Decreased Insulin Binding and Antilipolytic Response in Adipocytes from Patients with Cushing’s Syndrome

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Human adipocytes from patients with chronic endogenous hypercortisolism (Cushing’s syndrome) showed a statistically significant decrease in insulin binding at low unlabelled-insulin concentrations but no change in receptor numbers (Cushing’s 180,000 ± 48,000 (3) receptors/cell and controls 189,000 ± 30,000 (7)) together with a fourfold decrease in apparent receptor affinity (ED50: Cushing’s 2.25 × 10^{-9} M and controls 0.57 × 10^{-9} M) and a decreased sensitivity to the antilipolytic effect of insulin. These events could represent the final situation of a chronic and endogenous regulation by high levels of cortisol of insulin receptors in human adipose tissue.

INTRODUCTION

In humans, insulin binding studies after in vivo glucocorticoid-treatment have provided controversial results in circulating cells (1–11) and no changes in isolated adipocytes (12). Direct in vitro effects of glucocorticoids on the human insulin receptor have been studied in a monocyte-like cell line (U-937) (13), cultured lymphocytes (IM-9 line) (4,14,15), cultured human urinary bladder carcinoma cells (JTC-32) (16), human fibroblast cultures (17), and in human adipose tissue in culture (18), also rendering ambiguous results.

The present study investigates the binding of insulin to isolated human adipocytes

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from patients with chronic endogenous hypercortisolism. Data from other authors (6, 19, 20) have, to our knowledge, been obtained from circulating human cells. In addition, we studied the antilipolytic action of insulin in these human adipocytes as a post-binding event probably involved in the process.

**MATERIALS AND METHODS**

Three female patients with chronic endogenous hypercortisolism (Cushing's syndrome) were studied. They were diagnosed on the basis of clinical signs and symptoms and evaluated by a routine endocrine-function test. Their ages ranged from 39 to 59 yr. All of them were within 15% of their ideal body weight. Seven other patients (2 male and 5 female) were also included as controls. This group comprised patients undergoing cholecystectomy for gallstones. Their ages ranged from 31 to 59 yr. None of the patients had known endocrine or malignant disorders. All the patients received a normal hospital diet. Studies were performed in the morning following an overnight fast.

The adipose tissue was obtained from the subcutaneous tissue of the right upper quadrant of the abdomen. Isolated adipocytes were prepared by collagenase digestion according to the method reported by Rodbell (21). There was no size difference in adipocytes from Cushing's syndrome and control patients.

Mono-[¹²⁵I]insulin was prepared (22) with specific activities of 200–300 μCi/μg using Na¹²⁵I (Radiochemical Centre, Amersham).

Human adipocytes (0.1–0.3 x 10⁶ cells/ml) were incubated at a final volume of 350 μl with mono-[¹²⁵I]insulin (0.1–0.2 x 10⁻⁹ M) at 30°C for 30 min in Krebs-Hepes buffer pH 7.4 containing glucose (3.3 mM), HSA (1%) (Behring Co., Marburg) and bacitracin (0.9 mM) (Sigma Co., St Louis) either in the absence or the presence of unlabelled insulin (Novo Ind., Bagsvaerd) at increasing concentrations from 0.25 x 10⁻⁹ M to 0.5 x 10⁻⁷ M. Adipocytes were separated from the medium by centrifugation through dinonyl phthalate according to the method of Gliemann et al. (23). The amount of ¹²⁵I-insulin bound in the presence of 0.25 x 10⁻⁶ M unlabelled insulin was considered as non-specific binding; this was not different between groups. Binding data are expressed as ¹²⁵I-insulin specifically bound per 10⁶ cells/ml. Insulin degradation at the time of binding was determined by the amount of TCA-precipitable labelled insulin remaining after incubation using appropriate controls where fat cells were omitted.

For the antilipolysis studies, 100 μl of isolated adipocytes (0.1–0.3 x 10⁶ cells/ml) were incubated with Krebs-Hepes buffer pH 7.4 without glucose and with 1% FFA-free BSA (Sigma Co., St Louis) and bacitracin (0.9 mM). The cells were incubated at 37°C either in the absence or in the presence of 10⁻⁶ M isoproterenol or 10⁻⁶ M isoproterenol plus different concentrations of insulin (1.25 x 10⁻¹¹ M–1.25 x 10⁻¹⁰ M) for 120 min. The glycerol content was determined by the method of Lambert and Neish (24) with some of our own modifications (25). Glycerol data are expressed as nmol/10⁵ cells/2 h.

The results were expressed as mean ± SEM. For statistical comparison student's “t”-test was used.