Biomedicine & Pharmacotherapy 101 (2018) 155–161

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

# Investigation of insulin resistance in the popularly used four rat models of type-2 diabetes

Pin-Chun Chao[a,1], Yingxiao Li[b,c,1], Chin-Hong Chang[d], Ja Ping Shieh[e], Juei-Tang Cheng[c,f,*], Kai-Chun Cheng[b,**]

[a] Bachelor Program of Senior Services, College of Humanities and Social Sciences, Southern Taiwan University of Science and Technology, Yong Kang, Tainan City, 71005, Taiwan
[b] Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, 890-8520, Japan
[c] Department of Medical Research, Chi-Mei Medical Center, Yong Kang, Tainan City, 71003, Taiwan
[d] Department of Neurosurgery, Chi-Mei Medical Center, Yong Kang, Tainan City, 71003, Taiwan
[e] Department of Anesthesiology, Chi-Mei Medical Center, Yong Kang, Tainan City, 71003, Taiwan
[f] Institute of Medical Sciences, Chang Jung Christian University, Gueiren, Tainan City, 71101, Taiwan

ARTICLE INFO

*Keywords:*
Insulin resistance
T2DM
Rat models
Euglycemic clamp
HOMA-IR

ABSTRACT

Animal models are widely used to develop drugs for treating diabetes mellitus (DM). Insulin resistance (IR) is one of the main problems in type-2 DM (T2DM). Streptozotocin (STZ) is used to damage pancreatic cells for induction of DM. Many rat models were applied in research as T2DM. However, the degree of IR in each model is unknown. In the present study, IR and insulin signaling were compared in four models of type 2 diabetes: rats fed a fructose-rich chow for 8 weeks, rats feed high-fat chow for 4 weeks followed by injection with streptozotocin (35 mg/kg, i.p.), rats injected with a single low dose streptozotocin (45 mg/kg, i.p.), and rats injected with a single dose of nicotinamide followed by a single high dose of streptozotocin (60 mg/kg, i.p.). Values from these determinations in diabetic rats showing the order that insulin resistance is most marked in rats received fructose-rich chow followed by high-fat diet before STZ injection induced model (HFD/STZ rats), and rats injected with low dose of STZ but it is less marked in rats induced by nicotinamide and STZ. Additionally, insulin secretion was reduced in three rat models except the rats receiving fructose-rich chow. Western blots also showed the same changes in phosphorylation of IRS-1 or Akt using soleus muscle from each model. The obtained data suggest a lack of pronounced IR in the rats with acute diabetes induced by nicotinamide and STZ while IR is markedly identified in rats fed fructose-rich chow. However, the increase of plasma glucose levels in fructose-rich chow-fed rats was not so significant as other groups. Therefore, HFD/STZ rats is an appropriate and stable animal model which is analogous to the human T2DM through a combination of high-fat diet with multiple low-dose STZ injections.

## 1. Introduction

Diabetes mellitus (DM), which caused by either absolute (type-1 DM; T1DM) or relative (type-2 DM; T2DM) insulin deficiency, is a major metabolic disorder [1]. Official reports indicate that the incidence of DM is increasing [2]. Therefore, the development of new drugs for DM treatment is important. Various animal models of diabetes have been developed, with the aim of identifying mechanisms that may underlie hyperglycemia and of developing new agents for diabetes therapy.

Clinically, T2DM is more prevalent than T1DM [3]. In the pre-clinical stage of T2DM, IR is a syndrome (a set of signs and symptoms) resulting from defective insulin secretion, severe IR reduced the insulin activity. In early or progressing stage of T2DM, IR exists persistently and the amount of β-cell mass becomes inadequate, plasma glucose levels rise relatively rapidly. Advanced stage of T2DM is characterized as more severe β-cell damage for decreased insulin secretion to result in significantly increased glucose levels. Insulin resistance (IR) is one of the major characteristics of T2DM and presents as decreased insulin sensitivity in tissues. Thus, IR is an essential target for the development of therapeutic drugs for T2DM [4–7]. The induction of IR or a T2DM-like animal model is then widely desired. For this purpose, in addition

* Corresponding author at: Department of Medical Research, Chi-Mei Medical Center, Yong Kang, Tainan City, Taiwan.
** Corresponding author.
*E-mail addresses:* jtcheng@mail.cjcu.edu.tw (J.-T. Cheng), kc-cheng@m3.kufm.kagoshima-u.ac.jp (K.-C. Cheng).
[1] These authors contributed equally.

https://doi.org/10.1016/j.biopha.2018.02.084
Received 25 November 2017; Received in revised form 7 February 2018; Accepted 19 February 2018
0753-3322/ © 2018 Elsevier Masson SAS. All rights reserved.

to the genetically modified mice, food supplementation or specific diet-induced T2DM have been established, including methods using a high-fat diet (HFD) or a fructose-rich diet [8]. Both models are believed to approximate nutrient-induced diabetic disorders and have been widely used in basic research. Plasma glucose level and insulin level were significantly higher in high-fat diet fed rats, indicating the progressive worsening of insulin resistance [9]. On the other hand, rat fed a high-fructose diet showed the tendency of slight hyperglycemia at week 4 and a marked hyperinsulinemia after 8 weeks [10]. However, these diet manipulation models require a long time (at least several weeks) to induce diabetes. Therefore, a more rapid model using the administration of streptozotocin (STZ) and nicotinamide has been developed [11] because this model can be easily induced within just a few days. Due to the presence of hyperglycemia and/or hypoinsulinemia, this model has also been widely used in T2DM research. Additionally, the diabetic model induced by the injection of low STZ, is non-insulin-dependent, because non-fasting blood insulin levels did not significantly change throughout the 9-week-observation period after administration of STZ [12,13]. Therefore, it could be considered as a rat model of T2DM showing β-cell failure without obesity [14]. Then, rats received HFD before an injection of STZ at low dose applied to use a rat model of T2DM [15]. However, whether each model reproduces IR is unknown although these rat models were widely used in research. This uncertainty is particularly important because of the centrality of IR in T2DM.

In the present study, we thus reproduced these four rat models of T2DM that were popularly applied to investigate the IR using standard methods, including the values of HOMA-IR and hyperinsulinemic-euglycemic clamp. Additionally, insulin sensitivity and insulin signals were also compared in these models.

## 2. Materials and methods

### 2.1. Animals

Male Sprague-Dawley (SD) rats, weighing 260–280 g, were obtained from the National Laboratory Animal Center (Taipei, Taiwan). All rats were housed in plastic cages under standard laboratory conditions. Rats were maintained under a 12-h light/dark cycle (lights on at 07:00 am and lights off at 7:00 pm) and had free access to food and water. The animal experiments were approved and were conducted in accordance with local institutional guidelines for the care and use of laboratory animals at Chi-Mei Medical Center (No. 105051901), and the experiments conformed to the Guide for the Care and Use of Laboratory Animals, as well as the guidelines of thhe Animal Welfare Act.

### 2.2. Induction of T2DM by fructose-rich chow

To induce a T2DM -like model showing insulin resistance, rats were fed for four weeks on a 60% fructose chow (Harlan Research Diets, Madison, WI, USA), with an Fuel Energy of 3.6 Kcal/g, comprising 66.8% calories from carbohydrate, 20.2% from protein and 12.9% from fat, as described previously [16]. The control group was continued to receive the standard rat chow contained 56.74% carbohydrate, 29.8% protein, and 13.43% fat (total 3.35 Kcal/g) (LabDiet, St.Louis, MO, USA). Tolbutamide (10 mg/kg, i.p.) -induced hypoglycemia was employed to characterize the success of the induction in this model. A decrease in tolbutamide-induced action was observed in some rats after four weeks, and a loss and/or marked reduction in the responses occurred approximately eight weeks later in all rats. We then used these animals for further experiments in the present study.

### 2.3. Induction of T2DM by injection of streptozotocin with nicotinamide

As described in a previous report [11] with some modifications, overnight -fasted rats received an i.p. injection of nicotinamide (230 mg/kg) dissolved in 0.9% NaCl. After 15 min, STZ (60 mg/kg) dissolved in 0.1 mmol/L citrate buffer (pH 4.5) was administered intraperitoneally (i.p.). One week later, blood samples from each rat were used to assay the glucose and insulin levels. Hyperglycemia and hypoinsulinemia were induced to evaluate the success of this process as described previously [11] and no mortality was observed for this induction.

### 2.4. Induction of T2DM by injection of streptozotocin at low dose

According to a previous report [17], the freshly prepared solution of STZ (45 mg/kg) in 0.1 M citrate buffer, pH 4.5, was injected intraperitoneally into fasted rats at a volume of 1 ml/kg. After 48 h of STZ administration, rats with moderate diabetes having hyperglycemia (blood glucose over 250 mg/dl) were used for the experiment. 93% of the rats treated with STZ that achieved this cut-off glycemic values. No mortality was observed for this induction.

### 2.5. Induction of T2DM by HFD-fed and streptozotocin at low dose (HFD/STZ rats)

On caloric basis, the HFD consisted of Fuel Energy of 5.1 Kcal/g, comprising 61.6% calories fromfat, 18.1% from protein and 20.3% from carbohydrate (TestDiet, Richmond, IN, USA). After 4-week HFD feeding, rats were injected intraperitoneally with low dose of STZ (35 mg/kg) as described in previous report [18]. One week later of STZ injection, rats with diabetes showing hyperglycemia (blood glucose over 300 mg/dl) were applied for the experiment. 85% of the rats treated with HFD/STZ that achieved this cut-off glycemic values. Diabetic rats were allowed to feed HFD continuously during the study.

### 2.6. Laboratory measurements

The plasma glucose concentration was measured as described previously [19]. A blood sample (0.2 ml) was collected from the femoral vein of rats that were anesthetized with sodium pentobarbital (35 mg/kg, i.p.), and all efforts were made to minimize the animal suffering. The blood samples were then centrifuged at 13,000 rpm for 3 min, and an aliquot (15 μl) of plasma was added to 1.5 ml of glucose kit reagent (Biosystems S.A., Barcelona, Spain) and incubated at 37 °C for 10 min. The fasting blood glucose in all animals was measured weekly during the entire experimental period. The plasma glucose concentration was measured in an analyzer (Quik-Lab, Ames; Miles Inc., Elkhart, IN, USA). The plasma insulin concentration was determined using a commercially available enzyme-linked immunosorbent assay (Cat.10-1250-01, Mercodia, Uppsala, Sweden).

### 2.7. Assessment of insulin resistance

Homeostasis model assessment-insulin resistance (HOMA-IR) was used to assess β-cell function and insulin resistance (IR) from basal glucose and insulin. Samples from each rat were analyzed in triplicate, and the results were expressed as μU/mL of plasma insulin. HOMA-IR was then calculated as fasting glucose (mmol/L) × fasting insulin (μU/mL)/ 22.5 as described previously [19].

### 2.8. Hyperinsulinemic-euglycemic clamp

We performed hyperinsulinemic-euglycemic clamp as described in a previous report [16]. After overnight fasting, a cannula was implanted in the femoral vein of rats that were anesthetized with pentobarbital (35 mg/kg, i.p.) for the infusion of glucose and insulin, and another cannula was placed in the femoral artery for sampling. Before the experiment, the animals were placed in a restrainer for acclimation to the condition. At the beginning, the rats received 4 μU/kg/min infusions of regular human insulin (Novo Industrias, Bagsvaerd, Denmark). The

Download English Version:

https://daneshyari.com/en/article/8525480

Download Persian Version:

https://daneshyari.com/article/8525480

Daneshyari.com