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Chronic insulin infusion induces reversible glucose intolerance in lean rats yet ameliorates glucose intolerance in obese rats☆

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ABSTRACT

Background: Although insulin resistance (IR) is a key factor in the pathogenesis of type 2 diabetes (T2D), the precise role of insulin in the development of IR remains unclear. Therefore, we investigated whether chronic basal insulin infusion is causative in the development of glucose intolerance.
Methods: Normoglycemic lean rats surgically instrumented with i.v. catheters were infused with insulin (3 mU/kg/min) or physiological saline for 6 weeks. At infusion-end, plasma insulin levels along with glucose tolerance were assessed.
Results: Six weeks of insulin infusion induced glucose intolerance and impaired insulin response in healthy rats. Interestingly, the effects of chronic insulin infusion were completely normalized following 24 h withdrawal of exogenous insulin and plasma insulin response to glucose challenge was enhanced, suggesting improved insulin secretory capacity. As a result of this finding, we assessed whether the effects of insulin therapy followed by a washout could ameliorate established glucose intolerance in obese rats. Obese rats were similarly instrumented and infused with insulin or physiological saline for 7 days followed by 24 h washout. Seven day-insulin therapy in obese rats significantly improved glucose tolerance, which was attributed to improved insulin secretory capacity and improved insulin signaling in liver and skeletal muscle.
Conclusion: Moderate infusion of insulin alone is sufficient to cause glucose intolerance and impair endogenous insulin secretory capacity, whereas short-term, intensive insulin therapy followed by insulin removal effectively improves glucose tolerance, insulin response and peripheral insulin sensitivity in obese rats.
General significance: New insight into the link between insulin and glucose intolerance may optimize T2D management.

1. Introduction

Insulin resistance is a key factor in the pathogenesis of type 2 diabetes (T2D) and it is widely accepted that insulin resistance precedes the development of overt T2D [1]. Insulin resistance and hyperinsulinemia are intimately related and the prevailing point of view is that insulin resistance is the primary disturbance, with secondary appearance of elevated plasma insulin levels due to overproduction by pancreatic β-cells in response to poor peripheral tissue response. In contrast, there is evidence to support the role of insulin itself in the initiation and progression of insulin resistance [2–6] and the simple question of whether or not hyperinsulinemia can trigger and perpetuate insulin resistance remains controversial [7]. A clear understanding of the role of insulin itself in the pathogenesis of insulin resistance is especially important in light of the currently accepted clinical practice of early introduction of insulin therapy in pre-diabetic patients followed by progressive increase of the dose of insulin with time and deteriorating glucose control [8]. However, this approach may potentially worsen established insulin resistance and accelerate the development of T2D. Indeed, several studies in animals and humans indicate that hyperinsulinemia induces a reduction in whole-body insulin sensitivity [6,9,10], which may involve altered peripheral tissue insulin receptor expression [11] and/or function [12,13]. Conversely, amelioration of hyperinsulinemia with the insulin-reducing agent diazoxide, significantly increased insulin sensitivity in obese rats [14,15], however similar treatment did not alter insulin sensitivity in humans [16].

Conflicting reports of insulin's role in the development of insulin resistance may be due, in part, to the wide spectrum of models and

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experimental approaches employed that span *in vitro* studies in multiple cell types as well as *in vivo* studies in animals and humans. The major limitation of these studies is the short duration, model chosen, and mode of insulin delivery; all of which precludes clear interpretation of data and formulation of a reliable conclusion. Therefore, in order to investigate the role of chronic insulin exposure in the initiation and progression of glucose intolerance, we surgically instrumented normal lean rats for continuous intravenous infusion of insulin for 6 weeks. The lean rats were used to test whether chronic infusion of a moderate level of insulin is sufficient to trigger the development of glucose intolerance (Study 1). In a subsequent study, we further tested whether short-term intensive insulin infusion therapy of 7 days duration could boost insulin responses and improve glucose tolerance in rats with established insulin resistance (Study 2). Furthermore, we assessed post-receptor insulin signaling in key insulin-sensitive tissues (liver and skeletal muscle) to elucidate the molecular mechanism underlying the unexpected improvement in glucose tolerance in these rats.

2. Materials & methods

2.1. Animals & housing

This study was conducted with the approval of the University of Alberta Animal Policy and Welfare Committee which is in accordance with the National Institutes of Health guide for the care and use of laboratory animals. Experiments were conducted on 46 male Sprague–Dawley rats (250–300 g, 8–9 weeks of age, Charles River Canada), which were housed in a humidity and temperature-controlled room with an automatic 12 h light, 12 h dark cycle. In Study 1, rats were maintained on standard chow (5012, Lab Diet, MO, USA) throughout. In Study 2, all rats were maintained on a high fat, high sucrose diet (45% kcal fat; Research Diets, NJ, USA) for 8 weeks before surgical instrumentation to develop diet-induced insulin resistance.

2.2. Surgical instrumentation

Surgical preparation of rats for Studies 1 and 2 was identical. Anesthesia was induced with 4% isoflurane and maintained at ~1.5% for the duration of the surgical procedure. Upon induction of anesthesia, rats were transferred to a heated surgical table and an ophthalmic ointment was applied to the eyes (Tears Naturale, Sandoz); analgesia was also administered at this time (Metacam, 2 mg/kg b.w, i.m.). Body temperature was maintained at 37 °C.

Hair was removed from the left groin and midscapular region. Both areas were cleansed with 3× alternating applications of 10% povidone iodine solution (Betadine) and 70% ethanol. Aseptic technique was observed throughout the surgical procedure. An incision was made in the left groin and the left femoral vein was cannulated with a fluid-filled catheter (physiological saline, V3A tubing, i.d. 0.69 × o.d. 1.14 mm, #BB1785-V/3A, SCI Commodities, AZ, USA); the tubing was advanced such that the tip rested in the inferior vena cava, below the renal veins. After securing the catheter in place with 4–0 silk suture (Harvard Apparatus, MA, USA) and a drop of cyanoacrylate glue (Krazy Glue, gel formula), the cannula was tunneled subcutaneously to an incision in the midscapular region. The left groin incision was then closed (3–0 Chromic Gut, Johnson & Johnson, NJ, USA).

With the rat in the prone position, a sterile polysulfone button covered in Dacron and Mersilene mesh (Instech Labs, PA, USA) was sutured to the muscle overlying the shoulder blades (4–0 Silk, Harvard Apparatus, MA, USA). A sterile stainless steel spring (Instech Labs, PA, USA) was threaded onto the intravenous cannula and secured to the polysulfone button. The skin incision was then sutured around the button post (4–0 Vicryl, Johnson & Johnson, NJ, USA). Antibiotic ointment was applied to both groin and midscapular incision sites (Calmoseptine, Calmoseptine Inc., CA, USA) and the rat was allowed to recover on a warm heating pad.

2.3. Intravenous infusion protocol

Once the rats completely recovered from anesthesia, they were placed in specialized metabolic cages equipped with an infusion swivel (previously sterilized according to manufacturer specifications) and counter-balanced arm support (375/D/22 and SMCLA-META, Instech Labs, PA, USA). The stainless steel spring was fixed to the swivel and the intravenous catheter was connected to the infusion port. The infusion swivel was connected via sterile V3A tubing (see above) to a syringe pump (PHD 2000, Harvard Apparatus, MA, USA) and continuous infusion of sterile physiological saline commenced (0.9% NaCl, 0.56 mL/h). Saline infusion continued throughout the 7 day surgical recovery period, during which time, rats had free access to food and water. Baseline body weight and measurements of 24 h food intake were taken for at least 3–4 days and baseline glucose tolerance was assessed in the conscious rats by glucose tolerance test (GTT, described below). 1 day following recovery from the GTT, i.v. infusion of normal human insulin commenced (3 mU/kg/min at 0.56 mL/h, Novolin®ge Toronto, Novo Nordisk, Denmark) in half of the rats to raise plasma insulin to the degree observed in early insulin resistant states [17]. This dose and infusion rate of insulin does not produce hypoglycemia (17), nor does it result in high levels of urine ketones or altered plasma free fatty acid levels, as determined by assaying β-hydroxybutyrate and plasma free fatty acid content (described below). Although blood glucose levels were not continuously monitored to avoid unnecessary distress of the conscious animals under investigation, fasting blood glucose levels for all rats were well within the normal range (lean insulin-treated: 5.6 ± 0.4 mmol/L; Insulin Resistant-Treated: 5.1 ± 0.5 mmol/L). Saline infusion continued unaltered in control rats. Insulin or saline infusion continued for 6 weeks (Study 1) or 7 days (Study 2), with daily measurement of food intake. At the end of the infusion period, glucose tolerance was assessed in the conscious rats by GTT. Insulin infusion was stopped and switched to physiological saline in experimental groups for 24 h ("washout") and glucose tolerance was re-assessed.

2.4. Glucose tolerance test (GTT)

GTTs were performed in conscious rats, which were fasted for 5 h prior to testing. Insulin infusion was stopped 60 min before the first blood sample was taken. Baseline blood samples (~300 μL) were obtained via tail vein and blood glucose was measured with a glucometer (AccuCheck Aviva). An intraperitoneal injection of sterilized 50% dextrose (dissolved in physiological saline) was administered and blood samples were subsequently collected and blood glucose measured at 15, 30, 60, 90 and 120 min post-injection. Blood samples were chilled on ice; in-between data collection points, rats were returned to their home cage to minimize stress. At the end of the experiment, blood samples were centrifuged at 3000 rpm for 20 min and plasma was drawn off and stored at −80 °C for further analysis of circulating insulin levels (Meso Scale Diagnostics Kit, #K152BZC-3, Meso Scale Diagnostics, MD, USA).

2.5. Urine ketone body & plasma free fatty acid assessment

24-Hour urine samples were collected gravimetrically at 2, 4 and 6 week time-points during the insulin-infusion protocol in Study 1. Rats remained in their home metabolic cage and were not disturbed by the sample collection, as urine was funneled to specialized collection tubes connected to the base of the metabolic cage unit. Urine samples were centrifuged at 6000 rpm for 10 min to remove sediment and food particles. Supernatant was stored at −80 °C. Urine samples were later thawed on ice and ketone body (β-hydroxybutyrate) levels were assessed by a Ketone Body Colorimetric Assay Kit (#700190, Cayman Chemical, Ann Arbor, MI, USA). For plasma free fatty acid assessment, tail vein blood samples taken pre-treatment and at 1, 2, 4 and 6 week time-points were centrifuged at 3000 rpm for 20 min. Plasma was