Chronic inflammation exacerbates glucose metabolism disorders in C57BL/6J mice fed with high-fat diet

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Abstract

Inflammatory stress is closely related to metabolic disease and insulin resistance. The precise cellular mechanism linking obesity and diabetes is largely unknown, but about 14–20% of obese individuals develop diabetes. In this study, we investigated whether chronic inflammation exacerbated glucose metabolism disorder by impairing β cell function in high-fat diet (HFD)-fed C57BL/6J mice. We used s.c. casein injection to induce chronic inflammation in HFD-fed C57BL/6J mice; 14 weeks on a HFD resulted in weight gain, hyperlipidemia, and low insulin sensitivity in these mice which nevertheless had normal blood glucose and serum inflammatory cytokines levels. Casein injection in the background of HFD elevated serum tumor necrosis factor α (TNF α) and serum amyloid A levels and increased TNF α and MCP1 expression in the adipose tissue, liver, and muscle of HFD-fed mice. Chronic inflammation induced by casein injection further decreased insulin sensitivity and insulin signaling, resulting in insulin deficiency and hyperglycemia in these mice. Islet mass and insulin content were markedly increased in HFD mice. However, in contrast with HFD-fed alone, chronic inflammation in HFD-fed mice decreased both islet mass and insulin content, reduced the genetic expression of insulin synthesis and secretion, and increased β cell apoptosis. We conclude that chronic inflammation exacerbated glucose metabolism disorders by impairing β cell function in HFD-fed C57BL/6J mice, suggesting that this mechanism may operate in obese individuals with chronic inflammation, making them prone to hyperglycemia.

Key Words

- ▶ glucose metabolism
- ▶ inflammatory diseases
- ▶ insulin
- ▶ apoptosis
- ▶ pancreas

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Introduction

Obesity is an independent risk factor for type 2 diabetes (T2DM). However, it is estimated that currently 14–20% of Chinese with obesity suffer from diabetes, which leads the question of why all obese patients do not develop T2DM (Chen 2008). Decoding the relationship between obesity and diabetes requires complex interdisciplinary research

on the cellular mechanisms that link the two conditions (Prentki & Nolan 2006, Eckel *et al.* 2011).

T2DM is characterized by inadequate pancreatic β cell compensation in the presence of chronic insulin resistance. Numerous studies have shown that insulin resistance is the central component of the metabolic

syndrome and an important pathophysiological factor in the initiation and development of T2DM. However, a compensatory increase in insulin secretion makes most obese insulin-resistant individuals normoglycemic. Hyperglycemia is due to relative insulin deficiency, implying that T2DM develops in obese insulin-resistant subjects with the onset of β cell dysfunction (Ferrannini & Mari 2004, DeFronzo 2009). Pancreatic β cell function declines in chronic insulin resistance as people progress from normal to impaired glucose tolerance and then to diabetes. T2DM development begins when β cell dysfunction first appears and before the onset of prediabetes, when glycemia is still within the normal range (Godsland *et al.* 2004). Much less is known about the causes and mechanisms of progressive deterioration in β cell function leading to impaired glucose tolerance and diabetes (Xiang *et al.* 2010a).

Obesity, insulin resistance, and T2DM are closely associated with chronic inflammation characterized by abnormal cytokine production, increased acute-phase reactants and other mediators, and activation of a network of inflammatory signaling pathways (Hotamisligil 2006). The expansion of adipose tissue depots in obesity leads to elevated levels of adipocyte-derived circulating inflammatory cytokines, such as interleukin 6 (IL6), serum amyloid A (SAA), and tumor necrosis factor α (TNF α). Many lines of evidence have shown that chronic activation of inflammatory pathways within insulin-target tissues can lead to obesity-related insulin resistance (Boden & Shulman 2002, Schenk *et al.* 2008). Consistent with this, elevated levels of the inflammatory cytokines TNF α , IL6, and C-reactive protein (CRP) have been shown in individuals with insulin resistance and diabetes (de Luca & Olefsky 2008, Wang *et al.* 2008).

Given that T2DM is characterized by hyperglycemia, dyslipidemia, and increased circulating inflammatory factors (so-called low-grade inflammation), elevated inflammatory cytokines may play a role in the process of obesity-related β cell destruction. S.c. casein injection is an established method for the induction of chronic systemic inflammation in mouse models. This has been used in many studies of atherosclerosis and liver steatosis (Miura *et al.* 1985, Wal 2002, Ma *et al.* 2008). In comparison with other sole cytokine or lipopolysaccharide (LPS)-induced mouse models, casein injection induces a lower degree inflammatory stress characterized by increased multiple cytokines (IL1 β , TNF α , and IL6) and SAA (like CRP in human) levels in serum, which is more likely to mimic chronic systemic inflammatory state observed in patients with inflammatory stress (Zhang *et al.* 2008). The current

study was undertaken to investigate whether chronic inflammation induced by casein injection exacerbated pancreatic β cell dysfunction resulting in hyperglycemia in high-fat diet (HFD)-fed C57BL/6J mice and to explore its underlying mechanism.

Materials and methods

Animal model

Animal care and experimental procedures were performed with approval from the Animal Care Committee of Chongqing Medical University. Eight-week-old male C57BL/6J mice were fed with a normal chow diet (NCD) or a HFD or HFD plus s.c. injection of 0.5 ml 10% casein (HFD + casein) for 14 weeks (HFD: TD88137, 60% energy by fat; Harlan Laboratories Inc., Madison, WI, USA). Mice were injected on alternate days and were culled 14 weeks after the first injection. At termination, blood samples were taken for cytokines and lipid assays, and tissue samples were collected for further detection. For biochemical analysis of insulin signaling, another set of the mice in each group was fasted overnight and injected i.p. with insulin at a dose of 5 mU/g body weight (Sigma) for 10 min before adipose tissues, liver, and skeletal muscle were taken and snap-frozen in liquid nitrogen immediately after resection and stored at -80°C (Zhang *et al.* 2012).

Serum analysis

Serum TNF α and SAA protein were measured by ELISA Kits (HuaMei Biotech, Wu Han, China). Serum triglycerides (TG) concentrations were determined enzymatically with commercial kits (Jiancheng, Nanjing, China). Serum free fatty acids (FFA) concentrations were determined calorimetrically using commercial kits (Applygen Technologies, Beijing, China). Insulin levels in the serum were detected by ELISA Kits (Millipore, Billerica MA, USA).

Quantitative measurement of TG and FFA in adipose tissue

Quantitative measurements of TG and FFA were performed using commercial kits (Jiancheng and HuaMei Biotech). Briefly, white adipose tissue (epididymal fat pads) were collected and lipids were extracted by the addition of 1 ml solvents (TG, heptane/isopropanol = 2/3.5 and FFA, chloroform/heptane/methanol = 5/5/1). The lipid phase was collected and vacuum dried. The concentration of TG and FFA was analyzed using standards and normalized by total protein from tissues.

Glucose tolerance tests

Before the glucose tolerance tests (GTT), mice were starved overnight but allowed free access to water. Glucose tolerance was tested by the i.p. injection of 2 mg D-glucose/g body weight (Sigma). Blood glucose concentrations were determined in blood, which were taken from the cut tail tip, before and 15, 30, 60, and 120 min after the administration of glucose. The glucose concentrations were determined using an ACCU-CHEK Advantage blood glucose meter (Roche).

Insulin tolerance tests

Before the insulin tolerance tests (ITT), mice were starved for 4 h but allowed free access to water. Insulin tolerance was measured by i.p. injection of 1 mU insulin/g body weight (Sigma). The glucose concentrations in blood were determined using an ACCU-CHEK Advantage blood glucose meter (Roche), which were taken from the cut tail tip, before and 15, 30, 60, and 120 min after the administration of insulin.

Histology and immunohistochemistry

Pancreas from C57BL/6J mice were sequentially fixed, dehydrated, infiltrated, and cut into 5- μ m paraffin-embedded tissue sections. Sections were stained with hematoxylin-eosin. Sections were cut from embedded pancreatic slices and deparaffinized in dimethyl benzene. The immunohistochemistry procedures were set up according to the manufacturer's instructions (Zsbio, Beijing, China). Sections were blocked using 3% hydrogen peroxide and 10% serum, and then incubated with primary antibody (anti-insulin and anti-CD68, Bioss (Beijing, China)). Avidin anti-rabbit antibody served as the secondary antibody followed by the addition of HRP anti-avidin antibody. The HRP activity was detected using a DAB solution (Zsbio). Finally, the reaction was stopped and sections were counterstained with hematoxylin. The microscopic images were taken by a Zeiss microscope and insulin-positive cells were considered as functional β cells. Using WCIF ImageJ Software (NIH, Bethesda, MD, USA), the relative islet area to total pancreatic area ratio was calculated by random selection from magnifying fields at 10 $\times$ objective. The islet mass was then evaluated by multiplying the area ratio with the pancreatic weight (Xiang *et al.* 2010b).

β cell apoptosis

Free 3-hydroxy strand breaks resulting from DNA degradation were detected by the TUNEL technique

according to the manufacturer's instructions (DeadEnd Fluorometric TUNEL System, Promega). To detect the β cells, 5- μ m slices from paraffin-embedded tissue were incubated with rabbit anti-insulin antibody, followed by detection using the fluorescently labeled secondary antibody. The staining with DAPI was used for visualization of nuclei. The samples were immediately evaluated by fluorescence microscopy (Zeiss, Germany) for positively stained apoptotic nuclei. Positive cells costaining for insulin and TUNEL were designated as apoptotic β cells. Results were expressed as the percentage of apoptotic β cells, normalized by total insulin-positive cell number (Lacruz *et al.* 2010). To estimate β cell apoptotic rates, counts (at 40 $\times$ objective) from 500 β cells per pancreas section were analyzed under a Zeiss fluorescence microscope.

Real-time RT-PCR

Total RNA were isolated from pancreas homogenates from C57BL/6J mice using TRIzol reagent (Takara Life Technologies, Carlsbad, Japan). Real-time RT-PCR was performed in a Bio-Rad Sequence Detection System using SYBR Green dye (Applied Biosystems, Inc.) according to the manufacturer's protocol. All the primers (BGI, Beijing, China) were designed by Primer Express Software V2.0 (Applied Biosystems) (Table 1). To normalize expression data, β -actin was used as an internal control gene.

Western blot

Cytoplasmic and nuclear proteins were extracted from pancreas using a commercial kit. Sample proteins were separated by SDS-PAGE in a Bio-Rad Mini Protean apparatus and then transferred to a PVDF membrane.

Table 1 Mouse primers for real-time PCR

Gene	Mouse primers
<i>Bax</i>	F: 5'-GGCCTTTTGTCTACAGGGTTT-3' R: 5'-GTGTCTCCCAGCCATCCT-3'
<i>Bcl2</i>	F: 5'-CCTGTGGATGACTGAGTACCTGAA-3' R: 5'-CTACCCAGCTCCGTTATCCT-3'
<i>Pdx1</i>	F: 5'-CGCGTCCAGCTCCCTTT-3' R: 5'-CCACGCGTGAGCTTTGGT-3'
<i>Glut2</i>	F: 5'-TGGAAGGATCAAAGCAATGTTG-3' R: 5'-CATCAAGAGGGCTCCAGTCAA-3'
<i>Gk</i>	F: 5'-GCTTTTGAGACCCGTTTGTG-3' R: 5'-GCCTTCGGTCCCCAGAGT-3'
<i>Insulin</i>	F: 5'-CCATCAGCAAGCAGGTCAT-3' R: 5'-GGGTGTGTAGAAGAAGCCA-3'
β -actin	F: 5'-CGATGCCCTGAGGCTCTTT-3' R: 5'-TGGATGCCACAGGATCCAT-3'

Figure 1

Casein injection induced chronic inflammation in C57BL/6J mice. Mice fed with normal chow diet (NCD), high-fat diet (HFD), and HFD plus casein injection (HFD+casein) were culled 14 weeks after first injection. (A) The levels of SAA and TNFα in the serum of C57BL/6J mice were examined by ELISA. Results represent the mean $\pm$ s.d. ($n=6$). * $P<0.05$ vs NCD and

$P<0.05$ vs HFD. (B) The protein expression of TNFα and MCP1 in the adipose tissue, liver and muscle was detected by western blot. (C) Infiltration of inflammation cells in pancreas was detected by macrophage marker CD68 immunohistochemistry (original magnification $\times 40$), arrows show macrophages dyed brown.

The membrane was blocked with 5% (w/v) non-fat dried milk and incubated with primary antibodies (anti-TNFα, anti-MCP1, anti-insulin receptor substrate 1 (IRS1), anti-IRS2, anti-AKT, anti-p-AKT (ser473), anti-insulin, anti-β-actin, anti-β-tubulin, Santa Cruz Biotechnology, Inc.), followed by incubation with a secondary HRP-conjugated antibody. Finally, detection procedures were performed using ECL Advance Western Blotting Detection Kit (Amersham Bioscience). Band intensity volumes were measured by ImageJ Software.

Statistical analysis

Results are presented as means $\pm$ s.d. In all experiments, data were evaluated for statistical significance using

one-way ANOVA followed by Q-test. A difference was considered significant if the P value was <0.05 .

Results

Casein injection induced chronic inflammation in HFD-fed C57BL/6J mice

A chronic low-grade systemic inflammation was induced in C57BL/6J mice using a combination of a HFD and s.c. injection of 10% casein on alternate days for 14 weeks. There were significant increases of SAA and TNFα concentration in the serum of casein-injected mice compared with mice fed with HFD (Fig. 1A), suggesting that chronic inflammation was successfully induced in

Table 2 Effects of HFD and casein injection on metabolic parameters in C57BL/6J mice. TG and FFA levels in the serum and adipose tissue of C57BL/6J mice were measured as described in Materials and methods section and results represent the mean $\pm$ s.d. ($n=6$)

	NCD	HFD	HFD+casein
Body weight (g)	22.75 $\pm$ 1.27	27.03 $\pm$ 1.31*	25.73 $\pm$ 1.38*
Adipose/body weight (g/g)	0.014 $\pm$ 0.002	0.036 $\pm$ 0.01*	0.035 $\pm$ 0.004*
Serum TG levels (mmol/l)	3.84 $\pm$ 0.91	5.67 $\pm$ 0.84*	4.41 $\pm$ 1.02
Serum FFA levels (μg/ml)	1.52 $\pm$ 0.23	2.31 $\pm$ 0.55*	2.59 $\pm$ 0.10*
Adipose TG levels (mg/mg)	0.83 $\pm$ 0.27	2.42 $\pm$ 0.22*	2.19 $\pm$ 0.24*
Adipose FFA levels (ng/mg)	1.26 $\pm$ 0.53	12.60 $\pm$ 4.79*	11.79 $\pm$ 0.96*

* $P<0.05$ vs NCD.

C57BL/6J mice. We also detected the inflammatory cytokine productions in mouse adipose tissue, liver, and muscle. The protein expression of TNF α and MCP1 in the three types of tissues in HFD group slightly increased compared with NCD group (Fig. 1B), while inflammatory cytokines in the serum were kept in the normal levels (Fig. 1A). Casein-injected mice had a higher expression of TNF α and MCP1 protein compared with the other two groups (Fig. 1B), indicating that casein injection aggravated local inflammation in HFD-fed mice. Immunohistochemical analyses of pancreatic sections revealed that CD68, a specific marker of tissue macrophages, was increased in casein injection group (Fig. 1C). It indicated that casein injection also induced pancreatic inflammation in C57BL/6J mice.

Effects of HFD and chronic inflammation on metabolic parameters in C57BL/6J mice

HFD feeding for 14 weeks obviously increased body weight, adipose/body weight, serum and adipose TG, and FFA levels, and induced dyslipidemia in C57BL/6J mice. Compared with the mice fed with HFD, casein injection had no further effect on these parameters mentioned above (Table 2).

Chronic inflammation aggravated insulin resistance and glucose metabolism disorders in HFD-fed C57BL/6J mice

The fasting glucose levels were unchanged in HFD-fed mice compared with NCD-fed mice (Fig. 2A and C). After glucose challenge, blood glucose concentration of HFD group was persistently higher than that of the NCD group (Fig. 2A), suggesting that HFD feeding for 14 weeks induced glucose intolerance in mice. Insulin levels in serum were significantly increased in HFD-fed mice (Fig. 2D). After insulin loading, blood glucose levels decreased slowly and still higher in the HFD group (Fig. 2B), suggesting that HFD feeding reduced insulin sensitivity in mice. We also measured the major molecules involved in insulin signaling in insulin-sensitive tissues. HFD inhibited the levels of total IRS1, IRS2, and p-AKT:AKT ratio in the liver, muscle, and adipose tissue (Fig. 3), indicating that HFD impaired insulin signaling and induced insulin resistance in mice.

Casein injection further aggravated impaired glucose tolerance, showing higher blood glucose levels after glucose injection and lower ITT slope after insulin loading in the casein plus HFD group (Fig. 2A and B). Casein-injected mice had a notable increase in fasting glucose levels (Fig. 2A and C) and decrease insulin levels in serum

Figure 2

Chronic inflammation further impaired glucose tolerance and decreased insulin sensitivity in HFD-fed C57BL/6J mice. (A) Glucose tolerance tests (glucose-stimulated blood glucose concentrations, GTT) performed after 14 weeks of treatment in C57BL/6J mice starved overnight. (B) Insulin tolerance tests (insulin-stimulated blood glucose concentrations, ITT) performed at the end of experiments in C57BL/6J mice starved for 4 h. Mice were starved overnight before executed and the blood was collected for fasting blood glucose (C) and serum insulin (D) detection. Results represent the mean $\pm$ s.d. ($n=6$). * $P<0.05$ vs NCD and # $P<0.05$ vs HFD.

Figure 3

Chronic inflammation aggravated the impairment on insulin signaling in insulin-targeted tissues of HFD-fed mice. The protein expression of IRS1 and IRS2 (A), p-AKT (ser473), and AKT (B) in the adipose tissue, liver, and muscle was detected by western blot. To analyze the p-AKT level, C57BL/6J mice were fasted overnight and injected i.p. with insulin for 10 min before

adipose tissues, liver, and skeletal muscle were excised. The histogram represents mean $\pm$ s.d. ($n=3$) of the densitometric scans for protein bands from three experiments, normalized by comparison with β -actin in adipose and liver and tubulin in muscle, and expressed as a percentage of NCD.

* $P<0.05$ vs NCD and # $P<0.05$ vs HFD.

(Fig. 2D), implying that chronic inflammation impaired β cell function and induced hyperglycemia. Likewise, casein injection further downregulated IRS1, IRS2, and p-AKT protein expression (Fig. 3), and aggravated impaired insulin signaling induced by HFD.

Chronic inflammation exacerbated pancreatic β cell dysfunction in HFD-fed mice

Results from pancreatic histomorphology (Fig. 4A, B and C) and quantitative analysis of insulin in pancreas (Fig. 4D) showed that islet mass and insulin content were markedly increased in HFD-fed mice compared with NCD-fed mice. However, casein injection impaired β cell function by reducing islet mass and insulin content in HFD-fed mice (Fig. 4A, B, C and D). We further determined the expression of key participants in β cell functional integrity, namely pancreatic duodenal homeobox 1 (PDX1), glucokinase (GK), glucose transporter 2 (GLUT2), and insulin. The mRNA expression of *Pdx1* and *Gk* had no obvious change, while both *Glut2* (*Slc2a2*) as well as insulin mRNA levels were significantly increased in HFD group (Fig. 4E), indicating that a HFD for 14 weeks had no effect on insulin biosynthesis but enhanced insulin secretion. The mRNA expression of *Pdx1*, *Glut2*, *Gk*, and insulin were significantly downregulated in casein-injected mice (Fig. 4E), indicating that chronic

inflammation reduced insulin biosynthesis and secretion in HFD-fed mice.

Chronic inflammation increased β cell apoptosis in HFD-fed mice

Data showed that apoptotic β cell numbers had no difference between HFD group and NCD group (Fig. 5A and B). However, casein injection notably increased β cell apoptosis (Fig. 5A and B) and upregulated pancreatic *Bax/Bcl2* mRNA expression in mice fed with HFD (Fig. 5C), suggesting that chronic inflammation exacerbated β cell apoptosis in HFD-fed mice.

Discussion

Chronic systemic inflammation plays an important role in the pathogenesis of multiple metabolic disorders, including insulin resistance, T2DM, and obesity. Most obese individuals do not develop diabetes because β cells initially compensate for insulin resistance. Clinical studies have identified elevated serum levels of TNF α and IL6 as risk factors for subjects developing into T2DM (Spranger *et al.* 2003). We presume the progression of obesity-related β cell dysfunction may be related to a state of chronic inflammation.

Figure 4

Chronic inflammation exacerbated pancreatic β cell dysfunction in HFD-fed mice. (A) Hematoxylin–eosin (HE) staining in pancreas including the islet mass (boxed, arrows). (B) Insulin immunohistochemistry. Magnification $\times 10$ (top panels) and $\times 40$ (bottom panels). (C) Islet mass of C57BL/6J mice was calculated as described in Materials and methods section. Values are mean $\pm$ s.d. ($n=6$) in each group. (D) The protein levels of insulin in

pancreas of C57BL/6J mice were detected by western blot. The histogram represents mean $\pm$ s.d. ($n=3$) of the densitometric scans for protein bands from three experiments, normalized by comparison with β -actin. (E) The mRNA expression of *Pdx1*, *Glut2*, *Gk*, and insulin in pancreatic islet of C57BL/6J mice was detected by real-time PCR. Results represent the mean $\pm$ s.d. ($n=6$). * $P<0.05$ vs NCD and # $P<0.05$ vs HFD.

A HFD in which fat contributes 58% of daily energy intake fat in a C57BL/6J mice model is a robust model for the study of insulin resistance, impaired glucose tolerance, and T2DM (Winzell & Ahren 2004). Mice on long-term

HFD revealed β cell dysfunction and diminution of glucose-induced insulin secretion and developed glucose intolerance as a result of insulin resistance (Collins *et al.* 2010). In this study, C57BL/6J mice on a HFD for 14 weeks

Figure 5

Chronic inflammation aggravated β cell apoptosis in HFD-fed mice.

(A) Apoptosis of insulin-expressing cells on islet sections was determined by the TUNEL assay. Representative examples of pancreatic islets stained by immunofluorescence for insulin (red), marker of cell apoptosis TUNEL (green), and nuclear stain DAPI (blue) imaged at 100 $\times$. (B) The

percentage of apoptotic β cells was calculated as described in Materials and methods section. Values are mean $\pm$ s.d. ($n=6$) in each group.

(C) Pancreatic Bax and Bcl2 mRNA expression of C57BL/6J mice was detected by real-time PCR. Results represent the mean $\pm$ s.d. ($n=6$).

* $P<0.05$ vs NCD and # $P<0.05$ vs HFD.

revealed characteristic of obesity and hyperlipidemia. HFD-fed mice revealed glucose intolerance and decreased insulin sensitivity, accompanied by impaired insulin signaling suggesting that a 14-week HFD induced insulin resistance in C57BL/6J mice. In our experimental setting, there were no change in serum SAA and TNF α levels but increase in TNF α and MCP1 expression in the adipose tissue, liver, and muscle in HFD-fed mice compared with NCD-fed mice, in agreement with previous reports on overexpression of TNF α in different white adipose tissue depots of obese individuals and a normal circulating serum TNF α level (Hotamisligil *et al.* 1995).

The bacterial endotoxin, LPS, induced inflammatory stress is presented during endotoxic septic shock, a condition that often leads to multiple organ failure and mortality (Zhang *et al.* 2008). LPS injection results in robust CNS-controlled sickness behaviors accompanied by increases in inflammatory cytokines (IL1 β , TNF α , and IL6) in the blood and brain. Compared with the administration of LPS, the inflammation induced by casein is characterized by an increased SAA, which is well documented as a good marker of chronic low-grade systemic inflammation.

S.c. injection of casein in NCD alone-fed C57BL/6J mice had no obvious effect on metabolic parameters, such as body weight, serum FFA, and insulin levels (data not

shown). In this study, we used casein injection in HFD-fed C57BL/6J mice to investigate the role of inflammation in obesity-related β cell dysfunction. Serum TNF α and SAA levels were significantly increased after casein injection for 14 weeks. Moreover, TNF α and MCP1 expressions in the adipose, liver, and muscle were upregulated in casein plus HFD group compared with HFD group, suggesting that casein injection successfully induced chronic systemic and local inflammation in HFD-fed mice.

In the obesity-induced metabolic disorder, FFA, which is commonly elevated in obese individuals, may drive a compensatory increase in β cell mass and function followed by attenuation as T2DM develops (El Assaad *et al.* 2003, Maedler *et al.* 2003). Investigation of the effects of HFD and chronic inflammation on metabolic parameters in C57BL/6J mice revealed that HFD feeding for 14 weeks markedly increased serum TG and FFA levels in C57BL/6J mice, while casein injection did not increase serum TG and FFA contents in HFD-fed mice. We also investigated the effects of HFD and chronic inflammation on blood glucose and serum insulin in C57BL/6J mice. The data showed that HFD mice maintained normoglycemia in the presence of impaired GTT and higher serum insulin levels, whereas mice in the HFD plus casein injection group had notable hyperglycemia and low serum insulin

levels, implying that chronic inflammation accelerated deterioration of β cell function. Compared with HFD-fed mice, casein-injected mice revealed parallel serum FFA levels and marked β cell dysfunction, suggesting that chronic inflammation is an independent risk factor in the destruction of pancreatic β cells.

The dysregulation of IRS1 and IRS2 and the inhibition of its signaling downstream are the primary mechanisms of chronic inflammation-induced insulin resistance. In this study, casein injection notably diminished the IRS1, IRS2, and p-AKT levels in the liver, muscle and adipose tissues of mice, and revealed obvious insulin resistance. Research showed that IRS1 was the principal mediator of hepatic insulin action that maintains glucose homeostasis, especially during nutrient excess. IRS1-deficient liver showed poor regulations of the key gluconeogenic genes and impaired glucose tolerance and insulin sensitivity. Moreover, IRS1 was required to suppress hepatic glucose production during hyperinsulinemic-euglycemic clamp (Guo *et al.* 2009). In this study, inflammatory stress induced by casein may affect hepatic glucose production and gluconeogenesis by inhibiting hepatic IRS1.

Data on the augmentation of islet mass and pancreatic insulin content in HFD-fed mice compared with NCD-fed mice showed that islet mass and pancreatic insulin content were markedly increased in HFD-fed mice, consistent with previous studies (Collins *et al.* 2010, Li *et al.* 2011). Islet adaptation to obesity is well established in rodents, where islets compensate for insulin-resistant states by increasing β cell mass and function to maintain normoglycemia (Sachdeva & Stoffers 2009). In this study, we found that chronic inflammation impaired β cell function by reducing islet mass and pancreatic insulin content in HFD-fed mice. To explore the underlying mechanism of inflammation-mediated impairment in pancreatic islets of HFD-fed mice, we determined the expression of key participants in insulin synthesis and secretion. *Pdx1* acts in β cells as a house-keeping transcription factor for insulin gene expression (Melloul 2004). *Gk*, *Glut2*, and insulin are the three key components of the glucose-sensing machinery responsible for glucose-inducible insulin release (Tiedge & Lenzen 1991, Burcelin *et al.* 2000). HFD feeding did not significantly affect pancreatic *Pdx1* mRNA levels, but obviously increased *Glut2* and insulin mRNA expression associated with elevated serum insulin concentrations. Therefore, hyperinsulinemia in obese individuals may correlate with improved β cell insulin secretion. In contrast with HFD-fed group, casein injection significantly reduced pancreatic *Pdx1*, *Glut2*, *Gk*, and insulin mRNA levels and serum

insulin levels, indicating that chronic inflammation exacerbated HFD-induced islet β cell dysfunction and accelerated the progression of obesity-related T2DM.

Recent evidences have suggested that increased apoptosis of pancreatic β cells could explain insulin deficiency (Butler *et al.* 2003, Meier *et al.* 2005, Jurgens *et al.* 2011). However our data did not demonstrate that HFD feeding by itself for 14 weeks increased β cell apoptosis. It is possible that casein injection could augment β cell apoptosis in the presence of HFD with upregulated *Bax/Bcl2* mRNA levels, suggesting that chronic inflammation could exacerbate β cell apoptosis in HFD-fed individual.

Taken together, our results demonstrated that chronic inflammation exacerbated HFD-related β cell dysfunction and apoptosis, resulting in glucose metabolism disorder. Obese patients with chronic inflammation are more prone to develop into T2DM much earlier.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Y W, T W, and J W carried out experiments and researched data, L Z performed the data analysis, Q L researched data, Z V reviewed manuscript and contributed discussion, J F M refined the manuscript, S H P reviewed manuscript, Y C designed the project and experiments and wrote manuscript, and X Z R designed the project and reviewed manuscript.

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