Published in final edited form as:

Crit Care Med. 2010 June ; 38(6): . doi:10.1097/CCM.0b013e3181de8b9e.

Intensive insulin therapy improves insulin sensitivity and mitochondrial function in severely burned children

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Abstract

Objective—To institute intensive insulin therapy protocol in an acute pediatric burn unit and study the mechanisms underlying its benefits.

Design—Prospective, randomized study.

Setting—An acute pediatric burn unit in a tertiary teaching hospital.

Patients—Children, 4–18 yrs old, with total body surface area burned 40% and who arrived within 1 wk after injury were enrolled in the study.

Interventions—Patients were randomized to one of two groups. Intensive insulin therapy maintained blood glucose levels between 80 and 110 mg/dL. Conventional insulin therapy maintained blood glucose 215 mg/dL.

Measurements and Main Results—Twenty patients were included in the data analysis consisting of resting energy expenditure, whole body and liver insulin sensitivity, and skeletal muscle mitochondrial function. Studies were performed at 7 days post-burn (pretreatment) and at 21 days postburn (posttreatment). Resting energy expenditure significantly increased posttreatment (1476 ± 124 to 1925 ± 291 kcal/m²-day; $p = .02$) in conventional insulin therapy as compared with a decline in intensive insulin therapy. Glucose infusion rate was identical between groups before treatment (6.0 ± 0.8 conventional insulin therapy vs. 6.8 ± 0.9 mg/kg-min intensive insulin therapy; $p = .5$). Intensive insulin therapy displayed a significantly higher glucose clamp infusion rate posttreatment (9.1 ± 1.3 intensive insulin therapy versus 4.8 ± 0.6 mg/kg-min conventional insulin therapy, $p = .005$). Suppression of hepatic glucose release was significantly greater in the intensive insulin therapy after treatment compared with conventional insulin therapy (5.0 ± 0.9 vs. 2.5 ± 0.6 mg/kg-min; intensive insulin therapy vs. conventional insulin therapy; $p = .03$). States 3 and 4 mitochondrial oxidation of palmitate significantly improved in intensive insulin therapy (0.9 ± 0.1 to 1.7 ± 0.1 $\mu\text{m O}_2/\text{CS}/\text{mg protein}/\text{min}$ for state 3, $p = .004$; and 0.7 ± 0.1 to 1.3 ± 0.1 $\mu\text{m O}_2/\text{CS}/\text{mg protein}/\text{min}$ for state 4, $p < .002$), whereas conventional insulin therapy remained at the same level of activity (0.9 ± 0.1 to 0.8 ± 0.1 $\mu\text{m O}_2/\text{CS}/\text{mg protein}/\text{min}$ for state 3, $p = .4$; 0.6 ± 0.03 to 0.7 ± 0.1 $\mu\text{m O}_2/\text{CS}/\text{mg protein}/\text{min}$, $p = .6$).

Conclusion—Controlling blood glucose levels 120 mg/dL using an intensive insulin therapy protocol improves insulin sensitivity and mitochondrial oxidative capacity while decreasing resting energy expenditure in severely burned children.

Keywords

burns; child; pediatrics; insulin; hyperinsulinism; hyperglycemia; critical care

Hyperglycemia is a common sequelae of the metabolic response after thermal injury (1, 2). The cause is uncertain, but gluconeogenic hormones such as glucagon, cortisol, and catecholamines are elevated postinjury and likely play a role in increased plasma glucose (3). In addition, insulin resistance takes place in the skeletal muscle, which leads to a decrease in peripheral glucose uptake and an increase in hepatic glucose production and release (4).

Mitochondrial dysfunction has been implicated as a possible cause of insulin resistance in several different patient populations. Studies in the uninjured population, which include patients with type 2 diabetes and insulin-resistant elderly individuals displayed a reduction in skeletal muscle mitochondrial activity (5, 6). Thermally injured mice experience a downregulation of genes controlling mitochondrial function as well as carbohydrate and lipid metabolism (7). Changes in mitochondrial ultrastructure (8) and deficiencies in mitochondrial function (9) may be related to the alterations in insulin sensitivity after burn injury.

Hyperglycemia resulting from insulin resistance may be a clinical liability in the critically ill patient, which has led to the implementation of intensive insulin therapy protocols in adult intensive care units worldwide (10). Numerous studies have demonstrated improvements in clinical outcomes and mortality in intensive care unit patients who were treated with a tight glycemic control protocol (11–13). Furthermore, we have shown that continuous infusion of insulin stimulates muscle protein synthesis and improves lean body mass (14) without increasing hepatic triglyceride production (15) in severely burned children and adults.

However, the use of continuous infusion of insulin is not without concern. In review of the literature, chronic hyperinsulinemia can lead to further insulin resistance. This phenomenon has been illustrated in cultured animal (16, 17) and human cell lines (18) as well as in patients with cirrhosis of the liver (19) and healthy volunteers (20). Consequently, our aim was to determine the effect of tight glycemic control using an intensive insulin therapy protocol on mitochondrial function and insulin sensitivity in severely burned children.

MATERIALS AND METHODS

Patients and Clinical Care

This study was approved by the Institutional Review Board of the University of Texas Medical Branch. Informed written consent was obtained from each patient's guardian with assent of patients aged ≥ 7 yrs before enrollment into the study. This was a prospective, randomized clinical study.

Children, aged 4–18 yrs, with total body surface area burned $\geq 40\%$ requiring skin grafting, who arrived to the Shriners Hospital for Children Galveston within 1 wk after injury, were eligible for enrollment. All patients were treated in an identical surgical manner by the same team of burn surgeons. Standard treatment included early excision of the burn wound, systemic antibiotic therapy, and continuous enteral feeding (21). Children were >4 yrs as a result of limitations in sample collection and disproportionate noninsulin-sensitive glucose use by the brain in smaller children.

Patients were fed with Vivonex Total Enteral Nutrition (Sandoz Nutritional, Minneapolis, MN) through a nasoduodenal tube. The daily caloric intake was initially calculated to deliver 1500 kcal/m² body surface area + 1500 kcal/m² total body surface area burned. As soon as feasible typical within the first several days after admission, the resting energy expenditure was directly measured in each patient and they were then fed 120% of their measured caloric needs. Enteral nutrition was started at admission and continued until all burn wounds were determined as 95% healed by an attending physician.

Study Design

Two metabolic studies were performed in the acute recovery period, the first several days after the initial excision and the second after approximately 2 wks of therapy. Resting energy expenditure (REE), insulin-stimulated glucose uptake, hepatic glucose release, and skeletal muscle mitochondrial activity were the main outcome variables. Immediately after the first metabolic examination, patients were randomized to receive treatment using either an intensive insulin therapy protocol (Fig. 1) or conventional insulin therapy protocol (Fig. 2). Patients on the intensive insulin therapy protocol were placed on a continuous insulin infusion, which was titrated to maintain blood glucose levels between 80 mg/dL and 110 mg/dL. Blood glucose was monitored hourly using an Accucheek Advantage glucometer (Roche Diagnostics, Mannheim, Germany). Patients on the conventional insulin therapy protocol had blood glucose maintained <215 mg/dL with intravenous insulin administered using a sliding scale. Blood glucose was monitored every 2 hrs using the same method described previously in this article. Patients remained on their respective treatment protocols for approximately 10–14 days and underwent a second metabolic study at approximately 21 days after burn injury (Fig. 3).

Each metabolic study took place in the morning after a 4-hr fast with intravenous fluids of 0.9 normal saline and 8 hrs without blood, blood products, or albumin transfusions. Insulin therapy was discontinued 5 hrs before the initiation of the study to allow for fasting. Metabolic studies were performed approximately 4 days after the child's first (post-burn day 7) and third (postburn day 21) excision and grafting procedures. This allowed for the metabolic changes occurring with surgery to return to baseline as well as for the use of existing arterial and venous catheters.

Subjects were randomized to the insulin study as part of a hospitalwide multistudy randomization and thus did not receive any other study medications used in our institution such as fenofibrate, high-dose propranolol, growth hormone, or oxandrolone.

Measurement of Serum Glucose and Insulin

Fasting serum levels of glucose were determined (Stat-5 analyzer; Novel Biomedical, Waltham, MA) at 4 AM on the morning of the metabolic studies. In addition, serum levels of insulin were drawn every other day during the treatment period. Fasting insulin levels were determined using radioactive immunoassays (Diagnostic Laboratories, Los Angeles, CA). Non-fasted insulin levels were measured using enzyme-linked immunosorbent assays (Diagnostic Systems Laboratories, Inc, Webster, TX).

Indirect Calorimetry

Indirect calorimetry was performed at 5 AM on the day of the metabolic studies while the patients were asleep and had been fasted for at least 4 hrs. The main outcome measurement was REE, which was determined using a Sensor-Medics Vmax 29 metabolic cart (Yorba Linda, CA). Subjects were tested in a supine position while under a large, clear, ventilated hood. The REE was calculated from the measured rates of oxygen consumption and carbon dioxide production using an equation described by Weir (22). All REE measurements were

made at ambient temperatures of 30°C, which is the standard environmental setting for all patient rooms in our acute burn intensive care unit.

Isotopic Tracer Study

The details of the tracer study protocol are illustrated in Figure 4. Background blood samples were drawn before the start of the 4-hr basal period. A primed (47.1 $\mu\text{mol/kg}$), constant infusion of 6, 6- d_2 glucose was given intravenously at a rate of 1.110 $\mu\text{mol/kg}\cdot\text{min}$ and maintained for 8 hrs. A hyperinsulinemic–euglycemic clamp followed the 4-hr basal period. During the clamp, a primed (1.5 mU/kg), constant infusion of insulin was given intravenously at a rate of 1.5 mU/kg·min for the following 4 hrs, whereas 20% dextrose was simultaneously infused to maintain blood glucose levels between 80 and 90 mg/dL. Blood glucose levels were measured every 10 mins during the 4-hr clamp using an Accucheck Advantage glucometer. Arterial blood samples were taken 10 mins apart in triplicate at the end of each period. Under local anesthetic with lidocaine and general administration of ketamine and versed, a 50-mg muscle biopsy from the vastus lateralis was taken using a Bergstrom biopsy needle (Stille, Stockholm, Sweden). The muscle sample was immediately placed into cold buffer solution to stop mitochondrial activity and enzyme destruction.

Sample Analysis

Glucose Enrichment—Arterial samples were collected in lithium heparin tubes, centrifuged, and frozen at -80°C until analysis as previously described (23). Using gas chromatography–mass spectrometry, the penta-acetate derivative was examined at the 200/202 mass fragments.

Mitochondrial Oxidation Rates—Oxygen consumption in saponin-skinned muscle fibers was measured polarographically using a Clark-type electrode (Hansatech, Norfolk, UK) in a water-jacketed glass chamber at 30°C. These measurements enable the comparison of mitochondrial respiratory states by expressing the rate of use of O_2 when adenosine diphosphate is available (state 3 respiration or coupled) in relation to the corresponding rate when adenosine diphosphate is depleted (state 4 respiration or uncoupled). The muscle biopsies, 15–30 mg wet weight, were first washed and minced into small bundles in relaxing solution. The small bundles were incubated in saponin solution to permeabilize the sarcolemma membrane. The glucose oxidation capacity was measured with 2 mM malate and 10 mM pyruvate as substrates plus 0.5 mM adenosine diphosphate. To measure mitochondrial free fatty acid oxidation, pyruvate was replaced by 1 mM palmitoyl-L-carnitine. The final respiration activity was normalized by oxygen consumed per minute over citrate synthase activity/mg protein.

Calculations

Glucose Infusion Rate During the Hyper-insulinemic–Euglycemic Clamp—

Glucose uptake in mg/kg·min was calculated by measuring the amount of 20% dextrose infused over 1 hr to maintain a steady state of plasma glucose within the desired range of 80–90 mg/dL and was corrected for the actual plasma glucose and dextrose concentrations.

Endogenous Glucose Production—The rate of endogenous glucose release by the liver, representing both gluconeogenesis and glycogenolysis, was calculated by measuring the dilution of the infused tracer by unlabeled glucose. During the basal period, the calculation is:

$$\text{Rate of appearance (Ra)} \\ (\text{mg/kg} \cdot \text{min}) = \frac{\text{Rate of the glucose tracer infusion}}{\text{Arterial enrichment of glucose}} \quad \text{Equation 1}$$

where arterial enrichment is expressed as the tracer/tracee ratio. During the hyperinsulinemic–euglycemic clamp, endogenous Ra was corrected by subtracting the amount of unlabeled glucose infused to maintain euglycemia. The equation was:

$$\text{Total endogenous Ra} \\ (\text{mg/kg} \cdot \text{min}) = \frac{\text{rate of labeled glucose infusion}}{\text{arterial enrichment} - \text{unlabeled glucose infusion}} \quad \text{Equation 2}$$

Statistical Analysis

Data are presented as mean $\pm$ SD in the tables and mean $\pm$ SEM in the figures. We analyzed the data using parametric and non-parametric tests. The results were similar between the two methods. We chose to present the parametric results. Two-sided paired Student's *t* tests were used to compare data within groups. Comparisons between groups were made by unpaired Student's *t* tests. A two-way repeated measures analysis of variance was used to assess mean daily glucose levels in Figure 5. $p < 0.05$ was considered statistically significant. All statistics were performed using SigmaStat software package, version 2.03 (Systat Software, Inc., San Jose, CA).

RESULTS

The demographics of the patient populations were similar between treatment groups (Table 1). A total of 29 subjects were enrolled in this study. Five patients (one conventional and four intensive) were discharged before their second study. Four patients (two conventional and two intensive) were unable to have a second full 8-hr tracer study for technical or clinical reasons: two were undergoing peritoneal dialysis (one conventional insulin therapy [CON], one intensive insulin therapy [INT]), one septic on pressors (INT), and one with no arterial access (CON). None of the children who underwent the second study were receiving mechanical ventilation or septic at the time of study. None of the patients died during the study.

The clinical laboratory measurements on the morning of the metabolic studies are shown in Table 2. There was no significant difference in the basal fasting plasma glucose levels before treatment. Although not statistically significant, mean fasting glucose concentrations increased after treatment in CON and decreased after treatment in INT. There was no significant change in fasting insulin levels in both groups. There were a total of 2714 hourly glucose measures taken in the INT. Of those, 1511 were within the range of 80–110 mg/dL, which accounts for 56% of the treatment period. Additionally, the average was taken for each treatment day. Blood glucose levels were maintained <120 mg/dL in the INT during the treatment period and were significantly lower than CON ($p < .05$; Fig. 5). The calculated incidence of hypoglycemia among the hourly measurements during the treatment period was approximately 3%. All episodes were asymptomatic and treated by lowering the insulin infusion to baseline (0.4 mU/kg/min) and giving a bolus of 20% dextrose if necessary. Mean insulin levels calculated for the treatment period are shown in Figure 6. The average insulin concentration was double in the INT (96.9 ± 17.3 $\mu\text{U/mL}$) compared with the CON (46.6 ± 21.2 $\mu\text{U/mL}$) during the 10- to 14-day treatment period.

Resting energy expenditure was normalized for body surface area. Before treatment, REE was similar in both CON and INT. There was a significant increase in REE in the CON group (1476 ± 124 to 1925 ± 291 kcal/m²·day; $p = .02$), whereas the INT group had a slight decrease in REE (Fig. 7). Overall, CON had an increase in REE by 449 ± 217 kcal/m²·day, whereas INT showed a decrease in REE by 181 ± 96 kcal/m²·day ($p = .01$).

During the hyperinsulinemic–euglycemic clamp, there was no significant difference in the glucose infusion rate between groups before treatment. However, at the end of the treatment period, the glucose infusion rate was higher in INT as compared with CON (9.1 ± 1.3 versus 4.8 ± 0.6 mg/kg·min, respectively, $p = .005$; Fig. 8A). Overall, there was a significant change in glucose infusion rate at the end of treatment. Glucose infusion decreased by 1.19 ± 0.65 mg/kg·min in CON, whereas the corresponding value increased in INT by 2.23 ± 1.16 mg/kg·min; $p = .015$).

Before the treatment period, suppression of endogenous glucose Ra from the basal period to the clamp was not significantly different between CON and INT. However, after treatment, endogenous glucose Ra was suppressed to a greater extent in INT than CON (5.0 ± 0.9 vs. 2.5 ± 0.6 mg/kg·min; INT vs. CON; $p = .02$; Fig. 8B).

The total rate of glucose uptake during the clamp is an indicator of peripheral insulin sensitivity. The endogenous glucose Ra was added to the amount of glucose infused to calculate total glucose uptake. Total glucose uptake was significantly greater in INT after treatment as compared with CON ($p = .009$; Fig. 8C).

Mitochondrial activity was measured by means of quantifying the rate of oxidation. Muscle mitochondrial oxidation capacity was similar between groups before treatment. State 3 (coupled) oxidation with pyruvate as a substrate significantly decreased in CON after treatment ($*p = .01$), and no other significant differences were displayed during state 4 (uncoupled) oxidation after treatment in both groups (Fig. 9). There was a significant increase in palmitate oxidation in INT with both state 3 (coupled; $*p < .001$, Fig. 10A) and state 4 (uncoupled; $*p = .003$, Fig. 10B) respiration, whereas the CON group demonstrated a significant decrease in coupled palmitate oxidation after treatment ($\dagger p = .01$).

DISCUSSION

Critical illness and severe injury of all forms generally led to hyperglycemia secondary to the catabolic stress response. Hyperglycemia results from a decreased capacity of insulin to increase peripheral glucose uptake and lower endogenous glucose production (24). Clinical studies in other critically ill patients have shown that blood glucose concentrations can be maintained in the normal range with intensive insulin therapy and positive clinical results are obtained (11, 13). Specifically, severely burned children demonstrated improvements in infectious complications and mortality when treated with intensive insulin therapy during acute hospitalization (25). In the current study, we have shown that tight glycemic control with intensive insulin therapy can be effectively performed in severely burned children. Furthermore, we found that intensive insulin therapy improves insulin sensitivity and muscle mitochondrial oxidative capacity.

Our study was the first to examine the effects and mechanisms of tight glycemic control on insulin sensitivity in severely burned pediatric patients. We found that burned children treated with an intensive insulin therapy protocol had a 30% increase in the rate of insulin-stimulated glucose uptake, whereas the conventionally treated patients showed a 20% reduction. Previous studies have shown the average insulin-stimulated glucose uptake rate to be 10–15 mg/kg·min in nonburned, nondiabetic children (26). Both groups before treatment had glucose uptake rates <10 mg/kg·min, indicating insulin resistance. Intensive insulin

therapy improved insulin sensitivity to near normal values, whereas glucose uptake declined further from week 1 to week 3 in the conventionally treated patients. The persistence of insulin resistance in the conventional group is expected because insulin resistance begins to develop 1 hr after burn injury (27) and persists 100 days after a thermal injury (28).

Implementing an intensive insulin therapy protocol in an acute pediatric burn unit faces challenges in compliance with narrow target blood glucose ranges. On admission, we faced high blood glucose values secondary to the hypermetabolic stress response to injury. Then throughout hospitalization, daily burn care, including wound dressing changes, débridements, and frequent excision and grafting, led to more stress-induced responses, which further elevated blood glucose levels. Although our initial target blood glucose was 80–110 mg/dL, we were able to keep blood glucose levels between 80 and 120 mg/dL during 56% of the acute hospital stay. A study in tight glucose control in patients who underwent coronary artery bypass surgery defined good glucose control as glucose within their target range for >50% of the measured time (29). Other studies have shown that narrower target blood glucose ranges have led to lower compliance rates (30, 31). It is of importance to note that improvements in glucose metabolism and skeletal muscle mitochondrial activity were shown with just 50% compliance after treatment with an intensive insulin therapy protocol.

Furthermore, we used glucometers not only for point of care glucose values, but also for study values during our hyperinsulinemic–euglycemic clamp. Literature has shown that glucometer readings are directly affected by hematocrit levels (32–34). Compared with glucose analyzers, a lower hematocrit yields a higher plasma glucose level and vice versa (32). This poses a limitation not only to our study, but to the majority of intensive care units worldwide that use glucometers for point of care because critically ill patients often have variable changes in hematocrit levels, which could lead to invalid readings. However, it should be noted that in our study, hematocrit levels did not differ between groups before or after treatment and were above the Roche Diagnostics-recommended range for optimal device functioning (25% to 60%).

Studies have indicated that the severe catabolic state associated with burn injury persists up to 12 mos after injury (2, 35). Thus, the improved insulin sensitivity in the INT group was somewhat unexpected, because prolonged hormone therapy generally induces tachyphylaxis to the infused hormone rather than an increased stimulatory response. In animal studies, hyperinsulinemia created a dose-dependent loss in insulin receptors, a decrease in insulin-stimulated glucose transport, and a decrease in insulin degradation (16). Furthermore, normal volunteers showed a reduction in glucose use and overall glucose metabolism after 40 hrs of hyperinsulinemia as compared with after infusion with normal saline (36). Even the ability of exercise to increase insulin-stimulated glucose uptake is blunted in insulin-resistant type 1 diabetic subjects as compared with normal control subjects (37).

A hyperinsulinemic state would be expected to suppress endogenous glucose production by the liver (38). Children treated with an intensive insulin therapy protocol showed almost twice the suppression of endogenous glucose production as compared with children treated with the conventional therapy protocol. In addition, after adding the endogenous glucose produced by the liver to the amount of glucose infused during the hyperinsulinemic clamp, children treated with intensive insulin therapy continued to have a significantly greater glucose clamp infusion rate as compared with the CON group, thus indicating that peripheral response to insulin had improved as well. A similar study in adult trauma patients treated with intensive insulin therapy showed a reduction in endogenous glucose production as compared with control subjects (39).

Mitochondrial activity may play a role in the pathogenesis of insulin resistance. Studies in animal models have shown that burn injury causes a decline in mitochondrial function and the downregulation of gene activity in cardiac and skeletal muscle (7, 40, 41). We have recently found that mitochondrial oxidation is attenuated in burned children by approximately 70% of normal function 1 wk after injury (9). Postmortem evaluation of human liver mitochondria in critically ill adult patients had abnormalities in ultrastructure and deficiencies in activity, which significantly improved with strict blood glucose control (8). In our study, burned children similarly demonstrated the same level of impairment in mitochondrial oxidative function at 7 days postburn. Nonetheless, mitochondrial oxidation of palmitate improved to 50% of normal activity 14 days after intensive insulin therapy, whereas impairment of pyruvate oxidation remained the same.

We have previously shown evidence that impairment of the ability of mitochondria to oxidize fatty acids plays a role in the development of insulin resistance in pediatric burn patients (9). The selective improvement in palmitate oxidation could relate to the improved insulin sensitivity. This is best explained by stimulation of the transport of fatty acids into the mitochondria by carnitine palmitoyltransferase-1, which is involved in fatty acid oxidation (42). Simulation of the insulin resistant state by inducing hyperglycemia and hyper-insulinemia in healthy volunteers led to an almost threefold increase in skeletal muscle malonyl coenzyme A concentration (43). Malonyl coenzyme A acutely regulates fatty acid oxidation by inhibiting carnitine palmitoyltransferase-1 activity, thus leading to storage of long chain fatty acids (42). Therefore, it is possible that reversal of inhibited fatty acid oxidative capacity is linked to improved insulin sensitivity. Furthermore, treatment with peroxidase proliferator-activated receptor- α agonist in pediatric burn patients also improved insulin sensitivity as well as palmitate fat oxidation (44). This is somewhat paradoxical because the role of insulin is normally to limit fatty acid oxidative capacity (45, 46).

Resting energy expenditure (1), gluconeogenesis (47), and lipolysis (48) are elevated in thermally injured patients. Wolfe and colleagues (24) found that substrate cycling is increased in burn patients and leads to increased thermogenesis and energy expenditure. Gore et al (49) demonstrated that adenosine-5'-triphosphate and adenosine diphosphate concentration declined 72 hrs after burn injury in mice suggesting that energy expenditure is elevated, which leads to an increased consumption of adenosine-5'-triphosphate that exceeds the capacity of oxidative phosphorylation to produce adenosine-5'-triphosphate.

All children enrolled in our study were hypermetabolic before receiving treatment. However, 14 days after treatment, the CON patients had a significant increase in REE by 400 kcal/m²-day, whereas the intensive insulin therapy group showed a decrease in REE by 200 kcal/m²-day from day 7. It is possible that the dampening effect on REE seen after treatment with an intensive insulin therapy protocol may indicate a reduction in the overall stress caused by acute trauma such as a thermal injury, thereby lessening protein turnover, gluconeogenesis, and substrate cycling.

CONCLUSION

An intensive insulin therapy protocol in an acute pediatric burn unit can be instituted safely and effectively. Severely burned children, who were treated with an intensive insulin therapy protocol for 14 days, showed improvements in insulin sensitivity, mitochondrial oxidation, and a reduction in REE as compared with severely burned children with blood glucose levels ≥ 215 mg/dL. It is possible that improvements in mitochondrial function to oxidize fatty acids may attenuate the process of insulin resistance that takes place after an acute trauma. This is the first study to illustrate the outcomes of intensive insulin therapy on insulin sensitivity and mitochondrial oxidative capacity. Further studies looking at changes

in insulin signaling and fatty acid oxidation would provide further insight into the mechanisms by which tight glycemic control with constant infusion of insulin improves clinical outcomes after thermal injury.

Acknowledgments

We gratefully acknowledge Ann High-tower, Mary Finn, and the UTMB Metabolism Unit for their invaluable assistance. As well, we thank Dr Jong O. Lee, Dr Art P. Sanford, and the residents and fellows, the nurses, and respiratory staff at the Shriners Burns Hospital for Children Galveston for their clinical knowledge and care. Most importantly, we are indebted to the patients and their families for participating in our efforts.

Supported by the National Institutes of Health: R01-GM56687, "Modulation of the Post Burn Hypermetabolic Response" (DNH), T32-GM08256; "Postdoctoral Training in Trauma and Burns" (DNH), P50 GM60338; "Burn Center Grant: Assessment of Anabolic Agents and Exercise in Burn Children" (DNH), 5R01 DK034817-20; "Substrate Cycling in Burns" (RRW), Shriners Hospital for Children: 8940, "Mass Spectrometry Core Grant" (RRW).

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PHYSICIAN'S ORDER SHEET SHRINERS HOSPITAL FOR CHILDREN BURNS INSTITUTE			
☆ USE BALL POINT PEN - PRESS FIRMLY ☆		STAMP PATIENT'S NAME ABOVE	
<small>AUTHORIZATION IS GIVEN FOR DISPENSING BY NONPHYSICIAN STAFF NAME UNDER 3.B.1. FORMULARY SYSTEM UNLESS OTHERWISE SPECIFIED.</small>		<small>WEIGHT</small> <small>AGE</small>	<small>NOTE: ALL ORDERS MUST BE SIGNED BY PHYSICIANS. NURSES MUST ACKNOWLEDGE ORDERS WITH SIGNATURE, DATE AND HOUR!</small> <small>ALLERGIES:</small>
DATE	HOUR	ORDERS	SIGNATURE <small>DATE HOUR</small>
		Intensive Insulin Treatment-(IRB 454) <ol style="list-style-type: none"> Weight _____ kg. Baseline insulin rate _____ units per hour (0.05 units/kg/hour). DO NOT infuse insulin below baseline rate. Only regular insulin may be given intravenously. Infusion will be prepared by mixing regular insulin with 100 ml of 0.9 NS. Flush at least 25 ml of the solution through the line before connecting the infusion to the patient. Pt must remain on continuous enteral feedings. All medications should be mixed in 0.9 NS. Draw glucose levels one hour after insulin infusion is started and adjust the insulin dose based on the sliding scale to reach a goal of blood glucose between 80 and 110 mg/dL. Check blood glucose level every hour until normalized (three consecutive measurements > 80 mg/dL and <110 mg/dL). Once blood glucose levels are normalized (80 mg/dL to 110 mg/dL), check blood glucose every two hours. If blood glucose is less than 80, if insulin is stopped or if tube feedings have been discontinued for any reason (i.e. high residuals), start infusing D20 at 25 ml/hr and NOTIFY H.O. and Ricki Fram, M.D. at pager 643-4716. Then, check blood glucose levels Q 1 hour until the target range of 80 mg/dL-110 mg/dL is reached. Decrease D20 by 10 ml every hour until off if possible while keeping glucose at target goal, (80 mg/dL - 110 mg/dL) Adjust insulin infusion per sliding scale: <ul style="list-style-type: none"> If blood glucose is 80-110 mg/dL - no change. If blood glucose is 111-120 mg/dL, start infusion at _____ units per hour (0.05 unit/kg/hour). If blood glucose is 121-140 mg/dL, change infusion to _____ units per hour (0.1 units/kg/hour). If blood glucose is 141-160 mg/dL, change infusion to _____ units per hour (0.2 units/kg/hour). 	

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DATE	HOUR	ORDERS	SIGNATURE <small>DATE HOUR</small>
		<ul style="list-style-type: none"> If blood glucose is 161-190 mg/dL, change infusion to _____ units per hour (0.4 units/kg/hour). If blood glucose is 191-220 mg/dL, change infusion to _____ units per hour (0.6 units/kg/hour). If blood glucose is 221-260 mg/dL, change infusion to _____ units per hour (0.8 units/kg/hour). If blood glucose is greater than 260 mg/dL change infusion to _____ units per hour (1 unit/kg/hour) and NOTIFY H.O. and Ricki Fram, M.D. at pager 643-4716 Check blood glucose every hour until goal rate is achieved with three consecutive readings. Then accuchecks may be done every two hours. 	

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Figure 1.
Intensive insulin therapy protocol.

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AUTHORIZATION IS GIVEN FOR DISPENSING BY NONPROPRIETARY NAME UNDER S.B.I. FORMULARY SYSTEM UNLESS OTHERWISE SPECIFIED.		WEIGHT	AGE	NOTE: ALL ORDERS MUST BE SIGNED BY PHYSICIANS. NURSES MUST ACKNOWLEDGE ORDERS WITH SIGNATURE, DATE AND HOUR! ALLERGIES:	
DATE	HOUR	ORDERS		SIGNATURE	DATE HOUR
		Conventional Treatment Insulin Orders <ol style="list-style-type: none"> 1. Check blood glucose levels Q2 hours. 2. Patient to remain on continuous enteral tube feeds at ordered rate 3. If blood glucose is 80-215, no treatment is necessary. 4. If blood glucose is greater than 180, multistick urine and notify H.O. 5. If blood glucose exceeds 215 mg/dl, give 4 units IVP and check glucose in 30 minutes. If blood glucose is again 215 mg/dl, give an additional 4 units IVP and start insulin infusion at 2 units per hour. 6. Check blood glucose level every 30 minutes until a target goal between 80 and 215 mg/dl is reached, then return to accuchecks Q2 hours. 7. If blood glucose is between 250 and 300 give 6 units IVP and increase insulin infusion rate by 1 unit per hour. 8. Check blood glucose levels Q30 minutes until target goal is reached, then return to Q1 hour for 4 hours then Q 2 hours. 9. If blood glucose is less than 80 mg/dl or greater than 300 mg/dl, NOTIFY H.O. 			

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Figure 2.
Conventional insulin therapy protocol.

Figure 3.
Overall study design. *OR*, operation; *Post-Op*, postoperation.

Figure 4.
Infusion study design.

Figure 5.

Daily mean plasma glucose levels throughout the treatment period. Daily mean plasma glucose concentration in the intensive group and conventional group (mg/dL) during the treatment period. Blood glucose levels were checked hourly in the intensive insulin therapy group and every 2 hrs in the conventional insulin therapy group. * $p < .05$ between intensive insulin therapy group and conventional insulin therapy group.

Figure 6.

Mean insulin levels per treatment period. Average insulin concentration during treatment period (postburn day 7 through 21). Insulin concentration was measured every other day during the treatment period. The average insulin concentration for the conventional group was 46.6 ± 21.2 $\mu\text{U/mL}$, whereas the intensive group was 96.9 ± 17.3 $\mu\text{U/mL}$. Unburned pediatric values are represented with the black bar and range from 2 to 30 $\mu\text{U/mL}$.

Figure 7.

Resting energy expenditure (REE) before and after conventional or intensive insulin therapy protocol. REE was measured during each study (postburn day 7 and 21) in the fasting state. REE is normalized for body surface area and expressed in units of kcal/m²·day. * $p < .05$ in the conventional group at 7 days postburn vs. 21 days postburn.

Figure 8.

Peripheral, hepatic, and whole body glucose metabolism before and after treatment with conventional or intensive insulin therapy protocol. *A*, Glucose infusion rate required to maintain euglycemia during the hyperinsulinemic–euglycemic clamp before and after treatment (postburn days 7 and 21). $*p = .005$ between intensive group and conventional group at 21 days postburn. *B*, The change in suppression of hepatic glucose output during hyperinsulinemia as compared with the basal period before and after treatment is shown. $*p = .023$ between intensive group and conventional group at 21 days postburn. *C*, Total glucose uptake (endogenous and exogenous) during hyperinsulinemia expressed in units of mg/kg·min. $*p = .009$ between intensive group and conventional group at 21 days postburn.

Figure 9.

Changes in skeletal muscle mitochondria oxidation capacity of pyruvate before and after treatment with conventional or intensive insulin therapy protocol. *A*, State 3 (coupled) muscle mitochondria pyruvate oxidative capacity expressed in units of $\mu\text{mol O}_2/\text{mg protein}\cdot\text{min}$. $*p = .010$ in conventional group at 7 days postburn compared with 21 days postburn. *B*, State 4 (uncoupled) muscle mitochondria pyruvate oxidative capacity expressed in units of $\mu\text{mol O}_2/\text{mg protein}\cdot\text{min}$. There was no difference in either treatment group.

Figure 10.

Changes in skeletal muscle mitochondria oxidation capacity of palmitate before and after treatment with conventional or intensive insulin therapy protocol. *A*, State 3 (coupled) muscle mitochondria palmitate oxidative capacity expressed in units of $\mu\text{mol O}_2/\text{mg protein}\cdot\text{min}$. There was a significant decrease in coupled palmitate oxidation from 7 days postburn to 21 days postburn ($\dagger p = .010$) in the conventional group. The intensive group displayed a significant increase in coupled palmitate oxidation from 7 days postburn vs. 21 days postburn ($*p < .001$). *B*, State 4 (uncoupled) muscle mitochondria palmitate oxidative capacity expressed in units of $\mu\text{mol O}_2/\text{mg protein}\cdot\text{min}$. Uncoupled palmitate oxidation significantly increased in the intensive insulin therapy group after treatment ($*p = .003$).

Table 1

Patient demographics

Measurement	Conventional	Intensive
No. of subjects	11	9
Age, yrs	8 $\pm$ 4	6 $\pm$ 4
Sex, male:female	7:4	6:3
Total body surface area burned, %	68 $\pm$ 15	64 $\pm$ 15
Third-degree burns, %	59 $\pm$ 24	55 $\pm$ 24
Time from burn to admittance, days	3 $\pm$ 2	3 $\pm$ 1

Data are presented as means $\pm$ SD.

Table 2Clinical laboratory measurements^a

Measurement	Conventional Postburn Day 7	Conventional Postburn Day 21	Intensive Postburn Day 7	Intensive Postburn Day 21
Average heart rate, beats/min	148 ± 16	148 ± 15	156 ± 13	156 ± 13
Average hemoglobin, g/dL	11 ± 1	10 ± 1	11 ± 1	9 ± 1
Average hematocrit, %	32 ± 3	29 ± 3	30 ± 3	27 ± 4
White blood cell count, cm/m ²	12 ± 5	14 ± 3	13 ± 5	13 ± 2
Blood urea nitrogen, mg/dL	13 ± 4	15 ± 5	15 ± 8	10 ± 2 ^c
Creatinine, μmol/L	0.8 ± 1	0.4 ± 0.1	0.6 ± 0.4	0.26 ± 0.1 ^b
Alkaline phosphatase, U/L	112 ± 38	126 ± 33	132 ± 23	147 ± 30 ^b
Aspartate transaminase, U/L	95 ± 83	37 ± 24 ^b	118 ± 89	50 ± 28 ^b
Alanine transaminase, U/L	93 ± 72	63 ± 52	111 ± 62	69 ± 44
Gamma glutamyl transferase, U/L	47 ± 31	97 ± 27 ^b	106 ± 76 ^c	95 ± 30
Glucose, mg/dL	102 ± 6	121 ± 35	112 ± 22	104 ± 11
Insulin, μU/mL	6 ± 6	7 ± 5	4 ± 2	3 ± 2
Morning cortisol, nmol/L	17 ± 10	27 ± 10	27 ± 17	15 ± 11

^a Laboratory values were drawn between 4:00 AM and 5:00 AM the morning of the metabolic studies. All measurements are fasted. Data are means ± SD;

^b $p < .05$ and denotes a significant change from pretreatment values;

^c $p < .05$ and denotes a significant change between groups.