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Burn-induced alterations of chromium and the glucose/insulin system in rats

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Abstract

Our objective was to demonstrate a role of chromium (Cr) in response to severe burn. A third-degree burn involving 20% of total body surface was applied under anaesthesia in accord with ethical guidelines. Chromium concentrations in liver decreased progressively and were non-detectable on days 5 and 10 following injury. In quadriceps muscle, Cr concentrations increased 6 h after injury and then declined significantly within the first day and remained at these levels the following 9 days. Urinary Cr losses were also increased. Changes in kidney, brain and serum Cr were not significant. Non-fasting glucose rose 6 h after injury and then returned to levels measured before the burn. There was a significant rise in corticosterone reaching a maximum the first day after injury that was accompanied by significant increases in circulating insulin and glucagon that were maximal after 2 days. Changes in IGF-1 were not significant. In summary, changes in Cr concentrations were associated with an early hyperglycemia, hyperinsulinemia and increased secretion of stress hormones. These observations strongly suggest a mobilization and utilization of Cr following severe burn. Additional studies are needed to document that improved Cr status might lead to improved recovery following burn.
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Keywords: Chromium; Burn; Insulin; Glucagon; Corticosterone; Stress

1. Introduction

When faced by an injury, the body develops a stress response comprising hypercatabolism related to an alteration in tissue sensitivity to insulin [1,2]. Severe burn induces insulin resistance, hypermetabolism, alterations of glucose [3] and related hormonal changes [4]. Hyperglycemia is commonly associated with the hyper metabolic stress response in burned humans [5] and animals [6] and persistent hyperglycemia may adversely affect wound healing and immunity and lead to increases in the number of infections and mean stay in hospital [7]. These findings suggest that interventional strategies to normalize plasma glucose and insulin in critically injured burn patients may be warranted. Therapeutic approaches consisting of insulin therapy to counteract the burn-induced alterations of glucose metabolism have shown beneficial effects [8,9]. In burn-injured rats, insulin exhibits beneficial effects on the inflammatory response and acts as an antiproteolytic factor preventing increased protein breakdown in the muscle [10].

Since insulin is intimately involved in the nutrition of trauma patients, the management of burn patients by nutrition that improves insulin sensitivity and limits the stress response should be considered [11]. Among the nutritional factors modulating the glucose/insulin system, the essential trace element trivalent chromium (Cr) plays a key role in the regulation of glucose and insulin metabolism

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[12]. Response to Cr is not universal and many studies have not reported beneficial effects of Cr [13–15]. Chromium is a nutrient and not a therapeutic agent and only those people overt or marginally deficient in Cr will show a response to supplemental Cr. In the presence of Cr in a useable form, much lower amounts of insulin are required and the insulin sensitivity is improved [14]. Chromium metabolism is closely linked to that of insulin and factors that alter insulin usually lead to changes in Cr metabolism. The signs and symptoms of insulin resistance including hyperglycemia, hyperinsulinemia and decreased lean body mass are associated with poor Cr nutriture [16]. Glucagon also decreases when Cr status improves [17] and increases in cortisol are correlated with increases in urinary Cr losses [18]. All these variables are also impacted negatively by burn. Moreover, burn patients may be at higher risk of impaired Cr status since dietary intakes may be suboptimal as shown by the beneficial effects of supplemental Cr in human subjects [19,14,20] and also stress increases losses of Cr leading to possible signs of marginal deficiency and therefore, aggravating the burn-induced insulin resistance [21]. We postulated that negative effects occur in Cr status following severe burn. We used an experimental animal model of burn that allowed for assessment of kinetic changes in plasma, urine and tissue Cr levels with changes in the glucose/insulin system.

2. Materials and methods

2.1. Study design

Male Wistar rats weighing 250 ± 10 g were purchased from Charles River Laboratories (Les Oncins, France). Animals were housed individually in thermoformed polystyrene cages in accordance with standards accredited by the French Ministries of Agriculture and Environment. The cages were located in a room with a 12-h light:12-h dark schedule and a pressurized filtered air barrier. The temperature was controlled to 21 ± 1 °C for the duration of the experiment with a relative humidity of 55%. Water and litter were sterilized to limit bacterial contamination risk. A standard diet from SAFE (Epinay sur Orge, France) and tap water were given ad libitum.

A third-degree burn involving 20% of total body surface centered on the back was applied following the technique described by Walker and Mason [22] to 50 rats divided into nine groups and 5 rats served as the sham operated control group. Thermal burn and sample collection were performed under halothane anaesthesia (Belamont Laboratories, Boulogne Billancourt, France) to alleviate any possible discomfort to the rats. In the control group, rats received the same treatments except temperate water taking the place of boiled water. Animals were sacrificed 6 h after thermal injury, and after 1–6, 8 and 10 days. All procedures were in accord with guidelines of the National Institutes of Health and were approved by the French Army Ethical Committee.

2.2. Sample collection

Samples were collected after a 12-h overnight fast under halothane anesthesia and animals were sacrificed by section of major vessels before tissue sampling. Blood was collected from the vena cava using trace element free S-monovette syringes (Sarstedt Inc., Germany) and siliconized needles attached to polyvinylchloride tubing (Minicath, Deseret Medical Inc., Sandy, UT) and centrifuged for 30 min at $2000 \times g$. After centrifugation, serum was stored in polypropylene tubes at −20 °C prior to analyses.

Urine was collected directly from the bladder at sacrifice using siliconized needles attached to polyvinylchloride tubing (Minicath, Deseret Medical Inc.) attached to trace element free S-monovette syringes (Sarstedt Inc.) and stored at −20 °C before analyses. Liver, kidney, femoral quadriceps and brain were removed using plastic materials to minimize Cr contamination, weighed, immediately frozen in liquid nitrogen and stored at −80 °C until analyses.

2.3. Hormone analyses

Insulin, glucagon, corticosterone and IGF-1 were assayed using kits supplied by ALPCO Diagnostics (Windham, NH).

2.3.1. Chromium analyses

Serum and urinary Cr were analysed by graphite furnace atomic absorption as described [23,24]. A bovine serum sample whose Cr concentration had been verified by two independent methods was run as a quality control sample for the serum Cr determinations. For urinary Cr determinations, two pooled urine samples, whose Cr concentrations had been verified by four independent methods, were assayed at least two times daily to validate the analytical reliability of the urinary Cr concentrations [25]. Tissue Cr analyses and verification of the values were as described [26].

Statistical analyses of the data were performed by analysis of variance. Individual mean comparisons were identified with Duncan's multiple range tests (SAS Institute, Cary, NC). Values with different superscripts are significantly different at $p < 0.05$. Multiple superscripts are needed since multiple time points are being compared; all superscripts denote significant differences at $p < 0.05$.

3. Results

There were no changes in food intake, water consumption and body weight of the animals subjected to burn injury and the control sham treated animals. The most sensitive indicator of burn was a rapid increase and then a decline in quadriceps Cr concentrations. Chromium concentrations increased 6 h after injury, and then declined significantly