Biochemical and Biophysical Research Communications 458 (2015) 681–686

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Compensatory hyperinsulinemia in high-fat diet-induced obese mice is associated with enhanced insulin translation in islets

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ARTICLE INFO

Article history:
Received 30 January 2015
Available online 14 February 2015

Keywords:
High-fat diet
Mouse islet
Insulin translation

ABSTRACT

A high-fat diet (HF) is associated with obesity, insulin resistance, and hyperglycemia. Animal studies have shown compensatory mechanisms in pancreatic β-cells after high fat load, such as increased pancreatic β-cell mass, enhanced insulin secretion, and exocytosis. However, the effects of high fat intake on insulin synthesis are obscure. Here, we investigated whether insulin synthesis was altered in correlation with an HF diet, for the purpose of obtaining further understanding of the compensatory mechanisms in pancreatic β-cells. Mice fed an HF diet are obese, insulin resistant, hyperinsulinemic, and glucose intolerant. In islets of mice fed an HF diet, more storage of insulin was identified. We analyzed insulin translation in mouse islets, as well as in INS-1 cells, using non-radioisotope chemicals. We found that insulin translational levels were significantly increased in islets of mice fed an HF diet to meet systemic demand, without altering its transcriptional levels. Our data showed that not only increased pancreatic β-cell mass and insulin secretion but also elevated insulin translation is the major compensatory mechanism of pancreatic β-cells.

1. Introduction

Obesity and lifestyle-related diseases are rapidly increasing health threats in both developed and developing countries [1]. In Asian countries, the situation is particularly alarming and appears to be caused by changes in lifestyle, such as inadequate exercise and a westernized diet. For example, there was a marked increase in fat consumption in parallel with the prevalence of type 2 diabetes mellitus (T2DM) in the Japanese population between 1945 and 1980 [2,3].

There is accumulating evidence that high fat intake, especially a high saturated fat component, is associated with obesity, insulin resistance, and hyperglycemia in animal studies [4–6]. In addition, in humans, excess dietary fat consumption promotes insulin resistance [7] and T2DM [8,9]. T2DM is characterized by pancreatic β-cell failure and insulin resistance. The failure of pancreatic β-cells to secrete adequate amounts of insulin to compensate for insulin resistance ultimately leads to T2DM [10]. Some reports have analyzed the effects of a high-fat (HF) diet on pancreatic β-cells. For instance, there are reports that have shown increased β-cell volume [6,11], enhanced glucose induced insulin secretion together with augmented Ca^{2+} signals, and an increased exocytotic response of insulin granules after a high fat load [12]. However, other studies have shown functional deterioration of pancreatic β-cells. A reduction in the transcription of the glucose sensing genes glucose transporter 2 and glucokinase in the pancreas of rats fed an HF diet was also reported [13].

Abbreviations: AHA, L-azidohomoalanine; CHX, cycloheximide; HF, high-fat; ITT, insulin tolerance test; KRB, Krebs–Ringer buffer; MafA, musculoaponeurotic fibrosarcoma oncogene family, protein A; NC, normal chow; OGTT, oral glucose tolerance test; PAL, palmitate; PBS, phosphate-buffered saline; Pdx1, pancreatic and duodenal homeobox 1; RI, radioisotope; T2DM, type 2 diabetes mellitus.

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http://dx.doi.org/10.1016/j.bbrc.2015.02.024
0006-291X/

Pancreatic β-cells are specialized for the synthesis, storage, and secretion of insulin. Each step of this process should be tightly regulated to meet systemic demand. *In vitro*, the biosynthesis of insulin is controlled acutely at the translational level [14], and it can be increased more than 15-fold by glucose stimulation [15,16]. Despite the particular ability of pancreatic β-cells to produce insulin, there are few reports showing the effects of an HF diet on insulin synthesis. In this study, we investigated whether insulin synthesis was altered in correlation with an HF diet for the purpose of obtaining further understanding of the compensatory mechanisms of pancreatic β-cells.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice were purchased from CLEA Japan, Inc. The mice were maintained on a 12-h light and 12-h dark cycle. From the time of weaning (4 weeks of age), the mice were fed either a normal chow (NC) diet (4.8% fat; CLEA Japan, Inc.) or an HF diet (30.3% fat; Oriental Yeast Co.). Blood glucose and plasma insulin were measured as described previously [17,18].

This study was performed according to the guidelines of the Animal Ethics Committee of the Kobe University Graduate School of Medicine.

2.2. Oral glucose tolerance test and insulin tolerance test

An oral glucose tolerance test (OGTT) was conducted as described previously [19]. Briefly, the mice were fasted for 16 h and blood samples were collected before and after the oral administration of glucose (1.5 mg/g body weight). For the insulin tolerance test (ITT), the mice were administered 0.75 U/kg body weight of human regular insulin (Eli Lilly) intraperitoneally after a 4 h fast.

2.3. Islet isolation

Mouse pancreatic islets were isolated by collagenase digestion and Histopaque density-gradient centrifugation as described previously [20–22].

2.4. Histology and electron microscopy

For immunofluorescence, the tissues were cut after they were embedded in paraffin and stained with primary antibodies specific for insulin (Dako) and glucagon (Dako), and secondary antibodies conjugated to Cy3 and FITC (Jackson ImmunoResearch Laboratories). Pancreatic β-cell mass was quantified as described previously [18,20,23]. For electron microscopy, mouse pancreas was fixed in 0.1 M phosphate-buffered saline (PBS), 2% glutaraldehyde, and subsequently post-fixed in 2% osmium tetroxide for 2 h at 4 °C. Then, the specimens were dehydrated in a graded ethanol series and embedded in an epoxy resin. Ultrathin sections were obtained using an ultramicrotomy technique. Ultrathin sections stained with uranyl acetate for 15 min and a lead solution for 5 min were submitted to transmission electron microscope observation (JEM-1200EX; JEOL).

2.5. Measurement of islet insulin content

Islet insulin content was measured by placing 10 size-matched islets into a 1.5-mL tube containing a high-salt buffer, and insulin content was measured with a Mouse Insulin ELISA Kit T (Shibayagi Co.) as described previously [21]. Insulin levels were corrected to total DNA levels.

2.6. Cell culture

INS-1 cells were maintained in RPMI 1640 medium (Sigma–Aldrich), containing 10% fetal bovine serum. For the glucose stimulation study, INS-1 cells were preincubated with Krebs–Ringer buffer (KRB) containing 0.1% bovine serum albumin and 2.8 mM glucose for 2 h, followed by stimulation with the same solution containing either 2.8 or 16.8 mM glucose for 1 h. This incubation was performed with or without 5.0 ng/mL cycloheximide (CHX) (Sigma–Aldrich). For treatment with palmitate (PAL) (Sigma–Aldrich), 0.5 mM PAL was added to RPMI-1640 medium and the cells were incubated for 24 h.

2.7. Real-time RT-PCR

Total RNA was isolated from mouse islets or from INS-1 cells using an RNeasy kit (QIAGEN). Quantitative real-time RT-PCR was performed with the SYBR Green reagent (Promega) and evaluated using an ABI 7900 sequencer (Life Technologies). The relative abundance of mRNAs was calculated with cyclophilin mRNA as the invariant control. Details of the primers used for RT-PCR are provided in Supplementary Table 1.

2.8. Immunoblot analysis

Islets or INS-1 cells were lysed using sonication as described previously [22,24]. The lysates were probed with antibodies to insulin (Cell Signaling Technology) and β-actin (Sigma–Aldrich).

2.9. Insulin synthesis study

For INS-1 cells, they were treated with KRB containing either 2.8 or 16.8 mM glucose and treated with or without CHX as described in Cell culture. For the last 30 min, Click-iT® AHA (L-azidohomoalanine) (Life Technologies) was added and mixed gently. For islets, isolated mouse islets were transferred and incubated in a methionine-free medium (Sigma–Aldrich) for 30 min, following the addition of AHA for another 30 min. The incubations were terminated by washing the cells or islets with ice-cold PBS for 3 times. After the cells or islets were lysed, immunoprecipitation was performed with an anti-insulin antibody (Cell Signaling Technology #L6B10; Abcam #Ab7842). Eluted proteins from the precipitated samples were reacted with biotin-alkyne (Life Technologies) using a Click-iT Protein Reaction Buffer Kit (Life Technologies). After the reaction, acrylamide gel electrophoresis was performed and then probed with streptavidin-HRP (Cell Signaling Technology).

2.10. Statistical analysis

Data are presented as the mean ± standard error of the mean. We assessed the significance of differences between independent means by Student's t-test. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Mice fed an HF diet exhibit increased body weight, glucose intolerance, and hyperinsulinemia

Male C57BL/6J mice were fed with either an NC (4.8% fat) or HF diet (30.3% fat) from 4 weeks of age. Mice fed an HF diet displayed increased body weight compared with those fed an NC diet (Fig. 1A). Ad libitum blood glucose levels tended to increase in mice fed an HF diet, but not significantly so (Fig. 1B). Serum insulin levels were significantly increased at 12 weeks of age in mice fed