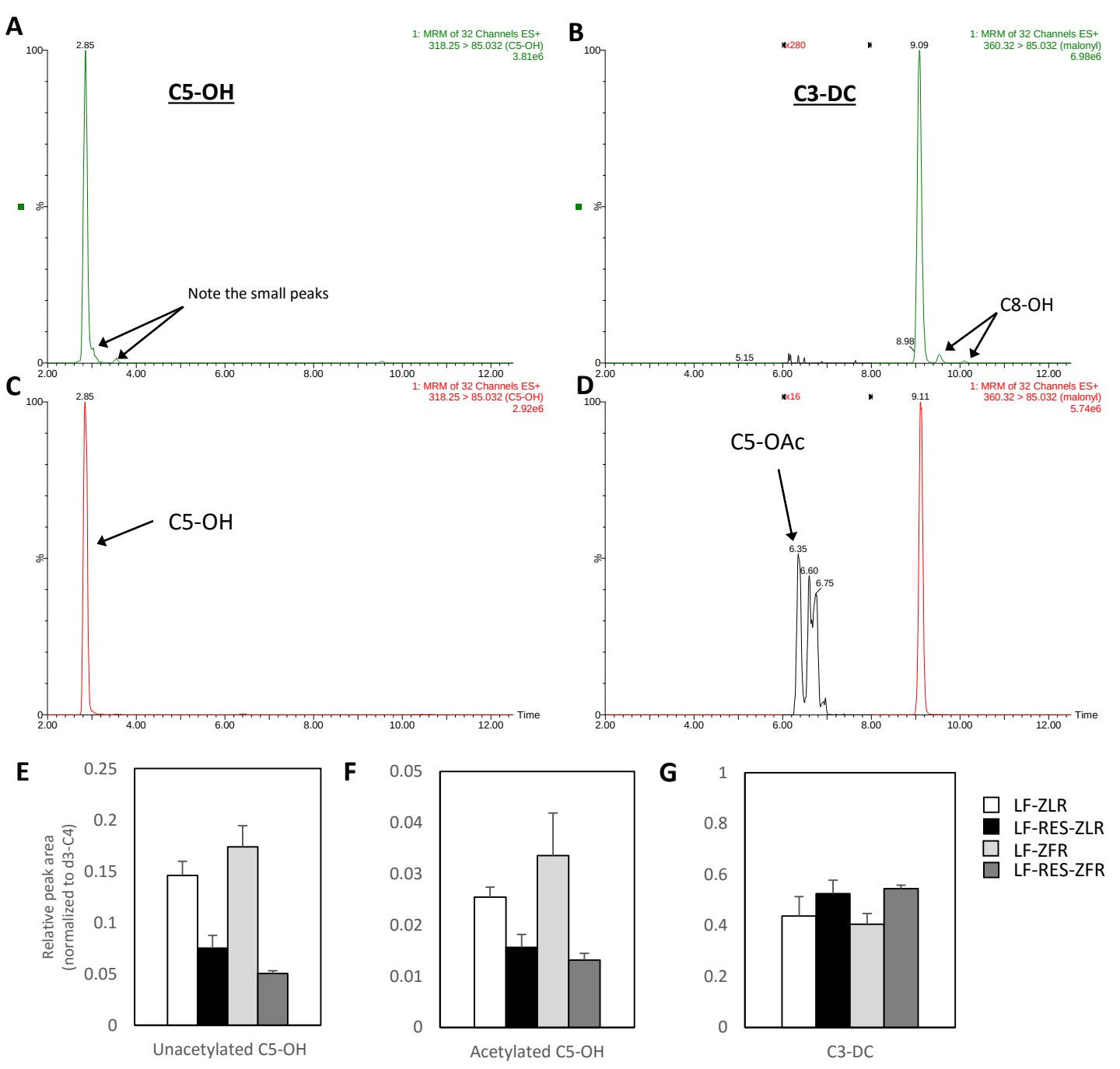

Supplemental Figure 1. Separation of skeletal muscle C5-OH and C3-DC carnitines by LC-MS/MS. To distinguish C5-OH from C3-DC the LC-MS/MS method of Maeda *et al*. [S1] was extensively modified. Acylcarnitines were derivatized to butyl esters, and in a subsequent reaction, to acetyl esters. The analytical platform was converted to a UPLC format using an Acquity UPLC HSS T3 column and the ion pairing reagent used was triethyl ammonium acetate. The carnitines were eluted with a linear gradient using water as solvent A and 95/5 v/v acetonitrile/water as solvent B starting at 20%-B. Panels **(A)** and **(B)** are the MRMs for 3-hydroxyisovaleryl/3-hydroxy-2-methylbutyryl carnitine (C5-OH) and malonyl carnitine (C3-DC) respectively. Panels **(C)** and **(D)** are the MRMs for C5-OH and C3-DC for the same samples after acetylation. Note the disappearance of the small peaks adjacent to the large peak at 2.85 min in panel **(A)** and the peaks labeled C8-OH in panel **(C)** (butyl esters of C8-OH are isobaric with the butyl ester of malonyl carnitine). Also, note the appearance of peaks in the MRM for malonyl carnitine in panel **(D)**. The region from 6-8 min in panel **(D)** has been magnified 16 times. The same region in panel **(B)** has been magnified 280 times in the unacetylated sample. These peaks labeled C5-Oac in panel **(D)** result from the acetylation of isomers of C5-OH, i.e., a mass shift of 42 amu. Panels **(E-G)** represent quantification of unacetylated C5-OH, acetylated C5-OH and C3-DC in 3 skeletal muscle samples per group. Note that the pattern for both unacetylated and acetylated C5-OH closely resembles the C5-OH/C3-DC data presented in Fig 5I but this is not the case for C3-DC. We interpret this to mean that C5-OH is the metabolite responsible for the group differences presented in Fig 5I.

Supplemental Reference

[S1] Maeda Y, Ito T, Suzuki A, Kurono Y, Ueta A, Yokoi K, et al. Simultaneous quantification of acylcarnitine isomers containing dicarboxylic acylcarnitines in human serum and urine by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid communications in mass spectrometry: RCM. 2007;21(5):799–806. doi: 10.1002/rcm.2905