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The acute effects of propranolol on metabolic dysfunction in severely burned pediatric patients

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The acute effects of propranolol on metabolic dysfunction in severely burned pediatric patients

by

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Dedication

This work is dedicated to the patients and families affected by burn trauma.

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The acute effects of propranolol on metabolic dysfunction in severely burned pediatric patients

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Severe burn injury is a profound and lasting form of trauma. Owing to advances over the past several decades, burn injury is no longer thought of as a mortal injury. However, significant morbidity still persists in the post-burn recovery period. Much of this morbidity is stimulated by the long-lasting catecholamine surge that develops after injury. This surge develops metabolic dysfunction that is categorized by significantly elevated energy mobilization through increased glucose production and lipolysis. These metabolic changes contribute to the morbidity associated with delayed recovery. Hyperglycemia, increased infections, insulin resistance, delayed wound healing, and organ dysfunction are all associated with metabolic dysfunction after burn injury. One strategy to curb this response is β-adrenergic blockade with the non-selective β-blocker propranolol. Studies over the last several decades have shown benefit of propranolol use in pediatric burned patients, but much is still unknown about the acute metabolic changes related to propranolol and burn injury. In this study we used stable isotope infusion techniques to analyze acute metabolism in the first few weeks after severe burn injury. Patients were randomized to receive propranolol or placebo and pathways related to lipid and glucose kinetics were analyzed. Propranolol significantly reduced glucose release, glucose

clearance, and FFA release during acute recovery. Propranolol also caused a significant increase in hepatic insulin sensitivity, improving central insulin resistance typically observed in severely burned patients. Lastly, we were also to show a significant correlation between changes in glucose kinetics and lipid kinetics. This correlation was reduced following propranolol administration, indicating this link could be mediated through catecholamines. Further studies are warranted to investigate the link between glucose metabolism and fat metabolism. Propranolol remains underutilized for treatment of severe burns in burn centers across the country. Here, we add evidence of the benefit of propranolol use in severely burned pediatric patients.

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List of Abbreviations

ATP	Adenosine Triphosphate
BPM	Beats Per Minute
CTRL	Control Patients
FA	Fatty Acid
FFA	Free Fatty Acid
GCMS	Gas Chromatography Mass Spectrometry
Gly	Glycerol
HR	Heart Rate
PPARa	Peroxisome Proliferator-Activated Receptor alpha
PROP	Propranolol
Ra	Rate of Appearance
Rd	Rate of Disappearance
REE	Resting Energy Expenditure
rhGH	Recombinant Human Growth Hormone
RHR	Resting Heart Rate
SD	Standard Deviation
TBSA	Total Body Surface Area
TG	Triglyceride
UCP1	Uncoupling Protein 1

CHAPTER 1

Introduction to Burn Injury

Burn injury is perhaps the most severe form of traumatic injury. The early and late effects are profound and debilitating. Delayed or inadequate treatment can be fatal. There are around 40,000 hospitalizations each year in the United States as a result of burn injury.[1] Many of these burn injuries are treated at burn-specific treatment centers. A recent review article analyzing the cost of burn injury treatments across the world helped shed light on the overall burden of burn injury on the health care market.[2] The average costs, outlined in the article, associated with burn injury treatment in the United States (based on available published data) during the last 20 years was over $120,000 per patient.[2] Based on the average hospitalizations per year, this equates to a burden of nearly 5 billion dollars a year in acute burn hospitalization costs (not including long-term costs associated with severe burns).

Burn injury was historically thought of as a mortal injury; however, sustained research efforts have led to advances in burn care that have reduced mortality rates significantly. A 2014 study analyzing mortality rates in pediatric patients showed that the chance of survival is > 92% with burns encompassing more than 55% of the total body surface area (TBSA).[3] This is a dramatic improvement considering a similar size burn 50 years ago resulted in death almost half of the time.[4] A recent study of outcomes determined that over the past 30 years, morality rates in all patients was reduced about 2% each year.[5] With this is mind it is perhaps not surprising that there has been a shift in burn research to focus more on strategies that reduce morbidity and hasten recovery.

PATHOPHYSIOLOGICAL AND METABOLIC RESPONSE

Chronic Adrenergic Stress and inflammation: Severe burns covering more than 20% of the body lead to a profound and persistent pathophysiological response.[6] There are several stages that occur after the initial insult. The first phase, generally within the first 24-48 hours after injury, is called the "ebb" phase. The ebb phase is characterized by reduced cardiac output and metabolic rate.[7, 8] Thereafter, the body rebounds and undergoes a prolonged hypermetabolic state. The "flow" phase occurs around 72 hours after burn injury and is characterized by increased cardiac work, increased metabolic rate, and a surge of hormones and cytokines including: epinephrine, norepinephrine, cortisol, glucagon, insulin, as well as pro- and anti-inflammatory cytokines.[9-11] This response is notable in terms of both its magnitude and persistence.[12, 13] Indeed, its resolution may take several years, likely driving the protracted recovery.

Hypermetabolism: One of the more influential stressors that follow severe burns is adrenergic stress, which can persist for three years after the initial insult.[12] Both epinephrine and norepinephrine remain elevated, stimulating adrenergic receptors throughout the body. Catecholamines, along with other stress signals like cortisol, are primary drivers of the hypermetabolic response, a hallmark of severe burns.[11] Generally, patients are considered "hypermetabolic" when the resting energy expenditure (REE) is 10% or more above normal.[6] Increased REE has been shown to increase upwards of 180% that of expected values following large burn injury.[14] The persistence of elevated metabolism and energy expenditure is maintained for up to a year or more.[15] Interestingly, although unsurprising, higher percentage of TBSA burns are associated with a longer persistence to the hypermetabolic and pathophysiological responses to burn injury.[12, 16, 17]

In addition to elevated REE, the hypermetabolic response to burns is associated with increased substrate mobilization and turnover, skeletal muscle catabolism, and poorer

clinical outcomes.[18, 19] Altered mitochondrial function likely plays an important role in driving this hypermetabolic response. Altered mitochondrial function has been documented in both skeletal muscle and adipose tissue.[17, 20-24] ATP production and consumption is increased significantly after large burns and accounts for a large portion of the increased energy expenditure.[25] However, mitochondrial ATP production alone is insufficient to explain the dramatic increase in oxygen consumption. Recent studies suggest that uncoupling proteins located in the mitochondria drive a thermogenic response that also contributes to hypermetabolism.[26-28]

Altered Cardiac Function: Cardiac dysfunction is another hallmark of severe burn injury. Cardiac dysfunction is displayed in a multitude of ways following burns. Catecholamine stimulation significantly increases heart rate, cardiac work, cardiac output, cardiac oxygen consumption, and overall cardiac stress.[8, 12, 13, 16, 29-36] In fact, heart rate, cardiac output, and cardiac oxygen consumption are significantly elevated for up to 2 years after severe burn injury.[30] A common occurrence seen at autopsy of burned patients is evidence of cardiac ischemia.[37] This suggests that burn-related stress may result in apoptosis and death in cardiomyocytes. Several studies have shown evidence of cardiac related hospitalizations occurring at a higher incidence in adults who recovered from large burn injuries as children.[33, 38] Additionally, a recent study analyzing patients 5 or more years (average time post-burn = 12 years) after recovering from severe burns showed significant cardiac dysfunction.[32] Patients had lower ejection fractions, diastolic dysfunction, and lower exercise tolerance compared to non-burned, age-matched individuals.[32] Exploring methods of reducing cardiac stress and dysfunction are warranted in burned patients.

Skeletal Muscle Wasting: Skeletal muscle catabolism is a significant contributor to morbidity after a severe burn. Skeletal muscle breakdown significantly increases in response to burns and contributes to delayed healing and recovery. The body's attempt to mobilize amino acid building blocks to support the healing process causes skeletal muscle

protein breakdown to greatly outweigh skeletal muscle protein synthesis.[10, 15] This process remains in a negative net balance for upwards of a year following injury.[39] As a result of skeletal muscle wasting, lean body mass (LBM) is reduced for several years after injury.[12, 40] Depletion of the bodies nitrogen pool is associated with immune dysfunction, decreased wound healing, increased risk of infection, and even death.[41, 42] Although likely an important adaptive response, severe catabolism in burned patients becomes maladaptive and inhibits recovery. Medical advances, discussed more later, have helped to overcome the necessity for profound substrate mobilization. Despite these advances, however, we still have not found a way to fully prevent muscle cachexia after severe burns.

Altered Adipose Tissue Metabolism: In addition to muscle catabolism, whole body adipose stores become depleted after burns. Much like skeletal muscle, reductions in subcutaneous adipose tissue stores may represent both a loss in absolute fat mass but also a redistribution of adipose tissue store Elevated lipolysis occurs early in the post-burn response. Catecholamines are strong stimulators of adipose tissue lipolysis and this effect is observed in burned patients. The breakdown of adipose tissue causes an increase release of glycerol and free fatty acids (FFA) into circulation. This chronic and robust response leads to significant hyperlipidemia. This effect is not limited to the circulation, where lipid uptake is increased in tissues such as muscle and the liver, likely contributing to steatosis after burns.

Altered Glucose Control after Burns: Glucose kinetics are acutely altered after burns, where insulin resistance and poor glucose control can persist throughout the recovery period after injury. Glucose concentrations in the blood have been shown to be elevated as early as one-day post-burn, and hyperglycemia has been documented 6 months after injury.[12, 43] Insulin resistance and hyperinsulinemia occur alongside hyperglycemia and last upwards of 3 years after burn injury.[12] Poor glucose control is thought to impact patient outcome after burn injury. Indeed, inability to adequately control

glucose in critically ill patients has been linked to increase morbidity and mortality.[44-46] The mechanistic basis for altered glucose kinetics in response to burns will be discussed further in Chapter 4.

THERAPEUTIC ADVANCES

Owing to significant improvements in burn care and markedly reduced mortality, researchers are now focusing much of their efforts on devising strategies aimed at blunting the stress response to burns and promoting recovery. Several important treatment strategies have been instrumental in allowing this shift to occur. These strategies include: early excision and grafting, improved nutritional support, increasing ICU room temperatures, exercise rehabilitation therapy, and pharmacotherapy.

Burn Resuscitation: Specialized care is essential after a severe burn. A critical aspect of burn care is adequate fluid resuscitation protocols during the early hours and days after injury.[47] Due to the systemic and complex nature of burn injury, proper fluid management and resuscitation in the initial hours is important to prevent further morbidity or mortality.[48-51] Organ perfusion can decrease significantly after severe burns, and over- or under-resuscitation can lead to additional morbidity or death.[52-56] There are several formulas utilized today, but the most widely used is the Parkland Formula.[47, 49, 50] These formulas guide fluid management based on the weight of the patient and the TBSA burned. Therefore, another essential aspect of fluid resuscitation is proper identification of the body surface covered in burns.[57, 58] Life-saving burn care starts from the early moments after burn injury. As proper fluid management has been researched over the past several decades, the problem of resuscitation has reduced significantly.

Burn Wound Excision: Early excision of the burn wound eschar and covering with skin graft is another essential component of burn care.[59] Wounds considered full-thickness, meaning they extend beyond the epidermis and dermis, possibly even into

muscle or bone, require surgical intervention for healing. Grafting requires either normal healthy skin or a skin substitute. If available, the patient's own non-burned skin (autograft) is the preferred method for covering. Early grafting helps speed up the wound healing process, reduces length of stay, decreases risk of infection, and improves survival.[19, 60-65] Additionally, early excision can help reduce inflammatory cytokines and possibly decrease cardiac dysfunction while improving skeletal muscle protein catabolism.[61, 63, 66] Advancements in surgical intervention to burn injury have significantly improved patient outcomes after severe burns.

Occlusive Wound Dressings: In addition to proper surgical wound care, adequate wound dressing has been an important advancement in the recovery from severe burns. The utilization of occlusive wound dressing was explored as a method to reduce the burden of hypermetabolism post-burn injury.[67] Using the same patients and altering metabolic studies over the course of recovery, Caldwell and colleagues were able to show that heat loss and overall energy expenditure was significantly reduced in patients with occlusive wound dressings when compared to studies without occlusive dressings.[67] This study helped to show that type of wound covering is important, but also show that maintaining wound coverage (and acting as a potential barrier to heat loss) showed reduced hypermetabolism in burned patients.

Nutritional Support: Once fluid resuscitation and surgical intervention has occurred, intensive care unit (ICU) nutritional supplement becomes important. Due to increases in REE, as discussed earlier, caloric intake must be increased above what is normally expected in similar sized non-burned individual. Additionally, burned patients hypermetabolic state causes muscle wasting. Aside from the caloric requirement, the macronutrient makeup of nutritional support is highly important (i.e. high-fat vs. low-fat).[9, 61, 68-71] Currently, a low-fat, high-carb diet is thought to be most effective providing adequate calories while reducing muscle catabolism.[68] Supplemental protein may also help increase whole-body and skin protein synthesis.[71, 72] Exact caloric

administration rates are still under investigation. Too little calories lead to significant weight loss while too many calories can exacerbate hypermetabolism and lead to fat deposition in the liver.[73-75] This aspect of burn care is still being actively researched.

Thermoregulation: Ambient temperature plays an important role in post-burn recovery.[76] Following significant loss of skin, the body's ability to thermoregulate is severely inhibited. In order to compensate the barrier loss, patient's bodies need to produce additional heat through increased metabolism.[61] This is perhaps one of the most important contributors to the post-burn hypermetabolic response and an essential aspect of burn care. During the earliest studies linking catecholamines (and ambient temperature) to the hypermetabolic response to burn trauma, elevation of room temperature was shown to significantly reduce the hypermetabolic response. Increasing the standard room temperature by 8 degrees Celsius caused a 30% reduction in energy expenditure.[11, 67] This change in burn care has been a key aspect of non-interventional methods to reduce hypermetabolism and improve outcomes in burn trauma.

It has recently been discovered that mitochondrial thermogenesis is another adaptive response to aid in increasing ambient body temperature.[22, 26, 27] In the process, white adipose tissue (WAT) is converted to more thermogenic form of adipose tissue, brown adipose tissue (BAT). This conversion causes a shift in mitochondrial function by reducing the amount of ATP production and increasing the heat production. The conversion, which is thought to be linked to adrenergic stress, causes an increase in uncoupling protein 1 (UCP1) and is believed to contribute to hypermetabolism.[26]

Rehabilitative Exercise Training: One of the last non-pharmacological approaches to burn care is the implementation of rehabilitative exercise training following discharge from the ICU. Rehabilitative exercise training is beneficial for improving muscle strength and mass, cardiopulmonary fitness, glucose control, and helping patients return to normal daily functions.[77-87] Despite the established benefit, however, exercise therapy is still underutilized in many treatment centers for severe burn trauma.[88] Recent studies

have explored the minimum time necessary to receive the most benefit. Current understanding suggests that 6-weeks of exercise therapy performed after hospital discharge is enough to significantly improve muscle strength, cardiopulmonary fitness, and LBM.[82] It is likely, however, that larger burn injuries necessitate additional exercise therapy when possible. Newer research is exploring a home-based exercise regimen for patients that do not have access to gym equipment, in order to still receive benefits from exercise training.[81]

Significant advancements have occurred over the past several decades to improve the standard of care treatment for severe burn injury. Due to the multitude of dysfunction that occurs, different approaches have to target different aspects of the burn response. Non-pharmacological approaches are the bare essentials for decreasing mortality and morbidity. Although beneficial, there are still many aspects of burn recovery that can be improved. The next step in improving burn care morbidity is exploring pharmacological treatments to further reduce detrimental effects of burn injury.

PHARMACOLOGICAL MODULATION OF THE STRESS RESPONSE TO BURNS

Thanks to intense research efforts, burn care has improved drastically over the last several decades. Mortality rates have dropped significantly, and burn injury as a whole is no longer considered a mortal injury. The focus of research has instead shifted to decreasing the persistent morbidity that hinders a patient's ability to return to normal daily life. The most impactful burn care research now surrounds pharmacological interventions.

Recombinant human growth hormone (rhGH): rhGH has been investigated in recent years due to its potential to improve wound healing and recovery.[89-91] Several beneficial effects have been observed with the use of rhGH treatment in burned patients. Overall growth of patients has been shown to increase, both height and weight, when compared to patients randomized to receive placebo.[90] Improvements were also seen in

LBM. Additionally, rhGH was shown to reduce the hypermetabolic response and reduce cardiac stress.[90] Despite the benefit, rhGH has been limited in widespread use over fears of morbidity and mortality increases in non-burned adults.[92] Further studies may be warranted to understand the benefits to pediatric burned patients.

Insulin Therapy: Due to increased morbidity and mortality correlating with hyperglycemia in burned and other critically ill patients, maintaining euglycemia has been an area of intense research efforts.[44-46, 93] Intensive insulin therapy (infusing insulin to maintain blood glucose levels between 80-110 mg/dL) has been utilized in critically ill patients.[44, 93] This phenomenon has been explored in burned patients at different burn centers across the globe. Insulin benefits go beyond maintaining euglycemia, but have been shown to reduce hyperlipidemia, improve body composition, and increase insulin sensitivity in otherwise insulin resistant patients.[94-98] Additionally, insulin is an anabolic drug and improves muscle protein accretion after burns.[96, 98] However, despite the multitude of benefits associated with intensive insulin therapy, tightly controlling blood glucose levels by the infusion of insulin is associated with an increased risk of hypoglycemia. Hypoglycemia (blood glucose <60 mg/dL) has been associated with increased mortality in burned and other critically ill patients.[99-101] The effects of hypoglycemia are not believed to outweigh the benefit of insulin therapy and therefore most burn centers do not maintain the strict low glucose guidelines associated with intensive insulin treatment. Investigating other methods or glycemic control may be necessary to further improve glucose control in burned patients.

Metformin: The use of metformin to control glucose in burned patients has recently been explored. Metformin is typically utilized in diabetic patients to reduce liver glucose production and improve insulin sensitivity in peripheral tissues.[102] Its utilization in burned patients seems promising, and several studies have tested its effectiveness at improving glycemic control. Importantly, metformin is believed to control glucose without the inherent risk of hypoglycemia associated with administration. In burned patients,

metformin administration has been shown to reduce fasting glucose, hypoglycemic episodes, and exogenous insulin requirements while at the same time increasing insulin sensitivity.[103-105] Larger, multicenter studies are needed to define the therapeutic potential of metformin use in severely burned patients.

PPAR-α agonists: Fenofibrate has also been used to improve glucose homeostasis in burned patients.[23, 24, 106] Fenofibrate is a peroxisome proliferator-activated receptor α (PPAR-α) agonist that is generally used in patients with hypercholesterolemia or dyslipidemia.[107] Fenofibrate works by activating enzymes involved in FFA oxidation. Fenofibrate has also shown to have anti-inflammatory effects. In burned patients, fenofibrate improves mitochondrial function, insulin sensitivity, and fasting glucose control.[23, 24] Fenofibrate's effect on glucose homeostasis is likely through secondary measures relating to insulin sensitivity and reducing FFA levels in tissue. Fenofibrate is a promising candidate for larger studies in burned patients moving forward.

Androgen therapy: Due to the severe cachexia associated with burn injuries, methods to improve muscle growth have been explored. Steroids are an appealing candidate due to the well documented ability to stimulate muscle growth and a significant amount of research into safety and use. Oxandrolone is the most researched steroid utilized in the treatment of burn injuries. Multiple studies have been conducted studying the short and long term administration to improve muscle loss associated with burn injury.[108-111] These studies have shown in, pediatric patients, that oxandrolone is not only safe to use for up to a year after injury, but its use improves muscle protein synthesis, body composition, muscle strength, LBM, bone mineral content, bone mineral density, pulmonary function, and can reduce length of stay.[108, 111-118] The ability to stimulate muscle grown and protein synthesis is augmented with exercise and greater than patients who only received exercise.[115] Previous studies hint at the benefit in pediatric patients, but additional studies are warranted to determine efficacy in severely burned adults.

β-blockade*:* Due to the significant and prolonged catecholamine surge that follows severe burns, propranolol is an ideal drug due to its catecholamine blocking activity. Propranolol is a non-selective β-adrenergic receptor antagonist, meaning it has the ability to block signal transduction through β1, β2, and β3 adrenergic receptors. Propranolol has been shown to be effective at improving cardiac function, reducing hypermetabolism, reducing the incidence of infections, and improving wound healing.[9, 11, 119-129] Additionally, propranolol has been shown to be safe at therapeutic doses in pediatric patients. Given the wide reaching effects of adrenergic stress following severe burns, non-selective β-blockade represent and attractive therapy.[121] A more in-depth look at propranolol will be continued in Chapter 2.

SUMMARY

Severe burn injury is a debilitating condition from which recovery is not readily achievable. The stress response to burns is widespread and long-lasting. Recovery from burns takes years, and many patients have morbidity that persists the rest of their lives. Thanks to significant advancements in acute care, severe burns are generally not viewed as a fatal injury. Research efforts now need to focus on reducing the stress response to burns while promoting recovery.

The earliest studies of severe burn trauma have established a link between the post-burn response and a significant elevation in catecholamines. The constant adrenergic stress observed in burned patients leads to a multitude of detrimental outcomes. Hypermetabolism, hyperglycemia, lipolysis, and insulin resistance all persist for months to years after injury. Although the persistence of these pathological responses has been observed, changes over time are poorly understood. Indeed, some of the more significant responses are related to increased lipolysis and hyperglycemia. Understanding how these processes change over time, and the effect of significant adrenergic stress are of critical

importance to improving our understanding of the pathophysiological responses, as well as improving overall clinical care. Additionally, it is poorly understood if a correlation exists between lipolysis and glucose metabolism.

Here, we try to answer several questions: 1) How are glucose and fat metabolism altered in the acute care setting with and without propranolol administration? 2) Are glucose and fat metabolic responses linked and can β-blockade improve these responses with early administration? If early metabolic dysfunction can be controlled, or improved, with drug administration, there could be a chance to reduce some of the severe morbidity associated with glucose and lipid metabolism. We will address these in 3 different aims:

Specific Aim 1: Determine the acute effects of propranolol on adipose tissue metabolism in patients with severe burns. Our hypothesis is that propranolol treatment will: decrease adipose tissue lipolysis and intracellular fatty acid cycling, reduce plasma FFA concentrations, and attenuate WAT browning. We will utilize stable isotope studies in burned patients and mitochondrial respirometry to address this specific aim.

Specific Aim 2: Determine the acute effects of propranolol on glucose kinetics in patients with severe burns. Our hypothesis is that propranolol will improve glucose control after burn. We will test this using stable isotope studies during the acute ICU stay to quantify hepatic and whole body glucose metabolism.

Specific Aim 3: Determine the relationship between lipid turnover and glucose control in patients with severe burns. We hypothesize that adipose tissue lipolysis will be significantly related to hepatic glucose release and insulin sensitivity in patients with severe burns.

CHAPTER 2

β-blockade Treatment Following Severe Burn Injury

β-BLOCKADE USE IN BURNED PATIENTS

Propranolol wasn't originally developed with burn injury in mind. In fact, propranolol was developed initially as a cardiac drug by James W. Black in the 1960s. Black and colleagues developed propranolol based on a different β-receptor antagonist, pronethalol, in order to reduce the toxic side effects observed with pronethalol usage.[130] The development of propranolol from its precursor pronethalol not only was able to reduce the toxic effects, but was shown to be more effective at lower doses.[130] Although not originally designed for the off-label use in burns, propranolol is overall a non-selective β-adrenergic receptor antagonist. The non-selective aspect refers to the mechanism of action that is not limited to β1 or β2 antagonistic properties, but rather the ability to block all β-receptors (β1, β2, and β3). There have been several "generations" of β blockers developed. The first generation, which include propranolol, are non-selective, and are not limited in blocking potential. Second and third generation β-blockers are more selective in nature based on the desired outcome.[131] The second generation class of β-blockers target the β1 receptor, which has higher distribution in cardiac tissue, leading to these drugs being more cardio-selective.[131] The third generation of drugs has different properties for α- and β-receptors and leads has anti-inflammatory and vasodilation properties.[131] These different mechanisms of action lead to different benefits when utilized in different situations. The non-selective aspect of propranolol, in addition to effectiveness at low concentrations, lead to an optimal drug for use in burn trauma.

Early studies. Studies identifying catecholamine release in burned patients date back to the 1950s and 1960s.[132, 133] Later studies began to elucidate how greater catecholamine levels were associated with hypermetabolism.[11, 134] The first study to test the effects of β-blockade on burned patients occurred in 1974.[11] Specifically, Wilmore and colleagues measured the response to infusion of different α- and β-blockers for 30 minutes or less. They tested the infusion of α-blockade using phentolamine against combination of α- and β-blockade (propranolol) and showed the combinational treatment reduced energy expenditure by nearly 20%, while α-blockade alone showed no change.[11] Since this early study, β-blockade has been investigated frequently in burned patients to identify the usefulness of reducing the systemic effects of catecholamines after thermal injury. Several studies published in the late 1980s continued to show benefit of adrenergic blockade in pediatric burn patients. In 1987, Wolfe and colleagues tested the immediate metabolic effect of propranolol in naïve burned patients.[18] Following a 10 minute infusion of 1 mg/kg propranolol, patients showed a 50% reduction in lipolysis with no effect on glucose production.[18] In 1988 Herndon and colleagues tested the effect of 2 mg/kg propranolol infusion for 5 days and showed a significant reduction in heart rate and rate pressure product by 20 and 36%, respectively.[135] Thanks to continued evidence of β-blockade in burned patients, studies began to explore the long-term administration of propranolol in patients with severe burns.[136] Below is a summary of the literature concerning the efficacy of propranolol treatment in burned patients.

Cardiac physiology. Cardiac changes related to burn injury are well documented.[30] Catecholamines stimulate sympathetic activation and subsequently increase cardiac function and stress significantly. In pediatric burned patients, heart rate, cardiac output, and rate pressure product are significantly elevated from 25% to more than 100% for up to 2-years post-burn.[30] Accordingly, restoring normal cardiac function will likely improve patient outcomes after severe burn trauma. Improvements in cardiac function are perhaps the most visible effect of propranolol treatment after severe burn

injury. Indeed, the efficacy of propranolol administration after burns is typically inferred by the reduction in resting heart rate (HR) that it elicits. Typically, propranolol is administered to burned patients at a dose that lowers resting HR by 15-20%. In pediatric patients, this typically occurs at around 4 mg/kg/day.[137] With a target HR reduction guiding drug administration, several important studies have evaluated how propranolol improves cardiac dysfunction.

Propranolol administered during the hypermetabolic response to burn injury helps to reduce resting HR, rate pressure product (RPP, myocardial oxygen consumption) and cardiac stress.[30, 121, 123, 126] A recent systemic review analyzed published data relating propranolol usage in burned patients (and more robustly in pediatric patients) showing that published data confirms the cardiovascular benefit of propranolol use without a reduction of negative side-effects related to reduced oxygen delivery, hypotension, or bradycardia.[138] Further, propranolol administration improves cardiac function without any detrimental effects on peripheral perfusion or exercise in severely burned children.[126, 139] Reducing cardiac stress likely helps reduce associated morbidities with burn injury and provides justification for propranolol administration after severe burns.

Infection. Several studies have cautioned the use of propranolol in burned patients due to an apparent increased rate of infection. To test this theory Jeschke and colleagues examined the effect of propranolol on the expression of inflammatory cytokines as well as incidence of infection and sepsis in patients with severe burns.[140] This study showed that there was no added risk of infection or sepsis in patients with severe burns receiving propranolol compared to those receiving a placebo. However, a small amount of pro-inflammatory markers were elevated with propranolol administration when compared to placebo.[140] Mechanisms and outcomes associated with this small increase were not examined further. Although this is a reason to monitor inflammation in burned patients during propranolol use, the lack of increased risk of infection does not support the hypothesis that propranolol may increase the risk of infection after severe burn injuries.

Organ dysfunction. Very few studies have specifically compared the occurrence of organ dysfunction and failure in burned patients receiving either propranolol or a placebo. A recent study underscored that respiratory, cardiac, hepatic, and renal failure all occur frequently in burned patients. [34] This study also noted that higher organ failure scores were associated with higher mortality rates.[34] Around the same time, another study determined that multiple organ failure (in children and adults) was almost 30%, consistently occurring in adults (26.7%) and children (27.8%).[141] This study also documented liver failure presenting in more than 25% of pediatric patients.[141] Perhaps the only study to analyze the effect of propranolol administration on organ failure rates was published in 2016 by Wurzer and colleagues. Using Denver 2 organ failure scores, this study discovered that although organ failure rates were not lower as a result of propranolol, they were not higher either.[126, 142] Additional studies exploring whether propranolol treatment prevents organ failure in response to burn trauma are warranted.

Wound healing. Several studies over the last decade have showed improved wound healing (donor site wounds and burn wounds) with administration of propranolol. Mohammadi et al. determined that propranolol usage reduced the time needed to fully cover 3rd degree burn wounds, and also reduced the total amount of graft needed for similar size burns.[143] Additionally, a 2015 study by Ali et al. showed that blood loss was reduced and donor site healing increased in patients receiving propranolol.[129] Wound healing plays a significant role in patient outcomes following burn trauma. Skin is the body's first line of defense for preventing water loss, preventing infection, and thermoregulation. Length of stay in burned patients is also directly linked to wound healing as patients are not discharged from the ICU until the majority of their wounds are healed. The findings described above provide strong evidence for the addition of propranolol to standard of care treatment for severe burn injury. Further multi-centered trials are needed to substantiate these data.

Lipid metabolism. Catecholamines are the principal positive regulators of adipose tissue lipolysis. Perhaps not surprisingly, the chronic secretion of catecholamines in response to burn trauma cause a significant lipolytic response.[120, 135, 144] This phenomenon is likely a natural adaptive response of the body to mobilize energy stores in response to burns. However, the prolonged nature of the response has a significant impact on body composition and potentially insulin sensitivity. Indeed, glycerol released from adipose tissue lipolysis likely contributes to elevated hepatic glucose production, insulin resistance, and steatosis in response to burn trauma.[23, 24, 145, 146] The ability of propranolol to reduce the lipolytic response to burns has been studied in several earlier studies. In 1987, Wolfe et al. analyzed lipid linked substrate cycling (triglyceride – fatty acid), as a possible contributory factor for the hypermetabolic state observed in burned patients.[18] In this study the researchers also administered propranolol to determine if these changes could be mediated with β-blockade. Following the steady-state determination of lipolysis and intracellular cycling, a propranolol infusion was administered for 10 minutes with a 90 minute study to assess the alteration due to β-blockade.[18] Propranolol had a significant effect which resulted in a decrease of glycerol appearance by half, a decrease of intracellular cycling by 70%, and no change in glucose metabolism.[18] In 1994, Herndon and colleagues examined the effect of different β-blockers on the lipolytic response in burns.[120] During his study, patients received a 5 day administration of β1-blockade (metoprolol) or non-selective β-blockade (propranolol) to determine if the effects were adrenergic receptor dependent.[120] Propranolol was found to decrease lipolysis by nearly 50% while metoprolol did not alter lipolysis.[120] This study was used to show that burn-induced, catecholamine driven lipolysis was propagated through the β2-adrenergic receptor, and not through the β1 receptor.

Glucose metabolism. Glucose metabolism is directly linked to morbidity and mortality in burned patients.[18, 45, 147] Hypermetabolism is a hallmark of burn injury and contributes to increased infection, decreased graft take, and fungal infections in

pediatric burned patients.[45, 148] Additionally, hyperglycemia has been linked to mortality rates in burned patients. Catecholamines are potent activators of hepatic gluconeogenesis and glycogenolysis. This effect if further increased with glucagon and cortisol, both of which are increased significantly post-burn. A significant limitation to burn care is based on hyperglycemia, decreasing the negative effects of hyperglycemia are of utmost importance to reducing burn related morbidity.

Interestingly, and poorly understood, is how a) lipolysis effects glucose kinetics, and b) how propranolol alters glucose metabolism. Lipolysis has been shown through several studies to alter hepatic glucose output. One mechanism through which lipolysis can alter glucose production in the liver is by providing gluconeogenesis substrates (e.g. glycerol) that can directly feed into the gluconeogenic pathway. As noted previously, glycerol appearance is significantly increased through catecholamine stimulation of adipose tissue. Additional mechanisms may exist through fat deposition in the liver following elevated lipolysis in burned patients. In 2006, Barrow and colleagues showed that catecholamine blockade has the ability to reduce hepatomegaly in pediatric burned patients. Throughout the course of ICU hospitalization, and with constant administration of propranolol, Barrow showed the pediatric patients had a negative change in liver weight per day (~1%/day) while patients not receiving propranolol had a 1.5%/day increase in liver weight.[127] This change correlated with a blunted increase in plasma TG, and a down-regulation of genes related to lipid metabolism only in patients receiving propranolol.[127] Altogether these studies suggest an effect of catecholamine induced lipolysis on glucose metabolism in severely burned pediatric patients.

Importantly, there haven't been any direct studies analyzing changes in glucose metabolism as a result of propranolol in the acute period post-burn. A key component of post-burn care is altering the hyperglycemic response, and pathways contributing to catecholamine stimulation of hepatic glucose production are poorly understood. We hope to address this gap in our current study.

Psychological. Several recent studies analyzed the effects of propranolol on reducing acute stress disorder (ASD) and post-traumatic stress disorder (PTSD) in pediatric burned patients.[149, 150] ASD was analyzed in more than 350 patients with significant TBSA burn injuries (>50% TBSA). Among the patients either in control or propranolol groups, there was no difference in the occurrence of ASD (5% vs. 8%, respectively).[149] PTSD analysis occurred in more than 200 pediatric patients after an average of >5 years post burn.[150] Although the occurrence of PTSD in the propranolol group was slightly less than that observed in the control group (3.5% vs 7.2%, respectively), there was no significant difference.[150] Overall these studies suggest that propranolol does not have a positive *or* negative effect on stress related psychological outcomes in pediatric burned patients.

The benefits of propranolol administration in severely burned patients are potentially several-fold. However, propranolol is not a standard of care in burn care. A recent survey of propranolol use in burn centers across the United States was conducted.[151] Out of all the responders (38 out of 123), only 60% said they utilized propranolol regularly in practice. If the ratios are representative, it means that only 74 of 123 burn centers in the US would prescribe propranolol to their patients. Despite a reasonable body of evidence suggesting that propranolol may be of benefit to those with severe burns, further robust evidence is required to underscore the efficacy of non-selective beta-blockade in patients with severe burn injuries.

CURRENT PROJECT

Although we have researched many areas of propranolol use in burned patients at our institution, there are still important questions that need to be answered – particularly as it relates to the impact of propranolol treatment on lipid and glucose metabolism following severe burns. To our knowledge, there haven't been any studies concurrently examining

the impact of propranolol treatment on glucose and lipid metabolism following severe burn trauma. Our objectives are to combine stable isotope and glucose clamp approaches to directly quantify the impact of propranolol therapy of whole body lipid and glucose metabolism in acutely burn-injured patients. We hypothesize that propranolol will attenuate lipid turnover in burned patients, and as a result of this, improve hepatic glucose metabolism and insulin sensitivity. We anticipate that these studies will further support the application of propranolol administration in patients with severe thermal injuries.

As the understanding of burn trauma and the benefits of catecholamine blockade have continued to be established, larger trials in pediatric burned patients have been initiated. Currently, a large multi-center trial is being conducted at pediatric burn centers to better explore the benefit of propranolol in severely burned children (NCT01957449). Additionally, Shriners Hospitals for Children – Galveston has received a NIH P50 grand award (P50GM060338), specifically awarded to fund a specialized treatment center. The patients in the current study come from the large specialized center grant geared at understanding the catecholamine induced effects of severely burned patients.

In burned patients, propranolol is typically administered at a dose approximately 4 mg/kg/day.[137] This method of dosing is based on the patients resting HR. Propranolol is titrated in order to reduce HR by 15-20%. An important aspect of analyzing propranolol use in patients is to determine if administration was efficacious with administration. In our current study, we measured the average dosage administered at time of the stable isotope studies performed to quantify lipid and glucose kinetics. Here we s present the data related to propranolol dose efficacy in terms of lowering heart rate in our patient cohort. Methods describing patient recruitment, treatment and randomization will be discussed in more detail in Chapter 3.

Administration of propranolol was measured daily and average dose leading up to study is noted in Figure 1. Average propranolol received during the 5 days leading up to the 1st acute study was 3.62 ± 2.0 mg/kg/day and 6.13 ± 2.7 mg/kg/day during the 2nd study. In order to achieve efficacy it is often required to continually increase propranolol doses over time as the body become more sensitized to the β-blockade.

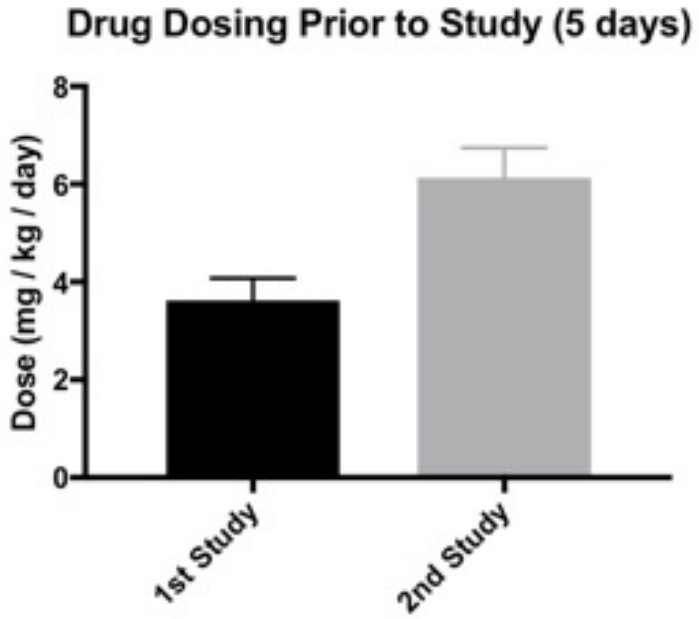

Figure 1: Average Drug Dosing Prior to Studies

The average daily dose was recorded in patients receiving propranolol at the time of metabolic study.

Average daily HR was analyzed throughout the longest measured study period (35 days). HR was significantly less throughout nearly the entire study period (See appendix for average time to study in patient groups). Figure 2 shows the change in average HR throughout the observed time.

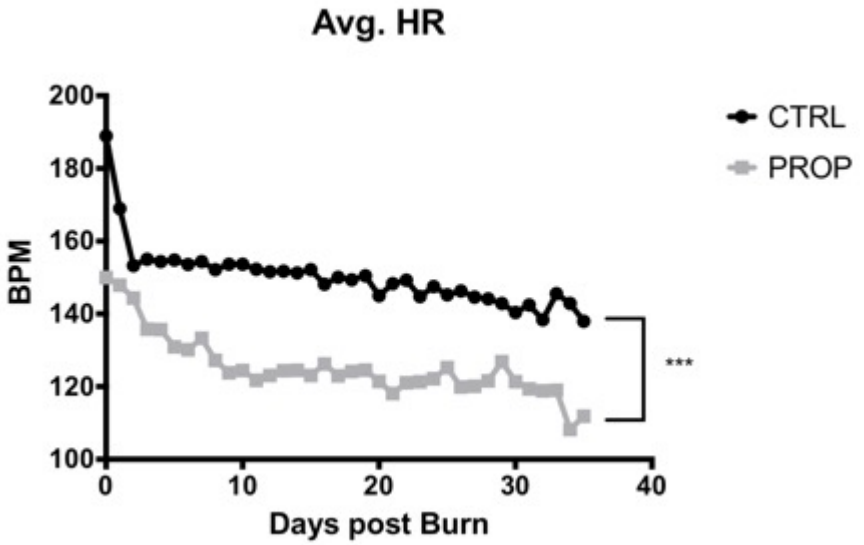

Figure 2: Average Daily HR

Average HR was measured in each patient daily. Propranolol group shows significantly lower HR throughout most of the observed time. ***, $p < 0.001$ between groups

HR as a percentage of maximum HR is presented as an indication of cardiac work (Figure 3). Maximum HR is believed to changed based on age and therefore observing HR alone may not be indicative of the absolute effort required due to elevated HR.

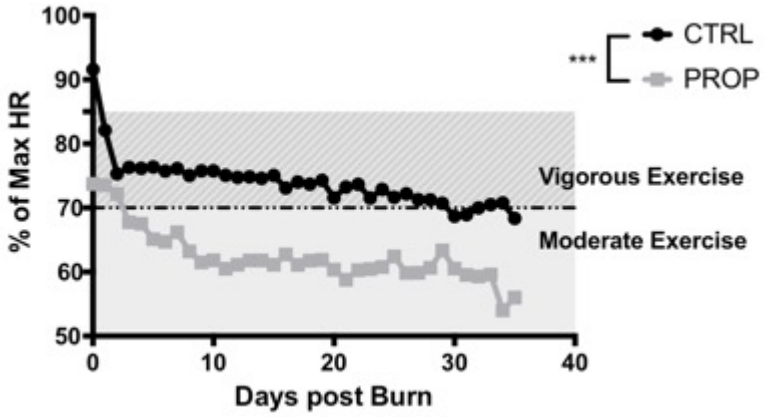

Figure 3: Percent Maximum HR

This graph displays the percent of maximum HR based on patient age and the Tanaka et al. age-predicted maximum formula (209 – 0.7 x age).[152] The shaded regions indicate the percentage of max HR typically associated with moderate (50-70%) and vigorous (70-85%) exercise (data outline by the CDC). These data show that while propranolol reduces cardiac stress, it does not fully normalize cardiac function in burned patients. ***, $p < 0.001$ between groups.

Resting energy expenditure(REE) was measured in our patient cohort (Figure 4). REE measurements are often used as a mechanism to determine overall metabolism and as it relates to burn injury, hypermetabolism. One of the proposed benefits of propranolol is the ability to reduce the REE in patients suffering from severe burn injury. We analyzed this in our patient cohort and found that propranolol was effective at lowering the REE over time, while REE was increased in the CTRL study group. The percent-predicted REE measurement in the CTRL group went from 124 ± 36 % during the first study to 159 ± 29

% at the second study period. PROP patients had a reduction of REE from 143 ± 34 % to 128 ± 26 % during the set of serial metabolic studies. Statistically, these findings indicate a significant change to do the interaction of time and drug (**, p = 0.002) and also show significant differences at the second time point between CTRL and PROP patients (*, p = 0.03). In terms of actual kcal/kg the REE measurement changed during the studies from 36.98 ± 13.1 kcal/kg to 52.17 ± 14.5 kcal/kg in the CTRL group and 40.04 ± 10.1 kcal/kg to 40.61 ± 12.0 kcal/kg in the PROP group. This shows that although there is a significant increase in REE in the CTRL group, the increase was not observed in the PROP group. This indicates significantly less energy needs when administered propranolol after severe burns.

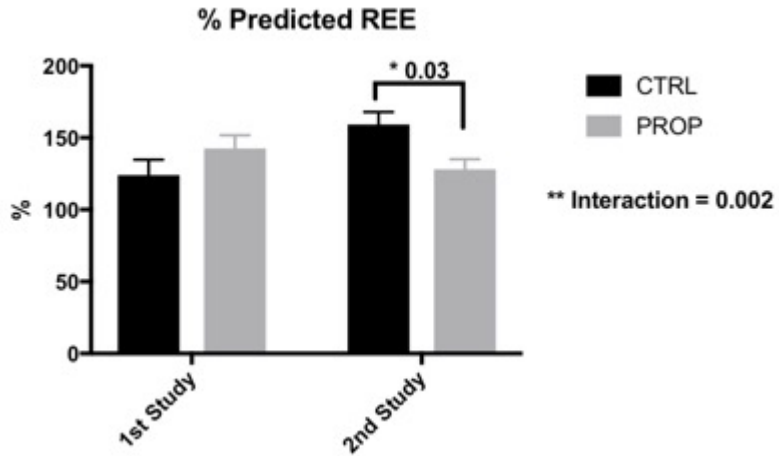

Figure 4: Percent Predicted REE

REE measurements were conducted during or near time of infusion studies. Data presented as percent predicted based on each individual patients and what the expected energy expenditure is based on the Harris-Benedict equation.[153] Significant differences were determined based on interaction of time and drug (**, p = 0.002) as well as between CTRL and PROP at the second study time point (*, p = 0.03).

Lastly, we measured RPP to further determine the efficacy of propranolol in our patient cohort (Figure 4). We found a significant difference in RPP between the placebo and propranolol treated patients. Specifically, administration of propranolol reduced the oxygen consumption (i.e. hypermetabolism) in the heart to a level that was similar to that observed in age-matched resting children.

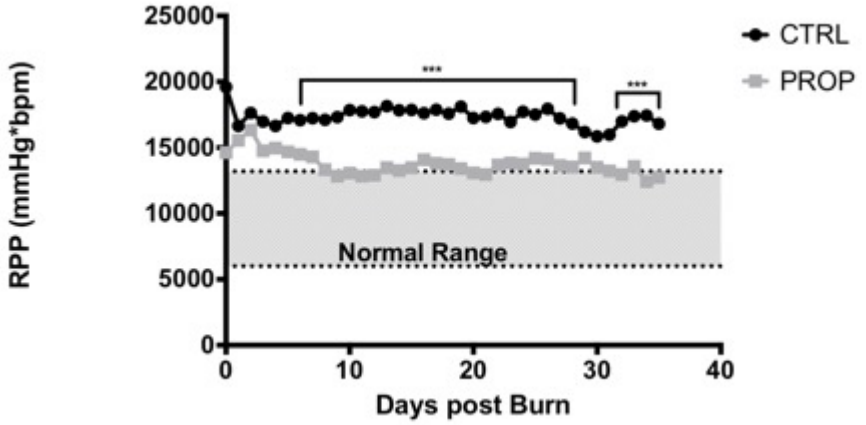

Figure 4: Rate-Pressure Product Changes

Rate-pressure product (RPP) was also analyzed to verify the effect of propranolol and show a reduction in the myocardial oxygen consumption. This is another indication of the efficacy of propranolol in our patient population. RPP was calculated by multiplying the systolic blood pressure (SBP) to the heart rate. ***, $p < 0.001$ between groups

SUMMARY

The current data underscore a beneficial effect of propranolol on cardiovascular function in burned patients. Here we present 3 simple, but largely confirming figures to show that patients in our drug group received and showed effect with the propranolol dose given. In this analysis, we see a 20% difference in average heart rate when comparing patients receiving propranolol or placebo. This data correlates previously published outcomes in pediatric burned patients.[10]

The data described within this chapter confirm that patients randomized to receive propranolol during their acute hospitalization received a significant dose of the drug that was efficacious in terms of improving cardiac function. In the proceeding chapters of this dissertation, the acute metabolic effects of propranolol administration will be determined in vivo in this patients who received a therapeutic propranolol dose.

CHAPTER 3

Changes in Lipid Metabolism and Mitochondrial Function After Burn Injury

INTRODUCTION

Burn injury has a profound effect on adipose tissue lipolysis and lipid redistribution. Catecholamines are potent stimulators of lipolysis, thus, the catecholamine surge in response to severe burns is thought to drive altered lipid metabolism commonly observed in burned patients. Indeed, Wolfe and colleagues compared lipid characteristics in burned and non-burned patients. They found that catecholamine levels were roughly 10 times higher than that of non-burned subjects.[18] During a stable isotope infusion study, the rate of appearance (Ra) of glycerol and FFA were 3-fold and 2-fold higher in burned patients.[18] Following baseline analyses, propranolol was infused to show a significant reduction in lipolysis (glycerol Ra) and intracellular cycling.[18] One caveat of this study is that the patients receiving propranolol were naïve to previous administration of the drug. This was the first study outlining that burn injury, and the catecholamine increase has the ability to significantly increase adipose tissue lipolysis.

Adipose tissue lipolysis is a normal physiological process. In diseased or injured states, adipose tissue can be a natural energy reserve that can be readily mobilized. Adipose tissue lipolysis is generally a normal acute response to increased stress or energy requirements. However, in burned patients, continued stimulation of lipolysis for extended periods can become a maladaptive response. Increased circulating products of lipolysis (glycerol and FFA) can affect other physiological processes in several tissues.

In addition to changes lipolysis, the fate of the products of lipolysis area also altered in burned patients. Glycerol liberated from lipolysis is released into circulation and is

generally believed to be the best marker of overall lipolytic rate. Since lipolysis of a TG molecule should yield one glycerol and 3 FFA, there should be a 3:1 ratio of FFA:glycerol. However, as witnessed in the aforementioned study by Wolfe and colleagues, glycerol is not released in a 1:3 ratio with FFA.[18] Instead, FFA is released at a rate of 1.6 times that of glycerol, indicating that some FFA is not released from the adipocyte.[18] Historical understanding suggested that the FFA were re-esterified into new TG molecules in a "futile cycle".[144] Recent studies suggest that this understanding may not be completely accurate.[21, 26, 27] New studies have shown that white adipose tissue may transition to a more thermogenic counterpart, brown adipose tissue.[21, 26, 27] This browning of adipocytes could provide another mechanism by which FFA could be utilized in the mitochondria of adipocytes and logically explain the mismatch in glycerol and FFA release during lipolysis.

The release of FFA and glycerol into circulation serve a number of purposes in response to burn injury. Glycerol utilization likely occurs in response to increased hepatic glucose mobilization and gluconeogenesis that results from burn trauma. Glycerol can directly enter the glycolysis-gluconeogenesis pathway through glycerol kinase and conversion to glycerol 3-phosphate in the liver. Glycerol likely contributes to hepatic glucose production following the significantly increased release from adipose tissue.[154] FFA may play a more detrimental role when released from adipose tissue. FFA and TG have both been shown to be significantly elevated in the response to burn injury.[12, 13] FFA has been shown to integrate into TG in the liver following severe burn injury, indicating that these may be linked in the early months post-burn.[155] Increased TG levels in blood have been associated with organ dysfunction and poor clinical outcomes.[156] Furthermore, fatty infiltration of the liver and skeletal muscle suggest that the post-burn lipolytic response can lead to ectopic lipid deposition.[23, 157] Ectopic lipid distribution has been shown to be closely associated with increased insulin resistance in skeletal muscle and the liver.[23, 158, 159]

Few studies have directly explored propranolol as a therapeutic strategy to reduce to lipolytic response severe burn injury. Wolfe and colleagues first explored reducing lipolysis over a 90-minute infusion study in which 7 patients were given propranolol at 1mg/kg over 10 minutes.[18] Propranolol was shown to reduce glycerol Ra by half and FFA by about 30%.[18] In 1994, Herndon and colleagues showed that β2 agonism was primarily involved in stimulating adipose tissue lipolysis.[120] In this study, patients were given metoprolol (β1-AR antagonist) or propranolol (β1- and β2-AR antagonist) and measured glycerol Ra.[120] Patients receiving propranolol had reduced glycerol Ra while those receiving metoprolol did not.[120] This study demonstrated that lipolysis in burned patients is stimulated with β2-AR activation. A 2001 study by Morio et al. showed that 3-week administration of propranolol could reduce palmitate uptake in splanchnic vessels, leading to decreased FFA uptake in the liver.[124] An additional study focused on the liver was carried out by Barrow and colleagues and reviewed 98 children with severe burns, 44 of which received propranolol administration.[127] Patients receiving propranolol had significantly smaller livers and also showed increased expression of genes related to lipid metabolism.[127] Lastly, a study examining the combined effects of rhGH and propranolol vs. control or rhGH alone showed that the group receiving propranolol had a lower Ra of palmitate when compared to the other groups.[119]

Adipose tissue mitochondria are also dysfunctional following severe burn injury. In particular, white adipose tissue physiology can change with increased adrenergic stress, similar to that observed in severe burn injury.[26] Following a surge of catecholamines, Sidossis and colleagues showed that morphological and physiological changes occurred in white adipose tissue through histological and functional analyses.[26] Perhaps the biggest changes are the increase in mitochondrial abundance and increased activation of uncoupling protein 1 (UCP1).[26] UCP1 increases mitochondrial thermogenesis that is thought to contribute to the profound hypermetabolic response to burns. These findings have since been confirmed in burned patients by others.[27] To our knowledge, there have

not been any studies directly assessing adipose tissue mitochondrial function in patients receiving propranolol.

Altogether our current understanding of burn induced lipolysis and response to propranolol administration remains limited. Specifically, all of the studies were done with relative short-term administration of propranolol and analyzed at a single time point only. It isn't known if these effects change over time. Furthermore, with our current standard of care treatment, including improvements to nutritional guidelines, it is not known if propranolol is still efficacious at reducing lipolysis and release of FFA and glycerol into circulation. Studies evaluating adipose tissue mitochondria in response to propranolol are also lacking. Understanding that mitochondria represent a major component of energy expenditure and to a certain degree insulin resistance, this information is important for determining the benefit of propranolol to burned patients. In this chapter, we are focused on exploring how propranolol alters lipid metabolism in serial studies performed on burn patients.

METHODS

Patients

This study was approved by the Institutional Review Board at the University of Texas Medical Branch and Shriners Hospitals for Children. Informed consent was obtained before any study related procedures occurred. Once enrolled, patients were prospectively determined to receive either propranolol or placebo. In order to qualify for this current study, additional inclusion criteria were used:

- Enrollment between 2012 - 2016
- Admitted to SHC-Galveston ≤ 7 days after burn injury
- TBSA ≥ 30%
- Patients had to receive 2 consecutive metabolic isotopic infusion studies
- Stable isotope studies occurred ≥ 7 days apart

Study values were validated by a blinded panel of experts prior to inclusion in data analyses. Propranolol was administered to reduce HR by 15-20% of baseline values (see Chapter 2). Surgical procedures, nutrition, and all other clinical care conducted in accordance with standard of care treatment at SHC-Galveston.

Patient demographics were compared based on age, TBSA, TBSA 3rd, length of stay (LOS), Burn to admit, admit to 1st study, and time between studies. Data are recorded as mean ± SD. For additional information on patient demographics, see Appendix Table 1.

Stable Isotope Studies

Stable isotope infusion studies were utilized to measure lipid kinetics. A visual outline of the study is shown in Figure 6. For purposes of this chapter, we will only be describing, in detail, the first 2 hours of the study, which directly relate to the basal/fasted

state and lipid kinetics. Patients were studied 3 days after surgery and fasted the night prior to study. At start of the study, background blood samples were collected to determine baseline levels of naturally occurring isotopes in the patient. After background samples, a prime of [1,1,2,3,3-$^{2}H_5$-glycerol] (18 μmol/kg) was given followed by constant infusion of [1,1,2,3,3-$^{2}H_5$-glycerol] (0.12 μmol/kg/min) and [U-$^{13}C_{16}$-palmitate] (0.02 μmol/kg/min) over a 2-hour period. Blood samples were regularly collected throughout the first 2 hours in order to measure and determine steady-state achievement with lipolysis. Blood samples were store appropriately after collection, either at room temperature or on ice. Blood samples were later analyzed in the lab through separation of blood and plasma via centrifugation.

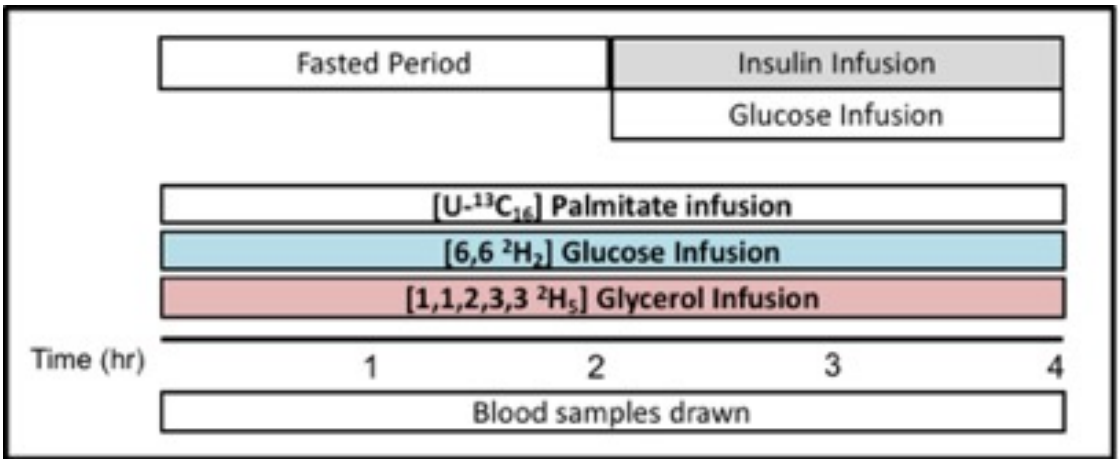

Figure 6: Stable Isotope Infusion Study Outline

Metabolic studies were utilized to determine lipid kinetics. Shown here is the 4-hour complete study, but only the first 2 hours were measured for lipid kinetics in the absence of a hyperinsulinemic-euglycemic clamp.

Sample Processing

Measurement of glycerol and FFA were done as previously reported.[160] 250 μL of each sample were placed into tubes with 250 μL of internal standard (heptadecanoic acid 0.23 μmol/mL in heptane) and ddH_20. Samples were then mixed with cold acetone and proteins were precipitated out of the mixture, mixed, and placed at -20C for 15 minutes. Samples were then centrifuged and supernatant was collected. Supernatant was mixed with equal parts hexane and ddH20 (3mL each) and shaken for 15 minutes. Samples were centrifuged again to separate solvent and aqueous layers. The upper mixture consists of palmitate and the lower aqueous layer contains the glycerol. Samples were dried, mixed with 250mL TBA-phosphate buffer and 250uL iodemethane-dichloromethane, and shaken for 15 minutes. Samples were then sonicated, mixed with 3mL hexane, and shaken again for 15 minutes. The solutions were then separated by centrifugation and the upper layer was collected and dried in a speedvac. 150mL heptane was added, samples vortexed, and then transferred to new vials for gas chromatography mass spectrometry (GCMS). This method was performed for each plasma sample collected during the studies.

GCMS was performed on an Agilent 6890. Mass-to-charge ratios of 253, 254, and 257 were used for glycerol enrichment. Mass-to-charge ratios of 270 and 286 were used for palmitate enrichment. Enrichments are expressed as tracer (labeled) to tracee (unlabeled) ratios (TTR).

Calculations

All calculations for lipid kinetics were performed during the last 15-30 minutes of the stable isotope study. This time period was chosen because it coordinates with steady-state achieved during the 2-hour study.

Glycerol and palmitate rate of appearance (Ra) was calculated by dividing the tracer infusion rate by the enrichment measured through GCMS. Enrichment measurements were

expressed as tracer to tracee ratios. These calculations give a general measure of adipose tissue lipolysis. Glycerol and palmitate concentrations are measured through GCMS. Intracellular cycling was calculated by subtracting FFA Ra from 3 times the glycerol Ra. This calculation accounts for the FFA that remains in the cell and is not released in the ratio equivalent to 3:1 FFA:glycerol.

Mitochondrial Function

Mitochondrial function was assessed in adipose tissue collected during early excision operations and correlating with acute metabolic studies. Tissue collection occurred in a subset of previously randomized patients to CTRL or PROP and were matched for similar demographics. No significant differences exist between the two groups analyzed for mitochondrial function. Adipose tissue was collected and analyzed for mitochondrial respirometry.

Mitochondrial function was analyzed using the Oroborus Oxygraph-2K (Innsbruck, Austria) as previously described.[17, 22] Respirometry was measured within 24 hours of tissue collection. Fresh adipose tissue was suspended in a preservation buffer (10 mM CaK2-EGTA, 0.1 μM free Ca2+, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM MgCl2, 5.77 mM ATP, and 15 mM creatine phosphate; pH 7.1) and processed as previously described.[22, 28] Tissue was gently pulled apart with forceps. A sucrose buffer with 5 μM saponin was added to the sample for 10-20 minutes at 4C. The samples were then quickly blotted dry, weighed, and transferred to the Oroborus O2K chamber, containing a respiration buffer of 0.5 mM EGTA, 3 mM MgCl2, 60 mM lactobionate, 20 mM taurine, 10 mM KH2PO4, 20 mM HEPES, 10 mM sucrose, and 1mg/ml BSA. To fully determine respirometry capacity, substrates and inhibitors were added to the respiration chamber in order to measure the mitochondrial response. Of

particular interest to our current study were analyses of OXPHOS (maximum respiration), State-4 (thermogenesis), and CCR (leak in the mitochondria, quality control).

Statistics

Statistical analyses were performed using GraphPad Prism 7.0d (GraphPad Software, La Jolla, CA, USA). Data was analyzed for normalized distribution prior to analyses. Demographic data was analyzed using t-Tests for normalized data and Mann-Whitney test for data that is not normal. Metabolic study and mitochondrial data comparisons were done using 2-way Repeated Measures Analysis of Variance (ANOVA) comparing the effect of drug and time. Sidak's multiple comparisons test was performed to determine variation between CTRL and PROP at each time interval. Significance was determined when the p value was less than 0.05.

RESULTS

Patient Demographics

Patients were enrolled and randomly separated to receive placebo (CTRL) or propranolol (PROP). Figure 7 shows patient consort diagram and outlines the process of including or excluding patients from the current study. Patient demographics were analyzed between the two groups and are presented in Table 1 as mean ± SD. No significant differences were found between CTRL and PROP group demographics. The average age of patients was 7.7 ± 6 and 9.7 ± 5 years for the CTRL and PROP groups, respectively. TBSA was similar between groups with average TBSA burned 59.7 ± 16.3 % in CTRL and 50.1 ± 16.8 in the PROP group. TBSA 3rd was 46.3 ± 24.1 % and 38.2 ± 22.2 % in CTRL and PROP, respectively. Length of stay was similar between groups as well with CTRL group averaging 42 ± 28 days and PROP group averaging 39 ± 26 days. Burn to admit was nearly identical between the groups, averaging 2.65 ± 1.6 days and 2.84 ± 1.9 days in CTRL and PROP, respectively. Admit to 1st study and time between studies was also nearly identical between groups, averaging around 7 and 9.5 days, respectively.

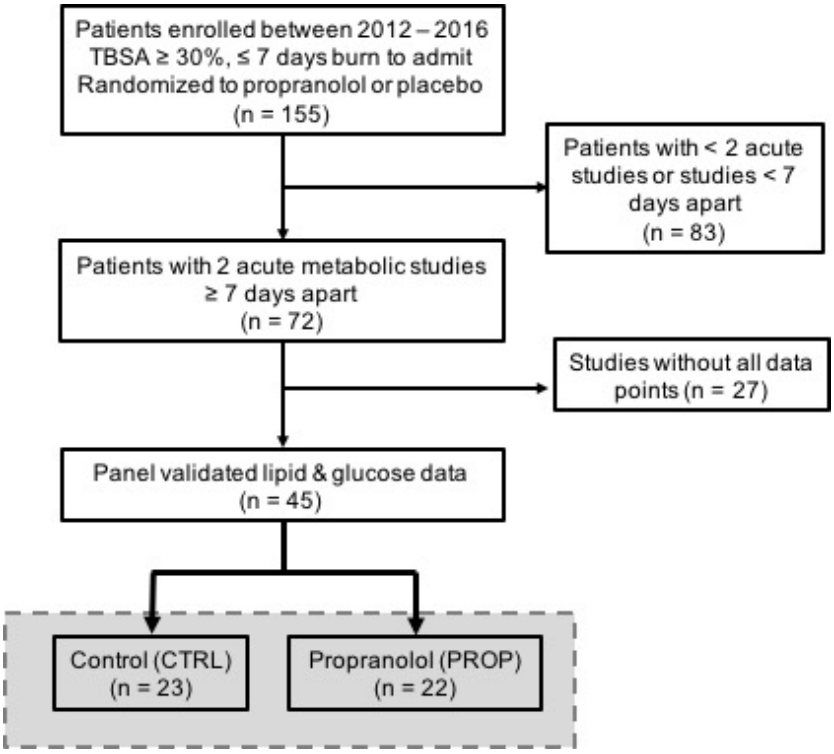

Figure 7: Enrolled Patient Consort Diagram

Patients admitted to SHC-Galveston and enrolled in research were randomized to receive either placebo (Control; CTRL) or propranolol (PROP). The inclusion and exclusion criteria are outlined here.

DEMOGRAPHICS	CTRL (n = 23)	PROP (n = 22)	Difference	p-value
AGE (YEARS)	7.70 ± 5.7	9.68 ± 4.9	ns	0.252
TBSA (%)	59.65 ± 16.3	50.05 ± 16.8	ns	0.078
TBSA 3RD (%)	46.25 ± 24.1	38.16 ± 22.2	ns	0.284
LOS (DAYS)	42.35 ± 27.5	38.74 ± 25.6	ns	0.674
BURN TO ADMIT (DAYS)	2.65 ± 1.6	2.84 ± 1.9	ns	0.734
ADMIT TO 1ST STUDY (DAYS)	7.40 ± 4.7	6.26 ± 3.1	ns	0.379
TIME BETWEEN STUDIES (DAYS)	9.60 ± 4.7	9.53 ± 4.8	ns	0.961

Table 1: Group Demographics

Patient demographics were compared between the two groups, CTRL and PROP. No significant different were observed between the groups. Although nearing significance, the change in TBSA is not considered to be clinically significant and likely has no effect on results. There were no corrections needed during final analyses based on patient demographics. Data are presented as mean ± SD.

Lipid Kinetics

Lipolysis was analyzed through measurements of glycerol Ra and palmitate Ra. Glycerol and palmitate concentrations were also determined in plasma. Lastly intracellular cycling was determined between the two groups. Glycerol Ra and concentration was measured in CTRL and PROP groups during the 1st and 2nd acute studies. Glycerol Ra is shown in Figure 8. Glycerol Ra in the CTRL group changed from 9.1 ± 4.3 μmol/kg/min in the first study to 9.8 ± 4.3 μmol/kg/min in the second study. In the PROP group Glycerol Ra was 8.3 ± 4.0 in the first study and 8.0 ± 3.9 in the second. Glycerol concentration was measured at 0.3 ± 0.5 μmol/mL and 0.4 ± 0.6 μmol/mL in the CTRL group at the first and second study, respectively. Glycerol concentration in the PROP group changed from 0.34 ± 0.4 μmol/mL to 0.25 ± 0.4 μmol/mL from the first to second study. Glycerol Concentration is displayed in Figure 9.

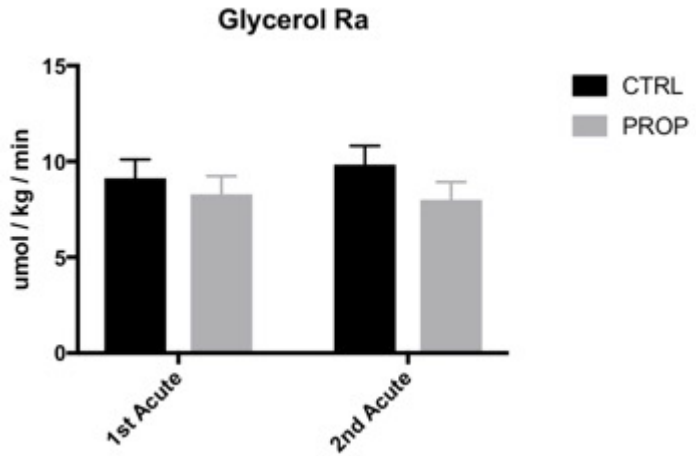

Figure 8: Glycerol Ra Changes

Glycerol rate of appearance measured during stable isotope infusion studies in the acute care setting. Glycerol Ra was similar between groups at each of the measured time points and changed similarly between studies.

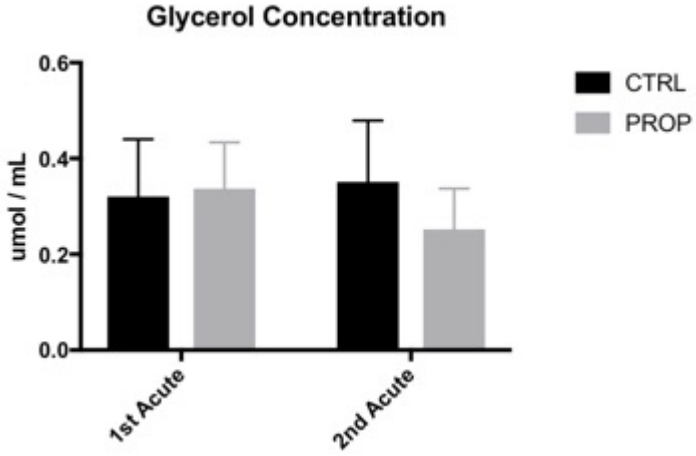

Figure 9: Glycerol Concentration Changes

Changes in glycerol concentration as measured via GCMS during acute metabolic studies. Glycerol concentrations were similar between groups and over time. A slight decrease was observed in the PROP group but this change was not found to be statistically significant.

Palmitate Ra and concentration was also measured in the CTRL and PROP groups. Palmitate Ra is shown in Figure 10. Palmitate Ra changed in the CTRL group 3.9 ± 1.8 μmol/kg/min in the first study to 4.8 ± 1.6 μmol/kg/min in the second study. Ra in the PROP group was reduced from 3.8 ± 1.8 μmol/kg/min to 3.6 ± 1.3 μmol/kg/min in the first and second study, respectively. Statistically, there is a significant interaction ($p = 0.05$) that occurs between time and drug group in the palmitate Ra response. Additionally, CTRL and PROP Ra are different at the second measured time point ($p = 0.05$). Palmitate concentration changes also showed significant differences between CTRL and PROP. Palmitate concentration increased in the CTRL group from 0.13 ± 0.05 μmol/mL to 0.17 ± 0.05 μmol/mL between acute studies and remained consistent in the PROP group at 0.15 ± 0.05 μmol/mL and 0.15 ± 0.06 μmol/mL in respective serial studies. Concentration changes are shown in Figure 11.

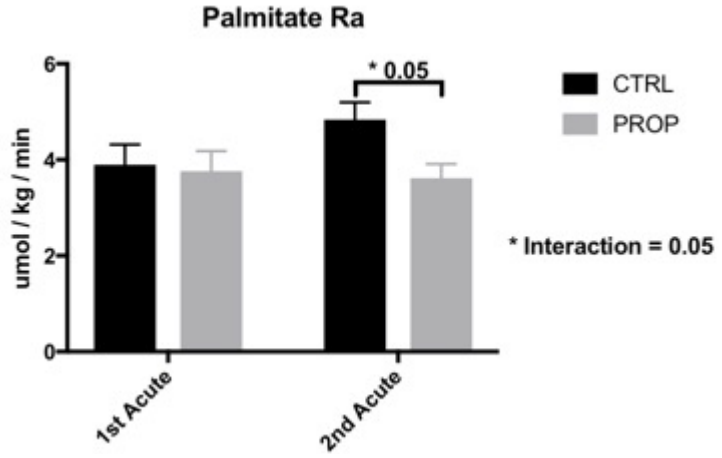

Figure 10: Palmitate Ra Changes

Acute metabolic changes in palmitate Ra are observed in burned patients. There was a significant interaction observed between time and drug groupings. Additionally, CTRL palmitate Ra is significantly higher than PROP Ra at the second time point.

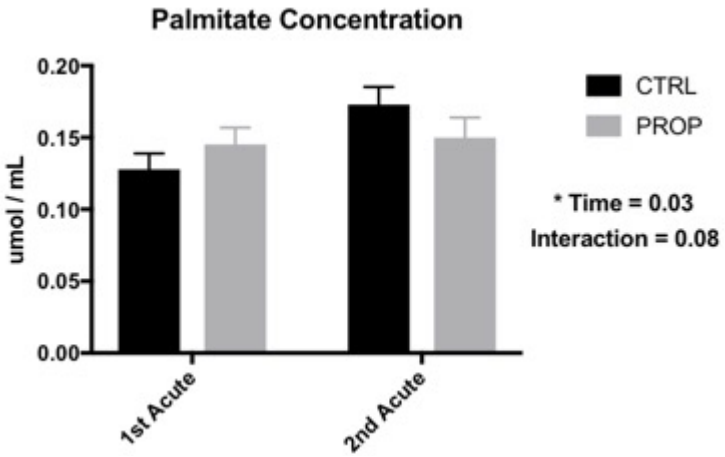

Figure 11: Palmitate Concentration Changes

Changes in palmitate concentration were measured during acute metabolic isotope studies. CTRL patients showed a slight increase in concentration while PROP remained relatively stable. Statistically, there is a significant effect of time on palmitate concentrations.

Intracellular (IC) recycling was also measured in CTRL and PROP patients. There were no significant differences observed between CRTL and PROP groups (Figure 12). CTRL IC recycling reduced from 10.9 ± 8.0 μmol/kg/min to 10.7 ± 9.4 μmol/kg/min while PROP increased from 8.4 ± 7.8 μmol/kg/min to 9.5 ± 7.5 μmol/kg/min between studies. Overall, PROP patients showed lower IC recycling at both time points, but these values did not appear to change over time and did not change significantly as a result of drug randomization.

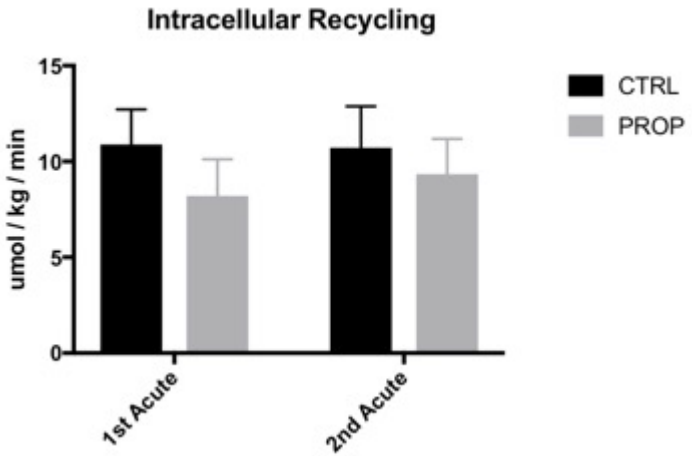

Figure 12: Intracellular Recycling

Intracellular recycling was calculated during the stable isotope studies. Although slightly lower in the PROP group, there were no statistically determined differences between groups.

Mitochondrial Function

Mitochondrial function was measured through the use of high-resolution respirometry. Mitochondrial function was determined through measurement of oxidative phosphorylation (Oxphos; max respiration), State 4 (thermogenesis), and coupling control ratio (CCR; quality control/leak). Oxphos was measured at time of first and second study in the CTRL and PROP groups. Measurement at the first study were similar between groups with values of 0.75 ± 0.4 O2 flux (ρmol/sec/mg) for CTRL and 0.69 ± .3 O2 flux in PROP (Figure 12). At the second study measurement, Oxphos was 2.77 ± 2.1 O2 flux in CTRL and 1.66 ± 1.2 O2 flux in the PROP group. Oxphos was significantly different over time (***, $p < 0.001$) and between groups at the second study time point (*, $p = 0.04$). Although approaching significance, drug was not determined to cause significant changes in Oxphos outcomes ($p = 0.09$). State 4 showed a similar pattern to that which was observed in Oxphos (Figure 13) and was significantly over time (***, $p < 0.001$) and at the second measured time point (*, $p = 0.05$). CTRL O2 flux increased from 0.44 ± 0.2 pmol/sec/mg

to 2.03 ± 1.7 ρmol/sec/mg between studies. PROP O2 flux increased as well, from 0.44 ± 0.3 ρmol/sec/mg to 1.15 ± 1.1 ρmol/sec/mg from first to second study. Lastly, we analyzed a marker of quality in the mitochondria by measuring CCR (Figure 14). CTRL and PROP were similar in the first study with measurements of 0.62 ± 1.4 ρmol/sec/mg and 0.58 ± 0.16 ρmol/sec/mg, respectively. Studies at the second time point were measured as 0.77 ± 0.16 ρmol/sec/mg in CTRL and 0.69 ± 0.16 ρmol/sec/mg in PROP. CCR was significantly different over time (**, p = 0.001), but no other aspects were different.

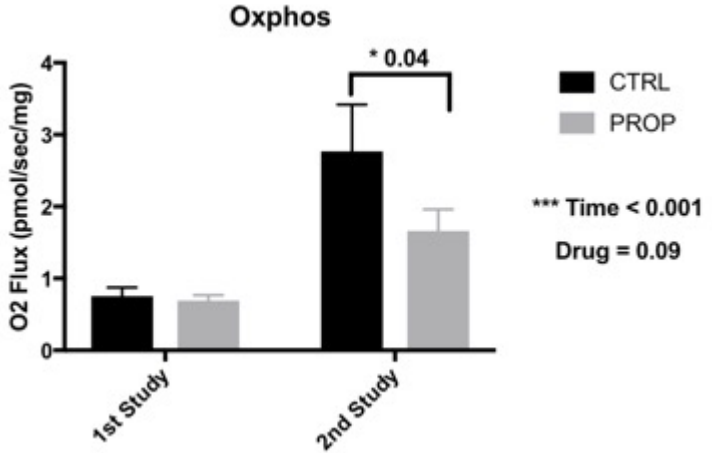

Figure 13: Oxidative Phosphorylation Changes After Burn

Oxphos was measured during the acute recovery from severe burns. Oxphos was significantly different based on time (***, p < 0.001) and at the second time point (*, p = 0.04). Despite the improvement, there were no significant changes observed due to drug administration.

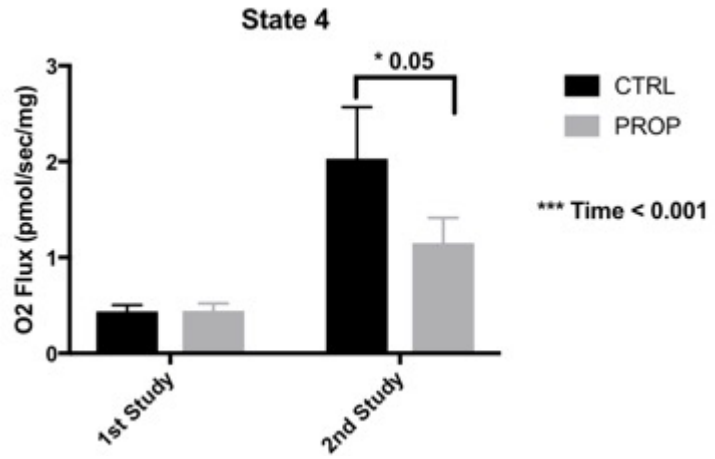

Figure 14: Mitochondrial Thermogenesis Measured After Burns.

State 4 mitochondrial function measured serially following severe burns. State 4 correlates with thermogenesis function in mitochondria. Significant differences were observed with time (***, $p < 0.001$) and between CTRL and PROP at the second study time point (*, $p = 0.05$).

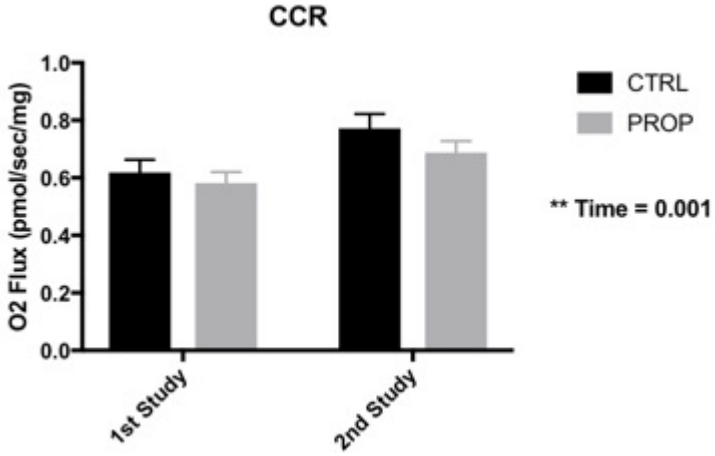

Figure 15: Mitochondrial Quality After Burns

Coupling controlled ratio (CCR), a marker of mitochondrial quality, was measured during the course of the study. Significant differences were found as a result of time (**, $p = 0.001$) but not due to any other variable. Quality was measured to be similar between CTRL and PROP groups.

DISCUSSION

Here, we are able to show benefit to patients receiving propranolol as it relates to lipid kinetics and mitochondrial function. The biggest differences observed related to FFA kinetics (i.e. palmitate) and mitochondrial function. Interestingly, we did not observe the same significant reduction in lipolysis with β-blockade that has previously been published, but this study does not refute previously claims of reduced lipolysis with propranolol use in severely burned patients.[18, 120, 144] indeed glycerol Ra was numerically lower in patients receiving propranolol, although this did not reach statistical significance. Overall, this data supports the use of propranolol in severely burned children as a safe and efficacious drug.

The rate of lipolysis in burned patients has been well documented in early studies of burn injury.[18, 120, 144] Catecholamines are potent stimulators of lipolysis, acting through the β-adrenergic receptors on adipose tissue and the resulting signaling cascade via cyclic AMP, PKA, and stimulation of lipolytic enzymes.[161] Through this process, TG stored in adipose tissue have FFA removed one by one. In order, adipose TG lipase, hormone-sensitive lipase, and monoglyceride lipase all act to remove a single FA from the attached glycerol. The resulting product is a single glycerol and three FFA molecules. Due to the nature that glycerol cannot be re-utilized in the fat cell, while FFA *can*, glycerol is subsequently released from the adipose tissue.

Despite previous studies showing a significant reduction in lipolysis with propranolol use, we do not see evidence of the same in this study. Glycerol Ra is reduced, slightly, in the PROP patients in our study, but this effect was not shown to be statistically significant. Additionally, we did not observe a change in glycerol Ra over time, likely indicating the continued increase of lipolytic pathways in the burned patients as the values observed in our study are still considerably higher than lipolytic rates in non-burned individuals.[18, 162] There are several reasons as to why we did not observe a reduction

in lipolysis with β-blockade. First, burned patients receiving propranolol show diminished efficacy over time.[123, 137] In our current study, we noted the necessary increase in PROP dosing between the two studies from ~4.5 to 6 mg/kg/day. A recent study by Guillory et al. determined that propranolol may need to be given at a dose up to 8 mg/kg/day to show effectiveness.[137] Second, and related to reduced efficacy of propranolol, it has been postulated that β-adrenergic receptors and prolonged catecholamine stimulation with or without blockade could cause a desensitization of receptors.[163, 164] In the current study, propranolol administration had occurred for around 6-7 days at the time of the first study. This could be long enough for desensitization to occur and feedback loops to alter lipolytic pathways. A recent study analyzing combinational treatment with oxandrolone and propranolol showed similar changes over time, but still showed decreased lipolysis during the first study (unpublished data). Lastly, one of the major differences between previous studies and the current study is the relative naïve response to propranolol in previous studies. In studies by Wolfe et al., propranolol was administered on naïve patients after baseline lipolysis measurements were conducted.[18, 144] This initial response could possibly be more profound than in our study with nearly a week of propranolol administration before the first study.

Despite similar levels of lipolysis in CTRL and PROP patients, we saw significant differences in palmitate Ra and serum concentration in this study. Analysis of palmitate Ra showed a significant effect due to the interaction of time and drug, and also significant differences between CTRL and PROP at the second study time point. This increase could be due to multiple factors. First, increased lipolysis could lead to elevated palmitate Ra levels. In our current study, we do see a slight increase in lipolysis in the CTRL group from the first to second study, but the change over time does not correlate with the level of increase observed in palmitate Ra release. Next, this increase could be due to a reduction of intracellular cycling. During the futile cycle associated with FFA-TG, FFAs are not released from the adipocyte but remain in the cell to be re-esterified into TG. This process

has been postulated to contribute to hypermetabolism in burned patients.[18] Recent evidence, however, suggests that changes in adipocyte physiology could utilize FFA for thermogenesis. The browning of white adipose tissue has been observed in man, and establishes a likely scenario for FFA utilization within adipose tissue.[21, 26, 27] A reduction in thermogenic utilization of FFA could contribute to release of palmitate. The cause is likely a combination of all three of these processes. We do not see a significant change in IC recycling over time, but just as we don't see a significant increase in lipolysis, there could still be contributions from multiple processes as to the reason we see increased palmitate Ra during the second study. IC recycling observed in this study remain fairly constant in each group between the two studies. There is, however, a decreased amount of IC cycling in the PROP group when compared to the CTRL group, although not significant. The increase in palmitate concentration in serum correlates with contributions from these aforementioned processes. Although the different is small, reducing FFA release and subsequent concentration in serum is highly important in the post-burn recovery. Increased FFA can be deposited into ectopic tissues and lead to morbidity associated with cellular function and insulin resistance. The ability of propranolol to show reduced FFA release over time helps advocate for administration in pediatric burn patients.

Overall, as it relates to lipolysis and lipid kinetics, we do see improvement in the propranolol group when compared to control patients. In this study, the only significant change we observed is in palmitate Ra and palmitate concentration changes. Due to the ability of FFA to affect other tissues in the body, we feel as though this finding alone would suggest further exploration of propranolol use in burned patients. With the amount of data already available for propranolol use in pediatric burn injury, these data adds to the body of evidence that propranolol can benefit burned patients.

Analysis of mitochondrial function also showed significant changes after severe burns. In the current study, we assessed several variables related to mitochondrial function. Oxphos (max respiration), State 4 (thermogenesis), and coupling-control ratio (CCR;

quality control marker) were all measured in burned patients at the two study intervals. Time had a significant effect in every analysis,. There is significantly diminished function after initial injury and through the first study. This response is reversed by the time of the second study with 2-3 times increase in both variables. Additionally, at the second time point we saw a significant difference between CTRL and PROP, with CTRL mitochondria measuring higher max respiration *and* thermogenesis. These indicate a higher threshold in the CTRL groups, possibly indicating higher energy expenditure and overall hypermetabolism. Apart from the change in time in the CCR analysis, there was no difference between groups. These results support a role for catecholamines in driving adipose tissue thermogenesis in burned patients.

In summary, we show that measures of adipose tissue lipolysis and mitochondrial function are improved in burned patients receiving propranolol. These results further suggest that propranolol is efficacious in burned patients. Further studies relating to propranolol dose and lipid metabolism in burned patients, accounting for other potential confounders such as sex, age and injury severity are needed to further define the efficacious potential of propranolol administration in patients with massive burns.

CHAPTER 4

Changes in Glucose Kinetics and Insulin Sensitivity After Severe Burns

INTRODUCTION

Changes in glucose kinetics after severe burn injury have been well documented. Hyperglycemia is a hallmark of burn injury and studies have shown that glucose is significantly elevated up to 6 months after initial injury.[12] There are several reasons why this occurs, but the response is ultimately driven by catecholamines inflammation, and endocrine dysfunction.[165, 166] In an effort to recover from the profound injury, the body stimulates energy metabolites that can be utilized for the healing process. Significant increase in hepatic glucose output is observed.[147] Increased glucose producing pathways in addition to insulin resistance permits the progression of hyperglycemia to linger in burned patients.[166]

Changes in blood glucose levels can have significant impact on critically ill patients, especially as it relates to burn injury. As stated before, today we view severe burn injuries as a non-fatal injury, owing to advances in burn care and research. However, hyperglycemia and poor glucose control are still associated with mortality in critically ill and burned patients.[44-46, 93, 166, 167] In addition to detrimental effects of hyperglycemia on mortality, hyperglycemia is associated with several other complications in burn injury. These complications include reduced graft success rate, bacterial infections, and fungal infections.[45, 148, 168] Ultimately, hyperglycemia is a well-documented detrimental response to severe burn injury.

Several strategies have been explored to properly control glucose metabolism in burned patients. Following the critical care study published by Van den Berghe and colleagues, stricter control of glucose was examined. In the Van den Berghe study,

critically ill patients in the ICU were given strict blood glucose guidelines to stay between 80-110 mg/dL.[44, 93] This "intensive insulin" regimen was shown to reduce mortality rates in the critically ill and led to several similar studies in burned patients. Studies following similar guidelines showed success at reducing hyperglycemia, but a negative response to such strict glucose control was the occurrence of hypoglycemia in those patients. Indeed, intensive insulin in severely burned pediatric patients showed benefit and reduced morbidity.[169] However, as mentioned before the use of insulin has drawbacks like the occurrence of hypoglycemia.[99] Overall, most of the studies focused on such strict glucose levels were determined to be too risky in the burn population and therefore intense insulin therapy was more or less abandoned in bur injury.

There is sparse data assessing the ability of propranolol to alter glucose metabolism in burned patients. That being said, there are a few studies that measured blood glucose and insulin administration. These studies, however, were not focused on understanding the effect of propranolol on glucose kinetics but rather a tangential finding of the study. A 2001 study by Herndon et al. assessed the influence of propranolol on skeletal muscle catabolism in severely burned patients. During this study, serum glucose was measured (non-fasted state) and was shown to reduce from 151 mg/dL to 115 mg/dL.[10] As mentioned above, this was a tangential analysis and not the focus of the study. In 2002 Hart and colleagues analyzed the effects of growth hormone, propranolol, and propranolol + growth hormone on various burn related outcomes.[170] The primary outcomes of the study were focused on burn-induced catabolism and reversing the negative protein balance seen in burned patients. After 10-day treatment with propranolol (12 patients), they found that insulin dosing in patients was not significantly increased compared to baseline levels (2 units/hour vs 1.5 units/hour, respectively, ns).[170] The investigation did not go any further.

It is interesting that larger, more direct studies have not been undertaken to further understand the impact, if any, propranolol may have on glucose kinetics and metabolism. In this chapter, we may be some of the first to look in-depth into propranolol administration

as a tool to help control glucose homeostasis. Although there doesn't appear to be a direct correlation between glucose metabolism and β-adrenergic stimulation, it does not mean that secondary mechanisms aren't involved in the overall hyperglycemic response and therefore could improve with β-blockade. Here, we help fill the gap in knowledge relating to propranolol administration and glucose kinetic responses in pediatric burned patients. Based on the multitude of benefits already observed with propranolol, we hypothesize that propranolol will be of benefit to burned patients by reducing overall glucose production (stimulated in part through catecholamines) and improving insulin sensitivity.

METHODS

Patients

Patients in this chapter are the same as were outlined, discussed, and analyzed in Chapter 3. Please refer to Chapter 3 for methods related to patient enrollment and randomization, Figure 6 for patient consort diagram, and table 1 for patient demographics. Data are recorded as mean ± SD. For additional information on patient demographics, see Appendix Table 1.

Stable Isotope Studies

Stable isotope infusion studies were utilized to measure glucose kinetics and insulin sensitivity. A visual outline of the study is shown in Figure 6. For purposes of this chapter, we will be describing the aspects of the study that directly relate to the basal/fasted state and clamp period associated with glucose kinetics. Patients were fasted the night prior to study. At start of the study, background blood samples were collected to determine baseline levels of naturally occurring isotopes in the patient. After background samples, a prime dose of [6,6-$^{2}H_2$-glucose] (20 μmol/kg) was given followed by constant infusion of [$^{2}H_2$-glucose] (0.44 μmol/kg/min) over a 4-hour period. Basal/fasted steady-state was achieved in the last 15-30 minutes of the first 2 hours of the study. During the second half of the study (hours 3 and 4) a constant infusion of insulin was administered at a dose of 1.5 mU/kg/min to test outcomes as part of a hyperinsulinemic-euglycemic clamp. In order to maintain euglycemia, exogenous glucose was administered (20% dextrose) and blood glucose levels were checked every 5 minutes for patient safety. Exogenous glucose was adjusted in order to maintain constant glucose similar to baseline levels that were determined prior to starting the infusion of insulin. Blood is sampled during the last 30 minutes of the study in order to confirm steady state achievement.

Sample Processing

Plasma glucose enrichment and TTR was analyzed a previously described.[171] Blood samples were derivatized with pentaacetate and analyzed using a glucose/lactate analyzer. The enrichments of [$^{2}H_{2}$-glucose] were represented as TTR. Samples were lastly run through GCMS and measured at mass-to-charge ratios of 242, 243, and 244.

Calculations

All calculations for basal glucose kinetics were performed during the last 15-30 minutes of the first 2 hours of a stable isotope study and the clamp period calculations were done during the last 15-30 minutes of the second 2-hour period. These time periods were chosen because they coordinate with steady-state achieved during the latter part of each 2-hour study.

There are several calculations used in relation to glucose kinetics. Endogenous glucose rate of appearance (Glucose Ra) during the basal period was calculated by dividing the infusion rate by the enrichment. Glucose Ra during the clamp is calculated by subtracting the exogenous glucose infusion rate from the whole-body glucose Ra. Glucose Rd is equal to the Glucose Ra + infused tracer at steady-state. Percent suppression is the percent change in Glucose Ra from basal to clamp steady-states. Lastly, glucose metabolic clearance rate was calculated as the ratio of glucose Rd to the mean plasma glucose measured during steady state of the clamp study period.

Clinical Insulin

Outside of metabolic infusion studies performed for research purposes, clinical insulin is also administered to help lower blood glucose levels in patients when blood glucose ≥ 180 mg/dL. For the purposes of this study, we measured clinical insulin administered during acute hospitalization, per patient. Outliers were excluded. Clinical insulin is presented as total insulin units given throughout the entire time period divided by the total number of patients receiving clinical insulin. Data presented at mean ± standard error.

Statistics

Statistical analyses were performed using GraphPad Prism 7.0d (GraphPad Software, La Jolla, CA, USA). Data was analyzed for normalized distribution prior to analyses. Demographic data was analyzed using t-Tests for normalized data and Mann-Whitney test for data that is not normal. Metabolic study and mitochondrial data comparisons were done using 2-way Repeated Measures Analysis of Variance (ANOVA) comparing the effect of drug and time. Sidak's multiple comparisons test was performed to determine variation between CTRL and PROP at each time interval. Significance was determined when the p value was less than 0.05. Unless otherwise noted, data shown as mean ± SD.

RESULTS

Patient demographics and characteristics were identical to those from Chapter 3. There is no difference between patient demographics as it relates to major variables affecting outcomes in burn research. To review date please refer to Figure 7 and Table 1 in Chapter 3 and Appendix Table 1 in the Appendix.

Glucose kinetics were significantly altered by propranolol treatment (Figure 16). In the basal period, Glucose Ra changed from 30.8 ± 10.3 μmol/kg/min in the first study to 31.5 ± 7.0 μmol/kg/min in the second study. Glucose Ra was relatively unchanged in the PROP group between studies measuring 25.8 ± 8.3 μmol/kg/min and 25.8 ± 6.5 μmol/kg/min in the first and second study, respectively. There was a significant (*, $p = 0.03$) effect of drug on the changes observed in Glucose Ra. Although not significant ($p = 0.07$), there was a difference between CTRL and PROP during the second study. A similar significant drug effect in Glucose Ra was found during the clamp period (*, $p = 0.01$). Glucose Ra (clamp) was measured as 10.3 ± 9.6 μmol/kg/min and 6.8 ± 4.0 μmol/kg/min in CTRL and PROP, respectively, during at the first study time point. During the second study period, CTRL and PROP Glucose Ra (clamp) was 8.2 ± 7.9 μmol/kg/min and 3.9 ± 3.3 μmol/kg/min.

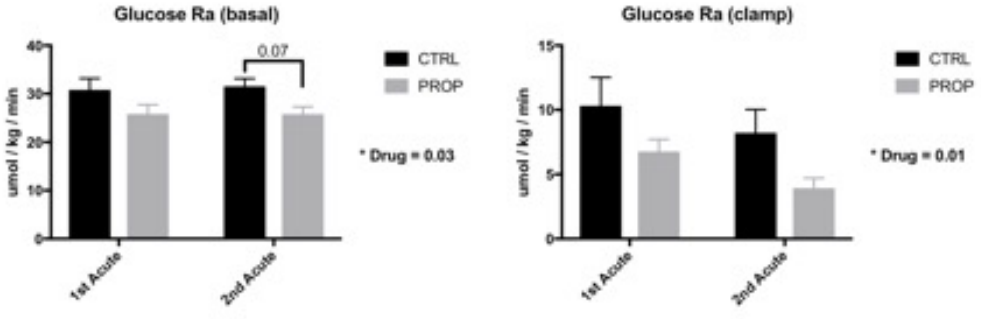

Figure 16: Glucose Ra Changes During Basal and Clamp Period

Changes in Glucose Ra in the basal and clamp period are shown here. Significant differences were observed in both the basal and clamp period ($p = 0.03$ and $p = 0.01$, respectively).

Glucose suppression was analyzed between the basal and clamp period in the CTRL and PROP groups (Figure 17). Percent suppression was not different as a result of time or drug. There was also no difference between drug groups at either of the time points. During the first study, percent glucose suppression was measured as 69.1 ± 0.2 % in the CTRL group and 71.7 ± 0.2 % in the PROP group. At the second time point, CTRL suppression was 73.7 ± 0.3 % and PROP suppression was measured as 84.7 ± 0.1 %. Although not significant, there was an increase in each group between the two time points and more of an increase measured in the PROP group when compared to the CTRL group.

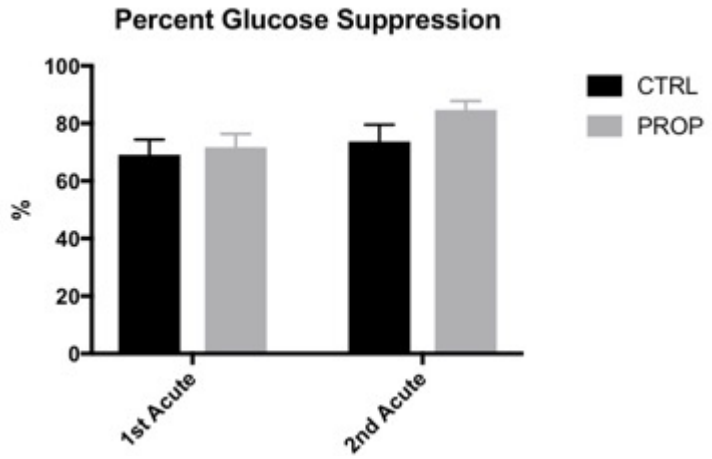

Figure 17: Percent Suppression of Hepatic Glucose Production

Glucose suppression (%) was measured as the difference in Glucose Ra between the basal and clamp periods. Higher percent suppression indicates a greater reduction in hepatic glucose production as a result of exogenous insulin administration. There were no significant differences measured.

Glucose Rd was measured in the basal and clamp period (Figure 18). Glucose Rd showed significant changes as a result of drug, but the clamp period showed a higher degree of significance due to drug (*, $p = 0.03$ vs ***, $p < 0.001$) and showed changes between groups at both the first (**, $p = 0.002$) and second (**, $p = 0.01$) study. Glucose Rd in the basal period was measured at 31.2 ± 10.3 μmol/kg/min and 26.3 ± 8.3 μmol/kg/min in the CTRL and PROP group, respectively. During the second study, glucose Rd was measured as 32.0 ± 7.0 μmol/kg/min in the CTRL group and 26.2 ± 6.5 μmol/kg/min in the PROP group. During the clamp period, glucose Rd was significantly different between CTRL and PROP during the first study (60.1 ± 33.8 and 34.2 ± 11.5 μmol/kg/min, respectively; **, $p = 0.002$) and the second study (60.6 ± 23.2 and 39.0 ± 12.8 μmol/kg/min, respectively; **, $p = 0.01$).

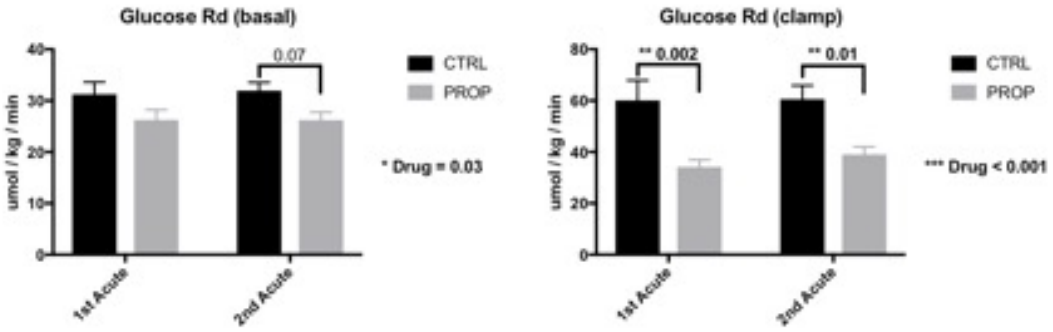

Figure 18: Glucose Rd During Fasted and Clamp Period

Glucose Rd was measured in the basal and clamp period for the 1st and 2nd studies. In the basal period, there was a significant effect of drug on the measured outcomes, $p = 0.03$. During the clamp period, there was a significant difference due to drug (***, $p < 0.001$) and at the first study period (**, $p = 0.002$) and second study period (**, $p = 0.01$).

As a marker of insulin resistance, glucose infused during the clamp period was measured as an average during the last 15-30 minutes of the hyperinsulinemic-euglycemic clamp, once steady state is achieved (Figure 19). During the first study, glucose infused averaged 74.6 ± 38.8 mL in the CTRL group and 52.8 ± 23.8 mL in the PROP group. During the second study, glucose infusion rates were similar (71.8 ± 36.5 mL vs 69.7 ± 34.1 in CTRL and PROP, respectively). There were no statistically significant differences observed between groups at either time point.

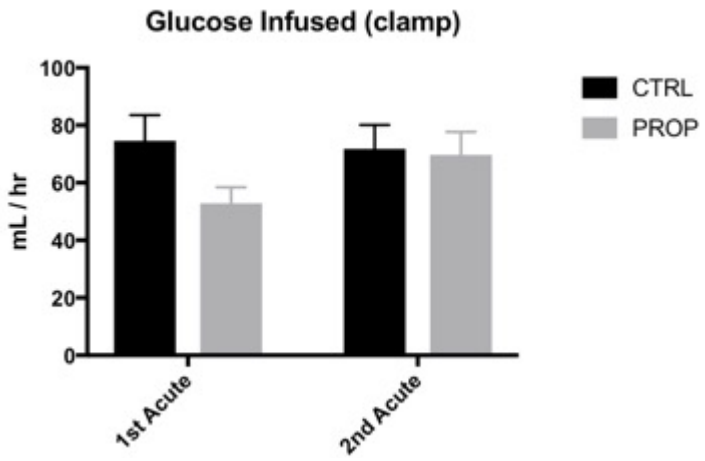

Figure 19: Glucose Infused During Hyperinsulinemic Clamp

Average glucose infused during the clamp period was measured. The average glucose given per hour was determined once steady state was achieved between insulin administration and dextrose 20% administration. No significant differences were observed.

Metabolic glucose clearance rate was also measured (Figure 20). Overall, there was a significant effect due to time (*, $p = 0.01$) and drug (**, $p = 0.002$). Propranolol showed significantly lower clearance rates at both time points. During the first acute study, CTRL had a clearance rate of 0.64 ± 0.35 (μmol/kg/min)/(mg*dL) while PROP showed a significantly lower rate of 0.34 ± 0.13 (μmol/kg/min)/(mg*dL); **, $p = 0.003$. During the second acute period, CTRL had a clearance rate of 0.71 ± 0.25 (μmol/kg/min)/(mg*dL) while PROP showed a significantly lower rate of 0.44 ± 0.19 (μmol/kg/min)/(mg*dL); **, $p = 0.007$.

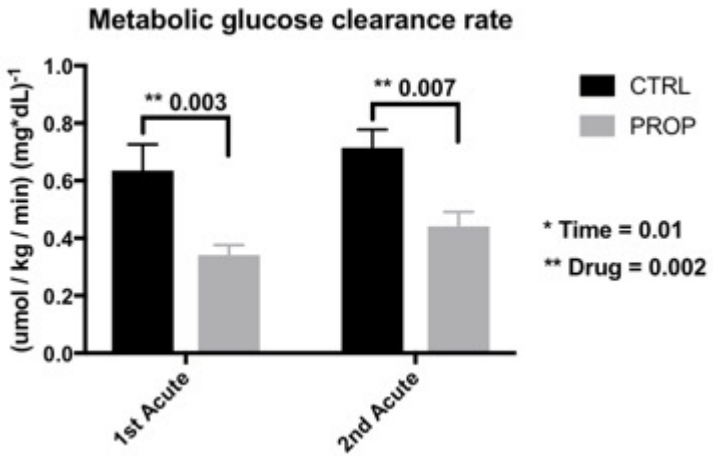

Figure 20: Metabolic Glucose Clearance Rate

Metabolic glucose clearance rates were measured during the clamp periods. Glucose Rd was divided by the average glucose infusion rate and act as a marker of whole body glucose uptake. This effect is significantly different over time and based on drug. Additionally, CTRL and PROP were significantly different at each time point.

Clinical insulin was measured and displayed as total units administered. Insulin is given when glucose reaches 180 mg/dL in an effort to reduce hyperglycemia. When accounting for outliers, both groups had nearly identical clinical insulin administered per patient (498.2 ± 124.5 Units, CTRL; 509.5 ± 141.7 Units, PROP). Total insulin units delivered were 9,965 units and 8,153 units in the CTRL and PROP groups, respectively (Figure 21). Daily 6am glucose values were also measured in our groups (data not shown). 6am Glucose was 143.3 ± 44.6 mg/dL in the CTRL group and 146 ± 32.14 mg/dL in the PROP group. No differences were determined between groups.

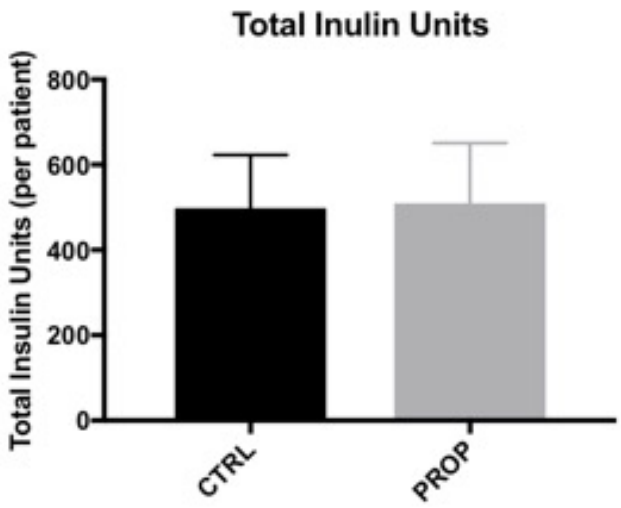

Figure 21: Clinical Insulin Received Per Patient

Clinical insulin administered during the time range of the acute metabolic studies was measured. Clinical insulin is delivered to reduce hyperglycemia and prevent morbidity associated with elevated blood glucose levels.

DISCUSSION

We set out to quantify the effect of propranolol on glucose metabolism in burned children. Glucose rate of appearance, rate of disappearance, and metabolic clearance rate were a significantly altered by propranolol administration. This is perhaps the first study to look in detail at the effects of propranolol on glucose metabolism during the acute recovery from severe burn trauma. We will discuss some implications of these findings and how this study benefits and/or detracts from our previous understanding of propranolol use in pediatric burned patients.

We found significant changes in endogenous glucose RA in the basal and clamp period. PROP significantly decreased hepatic (endogenous) glucose release. These differences were determined to be significant based on drug usage and was nearly significant at the second study time point, indicating that the effects of propranolol on glucose production are perhaps longer lasting, or at least as long as, the study period. There are several pathways contributing to excess hepatic glucose production in burned patients.[172] Mobilization of substrates available for gluconeogenic pathways are increased through other organ specific pathways like lipolysis and muscle breakdown. Reducing these pathways through blunted lipolysis, as previously shown with propranolol administration, is one method for reducing the hepatic glucose production. Additionally, catecholamines are stimulators of liver glucose production. Indeed, both glycogenolysis and gluconeogenesis are significantly increased with epinephrine stimulation.[173] Since these processes are propagated further with glucagon and cortisol (both which are significantly elevated following severe burns) it makes sense that hepatic glucose production would be significantly increased after burns. The changes in Glucose Ra from basal to clamp, however, were similar between the CTRL and PROP groups, suggesting that hepatic insulin sensitivity was not directly affected by propranolol administration.

Although slightly more suppression occurs in the PROP group vs the CTRL group during the second study, this effect was not found to be significant. Glucose Ra during the clamp was significantly affected by propranolol administration. Importantly, we show here that the excessive mobilization of glucose in the post burn period can be reduced with propranolol treatment. Percent glucose suppression is a method determining hepatic insulin sensitivity between the basal and clamp period. During the constant infusion of insulin at the clamp portion of the study, hepatic glucose production should really go to almost zero. Insulin is a mechanism of telling the liver that glucose levels are high enough and glucose uptake needs to help clear out the excess glucose. However, this doesn't necessarily occur as it should in burned patients. In the current study we did not see any significant differences based on drug or time. That being said, glucose suppression did increase more from the first to second study in PROP when compared to CTRL. There are likely other pathways (e.g. glucagon, cortisol) that continue to stimulate hepatic glucose production even in the presence of insulin (and β-blockers).

Glucose rate of disappearance also showed a significant response to propranolol treatment. In the clamp period, and indicative of whole body glucose uptake, there is a significant effect of PROP on glucose disposal, with PROP group showing significantly less Rd at both the first and second study period. The reduction can imply a few different things. First, with the reduced overall Ra, it makes sense that there is less Rd. indeed, glucose Rd is driven by hepatic glucose Ra. While lower glucose Rd may be indicative of lower peripheral insulin sensitivity, the fact that propranolol reduced glucose Ra in the clamp period likely explains lower glucose Rd in propranolol treated patients. Indeed, the infusion of glucose during the clamp periods was not significantly different between control and propranolol treated patients at either time point. There were also no changes associated with time or drug administration.

In order to further examine glucose metabolism in response to propranolol therapy, we measured the metabolic clearance rate of glucose. The metabolic clearance rate of

glucose is another method to measure whole-body glucose uptake, but takes into account the concentration of glucose in the blood, reducing any effects of clamping at lower or higher glucose and altering the clearance rates.[174-177] Higher metabolic clearance rate typically correlates with more peripheral insulin sensitivity. This method takes into account both the clearance levels of glucose (glucose Rd during clamp) as well as the blood glucose concentration at steady-state. This method is thought to be less variable than measuring glucose infusion alone in patients with varying base levels of euglycemia as it incorporates the blood concentration in addition to the overall clearance. We found that the CTRL group higher glucose clearance rates than the PROP group. Collectively, these data indicate that whole body glucose turnover is reduced in burned patients receiving propranolol.

As discussed earlier, few studies have determined the effect of propranolol on blood glucose metabolism in burned patients. In addition to analyses of metabolic studies, we measured clinical insulin and 6am daily glucose values. Clinical insulin is given to patients when approaching hyperglycemia. In our current study, we did not observe a difference in overall daily 6am blood glucose measurements or on clinical insulin administered throughout the course of recovery. There is sparse data linking β-blockade, and specifically non-vasodilating and non-selective blockade, with decreased insulin sensitivity in hypertensive or diabetic patients.[178, 179] In studies directly analyzing propranolol use and insulin sensitivity, Lithell and colleagues found using a hyperinsulinemiac clamp that propranolol reduced insulin sensitivity by up to 30%.[178, 179] These findings do not appear to have any similar effect on pediatric burned patients as we did not observe any increased insulin resistance in patients receiving propranolol.

In our analysis of the effect of propranolol on pediatric burn trauma we had several novel findings. First, we established a significant effect of propranolol on glucose Ra and Rd at both the basal and clamp periods. Second, we showed that glucose clearance rates were significantly higher in the control patients when compared to those receiving propranolol. While we show that hepatic glucose release is blunted by propranolol therapy,

we did not see direct evidence of improved hepatic insulin sensitivity in propranolol treated burn patients. Further, in line with blunted endogenous glucose production, whole body glucose clearance during an insulin clamp is reduced by propranolol therapy, indicating that propranolol reduces whole body glucose turnover in burned patients by lowering release from the liver. To our knowledge, this is the first study utilizing metabolic studies to determine changes in glucose metabolism during the acute recovery period.

CHAPTER 5

Correlation between glucose and lipid metabolism

INTRODUCTION

There are many contributors to hyperglycemia in burned patients. First, the overall catecholamine and cytokine surge during the hypermetabolic phase of burn injury contribute to glucose metabolic dysfunction.[12, 166, 172, 180] This surge directly stimulates gluconeogenesis and glycogenolysis, contributing to excess glucose production and hyperglycemia. Next, the pool of substrates to feed into those pathways is increased through pathways like lipolysis and skeletal muscle catabolism. Amino acid and glycerol release can quickly transition to gluconeogenic substrates, and these are significantly elevated as well after severe burns. Lastly, insulin resistance directly promotes hyperglycemia through the reduced clearance of glucose from circulation. Each of these processes alone may not be as difficult to manage as when all three are highly active, as is observed in burn trauma. Understanding ways to combat multiple pathways simultaneously, or through co-administration of drugs, may be a necessary step in reducing morbidity in burned patients.

As we've shown here, and through previous studies, lipid and glucose kinetics can both be improved through administration of propranolol. There are several ways in which this can occur. For example, a reduction of lipolysis and subsequent glycerol release from adipose tissue would reduce the substrate pool available for gluconeogenesis. Additionally, reducing FFA release into circulation, via lipolysis, could reduce peripheral and central insulin resistance by limiting FFA deposition into ectopic tissues. Lastly, β-blockade can directly decrease gluconeogenesis and glycogenolysis by decreasing catecholamine

stimulated processes in the liver. The overlap of these processes could occur with propranolol and we aim to analyze that in this chapter.

Here, we will determine the relationships between glucose and lipid kinetics in both the control and propranolol groups. Our first big question is: In the absence of additional drug intervention, is there a correlation between lipid and glucose kinetics? This is followed by an additional question: Do changes in glucose metabolism correlate with changes in lipid metabolism in patients receiving propranolol? Answering these two questions will help us understand if a link does exist between the multiple processes, as well as determine if propranolol affects these processes in a similar way.

METHODS

Patients

Patients used in this analysis were the same patients analyzed in Chapters 2, 3, and 4. Patient enrollment and demographics are identical.

Calculations

Data sets utilized in this chapter are the same that were calculated in Chapters 3 and 4. Lipid kinetic analyses included glycerol Ra, palmitate Ra, and IC cycling. From Chapter 4, we analyzed based on glucose Ra, glucose Rd, metabolic clearance rate of glucose, and exogenous glucose administered during constant infusion of insulin. Refer to previous chapter for description of calculations for each variable.

Statistics

Statistical correlations were performed using GraphPad Prism 7.0d. Before analyses were done, data sets were analyzed for Gaussian distribution (normality) with the D'Agostino-Pearson omnibus normality test. Following the normality test, a Pearson correlation coefficient was determined for groups with normal data and a nonparametric Spearman correlation was performed in non-normal data sets.

RESULTS

Correlations were first measured in the control group, to observe any similarities between data sets in the absence of interventional therapy. When comparing glucose Ra to lipid metabolic measurements, we found significant correlations with palmitate Ra during the basal periods at each time point (1st study, r = 0.4561, * p = 0.04; 2nd study, r = 0.7041, *** p = 0.0008) (Figure 22). We also saw significant correlations with the first and second study between glucose Ra and glycerol Ra during the basal period (r = 0.5853, ** p = 0.0085; r = 0.7572, *** p = 0.0002, respectively) (Figure 23). Lastly, we saw nearly significant changes at the first study between glucose Ra and IC cycling during the basal steady-state period (r = 0.4063, p = 0.08), but we did see significant correlation at the second basal period (r = 0.6093, ** p = 0.005) (Figure 24). Of note, but not surprising, we did not see any significant differences during the hyperinsulinemic clamp.

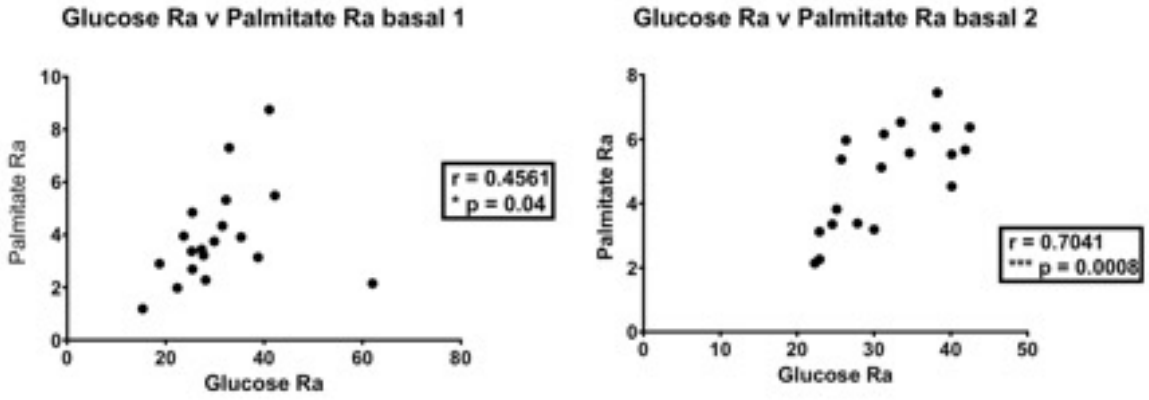

Figure 22: Glucose Ra vs. Palmitate Ra Correlation in CTRL Group

Glucose Ra was compared to Palmitate Ra in the basal/fasted steady-state period during each study. Correlation analysis showed significant connection between the two variables during both study periods, suggesting a possible link between the two.

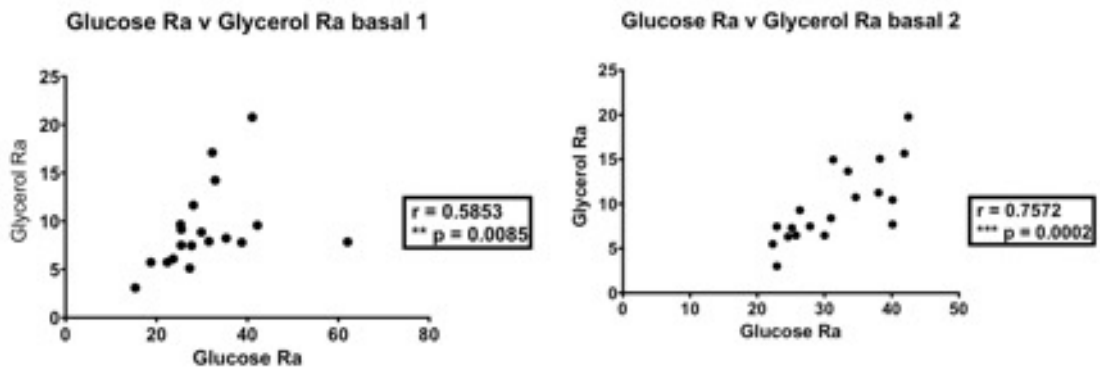

Figure 23: Glucose Ra vs. Glycerol Ra Correlation in CTRL Group

Glucose Ra was compared to Glycerol Ra in the basal/fasted steady-state period during each metabolic study. Significant correlation was determined between the two variables.

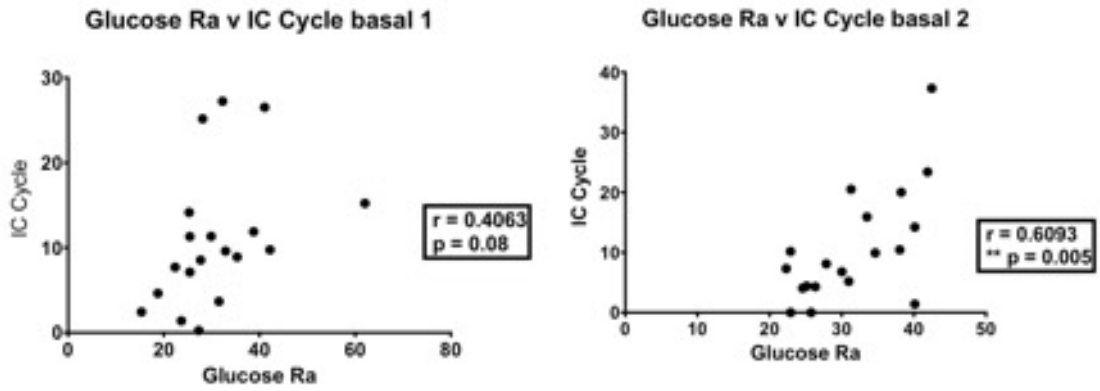

Figure 24: Glucose Ra vs. IC Cycle Correlation in CTRL Group

Glucose Ra was compared to IC cycling during each basal period of the metabolic studies. The correlation was only found to be significant during the second study and not in the first.

We also measured the correlation between glucose Rd and lipid kinetics. We saw similar correlation between Rd and lipid kinetics as we did with glucose Ra in the basal period. This is expected, as basal Ra equals Rd at steady-state equilibrium. Clamp measurements did not show any pattern of significant correlation as was observed in the fasted period (data not shown).

Metabolic clearance rate of glucose was compared to glucose and lipid kinetics during the hyperinsulinemic clamp period at both time points. Most comparisons showed no significant correlation. One interesting finding was a loose correlation between metabolic clearance of glucose and IC cycling. During the first study we observed correlation that was near significant ($r = 0.487$, $p = 0.06$) and significant correlation at the second study time point ($r = 0.55$, * $p = 0.036$) (Figure 25).

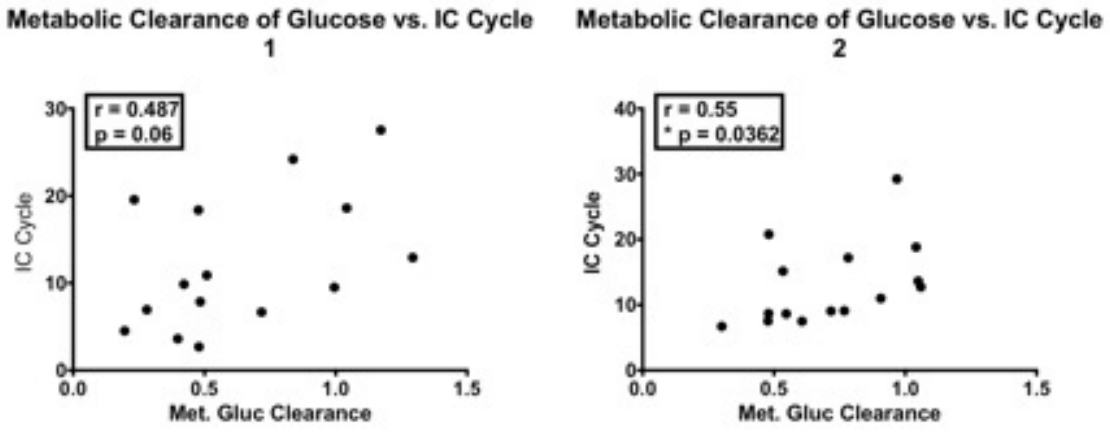

Figure 25: Metabolic clearance of glucose versus IC cycling in CTRL Group

Metabolic clearance of glucose was compared to intracellular cycling to determine any correlation. At the first time point, there was no significant correlation found, although it was close. At the second time point, there was a significant correlation between the two variables.

Glucose infused during the hyperinsulinemic-euglycemic clamp was compared against different lipolysis related variables. We were able to show significant correlation between plasma FFA levels (palmitate) and the glucose infused. Our data shows significant correlation during one of the time points observed, and near significance at the other. During the first study period, we determined that r = -0.409 with a p of 0.08. At the second study we determined a r of -0.44 and p of 0.05*. This data shows that when palmitate concentration is reduced, glucose required to maintain euglycemia is increased (Figure 26).

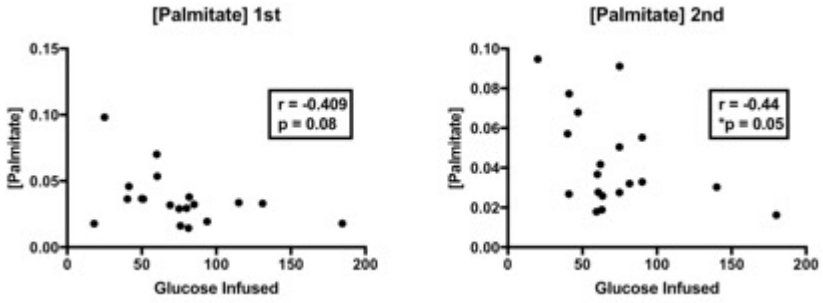

Figure 26: Glucose infused vs. Palmitate concentrations in CTRL Group
Glucose required to maintain euglycemia was measured against plasma palmitate concentration. There was significant correlation during the second study and near significant correlation during the first study.

After comparisons between variables in the control group, we similarly compared the propranolol group. In contrast to the control group, where less of a pattern observed in the propranolol group. In the fasted state, we saw significant correlation when comparing glucose Ra and Palmitate Ra (Figure 27). During the first study period, we found a correlation coefficient of 0.6511 and p value of 0.0034 (**). During the second period we also observed significance with a coefficient of 0.4727 and p value of 0.048 (*).

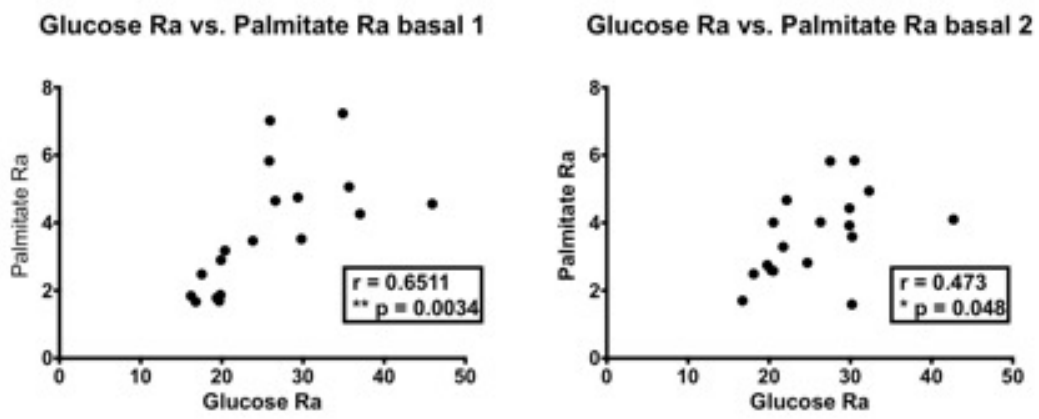

Figure 27: Glucose Ra versus Palmitate Ra during basal studies with propranolol
Glucose Ra was compared to Palmitate Ra during each of the two metabolic studies and during the basal period. There was significant correlation observed between these two variables.

Although there were not many other significant correlations determined between the lipid and glucose variables, we did observe highly significant correlation with IC cycling and Glycerol Ra during the clamp period of the second time point (Figure 28). When comparing glucose Ra and glycerol Ra we found a coefficient of 0.74 and a p value of 0.0004 (***). The correlation between glucose Ra and IC cycling measured 0.75 with a p value of 0.0003 (***). Lastly, we measured metabolic clearance rate of glucose in patients receiving propranolol. There were no significant correlations determined between the variables measured.

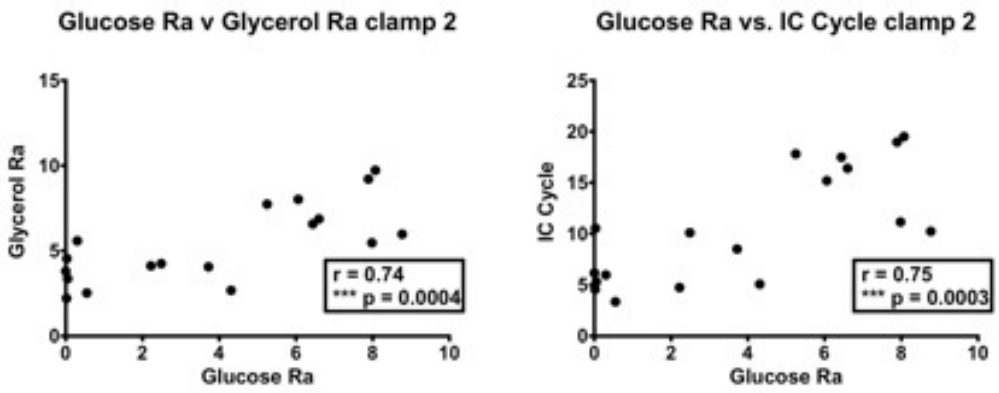

Figure 28: Glucose Ra versus Glycerol Ra and IC Cycle with propranolol administration

Glucose Ra was compared to glycerol Ra and IC cycling during the clamp period with propranolol administration. These findings were found to be significantly correlated between the variables.

DISCUSSION

For the first time, we measured correlation between lipid and glucose metabolism in burned patients. Interestingly, we found significant correlation in the control group in the fasted state. This is a very interesting that supports the hypothesis that altered glucose and lipid metabolism are linked in burned patients, and perhaps driven by catecholamines. As outlined earlier, there does appear to be some connections of gluconeogenesis, lipolysis, and insulin resistance. Here we provide preliminary results showing that this link could play an important role in post-burn recovery.

It is not surprising that we did not observe any significant correlation during the hyperinsulinemic clamp of control patients. As insulin is able to significantly inhibit lipolysis, removing lipolytic variables likely reduced the correlation observed. There was a loose correlation observed with the metabolic clearance of glucose and intracellular cycling. This is an interesting finding as recycling of FFA in the cell wouldn't necessarily correlate with insulin resistance. The data suggests that increase of glucose clearance correlated with increased FFA cycling, meaning a reduction in FFA release into circulation. This could perhaps suggest that IC cycling is linked to hypermetabolism in that elevated response to catecholamines causes more IC cycling to occur, possibly resulting in increased thermogenesis and heat production. Additionally, as we observe glucose Ra increase, IC cycling also increases, suggesting further that the catecholamine stimulated hypermetabolism affects glucose and lipid metabolic pathways. Over time, these variables could prove to be linked as a decrease in FFA cycling, and more released into circulation, could be related to insulin sensitivity with ectopic deposition of FFA in tissues. The short period of the clamp is likely an incidental finding and not important to the overall link between glucose and lipid kinetics.

Here, we present novel findings linking catecholamine driven hypermetabolism with glucose and lipid metabolism. Our data suggests that there is a link between the

elevation of lipolysis and the resulting hepatic glucose output. Catecholamines have the ability to directly impact both pathways and likely play an important role in this link, but it is interesting that we a significant effect on so many variables linking glucose and fat metabolism. In direct analysis of insulin resistance, we show that plasma FFA concentrations affect the exogenous glucose required to maintain normal glycemic control. Propranolol did not seem to affect both pathways in the same manner, as some of the correlations were diminished with propranolol administration, but our limited data needs to be followed up by larger studies in the future.

CHAPTER 6

Overall Conclusion and Discussion

In this project, we determined the acute metabolic of propranolol administration in patients with severe burns. There are several important implications from this study and strengthen the justification for use of propranolol in pediatric burned patients.

As important with any study, we validated the efficacy of propranolol through measuring HR (which constitutes the guidelines for administration post-burn). With average doses from 4.5 – 6 mg/kg/day, we saw a significant reduction in heart rate throughout the study period. More importantly, we were able to reduce the % maximum HR (a better representation of the changes in HR) from a state of "vigorous exercise" to only "moderate exercise", as outlined by the CDC guidelines for exercise HR ranges. We also showed that REE was decreased at the second study time with use of propranolol. Lastly, we showed that propranolol significantly reduced rate-pressure product, a marker of myocardial oxygen consumption, to the upper limit of normal ranges. HR, % max HR, and RPP were all significantly elevated throughout the duration of our study, confirming previous findings of propranolol use in burned patients.

After determining propranolol efficacy in two similar groups of patients, we assessed lipid and glucose kinetics during the acute recovery period of burn injury in patients randomized to propranolol or a placebo. Although we did not show identical results to previous studies of propranolol and lipolysis, we showed changes to FFA release and concentration in the post-burn recovery period. As mentioned, there are several reasons for the differences and are likely due to desensitization of β-AR and β-blockade, possibly upregulating other methods of lipolysis or leveling out completely. Decreasing FFA release is important as it pertains to burned patients as FFA can cause increases in insulin resistance and organ dysfunction. In this study, however, we noticed reduced insulin sensitivity in the

absence of increased serum FFA. This is a unique finding but also ties into the complex nature of severe burn trauma.

Adipose tissue mitochondrial function was also altered by propranolol therapy. Both maximum respiration and thermogenesis capacity were elevated in control patients not receiving propranolol. This likely relates to decreased energy expenditure and hypermetabolism in patients receiving propranolol. Mitochondrial function is moving closer to normal with propranolol administration (perhaps a better description would be normal post-burn).

Our last major analysis consisted of measuring glucose kinetics and insulin sensitivity. Propranolol reduced glucose turnover and in particular hepatic glucose output, without altering hepatic or peripheral insulin sensitivity. Propranolol did not have any negative effects on glucose metabolism or insulin sensitivity in our patient cohort.

Overall from our main study we were able to show that propranolol causes:

- **Significant reduction of FFA (palmitate) release compared to controls.** Palmitate Ra, and overall palmitate release from adipose tissue, was decreased in patients receiving propranolol. This is important for FFA disposal in ectopic tissues, liver function, and insulin resistance.
- **Significant change in palmitate concentration over time.** Similar to the previous point, changes in FFA release and serum concentration has impact on several aspects of metabolic function.
- **Blunted elevation of mitochondrial function (less energy expenditure).** Mitochondria from patients receiving propranolol had similar quality but less of an increase in oxidative phosphorylation and thermogenesis when compared to control patients. This likely contributes to the reduced energy expenditure that was observed through measuring REE.
- **Significant changes in mitochondrial function over time.** Time is an important variable in the post-burn recovery period.

- **Significant changes in Glucose Ra (basal and clamp) due to propranolol.** Propranolol significantly alters glucose Ra. All patients receiving propranolol had reduced glucose Ra when compared to control patients. This is important in reducing the hepatic glucose production after burn injury. Reducing glucose output could improve outcomes that are dysfunctional as a result of hyperglycemia.
- **Significantly reduced Glucose Rd in propranolol patients during a hyperinsulinemic clamp**. An interesting finding was the reduced glucose disappearance in propranolol patients. This could have several implications but is likely affected by a reduced glucose appearance.
- **Significant changes in metabolic clearance rate of glucose due to time and drug.** This finding reinforced what we measured in glucose Rd. Patients receiving propranolol had lower metabolic clearance rates of glucose, but this is largely due to reduced hepatic glucose output.

Overall, we have evidence to further support propranolol use in pediatric burned patients. Further studies are necessary to explore the novel findings we show here of a significant correlation between glucose and lipid pathways after severe burn injury. Propranolol is still not standard of care for most burn centers across the globe. Here, we further establish the benefit that pediatric burned patients would receive with acute propranolol administration.

Limitations and closing considerations. An important note to discuss as it relates to this study, and perhaps a limitation, is the differences from previous studies and the inability to fully compare this study to earlier studies. In the early work by Wolfe and colleagues, they used a short infusion on propranolol in naïve patients prior to analyzing the effects.[18] Additionally, patients were 20 days post burn at time of the study, missing earlier periods of burn recovery. In Herndon's 1994 study of propranolol vs. metoprolol,

patients were studied around 17 days post burn and given drug over 5 days.[120] Both of these studies similarly had different protocols for stable isotope infusion studies compared to the study we present here (different timelines). In the current study, patients were not naïve to propranolol at the initial study but had been on drug for 3 days to a week prior to the first acute study. This difference could cause us to miss changes observed in the early days after propranolol is administered in the acute recovery period. Additionally, the patients presented here were studied much earlier than in the prior studies (10 days average post-burn). After enrollment for patients in this study ended, propranolol has become standard of care at Shriners Hospitals for Children – Galveston. The benefit of propranolol seems abundant, and with the relative cost of the drug (100 tablets of 80mg propranolol is ~ $40), there are few reasons to not consider propranolol use after severe burns. With the previous studies outlining the benefit of blocking catecholamines post-burn, in addition to the data presented here, we hope to further convince burn care facilities to utilize propranolol after severe burn injury.

APPENDIX

Additional Figures

	CONTROL	PROPRANOLOL
Burn to Admit		
Min	0.00	0.00
Max	7.00	7.00
AVG	2.65	2.84
Burn to Drug		
Min		1.00
Max		8.00
AVG		4.21
Admit to Drug		
Min		0.00
Max		4.00
AVG		1.37
Burn to 1st Study		
Min	3.00	3.00
Max	25.00	14.00
AVG	10.05	9.11
Burn to 2nd Study		
Min	12.00	12.00
Max	32.00	33.00
AVG	19.65	18.63
1st to 2nd Study		
Min	7.00	7.00
Max	24.00	21.00
AVG	9.60	9.53

Appendix Table 1: Timing Table

This table outlines the timing when patients were admitted, started on drug, and the time between and up until each study.

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Vita

Robert "Patrick" Clayton grew up in College Station, Texas and graduated from A&M Consolidated High School in 2007. After graduating, Patrick attended Texas A&M University in College Station, TX and majored in Molecular and Cell Biology. He graduated in 2011 with a B.S. in Molecular and Cell Biology. During his last few years, Patrick worked in a chemistry laboratory and studied sporulating bacteria and x-ray crystallography. Upon graduating, Patrick enrolled in a PhD program, Cardiovascular Physiology, at the Texas A&M Health Science Center School of Medicine in Temple, TX. During his time at A&M, Patrick met his future wife, Holly Marshall, and subsequently transferred to UTMB to complete his studies as part of the new Human Pathophysiology and Translational Medicine (HPTM) program while his wife attended Physician Assistant school at the UTMB School of Public Health. Patrick joined Shriners Hospitals for Children – Galveston in the summer of 2013 and has been there throughout his graduate studies. While at UTMB, Patrick was awarded the prestigious John D. and Mary Ann Stobo Award in Oslerian Medicine from the UTMB McGovern Academy. He was also awarded the Ann Anderson Scholarship from UTMB GSBS. Additionally, Patrick was involved in several organizations on the campus of UTMB, helping to found and run the Society for Translational Research (2015-2017) and leading the Committee for Career Development 2016-2018). Upon graduation, Patrick will be branching out into pharmaceutical and medical device development as a Fellow at Fannin Innovation Studio in Houston, TX.

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17. Guillory AN, **Clayton RP**, Prasai A, Jay JW, Wetzel M, El Ayadi A, Herndon DN, Finnerty CC. Buprenorphine-SR alters hemodynamic parameters in a rat burn model. Journal of Surgical Research. Accepted March 2018.

Abstracts (First/Presenting author only)

1. **Clayton RP**, Guillory AN, El Ayadi A, Herndon DN, Finnerty CC. Characterizing changes in cardiac metabolism following burn injury. Poster presentation: 3rd Annual Clinical and Translational Research Forum; University of Texas Medical Branch, Galveston, TX. March 2015.

2. **Clayton RP**, Guillory AN, Jay JW, Herndon DN, Finnerty CC. Effect of analgesia on hemodynamic parameters in a rat model of burn injury. Poster presentation: 4th Annual Clinical and Translational Research Forum; University of Texas Medical Branch, Galveston, TX. 2016.

3. **Clayton RP,** Scofield HO, Hawkins, HK, Finnerty CC, Herndon DN. Age-associated complications of severe burn injury: a ten year review of patient autopsies from a single burn institute. Poster presentation: 39th Annual Conference on Shock, Austin, TX. 2016.

4. Guillory AN, Wurzer P, **Clayton RP**, Branski LK, Andersen CR, Herndon DN, Finnerty CC. Correlation of serum cytokine levels and markers of cardiovascular function in severely burned pediatric patients. Poster presentation (***presenter***): 39th Annual Conference on Shock, Austin, TX. 2016

5. **Clayton RP**, Hundeshagen G, Marques NR, Salter M, Guillory AN, Wurzer P, Kinsky MP, Herndon DN. Comparison of cardiac monitoring techniques in a long-term study of pediatric burn patients. Poster presentation: 49th Annual American Burn Association Conference. 2017.

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