Effects of Intermittent Cycle Exercise on Intramyocellular Lipid Use and Recovery

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ABSTRACT: The purpose of this investigation was to compare intramyocellular lipid (IMCL) changes in skeletal muscle in nine moderately trained subjects after 45 min of interval cycling and through 1 h of recovery. The exercise session was continuous with alternating cycling intensity achieving 50 (3 min) and 110% (2 min) of ventilatory threshold. Spectra from the vastus lateralis were acquired before, immediately after, and 60 min following exercise using a 1.5 T Signa whole-body magnet (point-resolved spectroscopy sequence, echo time 60 ms, transverse relaxation time 2000 ms, 128 acquisitions, and 20 mm^3 voxel). Immediately following exercise, IMCL concentration decreased 38% compared to pre-exercise levels ($P < 0.05$). Fitness level and baseline IMCL were not correlated with changes in IMCL following exercise ($P > 0.05$). In the 60-min recovery, IMCL was reduced 30% compared to baseline ($P < 0.05$) and did not recover. In contrast, a nonexercising control group showed no change in IMCL. Our results suggest that IMCL decreased significantly following 45 min of interval cycling, with little recovery in the hour following.

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Recent work with NMR has lead to the identification of two separate lipid compartments in skeletal muscle (1,2). The two compartments include intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL) locations. Although these pools contain predominantly TG [over two-thirds of the total FA in IMCL are thought to consist of three FA (18:0, 18:1, and 16:0)] (2), there are distinct metabolic differences between the locations. IMCL has a rapid turnover, and EMCL is thought to have a slow turnover and serve as a long-term storage depot (3). Differences in turnover may be related to location and metabolic enzyme activity (2). IMCL is stored in droplets (<200 Å radius) close to mitochondria and is associated with enzymes involved with FA esterification, transport, and hydrolysis. On the other hand, EMCL is located in more of an annular compartment oriented along muscle fibers and connective tissue (2).

Given the contribution of IMCL as a fuel source at rest, there has been increased interest in its role during exercise (4). Although much is known about muscle glycogen use during exercise, less is known about IMCL use. Until recently, plasma FFA from adipose stores were thought to be the primary fuel source during rest and mild exercise. However, recent work by Romijn *et al.* (4) and others (5,6) has suggested that FA from IMCL contribute significantly to fuel metabolism during moderate-intensity exercise.

Relatively few studies have used proton magnetic resonance spectroscopy (^{1}H MRS) to measure IMCL following exercise (1,7–11). This technique is noninvasive and has higher reproducibility than traditional biopsy studies (12). Most studies have focused on aerobic exercise, and fewer on anaerobic exercise. Larson-Meyer *et al.* (9) found a 25% decrease in soleus muscle IMCL concentrations in trained female subjects after 2 h of treadmill running at 67% of maximal oxygen uptake (VO_{2max}). Rico-Sanz *et al.* (11) observed a 32 and 19% decrease in IMCL concentration in the tibialis anterior and soleus muscles, respectively, in a group of male distance runners following 90 min of moderate-intensity (64% VO_{2max}) exercise. Krssak *et al.* (8) had trained subjects complete several discontinuous 45-min treadmill bouts to exhaustion at 65–70% VO_{2max} and found a 33% reduction in IMCL concentration in the soleus muscle. Taken together, these studies confirm that, under aerobic conditions with moderate-intensity exercise levels, IMCL is an important and measurable fuel.

In contrast, Rico-Sanz *et al.* (10) found no change in IMCL in a group of male athletes who performed several short sprint bouts with rest periods in between. It is likely that the strictly anaerobic type of exercise involved in this study relied less on lipid for fuel substrate compared with studies that have used longer-duration aerobic exercise in their investigations.

Additionally, little is known regarding whether IMCL is altered in a similar manner in sustained moderate- to high-intensity exercise. This is important because many exercise programs emphasize a combination of aerobic and anaerobic energy systems to improve both explosive and endurance exercise capacity. Fewer studies report recovery kinetics of IMCL following

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Abbreviations: aHSL, adipocyte hormone-sensitive lipase; AU, arbitrary units; BF, body fat; EMCL, extramyocellular lipid; IMCL, intramyocellular lipid; mHSL, muscle hormone-sensitive lipase; ^{1}H MRS, proton magnetic resonance spectroscopy; RER, respiratory exchange ratio; V_E, minute ventilation; VCO_2, carbon dioxide production; VO_2, oxygen consumption; VO_{2max}, maximal oxygen consumption; VT, ventilatory threshold.

exercise-induced perturbation. In one study of moderate exercise (8), after 5 h of recovery, IMCL recovered from 67 to 83% of baseline values.

Therefore, the purpose of the following study was to compare IMCL levels before and after 45 min of interval cycling in moderately trained subjects. Exercise consisted of a combination of anaerobic and aerobic intervals. ^{1}H MRS was used to measure IMCL at rest, following exercise, and 60 min into recovery.

MATERIALS AND METHODS

Nine moderately active, healthy young adult males were recruited from the University of New Mexico student and recreational community. All subjects exercised on average from two to five times per week. Criteria for inclusion in the study included having no cardiovascular or orthopedic limitations that would prevent completion of vigorous exercise. After being informed of the procedures involved and the possible risks associated with each procedure, participants signed an informed consent that was approved by the Human Research Review Committee at the University of New Mexico.

Study design. Subjects were evaluated for VO_{2max} and ventilatory threshold (VT) prior to inclusion in the study. The exercise trial was carried out within 7 d of the preliminary evaluation. To further minimize dietary variation, subjects were instructed to maintain similar eating habits in the week preceding the trial. Twenty-four hours before the exercise trial, subjects were instructed to refrain from exercise and from the consumption of alcohol and caffeine. Three hours before testing, subjects consumed a standard 400-kcal meal composed of 25% fat and 75% complex carbohydrates. The subjects reported to the magnetic resonance laboratory between 8:30 and 10:00 A.M.

After the initial resting ^{1}H MRS of the vastus lateralis, each subject performed 45 min of cycling on a Monark cycle ergometer (Varberg, Sweden). The subjects were placed in the magnet immediately following exercise and spectra were acquired. Additional spectra were obtained 60 min into recovery.

Preliminary metabolic testing. Measurements of VO_{2max}, VT, and body fat percentage (BF%) were conducted at the University of New Mexico Human Performance Laboratory. These measurements characterized the aerobic fitness of each subject and were used to prescribe exercise workloads to match relative exercise intensities for all subjects. VO_{2max} was determined using a cycle ergometer protocol, beginning with a light warm-up followed by progressive incremental workloads starting at 50 watts and increasing by 25 watts each minute. The criteria for test termination included a plateau in oxygen uptake with increasing workload and/or a respiratory exchange ratio (RER) greater than 1.15 (13). Heart rates were monitored using telemetry. Minute ventilation (V_E), oxygen consumption (VO_2), carbon dioxide production (VCO_2), and RER were monitored every 30 s using a Jaeger metabolic cart (Wurzburg, Germany). VT was determined through a series of graphs acquired from metabolic data and included V_E and ventilatory equivalents for (V_E/VO_2 and V_E/VCO_2) vs. workload (14). VT was defined as the point when V_E increased in a nonlinear fashion or when V_E/VO_2 began to rise without a concomitant rise in V_E/VCO_2. VT was verified by two independent investigators and was subsequently used to prescribe the exercise workload during the experimental protocol. BF% was estimated with the equation of Jackson–Pollock (15).

Exercise protocol. The 45-min exercise trial consisted of a 5-min warm-up followed by alternating 3-min intervals at 50% (easy) and 2-min intervals at 110% (hard) of the subject's VT. Therefore, each subject performed eight repetitions of 3 min easy and 2 min hard during the 45-min exercise trial. The exercise trial finished with 2 min of cycling at 110% of the subject's VT. This protocol was designed so that untrained subjects could complete the 45-min exercise protocol in a continuous manner and was part of a larger study.

MRS. ^{1}H MRS spectra were acquired using a 1.5 Tesla SIGNA whole-body imaging system (GE Medical Systems, Milwaukee, WI). High-resolution localizing images were initially acquired. Spectra were recorded from a 20 mm^3 region of interest in the subject's vastus lateralis using a general-purpose flexible extremity coil that was wrapped around the leg at the level of the region of interest. Voxel positions were carefully selected to avoid vascular structures and contamination by gross adipose tissue deposits. Localized proton spectra within muscle were collected using a point-resolved spectroscopy sequence (echo time 60 ms, transverse relaxation time 2000 ms, 128 acquisitions, water suppression and outer volume suppression).

^{1}H MRS was performed on the right vastus lateralis, one-third of the distance between the superior border of the patella and the crest of the ileum. This corresponded to a distance of between 12 and 20 cm superior to the superior margin of the patella. This location was marked on the thigh and used as a landmark for the center of the ^{1}H MRS voxel (Fig. 1). The subject's leg was rotated to be in alignment with the bore of the magnet using the longitudinal magnet alignment light (1).

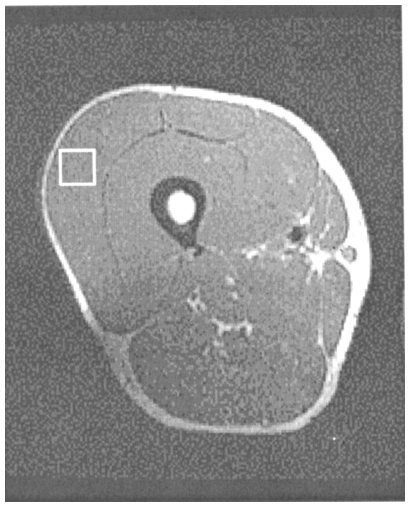

FIG. 1. Cross section of vastus lateralis. Voxel region of interest is highlighted in the scanning area.

To ensure that the leg was placed in the same position in the postexercise scan, anatomical landmarks, alignment lights, and padding were used to ensure relocalization in the same location within the magnet following exercise. The time between the end of exercise and the start of actual ^{1}H MRS data acquisition was approximately 15 min. This time included repositioning the subject in the magnet, acquiring a localizing thigh image, and shimming the voxel for optimal magnetic field homogeneity. Spectra were acquired immediately following and 60 min after exercise cessation.

Fitting of spectra. Spectra were analyzed using the Magnetic Resonance User Interface (MRUI) data analysis package (Leuven, Belgium). Initially, water filtering using the Hankel Lanczos Single Value Decomposition (HLSVD) filtering was performed to remove residual water resonances from the spectrum to flatten the baseline.

Time-domain fitting using Gaussian shapes was then performed on trimethylamine peaks (3.2 ppm), the creatine/phosphocreatine peak (3.03 ppm), the IMCL peak (1.28 ppm), and the EMCL peak (1.4 ppm) by AMARES (Advanced Method for Accurate, Robust, and Efficient Spectral Fitting). The total area under each peak was recorded for subsequent analysis. IMCL was expressed in arbitrary units (AU). In this study, peak fitting for IMCL has a within-subject variability of 6% on repeated scans.

Statistical analysis. Descriptive differences were analyzed with a Student's *t*-test. IMCL changes were tested with a one-way (within) ANOVA with repeated measures. Pearson product-moment correlation coefficients (R) were used to assess relationships between variables. An α level of $P < 0.05$ was considered significant. The Statistical Package for the Social Sciences (SPSS version 10.0; Chicago, IL) was used for all statistical analyses.

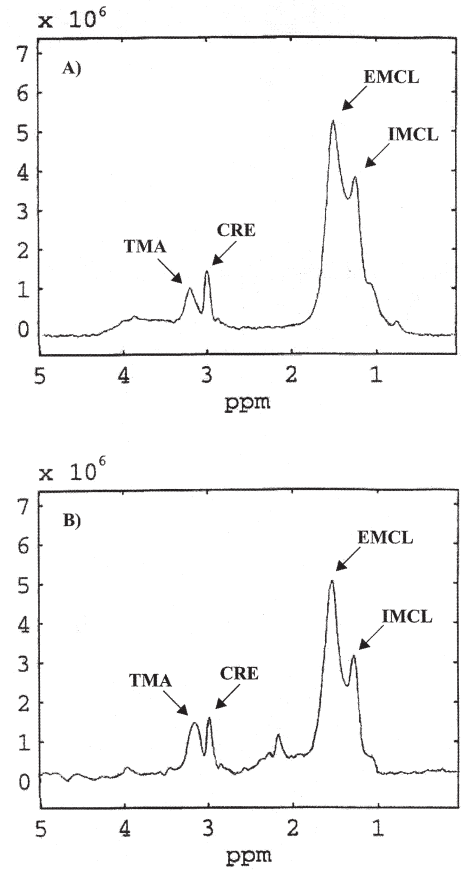

FIG. 2. ^{1}H Magnetic resonance spectroscopy of the vastus lateralis (A) pre-exercise and (B) postexercise following 45 min of continuous interval cycling. Abbreviations: TMA, trimethylammonium-containing compounds; CRE creatine and/or phosphocreatine; EMCL, $(CH_2)_n$ resonance of extramyocellular lipid pool; IMCL, $(CH_2)_n$ resonance of intramyocellular lipid pool.

RESULTS

Subject characteristics. Group characteristics of subjects ($n = 9$) were as follows: age 30.7 ± 4.9 yr; height 175.7 ± 7.5 cm; weight 74.0 ± 6.3 kg; BF% 11.9 ± 6.4; VO_{2max} 58.1 ± 14.9 mL kg min^{-1}; VT 225.0 ± 66.9 watts. Subjects were moderately trained as assessed by their VO_{2max} and self-reported physical activity (16). Body fat analysis showed their average body fat to be 11.9%.

In vivo *^{1}H MRS. In vivo* ^{1}H MRS from the vastus lateralis before and after exercise from one subject are shown in Figure 2. At rest there were no significant associations between IMCL and VO_{2max} ($R = 0.39$, $P = 0.30$), BF% ($R = -0.09$, $P = 0.81$), and VT ($R = 0.54$, $P = 0.13$). Subjects showed a significant decrease in IMCL immediately following exercise ($P < 0.01$). That is, the IMCL, expressed (in AU) as mean ± SEM, for pre-exercise was $(1.89 \pm 0.21) \times 10^7$, for postexercise was $(1.16 \pm 0.23) \times 10^7$, and after 60 min recovery was $(1.31 \pm 0.21) \times 10^7$; the differences between the latter two values and the pre-exercise IMCL value were both significant at $P < 0.05$. This finding was similar when examining the total area under the IMCL curve and quantifying IMCL relative to muscle creatine and H_2O levels ($P < 0.05$). The IMCL decrement immediately after exercise represented a 38% change (Fig. 3). However, there was no relationship between pre-exercise IMCL levels and the amount of IMCL change with exercise ($R = -0.05$, $P = 0.90$). Thus, subjects with greater IMCL levels at baseline did not show any greater change in IMCL levels than those with less IMCL at baseline. To examine IMCL recovery rates, ^{1}H MRS was obtained 60 min into recovery. At this time, IMCL had recovered to 30% of baseline values ($P < 0.05$ compared to baseline). Recovery rates were not greater in subjects who had greater baseline IMCL concentrations ($R = 0.52$, $P = 0.15$). No changes were noted in EMCL (data not shown, $P > 0.05$).

Immediately after exercise an unidentified peak presented at approximately 2.13 ppm (Fig. 1). During the course of recovery, this peak was present but decreased in magnitude over time.

DISCUSSION

This study compared IMCL changes in moderately trained subjects before and after a session of intense intermittent cycle ergometry. IMCL decreased significantly, and the

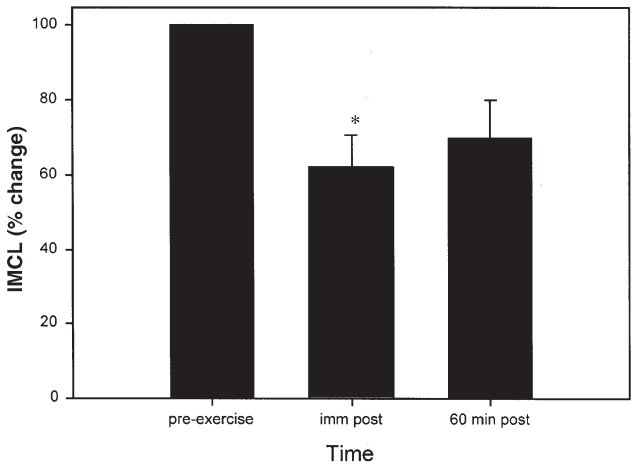

FIG. 3. Percent change in IMCL over time. Key: pre-exercise, immediately before exercise; imm post, immediately after exercise; 60 min post, 60 min after exercise cessation. Error bar represents SEM. Asterisk (*) indicates $P < 0.05$ compared to immediately before exercise.

magnitude of change immediately after exercise was 38% and did not recover significantly in the hour following exercise. No changes were noted for EMCL with exercise.

Until recently, most studies examining IMCL changes with a single bout of exercise have used muscle biopsy techniques. These studies found decreases (6) or no changes (17,18) in IMCL concentrations immediately following exercise. However, the biopsy technique has been criticized for its invasive nature and high variability with repeated sampling (12). For example, Wendling *et al.* (12) found as much as 26% variability in repeated biopsy IMCL sampling with exercise, justifying alternative methods of measurement of muscle lipid.

More recent studies have used ^{1}H MRS to measure IMCL at rest and with exercise. The majority of these studies have been completed with well-trained subjects using prolonged exercise protocols of submaximal workload. Recent work by Larson-Meyer *et al.* (9) studied IMCL changes in well-trained female endurance runners and triathletes following a 2-h treadmill run at 67% of VO_{2max}. IMCL decreased significantly (~25%) in soleus muscle immediately after exercise when subjects consumed either a moderate- or low-fat diet prior to exercise. Similar results were found by Rico-Sanz *et al.* (11) in a group of trained male distance runners following a 90-min treadmill run at 64% of VO_{2max}. A significant decrease in IMCL was found in the tibialis anterior (32%) and soleus (19%) muscles. The authors suggested that muscle fiber composition and muscle oxidative capacity played roles in differences in muscle lipid substrate use with exercise.

Our study is similar to previous studies that find decreases in IMCL in moderately trained subjects immediately following moderate- to high-intensity exercise (8,9,11). When IMCL changes from exercise were correlated with baseline IMCL or VO_{2max}, no significant associations were found. Thus, those subjects with greater baseline values of IMCL, or subjects with higher fitness levels did not show a different response for IMCL during exercise when compared to less fit subjects. To ensure that decrements in IMCL in the exercise treatment were from the exercise session, a resting male control group ($n = 4$) with similar ages, physical activity, and fitness levels were compared. This group completed the same experimental protocol as the exercise group, but instead of exercising for 45 min, this group rested quietly in the laboratory for 45 min. For the control group there were no significant changes in IMCL during the two time points measured (–2.1%) (pre- vs. 45 min post-rest period, $P = 0.90$). However, when the changes over time were compared between groups, the decrease in IMCL was significantly greater for the exercise group ($P < 0.05$). These results suggest that IMCL decreases during exercise and that FA from this depot are a significantly fuel source in skeletal muscle.

Our data are consistent with those of Romijn *et al.* (4), which suggest that IMCL may contribute as much as 50% of the total lipid used as a fuel source during moderate- to high-intensity exercise. It is likely that increased muscle hormone-sensitive lipase (mHSL) contributed to the activation of muscle lipolysis during exercise. Immunoblotting (19) and Northern blotting techniques have found protein and mRNA in muscle similar to that found with adipose tissue hormone-sensitive lipase (aHSL) (20). In adipose tissue, the activity of aHSL is regulated by sympathetic activity (21). During exercise, the plasma catecholamine concentration increases dramatically, inducing greater sympathetic activity (22). Although no blood samples were taken during the current study, it is probable that catecholamine levels were elevated during exercise (23), inducing the mHSL cascade.

Although its significance is not clear, a peak resonating around 2.1 ppm was present immediately after exercise. Previous work suggests that resonances in this area represent acetyl groups, possibly attached to carnitine groups (24). Increased acetylcarnitine formation during exercise could help explain how substrate use switches from predominantly carbohydrates to lipid during exercise (25). Less carnitine available during exercise would decrease FA shuttling into the mitochondria for oxidation, forcing the use of nonlipid fuel sources (26). However, more work is needed in this area before definitive statements can be made regarding the mechanisms controlling lipid use during exercise.

IMCL recovery was evaluated at 60 min after cessation of exercise. At this time, IMCL had recovered to 30% of the initial baseline value. These results are not surprising considering most published studies report that it takes several hours (8) to days (1) to replenish IMCL levels following exercise. Recovery rates of IMCL may be affected by substrate use and availability (4,17). Substrate availability (FFA from plasma TG) is regulated primarily by muscle lipoprotein lipase activity (27), which may not increase until 4 h after exercise (17). These data suggest that other mechanisms may contribute to the small recovery observed in the current investigation. Recent work by Ghou *et al.* (28) suggests that blood glycerol may contribute to IMCL replenishment and that there is a small depot of glycerol kinase in muscle available for FFA re-esterification. However, additional work is needed to clarify

whether FFA is re-esterfied in muscle following short-duration exercise and whether this mechanism is involved.

In conclusion, our results suggest that IMCL significantly decreases in response to interval cycling in moderately trained subjects. Changes in IMCL were not related to baseline IMCL levels or fitness. After 60 min of recovery, IMCL had not changed significantly, suggesting that IMCL does not significantly recover in the hour following exercise. Future work with extended follow-up as well as the study of potential mechanisms that contribute IMCL decrements during exercise is warranted.

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REFERENCES

1. Boesch, C., Slotboom, J., Hoppeler, H., and Kreis, R. (1997) *In vivo* Determination of Intramyocellular Lipids in Human Muscle by Means of Localized ^{1}H-MR Spectroscopy, *Magn. Reson. Med. 37*, 484–493.
2. Szcepaniak, L.S., Babcock, E.E., Schick, F., Dobbins, R.L., Garg, A., Burns, D.K., McGarry, J.D., and Stein D.T. (1999) Measurement of Intracellular Triglyceride Stores by ^{1}H Spectroscopy: Validation *in vivo*, *Am. J. Physiol. 276*, E977–E989.
3. Bessesen, D.H., Rupp, C.L., and Eckel, R.H., (1995) Trafficking of Dietary Fat in Lean Rats, *Obes. Res. 3*, 191–202.
4. Romijn, J.A., Coyle, E.F., Sidossis, L.S., Gastaldelli, A., Horowitz, J.F., Endert, E., and Wolfe, R.R. (1993) Regulation of Endogenous Fat and Carbohydrate Metabolism in Relation to Exercise Intensity and Duration, *Am. J. Physiol. 28*, E380–E391.
5. Hurley, B.F., Nemeth, P.M., Martin, W.H., Hagberg, J.M., Dalsky, G.P., and Holloszy, J.O. (1986) Muscle Triglyceride Utilization During Exercise: Effect of Training, *J. Appl. Physiol. 60*, 562–567.
6. Jansson, E., and Kaijser, L. (1987) Substrate Utilization and Enzyme in Skeletal Muscle of Extremely Endurance-Trained Men, *J. Appl. Physiol. 62*, 999–1005.
7. Brechtel, K., Niess, A.M., Machann, J., Rett, K., Schick, F., Claussen, C.D., Dickhuth, H.H., Haering, H.U., and Jacobs, S. (2001) Utilisation of Intramyocellular Lipids (IMCLs) During Exercise As Assessed by Proton Magnetic Resonance Spectroscopy (^{1}H-MRS), *Horm. Metab. Res. 33*, 63–66.
8. Krssak, M., Petersen K.F., Bergeron, R., Price, T., Laurent, D., Rothman, D.L., Roden, M., and Shulman, G.I. (2000) Intramuscular Glycogen and Intramuscular Lipid Utilization During Prolonged Exercise and Recovery in Man: A ^{13}C and ^{1}H Nuclear Magnetic Resonance Spectroscopy Study, *J. Clin. Endocrin. Metab. 85*, 748–754.
9. Larson-Meyer, D.E., Newcomer, B.R., and Hunter, G.R. (2002) Influence of Endurance Running and Recovery Diet on Intramyocellular Lipid Content in Women: A ^{1}H NMR Study, *Am. J. Physiol. 282*, E95–E106.
10. Rico-Sanz, J., Hajnal, J.V., Thomas, E.L., Mierisova, S., Ala-Korpela, M., and Bell, J.D. (1998) Intracellular and Extracellular Skeletal Muscle Triglyceride Metabolism During Alternative Intensity Exercise in Humans, *J. Physiol. 510*, 615–622.
11. Rico-Sanz, J., Moosavi, M., Thomas, E.L., McCarthy, J., Coutts, G., Saeed, N., and Bell, J.D. (2000) *In vivo* Evaluation of the Effects of Continuous Exercise on Skeletal Muscle Triglyceride in Trained Humans, *Lipids 35*, 1313–1317.
12. Wendling, P.S., Peters, S.J., Heigenhauser, G.J.F., and Spriet, L.L. (1996) Variability of Triacylglcerol Content in Human Muscle Biopsy Samples, *J. Appl. Physiol. 81*, 1150–1155.
13. Taylor, H.L., Buskirk, E., and Henchel, A. (1955) Maximal Oxygen Intake as an Objective Measure of Cardiorespiratory Performance, *J. Appl. Physiol. 8*, 73–80.
14. Caiozzo, V.J., Davis, J.A., Ellis, J.F., Azus, J.L., Vandagriff, R., Prietto, C.A., and McMaster, W.C. (1982) A Comparison of Gas Exchange Indices Used to Detect the Anaerobic Threshold, *J. Appl. Physiol. 53*, 1184–1189.
15. Jackson, A.S., and Pollock, M.L. (1977) Prediction Accuracy of Body Density, Lean Body Weight, and Total Body Volume Equations, *Med. Sci. Sports Exerc. 9*, 197–201.
16. Shvartz, E., and Reibold, R.C. (1990) Aerobic Fitness Norms for Males and Females Aged 6 to 75 Years: A Review, *Aviat. Space Environ. Med. 61*, 3–11.
17. Kiens, B., Lithell, H., Mikenes, J., and Richter, E.A. (1989) Effects of Insulin and Exercise on Muscle Lipoprotein Lipase Activity in Man and Its Relation to Insulin Activity, *J. Clin. Invest. 84*, 1124–1129.
18. Starling, R.D., Trappe, T.A., Parcell, A.C., Kerr, A.C., Fink, W.J., and Costill, D.L. (1997) Effects of Diet on Muscle Triglyceride and Endurance Performance, *J. Appl. Physiol. 82*, 1185–1189.
19. Holm, C.P., Belfrage, P., and Fredrickson, G. (1987) Immunological Evidence for the Presence of Hormone-Sensitive Lipase in Rat Tissue Other Than Adipose Tissue, *Biochem. Biophys. Res. Com. 148*, 99–105.
20. Holm, C.P., Kichgessner, T.G., Svenson, K.L., Fredrickson, G., Nilsson, S., Miller, C.G., Shivey, J.E., Heinzmann, C., Sparkes, R.S., Mohandas, T., *et al.* (1988) Hormone-Sensitive Lipase: Sequence, Expression and Chromosomal Localization to 19 Cent-q13.3, *Science 241*, 1503–1506.
21. Hales, C.N., Luzio, J.P., and Liddle, K. (1978) Hormonal Control of Adipose Tissue Lipolysis, *Biochem. Soc. Symp. 43*, 97–135.
22. Kjaer, M., and Secher, N.H. (1992) Neural Influence on Cardiovascular and Endocrine Responses to Static Exercise in Humans, *Sports Med. 13*, 303–319.
23. Christmas, M.A., Dawson, B., and Arthur, P.G. (1999) Effect of Work and Recovery Duration on Skeletal Muscle Oxygenation and Fuel Use During Sustained Intermittent Exercise, *Eur. J. Appl. Physiol. 80*, 436–447.
24. Kreis, R., Jung, B., Rotman, S., Slotboom, J., and Boesch, C. (1999) Non-invasive Observation of Acetyl-Group Buffering by ^{1}H-MR Spectroscopy in Exercising Human Muscle, *NMR Biomed. 12*, 471–476.
25. Holloszy, J.O., Kohrt, W.M., and Hansen, P.A. (1998) The Regulation of Carbohydrate and Fat Metabolism During and After Exercise, *Front. Biosci. 3*, D1011–D1027.
26. Winder, W.W. (1998) Intramuscular Mechanisms Regulating Fatty Acid Oxidation During Exercise, *Adv. Exp. Med. Biol. 441*, 239–248.
27. Oscai, L.B., Essig, D.A., and Palmer, W.K. (1990) Lipase Regulation of Muscle Triglyceride Hydrolysis, *J. Appl. Physiol. 69*, 1571–1577.
28. Guo, Z., and Jensen, M.D. (1999) Blood Glycerol Is an Important Precursor for Intramuscular Triacylglycerol Synthesis, *J. Biol. Chem. 274*, 702–706.

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