28 day bed-rest with hypercortisolemia induces peripheral insulin resistance and increases intramuscular triglycerides

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Abstract

Spaceflight represents a unique physiological challenge to humans, altering hormonal profiles and tissue insulin sensitivity. Among these hormonal alterations, hypercortisolemia and insulin insensitivity are thought to negatively affect muscle mass and function with spaceflight. As insulin sensitivity influences the accumulation of muscle triglycerides, we examined this relationship during hypercortisolemia and inactivity. Six young healthy volunteers were confined to bed rest for 28 days. To mimic the stress response observed during spaceflight, hypercortisolemia (20–24mg/dL) was induced and maintained by oral ingestion of hydrocortisone. On days 1 and 28 of bed rest, insulin sensitivity across the leg was assessed with a local (femoral arterial insulin infusion) 2 stage hyperinsulinemic-euglycemic clamp (stage 1: 35 µU/min/ml leg; stage 2: 70 µU/min/ml leg). Intramuscular lipid was measured with magnetic resonance spectroscopy. Following bed rest, there was a decrease in insulin sensitivity, as assessed by glucose uptake during hyperinsulinemia (from 9.1±1.3 (mean ± SEM) mg/kg.leg/min to 5.2±0.7 mg/kg.leg/min (P=0.015)). Intramuscular triglyceride increased from 0.077±0.011 to 0.136±0.018 (signal area of fat/signal area of standard; P=0.009). Intramuscular lipid content correlated with the glucose uptake at day 28, (R= −0.85; P=0.035). These data demonstrate that muscular inactivity and hypercortisolemia are associated with an increase in intramuscular triglyceride and skeletal muscle insulin resistance in previously healthy subjects.

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Introduction

Spaceflight results in a hormonal imbalance that predisposes astronauts to the loss of muscle mass and function. The existing evidence indicates that hormones important for muscle anabolism are either altered in production/concentration, such as testosterone (42), or by tissue action, as is the case with insulin (25,41). To further exacerbate the potential for muscle catabolism, spaceflight results in an increased production/excretion of cortisol (40). Thus, the prevailing hormonal influence favors muscle protein loss. This alteration in hormonal production and activity is particularly deleterious when combined with muscular inactivity. We have previously demonstrated that muscular inactivity predisposes the muscle to the catabolic effects of hypercortisolemia (17). Studies indicate that even short-term inactivity contributes to insulin resistance (26,39,43). Decreased insulin stimulated glucose uptake can be seen within 7 days of bed rest in healthy individuals, and within 24 hours when combined with fasting (4,26,44). In a patient population, hyperglycemia due to insulin resistance is directly linked to increased morbidity and mortality (48). During spaceflight, insulin resistance is most likely an important factor contributing to the loss of muscle mass and function.

The stress response, whether induced by illness or spaceflight, entails the increase in the counter-regulatory hormone cortisol (18). Cortisol release in healthy adults follows a diurnal pattern; however, in the stress state the diurnal pattern of cortisol release is lost, and concentrations remain elevated throughout the day (18,54). Cortisol has also been demonstrated to have detrimental effects on glucose tolerance (23,27,37). Whereas several studies have looked at the effect of bed rest/muscular inactivity alone on insulin sensitivity, none have examined the combination of inactivity and hypercortisolemia that is noted during spaceflight, as well as during hospitalization.

Further, little is known about the interaction of fat metabolism and insulin sensitivity during bed rest or hypercortisolemia. Multiple studies have found an interaction between altered fat metabolism and glucose uptake in patients with type 2 diabetes mellitus (T2DM) . This has been reviewed extensively, and the prevailing thought is that intracellular triglyceride products such as diacylglycerol (DAG) or ceramides directly effect the insulin signaling pathway, and that concentrations of these compounds are elevated secondary to increased concentrations of intramyocellular triglycerides (IMTG) and increased delivery of FFA to tissues (35). Although it has been determined that short term administration of cortisol increases plasma FFA (27) and regional and systemic lipolysis (14), an investigation of the effects of long-term hypercortisolemia on insulin sensitivity has not been accomplished.

We studied the effects of prolonged inactivity and hypercortisolemia on insulin resistance and intramuscular lipid in healthy volunteers. We hypothesized that the combination of inactivity and hypercortisolemia would decrease insulin sensitivity and that it would further be related to increased deposition of muscle triglycerides. To discern this interaction, we measured glucose uptake across the leg, plasma fat metabolism, and skeletal muscle triglyceride content and metabolites.

Methods

Subjects

Six healthy male (age 27±2 years) volunteers participated in this project . All volunteers provided informed, written consent and the project was approved by the institutional review board at the University of Texas Medical Branch (UTMB). Subject eligibility was assessed using multiple medical screening tests as previously described, and all lipid and hepatic tests

were within normal ranges (31). Additionally, a standard 2 hour oral glucose tolerance test was performed and all subjects had a normal glucose response to the challenge.

Admission and Pre- and Post-testing

The experimental protocol is depicted in Figure 1. Subjects were admitted and housed in the General Clinical Research Center (GCRC) at UTMB for pre-testing and five days of dietary stabilization prior to the start of bed rest. During this period, subjects were sedentary but remained ambulatory. The Harris-Benedict equation was used to estimate daily energy requirements as previously described (31,32). Subjects were placed on a three day rotating diet with daily nutrient intake evenly distributed between three meals (0830, 1300, 1830). Carbohydrate, fat and protein intake represented 59%, 27% and 14% of the daily energy intake respectively (16,31,32). Water was provided ad *libitum*. MRS spectroscopy was performed prior to bed rest after 2–3 days of diet stabilization and post-bed rest (day 29), immediately prior to the resumption of weight bearing.

Experimental Paradigm—Glucose clamp studies were performed on days 1 and 28 of bed rest. Subjects received 10–15 mg of oral hydrocortisone sodium succinate 5 times every day during bed rest (Days 2–27: 0800, 1200, 1600, 2000, 2400). This administration regimen resulted in circulating cortisol of approximately 20 µg/dl, while simultaneously removing the diurnal variation of cortisol secretion.

Infusion Protocol

At 2400 on both day 1 and 28, a constant peripheral venous infusion (18-guage polyethylene catheter: Insyte-W, Becton-Dickinson, Sandy, UT) of hydrocortisone sodium succinate (Pharmacia, Corp., MI), (60 µg/kg/h) was initiated to reduce fluctuations in plasma cortisol concentrations during measurement of insulin sensitivity. Peripheral blood samples for determination of plasma concentrations of cortisol, glucose and insulin were drawn through a contralateral 18-guage polyethylene peripheral venous catheter.

At 0700, 8 cm polyurethane catheters (Cook catheters, Bloomington, IN) were inserted into the femoral vein and artery of one leg under local anesthesia and maintained patent by normal saline. Femoral arterial and venous blood samples were obtained during the post-absorptive period between 0800 and 1100. Leg plasma flow was calculated from steady-state indocyanine green (ICG) concentrations and converted to leg blood flow using hematocrit as previously described (3,22).

Following the 3 hour basal period, the first step of a 2 step across the leg insulin clamp was initiated with a low level of insulin (0.38 µU/min/ml of leg volume) for 3 hours. *Insulin was infused directly into the femoral artery to minimize its systemic effects.* Systemic glucose was clamped between 85–95 mg/dL, checked every 10 min and maintained via peripheral infusion of 25% dextrose when appropriate. After 3 hours, the infusion rate of insulin was increased to 0.70 µU/min/ml of leg volume, while the whole blood glucose concentration was maintained. The calculation of leg glucose uptake was performed with glucose values from the last hour of each of the clamps by dividing the amount of glucose infused per min by 25% of the total body weight, to approximate the contribution of total mass by the leg.

At 0800, 1400 and 1700, muscle biopsy samples (approx. 50 mg) were taken from the lateral portion of the vastus lateralis using a 5 mm Bergstrom biopsy needle and local anesthesia (1% lidocaine) as previously described (2,30).

Bed rest—Subjects maintained strict bed rest throughout the study and were continually monitored by the scientific and GCRC nursing staffs. Subjects were encouraged to change

position periodically to alleviate positional discomfort and to eat. Bathing and hygiene activities and urine collection were performed during bed rest. Subjects were permitted to use a bedside commode for bowel movements, but the time out of bed was non-weight bearing and limited to approximately 5 minutes.

Sample Analysis

IMTG quantification—Samples were analyzed as previously described (9,10). Briefly, IMTG was measured from the soleus in 4 consistent voxels with a ^{1}H knee coil on a GE Advantage 1.5 Tesla whole body imager (General Electric, Milwaukee, WI). A tube of 20 % Intralipid$^©$ (IV high fat total parenteral feeding solution – Baxter Healthcare Deerfield Park, IL) was taped to the leg and placed inside the knee coil to obtain a standard external reference to normalize IMTG concentrations (33). Peak positions were determined by time domain fitting using jMRUi (24,49–51). The prior knowledge information used for the AMARES fits have been previously published by Rico-Sanz and our previous work (9–11,36). This process was repeated for the intralipid phantom. The TG levels were computed as a ratio relative to the Intralipid$^©$ standard using the following formula:

$$TG = [(PM/VM)/(PI/VI)]$$

where PM is the tissue lipid methylene peak area, VM is the total measured tissue voxel volume, PI is the Intralipid peak area, and VI is the Intralipid voxel volume. The results of this calculation are a ratio and expressed in arbitrary units.

Muscle DAG analysis—Muscle DAG palmitate concentrations were measured as previously described from frozen muscle tissue samples (46).

Glucose analysis—Plasma glucose was measured with an YSI 2300 Stat glucose/lactate analyzer (YSI, Inc. Yellow Springs, OH).

Insulin Analysis—Plasma was separated and stored at −80°C until analysis. After thawing, insulin levels were measured using RIA per the manufactures instructions (Diagnostic Laboratories, Los Angeles, CA). The coefficient of variation for these measurements in our lab is <10%.

Plasma Free Fatty Acid Analysis—Total free fatty acids were measured using a chromatographic kit per the manufactures instructions (Wako Diagnostics, Osaka, Japan).

Plasma Glycerol—Total free glycerol was measured using the free glycerol determination kit from Sigma (St. Loius, Mo), Catalog Number FG0100 per the manufactures instructions.

Statistics

Data reported are means ± Standard Error of the Mean. For IMTG and glucose uptake, a paired T-test was used to compare pre and post bed rest values in individuals pre and post-bedrest. DAG, plasma NEFA and plasma glycerol were analyzed using ANOVA with Tukey's post-hoc analysis. Pearson correlations with linear regression analysis were performed with IMTG and insulin resistance vs. bedrest. All statistics were performed on Sigma Stat (Version 2.01).

Results

Subject body composition

As has already been published, subjects lost an average of -2.8 ± 0.6 kg with more 1 kg of lean body mass loss in the lower extremities (29). There was a minimal decrease in body fat mass (-95.6 ± 288.1 g).

Glucose

Fasting glucose concentrations were 90 ± 7 mg/dl and 95 ± 5 mg/dl on study days 1 and 28, respectively ($p>0.05$).

Clamp insulin concentrations

Basal insulin concentrations obtained from the femoral vein were similar pre and post bed rest (11.4 ± 2.1 µIU/mL Pre and 14.2 ± 2.9 Post; $p=0.175$). Insulin concentrations (femoral vien) during the low level of the clamp were 37.5 ± 5.3 µIU/mL pre and 38.9 ± 5.5 µIU/mL post and during the high level of clamp were 70.6 ± 8.3 µIU/mL pre and 71.0 ± 6.2 µIU/mL post.

Leg glucose uptake

During the Stage 1 clamps on day 1 and day 28, there was no change in insulin stimulated glucose uptake (2.97 ± 0.37 mg/kg leg/min vs 3.13 ± 0.35; Pre vs. Post; $P>0.1$). There was a significant decrease in post-bed rest insulin stimulated glucose uptake during the stage 2 clamp on day 28 (Figure 2A; 9.1 ± 1.3 mg/kg leg/min vs. 5.2 ± 0.98; Pre vs. Post; $P=0.015$).

IMTG

The ratio of intracellular fat to external standard for pre-bed rest IMTG was 0.077 ± 0.016 arbitrary units (AU). Following bed rest, it significantly increased ($P=0.009$) to 0.136 ± 0.026 AU. All subjects had at least a 20% increase in IMTG following bed rest, and two individuals had more than a two-fold increase (Figure 2B).

Correlations

After bed rest, insulin sensitivity and IMTG were strongly correlated ($R= -0.85$, $P=0.035$, Figure 3).

Plasma NEFA

Plasma NEFA concentrations were unchanged before and after bed rest, although there was a tendency to increase at the high insulin concentration (Figure 4A). NEFA levels decreased in response to insulin equally before and after bed rest.

Plasma Glycerol

Plasma glycerol concentrations were unchanged in the basal and low insulin period of the clamp before and after bed rest (Figure 4B). The concentration of plasma glycerol was significantly greater following bed rest during the high insulin level phase of the clamp. Glycerol levels decreased in response to insulin similarly before and after bed rest.

Muscle DAG

The concentration of DAG palmitate did not change with increasing concentrations of insulin, and did not change with bed rest (Figure 5).

Discussion

Muscular inactivity and concomitant hypercortisolemia are commonly experienced by astronauts, as well as medical and surgical patients. We found that the combination of these two catabolic stressors induced peripheral insulin resistance and increased IMTG accumulation in previously healthy volunteers. Peripheral insulin resistance and hyperglycemia are of serious concern to both astronauts and patient populations, and may be related to altered fat metabolism. Our results indicate that impairment of muscle glucose uptake was significantly correlated with the increase in IMTG. However, the mechanism by which intracellular lipids contribute to insulin resistance is not clear, as we did not find increases in intramuscular DAG, or in fasted plasma NEFA or glycerol concentrations.

The individuals enrolled in this study were healthy, with normal pre-study blood test and screening results. Specifically, pre bed rest IMTG values (0.08±0.01AU) were identical to a previously studied cohort of 18–32 year old healthy volunteers (10). Plasma NEFA and glycerol concentrations were within normal range, as were pre bed rest measurements for insulin sensitivity. Thus, the demonstrated changes were attributable to the combination of bed rest and hypercortisolemia.

Our results suggest a relationship between the accumulation of IMTG and peripheral insulin sensitivity. Following bed rest the IMTG content increased in every subject by at least 20% and there was a strong correlation between the two following bed rest. We have recently shown that IMTG is elevated in insulin resistant burn trauma patients (9). Studies in burned animals have also shown that IMTG accumulates rapidly in animals with high cortisol concentrations (1). Thus, in multiple models, there is strong relationship between hypercortisolemia, insulin resistance, and IMTG.

IMTG is likely elevated as a result of both a decrease in metabolic rate, secondary to inactivity, and an increase in NEFA delivery due to the prevailing hypercortisolemia (14). Increased NEFA concentrations have been linked to insulin resistance in many models (5). Although the fasted values of NEFA were not different before and after bed rest, plasma NEFA and glycerol were higher during hyperinsulinemia, indicating that lipolysis was not suppressed to the same extent following bed rest and hypercortisolemia, and that adipose tissue as well as muscle tissue was experiencing insulin resistance. An overall decreased metabolic rate has been demonstrated following bed rest and lipid oxidation rates have also been shown to decrease by up to 90% following 7 days of bed rest (4). In our previous study of insulin resistant burn patients, decreased mitochondrial function and a decreased rate of lipid oxidation was linked to insulin resistance. This mechanism may have been present, in part, in our volunteers (8,13,34). It maybe that in ambulatory, healthy adults, several factors such as nutrition, activity and genetics influence IMTG pool size, but in illness, inactivity or stress, the pool size become more directly related to insulin sensitivity, since IMTG and glucose uptake were not linearly associated prior to bed-rest, but were strongly associated afterwards.

Whereas IMTG is often elevated in insulin resistance, several studies have indicated that increases in DAG are also associated with insulin resistance. DAG is thought to activate protein kinase C (PKC), which in turn inactivates the insulin receptor substrate-1 (IRS-1) by serine phosphorylation(21,35). However, despite parallel increases in insulin resistance and IMTG, we did not find any changes in DAG. These findings are similar to those in burn patients, where DAG concentrations were comparable to healthy controls (12). Further evidence is provided in a recent study by Dubé et al. where exercise training in obese older subjects resulted in an increase in IMTG and a decrease in DAG and ceramide (15). These

findings point to the complexity of insulin resistance and the idea that DAG may not be a primary causal/controlling factor.

While bed rest alone can mimic the physical inactivity associated with trauma or illness and is often utilized as a ground-based model for spaceflight, it cannot duplicate the complex hormonal and metabolic disruptions of the stress state (52,55). The chronic administration of exogenous cortisol in this study was designed to elevate plasma cortisol concentrations to the upper physiological range; consistent with values observed following moderate trauma (53,56), and certain aspects of spaceflight (57). Other investigators noted that insulin secretion in response to a glucose load increased after 20 days of bed rest, reflecting peripheral insulin resistance (26). Similar results were found after 7 days of bed rest (4) and 20 days of bed rest (58). These results in healthy volunteers are consistent with data from patients restricted to bed rest following elective surgery (28). Unfortunately, the across the leg clamp, while better for direct assessment of muscle insulin sensitivity, prevents a direct comparison of insulin resistance with previous whole-body determinations in studies investigating bed rest alone (29).

Excess cortisol secretion has been linked to insulin resistance in both observational and experimental studies. Increased cortisol concentrations have also been demonstrated in patients with type 2 diabetes mellitus (T2DM) (26,39). Studies in healthy volunteers indicate that cortisol increases plasma glucose concentrations via action on adipose, muscle and hepatic tissue, and may act by activating inflammatory pathways (6,7,20,23,27,37,45). As already published from this patient population, cytokines such as TNF-B and Il-6 did not increase, although we did not measure IL-1 TNF-alpha (47). It is unlikely that inflammation per see played a large role in the cortisol mediated insulin resistance in this patient model. While a shortcoming of this study was the absence of a bed rest alone group, it is clear that the combination of cortisol and bed rest is a model most relevant to patient and astronaut populations, and that an understanding of this combination has greater practical application.

We have previously shown that the combination of inactivity and stress facilitate a 3-fold greater loss of lean muscle mass than bed rest alone (7,37,38,45). It is likely that some of the mechanisms tied to disuse atrophy are also related to the decreased peripheral glucose uptake. The insulin like growth factor-1 pathway, with downstream signals PI3K, Akt and protein S6 kinase (ps6k) has been shown to be important to muscle hypertrophy (29). Decreased levels of Akt protein have been found with disuse (19). PI3K and Akt are also critical in the insulin signaling pathway to increase glucose uptake in muscle (21). This pathway is also affected by hypercortisolemia, and this combination may be responsible for the greater muscle loss in these subjects compared to those in bed rest alone (7,29,32,37). The loss of muscle protein may further affect insulin signaling by reducing the amount of metabolically-active tissue.

In conclusion, prolonged inactivity and hypercortisolemia induces insulin resistance in skeletal muscle which is associated with an increase in IMTG, with IMTG concentrations directly corresponding to leg glucose uptake. The increase in IMTG may be indicative of this combined insult and may not be related to DAG. Further exploration of the combined effect of inactivity and stress may lead to a better understanding of how to ameliorate these associated conditions.

Acknowledgments

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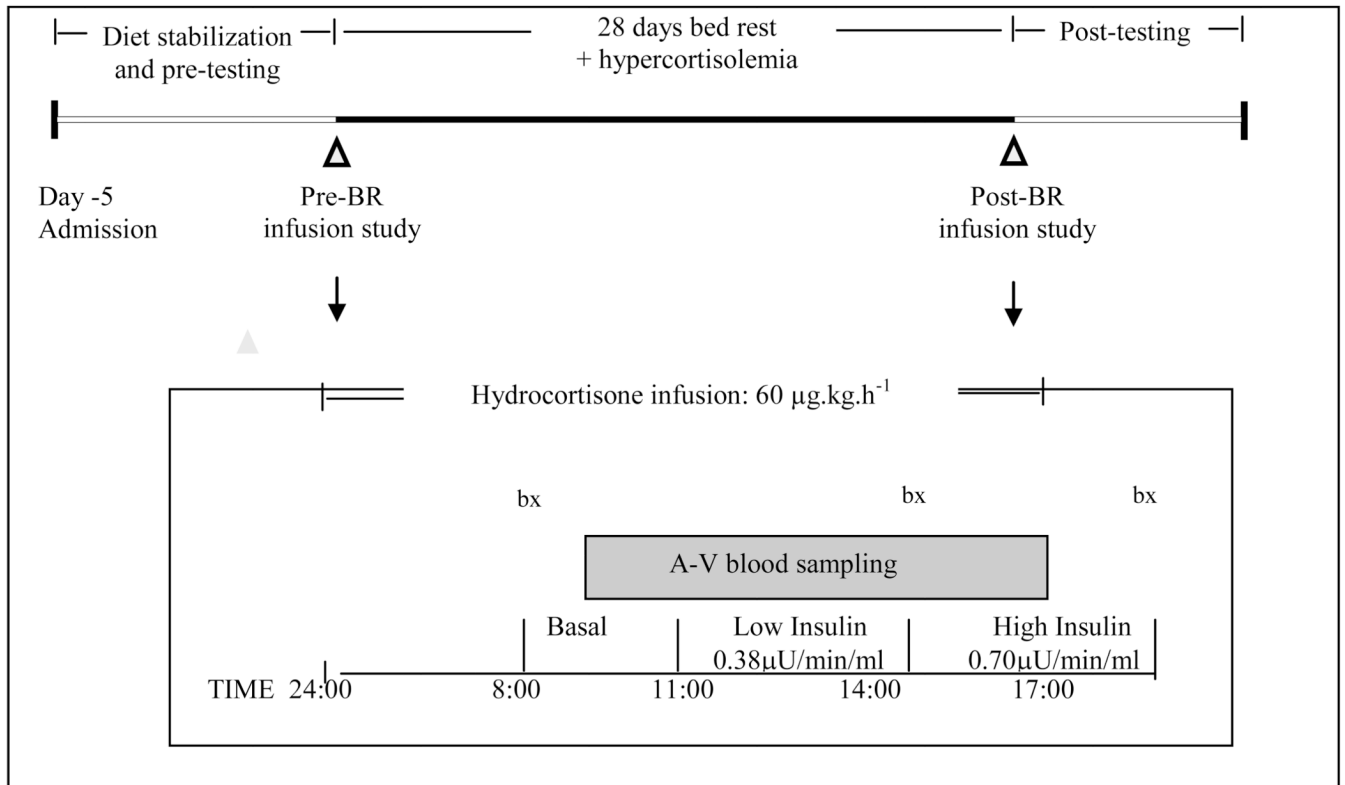
Figure 1.
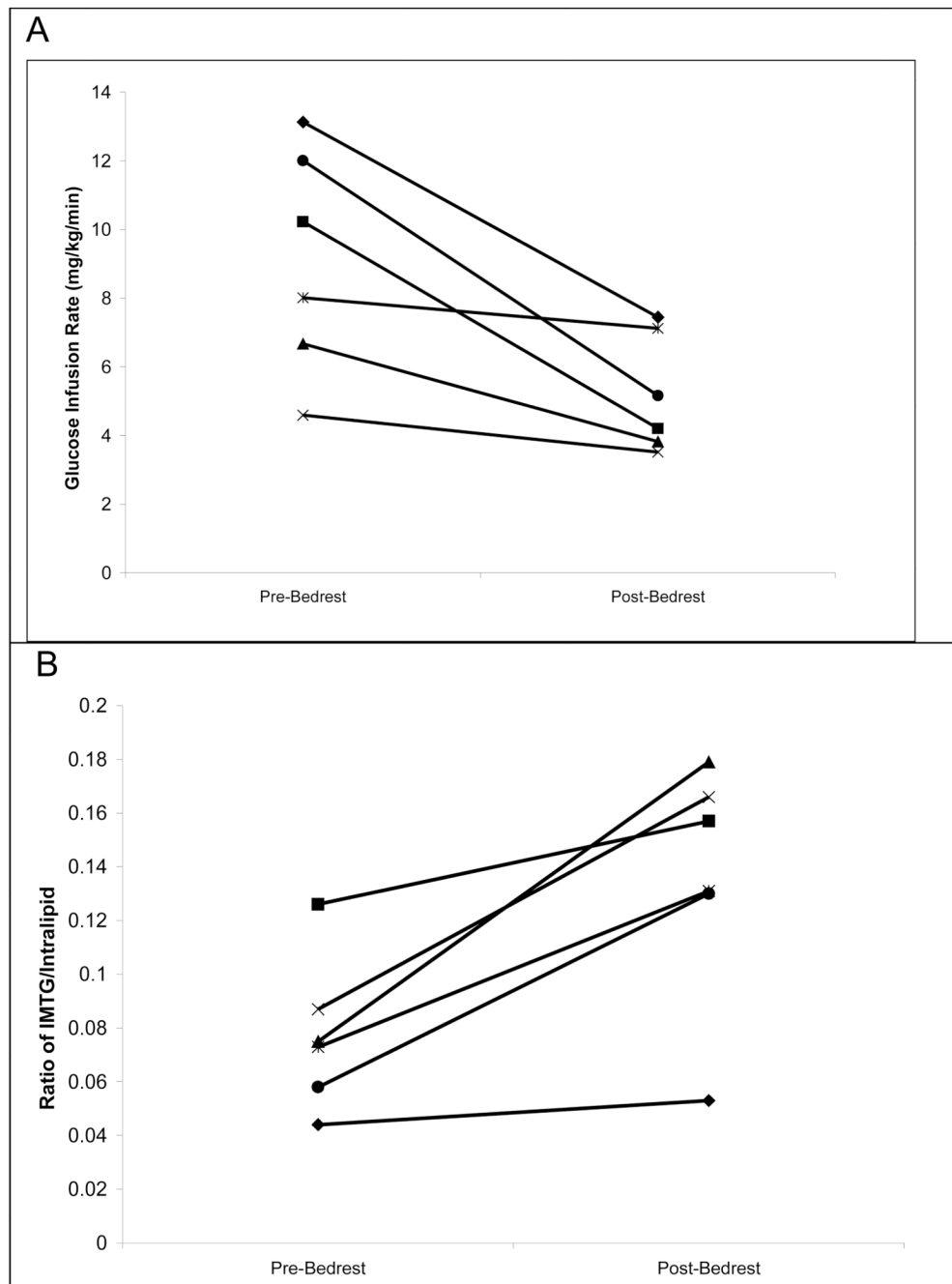Experimental and pre- and post- bed rest infusion protocol (expanded).

Figure 2.
Individual subject values of leg glucose uptake (A) and intramuscular triglycerides (IMTG; B). A) leg glucose uptake (as assessed by glucose infusion rate to maintain euglycemia) before and after bed rest in each individual patient. Uptake decreases significantly following bed rest; (P=0.015). B) IMTG in each individual patient before and after bed rest. IMTG increased in each subject and was significant for the group; P=0.009
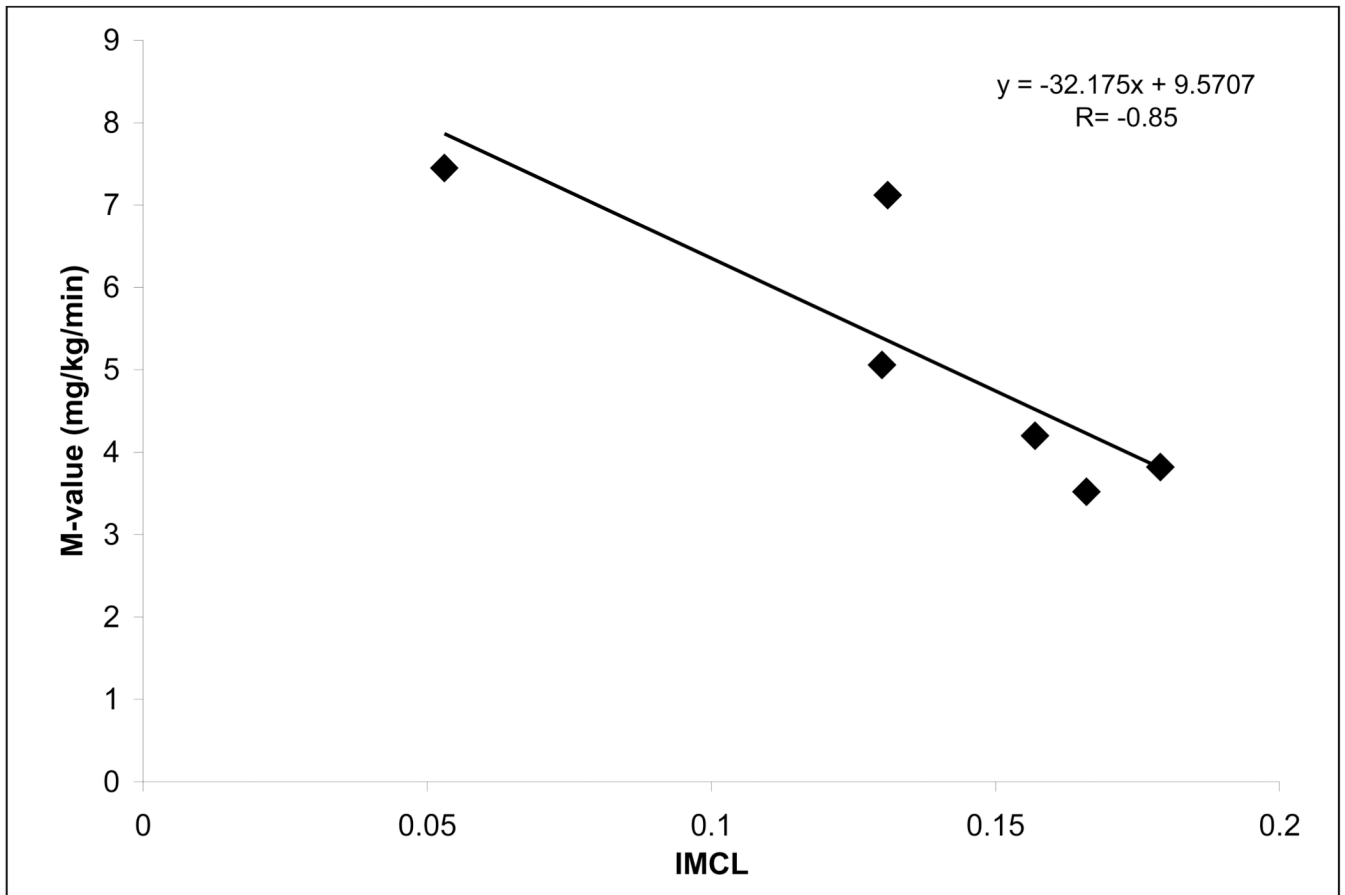
$$y = -32.175x + 9.5707$$
$$R = -0.85$$

Figure 3.
IMTG and glucose clamp correlations post bed rest. Leg glucose uptake (mg/kg/min) and IMTG (AU; pt value compared to standard) were significantly related following 28 days of hypercortisolemic bed rest with a R= −0.85; (P=0.035).
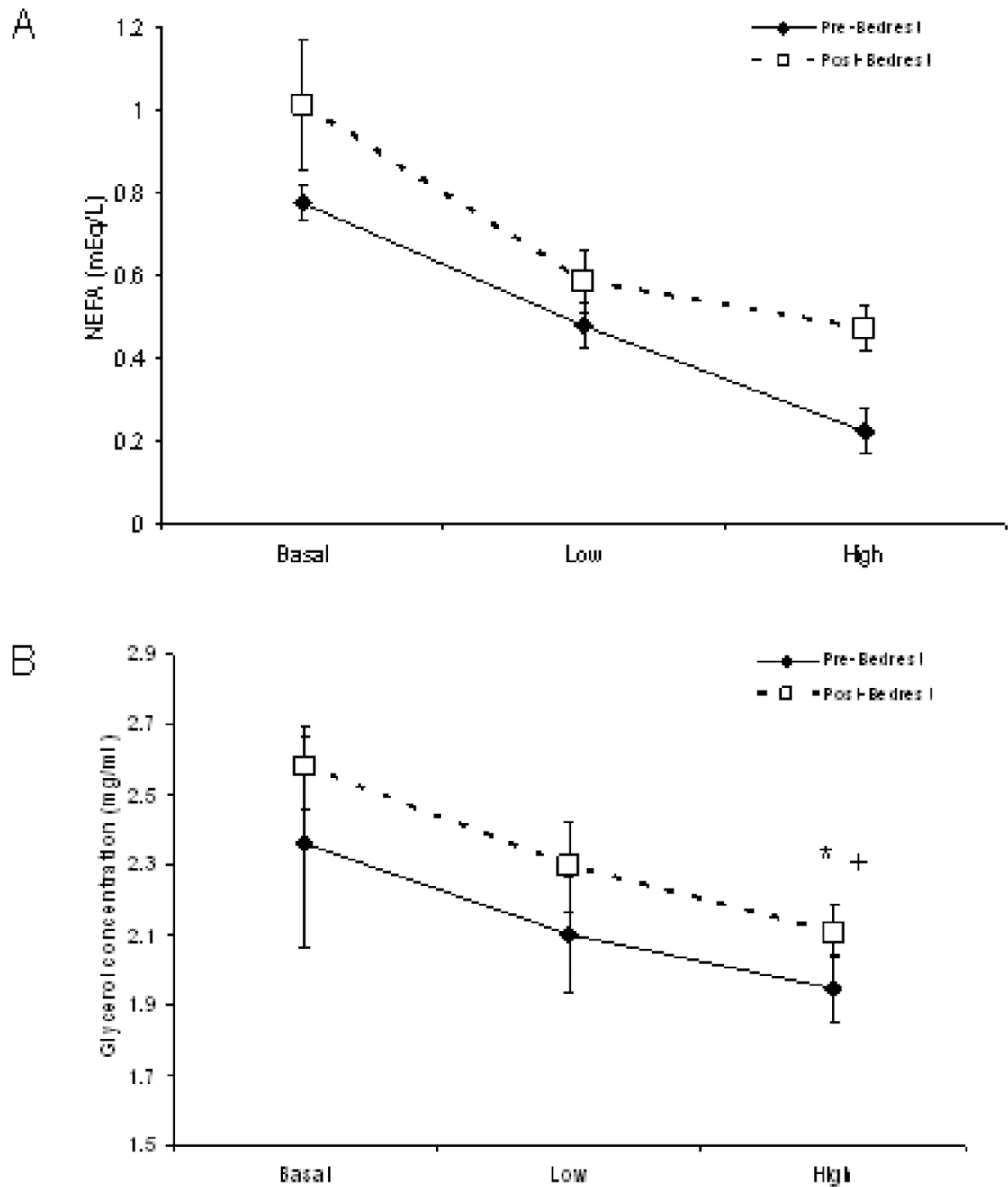
Figure 4.
Femoral vein plasma non-esterified free fatty acid (NEFA) and glycerol concentrations in response to 2 levels of intra-arterial insulin infusion. A) Plasma NEFA concentrations decreased in response to insulin equally before and after bed rest. B) Plasma glycerol decreased in response to insulin equally before and after bed rest. +; p =0.003 pre vs post at the high insulin level. *; p=0.01 high insulin vs basal, pre and post bed rest.
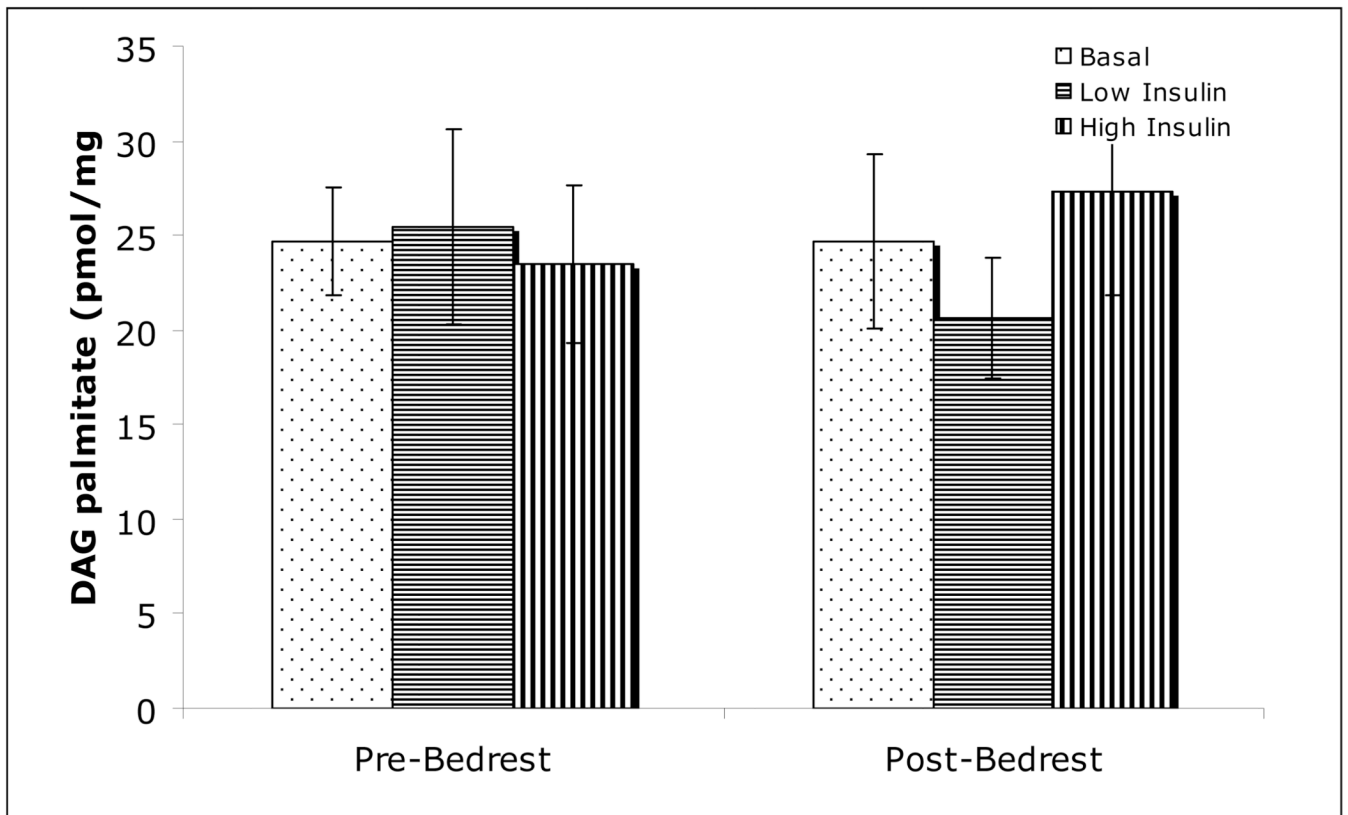
Figure 5.
Intramuscular diacylglycerol palmitoyl concentrations. Concentrations did not change with exposure to insulin, or following bed rest.