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Detrimental Effects of Inactivity on Insulin Action

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DETRIMENTAL EFFECTS OF INACTIVITY ON INSULIN ACTION

A Dissertation Presented

by

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DEDICATION

To my loving and patient husband who is my number one fan and supporter. Thank you not only for your emotional support, but also for your helpful advice during numerous stages of this project. I also dedicate this dissertation to my parents who have always supported me in all of my endeavors.

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ABSTRACT

DETRIMENTAL EFFECTS OF INACTIVITY ON INSULIN ACTION

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Inactivity reduces insulin action. Energy surplus causes similar reductions to insulin action. Unless energy intake is reduced to match low energy expenditure during inactivity, a concurrent energy surplus may account for the lower insulin action. This study evaluated the effect of inactivity (sitting) with and without energy surplus on insulin action. Fourteen young (26.1 ± 4.5 years (M ± SD)), lean (23.7 ± 7.1% fat), fit (VO_{2peak} = 49.1 ± 3.3 ml•kg^{-1}•min^{-1}) men (n=7) and women (n=7) completed each of 3, 24-hour conditions: an active condition (i.e. high energy expenditure with energy intake matched to expenditure) = ACTIV; 2) reduced energy expenditure (inactivity) with no reduction in energy intake (i.e. energy surplus) = INACTIV; 3) inactivity with energy intake reduced to match low energy expenditure = INACTIV LO-CAL. Insulin action was measured during a glucose infusion the following morning. Data were analyzed using linear mixed-effects models with planned contrasts. Compared to ACTIV, insulin action, defined as whole-body rate of glucose disappearance (R_d) scaled to steady-state plasma insulin, was reduced 39% in INACTIV ($p < 0.001$) and by 18% in INACTIV LO-CAL ($p = 0.07$). Insulin action was also higher in INACTIV LO-CAL compared to INACTIV ($p = 0.04$). These results suggest that 1 day of sitting elicits large reductions in

insulin action. Energy surplus accounts for half of the decline in insulin action, suggesting other factors are involved in the metabolic response to inactivity.

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CHAPTER 1

INTRODUCTION

It is well-established that structured exercise confers benefits to metabolic health and reduces risk for disease (29, 74, 95, 96, 101). Limited epidemiological data suggest that low-intensity activities of daily living, such as standing, ambulation, etc. (i.e. activity not defined as exercise), are also beneficial to metabolic health (59, 70). However, the relative importance of non-exercise physical activity, versus structured exercise, on health is less understood. Physical *inactivity* (i.e. considerable reductions in ambulation and standing), imposed by hindlimb suspension in rodents or bed rest in humans, clearly reduces insulin action (32, 90, 100, 119, 131, 133, 137) and impairs lipid metabolism (10, 14, 100).

Although a recent study examined the metabolic response to large reductions in daily walking (e.g. from10,000 steps/d to < 1500 steps/d) (114), no published studies have focused on the direct effects of more typical sedentary behaviors involving sitting (e.g. watching television, working on a computer, etc.). Understanding the metabolic impact of prolonged sitting has real-world relevance (54) since many people spend considerable amounts of time engaged in sedentary behaviors involving sitting (17, 97).

The underlying mechanism(s) for the impaired insulin action and lipid metabolism in response to physical inactivity are not well-characterized. A previously unexplored mechanism (i.e. energy imbalance) may be involved in the inactivity-induced decline in metabolic health. Less standing and ambulation reduces energy expenditure and leads to energy surplus unless energy intake is reduced to match the low expenditure.

Energy surplus independently reduces insulin action (4, 113) and impairs lipid metabolism (103). Therefore, the effects attributed to inactivity may actually be mediated by a concurrent energy surplus. However, no studies have determined the discrete effect of inactivity itself from the confounding impact of energy surplus by lowering energy intake to match the lower energy expenditure. Therefore, this study was designed to: 1) evaluate the metabolic impact of inactivity (sitting) and 2) to determine whether inactivity-induced declines in insulin action and lipid metabolism are attributable to energy surplus.

1.1 Specific Aims and hypotheses

To test the specific aims of this study, subjects completed 3 different conditions: 1) an active condition (i.e. high energy expenditure with energy intake matched to expenditure) = ACTIV; 2) reduced energy expenditure (inactivity) without a concomitant reduction in energy intake (energy intake > expenditure, i.e. energy surplus) = INACTIV; 3) inactivity with energy intake reduced to match the low expenditure = INACTIV LO-CAL. Specific aims and hypotheses of the study are presented below.

Aim 1: Determine the effect of 24 hours of inactivity, with no change to energy intake, on insulin-mediated glucose uptake (insulin action). We expected that inactivity would significantly reduce insulin-mediated glucose disposal (i.e. insulin action) relative to the active condition (i.e. INACTIV < ACTIV).

Aim 2: Determine the effect of 24 hours of inactivity, without the potential confounding effect of energy surplus, on insulin action. We expected that reducing energy intake to match low expenditure would attenuate, but not completely prevent, the

inactivity-induced decline in insulin action (i.e. INACTIV < INACTIV LO-CAL < ACTIV).

Aim 3: To determine the effect of inactivity on lipid metabolism. We expected that inactivity, with no change in energy intake, would impair: fasting lipid oxidation, fasting free fatty acid and triacylglycerol concentrations, and insulin-mediated suppression of lipolysis. However, we expected that reducing energy intake to match low expenditure would attenuate impairments to lipid metabolism relative to the active condition (i.e. INACTIV < INACTIV LO-CAL < ACTIV).

1.2 Significance

The aims of this study are to understand the metabolic response to 24 hours of sitting and to determine whether the deleterious effects of inactivity on that response are at least partially attributable to energy surplus. Results from this study will provide insights into factors that reduce insulin action and impair lipid metabolism following inactivity. Knowledge gained from this study is critically important to tailor appropriate public health recommendations to oppose the rapid and potentially frequent declines in metabolic health that result from sitting too much.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Associations of physical activity and health

It is well-established that exercise improves cardiometabolic health and reduces risk of disease (29, 74, 95, 96, 101). Epidemiological investigations have repeatedly demonstrated a negative association between physical activity level and/or fitness and all-cause mortality (13), cardiovascular mortality (13, 38, 104, 105), and incidence of type 2 diabetes (13, 38, 64, 95, 104, 105) independent of age or obesity status. Data from cross-sectional observations have shown habitually active individuals are more insulin sensitive (30, 102, 126), have elevated HDL cholesterol (66, 141, 151, 152), reduced LDL cholesterol and triacylglycerol levels (141, 151, 152), have a greater capacity to oxidize fatty acids (126) and exhibit enhanced triacylglycerol clearance (141) compared to their less active counterparts.

Maintaining the health benefits of exercise requires repeated bouts of sufficient frequency, intensity and duration (108, 132). Experimental data in both humans and rodents clearly indicate that the benefits of exercise are lost rapidly following exercise cessation. Exercise-induced increases in GLUT-4 protein and insulin-stimulated glucose uptake are completely reversed in isolated rat muscle 53 hours following cessation of running (84) or 40 hours after cessation of swimming (68). Insulin action, as assessed by an oral glucose tolerance test, is rapidly reduced in endurance-trained individuals upon cessation of exercise for 7-14 days (3, 62, 69). Glucose disposal rate assessed during a euglycemic-hyperinsulinemic clamp was reduced by 23% following 10 days without exercise in trained men (79). Similarly, 5 days of detraining in physically-trained

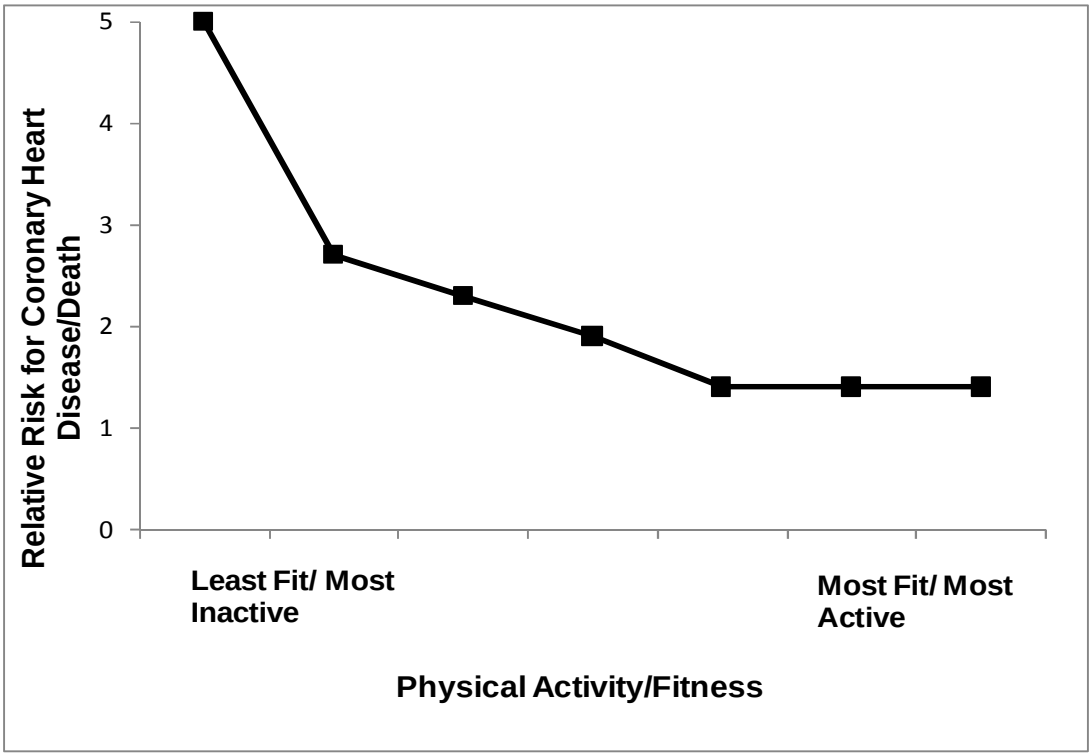
subjects elicited a 23% increase in the insulin concentration required to elicit 50% of maximal glucose disposal (102), indicative of reduced insulin sensitivity. Further, Burstein et al. (21) observed a significant decrease in glucose clearance rate in athletes 60 hours after the last exercise bout. Thus, exercise-enhanced insulin action is rapidly reversed by cessation of exercise in as little as several days.

A number of observational studies also suggest that, over time, reduced physical activity levels can lead to insulin resistance and type 2 diabetes and mortality associated with the metabolic syndrome (51, 82, 98), which is independent of age (147) and obesity status (20, 43, 64, 72, 91, 93, 95, 96, 147). Powell and Blair (120) estimated that 21% of deaths related to diabetes in 1988 were attributable to insufficient physical activity (less than 30 min/d or fewer than 5 d/wk of light or moderate physical activities). Thus, experimental and epidemiological evidence suggest reduced physical activity associated with exercise has deleterious effects on metabolic processes that over time increase the risk for disease (17). This knowledge has prompted current public health guidelines promoting at least 150 min/wk of moderate-intensity or 60 min/wk of vigorous-intensity physical activity to optimize metabolic health and reduce disease risk (57).

2.2 Impact of sedentary behavior and non-exercise activity on disease risk

Sedentary behavior associated with sitting and non-exercise activity involving standing (e.g. housework, stair-climbing, walking at home or work, etc.) also have effects on cardiometabolic health (54). Data from a number of epidemiological investigations indicate that the physical activity dose-response curve is steep with a significant increase in risk of disease and mortality in those individuals who are the least active (Fig. 2.1) (54, 56).

Figure 2.1 Relationship between physical activity and the risk for coronary heart disease/death (adapted from Haskell (56)).



In a recent meta-analysis of 23 studies, there was a 1.31-1.45 increase in risk of diabetes for sedentary individuals compared with their active counterparts (19). Powell and Blair (120) estimated that approximately 12% of deaths from diabetes in 1988 could be attributed to a sedentary lifestyle (defined as no leisure-time physical activity from self-report measures). Authors of a more recent prospective study of male physicians concluded that at least 25% of the incidence of type 2 diabetes could be attributable to a sedentary lifestyle (95). However, these associations are limited by different classifications and measurement of sedentary behavior. Until recently, many studies have not assessed time spent in sedentary behavior (e.g. via questionnaires on TV watching). Instead, low levels of self-reported leisure-time activities (i.e. lack of physical activity) are often used as a proxy measure of sedentary behavior. Thus, most

epidemiological studies have failed to distinguish the effect of sedentary behavior versus lack of physical activity. Recent data points to the importance of making the distinction between participation in physical activity and time spent in sedentary behaviors to provide independent measures of the activity spectrum (41, 83). Recently, several studies have suggested that sedentary behavior associated with sitting, distinguished from lack of physical activity (i.e. low levels of self-reported leisure-time activities), may have an independent effect on aspects of cardiovascular and metabolic health (9, 36, 37, 41, 71, 128). Sedentary behavior assessed by self-reported time spent watching television was positively associated with triacylglycerol levels and other risk factors for cardiovascular disease (83) and blood glucose levels in adults (35, 59, 83), independent of adiposity and time spent in physical activity (35, 37). Hu et al. (71) reported that for every 2 hr/d increase in television viewing time, risk of diabetes was increased by 14%. A recent study using more objective, accelerometer-based measures of physical activity and sedentary behavior reported a significant, positive association between sedentary time and 2-hour plasma glucose concentration following an oral glucose challenge, which was independent of age, sex, and waist circumference (59). It should be noted that television viewing time was taken to be representative of overall sedentary behavior in the majority of these studies. Although television viewing time has been shown to be most strongly associated with risk of type 2 diabetes compared to several other sedentary behaviors (i.e. sitting at work or other sitting) (71), television-viewing time constitutes only one component of sedentary behavior and it remains unknown the extent to which television viewing is representative of overall sedentary behavior (35). Nonetheless, these studies strongly suggest sedentary behavior involving prolonged sitting may be an important

modifier of metabolic health independent of obesity status and may mediate effects on metabolic function that are distinct from those of physical activity (35).

Moreover, these studies suggest that a significant reduction in risk of insulin resistance and type 2 diabetes can be gained by slight rightward shifts along the physical activity continuum (Fig. 2.1), highlighting the importance of daily non-exercise physical activity on cardiometabolic health. For instance, risk for development of type 2 diabetes was found to be highest in individuals who accumulated less than 500 kcals of activity per week; for each 500 kcal increment in weekly energy expenditure associated not only with sports activity, but also with walking and stair climbing, risk for diabetes was reduced by 6% (96). This association remained significant even after adjusting for obesity status, hypertension, and family history of type 2 diabetes (96). Similarly, a more recent study found that while brisk walking reduced the risk of diabetes by 34% for each 1 hr/d increment, even the activity associated with standing or puttering at home reduced diabetes risk by 12% for each 2 hr/d increment (71). Using accelerometry to objectively measure activity, Healy et al. (59) demonstrated a significant effect of low-intensity activity on 2-hour postprandial glucose concentration independent of time spent in moderate-vigorous activity.

Taken together, epidemiological evidence suggests that mortality and risk of disease cannot simply be ascribed to reductions in exercise. This underscores the significance of an inactivity physiology paradigm recently proposed by Hamilton and colleagues (53, 54) that sitting more and performing less non-exercise activity should be regarded as classes of behavior distinct from exercise that have independent effects on risk for disease (17). This paradigm emphasizes the importance of maintaining daily

non-exercise activity, which has real world relevance because for most individuals, the contribution of non-exercise activity to total daily energy expenditure is much greater compared to exercise (54, 150). Viewed in this context, understanding the underlying processes and mechanisms responsive to physical inactivity (i.e. reductions in contractile activity associated with standing, and ambulation) that promote disease is important (17). Whereas the metabolic adaptations to different amounts and types of physical activity have been relatively well-characterized, much less is understood regarding the response to increasing inactivity. It may be erroneous to assume that the adaptations to physical inactivity are merely opposite to the adaptations to physical activity (17, 54). In fact, in several well-controlled studies performed in rodents, reduced standing and ambulation had a much larger negative effect on lipoprotein metabolism than the positive effect of adding vigorous exercise training on top of normal daily activity levels (10, 54, 155). In addition, the molecular mechanisms underlying these changes in lipoprotein metabolism were distinctly different between inactivity and exercise training (10, 54, 155).

Limited experimental studies in both animals and humans have investigated the metabolic response to short-term and prolonged inactivity. Because the main focus of the present proposal is on the inactivity-induced changes in insulin action and glucose and lipid metabolism, the following review of the available literature is focused on the effects of inactivity on these metabolic parameters.

2.3 Experimental data in rodents and humans

One of the first studies to highlight the importance of standing and postural control was performed in mice whose hindlimbs were immobilized with plaster casts to significantly reduce contractile activity (131). In as little as 24 hours following

immobilization, insulin-stimulated glucose uptake and glycogen synthesis was significantly impaired in isolated soleus muscle (131). Similarly, hindlimb immobilization for 42-48 hours in rats reduced maximum insulin-stimulated glucose transport by 42% in isolated skeletal muscle (119). Thus, these studies provide direct evidence that insulin action can be rapidly reduced in response to inactivity in rodents.

Most of the available information in humans on the effects of inactivity (i.e. restriction of standing and ambulation), distinct from detraining (i.e. exercise restriction), comes from bed-rest studies with or without head-down tilt to mimic the effects of anti-gravity. Significant increases in fasting insulin concentrations were observed after bed rest (1, 8, 137, 144), suggesting a decrease in insulin action, although this has not been a consistent finding (32, 90, 116). While Acheson et al. (1) observed a significant increase in fasting insulin concentrations after 3 days of head-down bed rest, there was no change in fasting levels of this hormone after 3 days of bed rest in the study by Lipman et al. (90). In young, physically-active males, Stuart et al. (137) observed a 44% increase in fasting insulin concentrations after just 6 days of bed rest, whereas other studies employing similar (100) or more sustained (i.e. 2 weeks) bed rest protocols reported no change in fasting insulin levels (32, 116). In contrast, Bergouignan et al. (8) reported a 22% increase in fasting insulin concentrations after 1 month of bed rest in women. A more consistent finding in the literature, however, is impaired glucose tolerance and increased insulin response to an oral glucose load as a result of sustained bed rest in healthy subjects (14, 32, 116, 137). Both outcomes are indicative of reduced insulin action, which is usually attributed to a decreased peripheral glucose uptake (90, 100, 137).

Other studies using more direct assessments of insulin action support the finding of an inactivity-induced impairment in insulin action. One of the earliest studies reported a reduction in peripheral glucose uptake during a glucose infusion in as little as 3 days of bed rest in males (90). Further declines in peripheral glucose uptake were observed after an additional 11 days of bed rest (total 14 days) from 82% of control at 3 days to 56% of control after 14 days (90). Insulin infusion after bed rest also resulted in a smaller decrease in plasma glucose compared to control, further suggesting a reduction in insulin action following inactivity (90). Several studies employing the glucose clamp also report significant reductions in whole-body glucose uptake following 7 days of bed rest in men (100, 137).

While bed-rest studies have provided important information on the direct effects of restricted contractile activity on metabolic processes, this model has limited applicability for the majority of the general population who are not bed-ridden (54). Further, data from prolonged bed rest studies with head-down tilt may be confounded by shifts in fluid distribution, muscle atrophy, orthostatic intolerance, etc. (54).

Very few studies, however, have investigated the effects of non bed-rest inactivity models in humans. The limited data available support the general finding reported in bed rest studies that inactivity reduces insulin action. In a study utilizing 7 days of single leg casting to limit ambulatory activity, Richter et al. (125) observed reduced insulin-stimulated glucose uptake in the vastus lateralis of the casted relative to the non-casted leg. Compared to bed-rest induced inactivity, this model reduced insulin-stimulated glucose uptake to a lesser extent, which may be reflective of continued (albeit restricted)

muscle activity during ambulatory leg casting and/or less blunting of leg blood flow (100).

Limited data also point to the deleterious effects of significant reductions in non-exercise activity (e.g. standing, ambulation, and other activities of daily living). Recently, Olsen et al. (114) examined the metabolic effects of reduced daily steps (from 10,000/day to 1500/day) for 2 weeks in healthy, free-living, non-exercising men. Compared to baseline, insulin area under the curve in response to an oral glucose challenge was increased 57%, indicative of significantly reduced insulin action. Experimental reductions in ambulation and standing via prolonged sitting also impair insulin action. Compared to an active condition (i.e. high non-exercise physical activity) in which total sitting time was limited to less than 10 minutes per hour, sixteen hours of sitting resulted in a 30% reduction in insulin action as assessed by an oral glucose challenge (Hamilton and colleagues, unpublished observations). In summary, select non-bed rest inactivity paradigms in humans support the general finding of significant declines in insulin action following reductions in ambulation, standing, or other non-exercise activities.

2.4 Differential effects of inactivity in adipose tissue, liver, and skeletal muscle

It is likely that skeletal muscle accounts for the majority of the physical inactivity-induced decline in whole-body insulin action (84) since skeletal muscle accounts for 75-95% of insulin-stimulated glucose disposal in humans (6). Additionally, in isolated rat muscle, insulin-stimulated glucose uptake is significantly reduced after exercise cessation in as little as 40-90 hours after the last exercise bout (68, 75, 124). However, declines in liver (hepatic) insulin sensitivity may also contribute to reductions in whole-body insulin

action following inactivity. In addition to reduced skeletal muscle insulin sensitivity, Blanc et al. (14) observed less suppression of hepatic glucose production following 7 days of bed rest in women, suggesting impaired hepatic insulin sensitivity (14). However, no impairment in hepatic insulin sensitivity was observed in men in response to a similar inactivity protocol (14, 100, 137), suggesting potential sex differences in the physiological response to inactivity. Reduced adipose tissue insulin sensitivity has also been observed in response to inactivity. Mikines et al. (100) observed similar reductions in plasma free fatty acid and glycerol concentrations during a glucose infusion despite higher insulin concentrations following 7 days of bed rest indicative of impaired insulin suppression of lipolysis. In summary, reduced skeletal muscle insulin action likely accounts for much of the inactivity-induced decline in whole-body insulin action, but hepatic and adipose tissue insulin sensitivity may also be reduced.

2.5 Effect of physical inactivity on substrate metabolism

The ability to shift from oxidizing predominantly fat during fasting conditions and increase glucose oxidation and suppress lipolysis in response to insulin stimulation characterizes the metabolically healthy state, termed metabolic flexibility. In contrast, metabolic inflexibility is characterized by low fat oxidation during fasting conditions and impaired insulin-stimulated non-oxidative and oxidative glucose disposal. Originally, metabolic flexibility and inflexibility referred to substrate use profiles observed in skeletal muscle of lean and obese individuals (76). However, because skeletal muscle strongly influences whole-body metabolism, these terms can also be used to characterize whole-body substrate metabolism (136).

A number of reports suggest that inactivity is associated with a substrate use profile characteristic of metabolic inflexibility. In mice, hindlimb immobilization significantly reduced insulin-stimulated glycogen synthesis and glucose oxidation in isolated soleus muscle (131). Hindlimb unloading for 9 days reduced the capacity to oxidize long-chain fatty acids by 37% and increased reliance on carbohydrate utilization (5), supporting the hypothesis that fatty acid oxidation is reduced in response to inactivity. In humans, non-oxidative glucose disposal, which mainly reflects glycogen storage, was lower and glucose oxidation tended to be lower following 7 days of bed rest in humans during a glucose clamp at a relatively low insulin infusion rate (100). After bed rest, non-oxidative glucose disposal was also lower at high insulin infusion rates (100). Limited data in humans also suggests that fatty acid oxidation is altered following inactivity. After 7 days of head-down bed rest, fasting lipid oxidation was reduced by ~80% in both men and women (14). Similarly, in a study by Ritz et al. (127), fat oxidation during fasting was significantly lower and carbohydrate oxidation higher following long-term simulated microgravity (42 days). In addition, both fasting and insulin-stimulated levels of lipogenesis were significantly increased in women following 7 days of bed rest (14). However, not all studies suggest altered substrate oxidation following inactivity. Acheson et al. (1) reported that in response to 3 days of bed rest with head-down tilt, fat oxidation during fasting was significantly *increased*, while carbohydrate oxidation in response to a glucose load was unchanged. Despite this inconsistent finding, the majority of the human studies support the hypothesis that inactivity shifts fasting substrate metabolism toward increased reliance on carbohydrate utilization and reduced reliance on fat oxidation and impairs insulin-stimulated glucose

oxidation and suppression of lipolysis, all of which are characteristic features of metabolic inflexibility.

2.6 Mechanisms underlying inactivity-induced changes to insulin action

While the mechanisms responsible for the beneficial effects of exercise are well-studied, the cellular mechanisms involved in the rapid inactivity-related decline in insulin action are not well-characterized. Mechanisms to explain observed decrements in insulin action in response to inactivity may involve: changes in circulating levels of counterregulatory hormones (144); decreased blood flow to the inactive muscles (100); reductions in glucose transporters (GLUT-4) (138, 145); changes in the activity of enzymes involved in glucose metabolism such as glycogen synthase (100, 109); increased muscle glycogen concentration (44, 68, 75); and/or alterations in insulin receptor signaling (84).

Changes in the concentrations of counterregulatory hormones influencing glucose uptake (e.g. cortisol, growth hormone, glucagon, epinephrine) have been linked to reduced insulin action following inactivity. Increased plasma cortisol levels were linked to the reduction in peripheral glucose utilization in men following 30 days of bed rest (144). Increased growth hormone concentrations after 20 days of bed rest in humans have also been associated with decreased tissue sensitivity to insulin (144). However, other shorter duration bed rest studies (3-14 days) report unchanged levels of counterregulatory hormones (90, 100, 116), suggesting a possible time lag in the counterregulatory hormone response to inactivity. Because changes to insulin action in response to inactivity occur rapidly (119, 131) and can precede changes in

counterregulatory hormones, there is little evidence to suggest a significant role for these hormones in changes to insulin action (90).

Leg blood flow is reduced after 7 days of bed rest, which may decrease glucose availability to skeletal muscle and contribute to the reduction in glucose uptake following inactivity (100). Blood flow has been positively correlated with glucose uptake in skeletal muscle of rats (45). However, in humans, blood flow does not appear to independently mediate insulin-stimulated glucose uptake (110, 121). Thus, reduced blood flow may not be a plausible mechanism to explain inactivity-related declines in insulin action.

Lower insulin-stimulated glycogen synthase activity may contribute to reduced insulin action in skeletal muscle in response to inactivity (109) although evidence to support this is scarce (100). In rodents, activation of glycogen synthase by insulin was reduced following hindlimb immobilization (109), although this observation may be confounded by a slightly higher basal activity of the enzyme after immobilization (100). In humans, the activity of glycogen synthase during a euglycemic-hyperinsulinemic clamp was unaltered following 7 days of bed rest (100).

In rodents, insulin-stimulated glucose uptake is inversely related to muscle glycogen concentration (44, 65, 75, 124). Thus, glycogen may mediate reductions in insulin action following inactivity. However, insulin-stimulated glucose uptake was lower following 53 hours of restricted voluntary running in rats compared to 29 hours of restricted running despite no difference in muscle glycogen concentrations (84), which argues against a regulatory role of muscle glycogen in the inactivity-induced decline in insulin action (84).

More likely mechanisms to explain lower insulin action following inactivity are decreased glucose transport capacity (145), changes in insulin receptors, and/or alterations in insulin receptor signaling (84). A reversal of the adaptive increase in GLUT-4 transporters has been associated with the decline in insulin-stimulated glucose uptake following detraining in rodents (68, 75, 84, 124). However, Ploug et al. (119) reported no change in GLUT-4 protein concentration in rat hindlimbs following 42-48 hours of immobilization. Conversely, in humans both short-term (145) (6 days) and longer-term (138) (19 days) inactivity studies report significant reductions in GLUT-4 transporter levels. In a study by Vukovich et al. (145), lower insulin-mediated glucose uptake following inactivity was directly related to lower GLUT-4 transporter protein concentration.

Although not a consistent finding (119), results from a recent study by Kump and Booth (84) suggest that alterations in insulin receptor binding and/or signaling mediate the reduction in insulin-stimulated glucose uptake following reduced activity. Insulin binding, IRβ protein concentration, insulin-mediated IRβ tyrosine phosphorylation, and Akt Ser473 phosphorylation were reduced to sedentary values following 53 hours of prohibited running in rats. These changes coincided with the decline in submaximal insulin-stimulated glucose uptake, suggesting a potential mechanism for the reduction (84). Following 42-48 hours of hindlimb immobilization, however, Ploug et al. (119) reported no changes in insulin receptor binding or insulin receptor kinase activity. The discrepancy in the results between these two studies may reflect differences in the inactivity protocols, rat strains, or methodologies used to determine insulin receptor binding or insulin signaling. Regardless, the diversity of findings suggests that the

mechanism to explain the reduction in insulin action in response to inactivity is multifactorial and complex.

2.7 Effect of energy imbalance on the metabolic response to inactivity

Reductions in insulin action following inactivity may also be related to energy imbalance. Restriction of contractile activity associated with inactivity reduces overall energy expenditure and energy demand. Thus, energy surplus (greater energy intake relative to expenditure) and inactivity often coexist because maintaining energy balance during extended periods of low muscle activity requires a large reduction in daily energy intake.

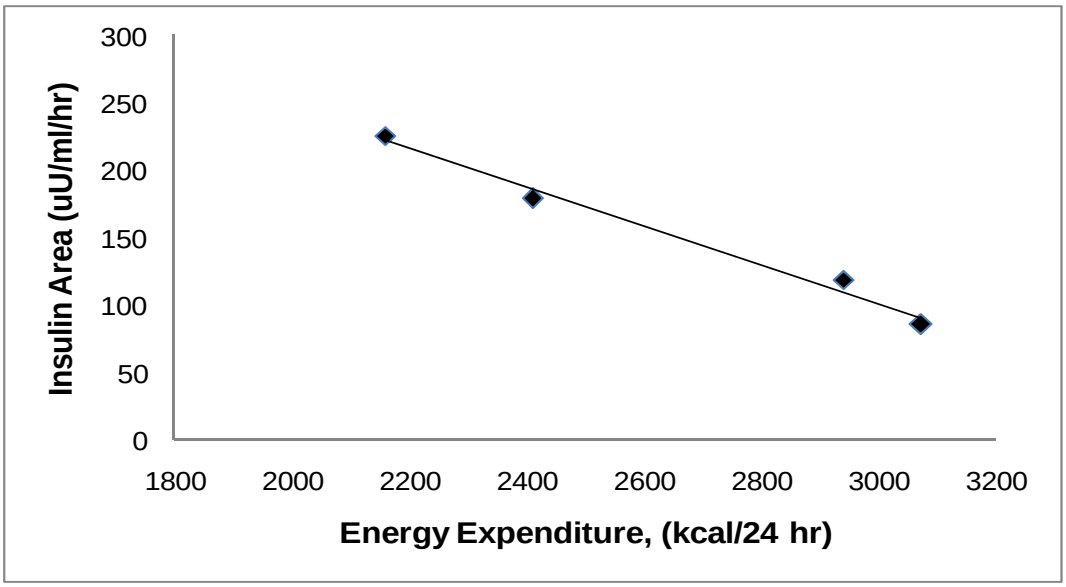
Thus, the effects of inactivity on glucose metabolism may be confounded by a greater supply of energy relative to decreased energy expenditure (i.e. energy surplus). Energy surplus is associated with insulin resistance and may play a role in the pathogenesis of type 2 diabetes (81). Chronic energy oversupply leads to body weight gain over time, which contributes significant risk for developing insulin resistance and type 2 diabetes (107). Reduced insulin action, however, appears be an early metabolic adaptation to energy surplus (111, 115) which occurs prior to significant changes in body weight (4, 52, 113, 146). The rapid changes to glucose metabolism as a result of energy surplus mimic the adaptations to inactivity. Thus, energy surplus may be a key modulator of the effects of physical inactivity on insulin action and substrate metabolism.

In obesity-prone rats, 3 days of overfeeding (an approximate doubling of caloric intake) impaired the action of insulin to suppress hepatic glucose production, and within 7 days the rate of glucose uptake in skeletal muscle was also significantly decreased (146). In our laboratory, we have demonstrated that a short period of energy surplus

coupled with a reduction in structured exercise is sufficient to reduce insulin action by ~30% in healthy, active humans (52). A bout of exercise performed in a state of energy surplus reversed the decline in insulin action, but did not return to baseline values (52). Thus, these results suggest an independent effect of energy surplus on insulin action (52). Therefore, it is plausible that the decline in insulin action due to inactivity may be, at least in part, due to an excess of nutrient availability relative to the reduced metabolic demand (energy surplus).

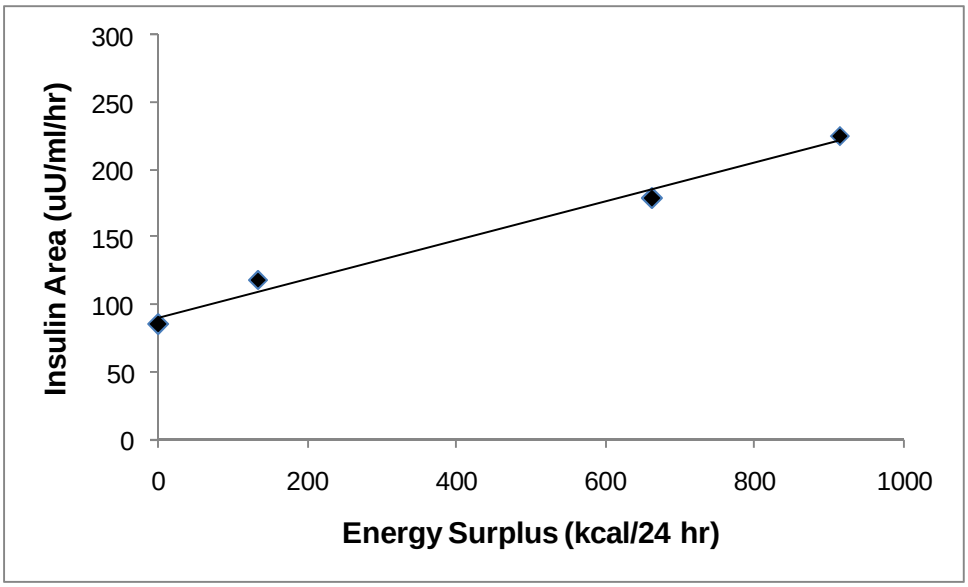
Although no studies to date have directly tested this hypothesis, results from a study in young men by Dolkas and Greenleaf (32) indirectly supports the hypothesis that energy surplus may mediate changes to insulin action in response to inactivity. The study authors compared insulin and glucose responses to an oral glucose tolerance test between 4 conditions: a 2-wk ambulatory control (total daily energy expenditure ((TDEE)) = ~3000 kcal); 2-wk bed rest with intermittent isotonic exercise (TDEE = ~ 2940); 2-wk bed rest with intermittent isometric exercise (TDEE = ~ 2410); and 2-wk bed rest with no exercise (TDEE ~2160 kcal). There was a linear inverse relationship between estimated energy expenditure and insulin area under the curve (r=0.99) (Fig. 2.2), suggesting energy expenditure is strongly related to the insulin action response to inactivity (32).

Figure 2.2 Regression of integrated area under insulin response curves during glucose tolerance tests on 24-hr energy expenditures for 3 bed-rest protocols and 1 ambulatory control. Redrawn from Dolkas et al. (32).



Alternatively, because energy intake was constant among the 4 conditions (3073 kcal/day), differences in energy imbalance ($energy\ intake - energy\ expenditure$) could also explain differences in the insulin response to oral glucose. In fact, a plot of insulin area and the difference between energy intake and energy expenditures in the 4 conditions indicates a clear positive relationship between the two variables (Fig. 2.3). The apparent relationship between insulin area in response to oral glucose and energy surplus suggests a role for energy surplus in mediating the change in insulin action in response to inactivity.

Figure 2.3 Relationship between area under insulin response curves during glucose tolerance tests and energy surplus (energy intake - energy expenditure) for 3 bed rest protocols and 1 ambulatory control. Adapted using results from Dolkas et al. (32).



However, data from one carefully controlled bed-rest study suggests that the relationship between energy surplus and the change to insulin action in response to inactivity may be more complex. Insulin-stimulated glucose uptake during a euglycemic-hyperinsulinemic clamp was reduced by approximately 25% following 3 days of bed rest coupled with a high-fat diet (135). Daily energy intake was reduced by approximately 500-600 kcals (from 1.6 x RMR to 1.2 x RMR), lower insulin action cannot be explained by energy imbalance. Conversely, when inactivity was coupled with a high-carbohydrate diet with the same reduction in energy intake, insulin-stimulated glucose uptake was not reduced, suggesting that dietary composition, rather than energy balance, modulates the inactivity-related response to insulin action, although this conclusion is tenuous since the role of energy balance was not directly tested in this study. Alternatively, because high-fat feeding for 3 days without restriction of physical activity did not impair insulin action (135), this study suggests that energy surplus may not fully explain the decline in insulin

action following inactivity. Instead, the story may be more complex and macronutrient composition may also play a role in the response to inactivity.

2.8 Role of dietary composition on insulin action

Some evidence suggests that macronutrient composition of the diet affects the insulin action response to energy surplus. In particular, a nutrient oversupply of fatty acids may exert a more potent negative effect on insulin action than an oversupply of carbohydrate. In lean, healthy, sedentary individuals, an increased availability of circulating fatty acids via lipid infusion (34, 40, 139) or an increase in dietary fat (92) impairs insulin action (34, 92), ostensibly via fatty-acid induced impairment in insulin signaling in skeletal muscle (154), although this is not a universal finding (42). In a study directly comparing the metabolic response to a high-fat (~55-60% fat) versus high-carbohydrate diet (62-64% carbohydrate) in humans, Bachman et al. (4) observed a significant reduction in insulin action after 3 days on the high-fat diet, whereas no differences were observed following the high-carbohydrate diet. However, the associated decline in insulin action in the high-fat condition is confounded by a greater energy intake relative to the high-carbohydrate condition (~ 767 kcal/day) (4), thus it is unclear from this study whether there is a true independent effect of increased dietary fat composition on insulin action. However, the results of a recent carefully-controlled study suggest there is no independent effect of an increase in dietary fat composition on insulin action. Following 6 days of a high-fat diet (75% energy as fat), insulin action assessed during a euglyemic-hyperinsulinemic clamp was not impaired relative to insulin action measured 6 days following an isocaloric low-fat diet (35% energy as fat) (22). In fact, during the last 30 minutes of the clamp, glucose disposal was greater following the high-

fat diet compared to the low-fat diet (22). Thus, when energy intake is carefully controlled, an increase in dietary fat composition appears to minimally affect insulin action.

Results from studies investigating the effects of a surplus of dietary carbohydrate on insulin action in humans are less clear. Following administration of 50% excess energy predominantly in the form of carbohydrate, plasma insulin concentrations were significantly higher and hepatic glucose production greater, indicative of reduced hepatic insulin sensitivity (129). However, four days of carbohydrate feeding did not affect the insulin or glucose response to an oral glucose load in lean or obese men and women despite an excess mean energy intake of ~1816 kcal/d and ~2100 kcal/d, respectively (103).

Thus, excess carbohydrate may affect insulin action differently than excess fat. Nonetheless, short-term overfeeding *without* alterations in dietary composition (proportional increases in fat, carbohydrate, and protein) has been shown to reduce insulin action in humans (106, 113). Therefore, the available data suggests that energy surplus exerts a clear negative effect on insulin action, irrespective of dietary composition.

2.9 Effects of energy surplus on substrate metabolism

Energy surplus also shifts fasting substrate oxidation toward increased carbohydrate oxidation and decreased fat oxidation. In Pima Indian men, 5 days of overfeeding (equivalent increases in macronutrients) reduced 24-hour fat oxidation and increased carbohydrate oxidation (86). In addition, four days of carbohydrate overfeeding has also been show to suppress lipid oxidation and increase plasma

triacylglycerol levels in both lean and obese subjects (103). Lower insulin-stimulated glucose uptake following energy surplus may also translate into reduced insulin-stimulated non-oxidative and oxidative disposal. However, although non-oxidative glucose disposal was reduced in response to an oral glucose load, oxidative glucose disposal increased following carbohydrate overfeeding (103). Similarly, a 62% increase in energy intake for 14 days reduced non-oxidative glucose disposal, but increased carbohydrate oxidation (106). Thus, though energy surplus reduces lipid oxidation during fasting conditions, insulin-stimulated carbohydrate oxidation appears unaffected. Nonetheless, increased plasma triacylglycerol levels and decreased fat oxidation are indicative of a metabolic state associated with the development of insulin resistance (123).

2.10 Mechanisms linking increased energy intake to decreased insulin action

A surplus of carbohydrate calories is stored as glycogen (129). Insulin action is inversely correlated with muscle glycogen (28). Thus, increases in muscle glycogen or changes in the activities of glycogen-dependent proteins (e.g. glycogen synthase) may be potential mechanisms to explain the reduction in insulin action following energy surplus. In a study by Mott et al. (106), a 62% increase in energy intake for 13 days reduced both fasting and insulin-stimulated glycogen synthase activity in skeletal muscle (106). Non-oxidative glucose disposal was also reduced in response to supra-physiological insulin levels, suggesting reduced insulin action. However, reduced glucose disposal was not related to changes in glycogen synthase activity or glycogen concentration, suggesting other mechanisms may be involved in the change to insulin action following energy surplus. Of note, however, subjects who exhibited the greatest decreases in glycogen

synthase activity also had the largest increases in fasting insulin concentrations following the overfeeding protocol (106). Thus, alterations in glycogen synthase activity may contribute to impaired glucose metabolism following energy surplus.

2.10.1 Nutrient fuel sensors involved in the regulation of energy balance and insulin action

Increased availability of both glucose and free fatty acids may initiate a cellular response that contributes to the reduction in insulin action. Nutrient oversupply of free fatty acids by lipid infusion impairs insulin-stimulated glucose uptake via effects on key components of the insulin-signaling cascade (81). Elevations in both glucose and free fatty acids have been shown to activate PKC isoforms (50, 73, 85, 88), which may lead to phosphorylation of serine/threonine residues on the insulin receptor and a subsequent impairment in insulin signal transduction (81). Thus, activation of PKC isoforms within skeletal muscle may be involved in reduced insulin-stimulated glucose uptake following energy surplus. Additionally, increased free fatty acids as well as glucose availability may also upregulate the hexosamine biosynthetic pathway (HBP) (58, 81). This pathway is hypothesized to serve as an energy sensor responsive to a surplus of calories which may modulate insulin action (111, 112, 149). Most of the glucose entering skeletal muscle is directed towards glycogen synthesis or glycolysis. However, a small percentage of glucose enters the HBP after conversion to fructose-6-phosphate. The enzyme fructose-6-phosphate-amidotransferase (GFAT) catalyzes the conversion of fructose-6-phosphate to glucosamine-6-phosphate and regulates flux through the pathway. The final step in HBP is the formation of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), a main substrate for protein glycosylation (111, 149).

Early observations that inhibition of GFAT could prevent hyperglycemia-induced insulin resistance suggested a role for the HBP as an energy sensor modulating insulin action (111, 149). Further, UDP-GlcNAc levels are also responsive to changes in nutrient intake. In rats, UDP-GlcNAc levels were 68% higher following overfeeding compared to controls (146). Conversely, calorie restriction reduces UDP-GlcNAc levels, further implicating its role as an energy sensor (46). Although HBP is most sensitive to changes in glucose, increased dietary fat availability also leads to increased flux through HBP and UDP-GlcNAc in skeletal muscle (58).

HBP activation via glucosamine administration increases UDP-GlcNAc concentrations and reduces insulin-stimulated glucose uptake (115, 149). Overexpression of GFAT in skeletal muscle and adipose tissue in mice also induces insulin resistance (63), further suggesting a link between HBP and insulin action (111). It is postulated that key proteins of the insulin-signaling pathway may undergo posttranslational modification via glycosylation with UDP-GlcNAc, potentially explaining a mechanism by which HBP activation reduces insulin-stimulated glucose uptake (111, 115). Activation of HBP has been shown to downregulate genes involved in oxidative phosphorylation and fatty acid oxidation in rat skeletal muscle (112), which may indirectly reduce insulin action.

Malonyl-CoA levels may also serve as a fuel sensor that may regulate substrate oxidation in response to energy surplus (111). Malonyl-CoA is an intermediate in fatty acid synthesis and inhibits carnitine palmitoyl transferase-1 (CPT1), which controls transport of free fatty acids into the mitochondria for oxidation. High concentrations of both glucose and insulin increases malonyl-CoA levels and suppresses lipid oxidation (111, 122). Decreased capacity to oxidize free fatty acids may increase the intracellular

pool of fatty acid moieties, which may impair insulin action via fatty acid inhibition of insulin signal transduction discussed above (81). In summary, increased energy intake may induce multiple and complex mechanisms involved in the regulation of energy balance and insulin action (111).

2.11 Summary

Data from hindlimb suspension studies in rodents and bed rest studies in humans indicate that inactivity exerts clear deleterious effects on insulin action and lipid metabolism. However, no published studies have examined the metabolic impact of a more relevant mode of inactivity, i.e. prolonged sitting in humans. Energy surplus causes similar impairments to insulin action and lipid metabolism. Thus, the effects attributed to inactivity may be confounded by a concurrent energy surplus. However, to our knowledge, no studies have investigated the combined effect of a high calorie diet and inactivity on insulin action. Therefore, the aims of this study were to evaluate the effects of short-term inactivity (sitting) with or without energy surplus on insulin action.

CHAPTER 3

METHODOLOGY

3.1 Overall design

An overview of the study design is shown in Figure 3.1. A cross-over design was used in which each subject completed 3 experimental conditions. Each condition required a 24-hour laboratory stay and the order of the conditions was counter-balanced across subjects. In 2 conditions, subjects remained seated for ~16 of the 24 hours to restrict physical activity (INACTIV and INACTIV LO-CAL). The 3rd condition served as an active condition in which subjects stood and performed activities of daily living for approximately 12 of the 24 hours (ACTIV). All meals (i.e. breakfast, lunch, and dinner) were provided throughout the 24-hour period. For ACTIV, energy intake approximated energy expenditure (i.e. energy balance). Energy intake in INACTIV was identical to that in ACTIV (energy intake > energy expenditure, i.e. energy surplus). In INACTIV LO-CAL, energy intake was reduced to more closely match the lower energy expenditure (i.e. energy balance) (Table 3.1). Insulin action was measured in the morning approximately 12 hours following the evening meal in each condition.

Figure 3.1 Overview of study design

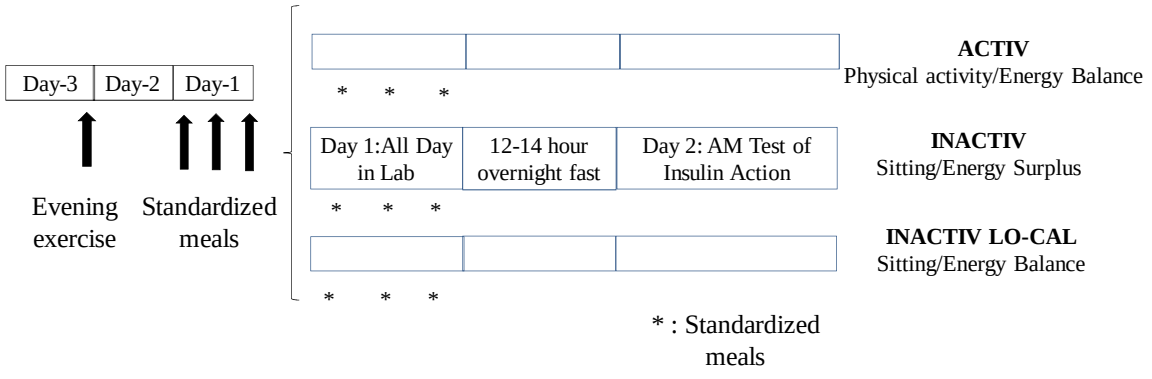


Table 3.1 Average energy intake and expenditure across conditions (M ± SD).

	Energy Intake (kcals/day)	**Energy Expenditure (kcals/day)**	**Energy Balance (EI-EE) (kcals/day)**
ACTIV	3106 ± 590[a]	2944 ± 462	162 ± 248[a]
INACTIV	3133 ± 583[a]	2195 ± 424[b]	938 ± 202[a]
INACTIV LO-CAL	2109 ± 428	2139 ± 427[b]	-30 ± 82

[a] Significantly different from INACTIV LO-CAL
[b] Significantly different from ACTIV

3.2 Subjects

Fourteen men (n=7) and women (n=7) between the ages of 20 and 32 years (26.1 ± 4.5) were recruited from the surrounding area by flyers and advertisements to participate in this study. Each subject completed all 3 trials except for one male and one female who only completed 2 out of 3 trials due to an adverse event and time constraints, respectively (ACTIV and INACTIV; and ACTIV and INACTIV LO-CAL, respectively). Subject characteristics are presented in Table 3.2. All volunteers were in good health, of normal body composition (23.7% body fat), and aerobically fit (VO_{2peak} = 49.1 mg•kg^{-1}•min^{-1}). All subjects were recreationally active, meeting the physical activity guidelines (i.e. at least 30 minutes of moderate exercise, 3 days a week or more) as determined by a generic physical activity questionnaire. All subjects were non-smoking, free of known disease (e.g. cardiovascular disease, type 2 diabetes, etc.), not following a very low or very high-carbohydrate diet (<30% or >70% carbohydrate, respectively), and were not taking any medications (e.g. metformin, insulin, statin drugs) or supplements (e.g.

chromium, vanadium, ephedra) known or suspected to alter carbohydrate or lipid metabolism. Four females were taking monophasic birth control. The study protocol was approved by the Institutional Review Board at the University of Massachusetts, Amherst prior to initiation of the study and all subjects gave their verbal and written informed consent before participating.

Table 3.2 Subject characteristics (n=14; 7M, 7F)

	Average (M ± SD)	**Range**
Age (years)	26.1 ± 4.5	19.8 – 32.2
Weight (kg)	69.5 ± 13.2	49.6 – 89.7
Height (cm)	170.9 ± 10.1	152.0 – 188.
BMI	23.6 ± 3.0	18.8 – 29.2
% Fat	23.7 ± 7.1	13.0 – 36.6
Lean mass (kg)	53.4 ± 13.4	35.6 – 78.0
VO_{2peak} ($mg \bullet kg^{-1} \bullet min^{-1}$)	49.1 ± 3.3	40.5 – 52.4
Physical activity (hrs/week)	2.8 ± 1.2	1.5 – 8.0

3.3 Preliminary testing

Prior to participating in the experimental protocol, body composition (fat mass, fat-free mass, and % body fat) was assessed by dual energy x-ray absorptiometry (DEXA) (Lunar, Madison, WI). Subjects completed a graded, continuous exercise test on a treadmill (LifeFitness 9100 HR, Schiller Park, IL) to assess peak oxygen consumption (VO_{2peak}). The test commenced at a low work rate (e.g. 5.0 miles per hour)

with incremental increases in treadmill grade (e.g. +2% every 2 minutes) and/or speed (e.g. +0.5 miles per hour) until a peak voluntary effort was achieved. Gas exchange measurements were obtained continuously throughout the test by open-circuit spirometry (TrueMax2400 Metabolic Measurement System, Parvomedics, Salt Lake City, UT). Heart rate was measured and recorded throughout the test by telemetry using a Polar monitor (Polar Electro Oy, Kempele, Finland). Peak effort was defined as achievement of at least two of the following criteria: 1) RER ≥ 1.10; 2) peak heart rate ≥ 95% of age-predicted maximum (220-age) and 3) a plateau in VO_2 as defined by an increase of < 150 $ml \cdot kg \cdot min^{-1}$ between the penultimate and final stage of the test. All of the subjects achieved at least two of these three criteria and all tests were considered a valid assessment of peak oxygen consumption.

Daily physical activity was assessed 2-4 days prior to each trial using an *activ*Pal professional physical activity monitor (PAL Technologies Ltd, Glasgow, Scotland). The *activ*Pal physical activity monitor is a single unit monitor based on a uni-axial accelerometer that is worn midline on the anterior aspect of the thigh. It produces a signal related to thigh inclination and can sense periods of walking, sitting and standing and is thus useful for measuring both activity and inactivity. The monitor also records step count and cadence. A software package (*activ*PAL Professional Research Edition) summarizes activity over 1 hour periods in graphical and numeric formats based on proprietary algorithms. The *activ*PAL has been shown to be a valid and reliable measurement tool for determining posture and motion during activities of daily living in a healthy population (48). Subjects were instructed to wear the monitor on the midline of the right thigh. These data were used to objectively quantify each subject's habitual

activity levels and energy expenditure during typical exercise and non-exercise days. The data were also used to determine any potential impact of prior physical activity and energy expenditure on outcome measures during the study. The *activ*Pal was also worn during all 24-hour laboratory visits. These data was used to quantify 24-hour energy expenditure during the 3 conditions (i.e. 8 A.M. – 8 A.M.).

3.3.1 Estimated energy expenditure and energy intake

To estimate total daily energy expenditure (TDEE) and energy intake in this sample, we have previously used the protocol described below (12, 52). Resting energy expenditure (REE) was measured in the morning after an overnight fast. Upon arrival, subjects lay supine in a quiet room for 30 minutes. A ventilated hood was placed over the subject's head and respiratory gases were collected using indirect calorimetry (TrueMax2400 Metabolic Measurement System, Parvomedics, Salt Lake City, UT) for 30 minutes. To estimate energy requirements for the day prior to each 24-hour laboratory visit, the REE was multiplied by an activity factor varying between 1.5-1.7 based on habitual physical activity as determined by questionnaire. Energy requirements estimated from REE using the appropriate activity factor are strongly correlated (r= 0.73) with energy requirements measured during 28 days of controlled feeding (78). Energy requirements during ACTIV and INACTIV were calculated by multiplying REE by 2.05 and requirements during INACTIV LO-CAL were calculated using a factor of 1.39 * REE based on estimates provided by Hamilton and colleagues (personal communication) using study designs similar to ours. Subjects were provided with all meals the day prior to, and the day of, each 24-hour laboratory visit in order to control energy intake. Meals consisted of commercially prepared frozen entrees and foods prepared and weighed in the

Energy Metabolism Laboratory (e.g. cereal, bagel, fruit, peanut butter, etc.). Subjects were asked to consume all food provided as discrete meals at certain times of the day (breakfast, lunch, dinner, etc.). Subjects were instructed to refrain from alcohol and caffeine for 24 hours prior to each 24-hour laboratory visit. Average daily macronutrient composition on the day prior to the 24-hour intervention was 55% carbohydrate, 29% fat, and 16% protein; average daily macronutrient composition during the 24-hour intervention was 54% carbohydrate, 29% fat, and 16% protein.

3.3.2 Control for menstrual cycle phase

Although the data are not consistent, some studies show that insulin sensitivity is lower in the luteal phase of the cycle compared with the follicular or menstrual phase (31, 142, 153). As previously mentioned, 4 females were taking monophasic birth control so all testing was conducted in a single cycle phase in these women. Based on self-reported onset and cessation of menses, one female completed all experimental conditions in the luteal phase; one completed 2 conditions in the luteal and the 3rd condition (ACTIV) in the follicular phase. For one woman with a history of irregular menstrual cycles, it was impossible to determine menstrual cycle phase during any experimental condition. However, because all but 2 female subjects completed all experimental conditions in a single cycle phase, results of this study were likely unaffected by variations in menstrual cyclicity.

3.4 Experimental protocol

Subjects completed three, 24-hour visits to the laboratory in a counter-balanced order with at least a week between visits. Three days prior to arrival in the laboratory

(Day -3), subjects were asked to perform 30 minutes of moderate exercise (e.g. jogging, cycling, etc.) at approximately 8 P.M. Subjects were instructed to perform the same exercise bout (i.e. same mode, duration, and intensity) before each visit. For the 2 days prior to each 24-hour intervention, subjects were instructed to refrain from any structured exercise (i.e. no physical activity beyond activities of daily living). All meals were provided the day prior to each 24-hour visit (Day -1) and were standardized across conditions. Average energy intake on Day -1 was 2321 ± 506 kcal/day. Daily macronutrient composition was 55% carbohydrate, 29% fat, and 16% protein. On the morning of Day 1, approximately 12 hours after the evening meal, subjects reported to the Energy Metabolism Laboratory for the 24-hour visit. Upon arrival, subjects were seated and a butterfly needle was inserted into a forearm vein and 5 ml of blood was drawn for the measurement of fasting plasma triacylglycerol concentration. Subjects were then given a standardized breakfast to eat and were shown to their designated room equipped with a chair, desk, and futon. Subjects were provided access to a computer with internet service, books and magazines, or movies throughout the day and evening. A standardized lunch and dinner were given at approximately 12 P.M. and 5 P.M., respectively. After an overnight stay in the laboratory, insulin action was assessed in the morning (Day 2) approximately 10-12 hours following the evening meal.

During ACTIV, total sitting time throughout the day was restricted and subjects sat for approximately 32% of total waking hours (Table 3.3), which is less than the amount of sedentary time in a typical individual (i.e. 55% of the waking day) (97). Subjects stood while reading, talking on the phone, or working on the computer, walked at a low to moderate intensity (≤ 3 mph) or performed activities of daily living (e.g.

sweeping, bending to pick up books, vacuuming, etc.). All subjects were instructed to perform standardized tasks and activities at specific times during the day based on an activity "menu". The energy expenditure goal for 24 hours was 2.05 * REE. To create the menu, low-intensity activities (≤ 3.8 METs) were chosen from a list of select household activities (e.g. sweeping, dusting, vacuuming, dish washing, etc.) with directly measured MET values from a study of 102 individuals (Freedson et al. unpublished data). Three additional activities with estimated MET values were selected from the Physical Activity Compendium (i.e. dart throwing, take out trash, put away groceries) (2). A sample schedule of activities is provided in Appendix A. During INACTIV and INACTIV LO-CAL, walking and standing was restricted and subjects spent approximately 98% of the waking day sitting (Table 3.3), which is much greater than the amount of time spent in sedentary behaviors in a large population sample in the United States (i.e. 55%) (97). A wheelchair was provided to transport the subjects within the laboratory and building. Energy expenditure during these two conditions was approximately the same (2195 ± 424 and 2139 ± 427 for INACTIV and INACTIV LO-CAL, respectively) and was significantly less than energy expenditure during ACTIV (2944 ± 462).

Table 3.3 Total sitting, standing, stepping, and sleep time during the 3, 24-hour conditions (M±SD).

	Sitting Time (hrs)	**Standing Time (hrs)**	**Stepping Time (hrs)**	**Sleep Time (hrs)**	**Total steps (steps/day)**
ACTIV	5.8 ± 1.7	9.8 ± 0.5	2.2 ± 0.4	6.1 ± 1.3	9913 ± 1669
INACTIV	16.9 ± 1.0[a]	0.2 ± 0.2[a]	0.1 ± 0.1[a]	6.8 ± 1.1[a]	264 ± 0.4[a]
INACTIV LO-CAL	16.8 ± 1.5[a]	0.3 ± 0.1[a]	0.1 ± 0.0[a]	6.8 ± 0.6[a]	251 ± 195[a]

[a] Significantly different from ACTIV.

3.4.1 Standardized meals

The energy content and composition of the evening meal (3 personal cheese pizzas and a Swiss cheese wedge) was identical both between subjects and across all conditions (1030 kcals, 39% fat, 18% protein, 43% carbohydrate) in order to standardize the effect of the previous meal on insulin action. The meal was also identical in content and composition to the meal consumed on the evening of Day -1 to allow for the comparison of fasting triacylglycerol concentrations from Day 1 to Day 2 without the potential confounding effect of the prior meal. The carbohydrate composition of breakfast and lunch was increased so that average daily macronutrient composition on Day 1 was 54% carbohydrate, 29% fat, and 16% protein. Breakfast and lunch consisted of solid foods (e.g. cereal, bread, deli meats), juice, or non-caffeinated soft-drink beverages. For all conditions, dietary composition, the timing of meals, and the time interval between the evening meal and the measurement of insulin action was held constant. However, the energy content of breakfast and lunch on Day 1 was lower in INACTIV LO-CAL compared to INACTIV and ACTIV. Total daily energy intake was the same in ACTIV and INACTIV (3106 ± 590 and 3133 ± 583, respectively), but energy intake in INACTIV LO-CAL was reduced to approximate the reduction in energy expenditure (2109 ± 428). Subjects were allotted 25 minutes to eat each meal.

3.5 Assessment of insulin action and other metabolic variables

Ten to 12 hours after the evening meal, insulin action was assessed using a 1-hour continuous infusion of 20% glucose that contained a 2% stable [6,6-^{2}H] glucose isotope tracer (Cambridge Laboratories, Andover, MA), as previously described (12, 134).

Indwelling catheters were placed in a superficial vein of each forearm for venous blood sampling and continuous infusion of the isotope tracer. Venous blood samples were collected to determine naturally occurring levels of isotopic enrichment prior to the infusion. These samples were also used to compare changes in fasting triacylglycerol levels from Day 1 to Day 2. A priming bolus of 200mg [6,6-^{2}H] glucose was given followed by a 90-minute infusion of 2.0% [6,6-^{2}H] glucose isotope at a rate of 3.0mg/min delivered by a peristaltic infusion pump (Harvard Apparatus Pump 22, Holliston, MA). Respiratory gases and venous blood samples were collected at 0, 75 and 90 min. At 90-min, the infusate was changed to 20% dextrose containing 2.0% [6,6-^{2}H] glucose delivered at a rate of 8.45mg/kg FFM/min for 60 min. Blood samples and respiratory gases were collected at 50, 55, and 60 minutes of the glucose/stable isotope infusion to determine glucose rate of appearance (R_a) and disappearance (R_d) as well as plasma concentrations of glucose and insulin. Glucose and insulin concentrations from minutes 50, 55, and 60 were averaged to determine the steady-state glucose (SSPG) and insulin (SSPI) concentrations. Insulin action was determined using the isotopically-determined glucose uptake scaled to steady-state insulin concentrations during the continuous infusion. This procedure for the assessment of insulin action was identical among the three different treatment conditions.

3.6 Blood collection and biochemical analysis

Venous blood was collected in vacutainers containing a glycolytic inhibitor (sodium fluoride) and potassium oxalate for analysis of glucose and glucose isotopic enrichment. In addition, vacutainers containing EDTA were used for the analysis of insulin, triacylglycerols, and free fatty acids. After collection, samples were immediately

centrifuged at 3300 rpm with a maximum force of 1380 x G for 10 minutes. Plasma aliquots were stored in 2ml cryotubes at -80° C until analysis.

Plasma glucose concentrations were determined by the glucose oxidase method using a GL5 Analox Analyzer (Analox Instruments, Lunenberg, MA). Insulin concentrations were determined using a radioimmunoassay kit specific for human insulin (Linco Research Inc. St. Charles, MO). Free fatty acid and triacylglycerol concentrations were determined with an enzymatic colorimetric assay kit (Wako Chemicals USA Inc., Richmond, VA, and Sigma Chemical, St. Louis, MO, respectively).

3.7 Plasma isotopic enrichment

Glucose isotopic enrichment was determined using liquid chromatography-mass spectrometry (LCMS) according to the methods outlined by McIntosh et al. (99). Serum samples (0.3 mL) were placed in 1.7-mL microcentrifuge tubes containing 1 mL of ice-cold acetone. Samples were vortexed and incubated for 10 minutes at -20°C; after chilling, samples were centrifuged for 2 min at 17,000*g* at 4°C. The supernatant was transferred to 12 X 75-mm borosilicate tubes (Fisher Scientific Inc., Pittsburgh, PA) and concentrated to dryness under ambient conditions. Once dry, samples were reconstituted with 0.3 mL 75:25 acetonitrile to water, vortexed, and transferred through a polyethersulfone filter (4 mm, 0.45 µm) into a glass HPLC vial and capped (Fisher Scientific Inc., Pittsburgh, PA). Vials were loaded into the autosampler compartment of the LC (Agilent 1100 series, Agilent Technologies, Santa Clara, CA) and set to an injection volume of 10 µL. The LC conditions were as follows: isocratic mobile phase 75:25 acetonitrile to water; flow rate of 1.0 mL•min^{-1}; column temperature 35°C.

Glucose eluted from the column at approximately 6 min, therefore, total run time on the column was set to 10 min. After the compounds were separated using a Shodex Asahipak NH2P-50, 4.6 x 250 mm column (Showa Denko America, Inc., New York, NY), they were ionized by electrospray in the MS (Esquire 6000 (Bruker Daltronics Inc., Billerica, MA) and detected under the following conditions: capillary, 5500 V; endplate offset, 500 V; nebulizer 30.0 psi; dry gas 10.0 L•min^{-1}; dry temperature, 300°C; scan range 100-210 *m/z*.

For chromatogram analysis (Bruker Data Analysis software, Bruker Daltronics, Inc.), the glucose peaks were isolated, integrated, and the average mass-to-charge ratio was generated. Isotopic enrichment of the [6,6-^{2}H] glucose (*m/z* = 205) was expressed as a percentage of the total glucose species (*m/z* = 203 + 204 + 205) as follows:

$$\% \text{ isotopic enrichment} = \frac{203}{203+204+205} * 100$$

3.8 Calculations

3.8.1 Isotope-derived glucose turnover

$$\text{Glucose rate of appearance } (R_a) = \frac{F - V\,[C1+C2)/2][(IE2-IE1)/(t2-t1)]}{[\frac{IE2+IE1}{2}]}$$

$$\text{Glucose rate of disappearance } (R_d) = Ra - V[\frac{C2-C1}{t2-t1}]$$

F represents the isotopic infusion rate. IE_1 and IE_2 are the isotopic enrichments (ratio of labeled [6,6-^{2}H] glucose to total plasma glucose) at time-points t_1 and t_2,

respectively. C_1 and C_2 are the concentrations of plasma glucose at t_1 and t_2, and V is the estimated volume of distribution for glucose of 180 ml/kg.

Whole body insulin action was defined as glucose R_d/SSPI, where SSPI is the mean plasma insulin concentration during the final stages of the infusion (12, 130).

Hepatic insulin action was defined as the percent suppression of basal hepatic glucose production (HGP) during the glucose infusion, where greater suppression indicates greater hepatic insulin action =

$$1 - \frac{HGPinfusion}{HGPfasting} * 100.$$

$HGP_{fasting}$ is equal to the basal rate of appearance while $HGP_{infusion}$ during the infusion is calculated as: $steady\ state\ glucose\ Ra - glucose\ infusion\ rate.$

3.8.2 Oxidative and non-oxidative glucose disposal

Glucose oxidative disposal was assumed to equal the carbohydrate oxidation rate during the continuous infusion of glucose. Non-oxidative glucose disposal, usually attributed to glucose storage, was expressed as a percentage of total glucose R_d and calculated as:

$$\frac{Glucose\ Rd - total\ carbohydrate\ oxidation\ rate}{Glucose\ Rd} * 100.$$

3.8.3 Metabolic flexibility

Whole-body substrate oxidation was estimated by calculating the respiratory exchange ratio (RER) in the fasted conditions and during the final 10 minutes of the glucose infusion using indirect calorimetry. Low RER values (i.e. 0.70-0.85) reflect a

greater reliance on fat oxidation where high RER values reflect greater reliance on carbohydrate oxidation (0.85-1.00). In subjects with "normal" insulin action, the elevated insulin concentrations during the infusion of glucose cause an abrupt increase in RER, indicative of a "switch" from primarily fat oxidation in the fasted state to primarily carbohydrate oxidation during the glucose infusion. The magnitude of the switch (Δ RER) was used as an index of metabolic flexibility.

3.8.4 Substrate oxidation

Fasting and insulin-stimulated carbohydrate and fat oxidation rates were calculated from the VO_2 and VCO_2 using the formulas of Péronnet and Massicotte (117):

Fat oxidation rate (g/min) = $1.6946\ VO2 - \ 1.7012\ VCO2$

Carbohydrate oxidation rate (g/min) = $4.5850\ VCO2 - \ 3.2255\ VO2$

3.9 Power and sample size analysis

The sample size required to test for significant differences in insulin action between group means was calculated based on mean differences and intra-individual variances from a study on the acute effects of energy surplus in a similar subject population (52). Since the most pertinent comparisons for this study were between INACTIV and INACTIV LO-CAL, the power calculations were based on a paired two-sample t-test (alpha level 0.05) of the two-sided null hypothesis that the difference between these two group means was zero. Based on this calculation, testing 13 subjects would provide 80% power to detect a 20% difference in insulin action between these two conditions. A 14^{th} subject was included for gender balance (i.e. 7 men, 7 women).

3.10 Statistical analysis

Differences in insulin action, glucose kinetics, substrate oxidation, and substrate and hormone variables between the three conditions were analyzed by means of linear mixed-effects models with planned contrast analyses using the R statistical software package, version 2.2.1 (R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2005, http://www.R-project.org). In addition, to determine which factor (inactivity *vs.* energy surplus) had a greater effect on insulin action we fit a model to test the separate effects of condition (inactive and active) and energy status (balance and surplus). All non-normally distributed data were log-transformed prior to analysis. Statistical significance was accepted at $p < 0.05$. Subject characteristics are presented as mean ± standard deviation; all other data are expressed as mean ± standard error of the mean.

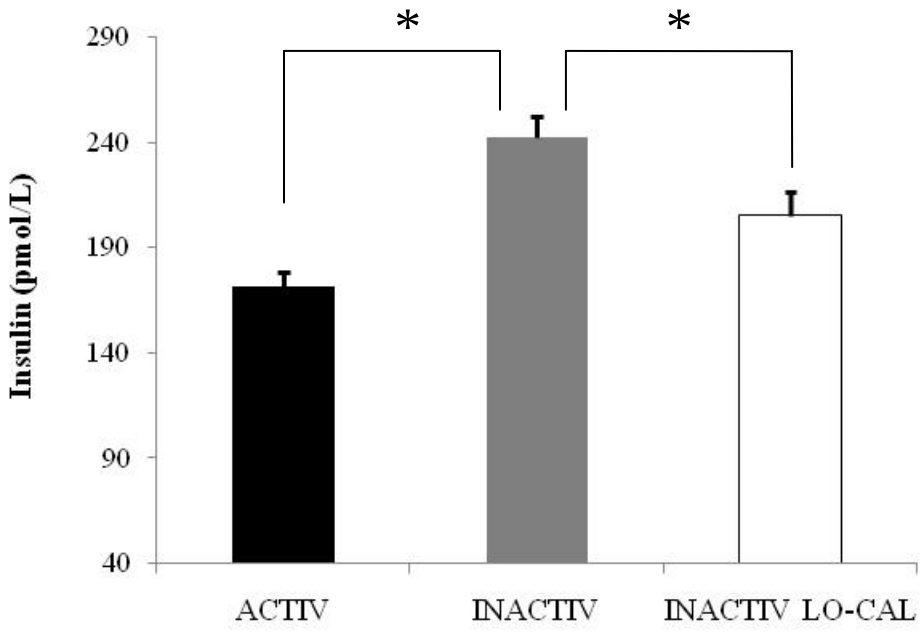
CHAPTER 4

RESULTS

4.1 Plasma glucose and insulin

Fasting plasma glucose concentrations were not significantly different among any of the 3 conditions (ACTIV = 4.91 ± 0.02 mmol•L^{-1}, INACTIV = 5.04 ± 0.03 mmol•L^{-1}, INACTIV LO-CAL = 4.91 ± 0.02 mmol•L^{-1}). Similarly, steady-state plasma glucose (SSPG) during the glucose infusion (ACTIV = 9.3 ± 0.1 mmol•L^{-1}, INACTIV = 9.4 ± 0.1 mmol•L^{-1}, INACTIV LO-CAL = 9.2 ± 0.1 mmol•L^{-1}) were also not different among the 3 conditions. Fasting insulin concentrations were greater in both INACTIV and INACTIV LO-CAL (47.6 ± 1.4 pmol•L^{-1} and 43.7 ± 1.9 pmol•L^{-1}, respectively) compared with ACTIV (39.9 ± 0.9 pmol•L^{-1}) although these differences were not statistically significant. Fasting insulin concentrations also were not different between INACTIV and INACTIV LO-CAL. Compared to control (ACTIV), steady-state plasma insulin (SSPI) concentrations were 41% higher in INACTIV ($p < 0.001$) and 20% higher in INACTIV-LO CAL (p=0.08) (Figure 4.1). Insulin concentrations during the glucose infusion were also 18% greater in INACTIV compared to INACTIV LO-CAL (p = 0.02) (Figure 4.1).

Figure 4.1 Steady-state plasma insulin (SSPI) concentrations during the continuous infusion of glucose across the 3 conditions. * $p < 0.05$.



4.2 Glucose turnover

Whole-body glucose rate of disappearance (R_d) and hepatic glucose production (HGP) before (fasting) and during the glucose infusion are shown in Table 4.1. During the infusion, total glucose R_d was significantly lower in INACTIV compared to both ACTIV ($p < 0.001$) and INACTIV LO-CAL ($p = 0.05$). Total glucose R_d was also lower in INACTIV LO-CAL compared to ACTIV although this difference was not significant ($p = 0.08$).

Table 4.1 Comparison of glucose turnover across the 3 conditions.

	Glucose R_d fasting	**Glucose R_d infusion**	**$HGP_{fasting}$**	**$HGP_{infusion}$**
ACTIV	21.5 ± 0.3	51.1 ± 0.6	24.0 ± 0.5	9.1 ± 0.6
INACTIV	21.7 ± 0.8	47.4 ± 0.8[a]	22.4 ± 0.8	6.3 ± 0.5
INACTIV LO-CAL	20.6 ± 0.5	48.7 ± 1.0[a]	21.7 ± 0.5	7.1 ± 1.0

Note: Glucose Rd, glucose rate of disappearance; HGP, hepatic glucose production.
Units for all variables are umol•L^{-1}•min^{-1}•$kgFFM^{-1}$
Data are mean and standard error.
[a] Significantly different from ACTIV

4.3 Insulin action

Insulin action, as defined by R_d/SSPI, was reduced relative to ACTIV by 39% in INACTIV ($p < 0.001$) and by 18% in INACTIV LO-CAL ($p = 0.07$) (Fig.4.2). R_d/SSPI was also higher in INACTIV LO-CAL compared to INACTIV ($p = 0.04$).

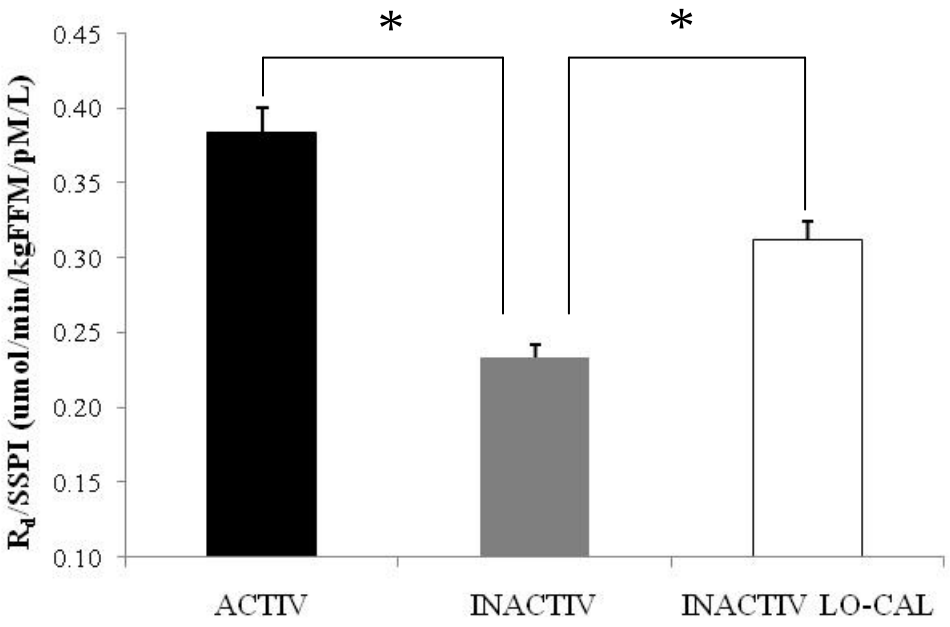
Because insulin action was lower in both INACTIV and INACTIV LO-CAL compared to ACTIV, we further examined which factor (energy status, i.e. balance *vs.* surplus, or inactivity) was the strongest contributor to insulin action. To do this, we fit a model using energy status and activity status (i.e. inactivity *vs.* activity) as factors. According to the model, only the effect of energy status was significant ($p < 0.05$), indicating that energy status has a more potent effect on insulin action compared to activity status.

To determine whether sex (male/female) or energy balance on Day -1 influenced the insulin action response, we performed a separate analysis incorporating these factors (i.e. sex, energy balance on Day -1) into the model. Unexpectedly, there was a significant effect of sex on insulin action, such that insulin action was higher in women compared to men. However, there was no sex by condition interaction suggesting the response to the intervention was the same regardless of sex. There was no significant effect of energy balance on Day -1 on insulin action ($p > 0.05$). Therefore, differences in insulin action between conditions cannot be explained by energy balance on Day -1.

The energy content and composition of the evening meal on Day 1 was the same for each subject across all 3 conditions (i.e. 1030 kcal). Therefore, the percent contribution of the evening meal to total daily energy intake (% contribution) varied

between subjects (i.e. higher % contribution in subjects with low total daily energy intake and vice versa) and between condition (i.e. higher % contribution in INACTIV LO-CAL compared to ACTIV and INACTIV). To assess whether % contribution of the evening meal had a significant impact on insulin action, we included % contribution of the evening meal into the model formula. There was no significant effect of % contribution of the evening meal on R_d/SSPI, nor did its inclusion in the model affect model outcomes.

Figure 4.2 Insulin action (R_d/SSPI) assessed during the continuous infusion of glucose across the 3 conditions. SSPI, steady-state mean of 50, 55, and 60 min plasma insulin concentrations. * $p < 0.05$.

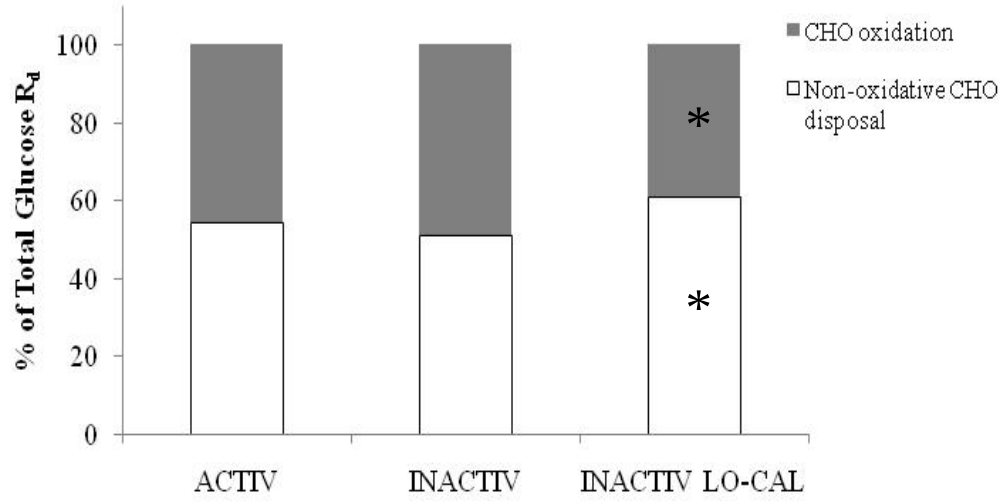


4.4 Partitioning of insulin-mediated glucose disposal

Non-oxidative glucose disposal, expressed as a percentage of total glucose R_d, was higher and percent oxidative disposal was lower in INACTIV LO-CAL compared to INACTIV ($p < 0.05$) (Figure 4.3). Non-oxidative glucose disposal was greater (61± 2.0% *vs.* 54 ± 1.3%) and oxidative disposal was lower (39 ± 2.0% *vs.* 46% ± 1.3) in INACTIV

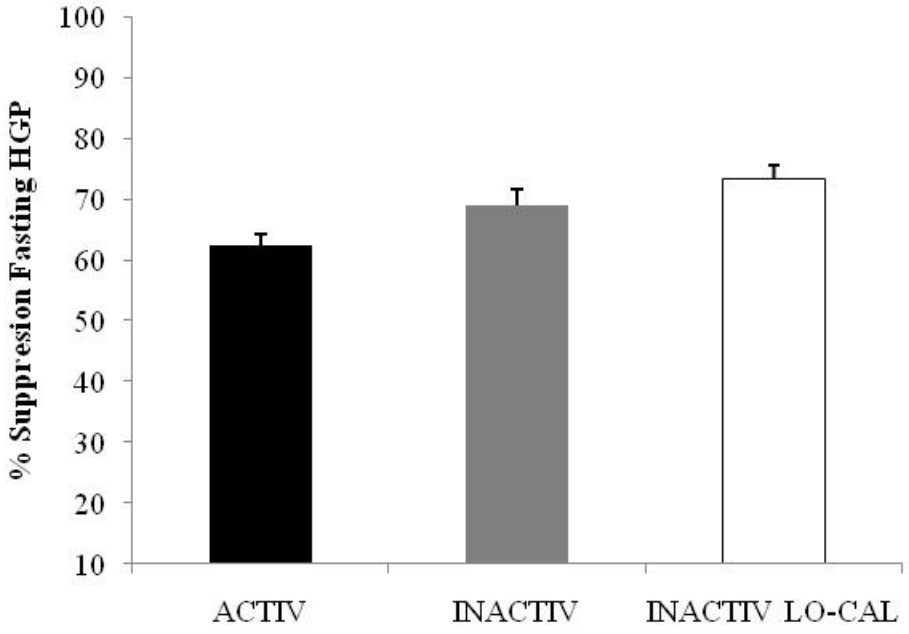
LO-CAL compared to ACTIV, respectively, although these differences were not significant ($p > 0.05$). There were no differences in non-oxidative or oxidative glucose disposal between ACTIV and INACTIV ($p > 0.05$).

Figure 4.3 Partitioning of insulin-mediated glucose disposal ($R_{d\text{infusion}}$) across the 3 conditions. The open (white) portion of the column reflects non-oxidative disposal and the gray portion reflects CHO oxidation. *, $p < 0.05$ when compared with INACTIV.



Basal glucose R_a ($HGP_{fasting}$) and hepatic glucose production during the infusion ($HGP_{infusion}$) were similar between the 3 conditions (Table 4.1). In all 3 conditions, the glucose infusion partially suppressed HGP, with residual HGP lowered to 27-38% of fasting values. Hepatic insulin action, defined as percent suppression of $HGP_{fasting}$ during the infusion, was not significantly different between the 3 conditions (Figure 4.4).

Figure 4.4 Hepatic insulin action (percent suppression of fasting hepatic glucose production during the glucose infusion) across the 3 conditions.



4.5 Markers of lipid metabolism

Fasting triacylglycerol (TAG) concentrations on Day 1 were similar across the 3 conditions ($p > 0.05$) (Figure 4.5). Fasting TAG concentrations on Day 2 (i.e. prior to the continuous infusion of glucose) were 27% lower in the active condition (ACTIV) compared to INACTIV ($p < 0.01$) (Figure 4.5). TAG levels were also 20% lower in INACTIV LO-CAL compared to ACTIV, although this difference was not significant ($p > 0.05$). There were no differences in fasting TAG concentrations on Day 2 between INACTIV and INACTIV LO-CAL ($p > 0.05$). To further examine whether there was an effect of intervention on fasting TAG concentrations, we examined the change in fasting TAG from Day1 to Day 2 (i.e. Δ $TAG_{fasting}$ (Day 2 – Day 1) (Figure 4.6). The change to fasting TAG levels were much smaller in INACTIV (+3%, $p < 0.01$) and INACTIV LO-CAL (-6%, $p = 0.10$) compared to ACTIV (-20%) (Figure 4.6). During the continuous

infusion of glucose, TAG concentrations were not different between conditions ($p > 0.05$) (Table 4.5).

Fasting and insulin-stimulated plasma free fatty acid (FFA) concentrations were similar among the 3 conditions. There were also no differences in fasting or insulin-stimulated lipid oxidation (LIPIDox ($mg \bullet min^{-1}$)) between conditions, although LIPIDox during the glucose infusion was slightly greater in INACTIV LO-CAL compared to INACTIV ($p = 0.08$) (Table 4.2). Similarly, respiratory exchange ratios (RER) were similar across conditions in the fasted state (Figure 4.7). However, during the glucose infusion, RER values were significantly higher in ACTIV ($p = 0.05$) and INACTIV ($p = 0.02$), indicating greater reliance on carbohydrate oxidation compared to INACTIV LO-CAL (Figure 4.7).

Table 4.2 Markers of lipid metabolism across the 3 conditions.

	$LIPIDox_{fasting}$ ($mg \cdot min^{-1}$)	$LIPIDox_{infusion}$ ($mg \cdot min^{-1}$)	Δ LIPIDox %	$FFA_{fasting}$ ($mmol \cdot L^{-1}$)	$FFA_{infusion}$ ($mmol \cdot L^{-1}$)	ΔFFA %	$TAG_{fasting}$ ($mg \cdot dL^{-1}$)	$TAG_{infusion}$ ($mg \cdot dL^{-1}$)
ACTIV	95.6 ± 3.1	60.8 ± 2.9	-39.9 ± 2.3	0.27 ± 0.01	0.08 ± 0.00	-68.4 ± 1.2	62.3 ± 2.2	62.8 ± 2.3
INACTIV	86.4 ± 3.3	57.8 ± 2.4	-33.7 ± 2.5	0.24 ± 0.01	0.07 ± 0.00	-68.6 ± 1.3	79.2 ± 2.9[a]	72.3 ± 2.9
INACTIV LO-CAL	98.9 ± 2.9	71.0 ± 2.9	-31.2 ± 2.2	0.31 ± 0.01	0.08 ± 0.00	-73.9 ± 1.1	74.8 ± 4.3	68.8 ± 3.3

Note: Δ, % Change (Infusion – Fasting); LIPIDox, rate of lipid oxidation; FFA, free fatty acid; TAG, triacylglycerol. Data are mean and standard error.

[a], Significantly different from ACTIV

Figure 4.5 Fasting TAG concentrations on Day 1 (in gray) and Day 2 (in black) across the 3 conditions. * $p < 0.05$ when compared with ACTIV.

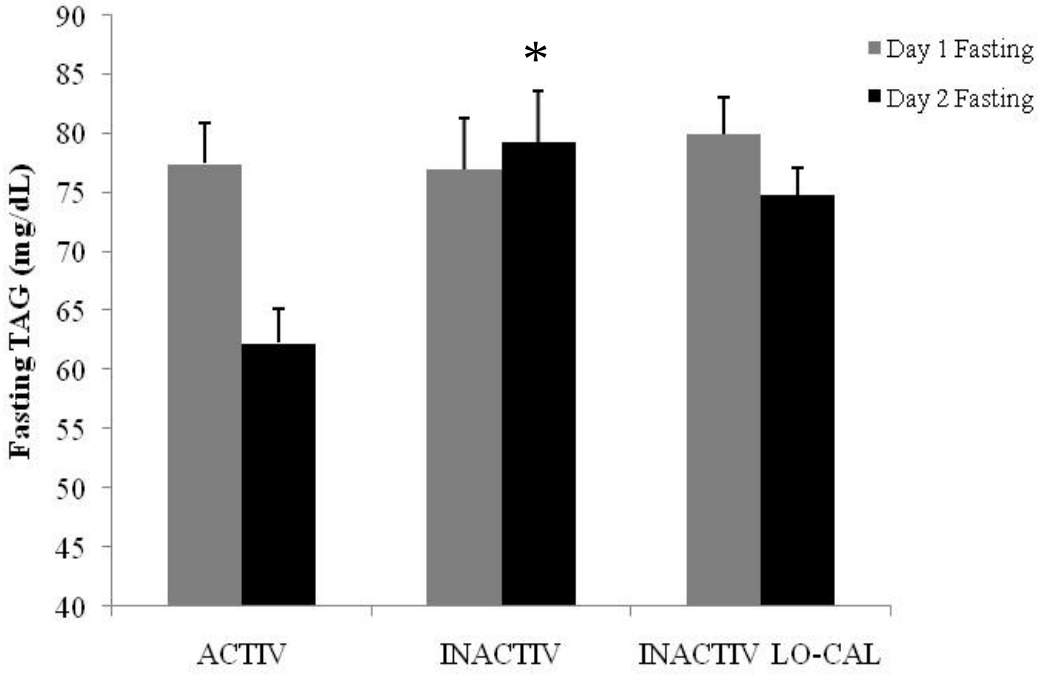
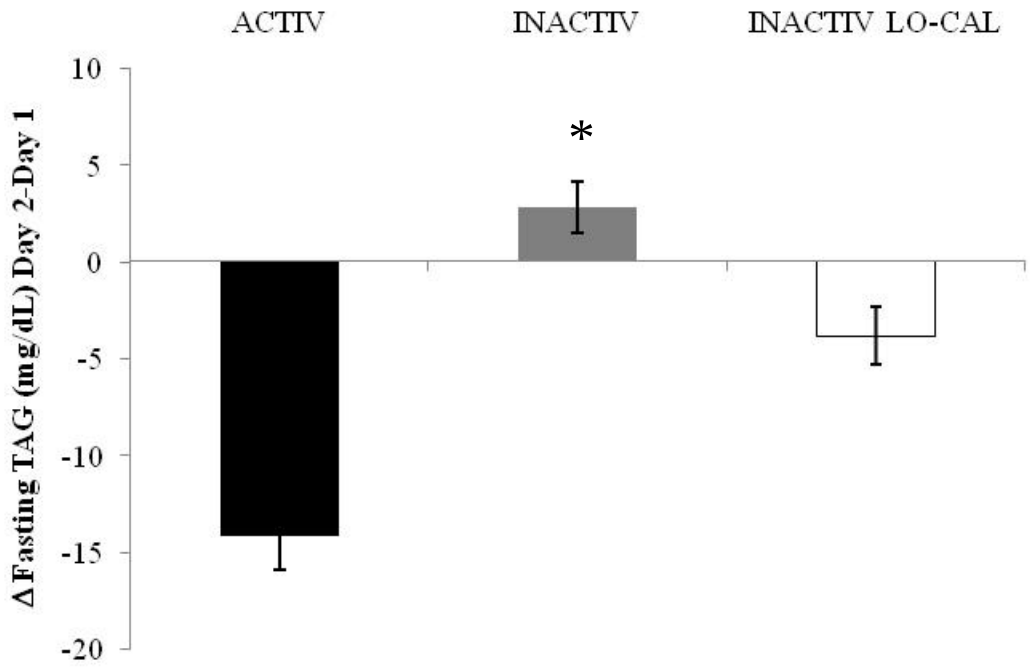
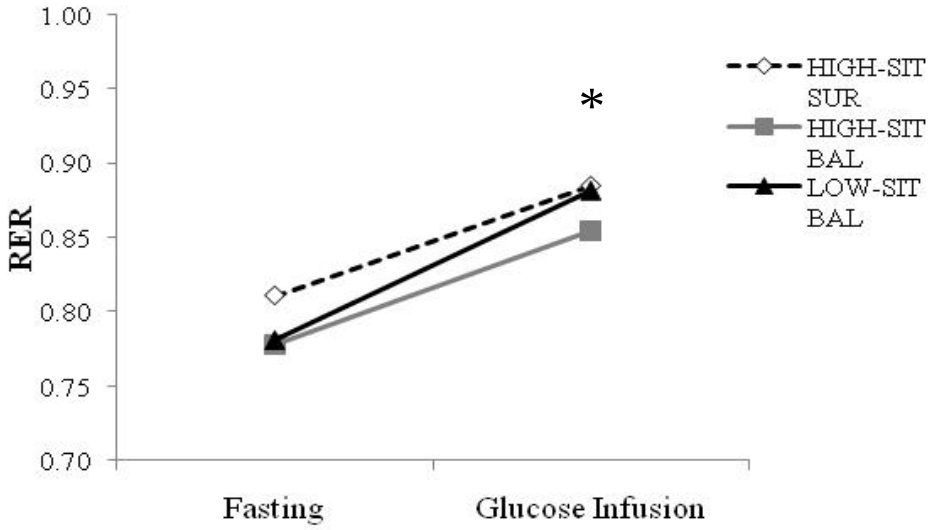


Figure 4.6 Change in fasting TAG concentrations from Day 1 to Day 2 across the 3 conditions.* $p < 0.05$ when compared with ACTIV.



To examine whether there were differences in the suppression of FFAs and LIPIDox by the elevated circulating insulin concentrations during the infusion, we compared the change in FFA from the fasted to insulin-stimulated state (ΔFFA) as well as the percent change in lipid oxidation rates (ΔLIPIDox) in all 3 conditions (Table 4.2). There were no significant differences in either variable (ΔFFA and ΔLIPIDox) between conditions. We also compared the change in the respiratory exchange ratio from the fasted to insulin-stimulated state (ΔRER) to examine differences in metabolic flexibility between conditions. Although there was a more dynamic increase in RER, as indicated by a steeper slope of the line in Figure 4.7., in ACTIV compared to INACTIV and INACTIV LO-CAL, there were no significant differences in ΔRER between conditions.

Figure 4.7 Respiratory exchange ratio (RER) across the 3 conditions. *, $p \leq 0.05$ when compared with INACTIV LO-CAL.



CHAPTER 5

DISCUSSION

The purpose of this study was to determine the metabolic response to one day of inactivity (prolonged sitting), with and without the potential confounding effect of energy surplus, in healthy, young adults. To test the specific aims, we compared the metabolic response to 24 hours of: 1) high non-exercise activity with energy intake = energy expenditure (ACTIV); 2) inactivity (sitting) with energy intake > expenditure, i.e. energy surplus (INACTIV); and 3) inactivity with energy intake = expenditure (INACTIV LO-CAL). The main findings of the study were: 1) compared to the active condition, 24-hours of inactivity, with no change to diet, significantly reduced insulin action; and 2) reducing energy intake to match low energy expenditure during inactivity (INACTIV LO-CAL) significantly attenuates, but does not completely prevent, the deleterious effect of inactivity on insulin action. Thus, mechanisms other than energy surplus are also involved in the inactivity-induced decline in insulin action.

Several studies in animals and humans have reported clear, deleterious consequences of inactivity on metabolic processes including insulin action (8, 14, 90, 100, 114, 119, 131, 137). The time course of metabolic changes is not clear from these studies due to different inactivity protocol durations. For example, studies in rodents report significant declines in insulin-stimulated glucose uptake in as little as 24-48 hours following hindlimb immobilization (119, 131). In humans, the earliest detectable reduction in insulin action has been observed following 3 days of bed rest (90, 133). Notably, the present study is the first to document considerable reductions in whole-body insulin action following just one day of inactivity in humans. Further, the magnitude of

the reduction in insulin action in such a short period of time is remarkable considering the similar declines (~30%) reported after longer periods (i.e. days - weeks) of inactivity (90, 100, 133). Therefore, reduced insulin action in humans appears to be a very early metabolic maladaptation to inactivity that is sustained over the course of at least several weeks of continued inactivity.

5.1 Metabolic response to inactivity versus exercise

Previous data on the regulation of lipoprotein lipase (LPL) activity suggest that the underlying mechanism(s) and the magnitude of the metabolic response to inactivity are not simply the opposite of the response to exercise (54). In the present study, the decline in insulin action in response to inactivity (~18-39%) was similar in magnitude to the increase in post-exercise insulin action observed in other studies (i.e. ~15-44%) (7, 67, 134). Thus, in contrast to LPL activity, it seems that the insulin action response to inactivity closely mirrors the response to exercise, although the mechanisms underlying those responses may be distinctly different. However, without direct comparisons between the metabolic effects of exercise and inactivity on insulin action in the same subject population, we cannot determine whether the magnitude of change in insulin action is different in response to similar increases and decreases in energy expenditure.

Another novel aspect of the current study is the inactivity paradigm used. Whereas previous studies in humans have mainly utilized bed rest as a model for inactivity, we used a prolonged sitting protocol to experimentally reduce standing and ambulation. Therefore, results from the present study are more relevant to the general population who spend considerable portions of the waking day engaged in sedentary

behaviors involving sitting (97). Recent epidemiological data suggest a positive relationship between time spent in sedentary behaviors and risk for insulin resistance and type 2 diabetes (35, 59, 60, 70). Results of this study provide additional evidence for a strong, cause-and-effect relationship between sedentary behavior associated with sitting and metabolic health outcomes reported in the epidemiological literature.

5.2 Mechanisms underlying inactivity-induced changes to insulin action

The mechanisms involved in the rapid, inactivity-induced decline in insulin action are not well-characterized. Potential factors that could account for lower insulin action following inactivity could involve reduced glucose transport, glucose oxidation and/or storage (i.e. non-oxidative glucose disposal). Defects in insulin-stimulated glucose disposal are often attributable to impaired non-oxidative glucose metabolism (16, 23, 118). Accordingly, several studies have reported selective impairments in glycogen synthesis and insulin-mediated non-oxidative glucose disposal following inactivity (100, 131). However, compared to the active condition, non-oxidative glucose disposal did not change following inactivity, suggesting that reduced insulin action in either of the inactive conditions is not attributable to impaired glucose storage. Several studies have reported a significant reduction in glucose transporters (GLUT-4) following inactivity (138, 145), which has been directly related to the inactivity-induced decline in insulin action (145). Although not measured in the present study, changes to glucose transport capacity may therefore be a more plausible mechanism for the inactivity-induced decline in insulin action. Other potential mechanisms are greater circulating levels of counterregulatory hormones (e.g. glucagon, epinephrine, cortisol) (144), decreased muscle blood flow (100), or increased systemic inflammation (11, 18). The data are

inconsistent, however, as to whether any of these factors play a major role in the insulin action response to inactivity. In summary, the diversity of findings suggests that the mechanism to explain the reduction in insulin action in response to inactivity is multifactorial and complex, but likely involves impaired glucose transport capacity.

5.3 Role of energy surplus on the insulin action response to inactivity

Considering the important effects of diet adds complexity to understanding the effects of inactivity on insulin action. Data from Stettler et al. (135) highlighted the importance of diet composition on whole-body insulin action following inactivity. In that study, the inactivity-induced decline in insulin action was prevented when the proportion of dietary fat was reduced from 45% to 15% of total calories. Results of the present study expand upon prior research by emphasizing the importance of a previously unexplored mechanism (i.e. energy surplus) on the metabolic response to inactivity. Holding macronutrient composition constant, energy surplus accounted for 53% of the deleterious effects of inactivity on insulin action. Taken together, these results suggest that energy surplus plays a key role in the metabolic response to inactivity, although diet composition may be important.

It is clear from studies in both animals (146) and humans (4, 52, 113) that energy surplus elicits decrements in insulin action prior to significant changes in body weight. In a previous study in our laboratory, insulin action was 30% lower after to 3 days of overfeeding (i.e. energy surplus) (52) in active men and women. The independent effects of energy surplus could not be determined since the 3-day overfeeding period was coupled with a reduction in structured exercise (52). To our knowledge, the present study

is the first to document the independent effect of energy surplus on insulin action when activity/energy expenditure is carefully controlled.

Further, results from this study provide insight into the duration of the metabolic response to energy surplus. No studies have examined the effects of energy surplus lasting less than ~2 days; thus, it is unclear whether changes to insulin action occur in response to acute (i.e. effect of the last meal) or more chronic changes to energy balance (i.e. days). The study design used in the present study provides insight into this uncertainty. To eliminate energy surplus during inactivity, we reduced the caloric content of breakfast and lunch. However, we standardized the energy content of the evening meal (i.e. 1030 kcals), temporarily restoring acute energy balance between conditions, which may have minimized differences in insulin action. Remarkably, despite the equivalent energy content of the evening meal, there were clear differences in insulin action between the two inactive conditions. Therefore, the metabolic effect of a surplus of calories at breakfast and lunch was sustained. While it is possible that a surplus of calories in the evening meal could have augmented differences between the inactive conditions, these results suggest that the metabolic response to energy surplus persists over the course of at least one day (i.e. 24 hours).

When considering the separate effects of activity and energy status on insulin action, only the effect of energy status was statistically significant. This result suggests that energy status, rather than activity, has a stronger independent influence on whole-body insulin action. However, although not statistically significant, the effect of inactivity *per se* is clearly physiologically relevant because inactivity independently accounted for

47% of the decline in insulin action. Thus, it is presumptuous to conclude from these results that energy status is more important than activity when both are clearly relevant.

5.4 Potential mechanisms involved in the metabolic response to inactivity and insulin action

There are several potential mechanisms to explain lower insulin action in the inactive, energy surplus condition. Non-oxidative glucose disposal was significantly lower following inactivity with no change to diet compared to inactivity with reduced energy intake. This observation provides evidence for impaired glucose storage as a mechanism to explain the energy surplus-mediated reduction in insulin action. Although not measured, differences in non-oxidative glucose disposal between the two inactive conditions could be explained by differences in muscle glycogen stores. Although net glycogen breakdown is negligible under conditions when demand for ATP is low (e.g. during inactivity) (23), significantly greater carbohydrate intake in INACTIV compared to INACTIV LO-CAL (426 g CHO vs. 280 g CHO) may have led to moderate, but important increases in muscle glycogen content in INACTIV. Elevated muscle glycogen levels would be expected to reduce insulin activation of glycogen synthase (15, 87, 94) providing a potential mechanism to explain the lower insulin-mediated glucose disposal following inactivity with no change to diet compared to inactivity with reduced energy intake. However, without direct measurements of muscle glycogen and/or glycogen synthase activity, we are unable to determine the mechanistic relationship between these factors and our results.

Impaired insulin signaling to glucose transport could also account for lower insulin-mediated glucose uptake following inactivity with no change to diet compared to

inactivity with reduced intake. Data from previous studies indicate that nutrient oversupply of glucose and/or fatty acids impairs key components of the insulin signaling pathway (50, 73, 81, 85, 88, 111, 115), potentially via upregulation of the hexosamine biosynthetic pathway (111, 115); upregulation of inflammatory pathways (11, 49, 55) and/or via accumulation of intracellular fatty acid moieties (81). In sum, multiple and complex mechanisms are likely responsible for the significantly greater reduction in insulin action following inactivity without change to energy intake.

5.5 Hepatic insulin action

Hepatic insulin action, as assessed by percent suppression of fasting hepatic glucose production (HGP) by the glucose infusion, was not significantly different between conditions. These results are consistent with studies showing that 7 days of bed rest did not change hepatic insulin action in men (14, 100, 137). In contrast, Blanc et al. (14) reported significant reductions in suppression of fasting hepatic glucose production after 7 days of bed rest in women, suggesting a potential sex difference in the response to inactivity. In the present study, although the pattern of the hepatic insulin action response to inactivity was different in women versus men (23% increase vs. 5% increase, respectively), these differences were not statistically significant. Although we may have lacked sufficient power to detect differences, the existence of sex differences in hepatic insulin action following inactivity in men versus women is dubious. Therefore, additional research is warranted to determine whether there are distinct gender differences in the response to inactivity and the mechanism to explain these differences.

The direction of the hepatic insulin action response was opposite to what we anticipated. Numerically, hepatic insulin action was lowest in the active condition,

highest following inactivity with no change to diet, and was intermediate following inactivity with reduced energy intake. However, since glucose Ra and Rd are not independent, less suppression of HGP (greater glucose production) in the active condition could be explained by the higher glucose Rd. Nonetheless, our results support the general finding that inactivity (lasting ≤ 7 days) has a negligible impact on hepatic insulin action, in stark contrast to the large reduction to whole-body insulin action.

5.6 Metabolic flexibility and lipid metabolism

In addition to deleterious effects of inactivity on insulin action, several studies also report detrimental effects of inactivity on lipid metabolism (8, 10, 155). For instance, in rodents there are striking increases in postprandial TAG concentrations within 4-12 hours of hindlimb unloading. These changes are attributable to large (i.e. ≥ 50%) reductions in lipoprotein lipase (LPL) activity (10, 53). In contrast to those previous studies, we did not observe changes to lipid metabolism (i.e. insulin-mediated suppression of lipolysis (Δ FFA), suppression of lipid oxidation (Δ LIPIDox)), after inactivity with or without energy surplus. These results suggest these aspects of lipid metabolism are relatively unaffected by 1 day of prolonged sitting. However, compared to the active condition, fasting TAG concentrations 24 hours after the intervention were greater in both inactive conditions (+27% INACTIV; +20% INACTIV LO-CAL), although the difference was only statistically significant in the condition when diet was not changed. In addition, the change in fasting TAG concentrations after the intervention (i.e. $Day\ 2 - Day\ 1$) was negligible in the inactive condition (+3 mg/dL, p=0.02) and in the inactive, reduced energy intake condition (-4 mg/dL, p>0.05) compared to the active

condition (-14 mg/dL). Bey et al. (10) also reported that fasting TAG concentrations did not change after 12-18 hours of hindlimb unloading in rodents. Thus, these results suggest that short-term (i.e. ≤ 24 hours) inactivity has little effect on fasting TAG compared to the dramatic changes to postprandial TAG levels reported by Bey et al. (10).

Insulin resistance is often associated with metabolic inflexibility (i.e. impaired "switching" from primarily lipid oxidation to primarily carbohydrate oxidation in response to insulin stimulation (77)). Therefore, we hypothesized that the shift in the respiratory exchange ratio (RER) from the fasting to insulin-stimulated state (ΔRER) would be blunted after 24 hours of inactivity. Despite a 22% lower ΔRER in both inactive conditions compared to ACTIV, indicative of lower metabolic flexibility, these differences were not statistically significant. Overall, these data suggest that 24 hours of inactivity has more subtle effects on lipid metabolism (with the exception of the postprandial lipid response) and metabolic flexibility compared with the larger changes to whole-body insulin action.

In contrast, other studies employing longer-term inactivity protocols (i.e. days, weeks) report significant decrements in markers of lipid metabolism (8, 14). For example, Bergouignan et al. (8) reported a 37% increase in fasting TAG levels and an 8% decrease in palmitate oxidation following one month of bed rest in healthy women. Blanc et al. (14) reported even more dramatic reductions in fasting lipid oxidation (i.e. ~90%) in response to a shorter period of bed rest (i.e. 7 days) in healthy women and men (14). Thus, the majority of the detrimental effects of inactivity on lipid metabolism, metabolic flexibility, and insulin-mediated suppression of lipolysis may take at least one week or longer to manifest compared to the rapid effects of inactivity on insulin action.

5.7 Importance of non-exercise activity on metabolic health

Epidemiological evidence to date indicates the importance of low-intensity, *non-exercise* activities associated with daily living (e.g. standing, ambulating, household chores, etc.) on metabolic health (59, 61, 70). An important finding of the present study was the 20% reduction in fasting TAG levels in response to one day of high, non-exercise activity. The magnitude of this change is similar to the ~30% reduction in fasting TAG commonly reported following prolonged (i.e. ≥ 2 hours) of moderate exercise (27, 39, 47, 140). Interestingly, exercise duration appears to have a more potent effect on fasting plasma TAG levels than exercise intensity when total energy expenditure is held constant (24, 25, 80, 143). Data from the present study suggest that a sufficient quantity of low-intensity (i.e. < 4 METs) activities of daily living appears to lower fasting TAG as effectively as a prolonged bout of moderate exercise. To our knowledge, this is the first experimental study to provide direct evidence for a beneficial effect of high, non-exercise activity on metabolic health.

Thus, data from the current study extend the findings from cross-sectional studies that the health benefits of high daily energy expenditure on health can be gained via increases in non-exercise activity.

5.8 Limitations and control for confounding variables

The use of healthy, normal-weight, active subjects may limit the generalizability of the study results to the general population. We chose to study the metabolic response to inactivity in recreationally-active, but not highly-trained, individuals to eliminate a potential confounding impact of detraining on these responses. This population was also

selected in order to minimize potential confounding effects of disease processes often observed in overweight individuals who have low levels of physical activity.

Another limitation of the study is the short-term (24-hour) period of inactivity since short-term metabolic adaptations to inactivity with concurrent energy surplus may not be reflective of longer term changes to insulin action. Lack of significant changes to lipid metabolism in response to 24 hours of inactivity certainly suggests this is true. However, the decline in insulin action was similar in magnitude compared to other studies that were longer in duration (90, 100, 133, 137). Further, compared to longer-term studies, examining these responses over 24 hours was advantageous because it allowed for greater control over study conditions and eliminated potential confounding factors such as muscle atrophy and/or changes in body composition.

Energy balance and/or the amount of physical activity performed on the days leading up to each 24-hour intervention may have influenced the response to inactivity. To control energy balance, subjects were given all food to eat each day prior to the intervention (i.e. Day -1). Based on estimated energy intake and expenditure, we calculated energy balance for Day -1, which did not differ between conditions ($p > 0.05$). Therefore, we are confident that energy balance on Day -1 did not impact the metabolic response to the 24-hour protocol. To eliminate the potential confounding influence of prior exercise, subjects were asked to refrain from structured exercise for 2 days prior to each intervention. Based on *activ*Pal data, energy expenditure as well as total sitting, standing, and stepping time on Day -1 were not different between conditions. Therefore, prior physical activity had negligible impact on the response to inactivity.

Finally, given the large differences in energy intake (~-1000 kcal/d) and similar *activ*Pal data (i.e. total sitting and standing time) between the two inactive conditions we are confident that, as designed, these two groups were in different energy states. It is possible we may have underestimated energy expenditure in the active condition. Data from Crouter et al. (26) indicate the metabolic equivalent of standing is less than what the *activ*Pal software assumes (i.e. 1.19 vs. 2.0 MET/hr, respectively). If actual energy expenditure was lower than estimated, differences in insulin action between the active and inactive conditions would likely be minimized. However, the fact that we observed such a large reduction in insulin action in the inactive versus active condition suggests that our estimates approximated true energy expenditure. It is possible though that the difference between energy intake and expenditure (i.e. energy balance) in the active condition may have been larger than estimated (i.e. +162 ± 248 kcal/d), which could have impacted our ability to detect significant differences in insulin action between this condition and the inactive condition with reduced energy intake. If anything, the effect of inactivity may be even larger than what we observed.

5.9 Summary and practical implications

In summary, results of this study are consistent with data from both animal (119, 131) and human (8, 14, 91, 100, 133) studies indicating the clear, deleterious effects of inactivity on insulin action. However, our results extend these findings by implicating the important role for energy surplus in the inactivity-induced decline in insulin action. Thus, the detrimental effects of inactivity on insulin action can be minimized if energy intake is reduced to match energy expenditure during inactivity. Still, 47% of the decline in insulin action was *not* attributable to energy surplus suggesting that other mechanisms

are involved in the process. Additional research is necessary to determine the cellular and molecular mechanisms mediating the direct effect of inactivity on metabolism. Future studies examining the mechanism for the reduction in insulin action following inactivity should carefully control energy balance.

The dramatic reduction in insulin action within just one day of prolonged sitting suggests the importance of maintaining daily non-exercise activity to minimize detriments to metabolic health. In this regard, it may be prudent to develop public health strategies aimed at limiting sitting and increasing daily non-exercise activity. Since adults spend the majority of waking hours (i.e. > 90%) engaged in sedentary behaviors or light-intensity activities (61), decreasing time spent sitting and/or increasing non-exercise activities could substantially raise total daily energy expenditure and lead to improved metabolic health (33, 54, 89, 97, 148).

APPENDIX A

SCHEDULE OF ACTIVITIES DURING THE ACTIVE CONDITION

Arrival in lab:	**8 - 9 AM**		
	Min		MET value
	0:00-5:00	Sit and eat	1.3
	5:00-10:00	Sit and eat	1.3
Hour	10:00-15:00	Sit and eat	1.3
Hour 1	15:00-20:00	Stand and eat	2
	20:00-25:00	tidy up room	2.5
	25:00-30:00	Dishes	1.8
~8:00 AM	30:00-35:00	sweep	3
	35:00-40:00	fold laundry	2.3
	40:00-45:00	walk to parking lot	3.8
	45:00-50:00	walk	3.8
	50:00-55:00	board games/cards	2
	55:00-60:00	board games/cards	2
		Avg.	2.26
	9-10 AM		
	0:00-5:00	work @ computer	2
	5:00-10:00	work @ computer	2
	10:00-15:00	work @ computer	2
Hour 2	15:00-20:00	work @ computer	2
~9:00 AM	20:00-25:00	vacuuming	3.5
	25:00-30:00	vacuuming	3.5
	30:00-35:00	board games/cards	2
	35:00-40:00	board games/cards	2
	40:00-45:00	board games/cards	2
	45:00-50:00	Sit	1.3
	50:00-55:00	Sit	1.3
	55:00-60:00	Sit	1.3
		Avg.	2.08
	10-11 AM		
	0:00-5:00	work @ computer	2
	5:00-10:00	work @ computer	2
Hour 3	10:00-15:00	work @ computer	2
~10:00 AM	15:00-20:00	work @ computer	2
	20:00-25:00	work @ computer	2
	25:00-30:00	sweep	3
	30:00-35:00	sweep	3
	35:00-40:00	take out trash	3

	40:00-45:00	tidy up room	3
	45:00-50:00	Sit	1.3
	50:00-55:00	Sit	1.3
	55:00-60:00	Sit	1.3
		Avg.	2.16
	11 AM - 12 PM		
	0:00-5:00	Sit	1.3
	5:00-10:00	Sit	1.3
	0:00 - 15:00	Sit	1.3
	15:00-20:00	Stand	2
	20:00-25:00	Stand	2
Hour 4	25:00-30:00	stand	2
~11:00 AM	30:00-35:00	stand	2
	35:00-40:00	stand	2
	40:00-45:00	dust	2.4
	45:00-50:00	dust	2.4
	50:00-55:00	vacuuming	3.5
	55:00-60:00	vacuuming	3.5
			2.14
	12 PM - 1 PM		
	0:00-5:00	tidy up room	3
	5:00-10:00	sit and eat	1.3
	10:00-15:00	sit and eat	1.3
Hour 5	15:00-20:00	sit and eat	1.3
~12:00 PM	20:00-25:00	sit and eat	1.3
	25:00-30:00	sit and eat	1.3
	30:00-35:00	sit and eat	1.3
	35:00-40:00	stand and eat	2
	40:00-45:00	fold laundry	2.3
	45:00-50:00	fold laundry	2.3
	50:00-55:00	sweep	3
	55:00-60:00	sweep	3
			1.95
	1-2 PM		
	0:00-5:00	work @ computer	2
	5:00-10:00	work @ computer	2
	10:00-15:00	work @ computer	2
Hour 6	15:00-20:00	work @ computer	2
~1:00 PM	20:00-25:00	work @ computer	2
	25:00-30:00	work @ computer	2
	30:00-35:00	work @ computer	2
	35:00-40:00	vacuum	3.5
	40:00-45:00	vacuum	3.5

	45:00-50:00	put away groceries	2.5
	50:00-55:00	put away groceries	2.5
	55:00-60:00	sweep	3
			2.42
	2-3 PM		
	0:00-5:00	sweep	3
	5:00-10:00	tidy up room	2.5
	10:00-15:00	tidy up room	2.5
Hour 7	15:00-20:00	work @ computer	2
~ 2:00 PM	20:00-25:00	work @ computer	2
	25:00-30:00	darts	2.5
	30:00-35:00	darts	2.5
	35:00-40:00	darts	2.5
	40:00-45:00	darts	2.5
	45:00-50:00	darts	2.5
	50:00-55:00	darts	2.5
	55:00-60:00	darts	2.5
			2.46
	3-4 PM		
	0:00-5:00	stand	2
	5:00-10:00	stand	2
	10:00-15:00	vacuum	3.5
Hour 8	15:00-20:00	vacuum	3.5
~ 3:00 PM	20:00-25:00	darts	2
	25:00-30:00	darts	2
	30:00-35:00	darts	2
	35:00-40:00	darts	2
	40:00-45:00	darts	2
	45:00-50:00	darts	2
	50:00-55:00	darts	2
	55:00-60:00	darts	2
			2.25
	4-5 PM		
	0:00-5:00	darts	2.5
	5:00-10:00	darts	2.5
	10:00-20:00	darts	2.5
Hour 9	20:00-25:00	darts	2.5
	25:00-30:00	darts	2.5
~4:00 PM	30:00-35:00	darts	2.5
	35:00-40:00	darts	2.5
	40:00-45:00	darts	2.5
	45:00-50:00	darts	2.5
	50:00-55:00	Sit/catheter insertion	1.3

	55:00-60:00	Sit	1.3
			2.28
	5-6 PM		
	0:00-5:00	sit and eat	1.3
	5:00-10:00	sit and eat	1.3
	10:00-15:00	sit and eat	1.3
	15:00-20:00	sit and eat	1.3
	20:00-25:00	stand and eat	2
Hour 10	25:00-30:00	stand and eat	2
~5:00 PM	30:00-35:00	work at computer	2
	35:00-40:00	work at computer	2
	40:00-45:00	work at computer	2
	45:00-50:00	work at computer	2
	50:00-55:00	work at computer	2
	55:00-60:00	work at computer	2
			1.77
	6-7 PM		
	0:00-5:00	play games	2
	5:00-10:00	play games	2
	10:00-15:00	play games	2
Hour 11	15:00-20:00	play games	2
~6:00 PM	20:00-25:00	play games	2
	25:00-30:00	play games	2
	30:00-35:00	play games	2
	35:00-40:00	play games	2
	40:00-45:00	play games	2
	45:00-50:00	walk	3.8
	50:00-55:00	walk	3.8
	55:00-60:00	Sit	1.3
			2.24
	7-8 PM		
	0:00-5:00	Sit	1.3
	5:00-10:00	play games	2
	10:00-15:00	play games	2
Hour 12	15:00-20:00	play games	2
~7:00 PM	20:00-25:00	play games	2
	25:00-30:00	play games	2
	30:00-35:00	play games	2
	35:00-40:00	play games	2
	40:00-45:00	play games	2
	45:00-50:00	play games	2
	50:00-55:00	Sit	1.3
	55:00-60:00	Sit	1.3

			1.83
	8-9 PM		
	0:00-5:00	walk outside	3.8
	5:00-10:00	stand outside	2
	10:00-15:00	stand outside	2
Hour 13	15:00-20:00	walk inside	3.8
~8:00 PM	20:00-25:00	work on computer	2
	25:00-30:00	work on computer	2
	30:00-35:00	work on computer	2
	35:00-40:00	work on computer	2
	40:00-45:00	work on computer	2
	45:00-50:00	Sit	1.3
	50:00-55:00	Sit	1.3
	55:00-60:00	Sit	1.3
			2.13
	9-10 PM		
	0:00-5:00	Sit	1.3
	5:00-10:00	Sit	1.3
	10:00-15:00	Sit	1.3
Hour 14	15:00-20:00	play games	2
~9:00 PM	20:00-25:00	play games	2
	25:00-30:00	play games	2
	30:00-35:00	play games	2
	35:00-40:00	play games	2
	40:00-45:00	play games	2
	45:00-50:00	play games	2
	50:00-55:00	play games	2
	55:00-60:00	play games	2
			1.83
	10-11 PM		
	0:00-5:00	sit	1.3
	5:00-10:00	sit	1.3
	10:00-15:00	sit	1.3
Hour 15	15:00-20:00	walk to bathroom	3.8
~10:00 PM	20:00-25:00	self care	2
	25:00-30:00	self care	2
	30:00-35:00	walk to lab	3.8
	35:00-40:00	stand	2
	40:00-45:00	stand	2
	45:00-50:00	sit	1.3
	50:00-55:00	sit	1.3
	55:00-60:00	sit	1.3

			1.95
	11-12 PM		
	0:00-5:00	play games	2
	5:00-10:00	play games	2
	10:00-15:00	play games	2
Hour 16	15:00-20:00	play games	2
~11:00 PM	20:00-25:00	Sit	1.3
	25:00-30:00	Sit	1.3
	30:00-35:00	Sit	1.3
	35:00-40:00	Sit	1.3
	40:00-45:00	Sit	1.3
	45:00-50:00	Sit	1.3
	50:00-55:00	Sit	1.3
	55:00-60:00	Sit	1.3
			1.53
	Total AVG		**2.08**

APPENDIX B

TABLES AND FIGURES

Table A.1 Energy balance and *activ*Pal data for Day -1 (M ± SD).

	Sitting Time (hrs)	**Standing Time (hrs)**	**Stepping Time (hrs)**	**Sleep Time (hrs)**	**Energy Expenditure (kcals/day)**	**Energy Intake (kcals/day)**	**Energy Balance (EI-EE)**
ACTIV	10.1 ± 2.1	3.8 ± 1.9	1.5 ± 1.0	8.3 ± 1.9	2497 ± 438	2420 ± 478	-77 ± 277
INACTIV	9.6 ± 2.5	3.7 ± 1.6	1.5 ± 0.5	9.1 ± 2.0	2311 ± 456	2157 ± 455	-87 ± 164
INACTIV LO-CAL	10.7 ± 2.2	3.1 ± 1.6	1.6 ± 0.8	8.6 ± 1.1	2430 ± 450	2338 ± 493	-92 ± 145

Figure A.1 Relationship between energy balance on Day -1 and insulin action measured 24 hours following the intervention.

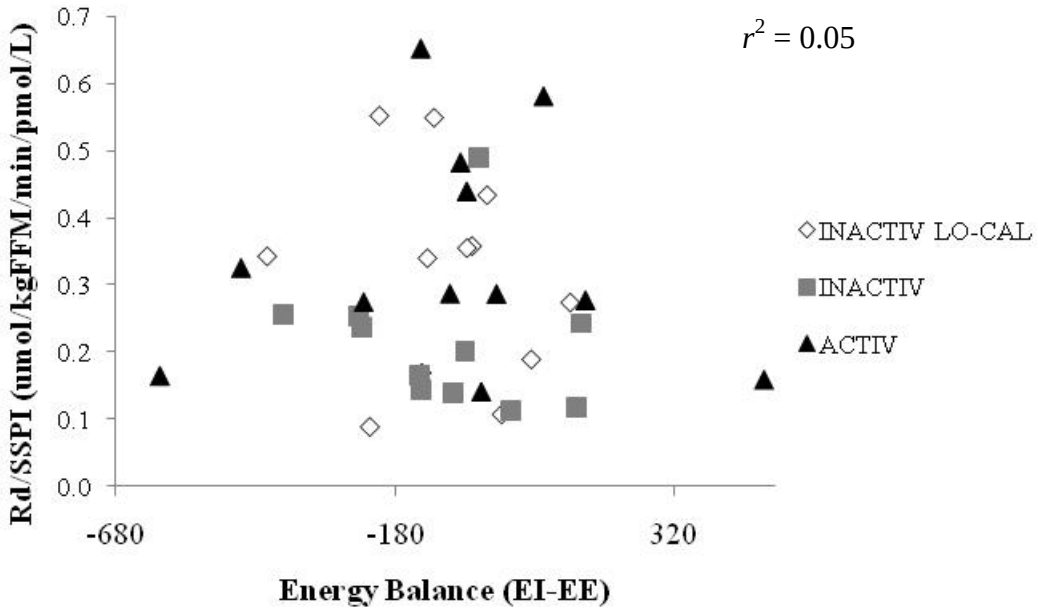


Figure A.2 Relationship between energy balance during the 24-hour intervention (Day 1) and insulin action measured the following morning.

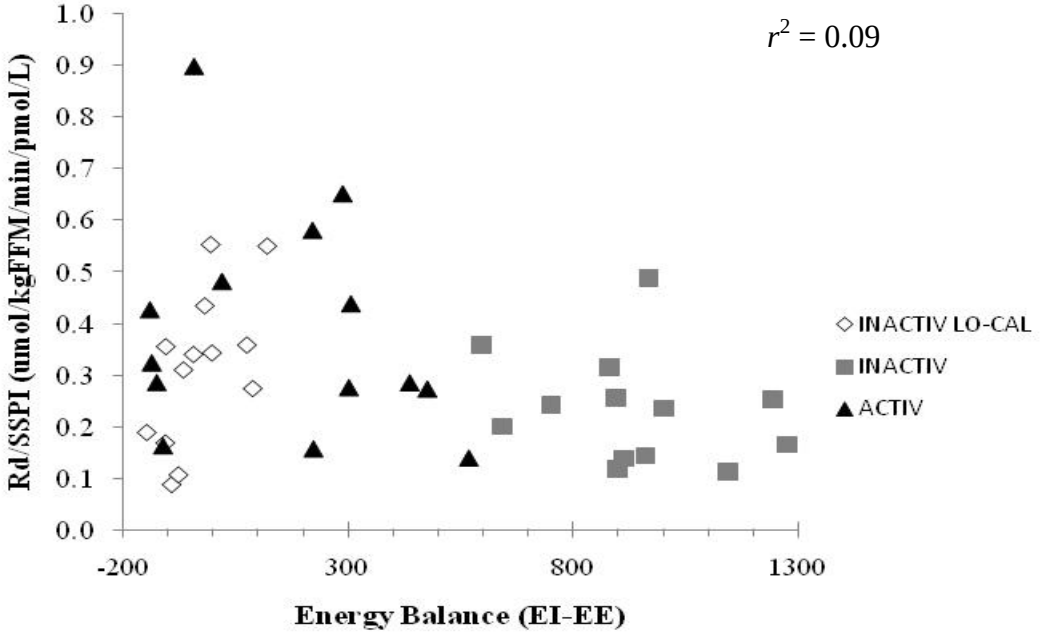


Figure A.3 Relationship between energy content of the evening meal (% total daily energy intake) during the 24-hour intervention (Day 1) and insulin action measured the following morning.

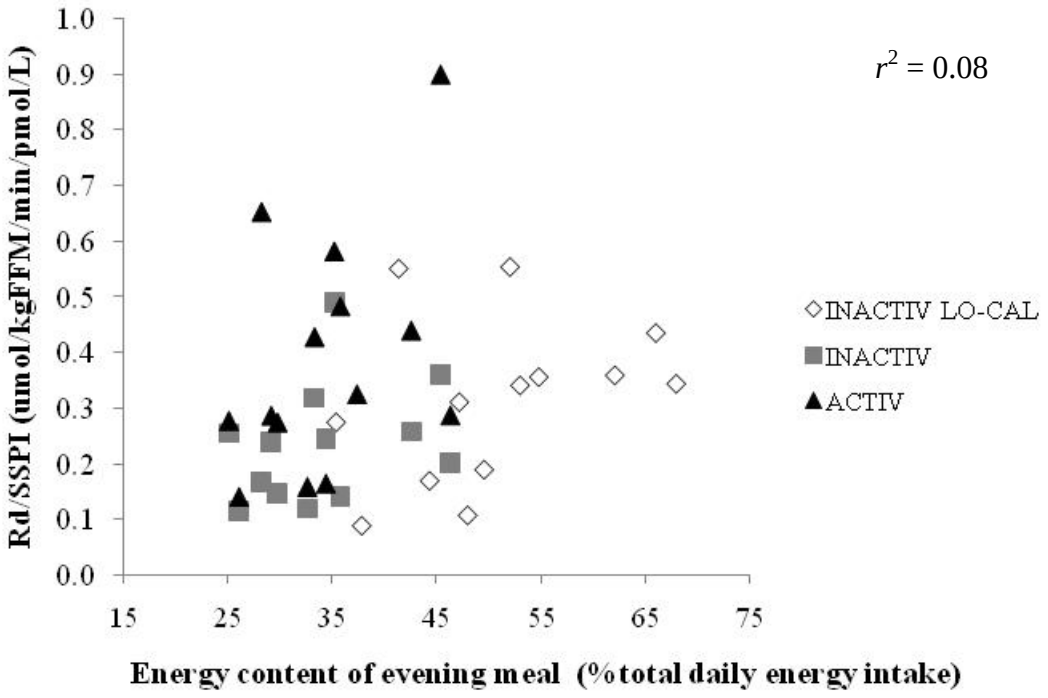
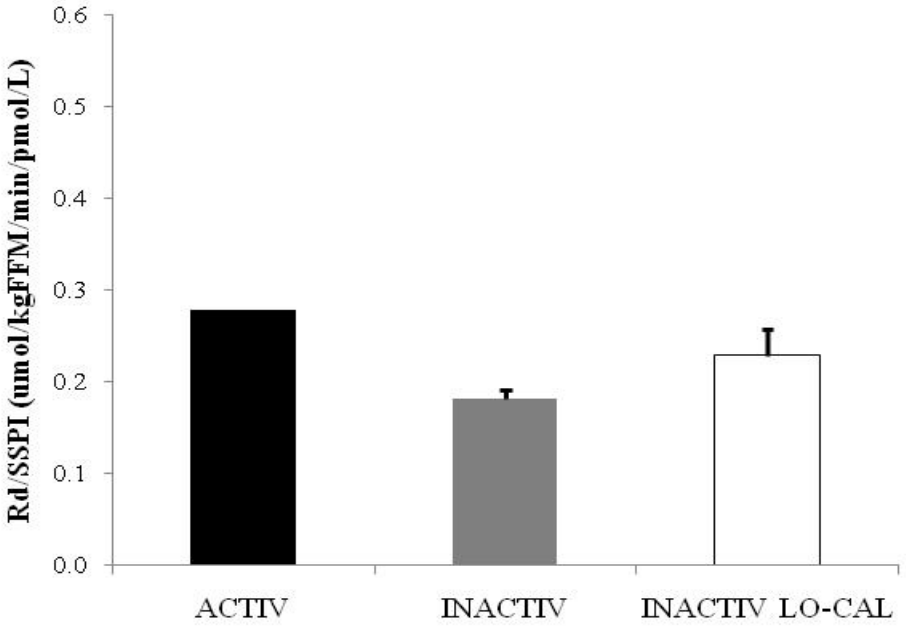


Figure A.4 Insulin action (R_d/SSPI) across condition in men (**a**) and women (**b**).

a.



b.

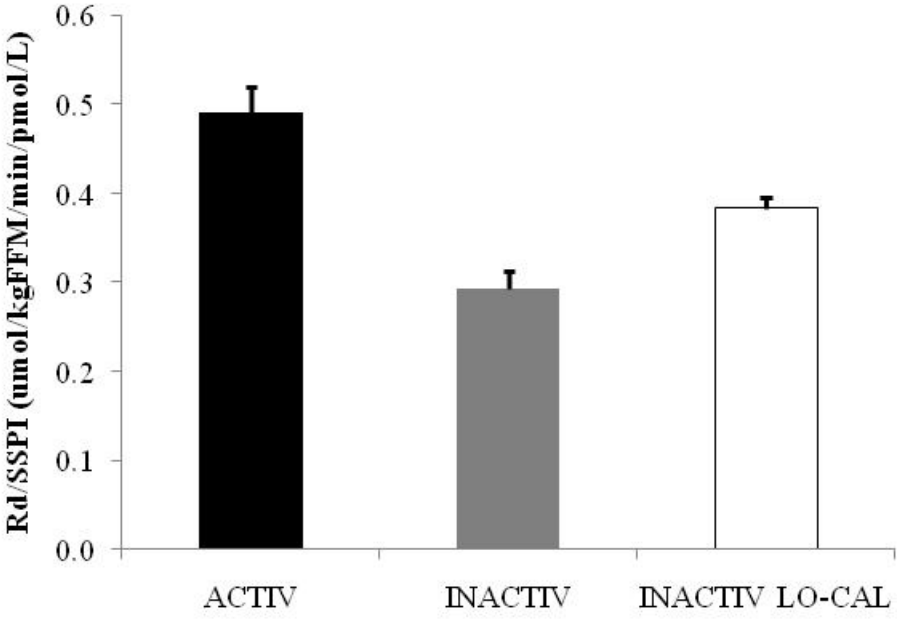
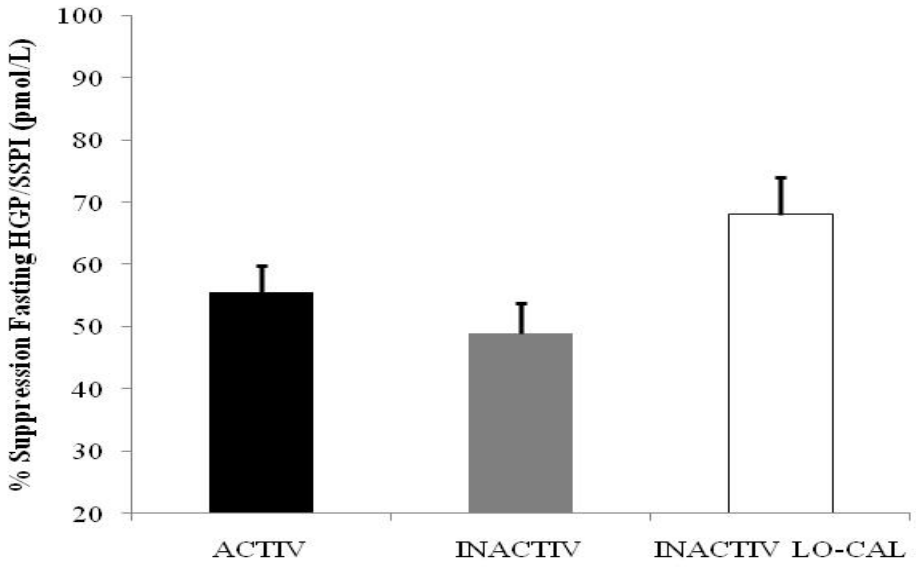
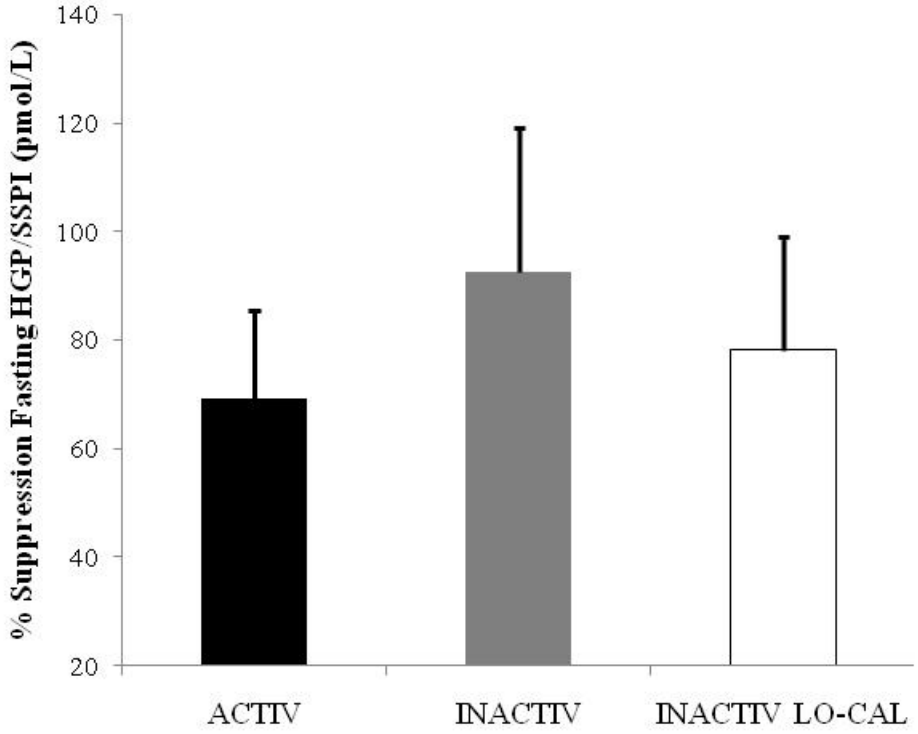


Figure A.5 Hepatic insulin action (% suppression fasting hepatic glucose production) in men (**a**) and women (**b**).

a.



b.



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