THE EFFECT OF A REDUCED-CALORIE DIET ON ALPHA-2 ADRENERGIC RECEPTOR RESPONSIVENESS IN ABDOMINAL ADIPOSE TISSUE IN OBESE MEN DURING EXERCISE

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Abstract:

There is at present an imperfect understanding of the effect of diet on availability of inhibitory receptors in fat cells during exercise among obese men.

Objective: The purpose of this study was to determine whether diet results in downregulation of alpha-2 adrenergic receptor (α_{2AR}) messenger RNA (mRNA), improving metabolism in exercise in obese men.

Design: One group, pre-test, post-test design.

Measurements: Subcutaneous abdominal adipose tissue was tested for physiologic response, such as changes in catecholamines and other markers of lipolysis measured during periods of exercise, before and after a 12-week diet. Plasma markers of lipolysis/antilipolytic activity (catecholamines [adrenaline and noradrenaline], NEFA, lactate, glucose, hematocrit, or insulin levels) were analyzed at four points in time in order to determine the effect of exercise on $\alpha_{2\text{-}AR}$ and β-AR responsiveness to sympathetic stimulation.

Subjects: Otherwise healthy 18 to 45 year old obese men (defined as a body mass index (BMI) over 33 kg/m^2).

Results: The 12-week reduced calorie diet did not result in improved metabolism. Instead, upregulation of alpha-2 adrenergic receptor (α_{2AR})

messenger RNA (mRNA) was observed. On average, $\alpha_{2\text{-AR}}$ mRNA levels (ratio of $\alpha_{2\text{-AR}}$to cyclophilin) in subjects increased by 0.022–0.023 after the diet. The average differences in of $\alpha_{2\text{-AR}}$ mRNA and β-AR mRNA measured before and after diet were both insignificant (M = 0.015) t(4) = –0.911; $P >$ 0.05; (M = 0.0139; t(4) = 0.077; $P > 0.05$).

Conclusion: The observed direction of change in $\alpha_{2\text{-AR}}$ mRNA levels, when viewed together with the stability of β-AR mRNA levels, suggests that upregulation of $\alpha_{2\text{-AR}}$ rather than downregulation occurred. Downregulation would account for decreased lipolytic activity during exercise, future study is needed.

Keywords: Obesity; Alpha-2 Adrenergic Receptor; Subcutaneous Adipose

Introduction

At the current rate of escalation, obesity will soon be the leading cause of preventable death in United States. Obesity results in considerable morbidity and mortality. Current research is focused on influences, on multiple factors that are thought to be the origin of obesity. The three most studied and commonly accepted categories are metabolic, genetic, and environmental factors. The structure and function of adipose tissue as it relates to its endocrine role is of particular interest to current research. Both white and brown fat have prompted considerable interest, as both act as lipid stores. White fat is more hormonally active than brown fat and is implicated in the pathophysiology of obesity by increases in the size and number of cells. There are many hormonal, central nervous system (CNS), and sympathetic nervous system (SNS) signals involved in the regulation of white fat equilibrium. There has been considerable research on adrenergic receptors (those receptors stimulated by the SNS), particularly the β-receptors on adipose cells, in the area of the SNS's modulation of lipid mobilization. β-receptors are those transmembrane proteins involved in the activation of lipolysis, whereas α-receptors appear to balance this equilibrium through an opposing, antilipolytic effect. Both types of receptors are stimulated by catecholamines released by the SNS, although they are

variably mediated and modulated by many factors, such as receptor subtype, receptor sensitivity to regulation, and affinity to stimulation at different levels of catecholamines. Although research on α-receptors in adipose cells is limited, an important finding has been the discovery of a stronger $\alpha_{2\text{-AR}}$ antilipolytic effect during exercise in men. The effect of environmental factors such as diet on metabolic factors, such as $\alpha_{2\text{-AR}}$ antilipolytic effect during exercise, has been studied in women. The current study was intended to extend prior research on the effect of diet on $\alpha_{2\text{-AR}}$ in the abdominal adipose tissue of obese men, in whom it is known that antilipolytic effects tend to be greater than for obese women. Specifically, due to the significantly stronger $\alpha_{2\text{-AR}}$ antilipolytic effect in men, the gap in the science pertains to whether a period of caloric restriction can alter the expected increased antilipolytic effect of exercise in obese men.

Methods

We employed a single-group, pre-test, post-test design for our study. The intervention in this study was a 12-week reduced calorie diet (500 calories less than usual diet) followed by a 45 minute cycle ergometry. Approximate duration of the study for each subject was 14-18 weeks.

Maximal exercise test was performed five days prior to the beginning of the diet phase of the study.

Inclusion criteria: Subjects were otherwise healthy 18 to 45 year old obese men (defined as a body mass index (BMI) over 33 kg/m^2). A higher BMI level than the standard identification of obesity (BMI > 30) was used to ensure adequate abdominal skin fold thickness for obtaining a biopsy specimen, and to increase our likelihood of obtaining fat cells of sufficient size to ensure a representative sample for $\alpha_{2\text{-AR}}$ expression. Men over the age of 45 years were excluded from our study so as to control for the possible confounding effect of age on catecholamine levels during exercise among older men. Further, only nonsmokers were included in our study, so as to control for the possible confounding effect of smoking related increases in catecholamines during exercise.

Procedure days:

Days 1 and 3

Needle Biopsy: On procedure days 1 and 3, a small sample of fat was taken from each subject's abdomen. Standard sterile method was used. The abdominal area was anesthetized using lidocaine without added adrenaline in order to prevent the inadvertent introduction of extrinsic adrenaline into the

surrounding fat cells. A 16-gauge needle was used to obtain subcutaneous abdominal adipose tissue samples.

α2-AR, β-AR levels: α2-AR responsiveness was determined by obtaining SCAAT through needle biopsy. α2-AR, β-AR, hormone-sensitive lipase (HSL) and cyclophilin mRNA - levels were obtained from biopsy material by RT-PCR. Total RNA was isolated from subcutaneous abdominal fat using Trizol reagent (Invitrogen, Carlsbad, CA) and quantitated. A DNA-free kit (Ambion, The Woodlands, TX) was used to treat RNA with DNase I. The RNA was then reversed transcribed into cDNA using a high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA) in a volume of 100 μL. Ten microliters of the cDNA was subsequently amplified using predeveloped TaqMan gene expression assays from Applied Biosystems for **$\alpha_{2\text{-AR}}$** mRNA (Hs00265081_s1), **β-AR** mRNA (Hs00240532_s1) and the internal control **cyclophilin** mRNA (Peptidyl prolyl isomerase A (PPIA)) (Hs99999904_m1) in a 50-μl volume, using conditions as required by Applied Biosystems. All reactions were conducted in 96 well plates. Samples were analyzed using the 7900HT sequence detection

system (Applied Biosystems), mRNA levels were quantitated using the standard curve method. Each sample was run in triplicate, cyclophilin mRNA level was utilized to normalize the $\alpha_{2\text{-AR}}$ data. As a housekeeping gene, cyclophilin allows measurements of other mRNA levels to be expressed as a ratio of that mRNA level and cyclophilin mRNA level. In this manner, results may be interpreted by using a stably expressed gene to normalize for differences in the amount of starting complementary DNA (i.e., expressing the complementary base pair for the mRNA being acted on by a reverse transcriptase enzyme) between samples.

Days 2 and 4

Exercise: On the second and fourth procedure days (i.e. the day immediately preceding the initiation of the diet, and on the final day of the diet, respectively), subjects performed cycle ergometry for approximately 45 minutes at 50% of their residual heart rate reserve.

Training Heart Rate = 50% Residual Heart Rate Reserve.

RHR = Resting Heart Rate, upon waking is best, otherwise recumbent 5 minutes.

MHR = Maximum Heart Rate, 220 – age.

HRR = Heart Rate Reserve, MHR – RHR.

THR = Training Heart Rate for this protocol, (HRR * 0.50) + RHR.

Subjects were then asked to lie down and rest for 60 minutes afterwards.

Blood samples: Blood samples were taken on the second and fourth procedure days. Blood was drawn both before and after subjects rode the exercise bicycle, as well as at sequential 30 minute resting intervals.

Lactate – was assessed in plasma and reported in mmol/L using an ABL 800 Radiometer (Radiometer America, Westlake, Ohio).

Glucose – plasma measurements were made using a glucose-oxidase technique on an ABL 800 Radiometer (Radiometer America, Westlake, Ohio) and reported in units of mg/dL.

Hematocrit – defined for our study as that percentage of whole blood consisting of red blood cells; reported as a percentage on Coulter LH 755 (Beckman Coulter, Inc., Fullerton, California).

Insulin - plasma measurements were obtained via RIA kits on an Immulite 2000 (Diagnostic Products Corporation, Los Angeles, CA) and reported in units of uIU/mL.

Non-esterfied fatty acids (NEFA) – were measured in the plasma through an enzymatic procedure using a Hitachi 717 chemistry analyzer (Boehringer-Mannheim Corp., Indianapolis, IN) and reported in mmol/L.

Adrenaline – electrochemical detection measurements were made in the plasma via High Performance Liquid Chromatography (HPLC) using a Waters 717 Plus HPLC autosampler containing a Waters 515 Pump and a Coulochem 3 Detector (Waters Corp., Milford, MA). Adrenaline measurements are reported in units of pg/mL.

Noradrenalin – was measured in the plasma by HPLC using electrochemical detection and reported as pg/mL. Noradrenalin levels were measured on a Waters 717 Plus HPLC autosampler with a Waters 515 Pump and a Coulochem 3 Detector (Waters Corp., Milford, MA).

Diet:

Subjects were asked to initiate a diet after the first set of procedure days. The diet consisted of a daily reduction of 500 calories compared to what subjects typically consumed in a day for a period of 12 weeks. Subjects were asked to complete a food diary for seven days prior to initiating the diet, as well as for each day during the diet.

Nutritionist visits – Subjects were asked to meet with a nutrition specialist about their target caloric intake. The nutrition specialist reviewed subjects' food diaries based on the results of their initial 7-day diet logs and counseled them as to their target calorie intake. Subjects met for follow-up appointments each two weeks during the diet in order to ensure that they were aware of the extent to which they were meeting their caloric intake goals.

Sample Size Calculation:

Setting the alpha level at 0.05 a priori for a dependent samples t-test, we calculated that a sample size of 10 subjects would provide a 99% chance of detecting a 3% difference between pre and post measures of $\alpha_{2\text{-AR}}$ mRNA normalized to the cyclophilin mRNA level (μg of $\alpha_{2\text{-AR}}$/μg of cyclophilin). This assertion was based on the results of a similar empirical study of $\alpha_{2\text{-AR}}$/cyclophilin mRNA among women before and after diet in which a 3% difference in means was detected [1]. To calculate effect size, we divided the

difference (.03 $\alpha_{2\text{-AR}}$/cyclophilin) by the square root of the sum of the standard deviation for the first measure squared (0.01^2) plus the standard deviation for the second measure squared (0.02^2) divided by 2 to arrive at an effect size of 1.9. The use of empirical data as a basis on which to calculate required sample size allowed for a smaller necessary sample than for inputs of more generalized estimated effect sizes (e.g., small (0.2), medium (0.5), large (0.8)). We used the ratio of $\alpha_{2\text{-AR}}$to cyclophilin as a basis for our power calculation because it was the main outcome variable, and a conservative choice on which to estimate statistical power of the study. Because each of the outcome variables under investigation was measured at several points in time, the number of observations was increased, thus increasing the statistical power of the study.

Means and standard deviations are reported for all pre and post diet biopsy data. These data were further analyzed by paired t-tests for dependent samples. Paired t-tests were utilized with Bonferroni adjustments to determine the effect of diet on any each variable (e.g., Noradrenaline) after exercise (Figure 1, Time 1 and Time 5).

A single-group repeated measures ANOVA was conducted to examine responsiveness at different points in time. In order to identify significant changes in responsiveness from one exercise session to the next,

paired T-tests were conducted on biopsy data at each of the four time points in the first exercise session and matched to its counterpart in the second session. $P < 0.05$ was considered statistically significant.

Statement of Ethics

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Approval by the Wilford Hall Medical Center Institutional Review Board (IRB) and the Touro University International IRB were obtained prior to beginning the research.

Results

Participants lost an average of 4 lbs (SD = 2.4) after the 12-week diet intervention. They began the study ranging from a minimum weight of 216 lbs to a maximum of 295 lbs; the BMI ranged from 33 to 40, and the average BMI was 35.6 (SD = 2.35) before starting the diet.

Bivariate Analyses.

Although weight loss was not an outcome variable in our investigation, weight loss is indeed an indicator of the effect of the reduced-

calorie diet intervention over the 12-week study period. A paired-samples t-test revealed that the post 12-week diet BMI of subjects was significantly lower than the pre 12-week diet BMI ($t(5)= 4.08$; $P <0.05$).

The first alternative hypothesis, "a 12-week RCD significantly increases plasma non-esterfied fatty acids NEFA levels (and therefore lipolysis) in subcutaneous abdominal fat during exercise in obese men," was not supported. No significant differences were found between pre-intervention and post-intervention NEFA levels as measured at several points surrounding exercise (i.e. before exercise, after exercise, after 30 minutes of rest, and after 60 minutes of rest) (Table 1).

The results shown in Table 1 indicate that mean NEFA levels measured before the diet were not significantly different than those obtained after the diet ($M = 0.180$; $M = 0.104$; $M = 0.126$; and $M = 0.046$ respectively from before exercise through 60 minutes of rest). Mean differences are also provided to clarify the direction of association between variables. NEFA tracked lower after diet than before for all time points: before and after exercise as well as 30 and 60 minutes after exercise.

As can be seen in Figure 2, after-diet measures of NEFA (an indicator of lipolysis) were generally lower than all before-diet measures for NEFA.

The second alternative hypothesis, "a 12-week RCD significantly reduces $a_{2\text{-AR}}$ mRNA levels, and therefore $a_{2\text{-AR}}$ responsiveness (e.g., the number of receptors available for activation to inhibit lipolysis), in subcutaneous abdominal fat in obese men," was not supported. The measurement of $\alpha_{2\text{-AR}}$ mRNA after the diet was not significantly higher than that obtained before the diet (M = 0.022). A paired-samples t-test revealed no significant difference in $\alpha_{2\text{-AR}}$ mRNA before and after the diet [$t(4) = -0.911$, $P > 0.05$]. Contrary to expectations, $\alpha_{2\text{-AR}}$ mRNA increased on average from 0.016 (90% CI +/- 0.038) to 0.038 (90% CI +/-0.156)(ratio of $\alpha_{2\text{-AR}}$ to cyclophilin, in micrograms) after the 12-week diet.

The ratios of $\alpha_{2\text{-AR}}$ mRNA to cyclophilin for each subject are reported in Table 2. Note that the mean $\alpha_{2\text{-AR}}$ mRNA levels before the diet and after the diet are reported as ratios. The standard deviation for each test is also included. Each abdominal fat sample was assayed in triplicate as per standard procedure for RT-PCR. Table 3 shows the mean of those measures before the diet and then again after the diet.

The third alternative hypothesis, "a 12-week RCD has a significant effect on β-AR or HSL mRNA levels," was not supported. As anticipated, β-AR mRNA levels on average did not change after the 12-week diet. A paired-samples t-test revealed no significant difference in the β-AR mRNA before and after the diet [$t(4) = 0.077$; $P > 0.05$]. The mean level of β-AR mRNA after the diet was not significantly higher than before the diet ($M = 0.0139$; $p>0.05$). Table 4 and 5 show the reported ratios of β-AR mRNA to cyclophilin reported as the β-AR mRNA measure for this study, for each subject before the diet and then again after the diet, with the standard deviation for the test which was assayed in triplicate as per standard procedure for RT-PCR and Table 5 the mean of those measures.

HSL was not measured with RT-PCR to confirm the results of the other assays due to a lack of remaining specimen following analysis of $\alpha_{2\text{-AR}}$ and β-AR mRNA. However, we believe that the results of HSL would have been inconsequential to the remaining analyses, given the results obtained with $\alpha_{2\text{-AR}}$ and β-AR mRNA.

The fifth alternative hypothesis, "a 12-week RCD has a significant effect on additional plasma markers of lipolysis/antilipolytic activity

(catecholamines—adrenaline and noradrenaline) or other associated plasma levels (lactate, glucose, hematocrit, or insulin levels) during exercise, and therefore these associated factors may confound the effect of $\alpha_{2\text{-AR}}$ responsiveness to sympathetic stimulation on lipolysis in subcutaneous abdominal fat during exercise in obese men," was not supported. As anticipated, none of the pairings for catecholamines: adrenaline and noradrenaline, lactate, glucose, hematocrit, or insulin levels measured at several points in time (i.e., before exercise, after exercise, after 30 minutes of rest, and after 60 minutes of rest) significantly changed before the diet and after the diet.

Multivariate Analyses.

To determine the effect of exercise on $\alpha_{2\text{-AR}}$ and β-AR responsiveness to sympathetic stimulation, plasma markers of lipolysis/antilipolytic activity (catecholamines [adrenaline and noradrenaline], NEFA, lactate, glucose, hematocrit, or insulin levels) at four exercise time points were analyzed with a repeated measures (RM) ANOVA during each exercise period. The fourth alternative hypothesis, "Exercise has a significant effect on plasma markers of lipolysis/antilipolytic activity (catecholamines—adrenaline and noradrenaline), NEFA, lactate, glucose, hematocrit, or insulin levels)," was

not supported. For most of the variables collected during the exercise–rest procedure the pairwise comparison of the repeated measures after Bonferroni adjustment did not differ significantly (adrenaline before and after the diet; noradrenaline after the diet; lactate before and after the diet, glucose before and after the diet, and insulin before and after the diet. However, after Bonferroni adjustment, during the exercise–rest procedure, a RM ANOVA of before diet noradrenaline exercise-measures, revealed at least one set of sample times that were significantly different during the specified period. Similarly, at least one set of sample times for after diet NEFA exercise-measures and at least one set in each, before and after diet, hematocrit measures, also indicated pairwise comparisons that were significantly different during the specified period.

After Bonferroni adjustment significance was reached for noradrenaline before the diet (before exercise as compared to after exercise ($P = 0.000$), after exercise as compared to 30 minutes ($P = 0.000$), and 60 minutes exercise ($P = 0.001$), of rest) and hematocrit both before the diet (after exercise as compared to 30 minutes of rest ($P = 0.035$)) and after the diet (before exercise as compared to after exercise ($P = 0.004$)). Pairwise comparisons reached significance for NEFA both before the diet (i.e., after

exercise as compared to 30 minutes ($P < 0.05$) of rest) and after the diet (i.e., before exercise as compared to 30 minutes ($P < 0.05$) of rest). The pair of repeated measures represents the difference between the before the diet measurements obtained for noradrenaline after exercise and after 60 minutes of rest (i.e., after completion of the exercise session) ($P > 0.05$). This difference was statistically significant, whereas the same measurement obtained after the diet was not statistically significant. Similarly, most of the NEFA and HCT repeated measures pairwise comparisons were not significantly different with some exceptions. Specific exceptions before the diet were after-exercise NEFA and HCT measurements as compared with measurements obtained after 30 minutes of rest (i.e., after completion of the exercise session) ($P > 0.05$). Note the slope of the curve for after diet NEFA during exercise is steeper than the curve for pre-diet exercise and remains relatively constant at a higher peak elevation during rest than the pre-diet rest (Figure 2). The post-diet NEFA curve also peaks earlier and remains elevated more so than the pre-diet curve that peaks late and drops quickly.

Viewing the noradrenaline data, a trend of an upward slope during the exercise phase before the diet was apparent (Figure 3). Both before- and after-diet noradrenaline levels declined sharply following exercise. Noradrenaline levels tended to recover to their pre-exercise levels within 30

minutes of rest, following exercise, and remain relatively constant for 60 minutes following exercise (Figure 3).

A strong correlation was observed between subjects' weight before and after the diet ($r = 0.99$, $P < 0.001$). A strong association was also observed between $\alpha_{2\text{-AR}}$ mRNA levels before and after the diet ($r = 0.97$, $P < 0.001$).

The central hypothesis for this study was that the average difference between subjects' before-diet $\alpha_{2\text{-AR}}$ and after-diet $\alpha_{2\text{-AR}}$ measurements would be significantly reduced following a 12-week diet.

Discussion

In the current study, the observed NEFA responses among subjects indicated that lipolysis occurred during each ergometer/rest procedure. The pattern of changes in NEFA during exercise supports the model for initiating lipolytic activity, even though there was no significant change caused by the diet as expected in the first hypothesis. Specifically, that lipolysis occurred supports previous research findings, indicating that this phenomenon occurs in obese subjects in response to the ergometer/rest procedure.

Reduced caloric intake and increased exercise are known to result in fat breakdown and weight loss in non-obese individuals. However, these outcomes are less predictable for obese individuals [2]. For example, fat breakdown is actually inhibited during exercise for obese men. The inhibition may be explained in part by the downregulation of $\alpha_{2\text{-AR}}$ mRNA that is seen after an RCD in women [1]. These researchers' findings suggest that an RCD might have a similar effect (decrease in availability of $\alpha_{2\text{-AR}}$) on lipolysis during exercise in men.

It is well established that NEFA is directly related to lipolysis via adrenergic receptor stimulation by SNS catecholamines, increasing cAMP, increasing activity of protein kinase A, phosphorylating HSL, and leading to the breakdown of triglycerides into products such as diacylglycerol, which results in interstitial fluid influxes of free fatty acid and glycerol [3]. The first hypothesis was based on the findings of Bartness & Bamshad (1998), Bougnères et al. (1997), Turtzo et al. (2001), Wolfe et al. (1987), and Youngstrom & Bartness (1998), which established a direct link between NEFA and lipolysis [4, 5, 6, 7, 8]. NEFA is released by adipose tissue in response to lipolysis, and as lipolysis increases, NEFA levels also increase. This

relationship was further described with the findings of Stich et al. (2002), which linked the possibility that metabolism might have been improved by diet in women [1]. In the current study, NEFA response to exercise was consistent before and after diet.

It is clear from the existing research that the SNS is highly integrated into the structure and function of adipose tissue [4, 6, 8, 9]. Knowledge of the SNS's effect on lipolysis when activated during exercise has been largely established in lean men [2]. Inhibition of that pathway via changes in $\alpha_{2\text{-AR}}$ responsiveness, and therefore likely $\alpha_{2\text{-AR}}$ availability, has been shown in obese men and women and are alterable by diet in women [1]. However, it cannot be concluded by findings in the current study that a change in $\alpha_{2\text{-AR}}$ after a 12-week diet was related to the diet or that $\alpha_{2\text{-AR}}$ significantly decreased, as anticipated in the second hypothesis. A paired samples t-test for before- and after-diet $\alpha_{2\text{-AR}}$was performed to test this hypothesis and revealed significance measures that were much greater than 0.05. Changing the alpha for this study would reduce the chance of rejecting the null hypothesis when it is false and making a type II error but would also reduce the chance of detecting a difference in the outcome measures. In addition, the *P* value for the t-test for $\alpha_{2\text{-AR}}$ was so high that this was not an option.

The diet alone could not be statistically shown to explain the alterations in $\alpha_{2\text{-AR}}$ availability for these subjects. It is possible that upregulation was responsible for the decreased lipolysis seen in this study in men, especially considering that diet did not have a significant effect on β-AR mRNA levels. Due to technical difficulties with the assays HSL mRNA could not be obtained for these samples.

Diet may indeed affect lipolysis, as evidenced by measurements of NEFA and $\alpha_{2\text{-AR}}$ mRNA in our study. These findings indicate that further study is necessary, allowing more careful control for threats to validity (e.g., increase in sample size) to ensure a significant change in lipolysis (NEFA and $\alpha_{2\text{-AR}}$ mRNA to show downregulation) and attempt to decrease the possibility of a change occurring by chance.

Approximately the same amount of β-AR was available for stimulation before and after the diet, which was consistent with the prediction of the third hypothesis. The third hypothesis was based on the findings of Reynisdottir et al. (1994), which described β-AR in obese subjects combined with the finding of Stich et al. (2002) [1, 10]. This finding is helpful in interpreting the slight increase seen in $\alpha_{2\text{-AR}}$. The stability of β-AR

mRNA measures in this study support the conclusion that upregulation of $\alpha_{2\text{-}AR}$ might have occurred instead of downregulation.

Catecholamine levels are known to increase in response to exercise as a part of SNS activity [11, 12, 13]. Our findings concerning the effect of exercise on several markers of lipolysis/antilipolytic activity (catecholamines—adrenaline and noradrenaline), NEFA, lactate, glucose, hematocrit, or insulin levels) were somewhat counterintuitive. The results indicate that some of these markers (hematocrit, NEFA, and the catecholamine noradrenaline) are excellent plasma markers of lipolytic activity.

Fat samples were collected before each exercise session. This proved to be effective in avoiding any confounding effect of these markers of lipolysis/antilipolytic activity on $\alpha_{2\text{-}AR}$ responsiveness to SNS stimulation. In essence, the method was sound for separating the concepts of diet as an effect on $\alpha_{2\text{-}AR}$ availability (biopsy of $\alpha_{2\text{-}AR}$ mRNA before and after diet) and exercise as an effect on lipolysis (primarily measured by NEFA before exercise, after exercise, and after 30 and 60 minutes of rest).

We expected that catecholamine levels released during exercise would not change substantially before and after the diet, as the body's response to

exercise would presumably be similar [1]. The catecholamines adrenaline and noradrenaline, on average, did not change when compared before and after diet at each individual time point; before exercise, after exercise, or at 30 minutes of rest. This indicates catecholamines do not mediate $\alpha_{2\text{-AR}}$ levels and therefore did not confound the effect of $\alpha_{2\text{-AR}}$ responsiveness to sympathetic stimulation on lipolysis. Indeed, catecholamines are among many factors known to stimulate fat cell lipolysis and antilipolytic activity on which diet might have had an effect. Other than the catecholamines adrenaline and noradrenaline, other associated plasma levels (lactate, glucose, hematocrit, or insulin levels) could have confounding effects on interpretation of $\alpha_{2\text{-AR}}$ responsiveness results. However, previous empirical evidence suggests that adrenaline in particular is more closely linked to the antilipolytic activity of $\alpha_{2\text{-AR}}$ than other biological amines when the SNS is activated [14.] Our findings suggest that catecholamines did not change substantially before and after the diet. For example, in our study no significant differences were detected in adrenaline levels before and after the diet, which is consistent with the findings of Stich et al. (2002) [1]. However, there was a statistically significant increase in noradrenaline.

The observed small, yet statistically significant increase in noradrenaline levels before and after the diet must be viewed in a holistic

context of catecholamine binding ability. Adrenaline is known to bind to α-AR at higher concentrations of catecholamines [15]. Both adrenaline and noradrenaline have a higher affinity for β-AR in general [15]. $\alpha_{2\text{-AR}}$ is stimulated at lower levels of catecholamines, and although β-AR is also stimulated, $\alpha_{2\text{-AR}}$ antagonism would be the likely result of slightly increased noradrenaline levels, especially given the ratio of $\alpha_{2\text{-AR}}$ to β-AR in adipose tissue. Indeed, $\alpha_{2\text{-AR}}$ antagonism is believed to be the basis for the lack of ability of obese men to lose weight during exercise [16, 17].

In the current study, the diet intervention resulted in lower blood glucose levels among obese men. Our findings support current guidelines specified in *Healthy People 2010* (U.S. Department of Health and Human Services [DHHS] 2000), which suggest that even a modest weight loss can result in improved glucose control in obese individuals[18]. It should be noted that the observed insulin levels among our study participants were also generally higher after the diet intervention than before the diet intervention, thus lending support for the assertion made in Healthy People 2010 that weight loss can improve insulin resistance [18].

The results of the current study contribute to the body of knowledge that has potential for the future treatment of obesity, as well as for the

discovery of a predictor of the effectiveness of diet on weight loss in obese men.

Obesity is a common medical condition that results in severe and life-threatening comorbidities and complications. Research on the physiologic and pathophysiologic mechanisms of obesity is needed to understand the disease and to develop preventive strategies and curative treatments. The metabolic aspects of the multifactorial theory of obesity are of particular importance in the overall theory and were the focus of this study.

The interrelationships among the three main factors of the multifactorial theory—metabolic, genetic, and environmental factors—remain important to understand this extremely complicated disease.

Conclusions.

In the present study, our findings that, a) $\alpha_{2\text{-AR}}$ mRNA levels measured before and after the diet increased slightly, contrary to expectations, and b) the observed stability of the β-AR mRNA levels, support the notion that upregulation of $\alpha_{2\text{-AR}}$ occurred. Since $\alpha_{2\text{-AR}}$ mRNA levels increased slightly, it is possible that upregulation was responsible for the observed decrease in

lipolytic activity, considering that approximately the same amount of β-AR was available for stimulation before and after the diet.

Implications and Directions for Future Research.

The observed lack of significant change in α2-AR availability in this study was more likely to have been a function of limited statistical power associated with small sample size than by virtue of the intervention itself – i.e., duration of the diet and amount of weight loss. Investigators who wish to conduct experiments of similar design in the future for the purpose of identifying significant changes in α2-AR availability must take into account the possibility of mortality as a threat to internal validity.

Unforeseen technical difficulties in the laboratory that occurred during this investigation limited the total number of observable samples we could obtain. The dropout rate was also somewhat high in our study, and thus our ability to generalize the findings beyond the setting is constrained. Nonetheless, our counterintuitive observation that antilipolytic activity increased among obese men during exercise following a diet warrants further attention. Further study should be devoted to investigating whether lipolytic activity potential during exercise tends to decrease over time for obese men.

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Table 1.

*Comparison of before- and after-diet plasma NEFA (lipolysis) during exercise**

Exercise Status	**NEFA t(4)**
Before exercise	1.49
After exercise	0.533
After 30 minutes of rest	0.647
After 60 minutes of rest	0.452

* Variables obtained during exercise/rest procedure before and after diet.

† *P* significant at <0.05.

Table 2.

$\alpha_{2\text{-}AR}$ mRNA normalized to Cy mRNA at rest

Diet Status	**α2-AR mRNA : Cy mRNA at rest**	
	Ratio*	**RT-PCR Assay SD**
Before diet at rest	0.0151	0.0007
	0.0025	0.0001
	0.0069	0.0014
	0.0063	0.0013
	0.0467	0.0235
After diet at rest	0.0068	0.0003
	0.0031	0.0013
	0.0110	0.0009
	0.0014	0.0120
	0.1687	0.0003

590 * Results are expressed as the ratio of $\alpha_{2\text{-}AR}$ to cyclophilin, in micrograms.

Table 3

Mean of Ratios: α2-AR mRNA normalized to Cy mRNA at rest

	Mean*	**SD**
Pre-diet at rest	0.016	0.018
Post-diet at rest	0.038	0.073

* Mean indicates (Mean of ratios in Table 2)

Table 4

595 *β-AR mRNA normalized to Cy mRNA at rest*

Diet Status	**β-AR mRNA : Cy mRNA at rest**	
	Ratio*	**RT-PCD Assay SD**
Before diet at rest	0.0852	0.007
	0.1149	0.0065
	0.1392	0.0034
	0.1644	0.007
	0.1896	0.0451
After diet at rest	0.2963	0.0291
	0.0572	0.0031
	0.1982	0.0058
	0.0612	0.0036
	0.0561	0.0087

* Results are expressed as the ratio of β-AR to cyclophilin, in micrograms.

Table 5

Mean of Ratios: β-AR mRNA normalized to Cy mRNA at rest

	Mean*	**SD**
Pre-diet at rest	0.14	0.041
Post-diet at rest	0.14	0.109

* Mean indicates (Mean of ratios in Table 4)

600

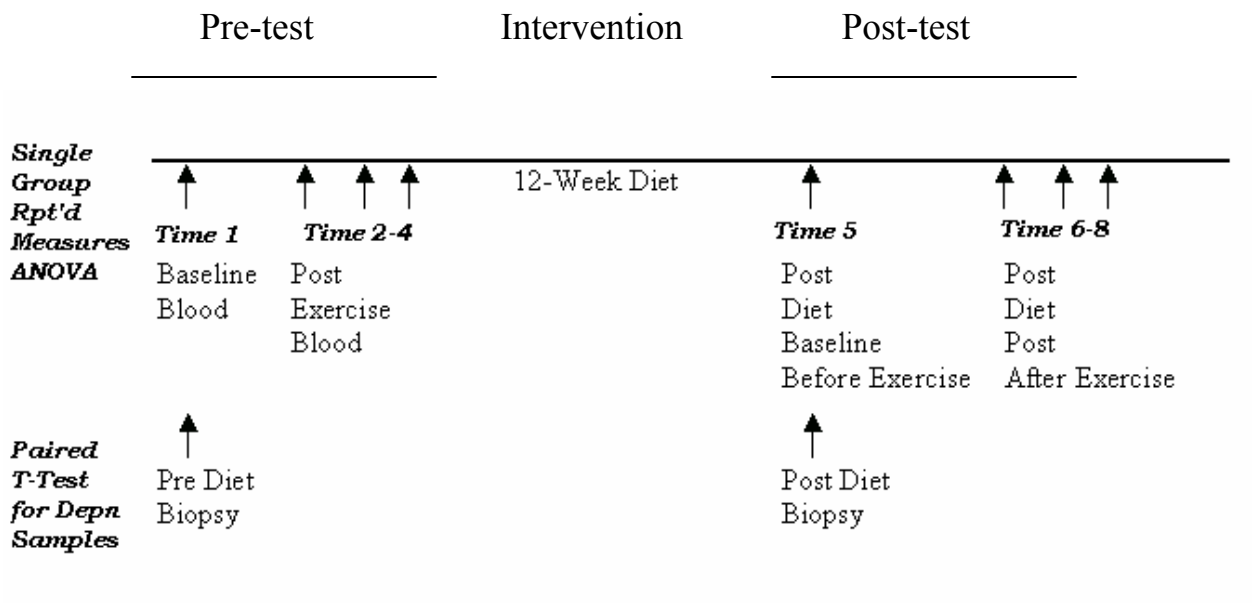

Figure 1

Statistical Analysis

Graphical representation for choice of statistical tests.

605

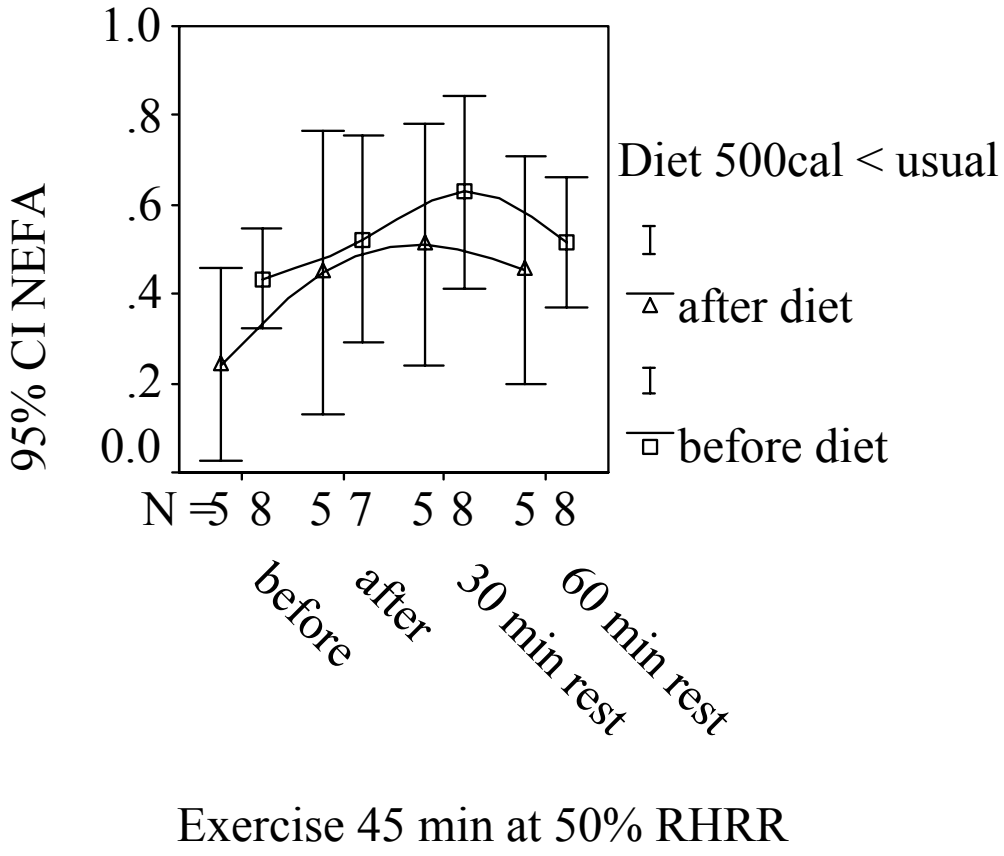

Figure 2

NEFA

A graphical indication of NEFA in mEq/L (lipolysis) response to exercise

610 *before and after diet.*

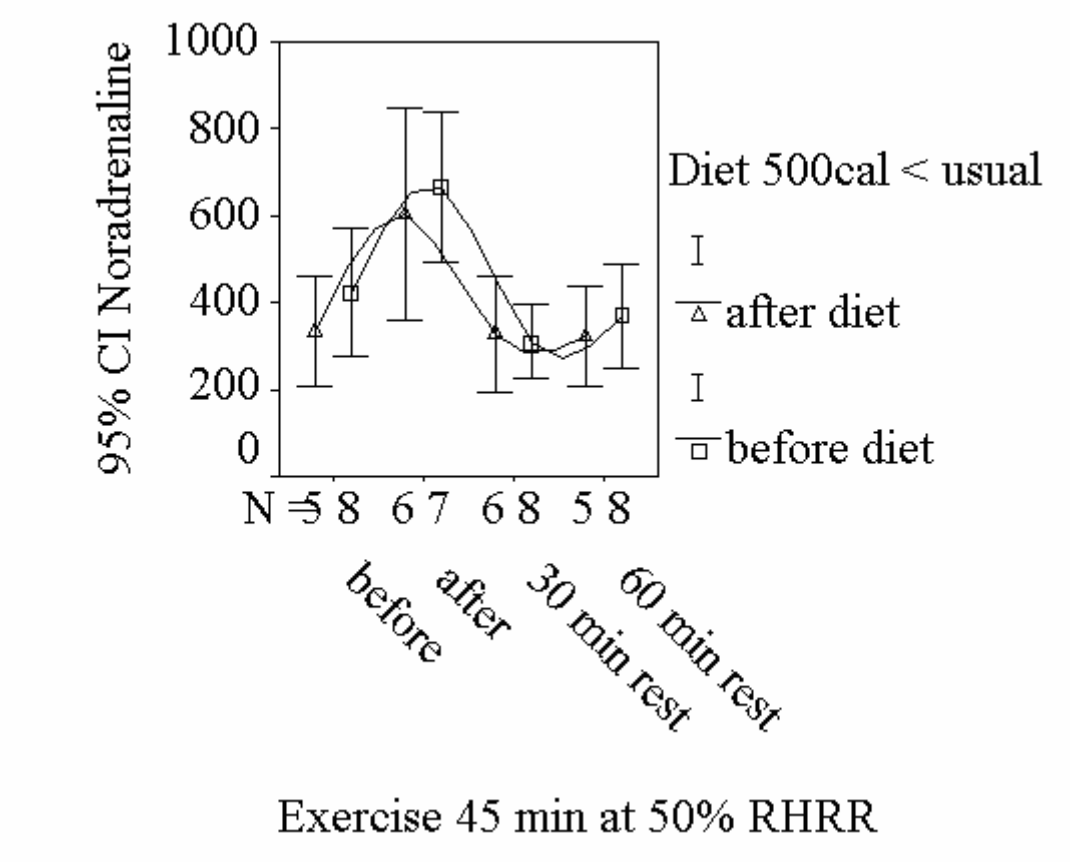

Figure 3

615 *Noradrenaline*

Noradrenaline in pg/ml reveals a graphical indication of lipolysis during exercise.