Obesity Research & Clinical Practice (2016) 10, 207—210

SHORT REPORT

Blood free fatty acids were not increased in high-fat diet induced obese insulin-resistant animals

Junfeng Jiang[a,b], Yongjie Wu[a], Xiaoxia Wang[a], Li Lu[a], Li Wang[a], Baolai Zhang[a], Mingxia Cui[a,*]

[a] *Institute of Pharmacology, School of Basic Medical Science, Lanzhou University, Key Laboratory of Preclinical Study for New Drugs of Gansu Province, Lanzhou, China*
[b] *Gansu Provincial Cancer Hospital, Lanzhou, China*

Received 18 February 2015; received in revised form 23 May 2015; accepted 20 June 2015

KEYWORDS
Insulin resistance;
Free fatty acids;
Obesity;
Ciliary neurotrophic factor;
Sibutramine

Summary
Objective: The close connection between high blood FFA and insulin resistance (IR) in obese individuals is well-known. The purpose of this study was to identify whether the blood FFA increased in obese-IR animals.
Methods: Obese-IR animal models were established using high-fat diet (HFD) or HFD and streptozocin, and treated with drugs.
Results: The serum FFA of obese-IR animals was not increased, even significantly lower than that of normal animals, and were not significantly decreased when insulin sensitivity and obesity-related indices were ameliorated after treatment.
Conclusion: The results suggest that blood FFA are unlikely the link between obesity and insulin resistance.

Obesity and insulin resistance (IR) are associated with several metabolic abnormalities [1]. Many studies have previously shown that there is an intrinsic relationship between obesity and IR [1—3].

Some studies suggest that the most likely factor by which IR in obese individuals develop is due to elevated levels of serum free fatty acids (FFA) [4]. However, there is still no complete consensus yet. Not all obese have IR or diabetes. McLaughlin and colleagues found that plasma FFA concentrations were not significantly increased in all obese individuals, but were significantly higher in patients with insulin-resistance compared to those that were

* Corresponding author at: Institute of Pharmacology, School of Basic Medical Science, Lanzhou University, No. 199 Donggang Xi Road, Lanzhou 730000, China. Tel.: +86 13893337604.
E-mail address: cmxlz@163.com (M. Cui).

http://dx.doi.org/10.1016/j.orcp.2015.06.005
1871-403X/

insulin-sensitive [5]. Johns et al. [6] and Karpe et al. [7] also suggested that serum FFA are unlikely the link between obesity and insulin resistance. The present studies support this view.

In the first experiment, a total of 60 male SPF Wistar rats, 170—210 g, were purchased from Shanghai Experimental Animal Center of Chinese Academy of Sciences (Shanghai, China). All animals were acclimated for a week with a normal diet and randomly divided into two groups: normal control ($n = 10$) and streptozotocin (STZ, $n = 50$) injection groups. The 12-h fasted rats were injected via tail vein with 25 mg/kg of STZ (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 0.05 mol/L citrate buffer (pH 4.4). Normal rats were injected with identical volumes of citrate buffer. 15 days later, a glucose tolerance test (GTT) was conducted as previously described [8] and the area under curve (AUC) of serum glucose was calculated on all the rats. Rats injected with STZ that had AUC values $\geq$ mean + 2SD of AUC values of normal rats ($n = 43$) were used for the remainder of the experiments. 10 of these STZ rats were fed with a normal diet and were selected by using randomised block design based on body weight and AUC values. The remaining rats were fed with a high-fat diet (HFD, containing 10% fat, g/g, Suzhou Shuangshi Laboratory Animal Feed Science Co., Ltd., Suzhou, China) for 3 months in order to establish the obese insulin-resistant model. These rats were subsequently treated with sibutramine (Taiji Group Chongqing Fuling Pharmaceutical Factory Co., Ltd., Chongqing, China) 8 mg/kg/day by oral gavage (STZ + HFD + S8, $n = 9$) or vehicle (STZ + HFD, $n = 10$) for 10 days (flow chart as shown in Fig. 1). Body weight and diet intake were measured daily during treatment, and a GTT was performed after 7 days of treatment. At the end of the experiment, rats were fasted overnight and then anaesthetised. Blood samples were obtained from the abdominal aorta and the serum was maintained at −20 °C until assayed. Serum FFA levels were determined by using calorimetric FFA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Fasting serum insulin (FINS) was detected by a rat/mouse insulin ELISA kit (Linco Research, Inc., St. Charles, MO, USA) and fasting blood glucose (FBG) was measured by calorimetry. Fasting insulin resistance index (FIRI) was obtained from the following equation: FIRI = FBG (mmol/L) × FINS (mIU/L)/25. The data is presented as mean ± SD. All animal procedures were conducted in compliance with the guidelines for animal care and use of Key Laboratory of Preclinical Study for New Drug of Gansu Province, Lanzhou, China. Statistical comparisons between groups were carried out using ANOVA

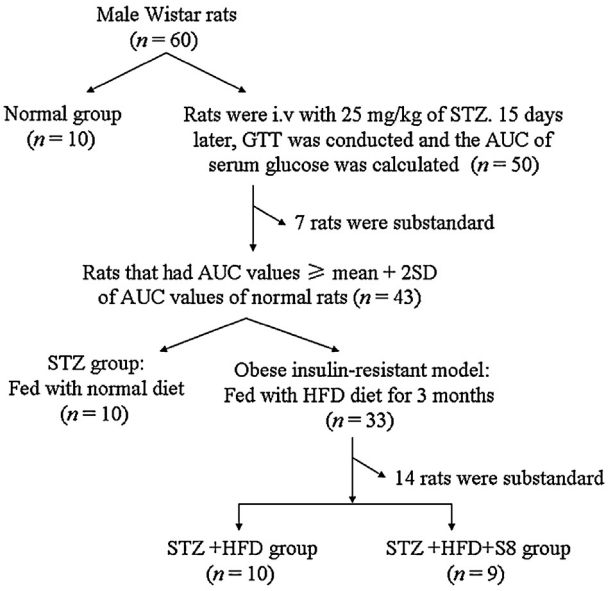

Figure 1 Flow chart of experimental procedure.

followed by Bonferroni's *post hoc* test. A value of $p < 0.05$ was considered to be statistically significant as shown in Table 1. The body weight, FINS, FIRI, and AUC_{0-2h} of serum glucose were significantly greater in the STZ + HFD group as compared with those in the normal and STZ groups ($p < 0.05$ or 0.01). Surprisingly, the serum FFA levels in the obese model group were lower than that of the STZ group and normal group (all, $p < 0.05$). After sibutramine treatment, the body weight, FBG, FINS, FIRI, and AUC_{0-2h} values of serum glucose were all significantly decreased (all, $p < 0.01$), while the serum FFA concentration was significantly increased ($p < 0.05$) (Table 1).

In another experiment, materials and methods are previously described in detail [9]. Briefly, male C57BL/6J mice of 9-week-old were fed with high-fat diet (HFD, 10% fat, 2% cholesterol, and 0.4% sodium cholate, g/g) for 18.5 weeks to establish obese insulin-resistant model. Then model animals were treated with sibutramine 8 mg/kg/day i.g. (HFD + S8) or recombinant human ciliary neurotrophic factor (rhCNTF) 0.3 mg/kg/day s.c. (HFD + C0.3) for 10 days. RhCNTF was a gift from Lanzhou Institute of Biological Products (Lanzhou, China). The purity of this protein is higher than 95%. In this study, serum FFA levels were detected according to the same methods. Obesity-related indices including body weight, FINS, FBG, and FIRI which have been previously published are cited in this paper for clarity. As shown in Table 2, compared to normal mice, the HFD mice had significantly higher visceral fat weight, FINS, FBG, and FIRI (all, $p < 0.01$). However, the serum FFA levels were