W, Ma L, Ding M, et al. (2014) High-fat diet from perilla oil induces insulin resistance despite lower serum lipids and increases hepatic fatty acid oxidation in rats. *Lipids in Health and Disease* 13: 15. doi: 10.1186/1476-511x-13-15  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)
40. Rachek LI (2014) Free fatty acids and skeletal muscle insulin resistance. *Prog Mol Biol Transl Sci* 121: 267–292. doi: 10.1016/b978-0-12-800101-1.00008-9  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)

41. Xiong J, Shang W, Wu L, Chen R, Liu W, et al. (2014) Glucose dominates the regulation of carboxylesterases induced by lipopolysaccharide or interleukin-6 in primary mouse hepatocytes. *Life Sci* 112: 41–48. doi: [10.1016/j.lfs.2014.07.019](https://doi.org/10.1016/j.lfs.2014.07.019)  
[View Article](#) • [PubMed/NCBI](#) • [Google Scholar](#)