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The Respiratory Exchange Ratio Is Higher in Older Subjects, but Is Reduced by Aerobic Exercise Training

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Background: Previous studies have suggested reduced fat metabolism in older subjects. However, corrections for their reduced maximal oxygen consumption and the effects of training and substrate availability have not been fully examined.
Objectives: Fat metabolism (FM) in older subjects (n = 14, 75 ± 7 yrs), and the effects of exercise training were compared with FM in younger subjects (n = 16, 22 ± 3 yrs).
Materials and Methods: All subjects completed a maximal exercise test and a sustained submaximal run at 70% of their maximal capacity. The respiratory exchange ratio (RER) and blood substrate levels were determined. Older subjects were re-tested after training.
Results: Young subjects had higher oxygen consumption (VO_2) peak (36.3 ± 6.7 *vs.* 23.7 ± 6.2 ml/kg/min) and lower slope of RER *vs.* VO_2 than older subjects. However, the slope of the RER *vs.* VO_2 relationship was not different between younger and older subjects, after correction for their VO_2 peaks. Younger subjects had longer sustained exercise times (45.5 ± 17.6 min) than the elderly (30.2 ± 14.0 min), pre-training. Post-training, there was a significant increase in VO_2 peak (25%) in older subjects ($P = 0.001$) and submaximal exercise time (30.2 ± 14.0 *vs.* 58.3 ± 27.3min, $P = 0.020$). Respiratory exchange ratio was reduced during both exercises after training (0.90 ± 0.03 *vs.* 1.00 ± 0.03, $P = 0.04$).
Conclusions: The RER of older subjects was not different from that of younger subjects, after correction for the VO_2 peak. The VO_2 peak, sustained exercise time, and RER decreased after training in older subjects, indicating increased fat metabolism.

Implication for health policy/practice/research/medical education:
The present study demonstrates the importance of maximal oxygen consumption in determining fat metabolism in the elderly. When this is considered along with the other reported benefits of relatively high maximal oxygen consumption in the elderly emphasizes the importance of regular physical activity to health and wellness.

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1. Background

The world's older populations are growing (1). Aging is associated with changes in body composition and impaired glucose and fat metabolism (FM) (2-7). Maximal oxygen consumption (VO_{2max}) also decreases with age (8-10), and as FM is associated with VO_2, FM may similarly decrease. It has been shown in some studies that FM is

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lower in older populations compared to younger adults (11-14). Inactivity may also impair FM and oxygen consumption (VO_2) peak (15). Alternatively, studies may have failed to document a decrease in FM in older subjects, particularly after correction for the VO_2 peak (16). As muscle plays a major role in FM, attention has been paid to study the effect of muscle exercise and training on FM (11, 17). One reason FM could be reduced in elderly subjects is the inadequate availability of fat substrate from blood. Some previous studies have suggested that lipolysis is reduced in elderly individuals and that this contributes to the lower FM reported in the elderly (18). However, other studies suggest that lipolysis is not a limiting factor (11, 19).

2. Objectives

There is sufficient evidence to conclude that FM is reduced in older persons. However, whether FM is reduced in the elderly even after correction for the age-associated reduction in maximal aerobic power, whether this reduction is due to reduced lipolysis, or whether it is driven by age-associated inactivity, has not been fully explored. In this study, we investigated FM and lipolysis of inactive older persons, before and after exercise training, compared with younger subjects. In addition to a graded maximal exercise, this study also included, for the first time, a sustained submaximal exercise test. The study also examined the effects of reversal of inactivity in the elderly, by correcting it with a unique high-intensity aerobic exercise program.

3. Materials and Methods

This study was approved by the Institutional Review Board and subjects signed an informed consent.

3.1. Subjects

Samples of convenience of young and independently living older subjects were recruited from the local community. Subjects who could not safely complete the protocols, as judged by their personal physicians, or participate in exercise sessions of 30 min or more once per week, were excluded. The average age was 22 ± 3 and 75 ± 7 yrs, height 1.71 ± 0.09 and 163.0 ± 0.11 cm, weight 74.35 ± 17.6 and 68.8 ± 15.1 kg, and body mass index 24.9 ± 3.7 and 25.1 ± 25.1 for younger and older subjects, respectively.

3.2. Protocol

In the initial session, subjects signed the informed consent and physical characteristics and a nutrition and physical activity questionnaire were completed. In the next session, a voluntary maximal (VO_2 peak) treadmill test was performed. In a third session, a sustained submaximal exercise test, to exhaustion at 70% of their individual VO_2 peak, was performed. Older subjects then participated in a supervised 12-week training program for 1 hour, 3 times per week, on a treadmill and testing was repeated. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), and the respiratory exchange ratio ($RER = VCO_2/VO_2$) were measured. Blood samples for determination of free fatty acids (FFA) and triglycerides (TG) were drawn from an antecubital vein pre- and post-exercise after all tests.

3.3. Physical Activity

To verify that the subjects were inactive, their physical activity levels were assessed using the Yale Physical Activity Survey (20). This is an interviewer-administered questionnaire that is used to estimate energy expended (kcal/week) above rest.

3.4. Nutritional Analysis

To match groups by diet, each subject completed a 3-day dietary record (2 weekdays and 1 weekend day) to determine the total caloric intake and the percentage of carbohydrates (CHO), fat, and protein in their diet, as well as micronutrients (Nutritionist Pro software, Axxya Systems LLC.).

3.5. Treadmill Tests and VO_2 and VCO_2 Measurements

During the treadmill test, young subjects started walking on a treadmill (Trackmaster™, JAS Fitness Systems, and model 225/R) set at 0% grade at 3.2 kph, then at 4.8 kph at 0% grade, after which the grade was increased in 2% increments every 3 minutes until voluntary exhaustion. Elderly subjects walked for 3 min at their comfortable speed (3.2 kph), and the grade was increased 2% every 3 min until voluntary exhaustion (21). Heart rate (HR) (Quinton Q750, A-H-Robins company) and blood pressure (BP) (Tango+, Sun Tech Medical, Inc.) were taken and VO_2 and VCO_2 (STPD), and RER were measured using a commercial metabolic cart (MedGraphics® Cardi O_2, Cardiorespiratory Diagnostic Systems) at the end of each stage of the treadmill test using standard methods (22). Blood samples were taken before and 5 min after exercise aseptically from an antecubital vein for TG and FFA (with EDTA) analysis. For the sustained submaximal test, subjects walked on a treadmill at the grade that resulted in 70% of their individual pre-training VO_2 peak until voluntary exhaustion. Older subjects repeated the same protocol post-training. Respiratory measures were determined as described above.

3.6. Blood Analysis

One blood sample was centrifuged at 750 × *g*, 3000 rpm, 4°C for 20 min, kept on ice, and analyzed at the Center for Laboratory Medicine, Department of Pathology, Kaleida Health, Buffalo, NY, for TG. Another sample (3 ml), collected in an EDTA-containing tube, was analyzed for FFA. It was centrifuged at 750 × *g* and 4°C for 20 min. Plasma aliquots were stored at -80°C until analysis. Free fatty acid was determined using a kit (Wako NEFA-HR, 2) obtained from Wako Diagnostics, (Wako Chemicals USA, Inc., Richmond, VA) according to the manufacturer's protocol.

3.7. Training Program

The unique high-intensity aerobic training program (35) consisted of intermittent walking on a treadmill for 1 hour, three times per week for 12 weeks. The intensity of the aerobic training increased, from 50% of the individual subject's VO_2 peak, in increments of 10% of the VO_2 peak every two weeks, reaching 100% in week 11. During the first week, the subject exercised at each intensity for 2 min and rested for 2 min. During the second week, the subjects exercised for 4 min and rested for 2 min. The subject sat on a stool during the rest periods. Measurements of heart rate and rating of perceived exertion were taken at the end of each exercise bout during the training.

3.8. Statistical Analysis

The data were analyzed using the SigmaStat Statistical Software for windows, Version 3.5 (Systat Software Inc., San Jose, CA). This study had a balanced number of male and female subjects in each group and the subjects acted as their own controls. Data comparing older and younger subjects were analyzed by analysis of variance (ANOVA). For elderly pre- and post-training, repeated-measures analyses were used. When significant differences between experimental groups were detected, the Student-Newman-Keuls test, as a post hoc test, was performed to test the significance of the differences between means. The statistical comparison was considered significant at α level 0.05.

4. Results

4.1. Physical Characteristics

Men had higher VO_2 peak values and longer endurance times than did women. However, their metabolic and blood data were comparable after correction for VO_2 peak, and the data for men and women were then combined. Although older subjects had less lean body mass, their BMI was not significantly greater than that of younger subjects.

4.2. Diet and Energy Expenditure

Total caloric intake of older subjects (1598 ± 442 kcal, 35%, $P = 0.001$) was lower than that of younger subjects (2470 ± 777 kcal). In addition, diet compositions of older subjects were higher in protein (-18 ± 4 *vs.* 15 ± 5, 20%, $P = 0.027$) and lower in fat (26 ± 5 *vs.* 30 ± 8, 13%, $P = 0.001$); however, there was no difference in carbohydrates (53 ± 5 *vs.* 53 ± 10, $P = 0.967$). Total caloric intake and food components were not affected by exercise training in the older subjects ($P = 0.685$ to 0.895). The daily estimated energy expenditure due to physical activity was not significantly different between younger (669 ± 368 kcal/day) and older (872 ± 484 kcal/day, $P = 0.511$) subjects. Exercise training increased caloric expenditure (103 to 258 kcal per session), but did not affect the normal daily activity of the older subjects (704 ± 327 kcal/day, $P = 0.082$).

Table 1. Maximal Exercise Responses and Sustained Sub-Maximal Exercise Responses of the Young and Elderly Subjects

Maximal Exercise Responses					
	Young, Mean ± SD	**Elderly Pre-Tr [a], Mean ± SD**	**Elderly Post-Tr [a], Mean ± SD**	**P [b]**	**P [c]**
Walking time, min	22.9 ± 5.4	18.1 ± 6.7	20.0 ± 7.8	0.04	0.005
Treadmill grade (%)	18 ± 5	14 ± 6	16 ± 7	0.06	0.004
VO2max, ml/kg/min	36.3 ± 6.7	23.7 ± 6.2	29.5 ± 4.6	0.001	0.004
RPE [a]	9 ± 2	6 ± 3	5 ± 2	0.01	0.45
Maximal HR [a], beats/min	178 ± 16	142 ± 19	138 ± 27	0.001	0.14
Maximal SBP [a], mmHg	179 ± 27	169 ± 22	161 ± 21	0.858	0.64
Maximal DBP [a], mmHg	77 ± 20	89 ± 29	69 ± 14	0.18	0.06
Sustained Sub-Maximal Exercise Responses					
	Young, Mean ± SD	**Elderly Pre-Tr [a], Mean ± SD**	**Elderly Post-Tr [a], Mean ± SD**	**P [b]**	**P [c]**
Walking time, min	45.5 ± 17.6	30.2 ± 14	58.3 ± 27.3	0.025	0.02
VO2max, ml/kg/min	25.4 ± 4.7	16.59 ± 4.3	16.58 ± 4.3	0.001	>1.00
RPE [a]	5 ± 1	3 ± 1	3 ± 1	0.029	>1.00
Average HR [a], beats/min	158 ± 8	122 ± 18	107 ± 16	0.001	0.04
Average SBP [a], mmHg	134 ± 10	146 ± 10	144 ± 8	0.027	0.98
Average DBP [a], mmHg	77 ± 5	80 ± 18	78 ± 10	0.248	0.82

[a] Abbreviations: DBP, Diastolic blood pressure; HR = Heart rate; O2 = Oxygen; Post-Tr = Post-training; Pre-Tr = Pre-training; RPE = Rating of perceived exertion; SBP = Systolic blood pressure
[b] Old *vs.* young
[c] Old pre- to post-training;

4.3. Exercise Performance

Older subjects achieved their VO_2 peak in a shorter time (18% sooner) and reached a lower final grade (28% lower) compared with the young subjects. As expected, the VO_2 peak of older subjects was significantly lower (35%) than that of younger subjects. Maximal heart rate (HR) and resting systolic blood pressure (SBP) were lower in older compared with younger subjects, but they reached 84% and 83% of their predicted maximal HR, respectively. Other tested parameters were not affected by age of the subject, and are shown in *Table 1*.

The RER of the older subjects was significantly higher than that of younger subjects (*Figure 1*). As RER is associated with VO_{2max}, the RER values were expressed as a function of VO_2 peak and are plotted in *Figure 2*. As shown in *Figure 2*, the RERs of younger and older subjects were not significantly different at the same percentage of VO_2 peak. During the sustained submaximal exercise test, exercise duration was significantly shorter in older than younger subjects (34%), in spite of the lower VO_2 (35%), HR (23%), and RPE (40%) in the older subjects (*Table 2*). However, SBP in older subjects was higher (12%), whereas diastolic blood pressure (DBP) was similar, compared with the younger subjects. The RER in older subjects was not significantly different from that of younger subjects (*Figure 3*) while they were walking at the same percentage of the VO_2 peak.

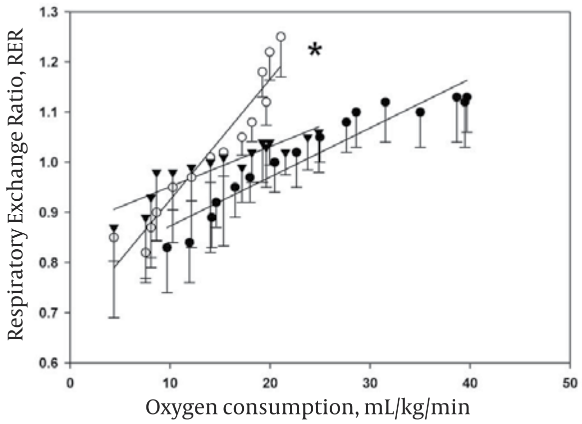

Figure 1. Effect of Oxygen Consumption (Absolute Values Per kg Body Weight) on RER During the VO2 Max max Test
Respiratory exchange ratio is plotted as function of oxygen consumption (ml/min/kg/min) for young (●) and elderly subjects prior to (○) and after (▼) exercise training.
* Significant difference between elderly pre-training and young subjects Indicates that values offor elderly subjects were significantly different from those of younger subjects ($P \le 0.05$).

4.4. Aerobic Training in Elderly Subjects

Fourteen of the 16 older subjects completed the 12-week program and attended over 90% of the sessions. The exercise intensity increased from 50% to 100% of the pre-training maximal over the 12 weeks. The average HR during exercise over the 12 weeks increased from 88 ± 10 to 102 ± 12 beats/min. The average rate of physical exertion (RPE) increased from 1.5 ± 1.0 to 2.1 ± 1.5 over the 12 weeks of the program.

On the post-maximal treadmill test, older subjects exercised longer (29%) and at higher grade (33%), which resulted in a 25% increase in the VO_2 peak (*Table 1*). Resting and maximal HR and SBP did not change significantly with training; however, resting DBP was reduced (*Table 1*).

Elderly subjects walked for a shorter time than younger subjects on the sustained submaximal test pre-training (36%); however, they walked 60% longer on the post-test than the younger subjects (*Table 1* and *Figure 3*). Similarly, RER was significantly higher in the pre-test in older compared with younger subjects; however, aerobic training

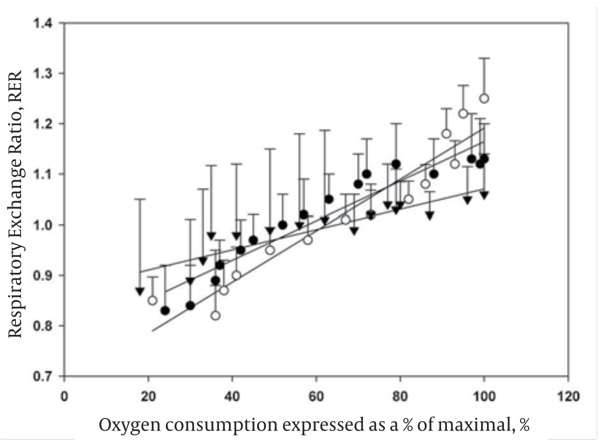

Figure 2. Effect of Oxygen Consumption (Relative Values Expressed as a Percent of VO2 Maxmax) on RER During the VO2 Max max Test on RER.
Respiratory exchange ratio is plotted as a function of oxygen consumption after correction for maximal oxygen consumption (% maximal) for young (●) and elderly subjects prior to (○) and after (▼) exercise training.
* Significant difference between elderly pre-training and young subjects ($P \le 0.05$).

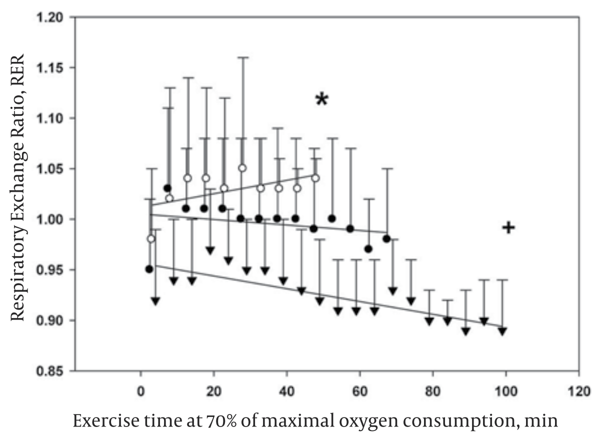

Figure 3. Effect of Oxygen Consumption on RER During the Submaximal Test
The Rrespiratory exchange ratio is plotted as a function of submaximal exercise time (min) with the subject walking at 70% of maximal oxygen consumption for young (●) and elderly subjects prior to (○) and after (▼) exercise training.
* Significant difference between elderly pre-training and young subjects. + Significant difference between elderly pre-training and post-training subjects ($P \le 0.05$).

significantly decreased RER in older subjects, and in fact, their values were lower than those of the younger subjects (*Figure 3*). These data suggest that older subjects had lower FM than younger subjects pre-training; however, exercise training significantly increased FM. Although TG values were higher in the elderly than in the young pre-exercise (89 ± 30 *vs.* 114 ± 54 μM), this difference was not significant (P = 0.097). Post-exercise TG levels were higher in both younger (10 ± 4 μM) and older subjects (16 ± 8 μM) after both the maximal and submaximal tests, with the levels being significantly greater in the elderly (P = 0.038). Training of elderly did not significantly affect the pre-post exercise change in TG in either max- or sub-max exercise. Pre exercise (resting) FFA was not significantly different between younger and older subjects (1003 ± 552 *vs.* 1038 ± 741 μM, P = 0.88), or in older subjects after training (1068 ± 863 μM). Although FFA increased more in older subjects after the VO_{2max} test, the difference was not significant (795 ± 437 *vs.* 238 ± 119 μM, P = 0.09). Similarly, FFA increased after the sub-max test in both younger and older subjects (1891 ± 946 and 1634 ± 849 μM, respectively, P = 0.31). After training, FFA in older subjects increased, similarly to pre-training for both the max- and sub-max tests (1182 ± 941 and 779 ± 390 μM, P = 0.21 and 0.48, respectively). For the submaximal exercise test, there were no changes in FFA and TG pre- to post-training.

5. Discussion

As expected, the present study demonstrated that RER increased as a function of exercise intensity (VO_2) in both younger and older subjects, but that the increase was higher in older subjects. The maximal aerobic power of older subjects was also significantly lower than that of younger subjects. Importantly, when FM (RER) was expressed as a percentage of VO_2 peak, the differences between younger and older subjects were not evident. Subjects in this study, for the first time, also completed a sustained submaximal exercise test to exhaustion, at 70% of the VO_2 peak, on a treadmill, which confirmed elevated RER in the older subjects during an endurance exercise, suggesting a reduced FM. However, by increasing activity level through exercise training, FM was increased in the older subjects, as was their exercise performance. There was no difference in body mass indices; however, the elderly subjects had a higher percentage of fat and less lean body weight, which probably contributed, in part, to their lower VO_2 peak and FM. The total daily energy expenditure due to physical activity of elderly subjects was similar to that of the younger subjects, and was not affected significantly by training. Therefore, activity level could not account for the lower FM in older subjects. However, lower muscle mass and VO_2 peak could result in lower FM. Elderly subjects consumed a diet that was 35% lower in total calories and contained 13% lower fat and 20% higher protein, compared to the younger subjects. These data suggest that diet may play a role in the observed difference in RER between older and younger subjects in this study. However, the difference in RER disappeared when values were expressed as a percentage of the VO_2 peak, suggesting that fat intake was not the cause of the higher RER, but that their low VO_2 peak was. In addition, the data obtained on energy substrate levels in blood argue against the suggestion that fat availability was lower in older subjects, since there were no differences between older and younger subjects' substrate levels in the blood. Furthermore, exercise training reduced RER, in spite of absence of changes in dietary intake and blood levels of fat.

5.1. Fat Oxidation as a Function of Absolute VO_2

The results from the present study demonstrated that FM, as assessed from RER, decreased as a function of exercise intensity (VO_2) in both younger and older subjects, which is in agreement with another study (23). The higher RER in older subjects is most likely caused by age-related changes in the respiratory capacity of skeletal muscles (19). When FM was expressed in absolute values of VO_2, the data from the present study agrees with that of previous studies (11, 14, 18) in which older subjects, compared to younger subjects, had reduced FM. Some studies have failed to demonstrate a reduced fat metabolism in older subjects (5, 16, 19, 24), but the overall evidence suggest that it is reduced, particulary as shown in the endurace tests in this study.

5.2. Maximal Aerobic Power

Both RER and FM at specific exercise intensities are determined, in part, by the maximal aerobic power, which is reduced in older subjects, as was shown in this and in previous studies (11, 14, 18). The decline in the VO_2 peak seems to be due to both central and peripheral adaptations. Reductions in maximal heart rate (HR_{max}), cardiac output, and lean body weight also play an important role (9, 25). The higher the VO_2 relative to the maximal VO_2, the less fat that is metabolized in both fit and unfit young subjects (21). It is unclear if FM in older subjects would be lower or similar to unfit young subjects when expressed as a percentage of the VO_2 peak. It is well established that VO_{2max} is lower and lactate is higher during cycling in untrained cyclists, due to the limited muscle mass used, high force of turning the crank, and relative lack of fitness (22). All of these factors could reduce fat oxidation on a cycle ergometer in both young and older subjects. The present study was conducted on a treadmill to eliminate the factors that influence cycling, particularly as many older subjects are accustomed to walking, but not to cycling. Previous studies have shown a marked reduction in activity in older subjects; however, those that are moderately or highly active have higher VO_2 peak levels (8, 26). In addition the VO_2 peak, lean body weight and intrinsic capacity of muscle for FM can be increased by exercise training (12). Physical activity, particularly fast walking, has been shown to be closely associated with body fat mass, but not total energy intake (27). Submaximal exercise training increases FM, resulting in increased aerobic capacity and reduced adiposity in older subjects (28). The increase in the VO_2 peak in the present study is in agreement with previous studies (approx. 10%–20%) (6, 29, 30).

5.3. Fat Oxidation Corrected for VO_{2max}

In the present study, when RER was expressed as a percentage of maximal aerobic power, RER in older inactive subjects was similar to that of the inactive younger subjects. This result is in agreement with some studies (18, 28), but not with another study where the authors used a cycle ergometer (19) in their testing. It would appear that during walking, RER is not elevated in healthy older subjects, when corrected for the reduction in their VO_2 peak.

5.4. Role of Body Characteristics, Activity Level, and Diet on Fat Oxidation

The present study revealed that lean body weight was lower in older than in younger subjects, which is in agreement with previous studies (31). The lower lean body mass would affect VO_2 peak and FM. Daily energy expenditure due to physical activity, and dietary fat intake did not play a role in the lower FM in older compared with younger subjects in this study. The potential influence of daily activity on FM in elderly is further demonstrated by the observation that after training the FM in older active subjects was higher than that of inactive younger subjects. The lower RER during the endurance walk, suggesting higher FM, was associated with the elderly subjects walking significantly longer. To our knowledge, this is the first study to examine FM pre- and post-training during a sustained submaximal exercise test (endurance) on a treadmill.

5.5. Availability of Fats from Blood

Some previous studies have suggested that lipolysis is reduced in older individuals and that this contributes to the lower FM reported in older subjects (18). However, other studies suggest that lipolysis is not a limiting factor (11, 19). The present study supports the conclusion that lipolysis is not reduced in older subjects and that the uptake of fat and its metabolism probably accounts for the reduced FM. In one study, sedentary elderly subjects had reduced plasma FFA release compared with fitter older subjects (26). However, the rate of delivery of fatty acids into systemic circulation was reported to be similar in older and younger subjects, and even slightly higher in older subjects, after adjustments for fat-free mass (18). Lipolytic rates and FFA availability do not appear to be rate limiting in the elderly subjects in this and other studies (18, 19). Therefore, a decrease in the capacity of muscle to oxidize fat, and/or a decrease in its capacity to transport long-chain fatty acids is what probably accounts for the reduced FM (11, 18). Some factors that may be responsible for reduced fat oxidation could be a diminished amount of oxidative enzymes, an increased glycolytic flux, inhibiting fatty acid transport into the mitochondria, or a diminished (possibly beta-adrenergically-mediated) activation of fatty acid transport (18). Many of these parameters have been shown to be affected by exercise training and may have been responsible for the increased FM after training. Another explanation for low FM in older subjects could be low intramuscular fat stores, as a result of reduced fat-free mass or fat uptake, as shown in this study, and storage in muscle, as shown previously. However, increases in both intramuscular and liver fat have been reported in older subjects, and are associated with insulin resistance, plasma lipids, and body fat (32). Other studies have shown that intramyocellular lipid content was higher in elderly than in younger subjects (32, 33). Therefore, the factor limiting fat oxidation does not seem to be substrate availability from blood or muscle, but rather could be related to the fate of fat.

5.6. Effects of Training on Fat Oxidation

The high-intensity intermittent exercise training model used in this study was well tolerated and enjoyed by older subjects (35), with 90% compliance during training and significant improvements in the VO_2 peak. These improvements were greater than previously reported for other training studies (6, 29, 30). The program used in this study was relatively high-intensity (up to 100% of pre-training VO_{2max}), but intermittent, thus keeping oxygen consumption and HR elevated for the full hour of the program. This is in contrast to other reported training models, where continuous low-level exercise was used, at lower levels of oxidation and HR, for only 20 to 30 min (6, 29, 30). It is well established that increased physical activity (training) increases both VO_{2max} and FM in young subjects (5, 19, 34). However, it is unclear if this would also occur in older subjects, as the limited numbers of studies of this aspect have failed to demonstrate an improvement in subjects, with higher activity levels (23), or after training (16). Exercise training does not influence the decline in maximal heart rate with aging, while body weight and percentage body fat, and thus lean body mass, can be maintained to some degree by exercise (9). In summary, the reduced FM reported in elderly subjects appears to be a consequence of their reduced maximal aerobic power, secondary to reduced activity and loss of muscle mass. There does not appear to be a reduction in the availability of fat from blood, and other studies have shown intramuscular stores are not limiting either. Reversing inactivity with an exercise program improved both VO_{2max} and FM, also suggesting their interrelationship.

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