PRELIMINARY REPORT

Skeletal Muscle Cell Lipid and Oxygen Supply

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In a group of 12 normal-weight, normotensive, nondiabetic adult females, the intramyocellular lipid (IMCL) to creatine ratio of the soleus muscle was determined using localized ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) and related to skeletal muscle blood (and oxygen) supply (as assessed by near infrared spectroscopy [NIRS] of the forearm). A significant positive association was found between IMCL content and reoxygenation rate of forearm muscle hemoglobin (Hb) after 1 minute of ischemic exercise ($r = .70$, $P = .01$). The relative efficiency of skeletal muscle oxygen supply may be a determining factor of IMCL content in skeletal muscle.

INSULIN RESISTANCE is associated with diminished muscle perfusion.[1] Studies using localized proton magnetic resonance spectroscopy (^{1}H-MRS) or biopsies have also confirmed a relationship between elevated intracellular triglyceride content of skeletal muscle (intramyocellular lipid [IMCL]), whole body insulin resistance,[2] and other features of insulin resistance such as central obesity.[2,3] The aim of this study was to seek an association between two recognized features of insulin resistance by using near infrared spectroscopy (NIRS) to measure muscle oxygen delivery in vivo and relating this index of muscle perfusion to IMCL levels.

We studied 12 normal-weight (body mass index, 22.3 kg/m^2 ± 2.2 SD; waist to hip ratio, 0.71 ± 0.05), normotensive, nondiabetic (fasting glucose level, 5.02 mmol/L ± 0.32) females (age 22 to 35 years).

Proton MRS is a noninvasive technique that can be used to measure IMCL from a reasonably large and representative sample of muscle without contamination by extramyocellular lipid.[4,5] Subjects lay supine within the scanner with their leg positioned such that the soleus muscle fibers were oriented parallel to the axis of the static magnetic field. Scout images were taken (1.5-Tesla magnetic resonance scanner; Philips Medical Systems, The Netherlands) to optimize the position of a 1.5 × 1.5 × 5.0 cm^3 voxel within the right soleus muscle. Spectra were obtained using a point-resolved spectroscopy sequence with TE/TR = 32/5,000 ms, 64 measurements, and 1,024 data points. The differing physicochemical properties of lipid within and without the muscle cell cause the IMCL protons to resonate at a different frequency to lipid outside the muscle cell. The peak intensities of the creatine signal (3.02 ppm) and IMCL signal (1.32 ppm) were measured and expressed as a ratio.[6]

NIRS has been validated as a useful noninvasive and reproducible method of monitoring skeletal muscle oxygenation.[7] A transmit/receive device (RunMan; NIM Inc, Philadelphia, PA) placed over the forearm muscle (flexor digitorum superficialis) of the subject's dominant arm emits light into the muscle and measures the reflected light at 760 and 850 nm. The difference in reflected light intensity at these two wavelengths gives a relative measure of the level of hemoglobin (Hb) oxygenation within the postcapillary venules. After 60 seconds of finger flexion exercise, a cuff was inflated around the subject's upper arm to 20 mm Hg above systolic blood pressure as exercise continued for a further 60 seconds. The cuff remained inflated for 10 seconds after cessation of exercise (the point of maximal Hb deoxygenation) and then rapidly released. Half-time Hb reoxygenation of the muscle (used as a relative measure of oxygen and blood supply to the tissue) was determined from the point of maximal Hb deoxygenation and time taken to total metabolic recovery after cuff release.

More than 2-fold interindividual variation was found in the rate of reoxygenation of the forearm muscle after ischemic exercise. Pearson correlation analysis of the IMCL:creatine ratio against postischemic muscle reoxygenation rate (Fig 1) showed a positive correlation (n = 12, $r = .70$, $P = .01$).

The results of our study in healthy normotensive young women point to a possible role of skeletal muscle perfusion in

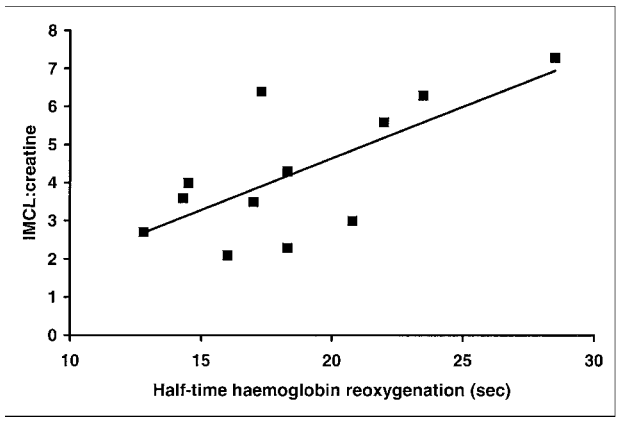

Fig 1. IMCL:creatine ratio in relation to half-time reoxygenation rate of postischemic forearm flexor muscle.

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the accumulation of IMCL. Animal studies investigating the metabolic effect of hypoxia on isolated cardiac muscle tissue have found that hypoxic tissue tends to accumulate more intracellular triglyceride than normally perfused tissue, while nonesterified fatty acid uptake remains unchanged.[8] These findings, coupled with our results, give grounds for speculation that IMCL content is influenced by oxygen supply. A relatively reduced blood, and hence oxygen, delivery might limit the capacity of skeletal muscle cells to oxidize fatty acids and result in lipid storage and accumulation of IMCL. Methodologic limitations prevented the measurement of IMCL content and oxygen delivery using the same muscle group. We have made our conclusions based on the assumption that the human forearm is a well-established model for flow studies[1,9,10] and that blood flow and oxygen supply to the forearm represent whole body skeletal muscle perfusion. The presence of a correlation despite possible differences in muscle fiber type distribution of the two muscles is an interesting finding that deserves further investigation. To conclude, we have demonstrated IMCL accumulation in subjects with relatively poor skeletal muscle oxygen supply, suggesting that efficiency of oxygen delivery may be a determinant of IMCL level. The coexistence of these phenomena and the association of each with insulin resistance support a pathogenic role for muscle oxygen supply in this condition.

REFERENCES

1. Fossum E, Hoieggen A, Moan A, et al: Relationship between insulin sensitivity and maximal forearm blood flow in young men. Hypertension 32:838-843, 1998

2. Forouhi NG, Fenkinson G, Thomas EL, et al: Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. Diabetologia 42:932-935, 1999

3. Phillips DIW, Caddy S, Ilic V, et al: Intramuscular triglyceride and muscle insulin sensitivity: Evidence for a relationship in nondiabetic subjects. Metabolism 45:947-950, 1996

4. Boesch C, Slotboom J, Hoppeler H, et al: In vivo determination of intramyocellular lipids in human muscle by means of localized ^{1}H-MR-spectroscopy. Magn Reson Med 37:484-493, 1997

5. Thompson C, Swan V: Measuring muscle metabolism. Todays Life Sci Nov/Dec:33-36, 1998

6. Jacob S, Machann J, Rett K, et al: Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. Diabetes 48:1113-1119, 1999

7. Mancini D, Bolinger L, Li H, et al: Validation of near-infrared spectroscopy in humans. J Appl Physiol 77:2740-2747, 1994

8. Balschi JA, Hai JO, Wolkowicz PE, et al: ^{1}H NMR measurement of triacylglycerol accumulation in the post-ischaemic canine heart after transient increase of plasma lipids. J Mol Cardiol 29:471-480, 1997

9. Steinberg H, Chaker H, Leaming R, et al: Obesity/insulin resistance is associated with endothelial dysfunction. J Clin Invest 97:2601-2610, 1996

10. Sahlin K: Non-invasive measurements of O_2 availability in human skeletal muscle with near-infrared spectroscopy. Int J Sports Med 13:S157-S160, 1992 (suppl)