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Abstract

Only half of pre-diabetic, subjects who are obese who underwent exercise training without weight loss increased their insulin responsiveness. We hypothesized that those who improved their insulin responsiveness might have pre-training characteristics favoring a positive response to exercise training. Thirty non-diabetic, subjects who are obese volunteered for eight weeks of either strength training or endurance training. During training, subjects increased their caloric intake to prevent weight loss. Insulin responsiveness by euglycemic clamps and muscle fiber composition and expression of muscle key biochemical pathways were quantified. Positive responders initially had 52% higher intermediate muscle fibers (fiber type IIa) with 27% lower slow twitch fibers (type I) and 23% lower expression of muscle insulin receptors. Whether after weight training or stationary bike training, positive responders' fiber type shifted away from type I and type IIa fibers to an increased proportion of type IIx fibers (fast twitch). Muscle insulin receptor expression and GLUT4 expression increased in all trained subjects, but these moderate changes did not consistently translate to improvement in whole body insulin responsiveness. Exercise training of previously sedentary subjects who are obese can result in muscle remodeling and increased expression of key elements of the insulin pathway, but in the absence of weight loss, insulin sensitivity improvement was modest and limited to about half of the participants. Our data suggest rather than responders being more fit, they may have been less fit, only catching up to the other half of subjects who are obese whose insulin responsiveness did not increase beyond their pre-training baseline.

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A. Introduction

Eat less and exercise more! This prescription may go all the way back to physicians of ancient times such as Hippocrates and Galen (1). With the growing global epidemic of obesity and type 2 diabetes, it is surprising that we do not better understand the mechanisms of action of the two components of this widely accepted dictum (2). Clinical trials using a combination of aerobic and strength training with weight loss have consistently demonstrated mitigation in the severity of insulin resistance in the metabolic syndrome and improved glycemic control in type 2 diabetes (3-5).

There are key elements within skeletal muscle that are known to be activated by exercise training, whether a program is primarily endurance and aerobic or the training is resistance and strength focused. Aerobic training in healthy young people acts to increase mitochondria in muscle by activation of AMP-dependent protein kinase (AMPK) and subsequent increase in and activation of peroxisome proliferator activated-receptor-gamma coactivator-1 alpha (PGC-1α) which in turn regulates mitochondrial gene expression from mitochondria and the cell nucleus (6). Strength training in normal subjects causes muscle hypertrophy by activation of protein synthesis through phosphorylation of the mammalian target of rapamycin (mTOR) and its phosphorylation and modification of the activity of downstream inhibitors and stimulators of protein translation initiation (7). Weight loss by caloric restriction without exercise improves insulin responsiveness, but the mechanisms of the impact of weight loss on insulin action have been elusive (8;9).

To our surprise, only about half of our obese participants improved their insulin responsiveness after either aerobic (10) or strength training (11) when weight loss was prevented by keeping energy intake and expenditure in balance. Our previous study designs were intended to determine the pure impact of these two types of training programs on the insulin resistance of obesity, without confounding the data with weight loss-related changes in insulin pathways. These two different training programs each were effective at increasing endurance and strength, respectively, but the average impact on insulin responsiveness was no effect. Even though our previously sedentary subjects who are obese seemed very similar to each other pre-training, we questioned whether there were any characteristics that could differentiate between those who would improve their insulin responsiveness with intense supervised exercise training. When we retrospectively evaluated our data describing muscle fiber composition and expression of multiple intracellular proteins before and after exercise training in pre-diabetic, subjects who are obese, we found major differences in the pre-training muscle characteristics between positive and negative responders.

B. Methods

Subject Selection

Thirty sedentary subjects with obesity (BMI > 30 kg/m2) and a family history of type 2 diabetes were recruited for three separate eight week duration exercise training programs. Each subject provided written informed consent approved by the East Tennessee State University Institutional Review Board. All of these subjects met the criteria for "metabolic syndrome" as set forward by the International Diabetes Federation (IDF) (12). There were ten women and twenty men who completed the programs. The programs retrospectively analyzed were performed over a four year period. The first phase results previously reported (11) were from men and women undergoing progressive resistance exercise training and included lean sedentary controls. The second phase was also men and women with lean controls who trained on stationary bikes for eight weeks with increasing duration and intensity (10). The third phase was the same design as the first with increasing intensity resistance training over eight weeks, but was restricted to men with the metabolic syndrome, ten of whom completed the protocol. All of the data from the subjects who are obese are pooled together because, to our surprise, the muscle changes were the same with bike training as with resistance training. This was not the case with the lean controls in Phase Two, where their changes in muscle fiber content showed a shift toward type I fibers and away from type IIx fibers as might be expected, in contrast to the opposite shift in the subjects who are obese after bike training (10). For comparisons, a total of sixteen sedentary lean controls with no family history of diabetes were evaluated for insulin responsiveness by the same euglycemic insulin clamp protocol. Each subject filled out a detailed Physical Activity Questionnaire prior to beginning the study.

Exercise training programs

Eleven of the thirty subjects who are obese trained on stationary bikes and nineteen performed resistance training. A three-day food diary was reviewed by a nutritionist to determine pre-training caloric intake. Body weights were recorded weekly and subjects counseled to adjust caloric intake for a deviation of greater than three pounds from the initial weight. Each subject was supervised for 45-60 minutes in the exercise laboratory five days per week, following specific protocols of weight lifting, core calisthenics, or stationary bike time and intensity as we have previously described (10;11). Estimated exercise-related energy expenditure began at 250 kcal per training day and peaked at 450 kcal per day in week seven for both protocols.

Subject Assessments

Body composition was measured by air displacement plethysmography (BodPod, Concord, CA). Glucose and insulin measurements were performed in a clinical laboratory from serum obtained after an overnight fast. Maximal oxygen consumption (VO$_2$max) was quantified using a TrueOne 2400 Metabolic Measurement System (ParvoMedics, Sandy, Utah) and a cycle ergometer. Strength was assessed isometrically using a custom-built lifting rack as previously described (11).

Euglycemic Hyperinsulinemic Clamp

After a 2-hour baseline period, a single infusion of regular insulin was performed at 40 $mU/m^2/min$ for 2 hours in order to achieve a physiological increment in insulin concentration of about 50 μU/mL (350 pmol/mL) as previously described (13). Blood glucose was determined every five minutes throughout with a 10% glucose infusion adjusted frequently to maintain the blood glucose at 85±5 mg/dL. The mean glucose infusion during the last 30 minutes of the insulin infusion was calculated as the steady state glucose infusion rate (SSGIR) expressed as mg/kg.min, where the whole body weight was used in the denominator (13).

Muscle Biopsies

Percutaneous needle biopsies of vastus lateralis were performed after an overnight fast and two hours of quiet recumbency using a Bergstrom-Stille 5 mm muscle biopsy needle with suction as previously described (14). The sample was divided in two, with one piece frozen immediately in liquid nitrogen for later analysis. The second piece for microscopy was mounted on cork under a dissecting microscope to orient the specimen for transverse sectioning and quickly frozen in a slurry of isopentane cooled by liquid nitrogen. These specimens were immediately frozen in an isopentane slurry and stored at -80oC until the day of sectioning with a Leica cryostat.

Quantification of Muscle Fiber Type Composition and Fiber Size

Fiber composition was determined using methods described by Behan et al. (15). This was a bright field staining method using labeled fast and slow myosin antibodies from Sigma-Aldrich (A4335) and Fisher (50-174-899, NC9996165, and NC9799788). All sections were coded and then quantified independently by 2 observers who were unaware of which subject the image represented. All fiber size data for the current study were calculated using the minimum diameter measured for each fiber (16). Fiber content is based on counting the number of fibers of each type in images from stained slides. At least 100 fibers were counted for determining percent fiber composition. Fiber diameter was measured in at least 30 fibers of each fiber type for each subject. Areas were the cross-sectional area per fiber of individual fibers of each fiber type, calculated using the measured minimal diameter.

Immunoblots

Immunoblots to assess the content of the insulin receptor beta subunit, IRS-1, GLUT4, phospho-AMPK, PGC-1α, ATP synthase, phospho-AKT, phospho-mTOR, and phopho-p70S6K1 were performed using muscle homogenates are previously described (11). Images were generated using a G-box from BioRad and were quantified using Quantity One software from BioRad. Sample homogenate protein content was quantified and sample loading consistency was confirmed by probing the blot with beta actin antibody.

Statistics

All data are displayed as mean ± standard error of the mean, except as explicitly indicated. Pre-training and post-training comparisons were made using the paired t test. Comparing data between two groups was performed using the independent t test except as noted.

Relationships between select variables were assessed using Pearson Product Moment Correlation. Statistical procedures were performed using SigmaPlot version 12.2 from Systat Software (San Jose, California). A *p* value less than 0.05 was considered significant.

C. Results

Exercise training as an intervention to decrease insulin resistance

Thirty subjects with obesity and insulin resistance volunteered to undergo either eight weeks of resistance training or eight weeks of endurance training and the results of most of these interventions have been previously published (10;11). A euglycemic insulin clamp study was performed on each subject before and at the end of training to quantitatively assess changes in insulin responsiveness. Sixteen sedentary control subjects' baseline SSGIR was 6.19±0.45 mg/kg.min (mean±SEM), whereas the thirty subjects who are obese averaged 2.33±0.20 mg/kg.min in their pre-training euglycemic clamp studies. Controls were seven males and nine females, mean age 38 (range 24-54), who were not obese (mean BMI 24.1), had no family history of diabetes, but had no regular exercise program for at least one year.

The insulin infusion at 40 mU/m^2/minute achieved an increment in serum insulin concentrations of 46±3 μU/mL (322±21 pmol/L) pre-training and 44±4 μU/mL (308±28 pmol/L) after training. In both of these reported studies, weight loss was not permitted. Even though some subjects improved their insulin responsiveness, many did not, resulting in the average response being no change to either resistance or bike training for the group of obese volunteers. A retrospective analysis of the data from these studies gave some interesting insights into the differences between those who improved and those who did not. We divided the subjects into quartiles based on the magnitude of the change in steady state glucose infusion rate (SSGIR) that occurred after training. The quartile data are displayed in Figures 1, 2 and 3 and in Tables 1, 2, and 3. The data were also analyzed by dividing between positive response and a negative response to training (Figures 4 and 5 and Tables 1, 2, and 3). The positive response group contained quartiles 1 and 2, whereas the negative response group consisted of the subjects of quartiles 3 and 4.

The persons who increased their insulin sensitivity the most shown as quartile 1 in Figure 1 started at 29% lower insulin sensitivity (Figure 2) than those who decreased the most (quartile 4), although the differences did not achieve statistical significance. However, the change in insulin responsiveness after exercise training did correlate inversely with the pre-training insulin responsiveness (R=-0.366, p=0.047), suggesting some subjects with lower baseline clamp steady state glucose infusion rates increased more than others.

Characteristics of subjects prior to training

Table 1 contains the characteristics of the obese individuals involved in these studies. There were multiple considerations that might have differentiated the subjects who increased their insulin responsiveness after training compared to those who did not. The data summarized in Table 1 show that there was no difference in age, BMI, body composition, aerobic fitness (VO$_2$max), or strength among the groups, either by quartile or by positive or negative response to training. Reported history of physical activity prior to training was not different.

No planned or structured recreational physical activity was reported by five of fifteen responders and seven of fifteen non-responders. The most common recreational activity was walking that averaged less than 60 minutes per week. For the previous 12 months, none reported engaging in swimming, hiking, aerobics, weight training, biking, jogging, martial arts, basketball, rowing, or horseback riding. Fasting insulin concentration was not different. Fasting glucose was not different among the quartiles, but did show a difference between the fifteen positive and negative responders.

The positive group contained six subjects who trained on stationary bikes and the negative group had five. There were six females in the positive group and four females in the negative group. These subgroups did not differ from the others in the groups.

The training programs were effective at increasing strength and endurance

Twenty of the subjects who are obese reported here were part of two previously published studies of eight weeks of progressive resistance training (11) or stationary bike training (17), and ten more were from currently unpublished resistance training. The subjects who are obese in the first reported study increased lean body mass, maximum power, and VO_2max (11). In the bike training study, subjects increased VO_2max but did not change power or lean body mass. Table 2 shows that there was a small increase in lean body mass in the subjects included in this analysis. There was no change in fat mass of in percent body fat (data not shown). Since Table 3 includes subjects from all three studies, the changes in power and VO_2max were significant when all were averaged together or in the groups with 15 subjects, but not in most of the quartiles with only seven or eight subjects. In all of these studies, every subject responded with increased strength after resistance training and VO_2max increased in each subject after endurance training.

Individual work load and change in insulin responsiveness

The stationary bike training was designed such that the effort involved in the training sessions was similar to that of those who underwent strength training. Post-training BMI was unchanged, 35.4 ± 1.0 kg/m^2 in the positive group and 35.2 ± 1.0 kg/m^2 in the negative response group. Body fat did not change in either group (positive responders pre-training $41\pm5\%$, post-training $42\pm9\%$; negative responders pre-training $40\pm7\%$, post-training $43\pm11\%$). Six of eleven (55%) stationary bike volunteers and eight of nineteen (42%) strength trainees were in the positive group, showing no advantage to either training mode. A conversion of training volume to estimated training energy for the strength trained subjects as described by Reis and coworkers (18) showed higher estimates of training energy for the stationary bike training, but there was no difference in mean total training energy expenditure between positive and negative responders (data not shown). These data suggest that positive responders did not train harder than non-responders.

Muscle fiber composition was different in pre-training biopsies

Figure 3 displays the pre-training muscle fiber composition of vastus lateralis divided by quartiles of change in insulin responsiveness after training. **Panels A** and **B** show transverse sections from one obese subject before and after strength training. These sections show visually an example of the decline in fiber type IIa that occurred in most of the positive

responders. **Panels C, D,** and **E** show the pre-training fiber composition of the subjects contained in each of the quartiles of change in insulin resistance after training. The content of type I and type IIa fibers appeared different in quartiles 3 and 4 compared with quartile 1 when analyzed by independent t tests, but by one-tailed ANOVA, these differences did not achieve statistical significance.

Figure 4 displays the fiber composition of the subjects who are obese prior to and at the end of training with the data divided according to positive or negative response to training. The pre-training muscle biopsies showed that those subjects who would eventually improve their insulin responsiveness after exercise training had 52% more IIa fibers and 27% less type I fibers that did the non-responders.

Panel A of Figure 4 shows the post-training fiber composition changed significantly in the positive group, but changed little in the negative group. What is very striking in these data is that the positive responders started very different from the negative group, but training made the positive group's fiber composition become essentially the same as where the negative group began. The biggest changes in positive responders were a 29% decrease in IIa fibers and a 39% increase in IIx fibers, likely representing a conversion of intermediate fibers to purely fast twitch fibers by either strength training or stationary bike training.

Muscle fiber size changes in response to exercise training of subjects who are obese

Muscle fiber size, shown in Figure 4, **Panel B** as cross-sectional area, appear smaller in positive responders' pre-training muscle biopsies in all three fiber types. The baseline fiber areas tended to be smaller in the positive group muscle biopsies, but only the type IIa fiber differences achieved statistical significance. The post-training biopsies, positive subjects increased the size of all three fiber types, but the non-responders did not alter the mean fiber areas after training. As with the fiber composition data shown in Figure 4 **Panel A,** training made the pre-training differences between the positive responder and negative responder fiber size disappear.

Exercise training-related changes in key components of the pathways of insulin action in muscle

Immunoblots were used to quantify and compare the expression of vastus lateralis muscle insulin receptor, insulin receptor substrate-1 (IRS-1), and GLUT4 expression, before and after exercise training. Figure 5, **Panels A** and **D** display the data from these studies. All of the data are expressed in relation to the baseline data from the sixteen sedentary, lean control subjects (10;11). Pre-training expression data showed no difference in GLUT4 or IRS-1 expression and a 43% higher expression of the insulin receptor in the negative group. Training resulted in modest increases in the insulin receptor and GLUT4 expression in muscle and paradoxically a modest decrease in IRS-1 expression only in the positive responders (Figure 5, **Panel D**).

Exercise training of subjects who are obese and changes in mitochondrial biogenesis

The immunoblot quantification data of the expression of activated AMPK, PGC-1α, and ATP synthase are shown in Figure 5, **Panels B** and **E**. In the pathway of mitochondrial

biogenesis, activation of AMPK drives PGC-1α expression, a key regulator of both nuclear and mitochondria-derived genes involved in mitochondrial expansion, and ATP synthase is a key enzyme component marker of mitochondria. The pre-training expression of phosphorylated AMPK was 57% higher in negative group muscle and most of these three components modestly increased or tended to increase in both positive and negative responders after training.

Changes in muscle protein synthetic pathways after exercise training

Phosphorylation status of AKT, mTOR, and p70S6K1 was quantified with digital image analysis of immunoblots using the specific antibodies listed in *Methods* above. These are three key steps in stimulation of protein synthesis. Phosphorylation of mTOR by activated AKT results in phosphorylation of p70S6K1, a key component of ribosomal translation initiation. **Panel F** of Figure 5 demonstrates that the pre-training expression of these activated proteins was not different between the positive and negative responders. Each of these components had increased expression after training, except p70S6K1 in the negative group where the increase did not reach statistical significance.

D. Discussion

When weight loss is prevented, changes after these activity interventions can be only attributed to the type and duration of exercise training. In the absence of weight loss, neither closely supervised strength training nor closely supervised stationary bike training resulted in consistent reversal of insulin resistance (10;11). The current study found that half of the subjects who are obese did improve their insulin responsiveness as measured in euglycemic insulin clamps. Of thirty non-diabetic, obese volunteers, fifteen responded by increasing their steady state glucose infusion rate (SSGIR) in euglycemic clamp studies, whereas fifteen decreased their SSGIR after training. Retrospective analysis of the anthropometric data and the characterization of muscle fiber content, muscle fiber size, and expression of key components of pathways of insulin action, mitochondrial biogenesis, and regulation of muscle protein synthesis revealed key differences between positive and negative responders in their pre-training characteristics and in their responses to training. Some characteristics of skeletal muscle from vastus lateralis biopsies were very different initially. There were more intermediate muscle fibers (IIa) and fiber cross sectional areas were smaller in the positive group subjects' baseline muscle. These characteristics changed dramatically with eight weeks of exercise training with what appears to be primarily a conversion of IIa fibers to IIx fibers and an increase in the size of type I, type IIa, and IIx fibers in the positive responders. These changes resulted in their muscle fiber composition becoming very much like the characteristics of the negative responder muscle which were unchanged by training.

The lower baseline SSGIR, fewer type I fibers, and lower expression of muscle insulin receptors and activated AMPK in the positive responder group suggested that their pre-training muscle characteristics put them at a modest metabolic disadvantage, compared with the negative responder group.

Statistical comparison of the positive and negative responder groups found no difference in the effort expended during training or in the history of previous recreational activity. There

was no significant difference in the age, gender, baseline BMI, body fat content, strength, aerobic fitness, or type of exercise training. There was no difference in the increase in strength (maximum power, rate of force development) or the increase in aerobic fitness (VO_2max) after training (data not shown). The increment in insulin concentration achieved during the euglycemic clamp studies was not different in either group before or after training.

Are there technical problems that could contribute to the apparent changes in fiber composition? The location of the needle biopsy along the vastus lateralis was consistently at the mid-point, but was done on the side contralateral to the baseline biopsy. Studies we performed on six cadavers (unpublished) found no significant difference in the fiber composition of the middle portion of vastus lateralis from the right side compared to the left side. Missing the vastus lateralis or going through it to the vastus intermedius is possible, but unlikely, and certainly did not happen systematically in these studies. Elder and coworkers reported extensive and laborious evaluation of fiber composition within the vastus lateralis from autopsies of four young adult, normal weight males (19). They found superficial sites had slightly more fast twitch fibers than deep sites in three of the four samples and there was some variability along the body of the muscle, but did not find any systematic differences based on depth or location for the vastus lateralis (19). The fast twitch content of the vastus lateralis was more consistent in different locations than they found for the soleus, biceps, or triceps from their subjects. Based on their statistics, they recommended averaging at least three sites to determine an accurate and reproducible fiber composition in the vastus lateralis. In studies like ours, multiple biopsies pre- and post- interventions would be challenging for the participants. We believe that small intrinsic variability of fiber composition within a muscle could not account for the differences that we observed. Naturally occurring differences along the body of the vastus lateralis would make statistical significance more challenging and increase the group size necessary to show real differences between groups or before and after an intervention.

The fiber composition was performed using light microscopy of slides stained sequentially with reagents that used commercially-available monoclonal antibodies. This method, described by Behan and coworkers (15), is relatively simple and reproducible, compared with the more challenging adjusted pH ATPase assays (16).

Previous reports of Type 2 diabetes patients showed improved glycemic control in exercise programs with weight loss (5;20;21). Combined aerobic and strength training was more effective than either aerobic or strength alone, but often the combined program involved more time exercising and accomplished more weight loss. Exercise interventions to prevent diabetes in pre-diabetic metabolic syndrome subjects or frailty in older subjects have consistently shown combined aerobic and strength training to have the largest improvement in insulin responsiveness (3;4;22-25). In most of these studies, improvement in insulin responsiveness paralleled the total amount of time devoted to exercise and the amount of weight loss. Weight loss and caloric restriction were critically important in the studies reported by Ross and coworkers (3) and by Villareal and coworkers (24). Ross found weight loss by diet only or by exercise improved insulin responsiveness. Exercise without weight loss did not significantly improve insulin responsiveness quantified in euglycemic clamps in

their report. In the study reported by Villareal, the insulin sensitivity index improved substantially in the diet only and the diet plus exercise groups but did not change in the control or the exercise without weight loss groups (24;25). Stensvold and coworkers did not allow weight loss in 33 metabolic syndrome volunteers assigned to 12 weeks of aerobic, strength, or combined exercise training (26). None of their interventions improved insulin responsiveness as quantified by HOMA (26). In summary, these previous studies found that exercise with weight loss improved insulin responsiveness, but for most subjects, weight loss was essential for the exercise training program to be effective at increasing insulin responsiveness, and thereby decreasing the diabetes risk. Our previously reported studies of aerobic only (10) or resistance only (11) training were designed to test the type of exercise as an intervention and avoided weight loss since weight loss alone without exercise training caused improved insulin responsiveness in most prior studies.

Elite runners typically have a much higher proportion of slow-twitch (type I) muscle fibers (27;28) and competitive weightlifters have a predominance of fast-twitch (type II) fibers (29), in contrast to the usual distribution of about 50% type I and 50% type II seen in normal healthy subjects. Type 2 diabetes and pre-diabetic metabolic syndrome subjects have higher type II fiber content (10;11;30), similar to weightlifters. Muscle fiber composition may be largely determined before birth (31;32), and the ability to modify the percent made up by slow twitch (type I) or fast twitch (type II) is limited. In contrast to our results, resistance training resulted in apparent shift from IIx to IIa in two previous reports (33;34). Andersen and Aagaard found a modest increase in IIa fibers and a corresponding decrease in IIx fibers in young men after three months of heavy load resistance training (33). Williamson and coworkers studied untrained older men before and after 3 months of progressive resistance training and found an increase in IIa fibers and a decrease in mixed IIa/IIx fibers (34). Our subjects appeared to shift intermediate fibers (IIa) to purely IIx. Our method relies on specific antibodies to slow-twitch and fast-twitch myosin heavy chains in contrast to the methods used by Andersen (33) and Williamson (34). Our results contrasting with Andersen's may be in part from differing histologic techniques, but might be more related to the differences between the subjects in these studies. Our subjects were all sedentary, obese, pre-diabetic men and women, in contrast to Andersen's lean young men (34) and Williamson's non-obese elderly men (33). Prolonged endurance training has also shown a decrease in IIx and an increase in number and size of type IIa fibers in older men and women (35). Our recent study of endurance training on stationary bikes found that non-obese, healthy control subjects decreased IIx and increased IIa fiber proportions suggesting a conversion of IIx to IIa fiber type (10). However, the subjects who are obese in this study appeared to alter their muscle fiber composition in the opposite direction, decreasing type I fibers and increasing IIx fibers after training (10).

In contrast to lean subjects, the mixed slow- and fast-twitch (type IIa) fibers appear to be the pool most likely to be modified in many subjects who are obese. The most common change in these subjects who increased their insulin responsiveness was conversion to fast-twitch, regardless of the type of exercise training used. In positive responders, fiber size started smaller but increased after training to become essentially the same as negative responders. The negative responder group subjects, however, did not change fiber composition or fiber size after exercise training.

Exercise-related changes in key muscle biochemical pathways (insulin action, mitochondrial biogenesis, and regulation of protein synthesis) are similar in both positive and negative responders, even though the positive group initially had lower expression of muscle insulin receptors and phosphorylated AMPK.

The increase in muscle fiber size that occurred in the positive responders may be the principle mechanism for the change in whole body insulin responsiveness. The muscle hypertrophy that occurred was calculated using the change in individual fiber type areas and the change in fiber proportions. The positive responders were estimated to increase muscle size by 18% whereas the negative responders decreased the average muscle size by 2%. The positive group averaged an increase in lean body mass of 1.1 kg, whereas the negative group averaged no change. These data support an explanation that hypertrophy of at least one fiber type and likely the increased size of all fiber types may account for a portion of the increased insulin responsiveness in these subjects who are obese after exercise training.

Those subjects who are obese who improved their insulin responsiveness after exercise training had a much higher content of the intermediate fibers (type IIa) in their pre-training muscle biopsies. These observations suggest that this pool may be the primary source of the changes that occur when exercise training-driven muscle remodeling occurs in these subjects. These data further suggest that exercise training without weight loss in obese, pre-diabetic subjects may come up against a "glass ceiling" in insulin responsiveness. It may be that those who did not improve their insulin responsiveness were already near the highest level they could achieve without weight loss. Because the positive group started somewhat lower in insulin sensitivity, training only enabled them to catch up to but not surpass the "non-responders".

The good news is that many people with the metabolic syndrome will benefit from exercise training alone. The bad news is that the exercise-induced muscle remodeling causes a limited increment in insulin responsiveness in the absence of weight loss.

The data generated in this study demonstrated that many sedentary, obese pre-diabetic subjects will reduce their insulin resistance after organized exercise training, but their response is largely dependent on the pre-training characteristics of their muscle. However, the clear inference from this report and those that preceded it, is that weight loss during an exercise training intervention is critically important to achieve a reduction in diabetes risk.

E. Practical Applications

These studies show that exercise as an intervention to decrease risk of diabetes and other cardiometabolic issues is not uniformly successful unless weight loss is also a goal of the program. Our data further suggest that when many sedentary obese participants perform traditional aerobic training, initially they need to build muscle, paradoxically causing a shift toward type IIx fibers and fiber hypertrophy, similar to what might be seen if this training were predominantly resistance.

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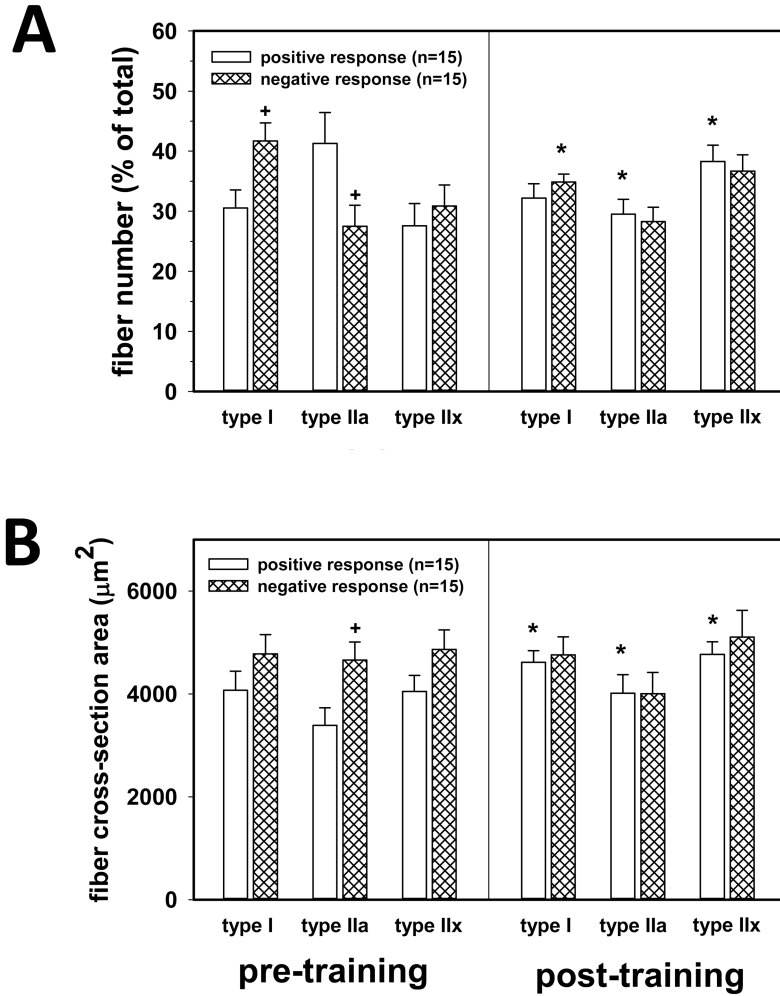
Figure 1. Change in insulin responsiveness of metabolic syndrome subjects after eight weeks of exercise training

Thirty subjects who met the criteria for metabolic syndrome underwent eight weeks of progressively increased intensity supervised exercise training, either predominantly resistance (twenty subjects) or predominantly aerobic bike training (ten subjects). Subject weights were monitored throughout the study and each participant was provided dietary counseling to prevent loss or gain in weight. The data shown here are the individual changes in steady state glucose infusion rates (SSGIR) between pre- and post-training euglycemic clamp studies with insulin infusions at 40 mU/m^2 per minute. The subjects were divided into quartiles according to the change in insulin sensitivity. The quartiles contained 8, 7, 7, and 8 subjects, the data for each were used for statistical comparisons. Quartiles 1 and 2 contained subjects whose second clamp SSGIR was equal to or greater than the first, and quartiles 3 and 4 contained all of the subjects whose SSGIR was decreased in the second clamp. One-tailed ANOVA did not find significant differences among the quartiles, but independent t tests showed p values less than 0.01 for quartile 2, 3, and 4 compared to quartile 1 subjects.
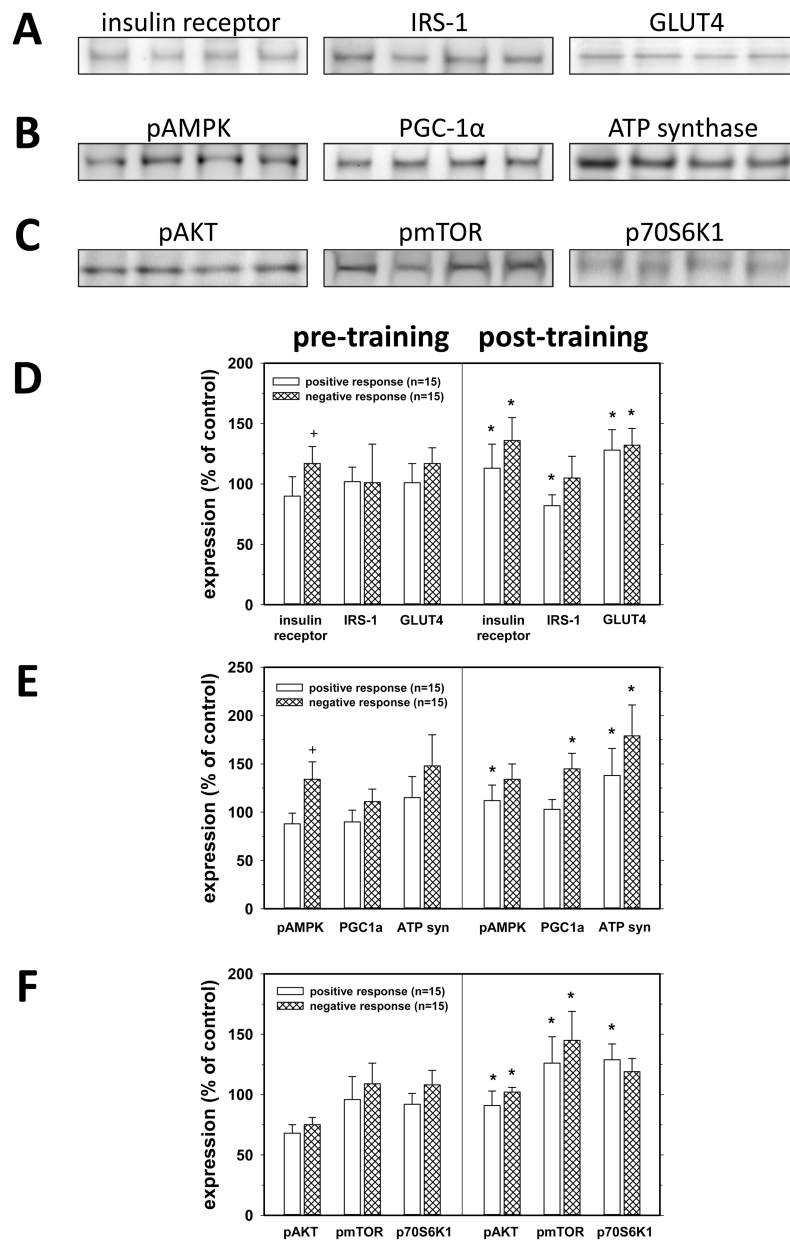
Figure 2. Pre-training insulin sensitivity of subjects comparing quartiles of change after training

The bars shown here show a trend toward the positive responders, quartiles 1 and 2, being more insulin resistant before training, but one-tailed ANOVA did not demonstrate significance. Comparing quartile 1 to quartile 4 by t test found p=0.09, with the mean of quartile 1 being 30% less than that of quartile 4.
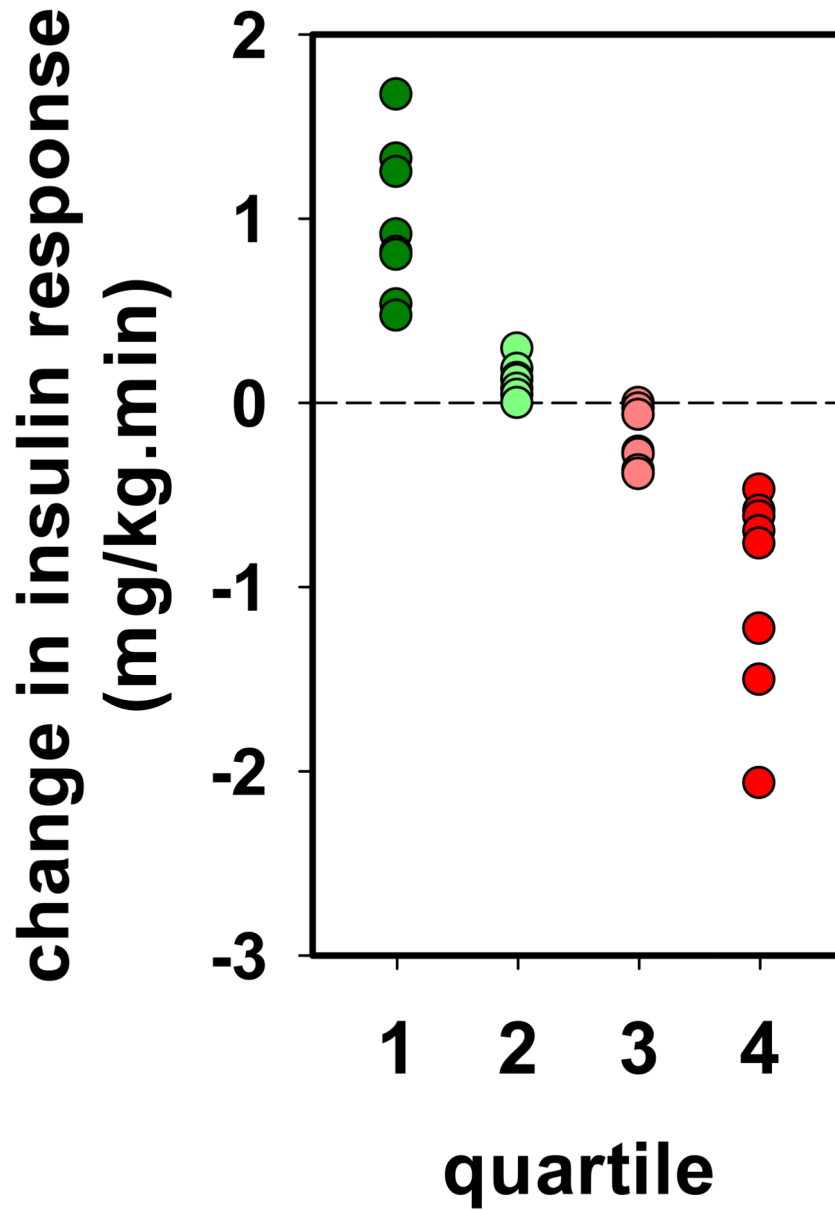
Figure 3. Fiber composition of pre-training muscle organized by quartiles of change in insulin sensitivity after training

Shown here are the percent content of fiber type I, type IIa, and type IIx, representing slow-twitch, mixed fast- and slow-twitch, and fast-twitch fibers determined by double staining with antibodies directed to slow-twitch myosin heavy chain and fast-twitch myosin heavy chain (15). **Panels A** and **B** show sample pre- and post- training muscle section images. Dark blue staining fibers were type I, red fibers were type IIx, and the purple staining fibers were type IIa. Examples of type I, type IIa, and type IIx are labeled in **Panels A** and **B**. These were from a 39 year old male (subject 29) who shifted from 39% IIa to 22% IIa fibers post-training. **Panels C, D,** and **E** show the fiber composition data for the quartiles of subjects determined by the post-training change in insulin sensitivity. Asterisks indicate quartiles different from quartile 1 by t test with p value less than 0.05.
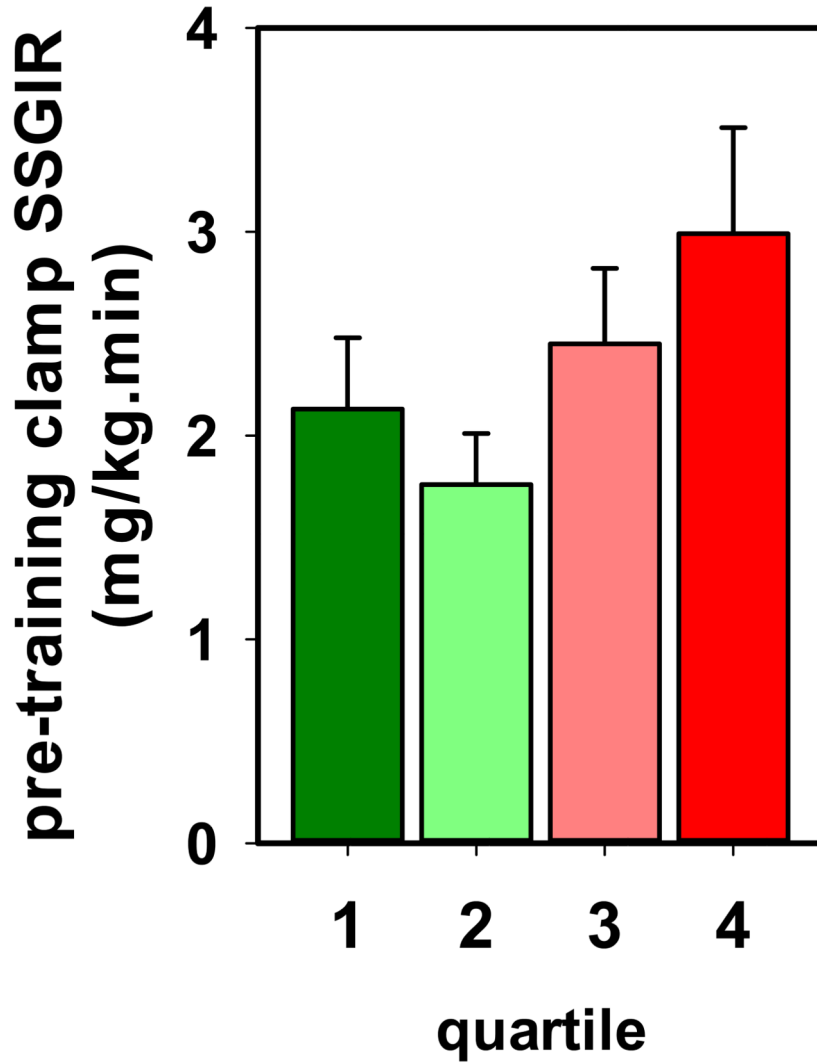
Figure 4. Fiber type composition and fiber size of vastus lateralis pre- and post-training in positive and negative responders

Pre-training, positive responders had fewer type I fibers and more type IIa fibers, but following training the fiber composition in both groups was the same (**Panel A**). This suggests that the positive responders converted type IIa fibers to type IIx fibers, whereas the negative responders decreased the proportion of type I fibers. **Panel B** represents the cross sectional areas determined in transverse cut sections using methods described by Dubowitz (16). The positive responder fibers were all smaller than the negative responders in the pre-training vastus lateralis biopsies, and post-training increased to the same size as the negative responders. The negative responder fiber size did not change after training. The plus sign indicates significant difference from positive responders in the pre-training biopsy ($p < 0.05$) and the asterisk indicates a significant change from the pre-training composition ($p < 0.05$).
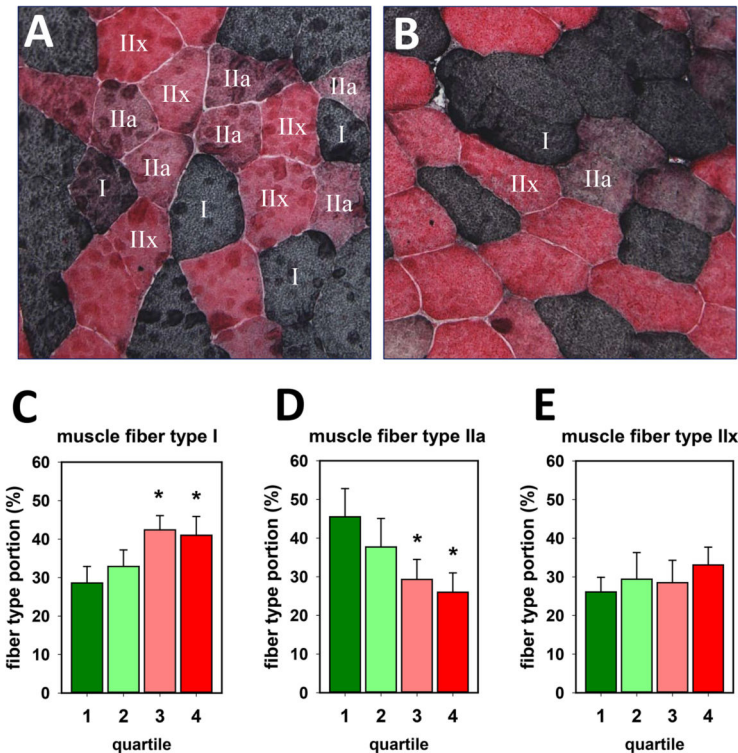
Figure 5. Muscle insulin pathway, mitochondrial biogenesis pathway, and protein synthetic pathway in response to exercise training in metabolic syndrome subjects

Immunoblots of muscle homogenate were quantified using digital image analysis, samples of each are displayed in **Panels A, B,** and **C**. These sample images contain alternating control and metabolic syndrome subjects. Each subject had 2 to 4 separate blots performed and averaged. Each series of blots was also probed with beta actin antibodies to confirm protein loading equivalence. Panel D displays results for key elements of the insulin pathway, **Panel E** shows key components of the mitochondrial biogenesis pathway, and **Panel F** shows components of the muscle hypertrophy pathway. All data was expressed in terms of the mean of the pre-training control subject values (n=16). These control subjects were sedentary volunteers who were not obese and had no family history of diabetes. They were the sedentary controls described in previous reports of resistance training (11) and stationary bike training (10) of metabolic syndrome subjects. In the pre-training biopsies, the insulin receptor expression was higher in non-responders. Post-training, insulin receptor expression increased in both responders and non-responders (**Panel D**). IRS-1 expression was lower in responders and GLUT4 expression was significantly higher in the non-responder subjects. The plus sign indicates significant difference from responders in the pre-training biopsy (p<0.05) and the asterisk indicates a significant change from the pre-training composition (p<0.05).

Panel E displays the mean and SEM for the expression in immunoblots of activated AMPK, PGC-1α, and ATP synthase (mitochondrial marker enzyme). In the pre-training biopsies, the activated AMPK (phospho-AMPK) was at higher levels in the non-responders. Post-training, phopho-AMPK was significantly increased in the responders, and expression of PGC-1α and ATP synthase were increased in the non-responders. The plus sign indicates significant

difference from responders in the pre-training biopsy (p<0.05) and the asterisk indicates a significant change from the pre-training composition (p<0.05).

Panel F displays the expression of activated AKT, mTOR, and p70S6K1 in pre-training and post-training biopsies of vastus lateralis in responders and non-responders as defined by improved insulin responsiveness quantified by euglycemic clamp studies. Activated AKT is an upstream regulator of mTOR activation and p70S6K1 is activated by phospho-mTOR and directly stimulates ribosomal protein translation. None of these parameters showed any difference between responders or non-responders before or after training. The asterisk in the right panel indicates a significant change from the pre-training composition (p<0.05).

Table 1

Subject Characteristics[*]

subjects	gender	age	BMI	body fat	prior activity	fasting glucose	fasting insulin
		years	kg/m²	percent	min/wk	mmol/L	pmol/L
all (n=30)	10F/20M	43±2	35.1±0.6	40.5±1.2	52±15	5.71±0.12	97±10

Quartiles

	age	BMI	body fat	prior activity	fasting glucose	fasting insulin
	years	kg/m²	percent	min/wk	mmol/L	pmol/L
quartile 1 (n=8)	39±5	34.4±0.4	40.8±2.3	35±26	5.58±0.17	74±13
quartile 2 (n=7)	43±3	36.0±1.6	39.1±2.6	65±30	5.39±0.14	115±14
quartile 3 (n=7)	46±3	36.5±1.5	41.4±2.4	51±36	5.94±0.22	79±23
quartile 4 (n=8)	44±3	33.6±1.1	40.7±1.2	57±31	5.91±0.32	128±25

Response

	age	BMI	body fat	prior activity	fasting glucose	fasting insulin
	years	kg/m²	percent	min/wk	mmol/L	pmol/L
positive (n=15)	41±3	35.2±0.9	40.0±1.7	49±20	5.49±0.11	91±11
negative (n=15)	45±2	35.0±0.9	41.0±1.2	54±23	5.93±0.19	107±18

[*] data is summarized as mean±standard error, prior activity is calculated from self-reported physical activity time in walking, jogging, and other exercise on standard questionnaires for the year prior to training.

Table 2

Training-related Changes in Body Composition[*]

subjects	LBM kg			fat mass kg		
	pre-	post-	p value	pre-	post-	p value
all (n=30)	62.2±2.0	62.8±2.1	0.005	42.7±1.7	43.2±1.8	0.622
Quartiles						
quartile 1 (n=8)	59.8±3.8	61.0±4.1	0.144	41.6±3.3	40.9±3.2	0.468
quartile 2 (n=7)	68.8±5.0	69.7±5.3	0.120	44.9±4.7	45.3±5.0	0.381
quartile 3 (n=7)	62.2±4.3	61.7±4.7	0.297	44.2±4.1	46.5±4.5	0.688
quartile 4 (n=8)	58.8±2.1	59.4±2.4	0.360	40.6±2.1	40.9±2.4	0.596
Response						
positive (n=15)	64.0±3.2	65.1±3.4	0.033	43.1±2.7	43.0±2.8	0.729
negative (n=15)	60.4±2.3	60.4±2.5	0.083	42.3±2.2	43.5±2.5	0.599

[*]
p value was estimated by paired t test

Table 3

Training-related Changes in VO$_2$max and Maximum Power*

subjects	VO$_2$max ml/kg.min			Power N/kg$^{2/3}$		
	pre-	post-	p value	pre-	post-	p value
all (n=30)	23.1±0.7	25.0±0.6	<0.001	139±5	153±6	<0.001
Quartiles						
	pre-	post-	p value	pre-	post-	p value
quartile 1 (n=8)	22.9±1.7	24.9±1.4	0.157	137±12	150±10	0.039
quartile 2 (n=7)	22.8±1.5	25.4±1.4	0.007	139±16	156±16	0.056
quartile 3 (n=7)	22.5±1.4	24.7±1.3	0.060	131±5	152±9	0.014
quartile 4 (n=8)	23.8±1.2	25.3±1.3	0.073	147±9	156±13	0.153
Response						
	pre-	post-	p value	pre-	post-	p value
positive (n=15)	22.9±1.1	25.1±1.0	0.006	138±9	153±9	0.002
negative (n=15)	23.2±0.9	25.0±0.9	0.006	140±6	154±8	0.004

*
power is static peak power in Newtons per allometrically scaled weight, p values were estimated using paired t tests