available at www.sciencedirect.com

ScienceDirect

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

Insulin resistance following thermal injury: An animal study

Xin-Long Chen[a,b], Zhao-Fan Xia[a,*], Dao-Feng Ben[a], Jian-Guang Tian[a], Duo Wei[a]

[a] Burn Center, Changhai Hospital, Second Military Medical University, Shanghai 200433, People's Republic of China
[b] Department of Burn, Pingjin Hospital, 300162 Tianjin, People's Republic of China

ARTICLE INFO

Article history:
Accepted 15 August 2006

Keywords:
Burn
Insulin resistance
Glucose tolerance tests
Insulin sensitivity index
Euglycemic–hyperinsulinemic glucose clamps

ABSTRACT

The aim of this study was to investigate the changes of glucose tolerance, insulin sensitivity, and euglycemic–hyperinsulinemic glucose clamps following a 30% TBSA full thickness third degree burn in rats. Sprague–Dawley rats weighing 160–170 g received 30% TBSA full thickness third degree burn by immersing the back of trunk for 12 s in a boiling water bath under anesthesia. Weight- and time-matched sham burn group (control) was treated in the same manner as the trauma group, except that they were immersed in a room-temperature water bath. After 12 h overnight fasting, plasma insulin concentration was determined by ELISA using rat-insulin enzyme immunoassay kit (SPI-BIO) and blood glucose was assayed by glucose analyzer at 3 days after burn. Insulin sensitivity index was calculated by using slightly modified formula. The rat was injected with 5% glucose (2 g/kg body weight, intraperitoneally) to observe the change of glucose tolerance at 3 days after burn. Euglycemic–hyperinsulinemic glucose clamps were performed at 4 days after burn. Insulin sensitivity index of burn group was significantly reduced compared with control group at 3 days after burn (0.58 ± 0.23 versus 1.23 ± 0.16, $P < 0.01$). The significant difference of glucose tolerance was observed between the two groups and the glucose infused rate measured in burned rats was significantly decreased compared with that in control at 4 days after injury (7.23 ± 1.35 versus 12.31 ± 0.54, $P < 0.01$). *Conclusion*: Burn causes the significant change of glucose metabolism and results in insulin resistance in rats.

1. Introduction

Insulin, the principal hormone maintaining glucose homeostasis, stimulates glucose transport into muscle, glycogen synthesis in the liver, and fat deposition in adipocytes [1,2]. Insulin resistance is a major metabolic abnormality following severe burn [3]. Insulin resistance after burn is indicated by elevated glucose production (gluconeogenesis) and blood glucose levels in association with normal or increased insulin levels. Vigorous nutritional support to optimize the hypermetabolic state and promote anabolism has not been effective. Attempts have been made to correct this burn stress-induced hyperglycemia and muscle wasting with pharmacological doses of insulin, but this treatment resulted in fatty liver and increased wasted substrate cycling, enhanced CO_2 production, and attendant complications of hepatic and respiratory failure [4,5].

The abnormal insulin function and glucose metabolism after burn have been found in human several decades ago. But further study still needs to conform the existence of insulin resistance following thermal injury *in vivo* in animal model through multiple approaches. The methods of glucose tolerance tests, insulin sensitivity index and euglycemic–hyperinsulinemic glucose clamps are testified to be optimal approaches to predict insulin resistance in diabetes and other diseases [6,7]. The hyperinsulinemic euglycemic glucose clamp technique is the "gold standard" for quantifying insulin sensitivity in vivo because it directly measures the effects of

* *Corresponding author*. Tel.: +86 21 25070600; fax: +86 21 65589829.
E-mail addresses: XiaZhaoFan@hotmail.com, cxlcxlzxy@hotmail.com (X. Zhao-Fan).
0305-4179/$32.00
doi:10.1016/j.burns.2006.08.017

insulin to promote glucose utilization under steady state conditions [8]. The plasma insulin concentration is acutely raised and maintained at high level by a primed continuous infusion of insulin. The plasma glucose concentration is held constant by a variable glucose infusion using the negative feedback principle. Under these steady-state conditions of euglycemia, the glucose infusion rate equals glucose uptake by all the tissues in the body and is therefore a measure of tissue sensitivity to exogenous insulin. We initiated this study based on this concept that it may be much more believable to confirm the existence of insulin resistance after burn by using multiple methods rather than one approach. The aim of the study was to confirm the association of insulin resistance with burn and provide multiple approaches of evidence indicating that insulin resistance exists in rats *in vivo* after burn in order to further investigate the exactly molecular mechanism of insulin resistance and correct this disorder.

2. Materials and methods

2.1. The animal model

The protocol for the studies was approved by the Institutional Animal Care Committee of the Second Military Medical University in Shanghai, China. The animal care facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Sprague–Dawley rats (Central Animal Laboratory of Second Military Medical University, Shanghai, China) weighing 160–170 g were housed in an environmentally controlled room with a 12 h light/dark cycle and fed with regular rat chow diet. The animals were randomized to each group after being accustomed to the environment for one week. After clipping of the hair, a 30% TBSA full thickness third degree burn injury was produced under anesthesia (2% pentobarbital 40 mg/kg i.p.) by immersing the back of trunk for 12 s in boiled water (100 °C). A weight- and time-matched sham burn group (controls) was treated in the same manner as the trauma group, except that they were immersed in room temperature water (28–30 °C). All biochemical experiments were performed at either day 3 for GGT or day 4 for euglycaemic–hyperinsulinamic clamp after burn or sham burn injury.

2.2. Glucose tolerance tests (GTT)

Overnight fasted (17:00–8:00 h) rats were anesthetized with 2% pentobarbital (40 mg/kg body weight, intraperitoneally) at 3 days after burn, and placed on heating pads maintained at 37 °C. After 30 min of anesthesia, the rats were injected with 5% glucose (2 g/kg body weight, intraperitoneally). Tail blood was collected at 0, 30, 60, 90 and 120 min after glucose injection. Blood glucose concentrations were measured using a glucose meter (Abbott Laboratories, MediSense Products, Bedford, MA, USA).

2.3. Insulin sensitivity index

After 12 h overnight fasting, blood sample was drawn in the morning for analysis of plasma concentration of glucose and insulin at 3 days after burn. Plasma Insulin concentration was determined by ELISA using rat-insulin enzyme immunoassay kit (A05105-96 Wells, SPI-BIO). Plasma glucose was assayed by glucose analyzer (Automatic analyzer, Hitachi, 7600-020). Insulin sensitivity index was calculated by using a slightly modified formula [7]. We subjected the fasting data to various transformations and ultimately defined insulin sensitivity index $ISI = \log[100/(\text{plasma glucose} \times \text{plasma insulin})]$. The comparison between ISI and the previous index of insulin sensitivity were performed and no statistical significance was found. Log transformation of these data was even more highly correlated with euglycemic–hyperinsulinemic glucose clamps.

2.4. Euglycemic–hyperinsulinemic glucose clamps

At 4 days after burn, the same animals after GTT were fasted for 5 h on the morning of the experiment and then placed in a restrainer to which they were accustomed. The rats were catheterized in the right jugular vein and the left femoral vein under anesthesia (2% pentobarbital 50 mg/kg i.p.). After an 80 min basal period, blood samples were collected from the tail tip for determination of the basal blood concentration of glucose. At time 0, regular human insulin was infused (200 mU/kg bolus, 20 mU/kg/min) through the left femoral vein catheter by using a microinfusion pump (Microinfusion Pump WZ-50C2, the Medical Instrument Factory of Zhejiang University, Zhejiang 310006, China). The insulin infusion solution was prepared by dilution of insulin with normal saline. Blood samples were subsequently drawn at 10 min intervals for the determination of blood glucose using a glucose meter (Abbott Laboratories, MediSense Products, Bedford, MA, USA). An infusion of 10% glucose was adjusted to maintain plasma glucose between 5.5 and 6.0 mmol/L through the right jugular vein catheter by using the same microinfusion pump. Steady state was ascertained when a fixed glucose infusion rate maintained blood glucose measurements constant for 30 min. This steady state was achieved within 90–120 min, at which time blood samples were collected for determination of blood concentration of glucose. The infused rate of total 10% glucose (mg/kg min) was measured in each rat.

3. Statistical analysis

Data were presented as means ± S.E.M. Statistical significance – $P < 0.05$ unless otherwise noted – was determined by *t*-test using SPSS11.0.

4. Results

4.1. Glucose tolerance tests (GTT)

The results of glucose tolerance tests (GTT) from two groups were shown in Fig. 1. At 3 days after burn, GTT were evidently attenuated in burn group in comparison with control group ($P < 0.01$). At 0, 30, 60, 90 and 120 min after glucose injection, blood glucose levels in the burn group were significantly increased compared with that in the control group