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Original article

Adiponectin is expressed in the pancreas of high-fat-diet-fed mice and protects pancreatic endothelial function during the development of type 2 diabetes

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Abstract

Aim. – Adiponectin levels in skeletal muscle and adipose tissue have been reported to be involved in insulin resistance in rats fed with a high-fat diet (HFD). Our objective was to explore whether adiponectin is also expressed in the pancreas and what its potential role is during the development of type 2 diabetes (T2D) in outbred CD-1 mice.

Methods. – Male 4-week-old outbred CD-1 mice were fed an HFD to induce a polygenic model of human T2D. Adiponectin expression was examined in mouse pancreas by quantitative real-time polymerase chain reaction (qPCR), western blots and immunofluorescence analyses. Human umbilical vein endothelium cells (HUVECs) were transfected with an adiponectin-expressing lentivirus to determine the effect of adiponectin on angiogenic function *in vitro*.

Results. – Feeding mice an HFD for 9 weeks resulted in constant hyperglycaemia, obesity, impaired glucose tolerance and insulin resistance. Additional hyperinsulinaemia emerged in mice fed an HFD for 18 weeks. Interestingly, aberrant expression of adiponectin was detectable in the pancreatic vascular endothelial cells (VECs) of mice fed with an HFD, but not in mice fed with regular chow (RC). Expression levels of pancreatic adiponectin varied during the development of T2D. This extraordinary expression of adiponectin in pancreatic VECs played a role in protecting endothelial function against potential damage by HFD. Our *in vitro* study has demonstrated that adiponectin promotes angiogenic function.

Conclusion. – These results reveal for the first time that adiponectin is expressed in pancreatic VECs of HFD-fed mice during the development of T2D as a protective adaptation in response to the HFD.

Keywords: Adiponectin; Type 2 diabetes; Pancreas; Vascular endothelial cells; High-fat diet

Abbreviations: AdipoR1, Adiponectin receptor 1; AdipoR2, Adiponectin receptor 2; AUC, Area under the curve; BW, Body weight; C/EBPα, CCAAT/enhancer-binding protein α; FBG, Fasting blood glucose; GFP, Green fluorescence protein; GTT, Glucose tolerance test; HFD, High-fat diet; HUVEC, Human umbilical vein endothelium cells; ITT, Insulin tolerance test; MANOVA, Multivariate analysis of variance; PPARγ, Peroxisome proliferator-activated receptor gamma; qPCR, Quantitative real-time PCR; RC, Regular chow; SEM, Standard error of mean; T2D, Type 2 diabetes; VEC, Vascular endothelial cells.

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1. Introduction

Type 2 diabetes (T2D) is a metabolic disease characterized by insulin resistance and pancreatic dysfunction. This metabolic disease results from the interaction of multiple genetic and environmental factors. T2D is a polygenic disease with multiple susceptibility loci, each of which contributes only a small increase in disease risk, but which together have a large physiological effect [1].

It is well accepted that a variety of adipokines, mainly produced by adipose tissue, are associated with insulin resistance and impaired insulin secretion, both of which contribute to T2D. Of these adipokines, adiponectin is often referred to as a "beneficial" adipokine possessing antidiabetic properties, including its "beneficial" effect on improving insulin sensitivity in skeletal muscle and the liver [2,3]. There is evidence to demonstrate that adiponectin is expressed not only in adipocytes, but also in mouse skeletal muscles, one of insulin's target organs [4]. In addition, adiponectin levels in skeletal muscle tissue are correlated with high-fat-diet (HFD)-induced insulin resistance during weight gain in rats [9]. As for the pancreas, studies of mouse cell lines have reported that the insulin receptor is expressed on pancreatic beta cells and its signaling in beta cells influences insulin gene expression and insulin content [5]. Studies with genetically engineered mouse models have reported that downstream signaling activity of the insulin receptor in pancreatic islet cells is required for the positive feedback needed to stimulate insulin synthesis, and severe insulin resistance in pancreatic islets results in reduced insulin content [6]. Indeed, the effect of adiponectin on the pancreas has been substantially investigated [7,8]. For example, adiponectin was found to significantly augment insulin release from cultured mouse pancreatic endocrine cell lines and from purified rat islets when incubated in culture media containing high concentrations of glucose [8,9]. However, no study has yet reported whether adiponectin is expressed in the pancreas. In humans, the adiponectin gene is located on chromosome 3q27, which contains certain genetic determinants for T2D [10,11]. Thus, the aim of the present study was to determine whether adiponectin is expressed in the pancreas and, if so, whether local adiponectin plays a role in the pancreas during the development of T2D.

Outbred CD-1 mice are closely analogous to their human counterparts in terms of genetics, and possess a large number of genetic loci that predispose them to impaired glucose tolerance (IGT) and T2D [12]. In this study, outbred CD-1 mice were fed with an HFD to develop a polygenic T2D model that closely mimics the aetiology of human T2D – namely, a polygenic inheritance and the most commonly involved environmental factor (an HFD). Using this mouse model, our study investigated whether adiponectin is expressed in the pancreas and what its potential protective role is during the development of T2D.

2. Materials and methods

2.1. Experimental animals

All experimental procedures were approved by the Animal Ethics Committee of Peking University Health Science Center and performed in accordance with the institutional guidelines. Male outbred CD-1 mice 3 weeks of age were purchased from Charles River Laboratories via Vital River Laboratories (VRL, Beijing, China). They were kept in a temperature-controlled environment on a 12-h light/dark cycle with free access to food and water. Before initiating the experiment, the mice were acclimatized for 1 week. At the age of 4 weeks, the mice (weighing 15.3 ± 0.6 g) were randomly assigned to either a control group ($n = 28$) and fed with regular chow (RC) or an HFD group ($n = 43$) fed with the HFD; this time point became week 0 of the experiment, which lasted 18 weeks in total. The composition of each diet is shown in the Supplementary material associated with this article online. Fasting blood glucose (FBG) levels and body weight (BW) were monitored weekly. Weeks 1, 9 and 18 were set as time points for observing serum insulin levels and performing glucose tolerance tests (GTT) and insulin tolerance tests (ITT). Ages of the mice at these three time points were 5, 13 and 22 weeks, respectively. Ten RC-fed mice and seven HFD-fed mice at week 1 and 10 RC-fed mice and eight HFD-fed mice at week 9 were sacrificed for further analyses. At week 18, there were eight mice in the RC group and 28 mice in the HFD group. Mice from each group at the three time points were sacrificed after saline perfusion, and epididymal adipose tissue, hind leg skeletal muscles (biceps femoris, semimembranosus and semitendinosus muscles), liver and pancreas from these mice were collected and prepared for mRNA detection, western blots and immunofluorescence staining.

2.2. Criteria for identifying T2D

At week 18, three criteria were used to identify T2D in the mouse model. The criteria were generated from the FBG, GTT, and ITT test results. To set these criteria, the sum of the mean value and 1.645-fold of its standard deviation (STD) from the corresponding result were used to represent the upper limit of the 90% confidence interval (CI) of each corresponding result for the control RC-fed mice. Specifically, as FBG levels fluctuated throughout the 18 weeks, the criterion for FBG was generated as a mean value plus 1.645 STD of the FBG levels monitored at weeks 16, 17 and 18 in the RC group (criterion 1). The mean FBG levels of the HFD-fed mice at the same time periods were then compared with this criterion. Criteria for the GTT and ITT results were generated from the mean value plus 1.645 STD of the respective area under the curve (AUC) values of the corresponding test in the RC group at week 18, which were defined as criterion 2 and criterion 3, respectively. Thus, the three criteria were FBG = 123 mg/dL, AUC of GTT = 40723.2 mg/mL × min and AUC of ITT = 6691.3 mg/mL × min. If a test result from an HFD mouse was greater than or equal to the corresponding criterion, it could be concluded that the result was outside the upper 90% CI of test results for the RC-fed mice. T2D was identified when the corresponding test results from an HFD mouse at week 18 met two of the three criteria.