VIGOROUS PHYSICAL ACTIVITY, HEREDITY, AND MODULATION OF RISK FOR OBESITY AND TYPE 2 DIABETES IN POSTMENOPAUSAL WOMEN.

by

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This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

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This is a part of a large randomized controlled trial designed to examine the effects of resistance training on bone density in postmenopausal women, The Bone Estrogen and Strength Training Study (BEST). The present study added fasting plasma glucose, insulin, and non-esterified fatty acids to existing measures in the main trial. In addition, an ancillary genetics study was added to the BEST trial for this work. Volunteer, one-year completers of the BEST Study were re-consented and genotyped for adrenergic receptor variants. I would like to acknowledge all of the BEST staff and participants for their dedication to this work and an extraordinary dissertation committee that was generous with time, knowledge, resources, and guidance. Support for this work has been provided by the National Institutes of Health (NIH AR 39559) and the 2005 Gatorade Sports Science Institute Student Grant Award. Research materials were also generously donated by Proctor and Gamble and Mission Pharmacal. It is not possible to acknowledge everyone who contributed to this dissertation, but the following list encompasses individuals that deserve special mention:

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“I dedicate this work to my family for their constant love and support throughout my education. They are the ultimate cheering section.”

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ABSTRACT

Both obesity and type 2 diabetes are significant health burdens in our society. The prevention of these conditions is vital to individual health and to the health care system, which is inordinately stressed by these chronic diseases. Due to variations in individual response to interventions, prevention strategies may require some tailoring based on heritable traits.

The objective of this study was to determine whether insulin sensitivity could be altered by resistance training, and further if body composition or insulin sensitivity response to resistance training in postmenopausal women may be influenced by adrenergic receptor genetic variants and gene-gene interactions.

Completers of a 12-month randomized controlled trial of resistance training in sedentary post-menopausal (PM) women, using or not using hormone therapy, were measured for fasting plasma glucose, insulin, and non-esterified fatty acids (NEFA) at baseline and one year. These biomarkers were used to compute models of insulin sensitivity. Body composition was measured by dual x-ray absorptiometry. Subjects were also re-consented for genotyping of adrenergic receptor (ADR) gene variants, ADRA2B $Glu^{9/12}$, ADRB3 Trp64Arg, ADRB2 Gln27Glu.

The resistance training intervention did not have an overall effect on insulin sensitivity in the largest sample and change in insulin sensitivity was largely dependent body composition. There were small favorable effects of genotype on initial measures of both body composition and insulin sensitivity in the ADRA2B Glu^{9+} carriers versus non-carriers. The effects of ADRA2B alone were no longer present following intervention,

but ADRB3 Arg^{64+} and ADRB2 Glu^{27+} contribute to improved insulin sensitivity with exercise, when accounting for body composition. ADRB2 Glu^{27+} was the key to improved biomarkers of insulin sensitivity when in combination with ADRA2B Glu^{9+} or ADRB3 Arg^{64+} and a model of insulin sensitivity was most improved by the combination ADRB3 Arg^{64+} by ADRB2 Glu^{27+}, compared to other ADRB3 by ADRB2 combinations.

This is the first trial of ADRA2B, ADRB3, and ADRB2 genetic variation combinations and resistance training in postmenopausal women relative to body composition and insulin sensitivity. Some specific genotypes were identified as responders and non-responders to exercise. These data support independent associations between body composition and insulin sensitivity and the ADR gene variants.

CHAPTER 1

INTRODUCTION

This study is part of a large randomized controlled trial in postmenopausal women, designed to examine the effects of resistance training on bone mineral density. The present study added measurements of fasting plasma glucose, insulin, and free fatty acids (biomarkers of insulin sensitivity), at baseline and 12 months, to existing measures from the Bone Estrogen and Strength Training (BEST) study. The author of this dissertation also contributed to the main intervention by performing anthropometric measurements for long-term follow-up, as well as design and execution of the ancillary genetics study. Adrenergic receptor (ADR) genotypes for the following ADR allelic variants were added to the BEST study for those re-consented for the ancillary study: ADRA2B $Glu^{9/12}$, ADRB3 Trp64Arg, ADRB2 Gln27Glu.

EXPLANATION OF THE PROBLEM AND ITS CONTEXT

The first manuscript examined the relationships between baseline body composition, adrenergic receptor variants, ADRA2B Glu9+, ADRB3 Arg64+, and ADRB2 Glu27+, and their gene-gene interactions, as well as body composition response to 12 months of resistance training. Cross-sectional and case matched studies have investigated inter-individual body composition variation with respect to these genes and have found varying results between populations when associating body composition or fitness to adrenergic receptors.[126, 175] However, the weight of the evidence falls in favor

of the ADRA2B Glu^{9+}, ADRB3 Arg^{64+}, and ADRB2 Glu^{27+} variants associating with obesity, compared to non-carriers of these variants. [126, 175]

Few have reported results for these adrenergic receptor variant gene-gene interactions or responsiveness to intervention.[27, 121, 126, 176] The interaction between ADRA2b and ADRB3 was cross-sectionally observed in obese middle-aged women.[27] Dionne et, al found that carriers of ADRA2B Glu^{9+} + ADRB3 Arg^{64+} were fatter than Arg^{64+} carriers alone. [27] Another trial, including an endurance training intervention in 70 older men and women, investigated the interactions of the ADRA2B Glu$^{9/12}$, ADRB2 Gln27Glu and ADRB3 Trp64Arg as modifiers of exercise induced weight loss.[121] Phares, et al found that the ADRA2B Glu^{9-} by ADRB3 Arg^{64+}, the ADRA2B Glu^{9-} by ADRB2 Glu^{27+}, and the ADRB3 Arg^{64+} by ADRB2 Glu^{27+} combinations lost significantly more total and trunk fat than other variant combinations. [121] These findings indicate that certain genotypes may be better responders to behavior or environment than others, since, for example, both obesity and enhanced fat loss are associated with ADRB3 Arg^{64+} genotype. The literature is lacking in confirmatory trials, thus these genotypes and gene-gene interactions in relationship to body composition require further investigation. There are no trials of these combinations with respect to resistance training interventions, this study will be the first.

The second manuscript examined the effects of 12 months of resistance training on biomarkers and models of insulin sensitivity, as well as examining the baseline contributors to initial insulin sensitivity. Aerobic training trials, of a variety of designs, have yielded improvements in insulin sensitivity,[78] but, despite a number of trials of

indicating improved insulin sensitivity in response to resistance training, there are few randomized controlled trials and fewer with female subjects. [17, 25, 31, 107, 108, 150, 179] It has been previously demonstrated that counter-regulatory responses to exercise are sexually dimorphic,[20-22, 38] thus the effects of resistance training on insulin sensitivity should be further investigated in postmenopausal women with and without hormone therapy. Additionally, if we are to prevent type 2 diabetes, rather than be forced to treat the frank disease, changes in biomarkers of insulin sensitivity in a healthy, but older, population must be done; most of the trials mentioned above involve populations that are already compromised by obesity or insulin resistance, [78, 177] others have been performed in youthful healthy populations that are not at risk.[24, 122] Our middle-aged study population was in general good health, non-obese, and demonstrated primarily normal fasting glucose levels.

The second manuscript also examined the dose of resistance training required for a positive impact on insulin sensitivity. The effect of dose of training on insulin sensitivity has not been well studied, particularly with respect to resistance training. Moderate endurance training, such as brisk walking for an hour per day has been shown to reduce risk of diabetes,[67] but it is not clear whether increased intensity or frequency can further reduce the risk. The Nurses' Health Study indicated that different intensities of endurance training did not produce significantly different reductions in type 2 diabetes risk if energy expenditure was equivalent.[68] Similarly, light or moderate resistance training paired with aerobic training resulted in similar improvements in insulin sensitivity in a randomized controlled trial in men,[25] but when compared to control, only

the moderate resistance training group demonstrated significant improvements in insulin sensitivity.[25] It is not yet clear whether varying doses of resistance training alone, in postmenopausal women, contribute to the variance in exercise induced changes in insulin sensitivity biomarkers. The BEST study will indirectly assess dose by utilizing measures of weight lifted and session attendance post-hoc.

Lastly, the impact of oral hormone therapy on biomarkers of insulin sensitivity was examined in the second manuscript. The literature, regarding the effect of HT on insulin sensitivity, is inconsistent. The Women's Health Initiative demonstrated a reduced risk of developing type 2 diabetes with HT use [96] and others have found that HT users were more insulin sensitive than non-users.[51, 158] However, The HERITAGE Family Study found that HT users and non-users were not significantly different either at baseline or post-exercise intervention in terms of fasting biomarkers of insulin sensitivity, plasma insulin and glucose.[50] In contrast, cross-sectional findings from the PEPI trial indicate that endogenous, bioavailable estradiol is associated with insulin resistance (odds ratio, 2.7; $p<0.001$).[76] Therefore, our study either accounted for HT status as a covariate or we stratified some analyses by HT to examine insulin sensitivity differences between HT users and non-users.

The third manuscript integratively examines how the adrenergic receptor variants may alter the body composition and the insulin sensitivity responses to resistance training. The adrenergic receptor variants of interest in this study, ADRA2B $Glu^{9/12}$, ADRB3 Trp64Arg, and ADRB2 Gln27Glu, have been associated separately with body composition [13-16, 27, 29, 42, 57, 59, 63, 74, 88, 95, 97, 100, 103-105, 112, 116, 119, 121, 138, 143, 157, 164, 167, 181] and

insulin sensitivity.[16, 32, 36, 74, 86, 88, 93, 94, 104, 115, 137, 138, 142, 146, 168, 170, 171, 181] Because body composition has such a great influence on insulin sensitivity, the adrenergic receptor variants may be working through changes in fat and lean tissue to affect changes in insulin sensitivity. Previously published cross-sectional or case match studies vary. Some show no effect,[29, 37, 57, 61, 63, 65, 72, 80, 99, 100, 105, 106, 110, 116, 131, 144, 147, 151, 157, 159-161, 163, 165, 178, 181] while others state that carriage of some of these adrenergic receptor gene polymorphisms are detrimental to insulin sensitivity.[16, 32, 74, 86, 88, 93, 94, 104, 115, 137, 138, 142, 146, 168, 170, 171, 181] Some of the inconsistent findings may be due to whether or not the modulating effects of the adrenergic receptor polymorphisms on insulin sensitivity also accounted for their modulating effects on body composition. The present study shows the modulating effects of adrenergic receptor gene variation on the body composition and on the insulin sensitivity responses to resistance training through progressively complex statistical models.

In addition, none of the few trials of physical activity alone, with out dietary-induced weight loss, which investigates the effects of ADRB3 Trp64Arg or ADRB2 Gln27Glu genotypes on the insulin sensitivity responses to physical activity [36] are randomized controlled trials, none include women with the ADRA2B $Glu^{9/12}$ variants. Cross-sectional studies of the ADRA2B $Glu^{9/12}$ variant in women have reported no genotypic differences in either insulin sensitivity or type 2 diabetes risk. [147, 159, 160, 163, 181] By contrast, short term endurance exercise interventions, including either the ADRB3 Trp64Arg or ADRB2 Gln27Glu variants have shown that homozygotes of either single nucleotide polymorphism (Arg64Arg or Glu27Glu) had unfavorable insulin sensitivity

responses to training. [75, 93] Therefore, the exact modulating effects of these adrenergic receptor genes on the insulin sensitivity responses to resistance training are unknown, which in turn, is an important knowledge gap to be filled by the present study.

GENERAL STUDY AIMS

The present study was designed to test the hypothesis that adrenergic receptor variation influences body habitus, insulin sensitivity, and their responses to resistance training. We hypothesized that strength training would improve body composition and insulin sensitivity in a dose-dependent manner, and that genetic variation in the adrenergic receptors would modulate the training-related changes in body composition and insulin sensitivity thereby partially accounting for the wide inter-individual variability in responses to training.

Baseline cross-sectional outcomes and 12-month longitudinal outcomes were included in this study. Based on the recruitment criteria, the baseline measures provided cross-sectional data in an inactive population. The one-year measures allowed us to look at the impact of being assigned to either resistance training exercise (EX) or control (NEX) on primary outcomes.

Measurements of fasting plasma glucose, insulin, and non-esterified fatty acids were made at baseline and at 12 months to quantify insulin sensitivity. These biomarkers were used individually as indicators of insulin sensitivity, as well as used in models of insulin sensitivity, such as the homeostasis model for insulin resistance (HOMA-IR), the quantitative insulin sensitivity check index (QUICKI), and the revised QUICKI (R-

QUICKI). Statistical analyses were also performed, without accounting for genetic variability in the adrenergic receptors, to determine the role of resistance training on biomarkers of insulin sensitivity, after statistically controlling for inter-individual differences in age, hormone therapy, initial biomarker values, initial body composition and changes in body composition. Adrenergic receptor allelic variation, with respect to ADRA2B $Glu^{9/12}$, ADRB3 Trp64Arg, and ADRB2 Gln27Glu, was assessed to quantify the extent to which body composition, insulin sensitivity, and their responses to resistance training may be modulated by the inherited variation in these genes.

The first aim was to evaluate the impact of genetic variation in adrenergic receptors, ADRA2B, ADRB3, and ADRB2, on body composition in sedentary and in resistance-trained postmenopausal women. The second aim was to evaluate the effect of strength training on biomarkers of insulin sensitivity and to explore the interaction between strength training-related changes in body composition and strength training-related changes in biomarkers of insulin sensitivity. The third aim was to evaluate the relationship between the dose of exercise completed, as assessed by exercise compliance, and the magnitude of the training-related changes in insulin sensitivity markers. The fourth aim was to evaluate the extent to which the adrenergic receptor polymorphisms modulated insulin sensitivity at baseline, as well as the insulin sensitivity responses to strength training. The final aim was to quantify the differences in all body composition and insulin sensitivity outcomes between hormone therapy (HT) users and non-users.

SIGNIFICANCE

Despite increased public awareness of Healthy People 2010 goals, such as increasing physical activity and improving body weight management, the prevalence of diabetes has reached an all time high in the United States (20.8 million),[2] and incidence rates continue to rise at a cost (direct and indirect) of $132 billion per year.[26] Type 2 diabetes accounts for 90% to 95% of these cases and typically begins with the loss of insulin sensitivity or insulin resistance.[26] Both insulin resistance and type 2 diabetes have been strongly associated with cardiovascular disease,[128] which is the leading cause of death in the United States.[73] Obesity and physical inactivity,[128] which precipitate insulin resistance, are leading causes of preventable death.[111] These reasons, coupled with recognition of the insulin resistant metabolic syndrome as a billable diagnosis, have made the primary and secondary prevention of insulin resistance and type 2 diabetes national priorities.

The rising prevalence and cost of type 2 diabetes, as well as its association with many other maladies, mandate targeted prevention options, such as physical activity programs tailored toward hereditary tendencies. There is a scarcity of randomized controlled trials investigating the effects of resistance training on biomarkers of insulin sensitivity, particularly in post-menopausal women, who are a at greater risk of type 2 diabetes and cardiovascular disease than pre-menopausal women due to the loss of endogenous ovarian estrogens and due to the increased deposition of intra-abdominal fat.[113, 156] Lastly, resistance training trials [18, 25, 122, 154] are also less common than endurance training trials[78] that are aimed at improving insulin sensitivity in post-

menopausal women. Furthermore, there are no previous reports in the literature, to our knowledge, which examine how genetic variation in the adrenergic receptors may modulate the body composition and the insulin sensitivity responses to resistance training.

All of the manuscripts included herein address the paucity of resistance training research in postmenopausal women, within the context of a randomized controlled trial. The second manuscript (Appendix B) evaluates the efficacy of resistance training for improving insulin sensitivity in individuals with normal and impaired fasting glucose, and it also identifies the degree of compliance to the prescribed dose of resistance training that is required to induce improvements in insulin sensitivity, a relationship rarely discussed in the literature.

The contributions of the specific genes to such inter-related outcomes as body composition and insulin sensitivity, within the same population, have only recently emerged.[29, 37, 57, 61, 63, 65, 72, 74, 75, 86, 88, 99, 100, 105, 106, 114-116, 131, 137, 138, 142, 151, 157, 161, 164, 165, 168-171, 178] In addition, the contribution of adrenergic receptor gene-gene interactions on either body composition or insulin sensitivity are just beginning to appear in the literature.[41, 99, 104, 110, 114, 121, 165-167] To fill these gaps in the literature, the first manuscript (Appendix A) investigated the single gene, as well as the gene-gene interactive, influences of the ADRA2b Glu^{9+}, ADRB3 Arg^{64+}, and ADRB2 Glu^{27+} genetic variants on body composition and on the body composition responses to resistance training. The third manuscript (Appendix C) examines the single gene, as well as the gene-gene interactions,

of the adrenergic receptor variants as modulators of the body composition and the insulin sensitivity responses to resistance training.

Since there may be many mediators and modulators of insulin sensitivity, this study has built onto a completed randomized controlled trial of exercise that targeted maintenance of bone density in postmenopausal women with strength training by exploring the behavioral mediators and the heritable modulators of insulin sensitivity. By capitalizing on a completed trial, it serves as an efficient and cost-effective approach to facilitate further research, and it contributes to a bourgeoning field investigating specific ancestral influences on the body composition and the insulin sensitivity responses to exercise that build on the former cross-sectional snap-shots of genetic tendencies. The long-term goal of this line of research is to develop targeted physical activity regimens that lead to optimal body composition and biochemical profiles for the primary and secondary prevention of insulin resistance and type 2 diabetes, in the at-risk population of postmenopausal women. To our knowledge, this is the first report of how the ADRA2B Glu^{9+}, ADRB3 Arg^{64+}, and ADRB2 Glu^{27+} genetic variants and their combinations may modulate the resistance training-related responses in body composition and in biomarkers of type 2 diabetes in postmenopausal women.

REVIEW OF THE LITERATURE

Cardiovascular disease and diabetes

Cardiovascular disease and type 2 diabetes are major health concerns in the Unites States and abroad,[4, 136] with common antecedents of obesity and physical inactivity.[90, 128, 173, 174] Although cardiovascular disease is still the number one killer in the United States, [73] type 2 diabetes is also a significant contributor to mortality.[2] However, where cardiovascular disease may be considered a "silent killer", i.e. the first sign of the disease may be a deadly cardiac event,[58, 84, 141] type 2 diabetes is accompanied by a host of maladies, particularly in advanced stages, such as retinopathy, neuropathy, nephropathy, and amputations.[2] Type 2 diabetes mellitus is also considered a significant contributor to cardiovascular disease (CVD).[2, 6, 30, 48, 55, 69, 70] The substantial evidence for the significant contribution of diabetes to cardiovascular disease includes cerebrovascular events, like stroke, [62, 89] as well as, general cardiovascular disease diagnosis, events, and mortality, [6, 30, 48, 55] particularly in women. [70] In recent years, diabetes has even come to be considered a cardiovascular disease risk equivalent. [55] Elevated risk of CVD may even occur prior to diagnosis of type 2 diabetes.[69]

The risk of CVD and diabetes is further elevated in postmenopausal women due to loss of cardio- protective estrogen,[113] increased central adiposity, [156] and decreased physical activity.[3, 11, 124, 153] Therefore, it is particularly important to address diabetic pre-cursors such as adverse body composition and physical inactivity in this population.

There is also a great social and financial burden associated with the treatment of diabetes (14 million hospital days, 30 million physician visits [152], $132 billion total direct

and indirect costs[2, 152]) and cardiovascular disease. Since the prevalence of type 2 diabetes has doubled over the last 15 years to 20.8 million,[44] and it is inextricably linked to cardiovascular disease, a greater understanding of type 2 diabetes and its prevention are critical for curtailing cardiovascular events.

Clinical interpretation of biomarkers of insulin sensitivity

Fasting plasma glucose levels are "normal" if <100mg/dl and are "impaired" if between 100-125mg/dl. Fasting plasma glucose levels >125mg/dl indicate provisional type 2 diabetes. If a repeat measure is also >125mg/dl, then type 2 diabetes is diagnosed, and appropriate treatment options should be employed.[2]

Fasting plasma insulin in a normal population ranges from 5-15uU/ml. Insulin may be elevated in the presence of normal fasting glucose, indicating some degree of insulin resistance. Fasting glucose levels may also be high and be accompanied by normal or lowered fasting insulin levels, indicating the inability of the pancreas adequately compensate for the peripheral tissue resistance to insulin by maintaining normal glucose levels with the upregulation of insulin production and release.[129] However, fasting measures of insulin and glucose largely reflect hepatic insulin sensitivity. By contrast, the hyperinsulinemic-euglycemic clamp, the oral glucose tolerance test, or the frequently sampled intravenous glucose tolerance tests are better indicators of whole body insulin sensitivity.[10]

Expected normal values for fasting non-esterified fatty acids range from 0.1-0.6 mEq/L. Fatty acids in circulation have also been associated with insulin sensitivity; higher levels of circulating non-esterified fatty acids inhibit proper insulin action at the

skeletal muscle and pancreas.[129] High levels of non-esterified fatty acids in circulation also reflect insulin resistance in fat tissue, as insulin is less effective at inhibiting lipolysis.

Models of insulin resistance and insulin sensitivity were computed in the present study utilizing the fasting measures above. The homeostasis model for insulin resistance (HOMA-IR), the quantitative insulin sensitivity check index (QUICKI), and the revised QUICKI (R-QUICKI) are all included. The formulas are as follows:

$$\text{HOMA-IR} = (\text{insulin}_{0\ (\text{uU/ml})} * \text{glucose}_{0\ (\text{mmol/l})}) / 22.5$$

$$\text{QUICKI} = 1/(\log(\text{glucose}_{0\ (\text{mg/dl})}) + \log(\text{insulin}_{0\ (\text{uU/ml})}))$$

$$\text{R-QUICKI} = 1/(\log(\text{glucose}_{0\ (\text{mg/dl})}) + \log(\text{insulin}_{0\ (\text{uU/ml})}) + \log(\text{NEFA}_{0\ (\text{mmol/l})}))$$

These models have been highly correlated with the minimal model of insulin sensitivity, from frequently sampled intravenous glucose tolerance tests, in healthy adults. The Pearson's correlation coefficient r for each is as follows: HOMA-IR r = -0.50 ($p=0.009$) QUICKI r = 0.51 ($p=0.007$), R-QUICKI r = 0.67 ($p<0.001$).[10] A higher HOMA-IR score is indicative of greater degrees of insulin resistance and lesser degrees of insulin sensitivity. By contrast, higher QUICKI and R-QUICKI scores are indicative of greater degrees of insulin sensitivity. Thus, training-related improvements in insulin sensitivity would be indicated by reductions in the HOMA-IR score and by increases in the QUICKI and the R-QUICKI scores.[10]

Body composition, insulin sensitivity, and risk of type 2 diabetes

Although most insulin resistant individuals are also overweight or obese, not all overweight/obese are insulin resistant.[128] In addition, insulin mediated glucose disposal is estimated to be impaired in as many as 25% of the normal non-diabetic population.[129] These ideas contradict the previously held belief that insulin resistance, and accompanying metabolic abnormalities, are simply manifestations of obesity.[128] Insulin resistance may be more highly associated with body composition rather than weight.

Previous weight loss studies, with or without physical activity, have demonstrated significant improvements in insulin sensitivity. [78, 83, 177] However, a recent review by Reaven et al has revealed that in healthy non-diabetic, normotensive individuals, approximately 50% of the variability in insulin mediated glucose disposal can be accounted for, equally, by overweight/obesity (25%) and physical inactivity (25%). [128] It is possible that lack of physical activity in weight comparable individuals may shift body composition toward greater fat and less lean mass, or a lower quality of lean mass, and thus lead to loss of insulin sensitivity or type 2 diabetes.

Reductions in fat mass and waist circumference have been associated with improvements in insulin sensitivity. [46, 133] More specific measures of regional fat, have been able to explain more of the variance in insulin sensitivity between individuals.[47, 79] Goodpaster et al studied nearly 3,000 older men and women and found that high levels of intramuscular or visceral abdominal fat increased the risk of type 2 diabetes, even in normal weight men and women.[47] Skeletal muscle triglyceride content also appears to be associated with insulin resistance, although the technology for in vivo measurements is

still evolving. [46]

Kelley et al further explored the subdivision of abdominal fat and found that the deep subcutaneous adipose tissue behaves similarly to visceral adipose tissue, and that both are functionally distinct from superficial subcutaneous adipose tissue in terms of their contributions to the metabolic profile and in response to the euglycemic-hyperinsulinemic clamp in lean and obese, glucose tolerant men and women. Deep subcutaneous adipose tissue was more strongly related to insulin resistance than superficial adipose tissue. [79]

The impact of body composition on insulin sensitivity is also demonstrated in intervention studies. Stewart et al[154] also used resistance training in combination with aerobic training versus control and found significant associations between favorable changes in body composition, particularly abdominal fat by MRI, and improved markers of insulin sensitivity, although there were no overall differences between groups. [154] Another study found that enhanced insulin sensitivity with training (aerobic, resistance, or control) was dependent on increased lean mass for the resistance trained group.[122]

Body composition and lifestyle

The increase in our national weight is of concern due to the growing associations between obesity and other chronic diseases with high morbidity, mortality, and cost, such as type 2 diabetes, cardiovascular disease, several cancers, and more.[1] The total costs of overweight and obesity combined was estimated at $92.6 billion per year in 2002.[33] Lifestyle factors, such as the lack of portion control, the high accessibility of food, and the lack of physical activity, as well as many other understudied lifestyle factors, have all

been associated with increasing weight in the United States and around the world.[77] Postmenopausal (PM) women are of particular interest, due to the increase in central adiposity after menopause [156] and due to age related reductions in physical activity.[3, 11, 124, 153] It has been estimated that lack of. physical activity and excess weight are responsible for 31% of the overall mortality in women. [71]

Traditionally, weight problems have been addressed with behavior modification programs, which include diet and exercise. However, many of these programs target weight loss, rather than favorable shifts in body composition. Nevertheless, large populations have been able to lose weight via diet and/or exercise, for at least a short period of time, without regain.[5, 120] In recent years, trials of physical activity have evaluated specific total and regional body composition outcomes. The summary of several aerobic and resistance training trials, allows us to conclude that physical activity promotes a favorable shift in body composition, towards decreased fat mass and increased lean mass, although some weight change usually accompanies the training.[172] It is important to consider that both fat and lean mass are lost, as companions, with weight loss programs,[34, 35] which may confer different health-related outcomes than programs that are capable of altering body composition without weight loss.

The BEST study was not aimed at weight loss, but rather the improvement of bone mineral density. In previous publications, we have demonstrated that one year of strength training increased bone mineral density (BMD),[45] increased lean soft tissue (LST), and decreased fat tissue (FT)[162] in postmenopausal women. Greater amounts of weight lifted were also correlated with greater increases in BMD and LST.[19, 162]

Despite population based evidence that diet and exercise induce weight loss or specific changes in body composition, body composition responsiveness to exercise programs often varies between individuals. Inter-individual variation is likely due to a complex interplay between genes, the environment and behavioral factors. [7] Based on associations between adrenergic receptors (ADR) and exercise, fat mobilization, and fat oxidation,[82] ADR genes are particularly interesting candidate genes, that may play a role in modulating and therefore possibly accounting for some of the inter-individual variability in the body composition response to exercise.

Type 2 diabetes prevention and lifestyle

Observational studies have shown that lifestyle choices, such as television watching or walking, can significantly impact risk of type 2 diabetes.[67, 68] So, it is encouraging that cost-effective lifestyle interventions have been effective in the delay or prevention of type 2 diabetes.[177] These lifestyle interventions, including weight loss and increased physical activity, have been conducted around the world with great success.[177] The most well-known study in the United States is the Diabetes Prevention Program (DPP), which was able to reduce the incidence of diabetes by 58% in over 3,000 individuals with impaired glucose tolerance.[54, 60, 83, 127] An important finding of the DPP was that the lifestyle intervention in these at-risk individuals was more effective than the drug metformin.[83]

Although these general lifestyle interventions have been successful, it is difficult to attribute the improvements in insulin sensitivity to a particular aspect of the interventions. The question of whether weight loss is the key element in diabetes

prevention, versus physical activity or shift in body composition, still remains. Since weight loss is accompanied by both fat and lean mass loss, [34, 35] teasing apart the contributions of weight loss versus body composition change are important to investigations of physical activity.

One study comparing lifestyle intervention components was conducted as a randomized controlled trial; it compared diet only, exercise only, and diet plus exercise. All of the groups demonstrated similar positive effects on diabetes prevention (~40%), however the absence of a control arm stills leaves us with questions.[117] An indication of the importance of physical activity and body composition change was delivered in a study of type 2 diabetics. Resistance training was paired with weight loss and compared to weight loss alone. The study found greater decreases in glycosylated hemoglobin, (HbA1c) in the resistance trained plus weight loss group than in the weight loss only group.[28] Clinical trials in diabetics without weight loss have demonstrated resistance training induced benefits as well.[18, 145] Cuff et al reported that resistance training added to endurance training-related improvements in insulin sensitivity. They also reported that the improvements in insulin sensitivity were significantly associated with reductions in both subcutaneous and visceral abdominal fat, as well as increases lean mass.[18] However, diabetics may respond differently to intervention than non-diabetics due to numerous metabolic differences found in the diabetic state.

Numerous trials of aerobic activity have demonstrated that physical activity is effective in type 2 diabetes prevention,[78] There are fewer trials evaluating prevention via resistance training.[78] There are even fewer diabetes prevention trials targeting body

composition change by weight training, rather than weight loss, with a robust randomized controlled design.[18, 25, 122, 154] The few available are detailed below.

In a randomized controlled trial investigating the relative contributions of fitness, measured by aerobic capacity, versus change in body composition to prevention of type 2 diabetes, Stewart et al found that total and abdominal fat reductions and increased lean mass were more strongly associated with improvements in diabetes risk factors, than improved fitness, in older men and women. Delecluse et al took a different tack in older men and compared endurance training to endurance plus resistance training for improvements in insulin sensitivity. The study randomized older men to the following exercise programs: 1.control, 2.endurance training only, 3.endurance plus low intensity resistance training, 4.endurance plus moderate intensity resistance training. In contrast to Stewart et al, they found that all exercise groups responded similarly with improved insulin response compared to controls, despite no significant changes in body composition over 20 weeks of training.[25] Similarly, a 6-month, which randomized young, non-obese women to the following 3 groups: control, endurance training, and resistance training, reported insulin sensitivity improvements after either endurance or resistance training, despite no training-related changes in total fat or abdominal fat (subcutaneous or visceral). However, when measures of glucose disposal were expressed per kilogram lean mass, the improvements did not remain in the resistance trained group.[122] These findings indicate that there are varying mechanisms between endurance and resistance training, for insulin sensitivity improvements, where resistance training improvements are reliant on change in body composition. The post-menopausal, non-obese, non-diabetic population,

has yet to be studied in a randomized controlled trial of resistance training only, for the prevention of type 2 diabetes.

Adrenergic receptor structure, function, and variations

Adrenergic receptors are G-protein coupled receptors found in cell surfaces. They are located in multiple cell types,[12, 23, 82, 87] however, for the purposes of this document, we will focus on fat and skeletal muscle cells. All adrenergic receptors are responsive to catecholamines, epinephrine or norepinephrine.[12, 82, 87, 149, 155] However, alpha adrenergic receptors (ADRA) tend to be inhibitory, as they are coupled to G_i-proteins which inhibit cAMP production through inhibition of adenylyl cyclase, inhibit calcium channels, and activate potassium channels. Beta adrenergic receptors (ADRB) tend to be stimulatory, i.e. coupled to G_s-proteins, which stimulate adenylyl cyclase.[82] In fat cells, catecholamine induction of the inhibitory ADRA leads to decreased lipolysis, while catecholamine induction of ADRB can lead to stimulation of lipolysis through hormone sensitive lipase, or other stimulatory pathways.[85]

Several common variants or polymorphisms in the adrenergic receptors have been identified. One type of polymorphism is a deletion of several base pairs, and hence deletion of multiple amino acids in the final protein sequence following translation, as in ADRA2B $Glu^{9/12}$. Another type of polymorphism identified in the ADR family is a single nucleotide polymorphism (SNP), or base pair substitution, which may result in the translation of synonymous amino acids, as in ADRA1B Gly183Gly, or non-synonymous amino acids, such as in ADRB3 Trp64Arg.[82]

Common non-synonymous variants include ADRA1a (Arg492Cys), ADRA2A (Asn251Lys), ADRA2B (Del 301-303, Glu9/12), ADRA2C (Del 322-325, Gly-Ala-Gly-Pro), ADRB1 (Ser49Gly, Gly389Arg), ADRB2 (Thr164Ile, Val34Met, Gly16Arg, and Gln27Glu), and ADRB3 (Trp64Arg).[82] These deletions or SNPs result in variations in the ultimate receptor, which may confer varied responsiveness to catecholamine enhanced environments, such as during exercise.[123] For example, the polymorphisms may up or down regulate the presence of a receptor in the membrane or enhance or diminish the signal transduction capability of the receptor. Since the beta adrenergic receptors tend to be preferentially recruited during exercise, the affects of polymorphisms in these receptors may be more evident with physical training. Alpha adrenergic receptors tend to be responsible for basal lipolytic activity and therefore their polymorphisms may exert greater effects in sedentary populations. This study focuses on ADRA2B $Glu^{9/12}$, ADRB3 Trp64Arg, and ADRB2 Gln27Glu only.

Adrenergic receptor variants and body composition

As described above, adrenergic receptors (ADR) are involved in fat mobilization and oxidation,[82] and thus variations in the receptors likely play a role in determining individual body composition, particularly since the alpha-adrenergic receptors (ADRA) tend to demonstrate inhibitory effects on lipolysis, while the beta-adrenergic receptors (ADRB) demonstrate stimulatory effects upon the same stimulus.[82] Here we will focus on the variants of interest, ADRA2B $Glu^{9/12}$, ADRB3 Trp64Arg, and ADRB2 Gln27Glu.

ADRA2B is not well studied, however there are a few cross-sectional and intervention trials involving body composition outcomes and/or physical activity. No

independent effects of ADRA2B on multiple body composition measures were found in healthy Caucasian women with a wide range of body weight,[27] in obese[163] and morbidly obese,[160] Chinese women, [182] or in young healthy Japanese males. [159] Although Finnish obese non-diabetic women compared to normal weight controls demonstrated similar allelic frequencies, basal metabolic rates in the homozygous Glu^{9} population were lower than in heterozygotes or homozygous wild type Glu^{12}.[59] In contrast, The Finnish Diabetes Prevention Study found an association between ADRA2B variant and waist circumference at baseline ($p<0.05$), however, following the lifestyle intervention, the relationship was absent.[146] Thus, we may conclude that the small effect of genotype on body composition was overcome by weight loss and increased physical activity. Another Finnish study over 10years, observed non-diabetic weight gain was significantly greater in Glu^{9} homozygotes than in the heterozygotes or Glu^{12} homozygotes. [147] They also found that non-diabetic women of either homozygote group had greater waist to hip ratios than the heterozygotes ($p<0.05$) at the 10 year follow up.[147] It appears the absence of active maintenance of healthy lifestyle behaviors, the ADRA2B variant may modulate total and regional body composition.

Since it is commonly believed that genes often do not exert affects on phenotype alone, gene-gene interactions are also of interest. Dionne et al, cross-sectionally evaluated the ADRA2B and ADRB3 interaction in obese middle-aged women and found that carriers of ADRA2B Glu^{9} + ADRB3 Arg^{64} had greater body fat than Arg^{64} carriers alone, [27] which suggests that carriage of the ADRA2B Glu^{9} may interact with ADRB3 Arg^{64} carriage to hence body fat. Lastly, an endurance training trial investigating ADRA2B

variants, found no independent association between ADRA2B and body composition, however ADRA2B interacted with ADRB2 or ADRB3 for significant associations with changes in both total and trunk fat. In this case non-carriage of the Glu^{9} variant (ADRA2B Glu^{9-}) with either ADRB3 Arg^{64+} or ADRB2 Glu^{27+} was advantageous, and individuals lost significantly greater % total and trunk fat.[121] These findings imply carriers of any of the variants have enhanced function of the receptors in the context of physical activity. Carriers of Glu^{9} variant may have greater ADRA activity, and thus may be more antilipolytic, while carriers of ADRB3 Arg^{64+} or ADRB2 Glu^{27+} may have greater ADRB activity and thus may be more lipolytic with physical activity. The optimal combinations, in an overweight population performing physical activity, appear to include "normal" antilypolytic function (i.e., non-carriage of the ADRA2B variant) combined with enhanced lipolytic activity due to carriage of the ADRB3 Arg^{64+} or ADRB2 Glu^{27+} variants, such that the balance is shifted toward lipolysis.

We have not yet seen report of lean soft tissue in relationship to ADRA2B genetic variation. Therefore, at this time, there is not sufficient evidence to conclude that there is an independent relationship between ADRA2B and body composition, with or without intervention. The relationship between ADRA2B and body composition or change in body composition may be dependent on other genes, [27, 121] and on metabolic or sympathetic activity. [146-148, 163, 181, 182]

ADRB3 has been associated with various measures of body composition, but studies have varied by age,[15, 143, 157] gender,[16, 42, 57, 104, 116, 167] and ethnicity, as well as body weight[13, 63, 74, 138] and activity levels[97]. However, others have not found independent

relationships between ADRB3 variants and initial body composition phenotypes.[37, 42, 57, 65, 75, 98, 116, 143, 144, 151, 161, 169]

A Spanish study found that carriage of the ADRB3 variant was a risk factor for obesity in sedentary individuals, but not in the active individuals when they assessed leisure time physical activity.[97] While one study of change in body composition with aerobic activity found a significant association between ADRB3 Arg^{64+} and change in total body fat (% and kg) and % trunk fat ($p<0.05$).[121] These studies imply that the ADRB3 variant may be responsive to behavior, i.e., it is important for weight gain in sedentary populations and weight loss or maintenance of healthy weight or healthy body composition in the context of physical activity.

Studies of ADRB2 Gln27Glu variants have also been inconsistent. Cross-sectional evidence has supported both Glu27 carriers [14, 29, 88, 95, 100, 112, 164] and non-carriers,[103, 105, 119] in obesity phenotypes. Weight loss and endurance exercise training studies of ADRB2 Glu^{27+} indicate that Glu27 carriers are at a disadvantage; in various trials they have gained more weight and subcutaneous fat with overfeeding, [164] they were weight loss resistant or slow to lose weight, [100] they had greater respiratory quotients and lower fat oxidation rates during the recovery from acute exercise, [95] and they lost less body fat after 20 weeks of endurance training.[41]

There are two studies that contrast these results, the first cross-sectional and the second an intervention without control or randomization. The cross-sectional study of French men, found that men with homozygous for the wild-type ADRB2 (Gln/Gln) genotype had higher weight, BMI, waist and hip circumferences, but the results only

persisted for the sedentary men.[105] In an intervention of aerobic training in men and women, ADRB2 Glu^{27} carriers were able to lose significantly more fat than non-carriers, particularly when combined with ADRB3 Arg^{64} carriers.[121] In addition, more trunk fat was lost when ADRB2 Glu^{27+} was in combination with ADRA2B Glu^{9-}. Due to the contradictory results from the intervention trials, it is not possible to conclude that carriage or non-carriage of the ADRB2 Glu^{27+} variant is advantageous, in terms of body fat loss with endurance training, at this time.

Adrenergic receptor variants and diabetes

Variation in biomarkers of insulin sensitivity comparing carriers and non-carriers of ADRA2b $Glu^{9/12}$, ADRB3 Trp64Arg, ADRB2 Gln27Glu, have been inconsistent in cross-sectional or case matched design. Carriage of the respective variants can be detrimental to insulin sensitivity, [16, 32, 74, 86, 88, 93, 94, 104, 115, 137, 138, 142, 146, 168, 170, 171, 181] or have no association with it. [29, 37, 57, 61, 63, 65, 72, 80, 99, 100, 105, 106, 110, 116, 131, 144, 147, 151, 157, 159-161, 163, 165, 178, 181] Few trials exist which test the effects of these genotypes on activity induced changes in insulin sensitivity, with out dietary induced weight loss;[36] none of these trials are randomized controlled trials.

The majority of cross-sectional trials have found no effect of ADRA2B on risk of type 2 diabetes or biomarkers of insulin sensitivity.[147, 159, 160, 163, 181] However, Glu^{9} carriage was associated with higher glucose in hypertensive men[181] (non-significant in women) and increased risk of type 2 diabetes in abdominally obese.[146]

The associations between ADRB3 variants and insulin resistance were evaluated in a recent meta-analysis, which indicated that individuals with the Arg^{64} allele were

more insulin resistant than wild-type homozygotes (Trp64Trp). However, the relationship did not remain for most populations when split into subgroups. The associations persisted for Asians, obese, and type 2 diabetics only.[180] In confirmation of the meta-analysis, other cross-sectional or case-controlled studies reported non-significant associations between the ADRB3 variants and insulin sensitivity, including hypertensives,[57] obese,[37, 63, 161] those with cardiovascular disease,[61] males alone,[80] children,[178] and Mexican-Americans.[65, 110] One endurance training trial, without accompanying dietary intervention, reported that the Arg64Arg genotype was less responsive to endurance training-related improvements in insulin sensitivity than the Trp64Trp and the Trp64Arg genotypes in young healthy Japanese males. [75]

Similar, generally negative findings have been reported for the ADRB2 Gln27Glu variant in cross-sectional studies; there were no significant associations between insulin sensitivity and the ADRB2 Gln27Glu genotype.[29, 72, 99, 100, 105, 106, 131, 165] In contrast, studies in African-American men, in Hispanic-American men, [86] in Hispanic-American women[86] and in Swedish women[88] reported that Glu27 carriage decreased insulin sensitivity. Also, subjects with ADRB2 Glu27Glu demonstrated lesser insulin sensitivity improvements with acute bouts of endurance exercise; [36, 94, 95] they also had higher baseline insulin levels compared to Gln27Gln subjects.[95]

To our knowledge, there are no reports of possible gene-gene interactions among these adrenergic receptor variants and insulin sensitivity.

Hormone therapy, cardiovascular disease, and diabetes

The influence of hormone therapy on cardiovascular disease is controversial. Despite previous evidence that hormone therapy (HT) may be beneficial for reducing the risk of cardiovascular disease,[49, 52, 66] the most influential publication in recent times has been the one reporting the early termination of the Women's Health Initiative HT trial in 2002.[132] The HT trial was terminated early due to the increased risk of cardiovascular disease events in the post-menopausal women assigned to HT. That publication has since been questioned by Gambacciani and Genazzani [39, 43] and others due to such subject characteristics, as older age, more years postmenopausal, and hormone therapy type and doses, rendering it impossible to extrapolate to all post-menopausal populations. The Women's Health Initiative (WHI) and the Nurse's Health Study have published more recent article stating with that early postmenopausal treatment with hormone therapy may be beneficial in reducing the risk of cardiovascular disease.[53, 64] Due to the notoriety of the initial findings for the WHI study, we anticipate that the controversy surrounding the use of hormone therapy and its effects on cardiovascular disease risk will continue for many years to come.

The influence of HT on insulin sensitivity and on the risk for type 2 diabetes has also not been clearly defined. The HERITAGE Family Study reported nonsignificant associations between hormone therapy and biomarkers of insulin sensitivity.[50] The large endurance training intervention found that fasting plasma glucose and insulin were not significantly different between those taking hormone therapy and those not taking hormone therapy at baseline or post-exercise intervention.[50] In contrast, other smaller

trials, in both impaired and normal glucose tolerant populations, have found that hormone therapy users were more insulin-sensitive than non-users.[51, 158] Here the large Women's Health Initiative (WHI) demonstrated a positive influence of hormone therapy on type 2 diabetes risk, i.e. reduced risk with HT use.[96] The opposite was demonstrated in the cross-sectional portion of the Postmenopausal Estrogen/Progestin Intervention Trial (PEPI), where insulin resistance was associated with estradiol (odds ratio, 2.7; $p<0.001$), even after accounting for indices of body composition and central adiposity.[76] In an age, weight, and BMI matched controlled trial of postmenopausal women, Ryan et al also found that women taking opposed or unopposed HT were more insulin resistant than those not on HT, demonstrated by 26% and 31% lower glucose utilization rates and 28% and 36% lower indices of insulin sensitivity ($p<0.05$).[135] Thus, the debate over hormone therapy use for protection against chronic conditions, or simply for the relief of menopausal symptoms without undue health risk, continues.

Physiologically, counter-regulatory hormonal responses to exercise and hypoglycemia are sexually dimorphic,[20-22, 38] indicating a role for female hormones in insulin sensitivity. Premenopausally, with estrogen (E2) intact, women have demonstrated lower sympathetic and other counter-regulatory responses to exercise and hypoglycemia than men.[20-22, 38] In addition, glucose production in response to hypoglycemic induction has also been blunted in women on estrogen (E2), necessitating greater glucose infusion, compare to women without estrogen and men. [139] Premenopausal women, compared to postmenopausal women and men, also have higher baseline levels of non-esterified fatty acids and significantly greater lipolytic response to

aerobic activity by 15min and through 90 min of exercise (higher levels of circulating glycerol, NEFA, β-hydroxybutyrate).[20] Blunted muscle sympathetic activity and lower circulating glycerol levels were also found following induced hypoglycemia in postmenopausal women treated with estrogen (E2),[139] while NEFA levels were not significantly different between estrogen treated women and those without estrogen treatment (although they were significantly higher in men). Even in light of this evidence, a greater understanding of the interactions between estrogen and insulin sensitivity biomarkers, such as glucose, insulin, and non-esterified fatty acids is needed, particularly in the context of physical activity.

Hormone therapy, body composition, and adrenergic receptors

Hagberg et al.[56] found that women on hormone therapy tend to have lower total body fat ($p<0.07$), but not regional body fat by DXA. However, postmenopausal participants of the HERTIAGE Family Study on estrogen therapy demonstrated significantly smaller waist to hip ratio and a tendency toward lower abdominal visceral fat, measured by computed tomography, than non-therapy users, at baseline. Following 20 weeks of aerobic training, estrogen users lost more abdominal visceral fat than non-users, although the difference was not significant.[50] In-vivo and in-vitro studies have shown that estrogen is directly involved with regulating lipolysis at the adipocyte level via ERα, although the estrogen induced upregulation of the antilypolytic α-adrenergic receptor appears to be limited to the subcutaneous fat.[118] Inhibition of epinephrine stimulated lipolysis by estrogen therapy in the subcutaneous fat of PM women has also been demonstrated.[91, 118]

An estrogen-induced downregulation of lipolytic activity in subcutaneous fat, by alpha adrenergic receptor upregulation, without concomitant alteration in beta adrenergic receptors (more heavily populating visceral fat) may shift the balance of lipolytic activity to the visceral fat to maintain the preferential deposition of fat subcutaneally found in the premenopausal state. In addition, this shift may increase the bioavailability of free fatty acids for metabolism, reflected in our higher levels of non-esterified fatty acids in women on hormone therapy. This theory appears to be in contradiction to the evidence above associating E2 with decreases in lipolysis, however the aforementioned studies were conducted with the acute induction of hypoglycemia, in which multiple counter-regulatory hormonal responses may play a role, [139] [118] and may vary compared to steady state lipolytic activity.

Responders and non-responders to lifestyle changes

Resistance training has been shown to be effective for favorable shifts in body composition, loss of body fat mass, gain of lean soft tissue,[162] as well as improvement in insulin sensitivity and type 2 diabetes management.[17, 18, 25, 31, 78, 107, 108, 122, 130, 134, 150, 179] However, study outcomes are based on populations enrolled, rather than individual outcomes; there is great inter-individual variability in response to environment or training.[125] Some individuals respond to resistance training with favorable shifts in body composition or insulin sensitivity, or both, (responders), while others may lose lean mass, gain fat, or decrease insulin sensitivity (non-responders), despite high intensity exercise. Many factors, such as age, sex, race, and pre-training phenotypes may be responsible for inter-individual variation in response to exercise,[125] however, heredity may also be a

factor.[8] It is possible that a complex interplay of many different genes, gene-gene interactions, and gene-environment interactions[7-9, 121] may explain some of the variation in the body composition and in the insulin sensitivity responses to resistance training.[36, 121] The present study will improve our understanding of how the individual genes, ADRA2B Glu[9/12], ADRB3 Trp64Arg, and ADRB2 Gln27Glu, and their gene-gene interactions may modulate the body composition and the insulin sensitivity responses to resistance training in postmenopausal women.

EXPLANATION OF DISSERTATION FORMAT

This dissertation was prepared in manuscript format. The "Present Study" (Chapter 2) summarizes methods, results, and conclusions relevant to all three manuscripts included in this document (Appendices A, B, and C). My original contributions to the large randomized controlled trial, involving many investigators and staff members, were the fasting plasma measurements of insulin sensitivity biomarkers (glucose, insulin, and non-esterified fatty acids) at baseline and one year, as well as the initiation of the ancillary genetics study. While the biomarker measurements involved the use of stored blood samples, the genetics study involved re-recruitment and re-consenting of participants of the main Bone Estrogen and Strength Training trial, as well as the collection of genetic material and subsequent allelic determination of ADRA2B Glu[9/12], ADRB3 Trp64Arg, and ADRB2 Gln27Glu, for each re-consented participant. My original contribution also includes statistical analysis to determine 12-month biomarker

changes and the relationships between changes in body composition, insulin sensitivity, dose of training, and heredity.

CHAPTER 2: PRESENT STUDY

INTRODUCTION

The more detailed descriptions of the present study methods, results, and conclusions are presented in the three manuscripts included in this document (Appendices A, B, C). In addition, the most important findings and conclusions are summarized below. All three manuscripts include both the cross-sectional and longitudinal findings of this project. The first manuscript targets the aim of evaluating the contributions of adrenergic receptor genotypes to body composition before and after the resistance training intervention (Appendix A). The non-genetic aims relating effects of resistance training and dose of training on insulin sensitivity are presented in the second manuscript, using the largest sample size (Appendix B). The third manuscript assessed the influence of adrenergic receptor heredity on biomarkers and models of insulin sensitivity at both the initiation of the trial and following the 12months of resistance training (Appendix C).

METHODS

Study design and population

For the present study, blood samples from one-year completers of the Bone, Estrogen and Strength Training (BEST) Study were analyzed retrospectively for fasting plasma glucose (FPG), insulin (FPI), and non-esterified fatty acids (NEFA). The main BEST trial for the effects of exercise and calcium on BMD included 266 completers of the one year intervention.[45] From a large subset of this sample, complete fasting plasma

glucose, insulin and NEFA data was available for use in this analysis (N=225). One-year completers that changed from no HT to HT or from oral HT to the patch (N=7), had FPG levels >125mg/dl at either baseline or 1 year (N=7), or insulin levels >12 standard deviations higher than the study population at 1 year (N=1) were excluded from the study. Due to significant differences between oral HT and patch users, patch users were also eliminated from the study (N=10). The largest final sample size included in this report is found in manuscript 2 (N=200) (Appendix B).

One-year completers were also re-recruited and re-consented to participate in the ancillary genetics study, in which new buccal cell samples were obtained for genetic analysis. We were able to re-consent 152 subjects from the main trial. Those with complete body composition measurements (N=148) were evaluated in the first manuscript (Appendix A). Some of those re-consented were using patch hormone therapy versus oral therapy and were eliminated, as were subjects with FPG values >125mg/dl. After these eliminations, we were able to analyze complete FPG, FPI, and NEFA and genetic data on a large subset (N=122) of re-consented subjects for the third manuscript (Appendix C).

The BEST Study was a prospective study of exercise and bone mineral density (BMD) in postmenopausal women. BEST randomized subjects to exercise (EX) and no exercise (NEX), hormone therapy status was self-selected in the postmenopausal women, giving four balanced groups: HT/EX; HT/NEX; NHT/EX; NHT/NEX. All participants agreed to the following: maintenance of current HT status, maintenance of baseline level of physical activity (controls), maintenance of dietary practices for the duration of the

study, acceptance of randomization to EX or NEX, and to consumption of calcium provided by the study (800 mg/d Citracal®, Mission Pharmacal, San Antonio, TX). Compliance with calcium supplements, monitored by pill counts, showed no significant differences among groups. Total serum estrone and estradiol was measured by radioimmunoassay (RIA) (Diagnostic Systems Laboratory; Webster, TX) for between group comparisons and monitoring HT compliance (intra- and inter-assay CV <10%).[45] In addition, dose, type, and mode of HT delivery were recorded.

Written informed consent was obtained from all participants prior to entering the study. Inclusion criteria were: age (40–65 years); surgical or natural menopause (3–10.9 years); body mass index (BMI) <33 kg/m2; non-smoker; no history of osteoporotic fractures and initial lumbar spine and hip BMD greater than Z-score of 3.0; undergoing HT (1–5.9 years) or no HT (>1 year); cancer free and treatment free >5 years, excluding skin cancers; no medications that alter BMD, no beta-blockers or steroids; calcium intake >300 mg/day; <120 min of physical activity per week, and no weightlifting or similar activity. An 8-week run-in phase to test adherence and encourage early drop out was used. All screening and baseline measurements were made during run-in. Follow-up measurements occurred at 12 months.[19, 45, 102, 109, 162]

Exercise intervention

The exercise group was required to perform supervised, progressive, high intensity weight lifting and moderate impact weight-bearing exercise for 75 minutes, 3 days/week. Two sets of 6-8 repetitions were done each day at 70-80% of the one-repetition maximum (1-RM) loads, per exercise. The eight weight training exercises

included: squats, leg press, weighted march, military press, seated row, latissimus dorsi pull-down, back extension, rotary torso. Attendance, loads, sets, and repetitions were monitored with logs checked regularly by trainers on site. Strength (1-RM) was measured every 6–8 weeks and the load increased to maintain loads. Mean exercise attendance was similar in EX/HRT and EX/NHRT (72%).[19, 45, 109, 162]

Strength and fitness assessment

Strength was assessed in the total population by the LIDO isokinetic dynamometer (Loredan Biomedical, Sacramento, CA) and additionally in the exercisers, by the one repetition maximum test (1RM)[45, 162], or the maximum weight that could be lifted one time with proper body alignment and technique.[162] The LIDO testing included assessment of the maximal isokinetic torque of the extensors and flexors of the back and right knee and hip.[45] The subjects performed 1RM tests for squats, leg press, military press, seated row, latissimus dorsi pull-down, back extension, rotary torso at baseline and one year. The facility and equipment used for strength measurements was consistent for each subject at both time points.[45, 162]

Body composition and diet assessment

Standard anthropometric (height, weight, waist and hip circumferences, skinfolds) and whole body composition (DXA by Lunar Radiation Corporation, Madison, WI) measures were performed in duplicate at baseline and 1year on 2 different days, within a 2 week period.[45, 92, 162] Means of the duplicates were used in all statistical analyses. Lean soft tissue (LST) mass was calculated by subtracting fat tissue (FT) mass, by DXA, from

soft tissue mass. The between duplicate CV was <1.8% for LST and FT,[162] therefore, means of the duplicates will be used in all statistical analyses. Abdominal fat was determined by manually adjusting the DXA frame to the intervertebral space between the first and second lumbar vertebrae and the iliac crest (AbROI); both lean and fat tissue measurements were then quantified by Lunar software.

Subjects received training regarding dietary portion sizes and proper recording of food descriptions in order to complete dietary records reflecting intake from 8 randomly assigned days, including 1 weekend day and 1-2 nonconsecutive weekdays (3 days at baseline, 2 days at 6months, and 3days at 12 months). Diet records were used to estimate fat, protein, and carbohydrate intake. Analysis of nutrient intake was performed using Minnesota Nutrient Data System versions 2.8-2.92.[45, 102] Subjects were not instructed to change their diets, but were asked to maintain their body weight.[102]

Blood analyses

Blood collection and storage

Blood was drawn as the first measure, following a 12hr overnight fast, at baseline and follow-up. Each collection was performed during the same phase of hormone cycles for women taking cyclic HT. Subjects were instructed not to take any medication, vitamins or minerals the morning of the draw. Both EDTA and sodium citrate treated vacutainers were used. Blood was centrifuged and aliquotted (500μL – 1mL each) into cryovials for immediate -80°C storage;[109] the samples used for the present study had never been thawed until use in glucose, insulin, or non-esterified fatty acid assays. The plasma samples were thawed, as needed, and vortexed to ensure homogeneity, prior to

any new measurements. EDTA treated samples were used for glucose analysis and sodium citrate treated samples were used to insulin and non-esterified fatty acid analyses. Neither anticoagulant interacted with their respective assays.

Blood drawing timing, with respect to last bout of physical exertion or activity, was not standardized. A statistical contrast was created to account for differences due to blood draw timing between those with blood draw dates within 6 days of last bout of physical activity and those with blood draw dates greater than one week since last bout of physical activity at baseline. Six statistical contrasts were created to account for differences between those with physical activity (PA) within and greater than 6days of their blood draw at one year. The contrasts were designed as follows: 1. No PA at all or PA at both baseline, B, and one year, Y1, within 6days of blood draw versus PA within 6 days of blood draw at one or the other time point, 2. No PA versus PA at baseline, 3. PA at both time points versus PA at Y1 only, 4. interaction of contrast 1 with random assignment to exercise (EX) or control (NEX) groups, 5. interaction of contrast 2 with random group assignment, 6. interaction of contrast 3 with random group assignment. These contrasts were utilized only for measures of glucose, insulin, and non-esterified fatty acids.

Glucose measurement

The Infinity™ Glucose Oxidase Liquid Stable Reagent (Thermo Electron Corporation, Louisville, CO) was used for glucose determination, following the recommended reagent ratio of 1:150 (5ul sample: 750ul reagent). The assays were run at room temperature with an incubation time of 25-40minutes. The glucose oxidase assay

absorbance was analyzed on the Beckman DU Series 600 Spectrophotometer (Beckman Instruments, Inc, Fullerton, CA) at a fixed wavelength of 500nm. According to the manufacturer assay linearity was 0-630mg/dL, with sensitivity of 0.002 DAbsorbance per mg/dL. Although the recommended protocol set an incubation time of 5min at 37ºC, prior to running participant samples, repeated experiments at room temperature determined that the reaction at room temperature peaked at 25min and remained stable for 1 hour in the high standard and up to 3.5 hours in the low standard (Low Standard = 50mg/dl, High Standard = 200mg/dl). All baseline and one year samples by individual were performed in triplicate within the same spectrophotometric carousel (6-wells) and reading. Intra- and inter-assay coefficients of variation were 5% and 8%, respectively.

Insulin measurement

A Human Insulin Specific Radioimmunoassay (RIA) Kit (Linco Research, Inc., St Charles, MO) was used to quantify fasting plasma insulin levels at baseline and following the one-year intervention in all groups. The assay utilized ^{125}I-labeled Human Insulin and a Human Insulin antiserum to determine plasma Insulin levels. The kit antibody was raised against purified Human Insulin; the standard and tracer were prepared from Human Insulin. The kit does not cross-react with Human Proinsulin (<0.2%) and is highly sensitivity (detects insulin at 2μU/ml at 100-μL). The intra and inter coefficients of variation, for insulin assays performed on both baseline and one year samples, were both 4% for insulin measurements.

Non-Esterified Fatty Acids measurement

Fasting plasma NEFA levels were analyzed pre and post intervention in all experimental groups via the NEFAc in vitro enzymatic colorimetric method, using the microtiter technique, i.e. 1/10 volume on 96well plates (Wako Chemicals USA, Inc., Richmond, VA). The assays began by adding acyl CoA synthatase (ACS) to the serum samples, which caused the fatty acids in the samples to acylate coenzyme A (CoA). The acyl-CoA produced by this reaction was oxidized by adding acyl CoA oxidase (ACOD) to generate hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase (POD) permitted the oxidative condensation of 3-methyl-N-ethyl-N- (b-hydroxyethyl)-aniline (MEHA) with 4-animoantipyrine to form a purple colored adduct which was measured colorimetrically at 550nm and 660nm on a plate reader. Optical densities read at 660nm were subtracted from those at 550nm, according to manufacturer protocol. Sample NEFA concentrations were calculated from the regression line generated by plotting the kit provided low, middle, and high standards, equivalent to 0.5mEq/L, 1.0mEq/L, and 1.97mEq/L, against the optical densities returned from the plate reader, for each plate.

None of our subjects were receiving heparin therapy or were diagnosed with conditions that may interfere with this assay (abnormal hemoglobin or bilirubin). The addition of other substances, glucose, uric acid, ascorbic acid, glutathione, cystein, anticoagulants and antiglycolytics, at various concentrations do not interfere with the measurement of NEFA in this assay, according to the manufacturer. Since the level of NEFA increases due to enzymatic action, as it is allowed to stand at room temperature, specimens were stored at -80°C until analyses were be performed. Overall intra and inter

CVs were 4% and 11%, respectively.

Insulin sensitivity models

The insulin sensitivity models utilized the fasting plasma measures of glucose, insulin, and non-esterified fatty acids. Three insulin sensitivity models were used in the present study and were computed according to Brady, et al[10] as surrogate measures of insulin action. The three models were: the homeostasis model for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and the revised QUICKI (R-QUICKI). High HOMA-IR scores indicate low insulin sensitivity, while high QUICKI or R-QUICKI scores indicate high insulin sensitivity.[10] The insulin sensitivity models equations are as follows:

$$\text{HOMA-IR} = (\text{insulin}_{0\ (\text{uU/ml})} * \text{glucose}_{0\ (\text{mmol/l})}) / 22.5$$

$$\text{QUICKI} = 1/(\log(\text{glucose}_{0\ (\text{mg/dl})}) + \log(\text{insulin}_{0\ (\text{uU/ml})}))$$

$$\text{R-QUICKI} = 1/(\log(\text{glucose}_{0\ (\text{mg/dl})}) + \log(\text{insulin}_{0\ (\text{uU/ml})}) + \log(\text{NEFA}_{0\ (\text{mmol/l})}))$$

Genotyping

Buccal cell collection

Collection of genetic material (buccal cell) was performed either in the lab during routine visits or by mail. If collection took place during lab visits, at the end of the 1hour visit, the subject was first consented, then given a pre-measured volume (10ml) of Scope mouthwash, with 15wt% alcohol content, (Proctor and Gamble, Cincinnati, OH) in a sterile collection cup. The subject was asked to swish the mouthwash vigorously for

45seconds, while being timed by a BEST staff member. Following the 45seconds, the subject was asked to expectorate the mouthwash back into the sterile collection cup, which was sealed and labeled by the BEST staff member with the subject identification number and date.

If buccal cell collection was performed by mail, the collection kit contained a sealed trial size bottle of Scope mouthwash with 15wt% alcohol content (Proctor and Gamble, Cincinnati, OH), a sealed, sterile collection cup with a 10ml fill line, instructions for collection, a Ziploc bag, and a prepaid return envelope. Participants were asked not to eat or drink for one hour before sample collection, fill the identification labeled, sterile, collection cup to the 10ml line with the provided Scope mouthwash, swish the mouthwash vigorously for 45sec and expectorate the mouthwash back into the cup, date the sample, and mail the container back to the laboratory in the pre-addressed postage paid packaging provided, similar to the methods described by Garcia-Closas et al.[40] One subject was mailed a sterile swab (Puritan) instead of mouthwash, due to an allergy to mint. The subject was instructed to open the swab packaging, scrape the inside of each cheek 6 times, seal the attached cap over the swab and mail the swab back to the laboratory in the pre-addressed postage paid packaging provided.

DNA processing

Samples received either in the laboratory or via mail were processed within 7 days of collection. The individual, whole mouthwash samples were vortexed, aliquotted into thirds, and placed into separate 15ml conical tubes. Centrifuged for 15min at 3500 rpm and discarded resultant supernatant.

Added 80ul 30mM NaOH to remaining pellet, vortexed and incubated until pellet was incorporated into solution with viscous appearance “slimed”, up to 30min at room temperature. Solution was neutralized with appropriate volume of 0.1N HCl (~22.5ul, volume 0.1N HCl needed to neutralize 80ul 30mM NaOH tested daily with pH strips prior to start of experiment) and vortexed. Sufficient PBS was added to bring total volume of solution to 400ul. Transferred solution to 1.7 ml microcentrifuge tubes. Then 20ul protease was added to the solution and tubes were incubated at 56ºC for 10min, after which the DNA extraction by the QIAampDNA Mini Kit (QIAGEN #51104, Valencia, CA) protocol was followed precisely. The manufacturer’s optional protocol steps were included.

The one buccal sample collected using a swab was cut off at the cotton tip. The tip was incubated with 2ul protease overnight at 56ºC and then processed according to the Argylla DNA MicroExtract Kit (Argylla Technologies, Tucson, AZ).

Two polymerase chain reaction (PCR) based assays were performed for quality control purposes, using an aliquot of the collected material. A 50ul PCR reaction utilizing the FastStart Taq DNA polymerase kit (Roche Penzberg, Germany), 558bp primers, and 2ul sample was performed. Amplicons of 558bp (1ul of 1:10 PCR product dilution) were separated on a 2% SFR agarose gel in 1XTBE, by electrophoresis (150v, 45minutes) with subsequent visualization by ethidium bromide staining against a 1kb ladder, to estimate quality and quantity of DNA collected from the buccal cells. DNA quantification was also performed by PicoGreen dsDNA Quantitation Reagent and fluorimetry with this aliquot, for subsequent normalization of DNA concentration in future allelic

determination. DNA. The extracted genetic material, not used in quality control procedures was stored at -20°C for future PCR and allelic determination.

Allelic determination for adrenergic receptors

ADRA2B, due to the insertion length for the Glu^{12}/Glu^{9} variant, could not be analyzed by Taqman technology, therefore, previously published Glu^{12}/Glu^{9} identification procedures by PCR and gel electrophoresis[147] were followed using primers designed for the long (112bp) and the short (103bp) variants (Midland Chemicals Midland, TX). Upon receipt, the primers were processed using the NAP-5 Column (Amersham Biosciences, Piscataway, NJ), for desalting and buffer exchange of the oligonucleotides, and evaluated on a Beckman DU Series 600 Spectrophotometer (Beckman Instruments, Inc, Fullerton, CA). Final concentration of the primers following desalting was 15-23uM. Gradient PCR according to the procedure above for quality control, was also run to determine the optimal thermocycling procedure specific to these oligonucleotides. The PCR reactions were 25ul reactions with 10ng DNA (2.5ul 10xPCRbuffer, 2ul primer, 1.5ul MgCl, 0.8ul 1:10 BSA, 0.5ul dNTP, 0.2ul Roche Fast Start Taq, 12.5ul MCB grade water). Thermocycling procedures were as follows: 1. initial denaturation at 94°C for 4min, 2. denaturation at 94°C for 30seconds, 3. annealing at 69°C for 30seconds, extension at 72°C for 30seconds, 5. repeat steps 2-4, 34times, 6. final extension at 72°C for 7min, 7. hold at 15°C until removed from thermocycler and refrigerated. PCR products were evaluated by 3% SFR agarose gel electrophoresis (80v,

2hr, 20min) and ethidium bromide visualization with a 10bp ladder. An example of the visualization and identification of the two lengths is presented in Appendix D.

ADRB3 Trp64Arg and ADRB2 Gln27Glu alleles were determined by using Assays-by-Design Service and TaqMan technology (Applied Biosystems, Foster City, CA). The Taqman PCR forward and reverse primers, and allelic probes were designed for the specific single nucleotide polymorphisms (SNPs) on the genes of interest. TaqMan PCR was performed under universal concentration conditions and universal thermal cycling parameters. TaqMan allelic determination was performed on the ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA).

Table 1 Adrenergic receptor allelic determination probes and primers

Gene	Allele	Probe	Forward 5’	Reverse 5’
ADRA2B	Glu^{9+}	103bp*	AGGGTGTTTGTGGGGCATCTCC	CAAGCTGAGGCCGGAGACACTG
	Glu^{9-}	112bp*		
ADRB3	Trp	VIC	GCAACCTGCTGGTC	ACGAACACGTTGGT
	Arg	FAM	ATCGT	CATGGT
ADRB2	Gln	VIC	CCTTCTTGCTGGCA	TGCCCACCACCCAC
	Glu	FAM	CCCAAT	AC

*ADRA2B allelic determination was performed by length of sequence visualized on agarose gel, not by a probe. Forward and reverse primers (Forward 5’ and Reverse 5’).

MAIN FINDINGS

First Manuscript

The associations between body composition and individual adrenergic receptor variants, ADRA2B, ADRB3, and ADRB2, and gene-gene interactions of these variants were examined in the first manuscript. The objective was also to determine whether allelic variation in ADRA2B, ADRB2, and ADRB3 could influence change in body composition by 1 year of resistance training (Appendix A).

The main effect of one year of resistance training exercise on body compositions was demonstrated by significant decreases in total body fat and abdominal fat ($p<0.05$), and an increase in lean soft tissue ($p<0.05$), without accounting for genotype.

The main effects of ADRA2b, ADRB3, and ADRB2 genotypes and gene-gene interactions on measures of body composition were examined at baseline and one year. At baseline, analysis of covariance models showed an association between ADRA2B Glu9+ and total lean soft tissue mass ($p=0.06$); ADRA2B Glu^{9+} carriers had greater lean soft tissue mass. In contrast, ADRB2 Glu^{27+} carriers had greater fat mass and less lean soft tissue mass than non-carriers both regionally and in the whole body ($p<0.10$). ADRB3 allelic variation and gene-gene interactions were not significantly associated with body composition at baseline for the whole population. Among controls only, 12 months of continued sedentary behavior was significantly associated with fat gain in ADRB3 Arg64 carriers; they gained significantly more % total body fat than non-carriers ($p<0.05$). A significant interaction between ADRA2B and ADRB2 was associated with change in % abdominal fat, however when split on intervention, the gene-gene interaction

persisted in controls only. Controls (non-exercisers) carrying either variant (ADRA2B Glu^{9+} or ADRB2 Glu^{27+}) gained % abdominal fat, while those that did not carry any polymorphisms, as well as those that carried both (ADRA2B Glu^{9+} and ADRB2 Glu^{27+}) lost relative abdominal fat over the 12months. In contrast, when focusing on exercisers only, the gene-gene interaction between ADRB3 and ADRB2 was significantly associated with % leg lean soft tissue ($p<0.05$): exercisers carrying both ADRB3 and ADRB2 variants (Arg^{64+} and Glu^{27+}) gained five times more leg lean tissue with the 12 month intervention than those that carried only the ADRB2 Glu^{27+} variant.

Second Manuscript

The second manuscript examined the relationship between insulin sensitivity and body composition, as well as insulin sensitivity response to resistance training and dose of training in post-menopausal women (Appendix B). This study found that the main effect of resistance training on insulin sensitivity was not significant. The largest determinants of insulin sensitivity change in individuals were initial and change in body composition, hormonal status, and initial values of insulin sensitivity biomarkers. However, although impaired fasting glucose was accompanied by higher initial values of fasting plasma insulin and NEFA levels, subjects with impaired fasting glucose, as a group, did not respond to the intervention differently than those with normal fasting glucose levels.

In addition to total fat mass, abdominal fat mass both at baseline and one year were significant, independent predictors of change in insulin sensitivity. Unexpectedly, hormone therapy was a significant determinant of baseline and change in NEFA levels

with resistance training, although higher levels of NEFA in oral HT users were not elevated above the normal range. The dose of exercise was also a significant predictor of change in insulin sensitivity. Exercisers that attended more than 2 out of 3 sessions per week throughout the year significantly reduced glucose (Standardized Beta: –0.193, $p<0.05$) and HOMA-IR scores ($p<0.01$).

Third Manuscript

The association between insulin sensitivity response to resistance training based on adrenergic receptor variants, ADRA2B $Glu^{9/12}$, ADRB3 Trp64Arg, and ADRB2 Gln27Glu, and gene-gene interactions, was examined in the third manuscript (Appendix C). Here we found that the main effect of exercise on body composition persisted in this smaller genotyped subsample, i.e. resistance training significantly improved body composition ($p<0.05$). However, in contrast to the larger group, in this subset, biomarkers of insulin sensitivity were altered by the intervention; controls (non-exercisers) experienced increases in circulating glucose ($p<0.05$), exercisers maintained initial glucose levels (between group $p<0.05$). Exercisers also significantly improved R-QUICKI scores (within exercise group $p<0.05$), but the R-QUIKCI difference between exercisers and controls did not reach significance at one year.

The only main effect of individual gene variation on insulin sensitivity biomarkers at baseline was detected as an association between fasting plasma glucose and ADRA2B (Glu^{9+} carrier standardized beta -0.13, $p<0.1$) using a general linear model accounting for age, hormone therapy, and body fat mass. However, fasting plasma glucose was associated with the gene x gene interactions for ADRA2B x ADRB3

($p<0.05$) and ADRB3 x ADRB2 ($p<0.1$), while ADRA2B x ADRB2 was associated with NEFA ($p<0.1$). ADRA2B x ADRB3 Glu^{9+}/Arg^{64+} glucose levels were higher than Glu^{9-}/Arg^{64+} ($p<0.05$) and Glu^{9+}/Arg^{64-} ($p<0.01$); Glu^{9-}/Arg^{64+} glucose was also higher than Glu^{9+}/Arg^{64-} ($p=0.06$). ADRB3 x ADRB2 Arg^{64+} /Glu^{27+} glucose levels appeared to be significantly higher than all three of the other genotypes, but specific between genotype differences were not significant. Although, ADRA2B x ADRB2 was associated with NEFA ($p<0.1$), between specific genotype differences were also not significant

Post-intervention, controls did not demonstrate any relationships between biomarkers of insulin sensitivity and ADR genes, but fasting plasma glucose decreased in exercisers carrying ADRB3 Arg^{64+} or ADRB2 Glu^{27+} more than in non-carriers (respective standardized betas: -0.72, $p<0.1$ and -0.30, $p<0.05$). ADRB2 Glu^{27+} carriers were additionally advantaged if exercising; they increased R-QUICKI scores to a greater extent than non-carriers. The gene-gene interactions alone, at one year, were not associated with changes in insulin sensitivity at one year, however, with the exception of ADRA2B x ADRB2 x exercise, they did interact with exercise for significant associations with insulin sensitivity. ADRA2B x ADRB3 x exercise was associated with change in fasting plasma glucose and change in NEFA ($p<0.1$). Although specific between genotype differences were not significant, ADRA2Bx ADRB3 Glu^{9-} x Arg^{64-} and Glu^{9+} x Arg^{64+} demonstrated expected intervention effects, i.e., exercisers decreased glucose and non-exercisers/controls increased glucose levels over 12 months. However, the ADRA2Bx ADRB3 Glu^{9-} x Arg^{64+} and Glu^{9+} x Arg^{64-} genotypes showed increased glucose levels regardless of intervention assignment. ADRB3 x ADRB2 x exercise was

also significantly associated with NEFA ($p<0.05$); Arg^{64-} x Glu^{27+} x exercise had the greatest reduction in NEFA levels and Arg^{64-} x Glu^{27-} x exercise increased NEFA levels with exercise ($p=0.07$).

CONCLUSIONS

This is the first trial of ADRA2b, ADRB3, and ADRB2 genetic variation combinations and resistance training in postmenopausal women relative to body composition and biomarkers of type 2 diabetes. The resistance training intervention did not have an overall effect on insulin sensitivity in the largest sample. Change in insulin sensitivity was dependent on hormonal status, body composition, and initial biomarker values.

There were small favorable effects of genotype on initial measures of both body composition and insulin sensitivity in the ADRA2B Glu^{9+} carriers versus non-carriers. Unfavorable effects of genotype on body composition, but not insulin sensitivity, were seen in carriers of the ADRB2 Glu^{27+} variant. ADRB3 Arg^{64+} carriage alone was not significantly associated body composition or insulin sensitivity at baseline, but in combination with ADRA2B Glu^{9+} it appeared to reverse the positive effects of the latter variant on insulin sensitivity.

Following the 12-month intervention, the effects of ADRA2B alone are no longer present. Instead, ADRB3 Arg^{64+} and ADRB2 Glu^{27+} each emerge as contributors to improved insulin sensitivity with exercise, despite no association of the genes with body composition change. It appears that ADRB2 Glu^{27+} is also the key to improved

biomarkers of insulin sensitivity when in combination with the other variants of interest (ADRA2B Glu^{9+} and ADRB3 Arg^{64+}), again, without concomitant associations with change in body composition. Lastly, the addition of ADRB3 Arg^{64+} to ADRB2 Glu^{27+} contributed to a more favorable overall indication of insulin sensitivity by R-QUICKI.

Overall, some specific genotypes were identified as responders and non-responders to exercise. Although, the contributions of specific genotypes to body composition did not directly parallel changes in insulin sensitivity, these data support ADR gene effects in body composition and insulin sensitivity independently. However, the data generally argue that despite modest gene effects, exercise is the major influence to affect positive changes in both body composition and insulin sensitivity.

LIMITATIONS

The use of fasting measures of glucose and insulin were not ideal, since their values largely reflect hepatic, rather than whole body insulin sensitivity, however, the inclusion of fasting non-esterified fatty acid measures provided an additional peripheral index of insulin sensitivity, adipose tissue. In addition, fasting biomarkers of insulin sensitivity have been reasonably correlated with more robust measures of insulin sensitivity.[101] The variation in blood draw timing in order to collect these fasting measures was also a limitation of the study, since improvements in insulin sensitivity following physical activity tend to be short lived, 3-6 days, [24, 81, 140]. In order to account for blood draw timing relative to the last bout of physical activity by individuals, we used robust statistical contrasts. In future trials, the use of the hyperinsulinemic-euglycemic

clamp method of measuring insulin sensitivity and systematic blood draw timing would be preferable.

The combination of normal fasting glucose and impaired fasting glucose groups was necessitated by the small sample size in the impaired group. Potential type 2 diabetics were eliminated and we accounted for potential differences in responsiveness between normal and impaired fasting glucose groups by including initial values of fasting plasma glucose, insulin, and non-esterified fatty acids in their respective analyses of biomarker change.

The small sample sizes of various hormone therapy regimens required the combination of therapies, however, patch users were removed to eliminate confounding variables between oral and patch hormone therapy. Ideally, hormone therapy groups would be large enough to compare differences between regimens and body composition or insulin sensitivity response to resistance training.

Lastly, due to the rare occurrence of certain genotypes, particularly in gene-gene interactions, the retrospective nature of this trial was a limitation. In the future, enrichment of specific genotypes during the selection process would enhance power to detect differences between groups.

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APPENDIX A: BODY FAT RESPONSE TO RESISTANCE TRAINING AND MULTILOCUS ADR GENOTYPES IN A POSTMENOPAUSAL POPULATION

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ABSTRACT

Body composition responsiveness to exercise programs varies between individuals. Due to the physiological link between adrenergic receptors (ADR), fat mobilization, and oxidation, we hypothesized that ADR genetic variants may play a role in the inter-individual variation in body composition response to exercise.

To determine whether allelic variation in ADRA2B, ADRB2, and ADRB3 influences the body composition response to 1 year of resistance training.

Completers of a large block-randomized trial of 12 months of resistance training in previously sedentary post-menopausal (PM) women, using or not using hormone therapy (HT), were genotyped for ADRA2B, ADRB2 and ADRB3 genes using buccal cell DNA (N=148). Exercise training sessions were supervised and recorded. Dual-energy X-ray absorptiometry was used to assess multiple measures of total and regional body composition at baseline and 1 year.

There were no significant differences between baseline characteristics of women randomized to exercise and control. Resistance training significantly decreased total body fat and abdominal fat ($p<0.05$) and increased lean soft tissue ($p<0.05$).

ANCOVA models at baseline showed an association between ADRA2B and total lean soft tissue ($p=0.06$), as well as ADRB2 and multiple total and regional body composition phenotypes ($p<0.10$); carriers of Glu27 exhibited greater fatness than noncarriers. There were no associations between ADRB3 variants or gene-gene interactions and body composition at baseline, however Arg64 carriers gained significantly more % total body fat compared to non-carriers in the control group

($p<0.05$). There was a significant interaction between ADRA2B and ADRB2 on change in % abdominal fat, however when split by intervention group the gene-gene interaction persisted for the controls only. The gene-gene interaction between ADRB3 and ADRB2 was positively associated with % leg lean soft tissue in exercisers, rather than controls ($p<0.05$), where carriers of both ADRB3 and ADRB2 variants gained five times more leg lean tissue than those that carried the ADRB2 variant alone. The main effect of exercise was significant across all body composition measures; changes were similar across all genotypes, except trunk fat ($p<0.05$).

In these post-menopausal women, the influence of ADR genes and gene-gene interactions on body composition varied based on group assignment and fat or lean depot. Specific genotypes were identified as responders and non-responders to exercise. When viewed as a whole group, body composition changes due to resistance training were largely independent of ADR genotypes.

Key words: body composition, body fat, genotype, resistance training, physical activity, post-menopausal

INTRODUCTION

Body composition responsiveness to exercise programs often varies between individuals. Inter-individual variation is likely due to a complex interplay between genes, the environment and behavioral factors. [2] Due to a physiological link between adrenergic receptors (ADR) and exercise, fat mobilization, and oxidation,[27] ADR genes are particularly interesting candidate genes, which may play a role in the inter-individual variation in body composition response to exercise.

Adrenergic receptors are G-protein coupled receptors located in the plasma membrane of several cell types, including fat cells.[3, 9, 27, 30] They are responsive to the catecholamines, epinephrine and norepinephrine.[3, 27, 30, 61, 63] The alpha adrenergic receptors (ADRA) tend to be inhibitory, while the beta adrenergic receptors (ADRB) tend to be stimulatory, relative to lipolysis.[27] Lipolysis can be induced in fat cells through catecholamine stimulation of ADRB and a secondary signaling cascade ultimately leading to activation of hormone sensitive lipase.[29]

Several common variants or polymorphisms in the adrenergic receptors have been identified, including insertion/deletion polymorphisms or single base pair substitutions.[27] These changes in sequence result in the translation of longer or shorter amino acid sequences in the case of insertion/deletion polymorphisms and in either synonymous amino acids or non-synonymous amino acid changes with base pair substitutions.[27] These polymorphic changes in the final amino acid sequence and ultimate G-protein coupled receptor may change the function of the receptors.

Previous studies have associated individual ADR polymorphisms with body composition or fitness, with mixed results across populations. [52, 80] Few have reported gene-gene interactions or responsiveness to intervention.[52, 80] Dionne et al, evaluated the interaction between ADRA2B and ADRB3 cross-sectionally in obese middle-aged women and found that carriers of ADRA2B Glu^{9} + ADRB3 Arg^{64} alleles had an increased %total body fat than Arg^{64} carriers on a ADRA2B Glu^{12} background. [10] More recently, Phares et al., looked at interactions of the ADRA2B, ADRB2 and ADRB3 in a trial of aerobic exercise training induced weight loss in 70 older men and women. These authors partially confirmed the aforementioned interactions on body fat mass by demonstrating that various combinations of non-carriers of ADRA2B Glu^{9} and carriers of ADRB2 Glu^{27+}, ADRB3 Arg^{64+} were associated with significantly greater decreases in fat mass than other variants.[51] Together, these data suggest an affect of genetic variation in the ADR genes on the lipolytic action of exercise on fat mass.

To determine whether the same genetic variants and gene-gene interactions would exert a similar influence on the body composition change induced by 12 months of resistance training in postmenopausal women, we genotyped participants in the Bone Estrogen and Strength Training (BEST) Study for ADRA2B, ADRB2, and ADRB3. We hypothesized that non-carriers of ADRA2B Glu^{9} and carriers of ADRB2 Glu^{27} and ADRB3 Arg^{64} variants would demonstrate greater fat loss in response to resistance training and that gene-gene interactions between these genotypes would prove to further enhance fat loss.

METHODS

Participants

Post-menopausal women were recruited from Southern Arizona and screened for the following criteria: age (40–65 years); menopause (3–10.9 years); body mass index <33 kg/m2; <120 min of physical activity per week, no weight gain or loss >30lbs, and no weightlifting. Subjects committed to maintaining hormone therapy status (confirmed by record of dose, type, mode of HT delivery, and blood estrogen levels), their weight, and diet during the trial. Baseline measurements were made during the run-in phase; follow-up measurements occurred at 12 months.[68] Women using steroids, beta-blockers, bone mineral density altering (BMD) medications, cancer within 5 years, or chronic health conditions were excluded from the study.[8]

The University of Arizona Institutional Review Board approved the study protocol and all subjects provided written informed consent prior to participation.

Study Design

For this study, women who completed one year of the Bone Estrogen and Strength Training Trial (BEST) were re-recruited and consented for DNA collection. The BEST study was a block-randomized resistance training trial in sedentary post-menopausal women, using or not using hormone therapy (HT), where subjects were randomized to exercise. Subjects were primarily of Caucasian origin. The 12 month intervention arm received vigorous training and monitoring. [8] The intervention was designed to increase bone density in exercisers and to maintain body weight in all subjects.

Dietary Stabilization and Monitoring

Subjects were asked to maintain their diets throughout the 12 month intervention. Three day diet records were collected at baseline and 1 year with a 2 day record collected at 6 months. The records were used to estimate dietary fat, protein, and carbohydrate intake, as well as other nutrients. Analysis of nutrient intake was performed using the Minnesota Nutrient Data System, versions 2.8-2.92.[18, 38]

Body Composition Measurements

Standard anthropometric (height, weight, waist and hip circumferences, skinfolds) and whole body composition (dual x-ray absorptiometry, DXA, by Lunar Radiation Corporation, Madison, WI) measures were performed in duplicate at baseline and at 1year.[18, 32, 68] Lean soft tissue (LST) mass was calculated by subtracting fat tissue (FT) mass, by DXA, from soft tissue mass. The between measure CV was <1.8% for LST and FT;[68] means of the duplicates were used in all statistical analyses. Abdominal fat was determined by from an operator set region of interest extending from the intervertebral space between the first and second lumbar vertebrae to the iliac crest.

Genotyping

DNA was obtained from buccal cells collected in mouthwash either during routine lab visits or via a buccal cell collection kit that subjects received by mail. Collection kits contained sealed 44 ml bottles Scope mouthwash (Proctor & Gamble, Cincinnati, OH), a sealed, sterile collection cup, instructions for collection, and a prepaid return envelope. Participants were asked not to eat or drink for 1hr before sample collection, to fill the cup to the 10 ml line with mouthwash, swish the mouthwash

vigorously for 45 sec, expectorate into the cup, and mail the container in the provided packaging.[15] Nearly 60% of the original completers of the trial were located and re-consented for the ancillary genetics study.

DNA extraction was performed by the QIAampDNA Mini Kit (QIAGEN #51104, Valencia, CA), and DNA quality and quantity was assessed by 558 bp polymerase chain reaction (PCR), separated on a 2% agarose gel by electrophoresis, and visualized by ethidium bromide staining. Allelic determination for adrenergic receptor variants (Gln27Glu β2-ADR, Trp64Arg β3-ADR, Glu^{12}/Glu^{9} α2b-ADR) was performed in two ways. Assays-by-Design Service and TaqMan technology (Applied Biosystems, Foster City, CA) designed PCR primers and allelic probes specific to ADRB2 Gln27Glu and ADRB3 Trp64Arg, shown in the table below. TaqMan PCR was performed under universal concentration conditions and universal thermal cycling parameters, with allelic determination on the ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). Taqman could not be used for the Glu^{12}/Glu^{9} α2b-ADR variant, due to the insertion length, therefore previously published ADRA2B Glu^{12}/Glu^{9} identification procedures by PCR and gel electrophoresis[59] were followed using primers designed for the long (112bp) and the short (103bp) variants (Midland Chemicals Midland, TX).

Variant	Probe	Forward Primer	Reverse Primer
A2B	Length 112/103	AGGGTGTTTGTGGGG CATCTCC	CAAGCTGAGGCCGGA GACACTG
B3			
Trp Arg	VIC FAM	GCAACCTGCTGGTCA TCGT	ACGAACACGTTGGTCA TGGT
B2			
Gln Glu	VIC FAM	CCTTCTTGCTGGCAC CCAAT	TGCCCACCACCCACAC

The frequencies of allelic variation presented in the results (carriers ADRA2B 31%, ADRB3 15%, ADRB2 40%) are within range of previous studies: ADRA2B,[10, 21, 51, 58, 59, 65, 70, 84] ADRB3,[1, 10, 12, 13, 17, 20, 23, 24, 26, 34, 36, 40, 45, 47, 48, 51, 53-57, 64, 69, 73, 75, 77-79, 81, 82] and ADRB2.[11, 16, 31, 33, 39, 41, 42, 44, 46, 49, 51, 74]

Exercise Training

Subjects were randomized within HT group to exercise (EX) or no exercise (NEX) groups. Exercise included supervised high intensity weight lifting and moderate impact weight-bearing exercise for 75 minutes, 3 days/week. Eight weight training exercises were performed per session, where individuals completed two sets of 6-8 repetitions at 70-80% of the one-repetition maximum loads per exercise. Attendance, loads, sets, and repetitions were recorded. Strength was measured every 6–8 weeks to increase loads and relative intensity. A detailed description of the exercise protocol has been previously published.[43]

Statistical Analyses

All statistical analyses were performed using SPSS v14.0 (SPSS Inc., Chicago, IL). The threshold of significance was set at p=0.10 to decrease type II error because of

sample size limitations for selected variables. A X^2 test was performed to determine if allele frequencies were in Hardy-Weinberg equilibrium. Allelic frequencies are presented as the percent of the subjects who completed 1 year and re-consented for the ancillary genetics study. All other data are expressed as mean ± standard error (SE) and/or p-value.

Within intervention group subject characteristics were compared using paired t-tests at one year. Between intervention group subject characteristics were compared using independent t-tests at 1 year.

Comparisons between genotype groups are confined to two genotype groups, i.e. carriers (ADRA2B Glu^{12}/Glu^{9} or Glu^{9}/Glu^{9}, also written as Glu^{9+}; ADRB3 Trp64Arg or Arg64Arg, also written as Arg^{64+}; and ADRB2 Gln27Glu or Glu27Glu, also written as Glu^{27+}) and noncarriers (ADRA2B Glu^{12}/Glu^{12} ADRA2B, also written as Glu^{9-}; ADRB3 Trp64Trp, also written as Arg^{64}; and ADRB2 Gln27Gln, also written as Glu^{27-}).

Associations between each gene or gene-gene interaction and body composition phenotype were analyzed separately. We used analysis of covariance (general linear model) including age and HT as covariates to obtain adjusted means, standard errors, partial Etas, power, and significance at baseline. The models were augmented to include covariates of exercise and baseline phenotype values for change at 1year. Linear regression models were used to obtain beta values and confirm significance. Additional analyses were completed within exercise and controls groups by analysis of variance to determine within genotype effects and within genotype to evaluate exercise effect.

RESULTS

Baseline Characteristics

Descriptive statistics at baseline and 1 year for the genetic subsample of the BEST study are presented in Table 1. There were no significant differences between those randomized to exercise and control at baseline. The women were postmenopausal, 56.1 ± 4.6 years of age, had a BMI of 24.9 ± 3.77 kg/m^2, and 54% of the women were on hormone therapy. The characteristics of the genotyped were not clinically different from those in the main study, however the genotyped group was slightly older and the body mass index was slightly lower (main study: 55.5 ± 4.8 years of age and BMI 25.3 ± 3.8 kg/m^2, $p<0.05$).

Main Effect of Exercise

There was no significant change in weight at 1year for either group and no significant difference in weight between exercisers and controls ($p>0.10$). The main effect of exercise was significant across all body composition measures, except trunk fat ($p<0.05$, Figure 4). The resistance training program altered body composition by significantly reducing total body fat and abdominal fat ($p<0.05$), and increasing lean soft tissue ($p<0.05$) in the genotyped subset. These changes are commensurate with the body composition changes of the main study ($p>0.05$). It should be noted that in the exercise sample, 19% did lose 2kg or more and 23% gained 2kg or more during the 1year intervention.

Allelic Frequencies

Genotype and allele frequencies are presented in Tables 2 and 3. Allele frequencies were in Hardy-Weinberg equilibrium across exercise and HT groups for ADRB2 and ADRB3 (Pearson Chi-Sq = 0.057, 0.097 and 0.16, 1.16; df = 1, respectively; $p>0.3$). While the carriage of the ADRA2B Glu^9 variant across intervention groups was not significant, ADRA2B Glu^9 variant carriage across HT groups was significantly different (Pearson Chi-Sq = 3.97; df = 1; $p<0.05$); the non-carriers were weighted more heavily toward no HT use, while the carriers were weighted more heavily toward HT use (non-carriers NHT 58%, HT 42%; carriers NHT 40%, HT 60%).

Gene-gene interactions must be interpreted cautiously given the low frequency of the ADRB3 Arg64 variant. In addition, exercisers re-consented for the ancillary genetics study more often than non-exercisers, as represented in Tables 1 and 2. In order to account for these differences, HT and exercise were used as covariates in all models, unless the analyses were split by those categorizations.

Effect of Adrenergic Receptor Variants on Baseline Body Composition

Associations between ADR genes and various body composition phenotypes at baseline are presented in Table 4. There was a weak association between the ADRA2B $Glu^{9/12}$ genotype and total lean soft tissue ($p<0.10$) and specifically arm and leg lean soft tissue ($p<0.10$); carriers of any Glu^9 allele were 1.4 ± 0.54 kg leaner than noncarriers of the Glu^9 allele. Associations between ADRB2 Gln27Glu polymorphism and multiple total and regional body composition phenotypes approached significance (Table 4 and

Figure 2). For example, carriers of any Glu^{27} allele in ADRB2 had greater fat mass than noncarriers. In contrast, there were no associations between ADRB3 Trp64Arg variants and body composition at baseline. Further, there were no gene-gene interactions at baseline.

Non-Genetic Determinants of Baseline Body Composition and Covariates

Interestingly, age was an independent, significant predictor of % total body fat and lean tissue in all models at baseline. We calculated body composition differences over a 10year span by using the unstandardized beta value and multiplying it by five years on either side of the average age in our study (56 years, Table 5.). The unstandardized beta values for age were similar across genes and therefore averaged for this projection. Based on this population, one may expect a 3% increase in total body fat and 3% decrease in lean soft tissue between the ages of 51 and 61years for sedentary individuals.

The effect of age in the post-intervention follow-up was not significant for most total and regional body composition measures. A small effect of age on leg lean mass for each gene and gene-gene combination was found (standardized beta range –0.167 to –0.207).

Adrenergic Receptor Variants as Determinants of Change in Body Composition at 1 Year

Table 6 presents the probability values for the multivariate associations between the ADR genotypes and various total and regional body composition variables at 1year, post-intervention. There were no significant associations between individual genes and

changes in body composition at one year. However, when the exercisers and the non-exercisers were separated, within the control group, carriers of any Arg^{64} allele in ADRB3 gained significantly greater % total body fat than non-carriers ($p<0.05$). Within genotype, there was a significant difference between exercise and control for ADRB3 Arg^{64} carriers and non-carriers ($p<0.05$). However, within carriers, controls gained weight while exercisers lost weight; within non-carriers, both exercisers and controls lost %total body fat (Figure 3).

Gene-Gene Interactions as Determinants of Change in Body Composition at 1 Year

ADRA2B and ADRB3 exhibited several gene-gene interaction effects. There was a trend toward gene-gene interactions within controls for changes in fat ($p<0.10$ %TBF and TBF grams, Figures 4, 5), while % total lean soft tissue and weight showed significant gene-gene interaction effects in both exercisers and controls ($p<0.10$, Figures 6,7). Changes in % leg fat demonstrated a gene-gene interaction within exercisers, rather than controls ($p<0.10$, Figure 8).

Lastly, ADRA2B and B3 did not exhibit different gene-gene interaction effects within controls or within exercisers. Within genotype, however, exercisers lost significantly more abdominal fat in the Glu^{9-}/Arg^{64+} genotype ($p<0.05$); the Glu^{9+}/Arg^{64-} genotype followed the same trend, but it was not quite significant ($p=0.11$, Figure 9). The difference between exercisers and controls was significant for change in % and grams total body fat, % total lean soft tissue, abdominal fat grams ($p<0.01$), and weight ($p<0.05$) within the Glu^{9-}/Arg^{64+} genotype; the Glu^{9+}/Arg^{64-} showed similar results for change %total body fat ($p<0.10$), % lean soft tissue ($p<0.05$), and abdominal fat ($p<0.10$). The

Glu^{9-}/Arg^{64-} and Glu^{9+}/Arg^{64+} did not demonstrate any significant effects of exercise on change in body composition in within genotype comparisons.

Surprisingly, there was a significant interaction between ADRA2B and ADRB2 on change in % abdominal fat ($p<0.01$), however when split by intervention, the gene-gene interaction persisted for the controls only. Exercisers lost abdominal fat regardless of genotype, however, non-carriers of both ADRA2B Glu^{9} and ADRB2 Glu^{27} alleles, as well as carriers of both ADRA2B Glu^{9} and ADRB2 Glu^{27} alleles lost abdominal fat independent of exercise status (Figure 10), while results from the other genotypes varied with exercise status.

The gene-gene interaction between ADRB3 and ADRB2 was positively associated with % leg lean soft tissue in exercisers, rather than controls ($p<0.05$), where carriers of both ADRB3 and ADRB2 variants gained five times more leg lean tissue than those that carried the ADRB2 variant alone (Figure 11). However, within genotype, differences relative to exercise were not significant for any of the four possible genotypes.

A final linear regression analysis including all genes and gene-gene interactions was used to compare the variance accounted for by the genes versus that by the full model including exercise and HT. The full model accounted for 19% of the variance in lean soft tissue, however for the remaining body composition variables the genes accounted for 5% or less of the variance.

DISCUSSION

Two themes predominate in the data presented here, gene-gene and gene-exercise interactions in body composition and change in body composition over a 12 month intervention trial. It is believed that individual genes do not operate in isolation to create and/or sustain body habitus as a complex phenotype. [35, 50] There is a complex interplay between stimuli, such as exercise, and individual response, whether it be internal balancing of genetic, hormonal, and other physiologic phenomena or strength and duration of external stimulus. Energy balance may influence genetic response and, conversely, genetic predisposition may energy balance.[50] Since the alpha-adrenergic receptors (ADRA) tend to demonstrate inhibitory effects on lipolysis, while the beta-adrenergic receptors (ADRB) demonstrate stimulatory effects upon the same stimulus,[27] it is relevant to study the interactions between genetic variants of each receptor type and the influence of co-carriage of allelic variation that may influence or attenuate gene-gene effects.

Although multiple allelic variations (or gene polymorphisms) have been discovered in both alpha- and beta-adrenergic receptors, the present study was confined to single variations in ADRA2B, ADRB3, and ADRB2 that previously have been shown to be associated with body composition and the response to endurance training, [52, 80] and therefore thought to be of functional significance. To our knowledge, this is the first study of ADRA2B, ADRB3, and ADRB2 genetic variation and resistance training in postmenopausal women.

The effect of exercise on body composition was significant in both the main BEST trial [68] and this ancillary genetics study, which contained a highly representative sub-sampling of the main trial. Both analyses demonstrated significant fat loss and lean soft tissue gain with exercise, and the magnitude of fat loss with exercise in the sub-sample was similar to the main trial (-1%), although controls in the sub-sample did not gain as much fat. Lean soft tissue increased by approximately 1kg in exercisers in the main trial and the subset, whereas controls in the subset gained 0.02kg overall versus a net effect of no change in the main trial. The differences between HT and NHT use among controls in the main trial and the present study are discussed below.

Overall our findings suggest that the strength of the exercise effect on body composition tended to overpower within intervention group effects of genotype, or within genotype effects of exercise. Given our sample size, this tended to mask the underlying effect modification on body composition by the ADR genotypes investigated. Exploratory analyses of gene effects by specific genotype or group assignment suggest that allelic variation in the adrenergic receptor gene and gene-gene associations do contribute to changes in body composition, both over time and with exercise though these effects may be modest in the presence of significant weight training exercise.

Individual Genes

ADRA2B is not well studied in obesity and physical activity trials. Other studies including the ADRA2B and body composition outcomes are primarily observational, while a few interventions involve diet and/or aerobic activity. In a cross-sectional study, Dionne, et al found no independent effect of the ADRA2B $Glu^{9/12}$ insertion

polymorphism on multiple body composition and fitness measures over a wide weight range in healthy Caucasian women,[10] which is in agreement with studies of other populations: obese [70] and morbidly obese persons,[66] Chinese women, [84] and young healthy Japanese males. [65] Heinonen found that among obese, non-diabetic Finnish women, compared to normoglycemic, normal weight controls, allelic frequencies were similar between groups, although basal metabolic rates in the homozygous Glu^{9} population were lower than in heterozygotes or homozygous wild type Glu^{12} individuals (mean age of 43years). No other obesity related phenotypes significantly differed by ADRA2B genotype.[21] However, in The Finnish Diabetes Prevention Study, an association was found between the $Glu^{9/12}$ genetic variant in ADRA2B and waist circumference at baseline ($p=0.052$). Although the relationship did not hold following lifestyle and diet intervention, Glu9 homozygotes in the high waist circumference group demonstrated an increased risk for type 2 diabetes.[58]

We also found no association between ADRA2B $Glu^{9/12}$ and waist circumference at baseline, but there was a significant interaction between the gene and exercise related to change in waist circumference following one year of resistance training ($p<0.05$). After stratifying by exercise, we found that ADRA2B tended to associate with an increase in waist circumference in the non-exercisers ($p<0.10$), but not in the exercise group (data not presented). ADRA2B Glu^{9} carrier controls gained in waist circumference, while non-carriers lost; carrier exercisers lost more in the waist than non-carriers, although the difference was not significant, given the small sample size.

In the Finnish studies, it was also observed that non-diabetic weight gain was significantly greater in Glu^{9} homozygotes than in the other genotypes among women with 10 years follow-up. [59] Either homozygote group in non-diabetic women had greater waist to hip ratios than the heterozygotes ($p<0.05$) at 10years.[59]

In the only trial of endurance training on change in body composition, in which ADRA2B was assessed, no independent association between ADRA2B $Glu^{9/12}$ and obesity phenotypes was found, however, the interaction between ADRA2B $Glu^{9/12}$ and ADRB2 Gln27Glu or ADRB3 Trp64Arg was significantly associated with change in % total body fat and trunk fat. Non-carriers of ADRA2B and carriers of either the ADRB3 or ADRB2 variants lost significantly greater % total and trunk fat.[51]

Our subjects, randomized to resistance training or control, showed a trend toward greater total and regional lean soft tissue in ADRA2B Glu^{9} carriers at baseline (Table 4), but the relationships were not present following the intervention (Table 6). It is difficult to say whether these baseline findings are similar to other studies, as lean soft tissue is seldom reported. However, this trial does confirm a lack of independent relationship between ADRA2B and body fat at baseline or body composition change with exercise.

Overall, the literature suggests that the relationship between ADRA2B and body composition or change in body composition appears to depend on other genes, [10, 51] and potentially metabolic or sympathetic activity. [58-60, 70, 83, 84] This study was not designed to examine the mechanism by which the genes may interact with exercise and change in body composition, and therefore is underpowered, but our results support further research

on the role of genetic variation in the ADR2B gene as a modifier of individual response to exercise.

In previous studies, genetic variation in ADRB3 has been associated with various measures of body composition. This association has been reported to vary by age,[6, 56, 64] gender,[7, 17, 20, 40, 48, 74] and ethnicity, as well as level of body fat[4, 22, 24, 54] and activity. [34] Similar to many other reports, we did not find an independent relationship between ADRB3 variants and body composition[14, 17, 20, 23, 25, 36, 48, 56, 57, 62, 67, 77] at baseline. However, change in % total body fat over 12 months was significantly associated with the Arg^{64} allele in our sedentary controls. Carriers of any Arg^{64} variant significantly increased % body fat in one year versus non-carriers. This relationship was not significant among all exercisers in our study when analyses were restricted to a given genotype. Among Arg^{64} carriers, exercisers lost body fat (%) while controls gained ($p<0.05$); whereas among the Arg $^{64-}$ genotype, both exercisers and controls lost total body fat (%) during the intervention. Similar to our results, a study of endurance training and change in body composition also found a significant association between ADRB3 and change in total body fat (% and kg) and % trunk fat ($p<0.05$), although they set the significance level at $p=0.01$ and therefore did not declare a significant association.[51] Since more stringent limits on probability values increases the possibility of type II error, we have chosen to interpret their results as significant, given our independent findings which decrease the risk of a false positive, or type I error.

Body composition has been inconsistently associated with ADRB2 variants. Cross-sectional evidence has supported obesity phenotypes in both Glu27 carriers [5, 11, 31,

[33, 37, 44, 71] and non-carriers,[39, 41, 49] as well as non-significant findings.[28, 46, 72, 76] In the present study, carriers of the Glu27 variant tended to be fatter with lower %lean soft tissue at baseline ($p<0.1$).

In intervention studies, we see many different aspects of obesity in relationship to ADRB2: fat loss, fat oxidation, weight loss resistance, and a greater propensity to gain weight when over fed. Among trials that involve endurance training and/or weight loss, the evidence more strongly supports carriage of Glu^{27} as a genetic barrier to changes in fat. The Quebec Overfeeding Study demonstrated that Glu27 carriers gained more weight and subcutaneous fat (NS abdominal fat) than non-carriers, following 100 days of overfeeding in young males.[71] In Japanese men, the ADRB2 Gln27Glu variant was associated with weight loss resistance or slow weight loss in a diet and exercise program approximately 2 years in duration.[37] An acute bout of exercise at maximal and submaximal intensities, demonstrated that Glu27 carriers had higher respiratory quotients during and following the bout, as well as lower fat oxidation in recovery.[33] In Caucasian women, The HERITAGE Study found that any carriage of ADRB2 Glu^{27} allele lost less fat after 20 weeks of endurance training compared to non-carriers of the ADRB2 Glu^{27} allele, while obese male carriers of the variant lost more fat, [16] suggesting gender specific effects.

In contrast, another study found that subjects that also carried the ADRB2 Glu^{27} allele were able to lose significantly more fat than non-carriers, particularly combined with ADRB3 carriers. [51] More trunk fat was lost with ADRB2 Glu^{27} carriage in combination with ADRA2B Glu^{9} non-carriage.

In our study, any body composition differences between ADRB2 Glu^{27} carriers and non-carriers present at baseline disappeared following one year of resistance training or control. Also. there were no significant associations between ADRB2 Gln27Glu and body composition changes when analyses within exercise or controls groups were performed. In contrast to the studies noted above, ADRB2 did not independently influence the body composition intervention results in our study. However, ADRB2 Gln27Glu did interact with other genes to influence body composition outcomes.

Gene-Gene Interactions

As stated previously the individual genes did not contribute a strong main effect on intervention response, however, ADRA2B $Glu^{9/12}$with ADRB3 Trp64Arg or ADRB2 Gln27Glu either exhibited or showed trends toward gene-gene interaction effects, and ADRB3 Trp64Arg and ADRB2 Gln27Glu combined to significantly influence leg lean soft tissue in exercisers when separated from controls. Because we observed more genotype effects stratified by exercise and controls groups than in the combined, adjusted group, we chose to explore gene-gene associations further by group (Figure 3-10). Gene-gene interaction effects on body composition by ADRA2B x ADRB3 and ADRA2B x ADRB2 included: within controls for changes in fat and specifically % abdominal fat, % total lean soft tissue, and weight; within exercisers for changes in % leg fat, % total lean soft tissue, and weight.

ADRA2B x ADRB3 did not exhibit different gene-gene interaction effects within groups with respect to abdominal fat. However within genotype, exercisers lost significantly more abdominal fat (g) in the Glu9-/Arg64+ genotype ($p<0.05$) and the

Glu9+/Arg64- genotype followed the same trend (p=0.11). ADRA2B x ADRB2, within the Glu9-/Glu27+ genotype, also demonstrated a trend toward "responsiveness" to sedentarism versus resistance training, i.e. a gain in %abdominal fat with sedentarism and a loss with resistance training (p=0.14). The overall association between change in % abdominal fat and the ADRA2B and ADRB3 interaction approached significance ($p<0.10$) and when split by exercise the association weakened (NEX p=0.228 and EX p=0.137). However, due to relatively low power and moderate effect sizes, the insignificant ADR2b x ADRB3 effect on change in grams abdominal fat split by exercise (NEX p=0.138, partial Eta 0.041, power .316, EX p= 0.136, partial Eta 0.031, power 0.318) suggest that with greater numbers a relationship between ADRA2B and ADRB3 would persist.

Our results seem to vary greatly depending on group assignment and individual genotype. They also vary in comparison to other studies, although some similarities are present. The most similar study for comparison with ours involves measures before and after endurance training in middle-aged men and women.[51] Our study found a significant within genotype effect of ADRA2B Glu9-/ADRB3 Arg64+ between control and exercise groups with respect to fat loss. Subjects with ADRA2B Glu9-/ADRB3 Arg64+ gained fat when sedentary, but lost fat with resistance exercise. Similarly, the ADRA2B Glu9-/ADRB3 Arg64+ subjects in the endurance training trial reported by Phares et al, lost significantly more %total body fat and % trunk fat. In addition, while both studies found that simultaneous carriage of variants for ADRB3 and ADRB2 enhanced changes body composition with exercise, Phares et al found these significant changes in % total and

trunk fat, whereas in our study ADRB3 x ADRB2 carriers had a significant advantage in increasing %leg lean soft tissue within the exercise group only, [51] an association that may reflect the type of intervention that was more weight bearing on the legs.

Interestingly, the significant interaction between ADRA2B and ADRB2 on change in % abdominal fat (Figure 10) in our population contradicts the findings for % trunk fat reported by Phares, et al. The fact that our exercisers lost %abdominal fat fits with the overall results of the trial. However, non-carriers and carriers of both ADRA2B Glu9 and ADRB2 Glu27 lost abdominal fat with or without resistance training exercise. Phares, et al found that non-carriage of ADRA2B Glu9 and carriage of ADRB2 Glu27 significantly enhanced trunk fat loss compared to all other multilocus genotype groups with endurance training.[51] Although, we found a trend toward the same genotype being more responsive to stimulus or lack of stimulus, it did not reach significance ($p=0.14$). We also did not find a significant loss of %trunk fat with exercise, to the contrary, our exercisers gained in trunk fat, relative to total body fat, due to losses in other regional depots. Our exercisers did lose grams of trunk fat, but the loss was not significant.

In a cross-sectional study of French men, Meirhaeghe et al, 1999 found that men with the ADRB2 Gln/Gln genotype had higher weight, BMI, waist and hip circumferences, however when the group was split into sedentary and physically active populations, the effect persisted for the sedentary men only.[41] A similar study conducted in a Spanish population, assessing leisure time physical activity in obese and normal weight controls, found that carriage of the ADRB3 variant was a risk factor for obesity in sedentary individuals, but not in active individuals.[34] Although Phares et al did not

compare exercisers with non-exercisers, they also found that carriers of variations previously thought to be associated with obesity phenotypes were in fact at an advantage in terms of body composition alteration in the setting of endurance training, particularly when accounting for gene-gene interactions, [51] an association that may reflect a more plastic genotype in terms of response to exercise stimulus.

Limitations

Two issues also need to be acknowledged before delving into individual gene and gene-gene interaction effects on body composition: the use or non-use of hormone therapy and sample size issues, particularly when examining at gene-gene interactions.

The potential influence of hormone therapy in postmenopausal women on body composition and gene response to behavior/environment may influence the interpretation of these results. In this study HT users were evenly split between resistance training and control groups. Although ADRB2 and ADRB3 variants were in Hardy-Weinberg equilibrium across intervention and HT groups, ADR2b variation was not equally distributed across HT groups. Evidence presented in a study of the determinants of body composition in postmenopausal women by Hagberg et al demonstrates that body composition is influenced less by HT than intense physical activity in postmenopausal women.[19] In addition, in an earlier publication we found that HT had only a small, clinically insignificant effect of preserving lean mass in sedentary controls in one year, with a large degree of inter-individual variation (mean and standard deviation in controls: 0.3±1.1 HT and –0.3±1.1 NHT), which was not observed in exercisers; no other significant effects of HT on body composition were found.[68] Since allelic frequencies of

variants did not allow for meaningful comparisons between four groups (NEX/NHT, NEX/HT, EX/NHT, EX/HT) and genotypic influences on body composition, hormone therapy was used as a covariate throughout analyses. We did not find a significant effect of HT on any fat or lean depots in the full model, nor did we find significant independent effects of HT in individual linear regression models at baseline or following 1yr of resistance training, except for one year change in % leg lean soft tissue with gene-gene interactions of ADRA2B x ADRB3 and ADRB3 x ADRB2. In addition, further analysis of these gene-gene interactions revealed that change in %leg lean soft tissue did not include interactions between exercise and hormone therapy or hormone therapy and gene-gene interactions.

As noted earlier, we must also use caution when interpreting gene-gene interactions, due in particular to the low frequency of the ADRB3 Arg64 variant and the fact that exercisers re-consented for the ancillary genetics study more than non-exercisers. Most of the sample did not lose weight (±2kg) and our study was characterized by small body composition changes giving less chance to detect genotype associations.

Both cross-sectional and intervention studies support our findings that genetic predispositions influence body composition within the context of behavior or other genes. In some of our analyses genetic influences were significant for controls, but not for exercisers or vice versa. In others, differences between exercise and controls were only present for specific genotypes. For ADRA2B and ADRB3 combinations, subjects with double non-carriage or double carriage seem to respond similarly without any influence

of behavior. However, at times, carriage of one or the other variant was advantageous (i.e., loss of fat or gain of lean) with exercise and detrimental with sedentary behavior (i.e., loss of lean and gain of fat). The same directional pattern was similar for ADRA2B combined with ADRB2 and change in % abdominal fat. Double carriers and double non-carriers both lost %abdominal fat with or without exercise, and carriers of one or the other responded with gain in controls and loss in exercisers. The gene-gene effect was only significant for the control group, however, within Glu9-/Glu27+ there was a trend toward a significant difference based on behavior.

The retrospective nature of our study was a limitation, as was our small sample sizes in gene-gene interaction groups and given the response variation based on ethnicity and age groups present in the literature, extrapolation to populations other than Caucasian post-menopausal women is not recommended. Non-responders were not accounted for by this set of genes, therefore exploration of other genes associated with physical activity and body composition is needed. In addition, more evidence is clearly needed to determine mechanisms and specific interactions between body composition change and genes. However, the idea of genetic responsiveness to stimulus or lack of stimulus was supported by our findings and responsiveness, rather than "bad gene" theories, should be researched further.

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TABLES AND FIGURES

Table 1. 1 Baseline subject characteristics and changes post-12 month intervention.

Characteristics	**Total (n=148)**	**Control (n=64)**	**Exercise (n=84)**	**p***
Age (years)	56.1 ± 0.37	56.4 ± 0.62	55.8 ± 0.46	0.398
Height (cm)	163.9 ± 0.53	164.0 ± 0.80	163.9 ± 0.72	0.923
Hormone Therapy (%)	54 %	53 ± 0.06	55 ± 0.06	0.844
Weight (kg)	66.9 ± 0.96	66.0 ± 1.36	67.6 ± 1.33	0.415
Post-Intervention Change	0.12 ± 0.22	0.25 ± 0.37	0.02 ± 0.27	0.620
Total body fat (%)	37.7 ± 0.54	37.44 ± 0.89	37.86 ± 0.68	0.707
Post-Intervention Change	-0.63 ± 0.21	0.09 ± 0.31	-1.19 ± 0.27†	**0.002**
Total body fat (kg)	25.38±0.68	24.95±1.03	25.71±0.91	0.577
Post-Intervention Change	-0.312±0.20	0.15±.032	-0.67±0.26	**0.046**
Trunk fat (%)	46.9 ± 0.40	46.59 ± 0.7	47.1 ± 0.52	0.586
Post-Intervention Change	0.25 ± 0.16	-0.14 ± 0.20	0.55 ± 0.21†	**0.030**
Trunk fat (kg)	12.04±0.36	11.77±0.055	12.24±0.48	0.528
Post-Intervention Change	-0.069	0.046±0.17	-0.16±0.15	0.364
Abdominal Fat (%)	10.3 ± 0.00	10.47 ± 0.29	10.3 ± 0.25	0.644
Post-Intervention Change	0.00 ± 0.00	0.06 ± 0.06	-0.10 ± 0.07	0.101
Abdominal Fat (kg)	2.73±.010	2.72±0.16	2.73±0.13	0.964
Post-Intervention Change	-0.023	0.05±0.04	-0.08±0.04	**0.021**
Lean Soft Tissue (%)	58.0± 0.51	58.3± 0.84	57.8 ± 0.67	0.64
Post-Intervention Change	0.70 ± 0.21†	-0.14 ± 0.31	1.33 ± 0.27†	**0.001**
Lean Soft Tissue (kg)	38.30±0.38	37.95±0.52	38.56±0.54	0.431
Post-Intervention Change	0.497†	0.024±0.15	0.86±0.11	**0.001**

Values are expressed as means ± SE.

* Probability for Student's t test comparing intervention groups

† Indicates significant change within group after intervention $p<0.05$.

Table 1. 2 ADR gene polymorphism frequencies for 1yr completers (N=148).

ADR gene polymorphism	**Allele Frequency**		**Genotype Frequency***		
ADRA2B $Glu^{12/9}$	Glu^{12}	Glu^{9}	Glu^{12}/ Glu^{12}	Glu^{12}/ Glu^{9}	Glu^{9}/ Glu^{9}
1yr	0.68	0.31	0.47 (70)	0.42 (62)	0.10 (15)
Exercisers			56%	55%	67%
ADRB3 Trp64Arg	Trp64	Arg64	Trp/Trp	Trp/Arg	Arg/Arg
1yr	0.85	0.15	0.85 (126)	0.15 (22)	0.00 (0)
Exercisers			56%	59%	0%
ADRB2 Gln27Glu	Gln27	Glu27	Gln/Gln	Gln/Glu	Glu/Glu
1yr	0.60	0.40	0.42 (62)	0.45 (67)	0.13 (19)
Exercisers			55%	54%	74%

*Values in parentheses are the sample sizes.
One subject could not be genotyped for the ADRA2B Glu^{12}/Glu^{9}

Table 1. 3 ADR gene polymorphism interaction frequencies for 1yr completers (N=148).

ADR gene polymorphism	**-/-**	**-/+**	**+/-**	**+/+**
ADRA2B X ADRB3	Glu^{9-}/Arg^{64-}	Glu^{9-}/Arg^{64+}	Glu^{9+}/Arg^{64-}	Glu^{9+}/Arg^{64+}
1Yr	0.40 (59)	0.07 (11)	0.45 (66)	0.07 (11)
Exercisers	59%	36%	53%	82%
ADRA2B X ADRB2	Glu^{9-}/Glu^{27-}	Glu^{9-}/Glu^{27+}	Glu^{9+}/Glu^{27-}	Glu^{9+}/Glu^{27+}
1Yr	0.17 (25)	0.31 (45)	0.25 (37)	0.27 (40)
Exercisers	56%	56%	54%	60%
ADRB3 X ADRB2	Arg^{64-}/Glu^{27-}	Arg^{64-}/Glu^{27+}	Arg^{64+}/Glu^{27-}	Arg^{64+}/Glu^{27+}
1Yr	0.34 (51)	0.51 (75)	0.07 (11)	0.07 (11)
Exercisers	55%	57%	55%	64%

*Values in parentheses are the sample sizes.
One subject could not be genotyped for the ADRA2B Glu^{12}/Glu^{9}

Table 1. 4 Probability values for the multivariate associations between the ADR genotypes and body composition at baseline.

ADR variant	**Body Weight (kg)**	**Fat Mass (kg)**	**Lean Soft Tissue (kg)**	**Trunk Fat (g)**	**AbFat (kg)**	**Arm Fat (kg)**	**Arm Lean (kg)**	**Leg Fat (g)**	**Leg Lean (kg)**
A2B	0.275	0.629	**0.059**	0.482	0.871	.477	**.058**	0.947	**0.068**
B3	0.516	0.405	0.976	0.453	0.506	0.393	1.00	0.461	0.596
B2	0.241	0.116	0.903	**0.075**	**0.100**	0.432	0.560	0.165	0.921
A2B X B3	**0.088**	0.138	0.122	0.181	0.663	0.143	0.302	0.158	0.134
A2B X B2	0.284	0.373	0.250	0.372	0.421	0.173	0.353	0.647	0.588
B3 X B2	0.777	0.614	0.739	0.514	0.399	0.956	0.404	0.734	0.596

ADR variant	**Total Body Fat (%)**	**Lean Soft Tissue (%)**	**Trunk Fat (%)**	**AbFat (%)**
A2B	0.906	0.809	0.225	0.694
B3	0.569	0.548	0.965	0.634
B2	**0.085**	**0.094**	0.127	**0.082**
A2B X B3	0.589	0.597	0.908	0.453
A2B X B2	0.639	0.694	0.521	0.795
B3 X B2	0.606	0.519	0.618	0.949

Full model includes covariates of age and hormone therapy status. Age was always significant in these models. Hormone therapy was not. A2B refers to the ADRA2B Glu^{12}/Glu^{9} insertion/deletion polymorphism, B3 refers to the ADRB3. Trp64Arg single nucleotide polymorphism (SNP), B2 refers to the ADRB2 Gln27Glu SNP. AbFat refers to abdominal fat.

Table 1. 5 Projected baseline body composition over 10 year span.

Depot	Unstandardized Beta	Age 51	Age 56	Age 61
% Total Body Fat	0.335	36.0	37.7	39.4
% Lean Soft Tissue	-0.303	59.5	58.0	56.5

Age was a significant predictor of body composition, independent of gene and exercise across all baseline regression models. average age was 56 years. Unstandardized Beta was similar across genes and therefore averaged.

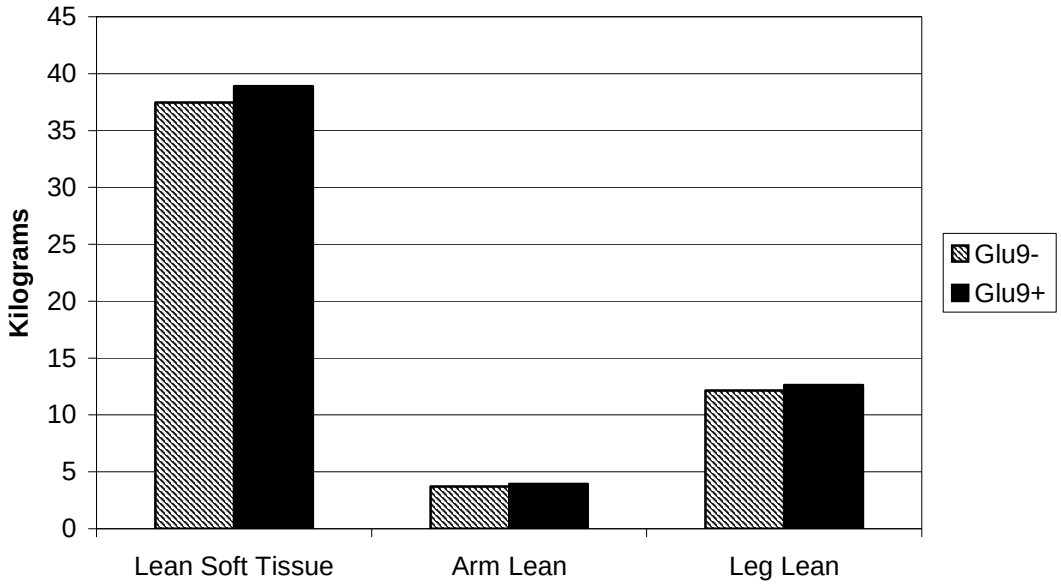
Table 1. 6 Probability values for the multivariate associations between the ADR genotypes and body composition post 1 yr intervention.

ADR variant	**Δ Body Weight (kg)**	**Δ Fat Mass (kg)**	**Δ Lean Soft Tissue (g)**	**Δ Trunk fat (g)**	**Δ AbFat (g)**	**Δ Arm Fat (g)**	**Δ Arm Lean (g)**	**Δ Leg Fat (g)**	**Δ Leg Lean (g)**
$\alpha 2_b$	0.950	0.989	0.758	0.807	0.712	0.822	0.936	0.959	0.493
β3	0.839	0.528	0.292	0.485	0.629	0.759	0.322	0.466	0.971
β2	0.396	0.852	0.875	0.525	0.593	0.233	0.941	0.889	0.612
$\alpha 2_b$ X β3	0.700	0.511	0.228	0.896	0.851	0.863	0.948	0.170	0.124
$\alpha 2_b$ X β2	0.470	0.800	0.220	0.449	0.279	0.521	0.117	0.698	0.323
β3 X β2	0.223	0.453	0.905	0.455	0.627	0.583	0.187	0.193	0.145

ADR variant	**Δ Total body fat (%)**	**Δ Lean Soft Tissue (%)**	**Δ Trunk Fat (%)**	**Δ Ab Fat (%)**
$\alpha 2_b$	0.871	0.732	0.505	0.771
β3	0.201	0.253	0.883	0.515
β2	0.645	0.465	0.268	0.592
$\alpha 2_b$ X β3	0.548	0.611	0.093	0.086
$\alpha 2_b$ X β2	0.833	0.712	**0.023**	**0.007**
β3 X β2	0.595	0.410	0.821	0.723

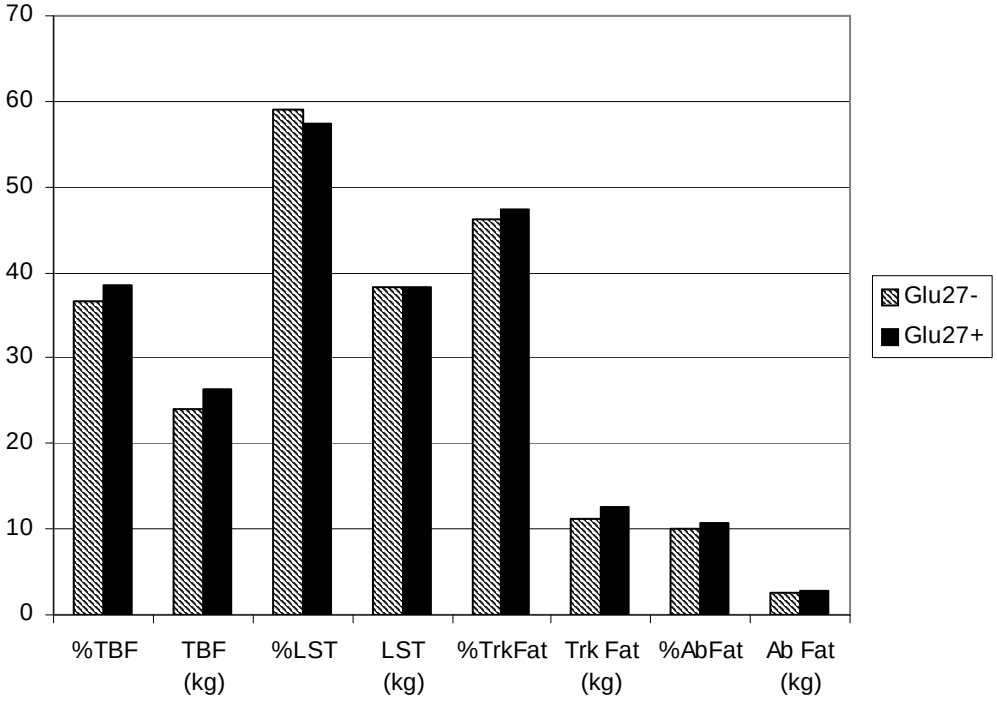
Full model includes covariates of age, hormone therapy status, exercise status, and baseline value of the body composition phenotype indicated. $\alpha 2_b$ refers to the Glu^{12}/Glu^{9} $\alpha 2_b$-ADR insertion/deletion polymorphism, β3 refers to the Trp64Arg β3-ADR single nucleotide polymorphism (SNP), β2 refers to the Gln27Glu β2-ADR SNP. AbFat refers to abdominal fat.

Figure 1. 1 ADRA2B lean tissue mass at baseline.



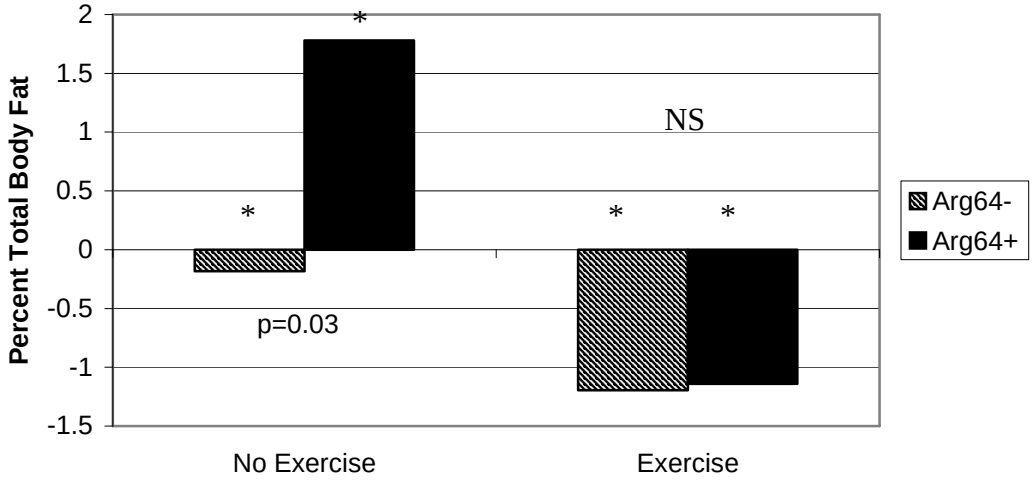
P-values for lean soft tissue, arm lean, and leg lean are the following 0.059, 0.058, 0.068, respectively. Covariates appearing in the model are evaluated at the following values: HRT Status at baseline = 0.54, Age (yrs) = 56.04.

Figure 1. 2 Baseline ADRB2 and Body Composition.



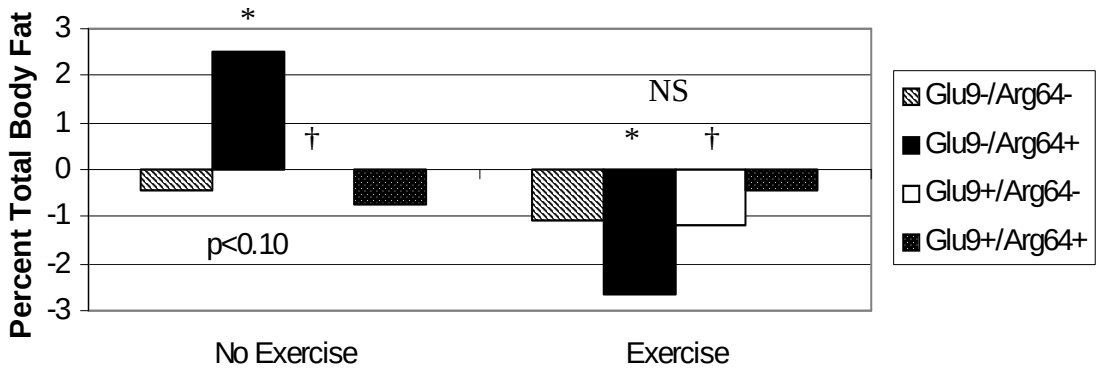
P-values are the following %TBF 0.085, TBF 0.116, %LST 0.094, LST (kg) 0.903, %Trk Fat 0.127, Trk Fat (g) 0.075, % AbFat 0.082, AbFat (g) 0.100. Covariates appearing in the model are evaluated at the following values: HRT Status at baseline = .54, Age (yrs) = 56.1.

Figure 1. 3 ADRB3 and Change in % Fat



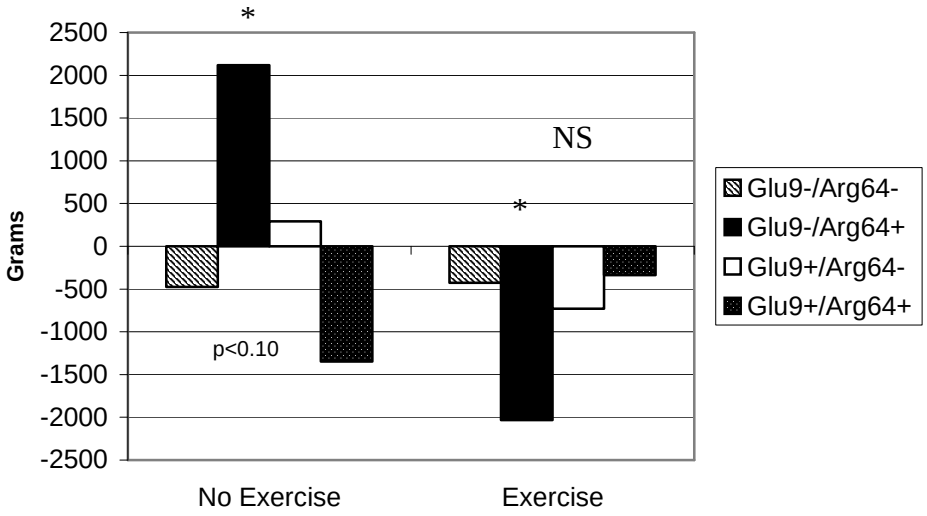
P-values refer to overall effects of gene or gene-gene interaction within intervention group by general linear models, not significant = NS. Within genotype effect between no exercise and exercise (NEX and EX), *p<0.05, † p<0.10.

Figure 1. 4 ADRA2b x ADRB3 and Change in % Fat



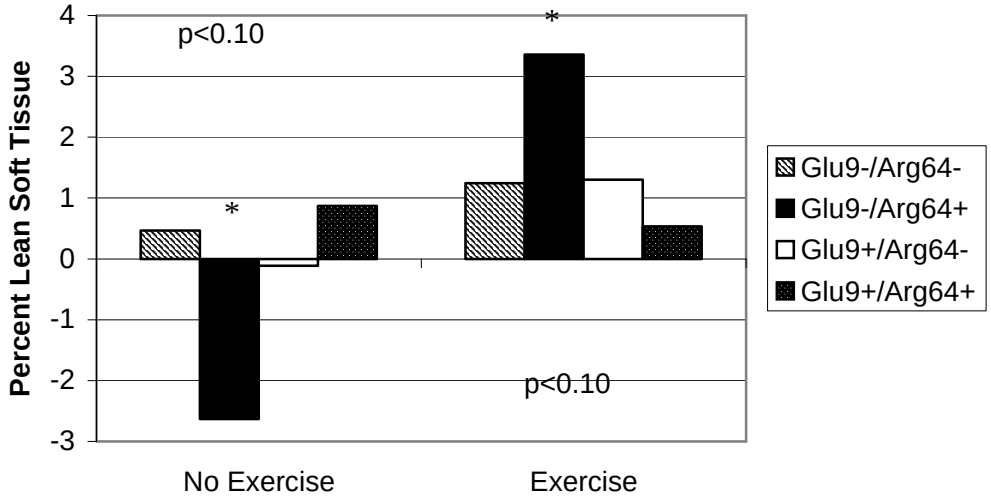
P-values present within the figures refer to overall effects of gene or gene-gene interaction within intervention group by general linear models, not significant = NS. Within genotype effect between no exercise and exercise (NEX and EX), *p<0.05, † p<0.10. (NEX Glu9-/Arg64- vs Glu9-/Arg+ p<0.05)

Figure 1. 5 ADRA2b x ADRB3 and Change in Fat (g)



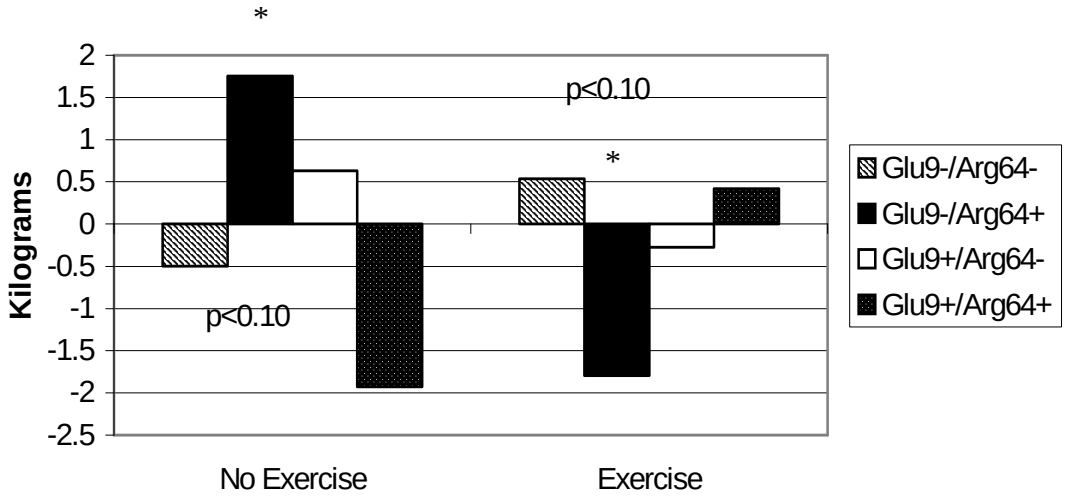
P-values present within the figures refer to overall effects of gene or gene-gene interaction within intervention group by general linear models, not significant = NS. Within genotype effect between no exercise and exercise (NEX and EX), *p<0.05, † p<0.10.

Figure 1. 6 ADRA2b and ADRB3 and Change in %Lean Soft Tissue



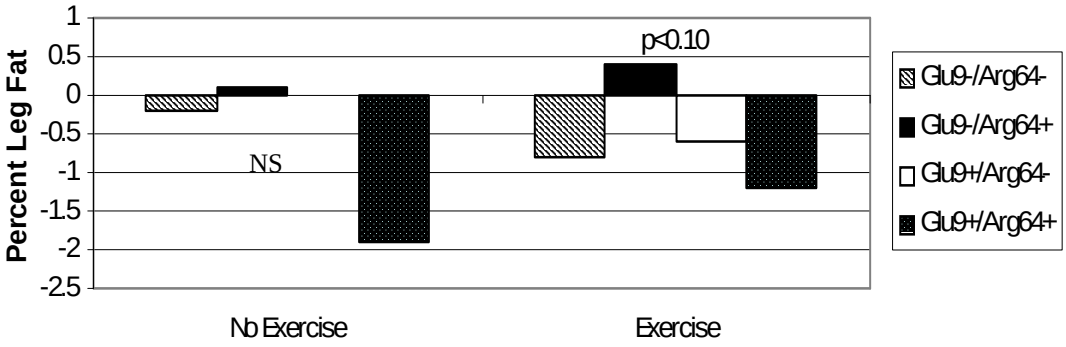
P-values present within the figures refer to overall effects of gene or gene-gene interaction within intervention group by general linear models, not significant = NS. Within genotype effect between no exercise and exercise (NEX and EX), *p<0.05, † p<0.10. Within NEX Glu9-/Arg64- vs Glu9-/Arg+ p<0.05.

Figure 1. 7 ADRA2b and ADRB3 and Change in Weight (kg)



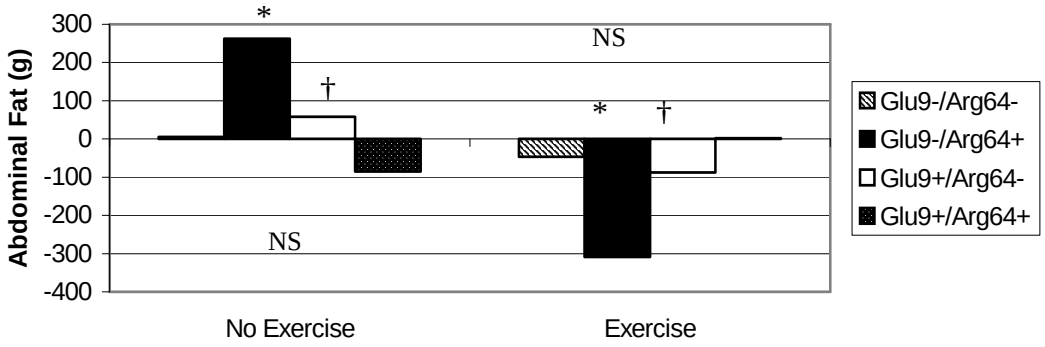
P-values present within the figures refer to overall effects of gene or gene-gene interaction within intervention group by general linear models, not significant = NS. Within genotype effect between no exercise and exercise (NEX and EX), *p<0.05, † p<0.10.

Figure 1. 8 ADRA2b and ADRB3 and Change %Leg Fat



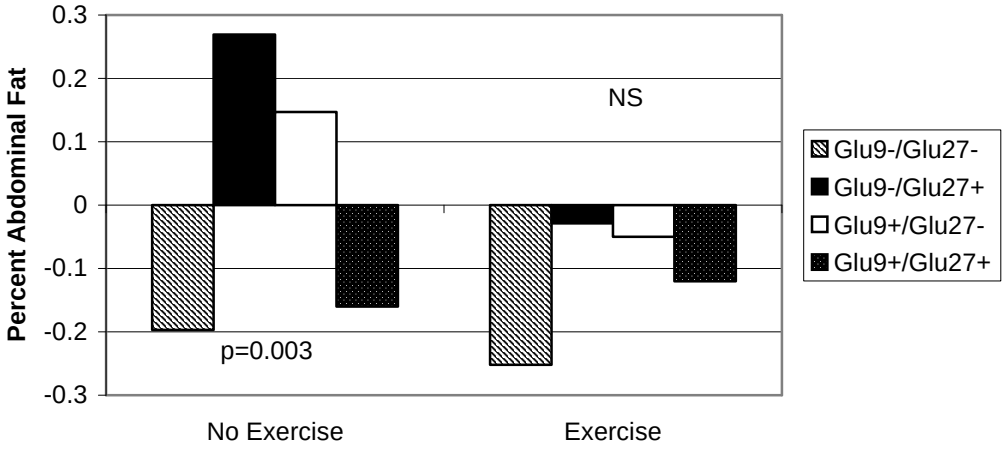
P-values present within the figures refer to overall effects of gene or gene-gene interaction within intervention group by general linear models, not significant = NS. Within genotype effect between no exercise and exercise (NEX and EX), *p<0.05, † p<0.10.

Figure 1. 9 ADRA2b and ADRB3 and Change in Abdominal Fat



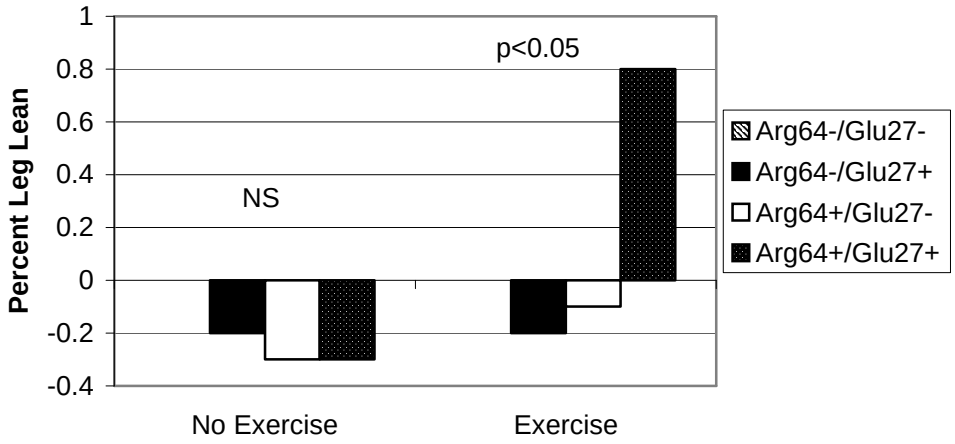
P-values present within the figures refer to overall effects of gene or gene-gene interaction within intervention group by general linear models, not significant = NS. Within genotype effect between no exercise and exercise (NEX and EX), *p<0.05, † p<0.10.

Figure 1. 10 ADRA2b and ADRB2 and Change in % Abdominal Fat



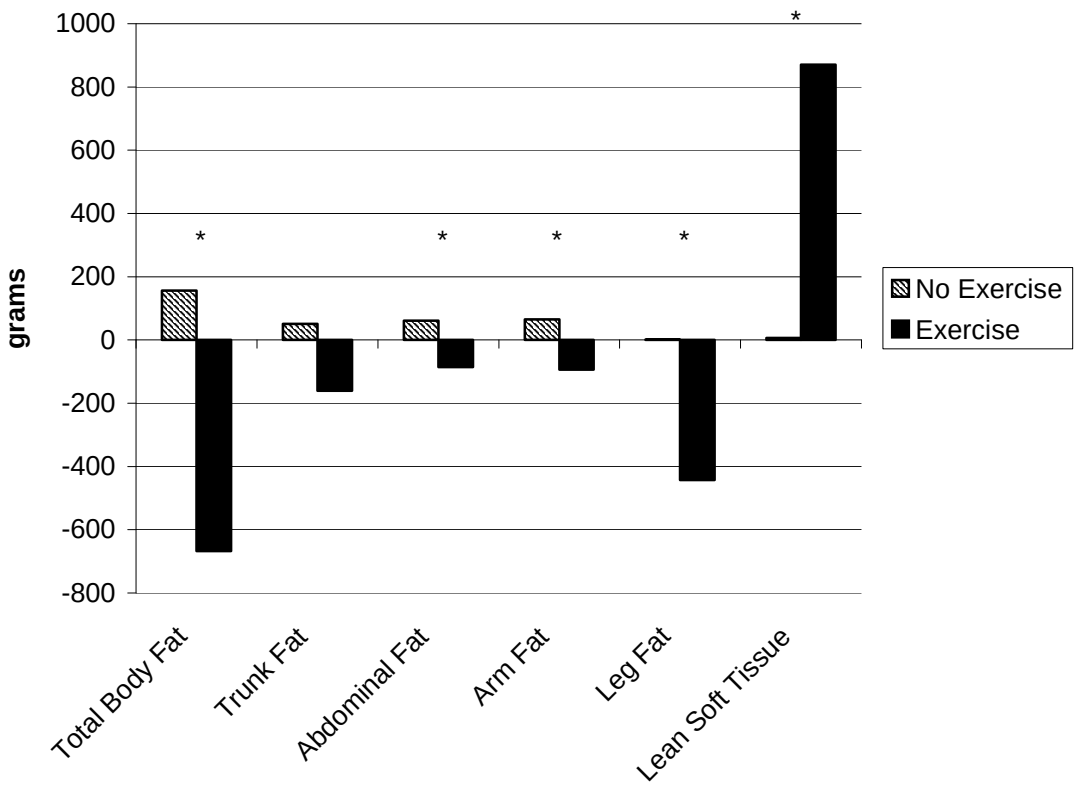
P-values present within the figures refer to overall effects of gene or gene-gene interaction within intervention group by general linear models, not significant = NS. Within genotype effect between no exercise and exercise (NEX and EX), *p<0.05, † p<0.10. Within controls Glu9-/Glu27- vs Glu9-/Glu27+ & Glu9-/Glu27+ vs Glu9+/Glu27+ p<0.1.

Figure 1. 11 ADRB3 and ADRB2 and Change in % Leg Lean Soft Tissue



P-values present within the figures refer to overall effects of gene or gene-gene interaction within intervention group by general linear models, not significant = NS. Within genotype effect between no exercise and exercise (NEX and EX), *p<0.05, † p<0.10.

Figure 1. 12 Main Effect of Exercise and Change in Body Composition



*p<0.05, General linear model with covariates appearing in the model evaluated at the following values: HRT Status at baseline = .54, Age (yrs) = 56.059537, Baseline Total Fat (gms) = 25381.15, Baseline Trunk Fat (gms) = 12035.75, Baseline Abdominal Fat from DXA in grams = 2730.28, Baseline Arm Fat 2399.73, Baseline Leg Fat 10013.71 Baseline Lean Soft Tissue in grams = 38296.85.
Note that No Exercise N=60 and Exercise N=80 for abdominal fat.

APPENDIX B: DIABETIC BIOMARKER RESPONSE TO RESISTANCE TRAINING IN POST-MENOPAUSAL WOMEN

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ABSTRACT

Obesity and physical inactivity are strongly associated with reductions in insulin sensitivity (IS) and subsequent maladies, including type 2 diabetes and cardiovascular disease. Post-menopausal (PM) women demonstrate increased susceptibility to both the risk factors and the disease states.

The objective of this study was to determine overall IS response to resistance training and IS response relative to the dose of training in PM women.

Fasting plasma biomarkers and surrogate models of IS were measured in completers of a large block-randomized trial of 12 months of resistance training (exercise, EX versus no exercise, NEX) in sedentary PM women, using (HT) or not using (NHT) hormone therapy, as part of the Bone Estrogen and Strength Training (BEST) Study. Plasma non-esterified fatty acids (NEFA) and fasting plasma glucose (FPG) were measured by colorimetric and spectrophotometric methods, and fasting plasma insulin (FPI) by radioimmunoassay. The homeostasis model for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and the revised QUICKI (R-QUICKI) scores reflecting IS were computed from these measures. Body composition was measured by dual x-ray absorptiometry. General linear models and linear regression were used to evaluate the intervention, effects of HT, body composition, and effects of initial FPG values.

The NHT/NEX group was older and had a greater number of years PM at baseline. NEFA levels were higher in HT groups. One year of exercise did not enhance IS in the whole group; however, exercise attendance was significantly associated with

glucose (Standardized Beta: –0.193, $p<0.05$), reflected in reduced FPG ($p=0.056$) and HOMA-IR scores ($p<0.01$) for exercisers who attended 2 out of 3 sessions per week. Baseline and change in IS were both largely explained by variation in fat mass, and more specifically, abdominal fat mass. Lean soft tissue was also positively associated with FPI and insulin change, but to a lesser extent than fat. HT was a significant determinant of baseline and change in NEFA levels with resistance training. Initial impaired FPG was accompanied by higher FPI and NEFA, but there were no significant differences between normal FPG and impaired FPG groups with respect to change in biomarkers or models of IS with body composition and change in body composition included in the model.

Resistance training did not improve IS. The strongest determinants of IS change were body composition, hormonal status, and initial values of IS biomarkers.

Key words: insulin sensitivity, weight lifting, physical activity, postmenopausal women, body composition

INTRODUCTION

Cardiovascular disease (CVD) is still the number one killer in the United States. [38] Postmenopausal (PM) women have an elevated risk for diabetes and CVD due to loss of cardio- protective estrogen,[56] increased central adiposity, [77] and decreased physical activity.[2, 6, 63, 75] It has been estimated that 31% of the mortality in women can be attributed to lack of physical activity and excess weight, with 59% of those deaths related to CVD.[37] Even healthy weight females have an increased risk of CVD (48%) if inactive.[46]

Whether or not diabetes is considered a CVD risk equivalent,[19, 30] there is substantial evidence for the significant contribution of diabetes to CVD diagnosis, events, and mortality, [4, 19, 27, 30] particularly in women, [36] including stroke.[32, 45] Since elevated risk of CVD may occur prior to diagnosis of type 2 diabetes,[35] it is important to address pre-cursors such as insulin resistance and adverse body composition.

Lifestyle interventions of both weight loss and increased physical activity have clearly demonstrated effectiveness in improving insulin sensitivity (IS);[82] however, it is often difficult to attribute IS improvements specifically to physical activity in these trials. One randomized controlled trial compared diet only, exercise only, and diet plus exercise with similar positive effects on diabetes prevention for both exercise and diet (~40%).[58] A similar trial of resistance training paired with weight loss versus weight loss alone in type 2 diabetics demonstrated greater decreases in HbA1c in the resistance trained plus weight loss than with weight loss only.[18] While numerous trials of aerobic activity alone have also demonstrated significant benefit in preventing type 2 diabetes,[42] there are fewer

trials evaluating diabetes prevention through resistance training,[42] especially randomized controlled trials,[8, 16, 61, 76] although clinical trials in diabetic populations have demonstrated resistance training induced benefits.[73]

Another important aspect of physical activity prescription for diabetes prevention is dose. The dose of activity required to improve IS has not been well studied with respect to aerobic or resistance training. Observational studies have shown that physical activity can significantly reduce risk of diabetes,[33, 34] but it is not clear whether increased intensity or frequency can further reduce the risk or if differences would be demonstrated in randomized controlled trials. One randomized controlled trial of moderate or light resistance training combined with aerobic training in males did not find significant differences between the combination of either intensity with aerobic training and aerobic training alone, both demonstrated significantly improved IS compared to controls. However, in this study, the effects of aerobic training versus resistance training cannot be truly teased apart. The dose of training required to produce significant differences in IS needs to be further explored.

The purpose of this study was to determine the response of insulin sensitivity to resistance training in postmenopausal women following 12-months of progressive resistance training, as part of the Bone Estrogen and Strength Training (BEST) Study. In addition, we evaluated the amount of resistance training to achieve IS benefits.

METHODS

Study Design

Blood samples from one-year completers of the Bone Estrogen and Strength Training Trial (BEST), a block-randomized resistance training trial in sedentary post-menopausal women who were using or not using hormone therapy (HT), were analyzed for fasting plasma glucose (FPG), insulin (FPI), and non-esterified fatty acids (NEFA). The training program consisted of vigorous, progressive resistance training and monitoring versus no training (control). HT users and non-users were evenly distributed across intervention groups.[9, 24]

Participants

Weight stable, sedentary post-menopausal women aged 40–65 years and 3–10.9 years postmenopausal, with body mass indices <33 kg/m^2 were recruited for the BEST study. Participants were instructed to maintain stable HT status, diet, and weight throughout the trial. [24, 50, 79] Details regarding dietary analyses have been previously published.[50] Measurements reported herein were taken at baseline and following 12 months of either control or resistance training. Exclusion criteria included medication use (i.e. steroids, beta-blockers, bone mineral density (BMD) altering medications), cancer within 5 years, or other chronic health conditions.[9]

Our main trial for the effects of exercise and calcium on BMD included 266 completers of the one year intervention.[24] From this sample, we were able to gather complete FPG, FPI, and NEFA data on a large subset (N=225) for use in this analysis. We excluded 25 one-year completers based on the following criteria: change from no HT

to HT or from oral HT to the patch (N=7), FPG levels >125mg/dl at either baseline or 1 year (N=7, glucose levels indicate undiagnosed provisional type 2 diabetes mellitus),[1] insulin levels greater than 12 standard deviations higher than the study population at 1 year (N=1). Lastly, due to significant differences between oral HT and patch users, patch users were eliminated from all further analyses (N=10). The final number of subjects included in this report is 200.

The participants were also classified based on glucose levels as normal fasting glucose (<100mg/dl) or impaired fasting glucose (100-125mg/dl), for subset analyses. Written informed consent was provided by all subjects prior to participation and study protocols were approved by University of Arizona Institutional Review Board.

Body Composition Measurements

Whole body and regional composition was measured by dual X-ray absorptiometry (DXA by Lunar Radiation Corporation, Madison, WI). Anthropometric measures of height, weight, waist and hip circumferences were performed according to industry standards.[47] Lean soft tissue (LST) mass was calculated by subtracting DXA fat tissue (FT) mass from soft tissue mass.[79]Abdominal fat was determined by an operator set region of interest that included the intervertebral space between the first and second lumbar vertebrae and the iliac crest. All body composition and anthropometric measures were performed in duplicate at baseline and 1 year on 2 different days, within a 2 week period.[24, 47, 79] Means of the duplicates were used in all statistical analyses.

Biochemical Assays for Biomarkers of IS

Blood samples were collected following an overnight fast and stored at -80ºC, as previously published.[54] EDTA treated samples were used for glucose analysis and sodium citrate treated samples were used for insulin and NEFA analyses. Neither anticoagulant interacted with their respective assays. All biochemical assays utilized fasting plasma samples at baseline and 1 year for all groups and were performed in duplicate for insulin and NEFA, and in triplicate for glucose. Baseline and 1 year samples for were run within the same assay for each subject. A Human Insulin Specific Radioimmunoassay (RIA) Kit (Linco Research, Inc., St Charles, MO) and gamma counter were used to quantify FPI levels. Intra and inter coefficients of variation (intra-CV, inter CV) were both 4% for insulin measurements. Determination of FPG concentration was performed by colorimetric assay via the glucose oxidase method and spectrophotometer (Thermo Electron Corporation, Pittsburgh, PA). Intra- and inter-CVs were 5% and 8% for glucose measures respectively. Fasting plasma NEFA levels were determined by the NEFA in vitro enzymatic colorimetric microtiter technique and plate reader (Wako Chemicals USA, Inc., Richmond, VA). Intra and inter CVs were 4% and 11%, respectively.

The homeostasis model for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and the revised QUICKI (R-QUICKI) were computed according to Brady, et al[5] as surrogate measures of insulin action:

$$\text{HOMA-IR} = (\text{insulin}_{0\ (\text{uU/ml})} * \text{glucose}_{0\ (\text{mmol/l})}) / 22.5$$

$$\text{QUICKI} = 1/(\log(\text{glucose}_{0\ (\text{mg/dl})}) + \log(\text{insulin}_{0\ (\text{uU/ml})}))$$

$$\text{R-QUICKI} = 1/(\log(\text{glucose}_{0\ (\text{mg/dl})}) + \log(\text{insulin}_{0\ (\text{uU/ml})}) + \log(\text{NEFA}_{0\ (\text{mmol/l})}))$$

High HOMA-IR scores indicate low insulin sensitivity, while high QUICKI or R-QUICKI scores indicate high insulin sensitivity.

Exercise Training

Hormone therapy users and non-users were randomized to resistance training (EX) or no resistance training (NEX). Exercise sessions were supervised and included high intensity weight lifting and moderate impact weight-bearing exercise for 75 minutes, 3 days per week. Two sets of eight weight training exercises (squats, leg press, weighted march, military press, seated row, latissimus dorsi pull-down, back extension, rotary torso) were performed for 6-8 repetitions at 70-80% of the one-repetition maximum (1RM) each session. Every 6–8 weeks strength was measured and weights increased to maintain loads of 70-80% 1RM. A detailed description of the exercise protocol has been previously published.[51]

Statistical Analysis

General linear models were used to compare oral versus patch HT users for all variables; covariates included age and total body fat (TBF) (g) at baseline, and age, baseline TBF (g), change in TBF (g), baseline values of the dependent variable, and exercise for change over the one-year intervention. Patch users displayed significantly lower NEFA than oral HT users at baseline ($p<0.05$) and a higher R-QUICKI score ($p<0.10$). Following the one-year intervention, patch users increased insulin ($p<0.10$) and

HOMA-IR scores (p=0.20) compared to oral HT users. Patch users were excluded from all further analyses due to these differences. HT use refers to oral HT for this report.

All baseline characteristics, except biomarkers of IS, between intervention groups were compared using one-way analysis of variance. Several analyses of biomarkers of IS included statistical contrasts to account for differences due to blood draw timing, since general linear models with covariates of age and weight showed insulin values were significantly different between participants with blood draws within 6 days of the last bout of activity versus drawn greater than one week after the last bout of activity. These contrasts are not included in analyses of the main effect of hormone therapy, exercise dose, or impaired fasting glucose due to small group numbers compared to number of comparisons. However, all analyses were originally performed with and without contrasts and the difference in results between the two procedures is negligible.

Baseline characteristics of IS were computed by analysis of covariance including a statistical contrast between blood draws greater than and less than one week at baseline to obtain adjusted means. Baseline linear regression models of glucose, insulin, and NEFA include the same contrast, as well as hormone therapy, age, and selected fat depot.

At one year, linear regression models include HT, age, exercise, baseline value of the dependent variable, baseline value of selected fat depot, change in selected fat depot, and 6 contrasts to account for differences between those with physical activity (PA) within and greater than 6 days of their blood draw (1. No PA or PA at both baseline, B, and one year, Y1, within 6days of blood draw versus PA within 6 days of blood draw at one or the other time point, 2. No PA versus PA at baseline, 3. PA at both time points

versus PA at Y1 only, 4. interaction of contrast 1 with random assignment to EX or NEX groups, 5. interaction of contrast 2 with random group assignment, 6. interaction of contrast 3 with random group assignment).

The selected fat depots in the linear regression models were also used to determine whether to use total body fat (g), % total body fat, or abdominal fat (g) as the covariate in future models for biomarkers of IS. From these models, we determined that both total body fat (g) and abdominal fat (g) account for significant variance in the models. Total body fat (g) and abdominal fat (g) are highly correlated at baseline (Pearson correlation 0.88, $p<0.01$) and change at 1year (Pearson correlation 0.94, $p<0.01$). Since abdominal data were incomplete for approximately 20 subjects, total body fat was used in all future models.

Analysis of covariance (general linear model) was used to evaluate the impact of exercise on one-year changes in fasting glucose, insulin, NEFA, and models of IS (HOMA-IR, QUICKI, Revised QUICKI). Models included covariates of age, hormone therapy, and baseline total body fat (g), baseline values of the dependent variable, and change in total body fat (g), plus the contrasts above. The general linear model was also used to explore the impact of oral HT on the biomarkers of IS. Models for the main effect of HT also included covariates of age, hormone therapy, and baseline total body fat (g), baseline values of the dependent variable, and change in total body fat (g). HT groups were also evaluated separately for the main effect of exercise in a similar fashion.

Analysis of covariance and linear regression models for NEFA and revised-QUICKI scores also included energy intake (kcals), protein (g), fat (g), and carbohydrate

(g), since these dietary variables were significantly associated with NEFA at baseline. Baseline models for NEFA used 3d diet records from baseline. One year models for NEFA used average values from 8d diet records (3d baseline, 2d at six months, 3d at 1year).

Dose of physical activity was evaluated by adding % exercise session attendance, tertiles of attendance, >70% attendance, total weight lifted, total squat weight lifted, total military press weight lifted, or 1RM-year total to individual regression models detailed above.

Statistical analyses were performed by SPSS v14.0 (SPSS Inc., Chicago, IL) with significance reported for both $p<0.10$ and $p < 0.05$ to minimize type II error.

RESULTS

The groups were evenly balanced between HT and random assignment to exercise or control (N = 55 HT/EX, 46 HT/NEX, 51 NHT/EX, 48 NHT/NEX). Baseline characteristics between intervention groups were not significantly different except for age, years postmenopausal (PM) and NEFA ($p<0.05$ for age: HT/NEX vs. NHT/NEX; Years PM: NHT/NEX vs. all other groups; NEFA: HT vs. NHT with or without EX). There were no significant differences between groups in terms of FPG and FPI, or surrogate models of IS. Body composition and dietary intake of total energy (kcal), protein, fat, and carbohydrates also did not differ between groups at baseline. The mean age of the total sample was 55.5 years. The average BMI and %TBF were close to limits set for overweight (25.4 kg/m2) and overfat (38.1%) categories.70 for this age group,

although, the mean caloric intake per day was not high 1763kcals. FPG, FPI, and NEFA values were 86.7mg/dl, 9.8 uU/ml, 0.39mEq/L, respectively (Table 1). HOMA-IR, QUICKI, and R-QUICKI surrogate models of IS scores were 2.1, 0.35, 0.41, respectively.

Effect of Resistance on Biomarkers of IS

There were no significant differences between exercisers and controls for changes in IS, when accounting for baseline values of the dependent variable, age, HT use, baseline TBF, change in TBF, and contrasts for blood draw dates. There was an increase in FPG with exercise ($p<0.1$), with a non-significant decrease in insulin in exercisers. Without further analysis, it may appear that whole body IS may be improved with resistance training in this group. However, the models of IS that take FPG and FPI into account (HOMA-IR and QUICKI) were not significantly affected by resistance training in this study. The revised-QUICKI model was also unaffected by exercise (Table 4).

Explanatory Variables for Biomarkers of IS

Baseline values of FPG, FPI, and NEFA were significantly intercorrelated. Baseline values of each biomarker were also significantly correlated with change in each of their respected values. Change in glucose and insulin levels were significantly intercorrelated, however change in NEFA did not correlate with change of either glucose or insulin levels (Table 5).

Baseline values of FPG, FPI, and NEFA were all significantly associated with baseline total and regional fatness. Greater fatness was associated with higher FPG, FPI,

and NEFA levels (standardized betas: 0.267, 0.425, and 0.280 respectively, $p<0.01$). Compared to total body fat, abdominal fat (g) explained more of the variance in both glucose and insulin, but not NEFA; relative total body fat (%TBF) was more influential for NEFA. Lean soft tissue mass was positively associated with baseline insulin values only. Baseline values of FPG were also explained by age. However, age was not a significant source of variation in insulin and NEFA levels at baseline. Hormone therapy was not significantly associated with glucose or insulin, but explained nearly as much of the variance in NEFA as explained by fatness (Table 2). Hormone therapy users had significantly higher circulating NEFA levels than non-users at baseline (Figure 1).

The changes in FPG, FPI, and NEFA at one year were explained primarily by initial values. Higher baseline levels of FPG, FPI, and NEFA were significantly associated with greater decreases in each variable. In all cases we accounted for exercise, age, body composition, and blood draw timing in the models (standardized betas for exercise, age, and body composition are -0.530, -0.440, -0.669, respectively, $p<0.01$, Table 4). All measures of initial fatness remained important for NEFA, but only fat mass (g) was significantly associated with insulin, while abdominal fat was significantly associated with glucose. Changes in fat were not significant for glucose, but explained a significant portion of the variance for insulin and NEFA (Table 4). Baseline LST was significantly associated with change in glucose, while change in LST was significantly associated with change in insulin. Age was significant only for change in glucose, while hormone therapy was significant only for NEFA changes (Table 4).

When groups were split by HT use or non-use, exercise did not affect changes in biomarkers of IS, after accounting for body composition. Baseline biomarkers of IS were more dependent on body composition in the HT users than in non-users, reflected in nearly double F statistics ($p<0.05$, Table 7). Changes in FPG, FPI, and NEFA were, again, heavily dependent on initial values. Baseline fat and change in fat were most influential for change in insulin in HT users, rather than non-users, however, the opposite was true for NEFA changes (Table 8).

Normal versus Impaired Fasting Glucose Response

Normal and impaired fasting glucose groups were defined by FPG levels alone; however, significant baseline differences in FPI and NEFA were found between glucose groups. All three surrogate models of IS were also significantly different between groups at baseline (Table 6). Change in biomarkers of IS evaluated by ANCOVA with covariates including age, HT, baseline TBF (g), change TBF (g), baseline dependent variable, and exercise, reveal no significant differences between groups relative to change in biomarkers or models of IS, when models were adjusted for body composition and change in body composition (Table 6). HT was not significant of change in insulin sensitivity biomarkers, except for NEFA and R-QUICKI ($p<0.05$).

Exercise did not significantly predict change in insulin sensitivity. If normal and impaired fasting glucose groups were split and exercise was used as the factor in ANCOVA models, glucose increased with exercise within the normal fasting glucose group ($p<0.10$), while insulin and NEFA were unchanged. NEFA decreased with exercise

within the impaired fasting glucose group ($p<0.05$), while glucose and insulin were unchanged.

Exercise Dose

When exercisers were evaluated separately (sub sample N= 106), exercise attendance was significantly associated with changes in glucose by linear regression (Standardized Beta: –0.193, $p<0.037$). Those who attended seventy-percent or more compared to those who attended less than 70% of the exercise sessions, experienced greater decreases in glucose ($p<0.06$) and HOMA-IR ($p<0.10$), but no change in insulin, NEFA, QUICKI or R-QUICKI models compared to <70% attendance. Total weight lifted, total squat weight lifted, total military press weight lifted, and tertiles of attendance were not significantly associated with changes in biomarkers or models of IS.

DISCUSSION

At first glance, our results differ with the current literature regarding improvements in IS with exercise. We did not demonstrate an overall effect of resistance training on fasting measures of IS in our population of PM women. Lifestyle modification studies have demonstrated significant improvements in IS with weight loss and increased physical activity. [82] Aerobic training trials, of a variety of designs, have also yielded positive results.[42] However, despite a number of non-randomized trials showing improved IS response with resistance training, there are few randomized controlled trials with which to compare our results and even fewer with female subjects. [7, 16, 20, 52, 53, 74, 83] Since counter regulatory responses to exercise and hypoglycemia are

sexually dimorphic,[11-13, 23] the initial discussion of resistance training effects on IS will be limited to studies in women. In addition, most of the aforementioned trials involved obese or insulin resistant populations. [42, 82] Our study population was in good health, non-obese, and had primarily normal fasting glucose levels.

Only two randomized controlled resistance training trials in PM women involving IS measures were found.[8, 76] The population utilized by Cuff et al was obese and included type 2 diabetes,[8] while the trial by Stewart, et al was performed in hypertensives,[76] many with metabolic syndrome. Both studies combined aerobic and resistance training programs. Cuff et al. compared aerobic training plus resistance training to aerobic training alone and control (randomized to 3 groups). Although their measure of IS was more sensitive than ours, the combination of groups makes it difficult to assign IS benefits to resistance training or aerobic training. However, there was greater benefit with aerobic training + resistance training compared to aerobic training alone ($p<0.10$).[8] Stewart et al[76] also used resistance training in combination with aerobic training versus control (randomized to 2 groups) in hypertensive women, but found no significant differences between groups for FPG, FPI, or the QUICKI model of IS. Similar to our findings, they did find significant associations between favorable changes in body composition, particularly abdominal fat by MRI, and improved markers of IS.[76]

Young, healthy women randomized to aerobic training, resistance training, or control demonstrated significant IS improvements with both aerobic training and resistance training compared to control, however the change was dependent on increased lean mass in the resistance trained group.[61]

Earlier pre-post trials of resistance training in PM women by Ryan et al [65, 68] demonstrated positive effects of resistance training alone and resistance training plus weight loss on IS; however, they did not account for changes in body composition. A more recent study by Ryan et al [66] did not find significant changes in IS in PM women with resistance training. Body composition was not associated with changes in IS in that study however the group did not significantly alter fat mass over the 6 month training period.

Our findings may be due in part to use of less sensitive measures of IS, lack of significant weight loss, or lack of obesity or large numbers of impaired fasting glucose subjects in our population. The mean initial BMI was 25.4 kg/m^2. Although some subjects lost (N=36) <2 kg of body weight or gained (N=54) >2kg of body weight, many subjects were weight stable (N=110). The mean change in weight was 0.22 ± 2.95kg, while the mean change in body fat and lean soft tissue were -0.01 ± 2.80kg and 0.40 ± 1.16kg respectively. Further, our population only included 11% impaired fasting glucose.

In a review of non-randomized and randomized controlled trials, IS was changed in the majority of the studies, independent of the change in body composition. However, measures of FPG and FPI were frequently unchanged, while area under the curve (AUC) in either or both could have changed significantly. The trials reviewed were primarily aerobic training studies in overweight or obese.[42] Resistance training trials have also shown insignificant changes in fasting measures of glucose and insulin, but significant reductions in AUC or infusion rates for insulin and/or glucose with training.[8, 16, 20]

One of the most interesting aspects of this study is that both baseline fat and change in fat are independently and significantly associated with change in IS biomarkers. Decreased fat mass and waist circumference have been directly associated with improvements in IS [25] in both aerobic and resistance training trials.[65] Although slightly more of the variance in measures of insulin could be accounted for by abdominal fat, the association between our measures of abdominal adiposity by DXA and total body fat were similarly associated with biomarkers of IS. Perhaps with a more specific measure of visceral fat, we would be able to explain more of the variance in IS response to resistance training, as found by Kelley et al[43] and Goodpaster et al.[26]

In a randomized controlled trial of young women, resistance training induced increases in IS were observed, but the change was dependent on increased lean soft tissue ($r=0.48$, $p<0.05$ for FFM and IS relationship).[61] In our PM women, we found LST mass to be positively, rather than negatively, associated with increased fasting insulin levels, when accounting for exercise status. This counterintuitive relationship may be due in part to a coupling of lean and fat mass in the absence of intervention, as has been theorized by Forbes et al.[21, 22] Change in LST (g) was not correlated with change in fat mass in this population. In addition although exercisers lost significantly more %TBF and gained significantly more LST ($p<0.05$), both exercisers and controls gained LST. The gain in LST in controls was accompanied by gain in fat mass. When LST and TBF mass were both included in a general linear model, fat mass remained significant, but LST was no longer independently associated with baseline or change in insulin ($p>0.05$), indicating that the positive association seen between insulin and LST is likely a reflection of the

association between insulin and fat. It appears that a "companionship" between lean and fat mass was demonstrated in controls, but the exercise intervention perturbed the relationship in exercisers.

Another important factor that may influence the results presented here is HT. Associations with HT were not present in insulin or glucose measures, which is in agreement with the HERITAGE Family Study.[28] Although the intervention involved endurance, rather than resistance training, the HERITAGE Family Study found that FPG and FPI were not different between those taking HT and those not taking HT at baseline and post-exercise intervention.[28] However, the literature regarding the effect of HT on IS is inconsistent.

Other trials have found that HT users were more insulin-sensitive than non-users.[29, 78] The Women's Health Initiative (WHI) further demonstrated a reduced risk of developing type 2 diabetes with HT use.[48] The opposite was demonstrated with baseline data from the Postmenopausal Estrogen/Progestin Intervention Trial (PEPI); endogenous, bioavailable, estradiol was linearly associated with insulin resistance (odds ratio, 2.7; $p<0.001$), even after accounting for body mass index and waist to hip ratio.[39]

When we split our women by HT use, we found that body fat explained more of the variance in glucose, insulin, and NEFA at baseline in HT users than non-users. Baseline fat and change in fat continued to be more influential on insulin change for HT users at one year. It is possible that altered fat metabolism in adipocytes due to estrogen binding of the alpha receptor (ERα) and subsequent upregulation of the antilypolytic α-adrenergic receptor[59] or blunted sympathetic activity in PM women treated with estrogen

(E2),[69] strengthens the relationship between insulin and fat in HT users.

In contrast, in this study, at baseline and one year, oral HT use was significantly associated with increased NEFA and decreased in R-QUICKI, an index of IS, independent of initial values of NEFA, exercise, body fat, macronutrient intake, and age. NEFA levels have been shown to influence IS[72] and as such have been recently incorporated into QUICKI model of IS[41] to derive the revised QUICKI model (R-QUICKI).[60] Perseghin et al found that the R-QUICKI model of IS was more highly correlated with euglycemic-hyperinsulinemic clamp measures of IS than HOMA-IR and QUICKI models. However, NEFA levels in healthy young women tend to be higher than in men and are similar to the levels found in our women on HT.[11] Since the majority our population demonstrated normal FPG and FPI levels, the mild elevation of NEFA by HT and subsequent lower R-QUICKI scores should not be considered clinically relevant. However, the incorporation of NEFA into the QUICKI model should be further investigated in the postmenopausal population.

Although the study was not designed to make specific conclusions based on transdermal versus oral or estrogen plus progesterone versus unopposed estrogen, we found differences between the patch and oral hormone therapy with respect to NEFA. Patch users displayed significantly lower NEFA than oral HT users at baseline ($p<0.05$) and a trend toward higher Revised QUICKI scores ($p<0.10$). Other biomarkers of IS tended to be different in patch versus oral HT users as well. Following the one-year intervention, patch users increased FPI ($p<0.10$) compared to oral HT users. Although data are not presented here, patch users were excluded from all further analyses due to

these differences.

Delivery of HT has been studied by others and postprandial lipid oxidation was higher,[17, 57] while NEFA during hyperinsulinemic-euglycemic clamp studies was lower[57] in transdermal versus oral HT users. These differences may be due in part to increases in fat mass with oral versus transdermal delivery.[17] Further exploration, of the type and delivery mechanism of hormonal therapy relative to its effects on IS, is needed.

The effect of dose of training on IS should be further explored. Although an hour of brisk walking per day can significantly reduce risk of diabetes,[33] it is not clear whether increased intensity or frequency can further reduce the risk. The Nurses' Health Study indicated that walking and vigorous physical activity produced similar reductions in type 2 diabetes risk if energy expenditure was equivalent, i.e. the product of metabolic equivalents (MET) per activity and time spent in activity.[34] A randomized controlled trial in men by Delecluse et al[16] found that light or moderate resistance training paired with aerobic training resulted in similar improvements in IS compared to aerobic training alone. When compared to control, however, only the moderate resistance training group demonstrated significant pre-post intervention improvements in IS.[16] The contributions of aerobic training versus resistance training could not be effectively separated in the study by Delecluse.[16]

In our study, the women who attended an average at least 2 out of 3 sessions per week for the 12 months demonstrated greater decreases in glucose ($p<0.06$) and HOMA-IR ($p<0.10$), but no change in insulin, NEFA, QUICKI or R-QUICKI models, compared to those who attended fewer sessions. This difference exists even when accounting for

body composition and baseline dependent variables.

Limitations

Use of FPG and FPI values, and HOMA and QUICKI indices, as opposed to the hyperinsulinemic-euglycemic clamp, can cause interpretive problems, as the values will largely reflect hepatic IS. However, hepatic and muscle sensitivity to insulin have been reasonably correlated[49] and the use of fasting plasma NEFA measures also provide an additional peripheral indicator of IS.

Interpretive problems can arise from combining normal fasting glucose and impaired fasting glucose groups, thus, we explored differences between these groups and found that while significantly different with respect to biomarkers of IS at baseline, changes in IS biomarkers were not significantly different between the groups, when accounting for HT, age, exercise status, body composition, and baseline dependent variables. Therefore, the inclusion of baseline values of dependent variables, in addition to other aforementioned covariates, in full group analyses should be sufficient to account for differences in normal fasting glucose and impaired fasting glucose populations in this study.

The timing of last bout of exertion to fasting blood draws was not standardized. Although few subjects had blood drawn ≤6 days following physical activity at baseline, at one year 70% of exercisers and 30% of non-exercisers had blood drawn within 6 days of their last bout of exertion. To ascertain the effect of blood draw timing on biomarkers of IS, analysis of covariance models were performed by day. There were no differences between glucose and NEFA values in those measured less than a week after exertion with

those measured greater than a week after last bout of exertion; however, insulin values were significantly different. Also, we found non-significant differences among subjects measured from day1 through day 6 following last bout of exertion. Previous studies found that improvements in IS following physical activity are short lived, lasting 3-6 days.[15, 44, 71] We used contrasts to account for blood draws within and greater than 6 days in our regression and analysis of covariance models for the whole group. Primary outcomes with and without timing contrasts were compared and the findings were similar.

A 1999 consensus statement of effects of physical activity and glucose tolerance also draws attention to the issue of blood draw timing.[42] The authors suggest that acute effects of exercise on IS are as important as the chronic effects. [42] Based on previous aerobic training trials, IS improvements may be acute only.[15, 44, 71] Resistance training trials appear to confirm this notion, by placing blood draw timing 24 h to 6 days following last bout of resistance training. [8, 16, 20, 61, 67 62, 64, 66] These findings suggest that the frequency of physical activity is critical for IS improvements, particularly in the absence of significant weight loss.[14, 44, 65, 71] In our study those who trained at least 2 out of 3 days per week had significantly improved FPG ($p<0.06$), although other measures were unchanged.

Some studies have shown an effect of diet on IS,[40, 55, 80, 81] although it is often difficult to tease apart effects from weight loss and those of diet content. Study of the effects of diet on IS is beyond the scope of this study; however, we found no significant differences between groups in terms of pre- and post-intervention intake of

carbohydrates, fats, and proteins, by the 3-day food diet records at baseline and the average of 8 days of food records throughout the year for change variables. In addition, since energy (kcal), protein, fat, and carbohydrate (g) were associated with NEFA values at baseline, these dietary variables were used as covariates in all analyses of NEFA or R-QUICKI.

In conclusion, our study indicates that the effect of resistance training on IS may be indirect and dependent on frequency of exercise in this population of PM women. Since resistance training in this population of women was significantly associated with positive changes in BMD, loss in total body fat, and increased lean soft tissue,[10, 24, 79] and also associated with improved bone and cardiovascular health and diabetes prevention or management,[31] resistance training should be considered as an adjunct to aerobic training and weight loss for overall primary and secondary prevention of osteoporosis, cardiovascular disease, and diabetes. This study lends further supports the recommendations of the American Diabetes Association to include aerobic and resistance training for the prevention and management of type 2 diabetes.[3, 73]

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insulin sensitivity and secretion during a stable-label intravenous glucose tolerance test. Metabolism. 45:254-260, 1996.

TABLES AND FIGURES

Table 2. 1 Baseline subject characteristics presented as means and standard errors.

	HT				NHT			
	EX (55)		**NEX (46)**		**EX (51)**		**NEX (48)**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age (yrs)*	55.17	0.48	54.36	0.64	55.23	0.69	57.40	0.75
Years PM*	4.83	0.31	4.98	0.44	5.75	0.41	7.40	0.49
Biomarkers of Insulin Sensitivity								
Glucose (mg/dl)	88.31	1.47	85.16	1.61	86.63	1.52	86.62	1.58
Insulin (uU/ml)	9.36	0.45	10.40	0.49	9.53	0.46	9.74	0.48
NEFA (mEq/L)*	0.44	0.02	0.41	0.02	0.34	0.02	0.38	0.02
HOMA-IR	2.04	0.11	2.18	0.13	2.05	0.12	2.10	0.12
QUICKI	0.35	0.00	0.34	0.00	0.35	0.00	0.35	0.00
Revised QUICKI	0.40	0.01	0.41	0.01	0.42	0.01	0.41	0.01
Body Composition								
Weight (kg)	68.80	1.76	67.07	1.65	67.95	1.44	67.20	1.55
BMI (kg/m^2)	25.36	0.56	25.29	0.63	25.38	0.43	25.40	0.57
WHR	0.78	0.01	0.78	0.01	0.78	0.01	0.79	0.01
TBF (kg)	26.32	1.24	25.36	1.28	26.31	0.99	25.99	1.23
% TBF	37.83	0.90	37.41	1.10	38.74	0.76	38.43	1.12
AbFat (kg)	2.80	0.18	2.96	0.19	2.76	0.16	2.88	0.19
LST (kg)	39.15	0.61	38.48	0.63	38.33	0.57	37.91	0.54
Dietary Intake								
Energy (kcal)	1779.35	58.75	1840.72	64.59	1719.07	57.68	1713.03	60.17
Protein (g)	70.90	2.90	74.50	2.90	67.92	3.09	71.07	2.92
Fat (g)	61.49	3.47	61.88	4.01	60.12	3.36	56.67	3.50
CHO (g)	236.71	8.27	246.09	9.35	227.88	7.45	230.90	9.07

*Mean difference is significant at the level $p < 0.05$ Age: HT/NEX vs NHT/NEX; Years PM: NHT/NEX vs. all other groups; NEFA: HT vs NHT with or without EX.

Table 2. 2 Baseline regression models (1-4) to compare effects of 1. total body fat, TBF (g), 2. %TBF, 3. abdominal fat (Abfat) (g), and 4.lean soft tissue (LST) (g) on glucose, insulin, and non-esterified fatty acids (NEFA).

Glucose				
Baseline	**TBF (g)**	**%TBF**	**AbFat (g)**	**LST (g)**
HT	0.051 (0.45)	0.064 (0.35)	0.036 (0.60)	0.047 (0.50)
Age	0.224 **(0.001)**	0.218 **(0.001)**	0.184 **(0.01)**	0.268 (0.00)
Selected Fat or Lean Depot	0.246 **(0.001)**	0.240 **(0.001)**	0.316 **(0.001)**	0.083 (0.23)
Adj. R^2	0.116	0.113	0.142	0.064

Insulin				
Baseline	**TBF (g)**	**%TBF**	**AbFat (g)**	**LST (g)**
HT	0.028 (0.68)	0.049 (0.46)	0.027 (0.69)	0.019 (0.80)
Age	-0.043 (0.52)	-0.056 (0.41)	-0.052 (0.44)	0.028 (0.69)
Selected Fat or Lean Depot	0.403 **(0.001)**	0.404 **(0.001)**	0.469 **(0.001)**	0.159 **(0.03)**
Adj. R^2	0.159	0.158	0.216	0.025

NEFA				
Baseline	**TBF (g)**	**%TBF**	**AbFat (g)**	**LST (g)**
HT	0.218 **(0.001)**	0.231 **(0.001)**	0.209 **(0.001)**	0.178 **(0.02)**
Age	0.041 (.0.56)	0.027 (0.69)	0.020 (0.79)	0.042 (0.59)
Selected Fat or Lean Depot	0.259 **(0.001)**	0.303 **(0.001)**	0.279 **(0.001)**	0.040 (0.64)
Adj. R^2	0.132	0.157	0.134	0.027

Values in the table are standardized betas (p-value). *Models for NEFA also include nutritional factors kcals, protein (g), fat (g), carbohydrate (g), due to significant associations with NEFA values. Baseline utilizes 3d diet record from baseline, change at one year utilizes average of 8d diet records over the year. Also adjusted for blood draw timing. Selected fat or lean depot refers to the column headings for total body fat, % total body fat, abdominal fat, and lean soft tissue. Adj. R^2 , adjusted r-squared.

Table 2. 3 Changes in body composition and biomarkers of insulin sensitivity for all subjects at one year (N=200).

Body Composition	Mean	SD	**Biomarkers of IS**	Mean	SD
Weight (kg)	0.22	2.96	Glucose (mg/dl)	1.25	8.29
BMI (kg/m^2)	0.18	1.18	Insulin (uU/ml)	-0.20	2.86
WHR	0.001	0.02	NEFA (mEq/L)	0.02	0.15
TBF (kg)	-0.011	2.80	HOMA-IR	-0.01	0.72
% TBF	-0.31	2.64	QUICKI	0.0003	0.01
AbFat (kg)	0.01	0.39	Revised QUICKI	0.01	0.05
LST (kg)	0.40	1.16			

N=117 waist to hip ratio, WHR, change. Regression models were used to estimate changes in biomarkers of IS in order to include contrasts for blood draw timing and exercise status.

Table 2. 4 One year change regression models (1-4) to compare effects of 1. total body fat, TBF (g), 2. %TBF, 3. abdominal fat (Abfat) (g), and 4. lean soft tissue (g) (LST) on glucose, insulin, and non-esterified fatty acids (NEFA).

Glucose				
1Y Change	**TBF (g)**	**% TBF**	**AbFat (g)**	**LST (g)**
HT	-0.023 (0.73)	-0.017 (0.80)	-0.039 (0.57)	-0.035 (0.59)
Age	0.165 **(0.02)**	0.173 **(0.02)**	0.150 **(0.04)**	0.177 **(0.01)**
Exercise	0.099 (0.13)	0.106 (0.11)	0.090 (0.18)	0.105 (0.12)
Baseline Dependent Variable	-0.527 **(0.001)**	-0.516 **(0.001)**	-0.547 **(0.001)**	-0.504 **(0.001)**
Baseline Selected Fat or Lean Depot	0.128 (0.06)	0.085 (0.21)	0.167 **(0.02)**	0.147 **(0.03)**
Change in Selected Fat or Lean Depot	-0.031 (0.63)	-0.011(0.86)	-0.050 (0.46)	-0.031 (0.67)
Adj. R^2	0.204	0.195	0.217	0.212
Insulin				
1Y Change	**TBF (g)**	**% TBF**	**AbFat (g)**	**LST (g)**
HT	-0.104 (0.12)	-0.097 (0.16)	-0.093 (0.19)	-0.113 (0.10)
Age	0.075 (0.28)	0.064 (0.36)	0.086 (0.24)	0.087 (0.22)
Exercise	-0.036 (0.58)	-0.026 (0.70)	-0.051 (0.46)	-0.093 (0.19)
Baseline Dependent Variable	-0.430 **(0.001)**	-0.421 **(0.001)**	-0.470 **(0.001)**	-0.369 **(0.001)**
Baseline Selected Fat or Lean Depot	0.160 **(0.03)**	0.136 (0.07)	0.149 (0.06)	0.121 (0.09)
Change in Selected Fat or Lean Depot	0.204 **(0.001)**	0.155 **(0.02)**	0.221 **(0.001)**	0.146 **(0.05)**
Adj. R^2	0.171	0.147	0.189	0.134
NEFA				
1Y Change	**TBF (g)**	**% TBF**	**AbFat (g)**	**LST (g)**
HT	0.218 **(0.001)**	0.227 **(0.001)**	0.234 **(0.001)**	0.206 **(0.001)**
Age	0.084 (0.19)	0.086 (0.18)	0.092 (0.19)	0.100 (0.13)
Exercise	0.041 (0.48)	0.056 (0.34)	0.055 (0.38)	0.022 (0.73)
Baseline Dependent Variable	-0.670 **(0.001)**	-0.673 **(0.001)**	-0.665 **(0.001)**	-0.640 **(0.001)**
Baseline Selected Fat or Lean Depot	0.182 **(0.01)**	0.157 **(0.02)**	0.168 **(0.02)**	0.107 (0.14)
Change in Selected Fat or Lean Depot	0.198 **(0.001)**	0.200 **(0.001)**	0.119 (0.06)	-0.012 (0.87)
Adj. R^2	0.434	0.429	0.395	0.378

Values in the table are standardized betas (p-value). *Models for NEFA also include nutritional factors kcals, protein (g), fat (g), carbohydrate (g), due to significant associations with NEFA values. Baseline utilizes 3d diet record from baseline, change at one year utilizes average of 8d diet records over the year. Adj. R^2, adjusted r-squared. (N=200 TBF, %TBF, N=181 AbFat).

Table 2. 5 Intercorrelations (Pearson) between baseline and changes in glucose, insulin, and NEFA (N=200).

Variable	B Glucose	B Insulin	B NEFA	Δ Glucose	Δ Insulin
Baseline Insulin (uU/ml)	**0.29****				
Baseline NEFA (mEq/L)	**0.16***	**0.25****			
Change in Glucose (mg/dl)	**-0.44****	-0.07	-0.04		
Change in Insulin (uU/ml)	-0.07	**-0.35****	-0.13	**0.24****	
Change in NEFA (mEq/L)	-0.01	-0.02	**-0.60****	0.09	0.06

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). B, Baseline; Δ, change at one year; NEFA, non-esterified fatty acids. No adjustments for blood draw timing were made to this analysis.

Table 2. 6 Baseline characteristics of subjects with normal fasting glucose (NFG) versus impaired fasting glucose (IFG) and change (Δ) at 1year.

	NFG (N=179)		IFG (N=21)	
Variable	**Mean**	**SE**	**Mean**	**SE**
Age (yr)	55.44	0.35	56.37	0.82
HT	51%		43%	
%TBF*	37.66	0.50	41.96	1.54
Glucose (mg/dl)	84.29	0.63	107.75	1.2
Δ Glucose (mg/dl)	1.295	0.575	0.824	2.141
Insulin (uU/ml)*	9.47	0.24	11.89	0.76
Δ Insulin (uU/ml)	-0.202	0.195	-0.182	0.590
NEFA (mEq/L)*	0.38	0.01	0.47	0.03
Δ NEFA (mEq/L)	-0.026	0.009	0.010	0.029
HOMA-IR*	1.97	0.06	3.13	0.20
Δ HOMA-IR	0.003	0.051	-0.064	0.162
QUICKI*	0.35	0.001	0.32	0.001
Δ QUICKI	0.000	0.001	0.001	0.003
R-QUICKI*	0.42	0.001	0.37	0.01
Δ R-QUICKI	0.007	0.003	0.001	0.006

*$p<0.05$. Baseline comparisons by independent Student's t-test. Change in biomarkers of IS by ANCOVA including covariates of age, HT, baseline TBF (g), change TBF (g), baseline dependent variable, exercise. No adjustments were made for blood draw timing in this analysis. HT was not independently significant in change models except for NEFA and R-QUICKI ($p<0.05$).

Exercise was not independently significant in change models. If normal fasting glucose and impaired fasting groups were split and exercise was used as the factor in ANCOVA models, glucose increased with exercise within the normal fasting glucose group ($p<0.10$), while insulin and NEFA were unchanged. NEFA decreased with exercise within the impaired fasting glucose group ($p<0.05$), while glucose and insulin were unchanged.

Table 2. 7 Baseline general linear models (1-3) with and without hormone therapy (HT) to compare effects of 1. total body fat, TBF (g), 2. %TBF, and 3. abdominal fat (Abfat) (g) on glucose, insulin, and non-esterified fatty acids (NEFA).

	Glucose		**Insulin**		**NEFA**	
Baseline	**NHT**	**HT**	**NHT**	**HT**	**NHT**	**HT**
TBF (g)	4.57	8.90	14.41	25.68	6.29	14.02
	(0.04)	(0.001)	(0.001)	(0.001)	(0.01)	(0.001)
Adj. R^2	0.095	0.134	0.138	0.206	0.038	0.139
%TBF	5.91	6.01	13.47	23.41	6.56	18.93
	(0.02)	(0.02)	(0.001)	(0.001)	(0.01)	(0.001)
Adj. R^2	0.107	0.109	0.131	0.191	0.041	0.181
AbFat	7.31	14.49	17.96	36.74	6.56	11.12
	(0.01)	(0.001)	(0.001)	(0.001)	(0.01)	(0.001)
Adj. R^2	0.103	0.180	0.183	0.282	0.033	0.103

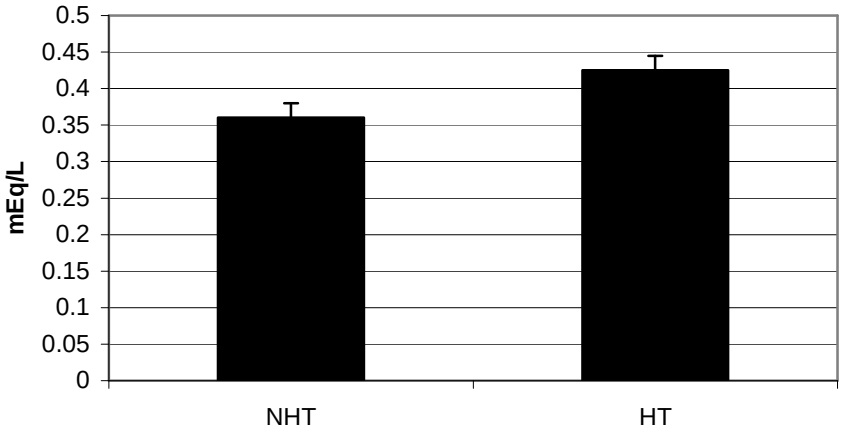
Values in the table are F-statistic (p-value). (N=200 TBF, %TBF; N=181 AbFat). Age included as a covariate in all models, macronutrients included for NEFA.

Table 2. 8 One year change general linear models (1-3) with and without hormone therapy (HT) to compare effects of 1. total body fat, TBF (g), 2. %TBF, and 3. abdominal fat (Abfat) (g) on glucose, insulin, and non-esterified fatty acids (NEFA).

	Δ Glucose		**Δ Insulin**		**Δ NEFA**	
1 Year	**NHT**	**HT**	**NHT**	**HT**	**NHT**	**HT**
B. Dependent	19.73 (0.001)	41.34 (0.001)	9.05 (0.001)	34.50 (0.001)	62.62 (0.001)	52.56 (0.001)
B. TBF (g)	3.51 (0.06)	1.67 (0.20)	1.27 (0.26)	3.81 (0.05)	7.43 (0.01)	3.00 (0.09)
ΔTBF (g)	0.57 (0.45)	0.99 (0.32)	4.16 (0.04)	7.36 (0.01)	9.30 (0.001)	3.36 (0.07)
Adj. R^2	0.147	0.283	0.066	0.328	0.447	0.416
B. Dependent	18.31 (0.001)	39.35 (0.001)	7.85 (0.01)	33.58 (0.001)	59.43 (0.001)	52.38 (0.001)
B. %TBF	1.64 (0.20)	0.38 (0.53)	0.51 (0.48)	3.47 (0.07)	4.67 (0.03)	2.69 (0.11)
Δ %TBF	0.51 (0.48)	0.03 (0.86)	2.50 (0.12)	4.20 (0.04)	9.01 (0.001)	3.83 (0.05)
Adj. R^2	0.130	0.266	0.045	0.304	0.432	0.416
B. Dependent	21.95 (0.001)	32.63 (0.001)	10.19 (0.001)	32.01 (0.001)	49.76 (0.001)	41.84 (0.001)
B. AbFat (g)	5.77 (0.02)	1.51 (0.22)	1.01 (0.32)	2.63 (0.11)	3.45 (0.07)	2.24 (0.14)
Δ AbFat (g)	0.19 (0.66)	0.71 (0.40)	4.67 (0.03)	7.01 (0.01)	5.43 (0.02)	0.60 (0.44)
Adj. R^2	0.178	0.274	0.089	0.365	0.384	0.380

Values in the table are F-statistic (p-value). (N=200 TBF, %TBF, N=181 AbFat). Age and exercise included as covariate in all models, macronutrients included for NEFA. B. dependent refers to the baseline value of the dependent variable. AbFat refers to abdominal fat. TBF refers to total body fat.

Figure 2. 1 Baseline Non-esterified Fatty Acids (NEFA) with (HT) and without oral hormone therapy (NHT) use.



$p<0.05$

APPENDIX C: DIABETIC BIOMARKER RESPONSE TO RESISTANCE TRAINING AND MULTILOCUS ADR GENOTYPES

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ABSTRACT

Decreases in insulin sensitivity mark the beginning of type 2 diabetes development, which affects 20.8 million people in the United States. Due to both the personal and social burdens associated with the diagnosis, and the increase in prevalence of diabetes throughout the US population, the prevention or delay of diabetes onset is a significant public health concern.

The objective of this study was to determine whether insulin sensitivity response to resistance training may be influenced by adrenergic receptor genetic variants and gene-gene interactions in postmenopausal women.

ADRA2B, ADRB2 and ADRB3 genotypes were determined using buccal cell DNA in a subgroup of completers of a well supervised, block-randomized trial of 1year of resistance training in sedentary post-menopausal (PM) women, using or not using hormone therapy (N=122). Fasting measures of plasma glucose (FPG), insulin, and non-esterified fatty acids (NEFA) were utilized to compute insulin sensitivity scores by the revised-QUICKI method at baseline and 1 year. Body composition was measured by dual x-ray absorptiometry.

There were no baseline, non-genetic, differences between intervention groups. Resistance training significantly improved body composition ($p<0.05$). Circulating glucose significantly increased in controls ($p<0.05$), while there was no change in exercisers (between group $p<0.05$). Exercisers, but not controls, significantly improved R-QUICKI scores ($p<0.05$), but between group differences did not reach significance. General linear models did not show significant relationships between individual

genotypes and biomarkers of insulin sensitivity at baseline, after accounting for age, hormone therapy, and body fat mass, except for FPG and ADRA2B (Glu9 carrier standardized beta -0.13, $p<0.1$). Following 1 year of training, controls did not demonstrate any relationships between ADR genes and biomarkers of insulin sensitivity in full models either, while resistance trained ADRB3 Arg64 and ADRB2 Glu27 carriers decreased FPG more than non-carriers (respective standardized betas: -0.72, $p<0.1$ and -0.30, $p<0.05$). ADRB2 Glu27 carriers also increased R-QUICKI scores to a greater extent than non-carriers.

At baseline, gene x gene interactions for ADRA2B x ADRB3 ($p<0.05$) and ADRB3 x ADRB2 ($p<0.1$) were associated with FPG; ADRA2B x ADRB2 was associated with NEFA. At one year, ADRAb2 x ADRB3 x exercise interaction was associated with change in FPG, as well as change in NEFA ($p<0.1$); Glu9X x Arg64X x exercise had the highest FPG value, while Glu9X x Arg64X x exercise had the lowest. ADRB3 x ADRB2 x exercise was also significantly associated with NEFA ($p<0.05$); Arg64X x Glu27X x exercise had the highest NEFA levels, while Arg64X x Glu27X x exercise had the lowest. ADRA2B x ADRB2 x exercise interactions were not observed for biomarkers of insulin sensitivity.

Small improvements in various measures of IS were demonstrated in postmenopausal women exercising and carrying ADRB3 and ADRB2 variants alone, but no individual gene effects were found in the sedentary group following the intervention. Without intervention, it appears that a small advantage in fasting glucose may be attributed to ADRA2B carriage.

Key words: insulin sensitivity, adrenergic receptors, genotype, resistance training, weight lifting, physical activity, postmenopausal

INTRODUCTION

Type 2 diabetes mellitus is one of the most significant health concerns of our time. It is a major contributor to cardiovascular disease (CVD),[1, 5, 24, 38, 41, 47, 48] which is the leading cause of death in the United States,[50] and carries high morbidity and mortality rates of its own.[1] Quality of life and overall health for individuals with type 2 diabetes is compromised, particularly in advanced stages, by retinopathy, neuropathy, nephropathy, amputations, and, of course, cardiovascular disease.[1] The social burden for care and financial resources is also astounding (14 million hospital days, 30 million physician visits [111], $132 billion total direct and indirect costs[1, 111]). Therefore, a greater understanding of the disease, its contributors, and effective prevention strategies is paramount.

With the increase in obesity in our society, the prevalence of type 2 diabetes has doubled over the last 15 years to 20.8 million.[34] Both adipose tissue volume and distribution has been linked with increased susceptibility for type 2 diabetes.[36, 98] It has been postulated that adrenergic receptors may play a role in general obesity and fat distribution, due to their lipolytic response to stimulus and potential variation in response.[58, 60, 90] Adrenergic receptor variation may contribute to the link between body fat and type 2 diabetes.

Both alpha and beta adrenergic receptors are located in adipose tissue [8, 17, 58, 62] and are responsive to catecholamines.[8, 58, 62, 114] The lipolytic function of alpha adrenergic receptors (ADRA) tends to be inhibitory[58, 60, 62, 114] while the beta adrenergic receptors (ADRB) are stimulatory[30, 58, 60, 62]. Several variants in the genes encoding these receptors have been identified[58] and may confer varied responsiveness to catecholamine enhanced environments, such as during exercise.[94] Typically the variants are single nucleotide polymorphisms or base-pair substitutions at particular points in the gene sequence, that create changes in amino acid translation.[58] Another type of variant is an insertion/deletion, where the difference in the code is due to addition or deletion of multiple base pairs, which translates to multiple amino acids.[58]

Common non-synonymous variants include ADRA1A (Arg492Cys), ADRA2A (Asn251Lys), ADRA2B (Del 301-303, Glu9/12), ADRA2C (Del 322-325, Gly-Ala-Gly-Pro), ADRB1 (Ser49Gly, Gly389Arg), ADRB2 (Thr164Ile, Val34Met, Gly16Arg, and Gln27Glu), and ADRB3 (Trp64Arg).[58] Since endurance training and beneficial changes in body composition have been associated with carriage of ADRB3 Arg64 and ADRB2 Glu27, and non-carriage of ADRA2B Glu9, as well as various combinations of these genes,[92, 96, 132] and since body composition is known to influence insulin sensitivity (IS),[36, 37, 55] we have chosen to evaluate the same variants in a setting of resistance training.

Variation in biomarkers of insulin sensitivity attributable to ADRA2B Glu$^{9/12}$, ADRB3 Trp64Arg, ADRB2 Gln27Glu by cross-sectional or case match studies are inconsistent. Some show no effect on insulin sensitivity,[22, 28, 42, 44-46, 49, 56, 71, 72, 78, 79, 84, 89,

[97, 106, 108, 110, 116, 118-120, 123, 124, 135, 139] while others state that carriage of the respective variants is detrimental to insulin sensitivity.[9, 26, 51, 61, 63, 65, 66, 77, 88, 101, 102, 104, 107, 128, 130, 131, 139] There are few trials of physical activity, with out dietary induced weight loss, which have investigated the effects of ADR genotypes on insulin sensitivity responsiveness to intervention, [27] none of which are randomized controlled trials.

Interventions with physical activity, without weight loss as a primarily outcome, with respect to ADRA2B variants, are absent from the literature. The majority of cross-sectional trials have found no effect of ADRA2B on risk of type 2 diabetes or biomarkers of insulin sensitivity.[108, 118, 119, 123, 139] Meanwhile, an acute bout of aerobic activity demonstrated higher insulin levels in ADRB2 Glu27Glu subjects,[65] while a 3 month aerobic intervention showed that ADRB3 Arg64Arg subjects were unable to alter fasting glucose levels with exercise.

The purpose of this study was to determine whether insulin sensitivity response to resistance training may be influenced by adrenergic receptor genetic variants and gene-gene interactions in postmenopausal women. A subset of completers in the Bone Estrogen and Strength Training (BEST) Study were re-consented and genotyped for ADRA2B, ADRB2, and ADRB3, following 12-months of progressive resistance training or control.

Based on the uncontrolled interventions above, with primary outcomes of various measures of insulin sensitivity, and the promising body composition changes demonstrated in other trials, we expected non-carriers of ADRA2B (Glu^{9-}) variant to show greater improvements in insulin sensitivity. We also expected the ADRB2

Gln27Glu carriers and the ADRB3 Trp64Arg carriers to have lower insulin sensitivity at baseline, but to be more responsive, i.e. increase insulin sensitivity, with exercise.

Gene-gene interactions of these genotypes are ADRA2BxADRB3, ADR2BxADRB2, and ADRB3xADRB2. We expected the following combinations to enhance insulin sensitivity based on both gene-gene interactions and body composition changes and individual genes and insulin sensitivity biomarker changes noted in the literature: Glu^{9-}/Arg^{64+}, Glu^{9-}/Glu^{27+}, and Arg^{64+}/Glu^{27+}.

METHODS

Participants

Post-menopausal women were recruited for the Bone Estrogen and Strength Training Trial (BEST) if they met the following criteria: 40–65 years of age with natural or surgically induced menopause for 3–10.9 years, and a body mass index <35 kg/m^2. The subjects were instructed to maintain their HT status, diet, and weight during the trial.[35, 75, 121] Details regarding diet records and analysis have been previously published.[75] Reasons for exclusion included medication use (i.e. steroids, beta-blockers, bone mineral density (BMD) altering medications), cancer within 5 years, or other chronic health conditions.[12]

Subjects who completed the 1 year intervention were asked to re-consent for the ancillary genetics study after several years of follow-up without intervention. Baseline and 1 year measurements are included in this report.

Study Design

Postmenopausal women, using or not using hormone therapy (HT) were randomized to progressive resistance training or control and carefully supervised for one year. HT users and non-users were evenly distributed across intervention groups.[12, 35] Retrospective analysis of fasting plasma glucose (FPG), insulin (FPI), and non-esterified fatty acids (NEFA) was performed on stored blood samples from one-year completers of the BEST study (N=265). Recent buccal cell collection was used for allelic determination in the population that re-consented for the ancillary genetics study (N=148) and paired with the fasting measures of insulin sensitivity (FPG, FPI, NEFA). Subjects using patch hormone therapy versus oral therapy were eliminated, as were subjects with FPG values >125mg/dl. After these eliminations, we were able to analyze complete FPG, FPI, and NEFA and genetic data on a large subset (N=122) of re-consented subjects for use in this analysis.

The participants with normal fasting glucose (<100mg/dl) and impaired fasting glucose (100-125mg/dl) were pooled for this analysis due to low subject numbers between genotypes. Written informed consent was provided by all subjects prior to participation.

Biological Measurements

Following an overnight fast, blood samples were collected and stored at –80°C until use, as previously published.[83] Glucose analysis was performed on EDTA treated samples due to interactions with other preservatives; insulin and NEFA measures utilized

sodium citrate treated samples. Samples were analyzed in duplicate for insulin and NEFA and triplicate for glucose.

A Human Insulin Specific Radioimmunoassay (RIA) Kit (Linco Research, Inc., St Charles, MO) and gamma counter were used to quantify FPI levels. Intra- and inter-coefficients of variation (intra-CV, inter CV) were both 4% for insulin measurements. A colorimetric glucose oxidase assay and spectrophotometer were used to determine FPG concentration (Thermo Electron Corporation, Pittsburgh, PA). Intra- and inter-CVs were 5% and 8% for glucose measures, respectively. An enzymatic, colorimetric microtiter technique and plate reader were used to evaluate NEFA levels (Wako Chemicals USA, Inc., Richmond, VA). Intra- and inter- CVs were 4% and 11%, respectively.

Computation of the homeostasis model for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and the revised QUICKI (R-QUICKI) was performed according to Brady, et al.[6] Low IS will be represented by high HOMA-IR scores or low QUICKI or R-QUICKI scores. The calculations were done as follows:

$$\text{HOMA-IR} = (\text{insulin}_{0\ (\text{uU/ml})} * \text{glucose}_{0\ (\text{mmol/l})}) / 22.5$$

$$\text{QUICKI} = 1/(\log(\text{glucose}_{0\ (\text{mg/dl})}) + \log(\text{insulin}_{0\ (\text{uU/ml})}))$$

$$\text{R-QUICKI} = 1/(\log(\text{glucose}_{0\ (\text{mg/dl})}) + \log(\text{insulin}_{0\ (\text{uU/ml})}) + \log(\text{NEFA}_{0\ (\text{mmol/l})}))$$

Measures of body fat were used as covariates in analyses, due to the associations between fat and biomarkers of insulin sensitivity. Total fat mass was measured in

duplicate at baseline and follow-up at 1 year by dual x-ray absorptiometry (DXA by Lunar Radiation Corporation, Madison, WI). [35, 64, 121]

Genotyping

If subjects were still making routine visits to the lab, collection of buccal cells was performed at the end of visits by vigorously swishing 10ml of pre-measured mouthwash for 45seconds and expectorating the mouthwash back into a cup. If subjects were no longer regularly interacting with the laboratory, collection of buccal cells was performed by mail with collection kits according to the methods described by Garcia-Closas, et at.[31]. Kits contained sealed 44ml bottles of Scope mouthwash (Proctor & Gamble, Cincinnati, OH), a sealed, sterile collection cup, instructions for collection, and a prepaid return envelope. Participants were asked not to eat or drink for 1hr before sample collection, to fill the cup to the 10ml line with mouthwash, swish the mouthwash vigorously for 45sec, expectorate back into the cup, and mail the container in provided packaging.[31]

The QIAampDNA Mini Kit (QIAGEN #51104, Valencia, CA) was used for DNA extraction and DNA quality and quantity was assessed by 558bp polymerase chain reaction (PCR), separated on a 2% agarose gel by electrophoresis, and visualized by ethidium bromide staining. Assays-by-Design Service and TaqMan technology (Applied Biosystems, Foster City, CA) designed PCR primers and allelic probes for Gln27Glu β2-ADR and Trp64Arg β3-ADR and TaqMan. PCR was performed under universal concentration conditions and universal thermal cycling parameters. Allelic determination for Gln27Glu β2-ADR and Trp64Arg β3-ADRwas performed on the ABI 7700 Sequence

Detection System (Applied Biosystems, Foster City, CA). Due to the insertion length for the Glu^{12}/Glu^{9} α2b-ADR variant, Taqman technology could not be used. Therefore previously published Glu^{12}/Glu^{9} α2b-ADR identification procedures by PCR and gel electrophoresis[108] were followed using primers designed for the long (112bp) and the short (103bp) variants (Midland Chemicals Midland, TX).

Exercise Training

Both resistance training (EX) and control groups (NEX) recorded physical activity patterns at baseline and one year; additional training logs documenting attendance, loads, sets, and repetitions were created for the EX group. Progressive weight lifting was designed to maintain an intensity of 70—80% one-repetition maximum for each exercise over the year. Moderate impact weight-bearing exercise was also included in the program. The 3 day per week training program was 75 minutes in duration and included 8 core weight training exercises. Each exercise was performed for 2 sets of 6-8 repetitions at each session. A detailed description of the exercise protocol has been previously published.[80]

Statistics

A X^2 test was performed to determine if allele frequencies were in Hardy-Weinberg equilibrium. Subjects from the Bone Estrogen and Strength Training Trial, were re-consented for the ancillary genetics study (148). Since counter regulatory responses to exercise and hypoglycemia are sexually dimorphic[14-16, 29] and the effect of HT on IS in the literature is inconsistent[39, 40, 53, 68, 117], we used HT as a covariate in all

general linear and regression models. HT patch users (N=10) were eliminated from this analysis due to significant differences in initial insulin sensitivity between oral and patch hormone therapy use. Those with incomplete insulin, glucose or NEFA data for baseline or one-year, or provisional type 2 diabetics or fasting plasma glucose higher than 125mg/dl, were also eliminated from this analysis (N=16). Results from the remaining subjects are presented herein (N=122).

Allelic frequencies are presented as the percent of the subject population with complete insulin sensitivity biomarker data, completing 1 year, and re-consenting for the ancillary genetics study. The percentage of each allelic frequency in the group assigned to resistance training are also reported. Other statistics are reported as mean ± standard error (SE), adjusted R-squared, and p-value.

Differences between group subject characteristics at baseline and one year were compared using independent t-tests, while change within intervention group subject characteristics were compared using paired t-tests at 1year (after adjusting for blood draw timing for biomarkers of insulin sensitivity).

Baseline measures of insulin sensitivity were adjusted by one contrast of physical exertion occurring within or greater than 6 days of the blood draw. For one year measures of insulin sensitivity, six contrasts were used to account for differences between those with physical exertion due to study testing or intervention program within and greater than 6 days of their blood draw (1. No exertion or exertion at both baseline, B, and one year, Y1, within 6days of blood draw versus exertion within 6 days of blood draw at one or the other time point, 2. No exertion versus exertion at B, 3. exertion at both time points

versus exertion at Y1 only, 4. interaction of contrast 1 with random assignment to EX or NEX groups, 5. interaction of contrast 2 with random group assignment, 6. interaction of contrast 3 with random group assignment). Contrasts were regressed onto insulin sensitivity measures and individual residuals were added to predicted means for each measure.

Although three genotypes are possible for each gene, wild type homozygote, heterozygote, and polymorphic homozygote, comparisons between genotype groups are confined to two genotype groups, i.e. carriers (ADRA2B Glu^{12}/Glu^{9} or Glu^{9}/Glu^{9}, also written as Glu^{9}+; ADRB3 Trp64Arg or Arg64Arg, also written as Arg64+; and ADRB2 Gln27Glu or Glu27Glu, also written as Glu27+) and noncarriers (ADRA2B Glu^{12}/Glu^{12} ADRA2B, also written as Glu^{9}-; ADRB3 Trp64Trp, also written as Arg64-; and ADRB2 Gln27Gln, also written as Glu27-). Gene-gene interactions are also based on these carrier/non-carrier groups.

Baseline general linear models for each biomarker of insulin sensitivity utilized each individual gene and gene-gene combination separately. Model 1 was bivariate with ADR genotype as an independent variable; model 2 is multivariate adjusted for age and hormone therapy; the full model (3) was additionally adjusted for of age, hormone therapy, and fat mass. All models were adjusted for blood draw timing.

One-year general linear models for biomarkers of insulin sensitivity and models of insulin sensitivity by gene and gene-gene interaction included covariates for blood draw timing, age, HT, baseline values of the dependent variable, baseline fat mass, change in fat mass, and exercise.

One-year general linear models were split by intervention group (EX/ NEX) and performed with serially with increasing complexity. Model 1 is bivariate with ADR genotype as an independent variable; model 2 is multivariate adjusted for age and hormone therapy; model 3 is also adjusted for baseline values of the dependent variable; the full model (4) is additionally adjusted for baseline body fat and change in body fat. Similar linear regression models were used to obtain beta values and confirm significance.

Following determination of significant gene-gene associations with insulin sensitivity biomarker outcomes by general linear models, between specific genotype differences were determined by analysis of variance post-hoc tests at both baseline and one year.

All statistical analyses were performed using SPSS v14.0 (SPSS Inc., Chicago, IL). Significance of both $p < 0.05$ and $p<0.10$ are reported.

RESULTS

Baseline subject characteristics between those randomly assigned to control and resistance training were not significantly different for age, hormone therapy use, body composition, biomarkers of insulin sensitivity, total energy or energy from macronutrients. The women were postmenopausal, average age of 56 years, with a mean BMI of 25kg/m^2 (Table 3); 53% were hormone therapy users.

Allelic and genotype frequencies are presented in Tables 1 and 2. Frequencies were similar to those previously reported in the literature.

Carriage of the ADRA2B variant across intervention groups was not significant, but HT non-use was higher in the non-carriers (59%), while use was higher in the carriers (63%) (Pearson $X^2 = 5.44$; df = 1; $p<0.02$). Allele frequencies were in Hardy-Weinberg equilibrium across intervention and HT groups for ADRB2 and ADRB3 (Pearson X^2 = 0.010, 0.002 and 0.031 1.01; df =1, respectively; $p>0.3$). Due to the low frequency of the ADRB3 Arg64 variant across groups, there are limitations in interpreting gene-gene interactions. Although intervention and genotype groups were in Hardy-Weinberg equilibrium, exercisers re-consented for the ancillary genetics study more often than non-exercisers (Tables 1 and 2). HT and exercise were used as covariates in all models, unless split by group, in order to adjust for these differences.

Main Effect of Exercise on Biomarkers of Insulin Sensitivity

Within this subsample of the main BEST trial, body composition among controls did not change significantly; in contrast, total body fat and abdominal fat were reduced in the exercise group ($p<0.05$ and $p<0.01$ respectively). Total body fat and abdominal fat were significantly different between exercisers and controls at one year. Lean soft tissue (%) was also significantly different between groups at one year. Within the exerciser group, a gain of approximately 1% lean soft tissue resulted from resistance training, which was also significant ($p<0.05$, Table 4).

Independent and paired t-tests showed that fasting plasma glucose was significantly increased in the control group (within controls $p<0.05$), but maintained in the exercise group (between group $p<0.05$) (Table 4). After adjusting for fat mass, change in fat mass, age, and HT in full general linear models, change in FPG remained

significantly different between EX and NEX groups ($p<0.05$). Although between group differences were not evident for the R-QUICKI scores at one year, within the exercise group the R-QUICKI score for IS was significantly improved. This significant difference in R-QUICKI score also persisted when covariates of age, HT, initial fat mass, and change in fat mass were included in a general linear model (i.e., $p>0.2$ for all covariates).

Main Effect of Genes on Biomarkers of Insulin Sensitivity

Basic baseline general linear models of each individual gene related to glucose, insulin, and R-QUICKI scores did not reveal any significant associations. Similarly, there were no associations between genes and biomarkers of insulin sensitivity, when models were adjusted for age and hormone therapy. When fat mass was included in the model, a relationship between ADRA2B and fasting plasma glucose emerged. Linear regression for this relationship indicated that carriers of the ADRA2B variant had lower glucose levels than non-carriers (Standardized Beta –0.13, $p<0.1$). Fourteen percent of the variance was accounted for in the full baseline model, including all covariates, for glucose and ADRA2B (13.6 adjusted R-squared, $p<0.1$).

Baseline full models, including age, hormone therapy, and total body fat, for gene-gene interactions demonstrated relationships between glucose and ADRA2B x ADRB3 ($p<0.05$) and ADRB3 x ADRB2 ($p<0.1$). ADRA2B x ADRB3 Glu9+/Arg64+ glucose levels were higher than Glu9-/Arg64+ ($p<0.05$) and Glu9+/Arg64- ($p<0.01$); Glu9-/Arg64+ glucose was also higher than Glu9+/Arg64- ($p=0.06$) (Figure 1). Although the over all gene x gene interaction was associated with glucose and ADRB3 x ADRB2 Arg64+ /Glu27+ glucose levels appeared to be significantly higher than all three of the

other genotypes, specific between genotype differences were not significant. Similar results were found for baseline NEFA; i.e. NEFA was associated with ADRA2B x ADRB2 ($p<0.1$), while specific Glu9/Glu27 differences were not significant.

General findings for the main effects of genotype and changes in insulin sensitivity biomarkers and models are based on full general linear models, including intervention group, as well as the other aforementioned covariates for complete models; findings are later split by intervention group.

Change in insulin at one year was not significantly associated with any of the genotypes by analysis of covariance. However, changes in other biomarkers of insulin sensitivity at one year were significantly associated with individual genes and gene-gene interactions when accounting for blood draw timing, age, HT, baseline values of the dependent variable, baseline fat mass, change in fat mass, and exercise.

None of the biomarker changes in insulin sensitivity or models of insulin sensitivity were significantly associated with ADRA2B, however, the R-QUICKI score change was associated with ADRA2B ($p<0.1$). ADRA2B non-carriers (Glu9-) increased their scores while carrier scores were unchanged.

Glucose change at one year was significantly associated with ADRB3 and ADRB2 ($p<0.1$). ADRB3 carriers (Arg64+) demonstrated greater increases in glucose than non-carriers, while ADRB2 non-carriers (Glu27-) increased glucose levels over one year more than non-carriers.

ADRB3 and ADRB2 were not associated with other biomarkers or models of insulin sensitivity individually, but there were interactions with other genes and exercise present in this analysis, as noted below.

Changes in the HOMA-IR and QUICKI models of insulin sensitivity were not significantly associated with individuals genes or gene-gene interactions. Within the ADRB2 models, the HOMA, QUICKI, and R-QUICKI scores were significantly associated with exercise, rather than the gene ($p<0.1$). Without assessing the interaction between exercise and gene-gene combinations, no associations between gene-gene interactions and IS variables were found.

Interactions between Exercise and Genes

R-QUICKI score change was associated with ADRA2B as well as the interaction between ADRA2B and exercise ($p<0.1$). As noted above, ADRA2B non-carriers increased their scores with no change in carriers; exercise appeared to decrease R-QUICKI scores regardless of genotype (Exercisers Glu9- -0.001 ± 0.008, Glu9+ -0.002±0.007; Controls: Glu9- 0.029±0.009, Glu9+ 0.002±0.009). Glu9- controls gained the greatest benefit in R-QUICKI scores over the year.

There was an interaction between exercise and ADRB2 and exercise and ADRA2B x ADRB3 for change in glucose as well ($p<0.1$). Adjusted means for ADRB2 carriers with and without exercise were 1.20±1.16 and –0.28±1.28mg/dl respectively, while adjusted means for ADRB2 non-carriers with and without exercise were 6.49±1.58 and –0.48±1.66mg/dl. Exercisers increased glucose levels with or without genetic

variance in ADRB2, however, non-carriers gained more. Non-exercisers with any genotype for ADRA2B x ADRB3 increased glucose over the year, while exercisers with neither or both variants (Glu9-/Arg64- and Glu9+/Arg64+) were able to decrease glucose levels. The difference between exercise and no exercise within the Glu9+/Arg64+ was significant ($p<0.05$), however, the number of subjects was small and this result should be interpreted cautiously.

There was a significant interaction between exercise and ADRA2B x ADRB3 with respect to change in NEFA ($p<0.1$). Non-exercisers either maintained or decreased NEFA levels, except for the ADRA2B x ADRB3 Glu9+/Arg64- genotype, again with a small sample size, while exercisers only with Glu9+/Arg64- and Glu9+/Arg64+ were able to decrease NEFA, Glu9-/Arg64+ NEFA levels increased with exercise. The difference between exercise and no exercise within the Glu9+/Arg64+ was significant ($p<0.05$); again, the number of subjects was small. However, there were no significant differences between specific genotypes within either exercise or no exercise groups. The interaction between exercise x ADRB3 x ADRB2 was also significant with respect to change in NEFA $p<0.05$). Non-exercisers decreased NEFA, except for those with the Arg64+/Glu27+ genotype. Exercisers maintained or decreased NEFA, except for those with the Arg64-/Glu27+ genotype, which increased over the year. Arg64-/Glu27+ genotype was the only genotype with a significant difference between exercise and no exercise ($p=0.08$), non-exercisers decreased NEFA, while exercisers increased NEFA.

Controls and Exercisers as Separate Groups

When one year changes in insulin sensitivity biomarkers for controls and exercisers were analyzed separately, (Tables 6 and 7) by individual gene, associations were no longer present in controls. The ADRA2B gene appeared to be associated with change in R-QUICKI score, but the association was explained by baseline fat mass and change in fat mass (i.e., the association was eliminated when measures of fat mass were added to the model).

Changes in glucose for resistance-trained subjects were significantly associated with ADRB3 and ADRB2. Regression models revealed the direction of the association (ADRB3 Standardized Beta –0.72, $p<0.1$; ADRB2 adjusted Beta –0.30, $p<0.05$); ADRB3 carriers lowered glucose levels more than non-carriers and ADRB2 carriers also lowered levels of glucose more than non-carriers. The full model, accounting for age, HT, initial glucose, initial and change in fat mass, for ADRB3 Trp64Arg carrier versus non-carrier explained 28% of the variance, while the full model for ADRB2 Gln27Glu carrier versus non-carrier explained 32% of the variance in change in glucose for resistance-trained subjects at one year. The ADRB2 gene alone explained 7% of the variance in glucose response.

R-QUICKI scores for resistance-trained subjects increased in ADRB2 carriers more than noncarriers (Beta 0.18, $p<0.1$), indicating more improved IS, only when baseline and change in fat mass were included in the model. Thirty-six percent of the variance in R-QUICKI response to resistance training was explained by the full model for ADRB2.

The samples sizes were too low for use of the robust model, therefore, gene-gene interactions are not reported separately by control and exercise in general linear models, but analysis of variance without covariates. There were no individual genotype differences between ADRA2B x ADRB3 genotypes with respect to changes in biomarkers or models of insulin sensitivity within the exercise group. Comparisons within the non-exercise group were not feasible, due to the low subject number in the Glu9+/Arg64+, no exercise group.

By analysis of variances, without covariates of age, HT, or body fat, glucose in exercisers decreased significantly in the Glu9-/Glu27+ (-2.45mg/dl) and Glu9+/Glu27+ (-1.85) versus Glu9+/Glu27-, which increased (5.43mg/dl, $p<0.1$). NEFA was significantly lowered in the ADRA2B x ADRB3 Glu9+/Glu27+ genotype (-0.09mEq/L) versus Glu9+/Glu27- (0.03mEq/L) ($p<0.1$).

Within in exercisers, ADRB3 x ADRB2 Arg64+/Glu27- (4.73mg/dl) and Arg64-/Glu27- (4.04mg/dl) had the highest increases in glucose compared to the Arg64-/Glu27+ (-1.71) and the Arg64+/Glu27+ (-6.21mg/dl), however only the difference between ADRB3 x ADRB2 -/- and -/+ was considered significant ($p<0.1$), due to sample sizes in the other groups. Also within exercisers, carriage of one or the other variant in ADRB3 x ADRB2 showed decreases in NEFA over the year, while double non-carriage or double carriage showed increases in NEFA.

However, only the difference between ADRB3 x ADRB2 Arg64-/Glu27- (0.041mEq/L) and Arg64-/Glu27+ (-0.060mEq/L) was significant ($p=0.07$). Lastly, the change in R-QUICKI score difference between ADRB3 x ADRB2 Arg64-/Glu27- (-0.006) and

Arg64-/Glu27+ (0.016) was significant ($p<0.1$), indicating an improvement in insulin sensitivity for the Arg64-/Glu27+ genotype.

DISCUSSION

The BEST trial is unique due to the strength of its design; it was a long-term, well-supervised randomized controlled trial of resistance training in postmenopausal women with equal representation of hormone therapy across groups. [13, 35, 74, 75, 121] We were fortunate to be able to locate and re-consent over 55% of the original completers of the trial for the ancillary genetics study approximately 10 years after its initiation, and 82% of those re-consented fulfilled the requirements for this analysis of biomarkers of type 2 diabetes in relation to adrenergic receptor variants. Since body weight and weight change can be a strong influence on biomarkers of type 2 diabetes,[21, 36, 59] another strength of the study was that all participants were encouraged to maintain their body weight, since the primary end points of the original trial were not related to resistance training induced weight loss, but resistance training induced changes in bone mineral density. [13, 35, 121]This is also the first study, to our knowledge, of ADRA2B, ADRB3, and ADRB2 genetic variation and resistance training in postmenopausal women relative to biomarkers of type 2 diabetes.

Finally, the subjects were a good representation of normal Caucasian, postmenopausal; they were somewhat overweight, [115] inactive, [2, 7, 95, 112] with generally normal fasting plasma glucose levels (93% with <100mg/dl, 7% with 100-125mg/dl, >125mg/dl excluded).[1] Their caloric intake was within normal ranges (1776.7 ± 440..8

kcals),[3] but their balance of macronutrients was shifted towards carbohydrates, so the represent women in the United States[3] (19% protein, 65 % carbohydrate, 16 % fat).

The frequencies of the three ADR genotypes included in this analysis were within range of previous studies: ADRA2B carriers, 32%,[20, 43, 92, 107, 108, 118, 123, 140] ADRB3 carriers 14%,[4, 20, 23, 26, 33, 42, 46, 51, 56, 69, 70, 77, 86, 88, 89, 92, 101, 102, 104-106, 116, 122, 125, 128-131, 135, 136] and ADRB2 carriers, 38%.[22, 32, 63, 67, 76, 78, 79, 85, 87, 91, 92, 126] The Arg/Arg genotype was absent from this sample; it has been reported as low as 1-3% in other studies, indicating the rarity of the genotype. [92, 128]

Main effects of exercise on biomarkers of insulin sensitivity

We demonstrated a small effect of resistance training on fasting measures of insulin sensitivity in our population of PM women. In particular, fasting plasma glucose was maintained with exercise, while it increased significantly in the control group within just one year (3.3mg/dl, $p<0.05$). If fasting glucose in the sedentary postmenopausal women continued to rise similarly each year, our control women would be diagnosed with impaired fasting glucose within 5 years and type 2 diabetes within about 13 years (NEX initial FPG mean = 84.75 ± 9.77 standard deviation), without accounting for likely increases in body fat with age, which may speed the development.

The improvement in the R-QUICKI model of insulin sensitivity within the exercisers is also promising. There are few randomized controlled trials with female subjects with which to compare our results, [10, 19, 25, 81, 82, 109, 137] however, there are several non-randomized trials of improved insulin sensitivity response to resistance training which lend support to our results. Although, most trials involve special populations, such

as the obese or insulin resistant persons, [54, 134] our study population included non-obese and persons with mild obesity (up to a BMI of 35kg/m^2) who were in good health.

Two randomized controlled trials of resistance training combined with aerobic training included post-menopausal obese [11] and hypertensive [113] women, and primary outcomes related to insulin sensitivity.[11, 113] There was greater benefit with aerobic training plus resistance training compared to aerobic training alone in one study ($p<0.10$).[11], while the other study found no significant differences in FPG, FPI, or the QUICKI model of insulin sensitivity between groups. However, in the latter trial, [113] there were significant associations between changes in fat mass, particularly abdominal fat mass, and insulin sensitivity improvements.[113] It is difficult to tease apart the contributions of aerobic versus the resistance training portions to improved insulin sensitivity in these trials. However, in a previous analysis of the BEST resistance training study, [133] we also found significant associations between fat mass, abdominal fat, and insulin sensitivity changes by surrogate markers.

In a study of young, healthy women, which separated the effects of aerobic and resistance training by randomizing to aerobic training, resistance training, or control, significant insulin sensitivity improvements in both exercise programs compared to control were demonstrated, but when adjusted for lean mass, the effect in the resistance trained group did not persist.[93]

Non-randomized, non-controlled resistance training trials in PM women by Ryan et al [98, 100], which did not account for changes in body composition, demonstrated positive effects of resistance training on insulin sensitivity, while in a more recent study by Ryan

et al, [99] in which fat mass did not change significantly over 6months of resistance training, significant changes in insulin sensitivity in PM women were not found. Other non-randomized, non-controlled resistance training trials have also shown insignificant changes in fasting measures of glucose and insulin, but significant reductions in AUC or infusion rates for insulin and/or glucose with training.[11, 19, 25]

When we adjusted for fat mass, change in fat mass, age, and HT, the significant difference between EX and NEX groups for FPG change persisted ($p<0.05$). The R-QUICKI pre-post intervention difference in exercisers was also independent of age, HT, initial fat mass, and change in fat mass ($p>0.2$ for all) as demonstrated in a general linear model when they were added to the model as covariates.

Main effects of genotype on biomarkers of insulin sensitivity

We found an association between ADRA2B and baseline levels of fasting plasma glucose. Glu9 carriers had lower levels of glucose than non-carriers ($p<0.1$), when accounting for age, HT, and fat mass. This finding contrasts with others who have found either no relationship, such as in cross-sectional studies of obese[119, 123] or a negative effect of Glu9 carriage on glucose in men[139] (non-significant in women).

A recent meta-analysis of ADRB3 variant associations with insulin resistance, which examined 40 trials and 56 subpopulations, indicated that individuals with the mutation were more insulin resistant than wild-type homozygotes. Subanalyses by subject characteristics showed the associations persisted for Asians, obese, and type 2 diabetics only; populations, such as represented in this analysis, no longer demonstrated an association between ADRB3 genetic variation and insulin resistance.[138] Several other

individual studies of various populations, including hypertensive,[42] obese,[28, 45, 120] those with cardiovascular disease,[44] males only,[56] children,[135] and Mexican-Americans,[46, 84] were unable to find associations between the ADRB3 mutation and insulin sensitivity in cross-sectional or case-controlled designs. Our study in primarily white postmenopausal women fell within this non-significant group, at baseline, regarding associations between ADRB3 and biomarkers or indices of insulin sensitivity. However, the interaction between ADRA2B and ADRB3 was significantly associated with fasting plasma glucose levels ($p<0.05$) (Figure 1); Glu9+ x Arg64+ demonstrated higher glucose values compared to all others. ADRB3 x ADRB2 was also associated with fasting plasma glucose levels ($p<0.1$), although no between specific genotype differences could be detected. Thus, although ADRB3 alone was not associated with initial values of insulin sensitivity, gene-gene interactions between ADRB3 and other adrenergic receptors were associated with measures of insulin sensitivity.

Several cross-sectional studies of the ADRB2 Gln27Glu single nucleotide polymorphism in various populations did not find significant associations between insulin sensitivity and genotype,[22, 49, 71, 72, 78, 79, 97, 124] while two cross-sectional studies found that carriage of the Glu variant decreased insulin sensitivity, one was in men and women in the African-American and Hispanic-American communities,[61] while the other represented Swedish women.[63] Similar to the findings with the ADRB3 genotype, our finding of no significant associations between ADRB2 alone and biomarkers or indices of insulin sensitivity agreed with the majority of prior trials. However, the combinations of ADRA2B and ADRB2 and ADRB3 and ADRB2 were associated with measures of

insulin sensitivity. The ADRB3 and ADRB2 interaction is noted above. The interaction between ADRA2B and ADRB2 was associated with NEFA levels at baseline, although no between specific genotype differences were detected.

Similar gene-gene interactions studies related to insulin sensitivity in the literature for these particular variants have not been published. The body composition studies by Dionne et al[20] and Phares et al[92] involving these ADR variants, would lend themselves well to retrospective analysis of IS, provided blood samples from the trial period are available.

Exercise x genotype effect on biomarkers of insulin sensitivity

One randomized controlled trial of diet and exercise in subjects with impaired glucose tolerance demonstrated increased risk of type 2 diabetes in abdominally obese carrying the Glu9 variant of ADRA2B.[107] Controls in the same trial also exhibited increased risk of type 2 diabetes with Glu9 carriage of the ADRA2B gene, even when adjusted for age, sex, weight change, waist circumference and fasting plasma glucose. An association between R-QUICKI and insulin sensitivity index was apparent in the full model with all subjects, including a gene by exercise interaction ($p<0.1$), however, when split by intervention group only controls appeared to exhibit an association between ADRA2B and R-QUICKI; when adjustments for body fat and change in body fat were made the association was eliminated. Exercisers did not exhibit any relationships between ADRA2B variation and insulin sensitivity biomarkers or indices.

In our primarily Caucasian postmenopausal population, we were able to demonstrate an association between greater decreases in fasting plasma glucose with

resistance training and carriage of the Arg variant of the ADRB3 gene, even when accounting for age, hormone therapy, baseline values of glucose, baseline body fat mass and change in fat mass with exercise. The only other trial we and Franks et al[27] were able to find including physical activity alone, without dietary intervention, and ADRB3 variation relative to insulin sensitivity was that by Kahara et al.[52] They found that fasting plasma glucose decreased significantly in Trp/Trp and Trp/Arg genotypes, but not in Arg/Arg in healthy Japanese males following 3months of endurance training. There study did not include a control group or randomization, nor investigation of gene x intervention interaction. Our study disagrees somewhat with that of Kahara et al.[52] since carriers were significantly different from non-carriers, however, we did not have Arg/Arg representation in our study to truly compare our results with theirs.

Although there were no associations between ADRB2 and insulin sensitivity biomarkers at baseline, we were able to demonstrate association between ADRB2 gene and glucose following the intervention ($p<0.1$), as well as a gene by exercise interaction ($p=0.06$), however, the relationship persisted for exercisers only when split by intervention group. ADRB2 Glu27 carriers decreased fasting plasma glucose levels more than non-carriers with resistance training. ADRB2 Glu27Glu subjects included in a recent trial[27, 66, 67]had higher baseline insulin levels. Following an acute bout of exercise, ADRB2 Glu27Glu subjects continued to have higher insulin levels compared to Gln27Gln subjects, indicating lower insulin sensitivity.[67] However, the gene x exercise interactions, were not tested. Long-term, physical activity interventions, without

concomitant dietary interventions, that have studied the intervention x gene effects of ADRB2 on insulin sensitivity, have not been published.

We have not seen gene by gene by exercise evaluations of these specific variants in other physical activity trials with outcomes of insulin sensitivity, however, we were able to show that ADRA2B x ADRB3 x exercise was significantly associated with changes in glucose following one year of resistance training ($p<0.1$). Change in NEFA was also associated with the ADRA2B x ADRB3 x exercise interaction ($p<0.1$). The ADRB3 x ADRB2 x exercise interaction significantly influenced change in NEFA levels at one year, as well. Within the context of exercise and ADRB3 x ADRB2, without accounting for covariates, there is an indication that carriage of the ADRB2 variant is the key to improved glucose, although double carriage of the ADRB3 and ADRB2 variants decreased glucose the most. NEFA and R-QUICKI changes indicate the same pattern (i.e. carriage of the ADRB2 Glu27 variant within the ADRB3 x ADRB2 interaction is key).

Limitations

Fasting measures of glucose and insulin values largely reflect hepatic insulin sensitivity and, therefore, can cause interpretive problems. However, hepatic and muscle sensitivity to insulin have been reasonably correlated by others.[73] To provide an additional peripheral index of insulin sensitivity, adipose tissue, we also included fasting plasma NEFA measures alone and as an integral part of the R-QUICKI model of insulin sensitivity. In future trials, more specific measures of insulin sensitivity, such as the hyperinsulinemic-euglycemic clamp, would be preferable to the fasting measures, but may be cost prohibitive.

The combination of normal fasting glucose and impaired fasting glucose groups was also carefully considered, due to potential differences in responsiveness between groups, particularly since initial values of FPG and FPI were strong predictors of change with intervention. Changes in models of insulin sensitivity were not significantly different between normal and impaired fasting glucose groups, when accounting for HT, age, exercise status, body composition, and baseline dependent variables, even though their metabolic profiles were significantly different at baseline. However, fasting glucose was decreased significantly more in the impaired group. When split by normal and impaired fasting glucose groups, exercise was not an independent predictor of change in FPG ($p>0.5$). Therefore, normal (93%) and impaired groups (7%) were combined for all analyses. Baseline values of dependent variables, in addition to the other covariates were always included.

Since, previous studies have found that improvements in insulin sensitivity following physical activity may be short lived, 3-6 days, [18, 57, 103] and our subjects with blood drawn ≤6 days following the last bout of physical activity were significantly different than subjects with blood drawn >6 days following the last bout of physical activity, we used statistical contrasts to account for the differences in blood draw timing. There were no significant differences among subjects measured from day 1 through day 6 following last bout of exertion and few subjects had blood drawn at baseline ≤6 days following physical activity. At one year, 70% of exercisers and 30% of non-exercisers had blood drawn within 6 days of their last bout of exertion, which was a favorable split.

In addition, primary outcomes with and without blood draw timing contrasts were not significantly different.

We were fortunate to be able to utilize such a robust trial, database and sample set, to enhance our understanding of the response variability due to heritage, however the retrospective nature of this ancillary study did not allow for a full complement of trial completers. It is also possible that there is a genetic selection bias related to willingness to re-consent for the ancillary study of genetics and markers of chronic diseases. Due to the low frequency of some alleles or gene-gene combinations, numbers of certain genotypes and gene-gene interactions were also limited.

In the future, enrichment of specific genotypes during the selection process, such as done by Mitchell et al[84] in sib pair design, and cross-sectionally by Umekawa et al[127] and Walston et al[130] would enhance power to detect differences between groups. To capture the intervention by genotype interaction, Macho-Azcarate et al[65-67] also enriched for specific genotypes and then performed acute bouts of exercise on the population. However, the ideal design, now that the foundation has been laid by previous researchers, would be genetic enrichment followed by longer term randomized interventions in various populations.

Conclusion

We have found that genetic contributions by these ADR gene variants is generally absent in a sedentary population, as indicated by baseline and control subject measures. Apparent effects of genotype on insulin sensitivity in controls were mediated by body fat mass. However, the same genotypes that conferred higher fasting plasma glucose levels

in other studies (ADRB2 Glu27 carriers and ADRB3 Arg64 carriers)**,** [9, 26, 51, 61, 63, 65, 66, 77, 88, 101, 102, 104, 107, 128, 130, 131, 139] were more responsive to resistance training in our study, which indicates general responsiveness to environment or behavior, i.e., increased glucose with sedentary behavior and decreased glucose with physical activity. Our study supports the contention that there are responders versus non-responders based on genetic variation.

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TABLES AND FIGURES

Table 3. 1 ADR gene polymorphism frequencies for 1yr completers (N=122).

ADR gene polymorphism	**Allele Frequency**		**Genotype Frequency***		
ADRA2B Glu^{12}/Glu^{9}	Glu^{12}	Glu^{9}	Glu^{12}/ Glu^{12}	Glu^{12}/ Glu^{9}	Glu^{9}/ Glu^{9}
1yr	0.68	0.32	0.48 (58)	0.42 (51)	0.11 (13)
Exercisers			52%	57%	62%
ADRB3 Trp64Arg	Trp64	Arg64	Trp/Trp	Trp/Arg	Arg/Arg
1yr	0.86	0.14	0.86 (105)	0.14 (17)	0.00 (0)
Exercisers			55%	53%	0%
ADRB2 Gln27Glu	Gln27	Glu27	Gln/Gln	Gln/Glu	Glu/Glu
1yr	0.62	0.38	0.38 (46)	0.49 (60)	0.13 (16)
Exercisers			54%	50%	75%

*Values in parentheses are the sample sizes.

Table 3. 2 ADR gene polymorphism interaction frequencies for 1yr completers (N=122).

ADR gene polymorphism	**-/-**	**-/+**	**+/-**	**+/+**
ADRA2B X ADRB3	Glu^{9-}/Arg^{64-}	Glu^{9-}/Arg^{64+}	Glu^{9+}/Arg^{64-}	Glu^{9+}/Arg^{64+}
1Yr	0.40 (49)	0.07 (9)	0.46 (56)	0.07 (3)
Exercisers	58%	22%	54%	87%
ADRA2B X ADRB2	Glu^{9-}/Glu^{27-}	Glu^{9-}/Glu^{27+}	Glu^{9+}/Glu^{27-}	Glu^{9+}/Glu^{27+}
1Yr	0.15 (18)	0.33 (40)	0.23 (28)	0.30 (36)
Exercisers	56%	50%	54%	61%
ADRB3 X ADRB2	Arg^{64-}/Glu^{27-}	Arg^{64-}/Glu^{27+}	Arg^{64+}/Glu^{27-}	Arg^{64+}/Glu^{27+}
1Yr	0.30 (37)	0.56 (68)	0.07 (9)	0.07 (8)
Exercisers	54%	56%	56%	50%

*Values in parentheses are the sample sizes.

Table 3. 3 Baseline subject characteristics for randomized groups.

	NEX (55)		EX (67)	
	Mean	SE	Mean	SE
Age	56.31	0.67	55.75	0.51
HT	0.51		0.55	
Weight (kg)	65.75	1.43	68.87	1.56
BMI (kg/m2)	24.52	0.51	25.37	0.49
%TBF	37.42	1.00	38.22	0.78
%LST	58.29	0.95	57.43	0.72
Abfat (kg)	2.78	0.16	2.84	0.16
Insulin Sensitivity Biomarkers[§]				
Glucose (mg/dl)	84.75	1.40	87.85	1.27
Insulin (uU/ml)	9.44	0.38	9.12	0.35
NEFA (mEq/L)	0.39	0.02	0.40	0.02
HOMA-IR	1.97	0.10	1.98	0.09
QUICKI	0.35	0.00	0.35	0.00
R-QUICKI	0.42	0.01	0.41	0.01
Energy (kcal)	1755.5	58.8	1794.2	54.7
Protein (g)	72.35	2.31	72.01	2.73
Fat (g)	57.08	3.46	61.84	3.19
Carbohydrates (g)	239.61	8.90	241.36	7.13

[*]$p<0.05$ for comparisons between No Exercise (NEX) and Exercise (EX). [§]adjusted means due to blood draw timing.

Table 3. 4 Changes at 1 year with and without resistance training.

	NEX (55)		EX (67)	
	Mean	SE	Mean	SE
Weight (kg)	0.39	0.39	-0.06	0.32
BMI (kg/m^2)	0.28	0.16	0.12	0.13
%TBF[*]	0.22	0.33	-1.07	0.29
%LST[*]	-0.24	0.33	1.16	0.29
Abfat (kg)[*]	51.6	44.7	-67.2	43.9
Insulin Sensitivity Biomarkers [§]				
Glucose (mg/dl) [*]	3.33[†]	1.16	0.22	1.10
Insulin (uU/ml)	-0.32	0.30	-0.01	0.35
NEFA (mEq/L)	-0.02	0.02	-0.02	0.02
HOMA-IR	0.01	0.08	-0.01	0.08
QUICKI	0.00	0.002	0.00	0.001
R-QUICKI	0.00	0.01	0.01[†]	0.004

[*]$p<0.05$ for comparisons between No Exercise (NEX) and Exercise (EX), [†]$p<0.05$ for within group comparisons pre- and post-intervention, [§]adjusted means due to blood draw timing.

Table 3. 5 Baseline associations between IS biomarkers and genotype.

Dependent and Independent Variables	**Model 1 (bivariate)**	**Model 2 (adjusted)**	**Model 3 (adjusted)**
Baseline Glucose mg/dl (fasting) %			
A2B genotype	0.00 (0.17)	3.2 (0.12)	13.6 (0.12) ‡
B3 genotype	0.0 (0.20)	2.8 (0.16)	12.9 (0.23)
B2 genotype	0.0 (0.40)	2.0 (0.31)	12.0 (0.64)
Baseline Insulin uU/ml (fasting) %			
A2B genotype	0.0 (0.64)	0.0 (0.57)	14.1 (0.67)
B3 genotype	0.0 (0.81)	0.0 (0.85)	14.2 (0.56)
B2 genotype	0.0 (0.59)	0.0 (0.56)	14.0 (0.95)
Baseline R-QUICKI %			
A2B genotype	0.1 (0.39)	3.9 (0.16)	18.9 (0.16)
B3 genotype	0.4 (0.30)	3.6 (0.20)	18.2 (0.29)
B2 genotype	0.0 (0.86)	2.3 (0.83)	17.6 (0.59)

Adjusted R^2 (p-value). Model 1 is bivariate with ADR genotype as an independent variable; model 2 is multivariate adjusted for age and hormone therapy; model 3 is additionally adjusted for body fat. All models are adjusted for blood draw timing.
* $p<0.05$ and ‡ $p<0.1$

Table 3. 6 Change in 1 year IS Biomarkers for Non-Exercisers only.

Dependent and Independent Variables	**Model 1 (bivariate)**	**Model 2 (adjusted)**	**Model 3 (adjusted)**	**Model 4 (adjusted)**
Change in glucose mg/dl (fasting) %				
A2B genotype	0.0 (0.51)	0.0 (0.48)	25.8 (0.93)	33.9 (0.76)
B3 genotype	0.0 (0.62)	0.0 (0.58)	25.7 (0.96)	34.0 (0.69)
B2 genotype	0.0 (0.89)	0.0 (0.85)	25.9 (0.74)	33.8 (0.88)
Change in insulin uU/ml (fasting) %				
A2B genotype	0.0 (0.45)	0.0 (0.45)	21.3 (0.61)	24.3 (0.80)
B3 genotype	0.0 (0.83)	0.0 (0.75)	21.0 (0.84)	24.2 (0.96)
B2 genotype	0.0 (0.56)	0.0 (0.61)	21.3 (0.62)	24.9 (0.53)
Change in R-QUICKI %				
A2B genotype	4.5 (0.03)*	9.6 (0.04)*	8.7 (0.06)‡	8.6 (0.91)
B3 genotype	0.0 (0.45)	1.7 (0.65)	1.7 (0.71)	3.0 (0.66)
B2 genotype	0.0 (0.87)	1.4 (0.77)	1.5 (0.88)	2.6 (0.98)

Adjusted R^2 (p-value). Model 1 is bivariate with ADR genotype as an independent variable; model 2 is multivariate adjusted for age and hormone therapy; model 3 is also adjusted for baseline values of the dependent variable; model 4 is additionally adjusted for baseline body fat and change in body fat due to sedentary behavior. All models are adjusted for blood draw timing. Baseline dependent values in models 3 and 4 were always significant, except for R-QUICKI. $^{*}p<0.05$, $^{\ddagger}p<0.1$

Table 3. 7 Change in 1 year IS Biomarkers for Exercisers only.

Dependent and Independent Variables	**Model 1 (bivariate)**	**Model 2 (adjusted)**	**Model 3 (adjusted)**	**Model 4 (adjusted)**
Change in glucose mg/dl (fasting)				
A2B genotype	0.0 (0.38)	0.0 (0.43)	25.1 (0.94)	23.0 (0.98)
B3 genotype	0.0 (0.98)	0.0 (0.95)	29.5 (0.06)‡	27.7 (0.06)‡
B2 genotype	6.8 (0.01)*	0.0 (0.01)*	33.3 (0.01)*	31.8 (0.01)*
Change in insulin uU/ml (fasting)				
A2B genotype	5.5 (0.18)	2.9 (0.18)	21.9 (0.16)	23.0 (0.15)
B3 genotype	2.9 (0.71)	0.2 (0.67)	19.4 (0.77)	20.2 (0.86)
B2 genotype	2.9 (0.71)	0.1 (0.72)	19.3 (0.84)	20.5 (0.60)
Change in R-QUICKI				
A2B genotype	0.0 (0.62)	0.0 (0.66)	32.1 (0.94)	32.8 (0.96)
B3 genotype	0.0 (0.88)	0.0 (0.79)	33.2 (0.34)	33.6 (0.40)
B2 genotype	1.8 (0.15)	0.00 (0.15)	34.1 (0.19)	36.0 (0.1)‡

Adjusted R^2 (p-value). Model 1 is bivariate with ADR genotype as an independent variable; model 2 is multivariate adjusted for age and hormone therapy; model 3 is also adjusted for baseline values of the dependent variable; model 4 is additionally adjusted for baseline body fat and change in body fat due to resistance training. All models are adjusted for blood draw timing. Baseline dependent values in models 3 and 4 were always significant. *$p<0.05$, ‡$p<0.1$.

Table 3. 8 Expected main effects of genotype on IS biomarkers at baseline and observed outcomes. Variant carriage (+), non-carriage (-).

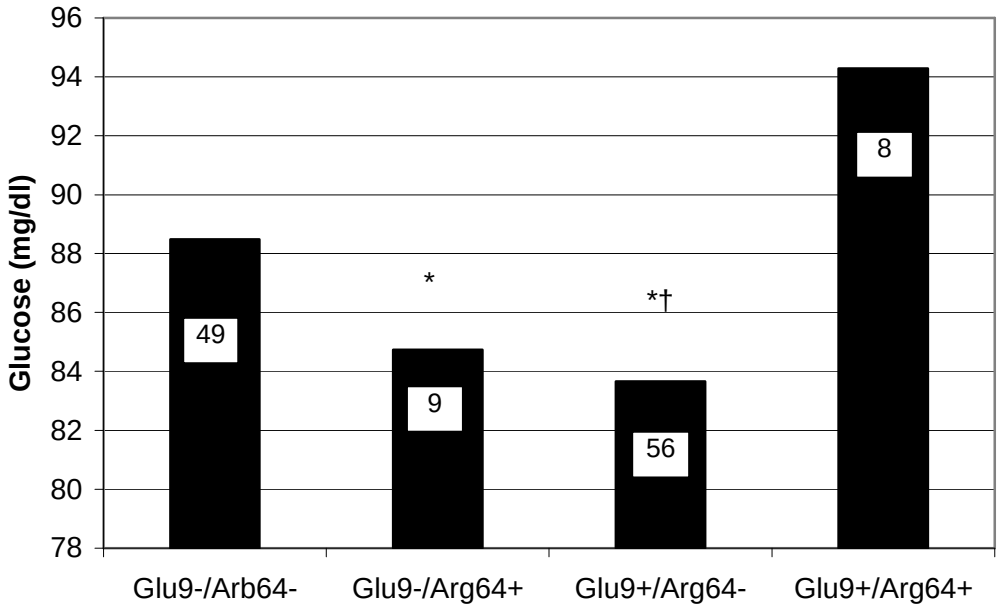
	Expected IS	Observed IS	Notes
ADRA2B (Glu^{9+})	↓	↑	Glucose lower in carriers, $p<0.1$
ADRB3 (Arg^{64+})	↓	↔	NS
ADRB2 (Glu^{27+})	↓	↔	NS
ADRA2BxADRB3 (Glu^{9+} x Arg^{64+})	↓	↓	+/+ hi glucose vs. +/- and -/+, $p<0.05$, NS -/- (Fig. 1)
ADRA2BxADRB3 (Glu^{9-} x Arg^{64+})	↔	↑	-/+ lower glucose than +/+, $p<0.05$, NS -/- and +/- (Fig. 1)
ADRA2BxADRB2 (Glu^{9+} x Glu^{27+})	↑	↔	Overall gene effect $p<0.1$, NS specific genotypes, but +/+ NEFA lower than -/-
ADRA2BxADRB2(Glu^{9-} x Glu^{27+})	↔	↔	Overall gene effect $p<0.1$, NS specific genotypes, but -/+ lower NEFA than -/-.
ADRB3xADRB2 (Arg^{64+} x Glu^{27+})	↑	↔	Overall gene effect $p<0.1$, NS specific genotypes, but +/+ highest glucose versus others.

Table 3. 9 Main effect of resistance training and main effect of genotype on IS, and expected IS outcomes based on their interactions. Variant carriage (+), non-carriage (-).

Genotypes	EX	Gene	Expected IS	Observed IS*†	Notes
ADRA2B (Glu^{9+})	↑	↓	↔	↔	NA
ADRB3 (Arg^{64+})	↑	↑	↑	↑	Glucose decreased, p=0.06
ADRB2 (Glu^{27+})	↑	↑	↑	↑	Glucose decreased, p=0.01, RQUICKI increased, p=0.1
ADRA2BxADRB3 (Glu^{9+} x Arg^{64+})	↑	↔	↔	↔	Glucose and NEFA p<0.1 for gene x gene x exercise, but no individual genotype difference
ADRA2BxADRB3 (Glu^{9-} x Arg^{64+})	↑	↑	↑	↔	same as above.
ADRA2BxADRB2 (Glu^{9+} x Glu^{27+})	↑	↔	↔	↑	+/+ glucose decreased less vs. Glu9- x Glu27+, overall gene x gene x exercise p<0.1; NEFA +/+ decreased vs. Glu9+ x Glu27- increase, p<0.1
ADRA2BxADRB2 (Glu^{9-} x Glu^{27+})	↑	↑	↑	↑	-/+ glucose decreased vs. Glu9+ x Glu27- increase, p<0.1
ADRB3xADRB2 (Arg^{64-} x Glu^{27-})	↑	↓	↔	↓	-/- glucose increased vs. -/+ p<0.1; -/- NEFA increased vs. -/+, p<0.1; -/- decreased R-QUICKI versus +/+ increase p<0.1.
ADRB3xADRB2 (Arg^{64+} x Glu^{27+})	↑	↑	↑	↔	NS

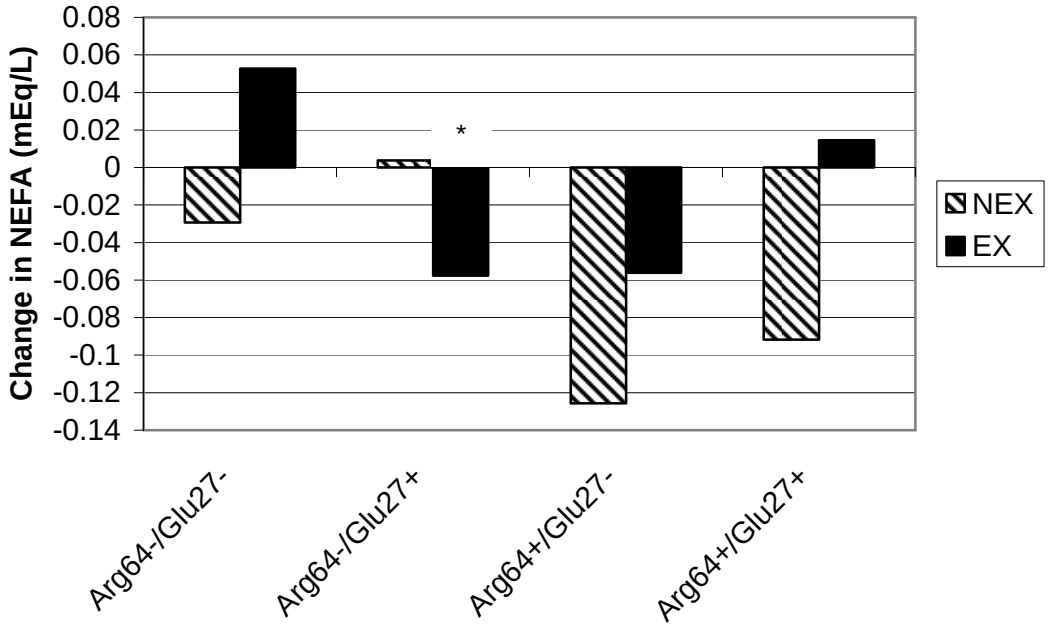
*Within exercise group only. No significant associations between genotype and biomarkers of insulin sensitivity (IS) were observed over the year for controls. †Gene-gene interaction general linear models indicate significance of interaction only, post-hoc tests with analysis of variance were used to determine significant differences between specific genotypes.

Figure 3. 1 ADRA2b x ADRB3 and Baseline Glucose Levels



gene x gene $p<0.05$. *$p<0.05$ +/+ versus -/+ and +/-; † $p=0.06$ -/- versus +/-

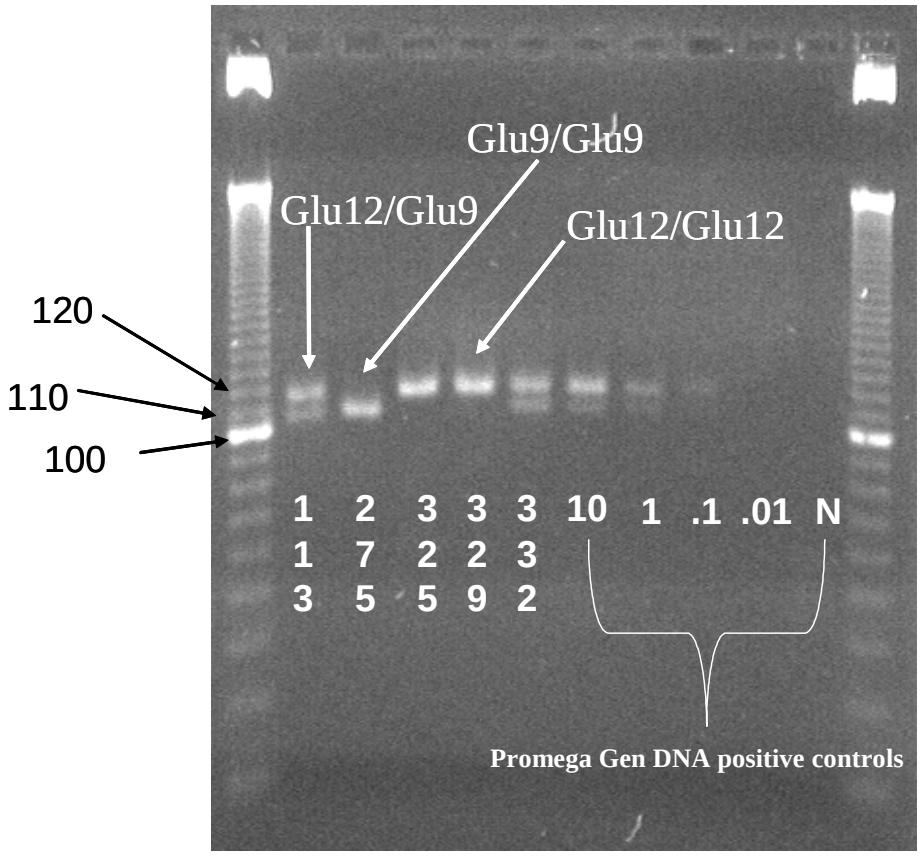
Figure 3. 2 ADRB3 x ADRB2 x exercise interaction and change in NEFA



gene x gene x exercise $p<0.05$. *$p=0.07$ -/- versus -/+

APPENDIX D:

Figure 4. 1 Example of BEST buccal samples genotyped for ADRA2B $Glu^{9/12}$ by gradient PCR with expected band sizes of 112bp/103bp.



DNA was extracted by QIAGEN for total 150uL of elution. Samples were run on 3% SFR agarose gel with 1XTBE. Each well contained 3 uL sample from a 25uL PCR reaction and 2uL blue juice. Left ladder is 3uL, right ladder is 2uL. Electrophoresis was run at 80V for 2hr 20min. Subject 113 is Glu9/12, 275 is Glu9/9, 325 and 329 are Glu12/12, and 332 is Glu9/12.