Intramyocellular lipid (IMCL) is associated with metabolic dysfunction in aging. Exercise is known to influence IMCL accumulation and was recently observed to have a direct association with vitamin D status (25(OH)D) in our lab. In addition, we observed an inverse association between IMCL and dietary unsaturated fatty acid (USFA) to saturated fatty acid (SFA) ratio that was independent of age, BMI, and physical activity. The purpose of this study was to further examine the relationship between IMCL, vitamin D status, and dietary fatty acid intake in older adults with and without the combined effect of an aerobic training (AT) program. Healthy participants (age≥60) were randomized into a program of 7-consecutive days of AT or were asked to maintain regular daily routine. Anthropometrics, dietary recalls, and 25(OH)D were measured. Magnetic resonance spectroscopy was performed to analyze IMCL noninvasively. Average age and BMI were 67.2±5 years and 25.4±4 kg/m², respectively. 25(OH)D was not associated with IMCL between groups or over time. Participants who completed the AT program (n=13) had lower IMCL at end study (0.28±0.19 au) than those who remained sedentary (n=9; 0.46±0.26 au). IMCL percent change decreased in participants who exercised (-17.01±73%) and increased in those who remained sedentary (4.9±35%). Dietary fatty acid intake and IMCL were significantly related in participants who were involved in the AT program; whereas this relationship was not found in those who maintained their regular daily routine. As polyunsaturated fatty acid to SFA ratio increased, IMCL decreased in the AT trained participants (n=13; p=0.003). These results suggest that AT combined with a diet higher in USFA may result in greater muscle lipid turnover in aging when compared to exercise alone. Future studies should examine the interaction between dietary fatty acid intake and exercise on muscle lipid turnover to determine if metabolic function is altered.