The effect of cholesteryl ester transfer protein on pancreatic beta cell dysfunction in mice

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Abstract

Background: Cholesterol accumulation causes pancreatic beta cell lipotoxicity and dysfunction. Cholesteryl ester transfer protein (CETP) plays an important role in blood lipid homeostasis. However, its role in tissue lipid metabolism remains unclear. We hypothesized that plasma CETP impact cholesterol homeostasis in the beta cells, thus damaging their functions.

Methods: The adipose tissue-specific CETP expression transgenic (aP2-CETPTg) mice, characterized by high CETP levels in the circulation, were used in this study. Pancreatic islet cholesterol and beta cell function were assessed in mice. We further measured mRNA levels of the genes involved in beta cell proliferation and differentiation, inflammation and cholesterol metabolism. TUNEL assay was applied to investigate beta cell apoptosis in islets.

Results: The aP2-CETPTg mice exhibited glucose intolerance, lower plasma insulin concentrations but increased insulin sensitivity compared with wild type mice. In addition, glucose-stimulated insulin secretion from isolated pancreatic islets significantly decreased, and free cholesterol significantly increased. Moreover, the number and size of islets from aP2-CETPTg mice were significantly decreased. Genes involved in beta cell proliferation, such as Pdx1 and BETA2, were down-regulated; genes involved in inflammation and ER stress, such as IL-1β, CHOP, and Xbp1 were up-regulated, in line with an increase of beta cell apoptosis.

Conclusions: Plasma CETP causes free cholesterol accumulation in islets which could contribute to beta cell dysfunction. Thus, CETP inhibition could be a novel protective strategy for dyslipidemia related to diabetes and obese.

Keywords: Cholesterol ester transfer protein, Free cholesterol, Beta cell function, Glucose metabolism

Background

The increased morbidity, mortality of type 2 diabetes mellitus (T2DM) makes it a crucial public health condition. Islet beta cell dysfunction and insulin resistance are major characteristics of pathophysiology of T2DM. Although insulin resistance occurs earlier during disease onset, beta cell dysfunction predates long before the occurrence of clinical hyperglycemia [1]. Many potential mechanisms for islet beta cell dysfunction have been proposed [2], however it still remains unclear. Lipotoxicity is regarded as one of the possible mechanisms which contribute to beta cell dysfunction. It has been suggested that increased free fatty acids accumulation in beta cells could reduce insulin secretion from beta cell, and induce beta cell apoptosis [3]. Recent studies have indicated that the accumulation of free cholesterol in beta cells may also contribute to lipotoxicity [4] and beta cell dysfunction [5].

Cholesteryl ester transfer protein (CETP) is a hydrophobic plasma glycoprotein that mediates the cholesteryl ester (CE) transfer from high density
lipoprotein (HDL) to very low density lipoprotein (VLDL) or low density lipoprotein (LDL) in exchange for triglycerides, leading to lower HDL concentrations and more small dense-LDL particles [6]. In human, CETP is highly expressed in adipose tissue and liver, where it can be secreted into the blood and then participates in cholesterol metabolism [7]. We speculated that circulating CETP may mediate cholesterol homeostasis in islet beta cells. Some studies indicate that CETP may have additional effects on glucose metabolism other than lipid metabolism. A sub-study of the Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILUMINATE) trial reported that treatment with CETP inhibitor (torcetrapib) improved glycemic control in atorvastatin-treated patients with T2DM [8]. Siebel et al. [9] found that CETP inhibitor increased postprandial insulin and promoted ex vivo beta cell glucose-stimulated insulin secretion, potentially via enhanced beta cell cholesterol efflux. Most clinical studies have shown that CETP mass was increased in patients with T2DM and obesity [10]. CETP activity was positively correlated with fasting blood glucose and HbA1c while negatively correlated with HDL-C in T2DM [11]. Few studies also reported diabetes may suppress hepatic CETP in obese [12, 13]. Our previous study showed that glucose uptake and intracellular cholesterol were increased when 3T3-L1 adipocyte was transfected with CETP [14]. All these studies indicated that CETP might play a role in glucose metabolism.

We previously generated adipose tissue-specific CETP expression transgenic (aP2-CETPTg) mice and demonstrated that this model have the following characteristics: 1) high circulating CETP secreted by adipose tissue; 2) elevated LDL and declined HDL levels compared with WT mice; 3) very low CETP expression in other tissues (including skeletal muscle, liver, lung, kidney, heart, and brain) [15]. Natural flanking region (NFR)-CETPTg mice is another CETP transgenic mouse model with human like CETP concentrations in the circulation and tissue distributions (liver, spleen, small intestine, kidney, and adipose tissue) [16]. Recently, Raposo et al. reported physiological levels of CETP (~2 μg/ml) did not affect glucose homeostasis and insulin secretion [17]. Interestingly, we previously found that over-expressed CETP might modulate glucose metabolism and insulin action in addition to its effects on lipoprotein metabolism in 3T3-L1 adipocytes [14]. In order to investigate whether circulating CETP (other than local CETP expression) and elevated CETP activity play roles in regulation of glucose metabolism and beta cell lipid accumulation, we used aP2-CETPTg mouse to explore the effects of CETP on mouse pancreatic beta cell lipid composition and beta cell function.

**Methods**

**Animals**

The NFR-CETPTg and aP2-CETPTg mice have C57BL/6J background [15]. Transgenic mice and non-transgenic littermates were fed standard rodent chow diet, housed in cages in a pathogen-free facility, and maintained on a 12 h light/12 h dark cycle. All experiments were performed on 10–16 week-old male mice. All animal experiments were conducted in accord with accepted standards of humane animal care, as outlined in the Ethical Guidelines, and were approved by the Research Animal Care Committee of Nanjing Medical University (IACUC-14030136).

**CETP activity measurement**

Mouse plasma CETP activity was measured by a fluorescence method as previously described [15]. Briefly, NBD-CE works as the donor, and ultracentrifugation separated human VLDL/LDL works as the acceptor. Reactions were performed in a 100 μl reaction mixture containing 3 μl plasma sample. Finally, fluorescence were measured by fluorescence microplate (SynergyMx, BioTek, United States) under 460 nm excitation wave and 530 nm emission wave. Plasma CETP activity was presented as detected fluorescence value (arbitrary units)/3 μl plasma).

**Glucose and insulin tolerance test**

Intraperitoneal glucose tolerance test (IPGTT) was performed on 16 h-fasted mice injected with D-glucose at 1 g/kg body weight. Blood glucose levels were measured at 0, 15, 30, 60 and 120 min after glucose injection. Plasma insulin during IPGTT was measured by Mouse Insulin ELISA kit (Millipore-linco, Billerica, MA, USA). Intraperitoneal insulin tolerance test (IPITT) was performed on 5 h-fasted mice injected with recombinant human insulin (Humulin, Eli Lilly and company, China) at 0.75 mU/kg body weight. Blood glucose levels were measured at 0, 15, 30, 60 and 120 min after insulin injection.

**Isolation of pancreatic islets**

Primary mouse islets were isolated by collagenase V (Sigma-Aldrich Chemie, Steinheim, Germany) digestion and filtration as previously described [18]. Islets used for glucose-stimulated insulin secretion measurement were cultured in RPMI-1640 (Gibco, California, USA) supplemented with 10 % FCS (Gibco, California, USA), 100 U/ml penicillin and 100 μg/ml streptomycin. Islets used for cholesterol measurement and real-time PCR were washed with PBS and frozen down immediately after isolation. Isolated islets number counting was conducted by two persons in a double-blinded fashion, referring to the method used by Ishikawa [19].
Glucose-stimulated insulin secretion assay
After overnight culture, islets were washed and used for glucose-stimulated insulin secretion (GSIS). Islets (eight similar-sized islets per well and eight wells per group) were firstly pre-incubated in Krebs-Ringer bicarbonate HEPES buffer supplemented with 0.2 % BSA for 1 h at 37 °C and 5 % CO2, then incubated with KRB/HEPES buffer supplemented with 0.2 % BSA containing 3.3 or 16.7 mmol/l glucose for 1 h [18]. After then, islets were incubated with acid ethanol (1.5 % HCl in 70 % Ethanol) overnight for insulin content measurement. The supernatants were collected after each treatment. Insulin levels were measured by the radioimmunoassay kit (BNIBT, Beijing, China). Insulin secretion ability is calculated by the relative secreted insulin to insulin content.

Plasma lipid measurement
Overnight fasting plasma were collected for plasma lipid measurement. The total cholesterol (TC), free cholesterol (FC) were assayed by enzymatic methods (Applygen Technologies Inc. Beijing, China), according to the instructions provided by the manufacturers. CE content is calculated by TC minus FC. Samples were split into two equal fractions to measure FC and TC separately. For TC measurement, CE was firstly transformed into free cholesterol. Finally, CE concentration is calculated by TC minus FC.

Islet lipid determination
Twenty similar-sized islets were washed five times with PBS and then cellular lipids were extracted by hexane: isopropyl alcohol (2:1 v/v) as described [20], using β-sitosterol (5 μg/ml) as an internal standard. Samples were split into two equal fractions to measure FC and TC separately. For TC measurement, CE was firstly transformed into free cholesterol. Finally, CE content is calculated by TC minus FC. To hydrolyze cellular cholesteryl esters prior to gas chromatography, 50 % KOH (200 μl) and methanol (3 ml) were added to dried aliquots and heated to 80 °C for 1 h in argon-capped tubes. After cooling, 3 ml H2O and 5 ml HPLC grade hexane were added. Samples were centrifuged at 1000 rpm for 5 min and supernatant containing cholesterol was removed and dried under nitrogen. Cholesterol mass was measured by injection of 1 μl aliquots into a 5890 Series II gas chromatograph with a Detector (FID DETECTOR) using a 15 m × 0.53 mm × 1.5 μm cross-linked HP-5 Trace Analysis (5 % Ph Me Siloxane) column. Gas chromatography was performed at an isothermal 260 °C, injection port temperature of 300 °C, and detector temperature of 300 °C with nitrogen carrier flow rate of 30 ml/min. Calibration was achieved by injection of a standard solution of cholesterol (100 μg/ml).

RNA extraction, reverse transcription and gene expression
Total RNA from isolated islets were extracted using the RNAiso Plus (Takara, Dalian, China) and reverse-transcribed into cDNA using the Prime Script RT reagent kit (Takara, Dalian, China). Data for triplex real-time PCR assay were collected with an AB 7500 Real Time PCR system (Applied Biosystems, Inc. United States). Reactions were performed with SYBR Green PCR Master Mix (Takara, Dalian, China) under following thermal cycling conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Conventional PCR reactions were performed in a 20 μl reaction mixture containing 0.5 μmol/L primers and 12.5 mg/L cDNA. Relative gene expression data were analyzed in the 2^ΔΔCT method, and the results were expressed as the expression fold over the control group. In exception, since there is no CETP mRNA expression in WT mice, the relative CETP mRNA of adipose tissue from aP2-CETPTg mice and NFR-CETPTg mice and of islets from NFR-CETPTg mice were expressed as 2^ΔCT. The following primer sequences were used:

- CETP310-A, 5′-TAGTGTTTACACCCCTCATCAGAAA GAAG- 3′;
- CETP310-B, 5′-CTCCATCTCCGTACTCTAACCACAA CTTC- 3′.
- Ki-67 forward, 5′-ATCCATTGACCCTCTTCTTAGG T- 3′;
- Pdx1 forward, 5′-GATGAAATCCACCAAGACTC- 3′;
- Pdx1 reverse, 5′-AGAATTCCTTCTCAGCTCCA - 3′.
- BETA2 forward, 5′-ACTCCAAGCCAGAAAAGCT- 3′;
- BETA2 reverse, 5′-ACTGGTAGGGAGTAGGATGCA- C- 3′.
- IL-1β forward, 5′-TGCAGAGTTCCCAACCTGATGTA- CATC- 3′;
- IL-1β reverse, 5′-GCTGCTGCTATGCTCTCCCCT- GAATT- 3.
- TNF-α forward, 5′-CCCTCAACTCATGATCTTCTT- 3′;
- TNF-α reverse, 5′-GCTACGACGTGGGCTACAG- 3′.
- IL-6 forward, 5′-CCAGAGGTTGAGTCTCCCT- 3′;
- IL-6 reverse, 5′-CTGGTTGCACTGACTCTTCCCT- 3′;
- IL-10 forward, 5′-GCTCTAATGACTCCGCAAG- 3′;
- CHOP forward, 5′-CATACACCACACACATGAA- 3′;
- CHOP reverse, 5′-CCGTTTTCCTAGTCTTTCCTTC- 3′.
- Atf6 forward, 5′-GACTCCACCATCGAGTGTG- 3′;
Histology and immunohistochemistry
Pancreata were isolated, fixed with 10 % buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for histological analysis. Pancreatic sections were scanned by Zeiss microscope at ×200 magnifications and islet size was analyzed with AxioVision software. For analysis of pancreatic islet apoptosis, double staining of in situ cell death detection fluorescence and insulin fluorescent immunohistochemistry were performed. The following primary antibodies were used: Rabbit anti-insulin polyclonal antibody, 1:50 (Proteintech Group, Inc. Chicago, USA); Secondary antibodies DyLight594 rabbit anti-goat IgG[H+L], 1:300 (MultiSciences Biotech, Hangzhou, China). The beta cell apoptosis was assessed by transferase-mediated dUTP nick-end labeling (TUNEL) assay (keyGEN, Nanjing, China) according to the manufacturer’s protocol. For the determination of the overall rate of apoptosis, each section stained for insulin, TUNEL, and DAPI were imaged at 400 magnification (40 × objective), and the total number of TUNEL-positive cells per field were quantified. To determine the frequency of beta cell apoptosis, the number of cells positive for TUNEL was quantified in each islet and expressed as percentage of the total number of beta cells (n = ~4000). Beta cells were counted in 16 week old of age male mice per genotype (n = 4–5).

Statistical analysis and calculation
Data are expressed as means ± SD. Differences between groups were calculated by Student’s t test. Data were analyzed using GraphPad Prism Software version 5.0, with significance defined as p < 0.05 (two-tailed). HOMA-IR is calculated by fasting plasma glucose (mmol/L) × fasting insulin (mIU/L)/22.5; HOMA-β is calculated by 20 × fasting insulin (mIU/L)/(fasting plasma glucose (mmol/L) – 3.5) (%).
measured insulin levels after glucose overload, and we found that aP2-CETPTg mice had less insulin secretion than WT mice, especially at 0 and 60 min (p < 0.05) (Fig. 3d). Homeostasis model assessment of beta cell function index (HOMA-β) of NFR-CETPTg and aP2-CETPTg mice were lower compared with WT mice (48.7 ± 26.2, 56.9 ± 24.0, 96.6 ± 31.6, respectively, p < 0.05), which indicated beta cell dysfunction in aP2-CETPTg mice. Furthermore, we found that islets from aP2-CETPTg mice had a significant reduction in glucose-stimulated insulin secretion in vitro compared with that from WT mice (p < 0.05) (Fig. 3e). Collectively, both NFR-CETPTg and aP2-CETPTg mice have impaired glucose tolerance and insulin secretion, but increased insulin sensitivity. Moreover, we also found that there was a CETP dose-dependent effect on glucose tolerance.

**aP2-CETPTg mice have fewer and smaller islets**

Islets from aP2-CETPTg mice exhibited less islet number (138 ± 14 vs.166 ± 10, p < 0.05) (Fig. 4d) and smaller size (6850 ± 6940 vs. 9780 ± 10,100 μm², p < 0.05) compared with WT mice, which was clearly reflected by frequency distribution and mean area quantification on the pancreatic H-E staining sections (10 sections from two separate levels per mouse, 9–10 mice per group) (Fig. 4a–c).

**aP2-CETPTg mice have higher apoptosis rate in beta cell**

We next sought to determine whether decreased insulin secretion and smaller sized islets in aP2-CETPTg mice result from increased apoptosis of beta cells? Immunofluorescence staining of pancreas sections were conducted to quantify apoptosis rate of beta cells in aP2-CETPT and WT mice. Results showed that the frequency of TUNEL-positive beta cell significantly increased in aP2-CETPTg mice (Fig. 5), indicating that circulating CETP could induce beta cell apoptosis. To further explain the reduction of islet mass, we next measured general proliferation marker Ki-67 and beta cell specific transcription factors, pancreatic and duodenal homeobox 1 (Pdx1) and BETA2. Ki-67 mRNA level was increased but the latter two both were suppressed in aP2-CETPTg mice (Fig. 6).

**Circulating CETP promotes free cholesterol accumulation in the islets**

To investigate the effect of CETP on islet lipid content, we utilized gas chromatograph to determine the lipid profile in the islets and found that FC (15.6 ± 3.60 vs. 10.3 ± 1.62 mmol/mg protein, p < 0.05), FC/CE ratio (2.44 ± 0.640 vs. 1.20 ± 0.430, p < 0.05) but not total cholesterol (22.0 ± 3.83 vs. 19.9 ± 2.13 mmol/mg protein, p = 0.216) were significantly increased in the transgenic mice islets compared with controls (Fig. 7a). In addition, plasma TC and CE were significantly decreased in aP2-CETPTg mice than WT mice (Fig. 7b), no difference was observed in FC (Fig. 7b) but FC/CE ratio induced (Fig. 7c). ACAT1 converts FC to CE, playing an important role in cellular cholesterol homeostasis, but ACAT1 mRNA in islets did not differ between the two groups (Fig. 7b). We further found that mRNA levels of cholesterol synthesis and uptake related genes, 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS), sterol regulatory element-binding protein-2 (SREBP-2), and LDL receptor (LDLR) were all significantly reduced (71, 41, 71 %, respectively) (Fig. 8).

**The effect of circulating CETP on the expression of genes participated in islet inflammation and ER stress**

Islet inflammation is emerging as an important factor that participates in the development of T2DM [21]. Thus, we sought to examine mRNA levels of IL-1β, TNF-α and IL-6, these representative pro-inflammatory cytokines that play key roles in modulating islet cytokine...
Fig. 3 (See legend on next page.)
release and impairs islet function [21]. The results indicated that IL-1β mRNA was significantly increased in islets of aP2-CETPTg mice, but without differences in TNF-α, IL-6 or IL-10 mRNA expression (Fig. 9a).

Free cholesterol accumulation in macrophages leads to CHOP-induced ER stress [22]. To investigate whether unfolded protein response is enhanced in the islets from aP2-CETPTg mice, we measured the mRNA expression of CHOP, X-box binding protein 1 (Xbp1) and activating transcription factor 6 (Atf6). Our data showed that the mRNA levels of CHOP and Xbp1 were also increased in aP2-CETPTg mouse islets (Fig. 9b), suggesting that circulating CETP could be cytotoxic to beta cells. Atf6 did not differ between the islets from aP2-CETPTg and WT mice (Fig. 9b).

Discussion
In this study, we demonstrated for the first time that circulating CETP, 1) exacerbate glucose intolerance, lower plasma insulin concentrations but increase insulin sensitivity; 2) increase free cholesterol accumulation in the islets; 3) reduce the expression of the genes which participate in beta cell proliferation and differentiation, resulting in fewer and smaller islet number and size; 4) induced the expression of the genes which are involved in inflammation, ER stress, and apoptosis; and 5) finally and most importantly, circulating CETP decreased insulin secretion from islet beta cells.

It is well known that insulin resistance and insulin deficiency are main factors of glucose intolerance. In our aP2-CETPTg mice, insulin sensitivity is increased, compared with WT mice. This could be due to two reasons, 1) adipocyte size becomes smaller [23], and 2) skeletal muscle takes up more glucose. Although aP2-CETPTg mice had increased insulin sensitivity, they displayed glucose intolerance and this could be due to beta cell dysfunction.

Circulating CETP influences insulin sensitivity and insulin secretion in a dose-dependent fashion. Recently, Raposo et al. [17] reported that CETP expression does not affect glucose homeostasis and insulin secretion. However, our GTT results clearly indicated that there is a CETP dose-dependent effect on mice glucose tolerance impairment and fasting insulin secretion dysfunction. Therefore, the inconsistent results between Raposo et al’s and ours’ could be mainly due to the different CETP levels in the blood. Raposo et al utilized a heterozygous CETP transgenic (NFR-CETP-Tg) mice model which is identical to our NFR-CETP-Tg mice, presenting human like plasma levels of CETP (~2 μg/ml). However, our aP2-CETPTg mice have significantly higher levels of CETP in circulation. Therefore, elevated CETP mass and activity in T2DM patients may bring additional damage to insulin secretion.

What is the mechanism for beta cell dysfunction in aP2-CETPTg mice? We hypothesized that free cholesterol accumulation in aP2-CETPTg mice islet induce beta cell dysfunction. It is known that high cholesterol levels in islets interfere with glucose metabolism by inhibiting glucokinase activity, the critical glucose-sensing component of the beta cell, and reducing the effectiveness of the insulin secretory apparatus [4, 24]. ATP-binding cassette transporter A1 (ABCA1) is a cellular cholesterol transporter which participates in cholesterol efflux, mice lacking beta cell ABCA1 exhibited impaired glucose tolerance and decreased insulin secretion due to abnormalities of cholesterol homeostasis in isolated islets [25]. SREBP-2 is another member of the membrane-bound transcription factor SREBP family, and plays a crucial role in the regulation of cholesterol metabolism [19]. Expression of nuclear human SREBP-2 in beta cells resulted in severe diabetes due to defects in glucose- and potassium-stimulated insulin secretion. Cholesterol is a well-known component of both secretory granules and plasma membranes, and cholesterol-rich microdomains in the plasma membrane are thought to be essential for normal cell function, including exocytosis [26, 27]. The cholesterol-rich lipid rafts in beta cell membranes contain calcium channels and the proteins that constitute the secretory apparatus which consists of soluble N-ethylmaleimide-sensitive factor (NST) attachment protein receptor (SNARE), soluble NST attachment
Fig. 4 (See legend on next page.)
protein-25 (SNAP-25), and synaptobrevin. All of these proteins participate in insulin secretion. The accumulation of cholesterol in beta cell interferes with the effectiveness of the insulin secretory apparatus then decrease insulin secretion. Conversely, lowering beta cell cholesterol levels with methyl-β-cyclodextrin [4, 28], which depletes membrane cholesterol, increases insulin secretion. Our results indicated that FC/CE of islets was increased in the islets from aP2-CETPTg mice when compared with WT mice. Moreover, the free cholesterol accumulation is not directly result from ACAT mRNA level changes. Enrichment of FC changes beta cell membrane lipid composition and this could lead to less efficiency in insulin secretion through exocytosis.

Our study showed that the size of islets from aP2-CETPTg mice were significantly reduced, which could be another reason for the decrease of insulin secretion. These morphologic variations of islets in aP2-CETPTg mice might be a consequence of cholesterol accumulation in the beta cells. Studies in macrophages demonstrated that free cholesterol induces apoptosis by causing stiffening of the ER membrane, with subsequent induction of the unfolded protein response [29]. Our data also showed that beta cell apoptosis was increased, in line with higher expression of ER stress markers, including CHOP and Xbp1. Cholesterol accumulation can decrease expression of the beta cell specific proliferation and differentiation genes, Pdx1 and BETA2, which may be another reason for the decrease of beta cell number and size. Interestingly, Ki-67 mRNA expression was upregulated in aP2-CETPTg mice, which may be a feedback regulation of increased beta cell apoptosis. Islet inflammation has been reported in T2DM, and individuals with elevated serum levels of certain pro-inflammatory cytokines show increased risks for developing T2DM [30]. IL-1β is well established as a pro-inflammatory cytokine involved with the demise of the beta cell [31]. Our results showed that IL-1β mRNA was significantly increased in islets of aP2-CETPTg mice.

The question arises as to how CETP might promote free cholesterol accumulation in pancreatic beta cells. CETP has been reported to exchange lipids between cell membranes and between cell membranes and lipoproteins. For example, CETP can mediate efflux of cholesteryl ester from biological membranes to lipoproteins [32, 33]. CETP can also facilitate HDL-cholesterol selective uptake by adipocytes [34]. It is conceivable that CETP could also facilitate cholesteryl ester efflux out of the plasma membrane of cell, thus causing free cholesterol accumulation in the cells. It has been suggested that CETP could enhance the exchange of lipids during the formation of a ternary collision complex consisting of donor and acceptor lipoproteins, bridged by CETP [35]. By analogy, CETP might bind the HDL to the plasma membrane of cell, and increase the collisions between the membrane and the lipoprotein, allowing lipids to be exchanged in the process. We speculate that circulating CETP could transfer cholesteryl ester but not free cholesterol out of the beta cell to lipoproteins in the
circulation, resulting in elevation of FC/CE ratio in the islets, and the precise mechanism needs further study.

Conclusions
In summary, our data show that circulating CETP have an impact on beta cell cholesterol homeostasis by accumulation of free cholesterol, thus reducing beta cell insulin secretion. These data provide further evidence to the concept that regulation of beta cell cholesterol homeostasis is essential for normal islet function. CETP inhibition could be a potential promising approach to improve beta cell function in T2DM by decreasing islet cholesterol accumulation and inflammation.

![Figure 6](image-url)
**Expression of proliferation and differentiation related genes in isolated islets from aP2-CETPTg mice.** KI-67 mRNA level was significantly upregulated in islets from aP2-CETPTg mice. The mRNA expression of Pdx1 and BETA2 were decreased compared with WT mice. Values are expressed as mean ± SD. *p < 0.05, **p < 0.01, and *** p < 0.001 compared with control group. 

![Figure 7](image-url)
**Lipid profile in plasma and islets from ap2-CETPTg mice.** a Total cholesterol, free cholesterol, and cholesteryl ester of islets isolated from aP2-CETPTg mice and WT mice (20 islets per mice, 5 mice per group) were extracted and measured as described in the Methods. b Plasma cholesterol profile in aP2-CETPTg and WT mice (n = 10 per group). c The FC/CE ratio in plasma and islets. d ACAT1 mRNA expression does not differ in the two groups. Values are expressed as mean ± SD. *p < 0.05 and **p < 0.01, ***p < 0.001, compared with control group.
Expression of inflammation and ER stress related genes in isolated islets from aP2-CETPTg mice.

**A.** As for the mRNA level of pro-inflammatory markers, IL-1β was higher in aP2-CETPTg mice than their control, but TNF-α and IL-6 mRNA expression did not differ between the two groups. For the anti-inflammatory marker, IL-10 mRNA level had an increasing tendency in aP2-CETPTg mice. 

**B.** Relative mRNA levels of CHOP and Xbp1 were increased in aP2-CETPTg mice compared with control group. Values are expressed as mean ± SD. *p < 0.05, and ***p < 0.001 compared with control group.

**Fig. 9** Expression of inflammation and ER stress related genes in isolated islets from aP2-CETPTg mice. A. As for the mRNA level of pro-inflammatory markers, IL-1β was higher in aP2-CETPTg mice than their control, but TNF-α and IL-6 mRNA expression did not differ between the two groups. For the anti-inflammatory marker, IL-10 mRNA level had an increasing tendency in aP2-CETPTg mice. B. Relative mRNA levels of CHOP and Xbp1 in isolated islets were increased in aP2-CETPTg mice. *n = 4–6 per group. Values are expressed as mean ± SD. *p < 0.05, and ***p < 0.001 compared with control group.*

**Abbreviations**

ABCA1: ATP-binding cassette transporter A1; Atf6: activating transcription factor 6; CETP: cholesteryl ester transfer protein; FC: free cholesterol; GSIS: glucose-stimulated insulin secretion; HDL: high density lipoprotein; HMGCoA: 3-Hydroxy-3-methylglutaryl-CoA synthase; IPGT: intraperitoneal glucose tolerance test; LDL: low density lipoprotein; LDLr: LDL receptor; Pdx1: pancreatic and duodenal homeobox 1; SNAP: soluble N-ethylmaleimide-sensitive factor attachment protein; SNARE: soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SREBP-2: sterol regulatory element-binding protein-2; T2DM: type 2 diabetes mellitus; TuNEL: terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; VLDL: very low density lipoprotein; WT: wild type; Xbp1: X-box binding protein 1; ATP-binding cassette transporter A1; Atf6: activating transcription factor 6.

**Ethics approval**

All animal experiments were conducted in accord with accepted standards of humane animal care, as outlined in the Ethical Guidelines, and were approved by the Research Animal Care Committee of Nanjing Medical University (IACUC-14030136).

**Consent for publication**

Not applicable.

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