Aerobic Exercise Training Increases Muscle Water Content in Obese Middle-Age Men

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ABSTRACT

**Purpose:** To determine if muscle water content ($H_2O_{\text{muscle}}$) expands with training in deconditioned middle-age men and the effects of this expansion in other muscle metabolites.

**Methods:** Eighteen obese (BMI = 33±3 kg·m$^{-2}$) untrained ($VO_{2\text{peak}} = 29±7$ mL·kg$^{-1}$·min$^{-1}$) metabolic syndrome men completed a 4-month aerobic cycling training program. *Vastus lateralis* muscle biopsies were collected prior and 72 hours after the completion of the last training bout. Water content, total protein, glycogen concentration and citrate synthase activity were measured in biopsy tissue. Body composition was assessed using DXA and cardio-metabolic fitness was measured during an incremental cycling test. **Results:** Body weight and fat mass were reduced -1.9% and -5.4%, respectively ($P<0.05$) while leg fat free mass increased with training (1.8%; $P=0.023$). Cardiorespiratory fitness (i.e., $VO_{2\text{peak}}$) exercise maximal fat oxidation (i.e., $FO_{\text{MAX}}$) and maximal cycling power (i.e., $W_{\text{MAX}}$) improved with training (11%, 33% and 10%, respectively; $P<0.05$). After 4-months of training $H_2O_{\text{muscle}}$ increased from 783±18 to 799±24 g · kg$^{-1}$ wet weight (2%; $P=0.011$) while muscle protein concentration decreased 11% (145±15 to 129±13 g · kg$^{-1}$ ww $P=0.007$). Citrate synthase activity (proxy for mitochondrial density) increased 31% (17±5 to 22±5 mmol · min$^{-1}$ · kg$^{-1}$ ww; $P=0.024$). Muscle glycogen concentration increased 14% (22±7 to 25±7 g · kg$^{-1}$ ww) although without reaching statistical significance when expressed per kg of wet weight ($P=0.15$). **Conclusion:** Our findings suggest that aerobic cycling training increases quadriceps muscle water while reducing muscle protein concentration in obese metabolic syndrome men. Reduced protein concentration co-exists with increase leg lean mass suggestive of a water dilution effect that however does impair increased cycling leg power with training. **Keywords.** Exercise training; muscle water content; muscle hypertrophy; aging
INTRODUCTION

More than 50 y ago, Pace and Rathbun first proposed that total body water (TBW) is a constant fraction of fat free mass (FFM) based on experiments in guinea pigs (25). This constant value (i.e., TBW:FFM = 0.73) latter confirmed in humans cadavers (37) allows the calculation of body composition when using dual X ray absorptiometry and hydro-densitometry (28). It is also the foundation for calculating body fat from measures of total body water by dilution methods (Fat = body mass – TBW/0.73; (36)). The use of this constant implies a fixed FFM to water content ratio (i.e., FFM hydration). More than half of FFM is skeletal muscle (31) and thus this constant also assumes fixed skeletal muscle hydration. In fact, muscle water content reported in resting healthy-young subjects coincides across studies (i.e., ~78±3%; (6-9, 13, 14, 20, 22, 29, 30)).

The water to FFM constant originates from experiments where animals or human cadavers were thoroughly homogenized, fat extracted, and water content determined by weighing homogenate aliquots before and after drying by evaporation or sublimation (i.e., freeze-drying). Similar processing of a muscle biopsy sample allows the measurement of muscle water content in vivo. Using this technique it has been shown that congestive heart failure patients increase leg muscle water content in comparison to age matched healthy controls (2) likely due to reduced venous return provoking edema. In healthy men, short-intense exercise increase muscle water content (30) likely due to increased transcapillary pressure. Conversely, submaximal prolonged exercise in a hot environment results in whole body dehydration and losses of muscle water content (6). We have recently shown that whole body dehydration by 4.2%, reduces muscle water content when 1 h of recovery is allowed to re-equilibrate among body fluids spaces (20).
While it is clear that muscle water content can transitorily change in response to a bout of exercise the effects of chronic exercise training on muscle water content are not well-defined. To our knowledge, only four studies in humans report the effects of training on muscle water changes. All of them report water content as a secondary finding to the study of muscle hypertrophy. One study uses NMR to estimate leg water changes after leg extension training in young subjects finding no effects (17). Three of them use direct measurement of water content in leg muscle biopsies (13, 14, 29). One is a cross-sectional comparison between competitive and recreational runners and thus subjected to the effects of different diets, muscle fiber composition, genetics and hormonal milieu between groups (29). The two remaining studies follow a repeated measures design in the same group of subjects before and after 12 weeks of cycle-ergometer training. However, while the first study conducted in old women finds increases in muscle water content with training (10) the subsequent study conducted in old and young men does not (11).

Despite the utmost relevance of water for muscle energetics during and after exercise (9, 10) the effects of chronic training on muscle water content remain unclear. A few aerobic training exercise sessions expand intravascular water (i.e., plasma volume expansion) allowing better cardiac function, cutaneous blood flow and sweat gland fluid supply (4). It is however unclear if water within muscles is also expanded with aerobic training to improve muscle energetics and/or contractile function. To study muscle water changes in a sedentary population initially seems pertinent to unveil if muscle conditioning includes muscle water expansion and how does it relates to other known muscle training adaptations (e.g., increase in mitochondrial density or glycogen content). We hypothesized that a health-oriented fitness-training program using aerobic interval exercise will increase muscle water content in initially sedentary,
metabolic syndrome men. We will relate the changes in muscle water content to muscle variables known to change with training (mitochondrial and glycogen content) in an attempt to establish possible links.

METHODS

Participants. Eighteen obese men between 34 and 64 years old (mean 54±8 yr old) completed the study. Participants were enrolled based on fulfilling ≥3 MetS criteria as per harmonized definition (1) using population Europid waist circumference cutpoints. Exclusion criteria included cardiovascular or renal disease, peripheral vascular disease and any disease associated with exercise intolerance. Body weight stability in the last six months (i.e., changes below 1% of initial body weight) was also a requirement. Participants reported not to be engaged in regular physical activity beyond walking less than 30 min per day in the past six months. The local Hospital’s Research Ethics Committee approved the study procedures and the informed consent documents. Subjects were informed of the purpose and risks involved in the study before signing the written consent. The study fulfilled the latest version of the Declaration of Helsinki.

Exercise training and dietary records. Subjects underwent supervised aerobic interval training (AIT) with a frequency of 3 times per week during 4 months. Training consisted on pedaling for 10-min as warm up at 70% HR$_{\text{max}}$ followed by 4 x 4-min intervals at 90% of HR$_{\text{max}}$ interspersed with 3-min active recovery at 70% HR$_{\text{max}}$ and a 5-min cool-down period for a total of 43 min. Exercise intensity was increased as training adaptations developed to maintain the target heart rate (Accurex coded, Polar, Finland). Participants were required to attend at least
85% of all the exercise sessions. Subjects were instructed to maintain their dietary patterns during the duration of the study. A three day dietary log was collected monthly and analyzed for caloric intake and macronutrient composition.

**Cardio-respiratory and metabolic fitness assessment.** Before and after 4 months of training we tested all subjects for weight, waist circumference, exercise maximal fat oxidation (FO\textsubscript{MAX}) peak oxygen consumption (VO\textsubscript{2peak}) and body composition. All tests were scheduled prior and at least 72 h after the last exercise training session to avoid measuring the acute effects of the last exercise bout rather than the chronic effects of the exercise-training program. In testing days, subjects arrived to the laboratory after an overnight fast. Upon arrival, subjects voided and their body weight was assessed (Hawk, Mettler Toledo, USA). Urine was analyzed for specific gravity (Usg, Uricon-NE, Atago, Japan) to ensure that subjects were euhydrated (Usg less than 1.015). Subjects rested in a stretcher during 20 min while resting ECG was examined (Cosmed T12, Italy). Then, exercise testing started in an electromagnetically-braked cycle ergometer (Cardiotest 100, Seca, Germany) with 3-8 submaximal 4 min stages to assess maximal fat oxidation (FO\textsubscript{max}). During FO\textsubscript{max} test oxygen consumption and carbon dioxide production was analyzed in a breath-by-breath mode (Quark b2, Cosmed, Italy). The test was discontinued when the respiratory exchange ratio exceeded 1. The last minute of each stage was averaged to calculate non-protein respiratory quotient and fat oxidation rate (11). Following, subjects recovered during 40 min while 250 mL of juice were ingested (125 kcals). Then, a graded exercise test (GXT) was conducted to volitional fatigue to determine subject’s peak aerobic power (VO\textsubscript{2peak}). After a 5-min warm-up at 100 W, participants began cycling at 125 W with increments of 25 W each minute. Gas exchange data were collected using an automated breath-
by-breath system and averaged every 15 s. A physician visually inspected the ECG tracing during the GXT.

**Body composition and biopsy tissue collection.** Before the above described test subjects arrived after an overnight fast prior and after training for body composition assessment and muscle biopsy collection. Percent body fat and right leg lean soft tissue mass were determined by dual energy X-ray absorptiometry (DXA Hologic Serie Discovery Wi QDR, Bedford, USA). Muscle biopsies were obtained before and at least 3 days after training from the vastus lateralis using the suction-modified Bergstrom technique (33). Skin was prepared with Povidone-iodine (Betadine, MEDA, France), followed by injection of 2% lidocaine without epinephrine (Braun 2%, Braun Medical, Spain). Then, the skin and underlying tissues were surgically opened (scalpel blade number 10, Braun, Germany) and muscle tissue obtained using a 4 mm internal diameter Bergstrom biopsy needle. Upon collection, muscle samples were immediately cleaned of connective tissue, divided into two pieces and rapidly frozen in liquid nitrogen for subsequent analysis of water and metabolite contents. The incision was closed using adhesive strips (Steri-StripTM, 3M, USA) covered with an adhesive dressing pad (TegadermTM+Pad, 3M, USA) and compressive dressing (IcoVenda, Novico medica, Spain).

**Muscle water content measurement.** All the samples from a given subject were analyzed in the same assay batch. Frozen samples were weighed on an electronic balance with a sensitivity of 0.1 µg (XB220A, Precisa, Switzerland). Elapsed time from sample removal from the freezer until weighing was recorded to permit correction for tissue water evaporation. Samples were freeze dried in a thermoelectric freeze-dryer (Cryodos-50, Telstar, Spain) for 6 h at -50°C and at a vacuum of 10^{-2} Torr. In brief, this apparatus freezes the liquid in the sample to -50°C to then sublimes it with a potent vacuum pump at a high flux rate (83 L · min^{-1}).
Samples were then re-weighed in the same precision scale to measure water content. Pilot data in our laboratory in fresh pig leg muscle indicated that $\text{H}_2\text{O}_{\text{muscle}}$ measurement was highly reproducible (i.e. 6% CV). Data in one subject that underwent 10 resting biopsies within 8 weeks in a euhydrated state ($\text{Usg} < 1.020$ and body weight $\pm 0.25$ kg) confirmed the high reproducibility of this technique in our laboratory in human muscle (i.e., 5% CV).

**Muscle metabolites analysis.** Glycogen concentration was determined from the measurement of glucose after acid hydrolysis analysis (26). Briefly, muscle samples (~20 mg) were homogenized using a glass-on-glass system on ice with deionized water. Then, samples were hydrolyzed in 2N hydrochloric acid and heated for 2 hours at 100°C (Tembloc, JP Selecta, Spain). Finally, samples were neutralized to pH 6.5-7.5 with 1N sodium hydroxide and glucose concentration was analyzed by colorimetric assay (Enzymatic Glucose Reagent, Thermo Scientific, Waltham, Massachusetts, USA). Muscle protein was assayed after tissue homogenization using modified Lowry technique with bicinchoninic acid (16). Citrate synthase activity was measured from a ~10 mg portion of muscle through the reduction of 5,5'-dithiobis(2-nitrobenzoic acid) by the release of CoA-SH in the cleaving of acetyl-CoA (32).

**Statistical analysis.** Data are presented as mean ± SD. Sample size was calculated based on muscle water increases in three pilot subject undergoing a similar training program. Power test revealed that at least 10 subjects were needed to reach significance for a statistical power at 80% ($\alpha=0.05$; (4)). Normally distributed data were analyzed using Student’s two tailed paired $t$ test (pre-to-post training comparison). Pearson product-moment correlation coefficient was used to establish linear correlations (dependence) between the changes with training in muscle variables and muscle water. Level of significance was set at $P < 0.05$. Cohen’s formula for effect size (ES; (4)) was used, and the results were based on the following criteria; >0.70
large effect; 0.30–0.69 moderate effect; ≤0.30 small effect. 95% confidence intervals are also presented. Data analysis was performed using SPSS software for windows (v.18, IBM., USA).

RESULTS

The group was quite homogenous regarding, body weight, BMI and waist circumference, (Table 1, pre-training column). Although their initial cardiorespiratory fitness (VO_2peak; Table 1) had a coefficient of variation of 24% they all were largely untrained according with the normative values for their age and gender (38).

**Cardio-metabolic fitness and exercise capacity.** VO_2peak increased significantly after 4 months of AIT by 3 mL·kg\(^{-1}\)·min\(^{-1}\) (95% CI=5 to 2 mL·kg\(^{-1}\)·min\(^{-1}\); ES=0.44; \(P=0.001\)). In turn, FOMax increased by 0.08 g·min\(^{-1}\) (i.e., 33%) after 4 months of training (95% CI=0.12 to 0.04 g·min\(^{-1}\); ES=0.78; \(P=0.003\)). During the GXT, cycling power output increased by 23 W (i.e., 10% from 232±67 to 255±75 W; Table 1) at the end of the training program (95% CI=32 to 14 W; ES=0.32; \(P=0.001\)).

**Anthropometry and body composition.** Although subjects did not undergo a hypocaloric diet, body weight was reduced by 1.85 kg (1.9%; from 95.2±9.7 to 93.4±9.6 kg) after 4 months of training (95% CI=-0.4 to -3.3 kg; ES=0.19; \(P=0.022\)). Furthermore, macronutrient distribution in diet remained constant throughout the 4 months of the study with 41±2% of energy intake from carbohydrates, 38±1% of fat (40% saturated) and 21±1% of protein. Because of the body weight losses, BMI was also reduced by a similar magnitude (from 33.4 to 32.7 kg·m\(^{-2}\); 95% CI=-0.2 to -1.3 kg·m\(^{-2}\); ES=0.30; \(P=0.013\)). Fat mass measured by
DXA was also significantly reduced with training (-1.7 kg; 95% CI=-0.17 to -3.28 kg; ES=0.37; \( P=0.044 \)) while whole body fat free mass was not significantly changed. However, sectional DXA analysis of the right leg revealed a significant increase of 1.8% in fat free mass (0.2 kg; 95% CI=0.31 to 0.05 kg; ES=0.10; \( P=0.023 \); Table 1).

**Muscle water content.** Prior to exercise *vastus lateralis* contained 363±36 mL of water per each 100 g dry weight muscle. After 4 months of exercise muscle water content (i.e., \( H_2O_{\text{muscle}} \)) increased by 12% to 404±69 mL · 100 g dw muscle\(^{-1} \) (\( P=0.019 \)). When the changes in water were expressed per wet weight (i.e., physiological muscle condition) the increases in \( H_2O_{\text{muscle}} \) were of 16 g·kg\(^{-1} \) increasing from 783±18 g·kg ww to 799±24 g·kg\(^{-1} \) ww (95% CI=27 to 5 kg; ES=0.76; \( P=0.011 \); Figure 1).

**Muscle metabolites.** Glycogen concentration increased 19% as a result of training (546±146 to 650±158 mmol·kg\(^{-1} \) dry weight) when expressed per dry weight (95% CI=180 to 27 mmol·kg\(^{-1} \) dw; ES=0.68; \( P=0.017 \)). However, when glycogen concentrations were expressed per wet weight the increases were of 14%, and did not reach statistical significance (from 22±7 to 25±7 g·kg\(^{-1} \) ww; 95% CI=7 to -1 g·kg\(^{-1} \) ww; ES=0.43; \( P=0.154 \); Figure 2). Total protein concentration was significantly reduced after the 4 months of training by 11% from 145±15 to 129±13 g·kg\(^{-1} \) ww (95% CI=-6 to -26 g·kg\(^{-1} \) ww; ES=1.17; \( P=0.007 \); Figure 2). Citrate synthase activity increased by 31% from 17±5 to 22±5 mmol·min\(^{-1} \)·kg\(^{-1} \) ww (95% CI=8 to 3 mmol·min\(^{-1} \)·kg\(^{-1} \) ww; ES=1.08; \( P=0.001 \); Figure 2).

**Metabolic syndrome components.** After 16 weeks of training, three out of the five components of metabolic syndrome significantly improved. Waist circumference (index of abdominal obesity) was reduced by 2% (i.e., 2 cm), fasting blood glucose by 7% (0.5 mmol·L\(^{-1} \))
and mean arterial pressure by 7% (8 mmHg). However, blood triglyceride and high-density lipoprotein cholesterol concentrations did not improve with training.

**Correlations of muscle tissue analysis.** Pearson coefficient correlation analyses were performed in selected variables using wet weight as physiological muscle conditions for expressing concentrations (glycogen and protein) content (water) and enzyme activity (citrate synthase; CS activity). The increase in \( \text{H}_2\text{O}_{\text{muscle}} \) was associated with reductions in protein, muscle glycogen concentration and citrate synthase activity (Table 2). Conversely, the changes in protein concentration were positively associated with changes in CS activity.

**DISCUSSION**

We trained eighteen obese, metabolic syndrome patients during 4 months (i.e., 48 sessions) using intense aerobic interval training program (34) and obtained the typical cardio-metabolic and body composition improvements previously reported in the literature (21). Our subjects lost body weight and fat mass (-1.9% and -5.4%, respectively; Table 1). They also increased their cardiorespiratory fitness, exercise maximal fat oxidation and cycling peak power by 11%, 33% and 10%, respectively (Table 1). The novel finding of our study is that skeletal muscle (i.e., *vastus lateralis*) water content (i.e., \( \text{H}_2\text{O}_{\text{muscle}} \)) increase while surprisingly, muscle total protein concentration decreased after 4 months of aerobic training.

DXA analysis reflected an increase in leg fat free mass suggestive of muscle hypertrophy despite reductions in muscle protein concentration. Other investigators have shown hypertrophic response to similar cycling endurance training programs in older women and men using
microscopic determination of myofibers diameter and magnetic resonance imaging of the leg (12, 14). Our data suggest that increases in muscle water content notably participates in the hypertrophic response to aerobic training in middle age (54±8 yrs old) initially untrained metabolic syndrome men. It also suggests that the mild hypertrophic response to aerobic training detected in our individuals is not due to muscle protein accretion. Furthermore, correlations (Table 2) suggest that the reduction in protein concentration is related to the gain in muscle water probably thru a dilution effect.

It seems contradictory that aerobic training would reduce muscle protein concentration while improving leg muscle power (i.e., peak cycling power; Table 1). It could be argued that the weight loss experienced by our subjects set them in a catabolic state, preventing muscle protein anabolism or muscle protein accretion. However, our subjects did not follow a hypocaloric diet and neither reduced the percent of protein ingested in diet (i.e., 21% of energy intake). Thus, the moderate weight loss experienced during training (i.e., 1.85 kg weight loss or 1.9% of body mass) was most likely induced by a lack of compensatory increase in calorie intake to match energy expenditure during training. In fact, most of the weight loss (93%; i.e., 1.73 out of 1.85 kg) could be accounted by reduction in fat mass tissue (Table 1) in the abdominal region as to judge by the reduction in waist perimeter. Harber and coworkers (12) subjected old women (71±2 yrs. old) to 12 weeks of aerobic training and also found increased skeletal muscle water content and reductions in muscle protein concentration despite null losses of body weight. Furthermore, they measured isolated fiber contractile function and found increased unloaded contraction velocity and absolute power in type I fibers (12). Our data amounts to theirs to suggest that aerobic training improves leg peak power ($W_{\text{max}}$; Table 1) despite reducing muscle protein concentration.
Although H$_2$O$_{\text{muscle}}$ gains have been reported as an acute response during the first stages of moderate (5) and intense exercise (30), we are the first to report an increased H$_2$O$_{\text{muscle}}$ 72 h after the final session of exercise of a four months long training program. We interpret this to suggest that gain in muscle water is another of the adaptations to endurance training. Aerobic or resistance training seems to reduce specific force (the ratio between isometric force and fiber cross sectional area) in both young (18) and old subjects (12, 14). Reduction in specific force evidences that the increase in size due to aerobic training is not entirely functional. Others have suggested that the reduced specific force is due to edema or swelling. Increased in H$_2$O$_{\text{muscle}}$ diluting myofibrillar protein could explain the reduced specific force after aerobic training reported in the literature. Our measurements of increased H$_2$O$_{\text{muscle}}$ support this view.

The gain in muscle water that we observed may have remodeled vastus lateralis fibers’ contractile performance. Dehydration of rat skinned isolated single fibers by bathing the muscle preparation in a hyperosmotic fluid (10% dextran) reduces the space between myofilaments (myosin and actin) and lowers maximal shortening velocity. The authors suggest that filament lattice compression likely affects the rate constant for cross-bridge detachment (19). In humans, 17 days of bed rest increased type I fiber unloaded shortening velocity although they reduce their peak isometric force (39). On the other hand, resistance training can increase muscle strength and cross sectional area without changes in myofilament spacing (3) when a reduction in this spacing was expected due to myofilament packing (27). It is then possible that the gain in muscle water reported presently constitute a training adaptation geared to increase the space between thin and thick filaments allowing faster cross bridge cycling. Vastus lateralis importantly contributes to cycling mechanics and the increase in cycling power in our data suggest improvement in the rate of force applied to the pedals from this muscle. Based on the
current literature, it is reasonable to hypothesize that the gains in muscle water content increased filament spacing allowing higher muscle power.

Our proxy marker of mitochondrial density (citrate synthase activity) increased 31% with the completion of the 48 exercise training sessions (Figure 2). Mitochondria constitutes approximately 4-6% of muscle tissue (15) and its proliferation could be contributing to the modest leg hyperthopy currently reported (Table 1). However, the decrease in protein concentration is counterintuitive in the face of an increased mitochondrial density. Sarcomeres have more protein concentration than mitochondria and thus a superior mitochondrial proliferation respect to sarcomere, could explain the reduction in total protein concentration. We did not separate the different protein fractions but Harber and co-workers reported that 12 weeks of aerobic training reduce myofibrillar protein (12) while not affecting sarcoplasmic protein concentration. Thus, the large increase in citrate synthase suggests gains in mitochondria that may have contributed to the reduced protein concentration.

On the other hand, the increased H$_2$O$_{\text{muscle}}$ with aerobic training may be influenced by the 14% higher muscle glycogen storage after training. The literature suggest that muscle glycogen may require water for its storage (10, 24). Other researchers have proposed that the combined increases in mitochondrial volume and glycogen stores could account for an significant portion of the increases in leg cross sectional area with exercise training (23). Water and glycogen were the two components of muscle that increased after training. The increase in muscle water content was inversely associated to typically regarded adaptations to endurance training such as increased muscle glycogen, protein concentration and citrate synthase activity (Table 2). This inverse association reveals the strong dilution effect that muscle water has in these muscle
metabolites. In fact, the changes in glycogen, protein and citrate synthase were positively associated among them, probably driven by the dilution effect of water in all of them.

In contrast, other investigators have reported no changes in $H_2O_{\text{muscle}}$ after training young individuals using resistance training (17). Competitive runners display lower muscle water content than recreational runners (29) arguing against muscle water expansion as an adaptation to endurance training. However, the same authors found that training for a marathon reduces slow and fast twitch fiber size (35). A deficit in nutritional water during the extraneous training for the marathon may refrain the otherwise natural muscle water expansion. Of note, our findings of increased $H_2O_{\text{muscle}}$ with training might be specific of very untrained individuals since our subjects’ VO$_2$peak ranked in the lower 20% percentile according to ACSM guidelines (38). Possibly, the initial muscle atrophy due to detraining and a sub-optimal muscle water content may set the scenario for the increases in muscle water content with aerobic training. In fact, muscle water content before training in the current metabolic syndrome men was 8% lower than in a group of young endurance trained cyclist recently tested in our lab (363±9 vs. 395 mL·100 g$^{-1}$ dw; (9)). Likewise, initial muscle water levels may explain why identical training increased muscle water content in old women (71.9±1.0% of water in muscle; (13)) but not in old men (77.8±0.9% of water in muscle; (14)). Our data suggest that muscle water expansion is not gender specific, since our men increased muscle water content, but influenced by pre-training water content (73.8% of initial muscle water in muscle in our data).

In summary, we found increases in muscle water as a result of an endurance training program in much deconditioned (VO$_2$ peak 28.8±7 mLO$_2$·kg$^{-1}$·min$^{-1}$) obese, metabolic syndrome men. The increases in muscle water were negatively associated with changes in protein and glycogen muscle concentration, suggesting a dilution effect. Nevertheless, glycogen
concentration tended to increase while muscle protein concentration decreased. Interestingly, the increases in muscle water rather than protein accretion can explain the mild leg hypertrophy observed after the endurance-training program (48 sessions). Two possible factors accounting for the increased cycling peak power despite reduced total muscle protein are improved neuromuscular function with training and/or myofilament remodeling linked to the gains in muscle water. Lastly, the diluting effect of muscle water expansion should be taken into account when expressing muscle metabolites or substrates per unit of wet weight (i.e., original physiological state).

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REFERENCES


FIGURE CAPTIONS

Figure 1. Changes in muscle water (H$_2$O$_{\text{muscle}}$) prior and after 4 months of aerobic interval training in metabolic syndrome patients. Data are presented as individual responses and means for 18 subjects. * Significantly different from pre-training values ($P<0.05$).

Figure 2. Changes in muscle glycogen, protein concentrations and citrate synthase activity expressed per kilogram of wet muscle (physiological conditions) prior and after 4 months of aerobic interval training in metabolic syndrome patients. Data are means ± SEM for 18 subjects. * Significantly different from pre-training values ($P<0.05$).
Figure 1

Muscle water content (g/kg ww)

- PRE-TRAINING
- POST-TRAINING

*
Table 1. Exercise and anthropometric changes with 4 months of aerobic interval training.

<table>
<thead>
<tr>
<th></th>
<th>Pre-training</th>
<th>Post-training</th>
<th>% change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO\textsubscript{2} peak (mLO\textsubscript{2}·kg\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>28.8±7</td>
<td>32.1±8</td>
<td>11%</td>
<td>0.001*</td>
</tr>
<tr>
<td>FO\textsubscript{max} (g·min\textsuperscript{-1})</td>
<td>0.24±0.09</td>
<td>0.32±0.11</td>
<td>33%</td>
<td>0.003*</td>
</tr>
<tr>
<td>Workload\textsubscript{max} (W\textsubscript{max})</td>
<td>232±67</td>
<td>255±75</td>
<td>10%</td>
<td>0.001*</td>
</tr>
<tr>
<td>Body wt (kg)</td>
<td>95.21±10</td>
<td>93.36±10</td>
<td>-1.9%</td>
<td>0.022*</td>
</tr>
<tr>
<td>Body Mass Index (kg·cm\textsuperscript{-2})</td>
<td>33.4±3</td>
<td>32.7±2</td>
<td>-2.2%</td>
<td>0.013*</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>109±5</td>
<td>107±5</td>
<td>-1.8%</td>
<td>0.016*</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>31.9±4.3</td>
<td>30.1±5.1</td>
<td>-5.4%</td>
<td>0.044*</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>59.9±9</td>
<td>60.3±8</td>
<td>0.5%</td>
<td>0.477</td>
</tr>
<tr>
<td>Right leg Fat Free Mass (kg)</td>
<td>9.84±1.84</td>
<td>10.02±1.85</td>
<td>1.8%</td>
<td>0.023*</td>
</tr>
</tbody>
</table>

Data are mean ± SD for 18 subjects. * Significantly different from Pre-training.
Table 2. Correlation (r Pearson) between changes (i.e., Δ) in muscle variables after 4 months of aerobic interval training.

<table>
<thead>
<tr>
<th></th>
<th>Δ H₂O_ms</th>
<th>Δ [Glycogen]</th>
<th>Δ [Protein]_ms</th>
<th>Δ CS_activity</th>
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<tbody>
<tr>
<td>Δ H₂O_ms</td>
<td>-------</td>
<td>-0.659</td>
<td>-0.687</td>
<td>-0.759</td>
</tr>
<tr>
<td>Δ [Glycogen]</td>
<td>-------</td>
<td>0.328</td>
<td>0.475</td>
<td></td>
</tr>
<tr>
<td>Δ [Protein]_ms</td>
<td>-------</td>
<td></td>
<td>0.680</td>
<td></td>
</tr>
<tr>
<td>Δ CS_activity</td>
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</tr>
</tbody>
</table>

Required coefficient of correlation for n=18 and P≤ 0.05 is 0.470