Published in final edited form as:

J Burn Care Res. 2008 ; 29(4): 585–594. doi:10.1097/BCR.0b013e31817db88f.

Insulin sensitivity is related to fat oxidation and protein kinase C activity in children with acute burn injury

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Abstract

Objective—Impaired fatty acid oxidation occurs with type 2 diabetes and is associated with accumulations of intracellular lipids, which may increase diacylglycerol, stimulate protein kinase C activity and inactivate insulin signaling. Glucose and fat metabolism are altered in burn patients, but have never been related to intracellular lipids or insulin signaling.

Methods—Thirty children sustaining >40% total body surface area burns were studied acutely with glucose and palmitate tracer infusions and a hyper-insulinemic euglycemic clamp. Muscle triglyceride, diacylglycerol, fatty acyl CoA and insulin signaling were measured. Liver and muscle triglyceride levels were measured with magnetic resonance spectroscopy. Muscle samples from healthy children were controls for diacylglycerol concentrations.

Results—Insulin sensitivity was reduced and correlated with whole body palmitate β -oxidation ($P=0.004$). Muscle insulin signaling was not stimulated by hyper-insulinemia. Tissue triglyceride concentrations and activated protein kinase C- β were elevated, whereas the concentration of diacylglycerol was similar to the controls. Free fatty acid profiles of muscle triglyceride did not match diacylglycerol.

Conclusions—Insulin resistance following burn injury is accompanied by decreased insulin signaling and increased protein kinase C- β activation. The best metabolic predictor of insulin resistance in burned patients was palmitate oxidation.

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Keywords

Insulin Sensitivity; fat metabolism; Protein Kinase C; Burn Trauma

INTRODUCTION

Previous studies have documented hyperglycemia, insulin resistance and increased concentrations of circulating free fatty acids post-trauma¹⁻³. Acute onset insulin resistance has also been documented in patients after surgical procedures, myocardial infarctions or stroke⁴. Insulin resistance and related liver dysfunction may be implicated in the increased morbidity and mortality of surgical and burned patients⁴⁻⁶. Trauma provides a unique opportunity to study the acute onset of insulin resistance in an altered physiologic state. Further, lean children often have not yet developed co-morbid conditions such as hypertension or hyper-lipidemia that may contribute to the susceptibility for development of insulin resistance post trauma. Thus, the rate of development of severe insulin resistance in these patients with no history of metabolic disorder can be remarkable.

Studies in the elderly and in type 2 diabetics have suggested that insulin resistance is related to alterations in mitochondrial fat metabolism^{7, 8}. The ability of mitochondria to oxidize long chain fatty acids is impaired in both elderly and diabetic patients, although it is not clear if this is due to failure of fatty acids to enter mitochondria through the carnitine palmitoyl transferase-1 (CPT-1) transporter, lack of mitochondria, or decreased respiratory capacity of the inner mitochondrial membrane^{7, 8}. One hypothesis is that impaired fatty acid oxidation results in the accumulation of intracellular triglycerides (TAG) and, thus, diacylglycerol (DAG) in the muscle, which in turn inactivates the insulin signaling pathway through activation of protein kinase C (PKC) and prevention of insulin receptor substrate-1 (IRS-1) signaling⁹. However, these pathways have never been examined in acute onset insulin resistance in humans, such as occurs after severe trauma.

The overall goal of this study was to attempt to understand some of the mechanisms involved in the development of insulin resistance following trauma. This study was designed to determine whole body glucose disposal and fat oxidation, and insulin signaling and PKC activation in the muscle of previously healthy burned children during both the basal and hyper-insulinemic state. Further, basal muscle tissue concentrations of DAG, TAG and liver triglycerides (LFAT) were also measured. Muscle tissue DAG concentrations were measured in healthy children in order to assess the difference induced by the burn injury, and other basal measurements were compared to literature controls.

METHODS

Study design

This was a prospective, cross sectional study with a patient and a control group. All protocols were approved by the IRB at the University of Texas Medical Branch. A legal guardian provided permission for participation of the child in the study. Assent was obtained in children aged 7–18 when medically and ethically possible.

Patient study

Thirty children aged 4–17 and >20 kg with >40% total body surface area burns that would require skin grafting, who arrived at our hospital within 96 hours of injury were eligible. Children with major electrical burns, renal or hepatic failure, iodine allergies, severe sepsis or who had required resuscitation from cardiac arrest were excluded. A diabetic family

history was obtained from the families, but is admittedly limited, as many of the families had limited healthcare, and may have included members with undiagnosed disease. 2 patients had a 1st degree relative with type 2 diabetes, and 2 had a second degree relative with diabetes. This is lower than the prevalence of children with a diabetic family history in the US. Of these 4 children, one of them developed type 2 diabetes within the recovery period of her injury and remains on medication at this time (4 years post-injury). This individual was also the only child who was obese prior to injury. 7 of the children were intubated at the time of study due to respiratory injury, and oxygen saturation was maintained above 96%.

Medical care was determined by faculty surgeons, fellows and residents according to clinical protocols that have been previously described¹⁰. Patients were fed with Vivonex T.E.N. © (Novartis, Minneapolis, Minnesota –82% carbohydrate, 15% protein, 3% fat) at 1.4 times their measured resting energy expenditure. The resting metabolic rate was determined once a week in the early morning at 30°C with a V-max 29 metabolic cart (Sensormedics, Yorba Linda, CA). The patients received nutritional supplements including a multi-vitamin, folic acid, zinc, and vitamin C.

A tracer metabolic study was performed within 10 days of injury and 4 days after the children's first excision and skin grafting operation to avoid acute operational-induced metabolic changes, yet make use of the existing venous and arterial access (Figure 1). To achieve a basal fasting state within reasonable clinical limits, each study was preceded by a 4 hour fast with IV fluids consisting of only saline, and 8 hours without blood, blood products or albumin transfusions. A 4 hour basal period was followed by a 4 hour hyper-insulinemic-euglycemic clamp. During the clamp period, insulin was infused at the rate of 1.5 mU•min⁻¹•kg⁻¹ into a vein and 20% Dextrose was simultaneously infused to maintain a plasma glucose concentration between 85–95 mg/dL. Muscle biopsies from the vastus lateralis were performed one hour into the tracer study, and at the end of each period. Room temperature was maintained between 85 and 95°F prior to and during the study. The total loss in a full size patient was 150 cc and 100 in the smallest child. The overall RBC loss in smaller children was offset in 2 ways—first smaller sample sizes were taken in the smaller children, and secondly, the volumes for the infusates was relatively greater (i.e. same volume regardless of the size of the child), giving a larger fluid bolus and thus increasing dilution during the study. The smaller sample sizes in the smaller children allowed for no repeats of any processing, were the sample to be altered in processing.

Background samples were taken prior to the start of a primed constant infusion of 6,6 D2 glucose at the rate of 0.444 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ that ran throughout the entire 8 hour study period. Arterial samples for glucose enrichment were taken 10 min apart in triplicate at the end of each 4 hour period. An infusion of U-¹³C palmitate was started at the rate of 0.08 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ after a 150 $\mu\text{mol}/\text{kg}$ NaH¹³CO₃ prime (dissolved in 0.45% saline) two hours after the start of each period, and 80 minutes were allowed to reach steady state before samples were taken for assessing palmitate turnover and oxidation. Four arterial, venous and breath samples were taken 10 min apart. A V-max 29 metabolic cart (Sensormedics, Yorba Linda, CA) was used during the sampling period to measure the volume of CO₂ expired. The same primed infusion of U-¹³C palmitate was again started 2 hours after the start of the clamp with an identical sampling schedule. LFAT and soleus triglycerides (IMCL) were assessed by magnetic resonance spectroscopy (MRS) as described previously the morning following the tracer study¹¹ except that the settings of the scans were altered to decrease the amount of time in the machine for the patients. For the liver scans, 128 scans were performed, rather than 256, and the repetition time 1188ms was rather than 2000ms. For the IMCL scans the repetition time was also decreased to 1188ms.

Control Study

Children undergoing open abdominal surgeries for non-metabolic illness such as hernia repair, orchidopexy, or ureter reimplantation were chosen as our control population. A small piece of the rectus abdominus or external oblique muscle was removed from the margin of the operative field for measurement of tissue DAG concentrations.

Seven children were enrolled as controls. The age range was 1–16 years, with a mean age of 6 ± 2 . 3 were Caucasian, 3 Hispanic and 1 African American and there were 6 males and one female. One child had a parent with type 2 diabetes, three children had a grandparent with type 2 diabetes, and the other three had no family history of diabetes. None of the mothers of the children had gestational diabetes during pregnancy. All of the children were lean and had no clinical signs of diabetes, such as polyuria, polydipsia or acanthosis nigricans.

Methods of Analysis

Plasma glucose concentration was measured on an YSI 2300 Stat glucose/lactate analyzer (YSI, Inc. Yellow Springs, OH). Plasma glucose was purified as described previously for measurement of enrichment, and the isotopic enrichment of the penta-acetate derivative was determined by gas chromatography mass spectrometry as described previously¹². Serum insulin concentrations were measured using radioimmunoassay (Diagnostic Laboratories, Los Angeles, CA). Plasma non-esterified Free fatty acids were measured with a standard kit (Wako Diagnostics, Osaka, Japan)

The insulin receptor (IR) tyrosine phosphorylation and IRS-1 tyrosine phosphorylation were measured from frozen muscle samples using Western-blot separation and antibody probing methods¹³. The same gels used for IR and IRS-1 were stripped, and re-probed for PI3K p85 content. The concentration of membrane bound, or activated protein kinase C- θ (PKC- θ) and protein kinase C- β (PKC- β) were measured from frozen muscle samples using Western-blot separation and antibody probing methods¹³.

LFAT and IMCL triglyceride measurements were processed with jMRUI software as previously described¹¹. DAG and TAG were measured as described previously¹⁴.

Plasma TNF- α , IL-1B, IL-6, resistin and adiponectin were measured using standard Elisa kits (Linco, St. Charles, MO).

FFA concentrations and enrichments were measured after purification as previously described¹⁵. Enrichment was measured with an HP/Agilent 6890GC/5973 Mass Spectrometer (Wilmington, DE) by monitoring the ions of the m/e 270 (M+0), 285(M+15) and 286 (M+16). Concentrations were measured on a HP- 5890 GC-FID with 3392A integrator or a HP-6890 GC-FID (Agilent technologies, Palo Alto, CA), using heptadecanoic acid as an internal standard.

Blood CO₂ samples were collected in 15 ml vacutainer tubes containing 7 drops of 85% phosphoric acid and breath samples were collected in plain 15ml vacutainer tubes. The samples were analyzed by means of an isotope ratio mass spectrometer (VG ISOGAS, Cheshire, England) and a standard curve prepared from phosphoric acid and NaH¹³CO₃ standard (Bayer, East Walpole, MA) was used to calculate the concentration in each blood sample.

Mathematical calculations

Glucose infusion rate in mg/kg/min was calculated by measuring the amount of 20% dextrose glucose infused to maintain plasma glucose between 80–90 mg/dL.

The rate of endogenous release of glucose by the liver in the basal state, representing both gluconeogenesis and glycogenolysis, was calculated by measuring the dilution of the infused tracer by unlabeled glucose. The same technique was used to measure the amount of unlabelled palmitate release. Thus during the basal period the calculation for both is:

$$\text{Rate of appearance} = \text{rate tracer infusion/arterial enrichment}$$

where arterial enrichment is expressed as tracer/tracee ratio. During the hyperinsulinemic-euglycemic clamp, the rate of endogenous release of glucose was calculated by subtracting the amount of unlabelled glucose infused to maintain euglycemia from the total rate of appearance. The units for glucose release are mg/kg/min. The palmitate results were divided by 33% (the contribution of palmitate to total long chain fatty acids) and thus the units for long chain FFA release are $\mu\text{mol/kg/min}$.

To calculate the rate of whole body fat oxidation, the following equation was used:

$$\text{Palmitate Oxidation} = - \frac{\text{Enrichment CO}_2 * \text{Volume CO}_2}{\text{Precursor Enrich} * \text{Acetate Corr} * (\%M + 16 * 16 + \%M + 15 * 15)}.$$

The precursor enrichment measurement was made prior to the infusion of the tracer and the acetate correction factor used for these calculations was 0.90, as determined by studies in a similar patient population of pediatric burns.

Statistics

All data are presented as the mean $\pm$ standard error of the mean. The difference between the basal and clamp periods was compared with a paired students T-test with an alpha value of 0.05. Pearson product correlation analyses were performed to determine the relationship between insulin stimulated glucose uptake and potentially related metabolites. All statistics were performed with Sigma Stat software package, version 2.03.

RESULTS

Basal measurements of hematologic, plasma lipids, renal hepatic function and metabolism are listed in table 1. The Resting energy expenditure (REE) was elevated, but not significantly so ($P=0.12$). AM cortisol levels were increased above normal, as has been previously shown, although adiponectin, resistin, IL-1 β , IL-6 and TNF- α were not significantly elevated.

Glucose metabolism

The mean insulin stimulated glucose uptake in mg/kg/min (M-value) in these patients was 5.4 ± 0.5 mg/kg/min of dextrose infused with a range of 1.9 to 11.5 (Figure 2). During the basal period the rate of hepatic glucose release was 4.86 ± 0.25 mg/kg/min and following hyper-insulinemia was significantly ($P<0.001$) suppressed 46% to 2.55 ± 0.23 mg/kg/min.

Lipid metabolism

Whole body palmitate oxidation in the basal state was 0.93 ± 0.09 μmol palmitate/kg.min and was significantly ($P=0.002$) suppressed by 50% during hyper-insulinemia (Figure 3A). Total release of long chain FFA was 10.9 ± 0.9 μmol FFA/kg.min and whereas insulin significantly ($P<0.001$) decreased FFA release, it was not fully suppressed (Figure 3B). The M-values correlated significantly with the basal rate of whole body free fatty acid oxidation ($P=0.01$, correlation coefficient=0.45) (Figure 3C).

A comparison of palmitate release and oxidation in burned children compared to those measured in healthy literature controls is shown in table 2. The concentrations of total long chain DAG were similar between burn and control patients (486 ± 103 BURN and 415 ± 22 Healthy pmol DAG/mg tissue; $p=0.09$), LFAT and IMCL measured with MRS were 0.27 ± 0.07 and 0.21 ± 0.06 ratio of standard to tissue fat respectively, in the burned patients, and were 3 times elevated compared to those of healthy young adults studied previously¹¹. Those studies with a * were conducted by the current investigators with similar methods.

Insulin Signaling Proteins

The tyrosine phosphorylation state, or activation of proteins involved in the insulin signaling cascade, including IR, IRS-1 and AKT and IRS-1 associated with PI3-K did not change following hyperinsulinemia (Figure 4A). However, the membrane bound, or activated PKC- β increased significantly following hyper-insulinemia ($P=0.008$)(Figure 4B).

DISCUSSION

This study was designed to attempt to understand the relationship between fat and glucose metabolism, as relates to insulin resistance and hyperglycemia following burns. We found that insulin sensitivity correlated with the basal rate of palmitate oxidation in children with burn injury implying a possible relationship between insulin resistance and an impaired ability to oxidize fatty acids. Further, we found that there was a lack of downstream insulin signaling and a significant increase in PKC- α activation in response to the acute elevation of insulin concentration. Whereas the IMCL and LFAT were increased compared to healthy young adults, the concentrations of intracellular skeletal muscle DAG was not elevated in burned compared to healthy children, implying that perhaps the concentrations of IMCL are not directly related to DAG, as has been demonstrated in patients with type 2 diabetes.

Whereas there was some variation in the insulin sensitivity of the burned children, the majority were insulin resistant based on values published in the literature from non-burned children during hyper-insulinemic euglycemic clamps. In lean, healthy children tested with a 1 mU/kg/min clamp, the glucose uptake was $15.6 \pm 2.3 \text{ mg/kg/min}$ ¹⁶. Healthy, obese adolescents given 1.3 mU/kg/min of insulin had a glucose uptake of $10.9 \pm 1.3 \text{ mg/kg/lean body mass}$ ¹⁷. A cohort of mixed lean and obese Caucasian children had a glucose uptake of $12.6 \pm 7.2 \text{ mg/kg/min}$ during hyperinsulinemia¹⁸. Based on these studies, the expected glucose uptake would be in the range of $10\text{--}15 \text{ mg/kg/min}$, with most of the children closer to 15, since they were generally pre-pubertal and lean. The wide range of values that we measured could be influenced not only by age, since adolescents have decreased insulin sensitivity with normal values near 10 mg/kg/min , but in the variation of the treatment patients received prior to arrival at our tertiary care center. However, the average glucose uptake in the burned patients was less than 6 mg/kg/min during the clamp indicating that the sensitivity to insulin in this patient population was reduced by more than 50% one week following the initial injury.

Whole body palmitate flux was comparable in the burned children in this study with our previous reports of $13.3 \pm 3 \text{ } \mu\text{mol/kg/min}$ in burned children and $14.5 \pm 1.1 \text{ } \mu\text{mol/kg/min}$ in burned adults¹⁹ and $8.1 \pm 1.9 \text{ } \mu\text{mol/kg/min}$ in trauma patients²⁰. These values are elevated compared to rates in healthy adults fasted for 24 hours ($6.7 \pm 0.7 \text{ } \mu\text{mol/kg/min}$) and are comparable to rates of lipolysis seen in adults fasted for 84 hours ($13.1 \pm 0.6 \text{ } \mu\text{mol/kg/min}$)²¹. Our previous work indicates that persistent beta-adrenergic stimulation is primarily responsible for the stimulation of lipolysis in burned children¹⁹.

Several studies have associated an increased availability of plasma FFA's with insulin resistance^{22–24}. Acute elevation of plasma FFA levels can induce insulin resistance^{25–27}.

Studies that have decreased plasma FFA's in volunteers and type II diabetics, with drugs such as acipimox, have shown an improvement in insulin sensitivity within three days,^{28, 29}. The improvement in insulin stimulated glucose uptake was associated with improved intracellular glucose metabolism. Thus, there seems to be a clear relationship between plasma FFA's and glucose metabolism^{30, 31}. The initial interpretation of the relationship between plasma FFA and insulin sensitivity was that increased fatty acid oxidation inhibited intracellular glucose oxidation and therefore glucose entry into the cell³². However, studies controlling fatty acid and glucose availability have failed to support this interpretation³³. In fact, more recently, it has become recognized that in diabetes, insulin resistance is associated with an impaired rate of fatty acid oxidation⁷. Consistent with that observation, in the current study we found that burned children with the lowest rates of fatty acid oxidation had the most impaired insulin sensitivity (figure 3C). This suggests that the relationship between FFA availability and insulin sensitivity is mediated by a mechanism other than a product of fatty acid oxidation.

Recently, the relationship between the accumulation of intracellular lipids and insulin resistance has received attention⁹. In that light, the amounts of both LFAT and IMCL in these burn patients were similar to those that we have found previously in elderly subjects with insulin resistance¹¹. Although we have not measured LFAT or IMCL in healthy children, we speculate that their values would be comparable to those in healthy young adults. If this is the case, measured LFAT and IMCL may be increased 3 to 4 fold in the children at one week post-burn. While this observation is consistent with the notion that accumulation of IMCL is responsible for insulin resistance, it is thought to be a source for DAG, which was not elevated.

Intracellular lipids can be elevated due to decreased oxidation of fatty acids, or increased delivery of FFA to the cell without compensatory increased in β -oxidation³⁴. Studies in animals indicate that mitochondrial function is impaired in tissues throughout the body, not just those tissues directly injured³⁵. This alteration appears to occur within 72 hours of burn. The mediator of this damage is unclear, although both TNF- α and FFA, potent disruptors of mitochondrial function, are elevated post-trauma³⁶, although we did not find elevations in these cytokines the day of our study. Thus, the increased intracellular lipids may partially be increased from mitochondrial damage.

We have recently documented altered insulin signaling in burn patients for the first time^{37, 38}. The signaling cascade should have been activated during the insulin clamp, as demonstrated by increased tyrosine phosphorylation of IR and IRS-1 and PI3-K association with IRS-1³⁹. However, we observed no response in tyrosine phosphorylation of the insulin receptor, IRS-1 tyrosine phosphorylation, or PI3-Kinase precipitation with IRS-1. This absence of muscle cell responsiveness to insulin was consistent with the impaired response of whole body glucose uptake. The abnormalities in insulin signaling found in burned children are similar to those in burned animals. Three days following a scald injury in rats, insulin did not induce stimulation of IR tyrosine phosphorylation, IRS-1 tyrosine phosphorylation or PI3-K activity in these animals compared to controls rats⁴⁰. Signaling downstream of PI3-K in rats was also attenuated by burn⁴¹. The lack of response of the intracellular insulin signaling pathway to insulin in week one of our studies is very similar to that seen in type 2 diabetes, in which there is a lack of insulin receptor phosphorylation, IRS-1 phosphorylation, PI3-K activation, and AKT activation⁴². We did not examine cell surface levels of GLUT-4 translocation in this study, however, when similar defects in insulin signaling are found in other models of insulin resistance, levels cell surface of GLUT-4 are also decreased..

Levels of PKC- β , but not PKC- θ , increased during insulin stimulation. PKC- β and PKC- θ are found in skeletal muscle⁴³ and both have been associated with insulin resistance in different settings, depending on the model, thus we examined both. PKC- β and PKC- θ are thought inhibit the migration of the muscle glucose transporter GLUT-4 to the cell surface by causing serine phosphorylation of the insulin receptor and IRS-1 and preventing downstream signaling⁴⁴. Animals overfed with either fat or glucose have increased concentrations of PKC during insulin stimulation and insulin resistance^{45, 46}. Increased concentrations of PKC are also found in diabetic patients and their offspring during hyperinsulinemia^{47, 48}. Therefore, the increased levels of PKC- β may have had contributed to the resultant impairment in insulin sensitivity in burn trauma patients as well.

Timing of the biopsy may not have been optimal to measure the peak of phosphorylation signaling, but should have been adequate to measure changes in DAG or PKC- β . Previous studies have shown that changes in peak insulin signaling are seen within 60 min of a hyperinsulinemic-euglycemic clamp⁴⁹. Other studies have found that phosphorylation of IRS-1 and activation of PKC's are detectable 3 hours after the initiation of hyperinsulinemia³⁹. However, under normal clinical conditions this patient population is continuously fed and, therefore, has constant stimulation of insulin secretion. Consequently, we measured insulin signaling at 4 hours post- insulin exposure to reflect the typical insulin stimulated state for this patient population, recognizing that we may not have captured the peak of phosphorylation. A study by Itani et utilized 2 biopsies following a high lipid infusion, and found decreased insulin sensitivity at 2 hours, but increases in DAG and PKC- β at 6 hours following the start of the lipid infusion. However, in our patient population, the first study was conducted 5–7 day's post-injury, during which time the patients were exposed to continuously high levels of FFA, and thus if the DAG were largely elevated, it likely could have been measured at any time point during the clamp.

Activation of PKC has been found to be responsive to concentration of intracellular lipids, including DAG⁴⁴. Thus, it is thought that although intracellular TAG's are related to insulin sensitivity, the actual mechanism is mediated by increases in DAG⁵⁰. However, in the current study, increased PKC were detected in the absence of increased long chain intracellular DAG concentrations. PKC can also be stimulated by ceramide, a fatty acid metabolite, or NFkappa- β from the inflammatory pathway and perhaps these pathways are activated following burn trauma^{50, 51}.

In conclusion, we measured several physiologic variables in burned patients that are altered in patients with type 2 diabetes. Our results support the likely relationship between the impairment of fatty acid oxidation and insulin resistance in burned patients, as is found in other models of insulin resistance and hyperglycemia. Additionally, impaired insulin signaling may be the result of increased levels of PKC- β . Finally, although the levels of intracellular TAG in the burned children generally relate to insulin resistance, our results fail to support the role of DAG in this regard. It could be the defects in insulin signaling are thus caused by other fat metabolites such as ceramides and this should be determined in future studies.

Acknowledgments

We would like to thank the patients and families for their participation in the study and acknowledge the contributions of the Shriners Hospital of Galveston research, clinical, respiratory and pharmacy staff, the UTMB Metabolism Unit staff.

Funding:

Shriners Hospital Grant 8490 to Robert R. Wolfe;

NIH R01 DK041317 “Substrate Cycling in Burns” to Robert R. Wolfe;

NIH R01-GM56687 “Modulation of the Post burn Hyper-metabolic Response” to D.N. Herndon.

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Figure 1. Tracer Infusion Study Design

The tracer study used 6,6 D₂ glucose, which ran for all 8 hours. There were four hours of fasted basal state, and 4 hour of hyper-insulinemic-euglycemic clamp. U¹³C palmitate was infused for the last two hours of each period, indirect calorimetry was performed at the end of each period, and biopsies were taken at 1,4 and 8 hours into the study.

Figure 2. Individual insulin sensitivity measurments

The individual measurements of insulin sensitivity are shown. The mean of 5.4 is indicated with the bar.

Figure 3. Palmitate metabolism

(A) Rates of release of free fatty acids in the basal and the hyper-insulinemic state. Release was reduced significantly during hyperinsulinemia ($P < 0.001$) (B) Rate of whole body palmitate oxidation in the basal and hyperinsulinemia state. Oxidation was significantly reduced during hyperinsulinemia ($P = 0.002$) (C) Insulin sensitivity correlated strongly with the whole body rate of palmitate oxidation ($P = 0.01$)

Figure 4. Intracellular insulin signaling before and after hyper-insulinemia

(A) Phosphorylation of the Insulin receptor (IR), Insulin Receptor Substate-1 (IRS-1), AKT and PI3-K associated with IRS-1 before and after hyperinsulinemia. There is no significant phosphorylation response in any of the proteins. (B) Membrane content of protein kinase C- β and θ before and after hyper-insulinemia. The content and thus activation of PKC- β was significantly elevated after hyperinsulinemia ($P=0.008$).

Table 1
Physiological measurements on study day

Demographics and clinical measurement on the study day are shown above. Data are Means $\pm$ SEM

Demographics		
Age (Years)	7 $\pm$ 1	
Male/female	22/8	
Percent total TBSA	66 $\pm$ 3	
Percent 3 rd degree TBSA	53 $\pm$ 5	
Days between burn and admit	2 $\pm$ 0.2	
Race (H/W/B)	24/4/2	
Hematology		
Hemoglobin (g/dL)	10.7 $\pm$ 0.3	
Hematocrit (%)	31.3 $\pm$ 0.6	
WBC (CMM)	10.5 $\pm$ 0.8	
Plasma Lipids		
Total Cholesterol mg/dL (mmol/L)	80 $\pm$ 4 (2.01 $\pm$ 0.10)	
Triglycerides mg/dL (mmol/L)	134 $\pm$ 11 (1.51 $\pm$ 0.12)	
HDL mg/dL (mmol/L)	12 $\pm$ 1 (0.31 $\pm$ 0.03)	
LDL mg/dL (mmol/L)	41 $\pm$ 4 (1.06 $\pm$ 0.10)	
VLDL mg/dL (mmol/L)	27 $\pm$ 2 (0.70 $\pm$ 0.05)	
Body Composition		
Percent Fat	18 $\pm$ 1%	
Percent Muscle	78 $\pm$ 1%	
Renal and Hepatic Function		
BUN mg/dL (mmol/L)	14.3 $\pm$ 1.1 (5.1 $\pm$ 0.4)	
Creatinine mg/dL (μ mol/L)	0.5 $\pm$ 0.05 (44 $\pm$ 4)	
Alkaline Phos U/L	101 $\pm$ 6	
AST U/L	68 $\pm$ 14	
ALT U/L	60 $\pm$ 10	
GGT U/L	61 $\pm$ 11	
Metabolic		
Resting Energy Expenditure kcal/day	1404 $\pm$ 92	
Predicted Energy Expenditure kcal/day	1184 $\pm$ 67	
AM cortisol μ g/dL (nmol/L)	23 $\pm$ 4 (634 $\pm$ 110)	
Fasted glucose mg/dL (mmol/L)	106 $\pm$ 4 (5.8 $\pm$ 0.2)	
Fasted insulin μ U/mL (pmol/L)	7 $\pm$ 3 (49 $\pm$ 21)	
Adiponectin ng/mL	4.6 $\pm$ 0.3	
Resistin ng/mL	20 $\pm$ 1	
TNF- α pg/mL	5.0 $\pm$ 0.9	
IL-1 β pg/ml	1.9 $\pm$ 0.3	
IL-6 pg/ml	130.5 $\pm$ 27.4	
C-reactive protein mg/L	17 $\pm$ 2	

Table 2
Glucose and Lipid metabolism compared to healthy literature controls

Measurements of lipid metabolism and tissue lipids are shown for literature controls, compared to the same measurements made in burned children. Basal FFA release, IMCL and LFAT are increased. Basal FFA oxidation and DAG concentrations are similar to healthy controls.

Measurement	Burn	Healthy Control
β -oxidation ($\mu\text{mol/palmitate/kg.min}$)	0.93 ± 0.09	$\sim 0.6 \pm 0.05^{52}$
Ra FFA ($\mu\text{mol/kg/min}$)	10.9 ± 0.9	$6.7 \pm 0.7^{*21}$
IMCL	0.21 ± 0.06	$0.08 \pm 0.01^{*11}$
LFAT	0.27 ± 0.07	$0.08 \pm 0.01^{*11}$
DAG (pmol DAG/ mg tissue)	486 ± 103	415 ± 22