DEVELOPMENT OF AN ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY METHOD FOR MEASUREMENT OF MYOFIBRILLAR PROTEIN SYNTHESIS: APPLICATION TO ANALYSIS OF MURINE MUSCLES DURING CANCER CACHEXIA

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Cachexia, characterized by loss of skeletal muscle mass, is a major contributory factor to patient morbidity and mortality during cancer. The intracellular regulation of muscle wasting is affected by the muscle's capacity for oxidative metabolism. However, there are no reports on the rate of myofibrillar protein synthesis (MPS) in skeletal muscles that vary in primary metabolic phenotype during cachexia, in large part because of the small size of plantaris and soleus muscle, and regional differences in larger muscles in the mouse. In this paper we describe a sensitive method for measurement of MPS in murine tissue and apply this measurement to analysis of myofibrillar protein synthesis in specific muscles of mice with and without colon cancer cachexia.

A mouse model of colorectal cancer cachexia ($Apc^{Min/+}$) and their controls (C57BL/6) were injected with a loading dose of deuterated phenylalanine (D_5F) and myofibrillar proteins extracted from skeletal muscles at 30 min. The relative concentrations of D_5F and naturally occurring phenylalanine (F) in the myofibrillar proteins and the amino acid pool from 5 mg wet weight of muscle were quantified on an ultra performance liquid chromatograph (UPLC), coupled to an electro-spray triple quadruple mass spectrometer operated in multiple reaction monitoring mode. The rate of MPS was determined from the ratio of D_5F: F in the protein fraction, compared to the amino acid pool.

The rate of MPS, measured in 2-5 mg muscle protein, was reduced by ~ 65% in cachectic $Apc^{Min/+}$ compared to the control C57BL/6 mice in the soleus, plantaris, oxidative (red) and glycolytic (white) regions of the gastrocnemius, and diaphragm. The rate of MPS was significantly higher in the oxidative vs. glycolytic gastrocnemius muscle.

A sensitive UPLC-MS method has been developed to measure the rate of MPS in mouse muscles. The method does not require chemical derivatization of the analyte and is sufficiently sensitive that it can be applied for analysis of MPS in 2-5 mg quantities of mouse gastrocnemius, soleus, plantaris, diaphragm and other muscle. This method could be further applied to other animal models for quantifying effects of cancer and cancer therapies on protein synthesis in cachexia.