Title: TRIGLYCERIDE LIPASE, NOT PHOSPHOLIPASE A_2, ACTIVITY IS ELEVATED IN SKELETAL MUSCLE FROM MALIGNANT HYPERTHERMIA SUSCEPTIBLES

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Introduction. Abnormally high free fatty acid (FFA) production has been observed in incubates of human whole skeletal muscle homogenates from malignant hyperthermia (MH) susceptibles (1). However, the source of these FFAs has not been defined. Lipid metabolism is reviewed in (2). Briefly, there are two major sources of FFAs: 1) phospholipid, by the action of phospholipase A_2 (PLA_2) activity and 2) triglyceride (TG), by the action of TG lipase. PLA_2 activity is associated with an increase in arachidonic acid (AA) in the FFA pool. AA, a fatty acid precursor of prostaglandins and leukotrienes, is not released to any great extent by TG lipase. Fatty acids derived from TGs would not readily build up in the FFA pool, as they are quickly removed by ß-oxidation, a major means of energy and heat production. Therefore, increased TG lipase activity, would cause a decrease in the fatty acid composition of the TGs, not a buildup in the FFAs. The present study examines the activities of PLA_2 and TG lipase in skeletal muscle used for testing MH susceptibility.

Methods. Approval by the Hahnemann University Human Studies Committee was obtained for this study. The preparation and stimulation of vastus lateralis muscle strips from patients referred for MH diagnosis was the same as previously described (1). Muscle strips were mounted at an initial resting tension of 2 g and stimulated at 0.2 Hz in a tissue bath containing Krebs solution at 37°C (pH 7.4) bubbled with O_2:CO_2 (95:5). Halothane 3% in O_2:CO_2 (95:5), when used, was then bubbled through the chamber. The halothane concentration in the gas phase was confirmed by gas chromatography. The muscle from each patient was then catagorized by the contracture response of 6-8 fiber bundles to halothane. The muscle was designated: 1) MH-, all contractures < 0.7 g; 2) MH+, any strip ≥ 0.7 g and average response < 1 g and ; 3) MH++, average response ≥ 1 g. Excess muscle from contracture testing was frozen in liquid N_2. To reduce the production of FFAs during lipid extraction (3), frozen samples were pulverized in a mortar suspended in liquid N_2, homogenized in methanol (4°C) and the lipids extracted and analyzed, as previously described for porcine skeletal muscle (4). Briefly, neutral lipids were separated on silica gel plates by one-dimensional thin-layer chromatography. FFAs and TGs were analyzed as methyl esters by gas chromatography using internal standards. These values were corrected for whole muscle protein. Data in Tables 1 and 2 were analyzed with a one-way analysis of variance ($p<0.05$) and Duncan's multiple-range test for each fatty acid type. Values are mean±SE for the indicated number of patients.

Results. Due to space limitations not all fatty acids analyzed are in Tables 1 and 2. There were no differences between MH-, MH+ and MH++ muscle in respect to AA production, the major product of PLA_2 activity (Table 1). TGs, the only other major source of FFAs in skeletal muscle, do not contain large amounts of AA (Table 2). Muscle highly responsive to halothane (MH++) in the contracture test exhibits greater TG breakdown, as judged by lower palmitoleic and oleic acid values compared to MH- muscle (Table 2).

Discussion. The FFAs in skeletal muscle from MH susceptibles have not previously been characterized by type, nor has PLA_2 activity been directly determined. The present investigation suggests there is no difference in PLA_2 activity in MH-, MH+ and MH++ muscle used in the contracture test, as judged by comparable free AA levels. In contrast, TG lipase activity is elevated in MH, as indicated by lower levels of fatty acids in the TGs from MH++ muscle. TGs, which are in abundance in skeletal muscle mitochondria, are a significant heat and energy source (5). In addition, some of the FFAs liberated by TG lipase could act in synergy with halothane to induce membrane leakage (6). Both of these effects of TG breakdown may be important in MH. These results support elevated TG lipase, not PLA_2, activity in humans with MH.

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TABLE 1. Free fatty acids - human vastus lateralis

		Free Fatty Acids (pmol/mg protein)				
Group	n	16:0	16:1	18:0	18:1	20:4
MH-	12	538±51	54±7	436±41	436±50	591±91
MH+	15	523±28	70±8	400±44	478±31	516±67
MH++	5	561±59	50±7	453±37	400±58	476±95

Abbreviations: 16:0, palmitic acid; 16:1, palmitoleic; 18:0, stearic acid; 18:1, oleic acid; 20:4, arachidonic acid.

TABLE 2. Fatty acid composition of triglycerides

		Fatty Acids (nmol/mg protein)				
Group	n	16:0	16:1	18:0	18:1	20:4
MH-	13	14±2	3.5±0.4	3.5±0.4	23±2	0.4±0.0
MH+	11	12±1	2.6±0.3	3.6±0.3	21±1	0.3±0.0
MH++	6	10±1	2.0±0.2*	3.2±0.4	17±1*	0.2±0.0

Abbreviations: see Table 1. *P<.05 less than MH-.