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Dietary methionine restriction: Effects on glucose tolerance, lipid content and micro-RNA composition in the muscle of rainbow trout

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ABSTRACT

Lean muscle mass plays an important role in overall health, as altered skeletal muscle metabolism can impact both the incidence and prevention of conditions related to metabolic health. Intriguingly, dietary methionine restriction (MR) has been shown to ameliorate this phenotype over time potentially through mechanisms related to changes in myogenic precursor cell (MPC) differentiation status. Recently the role of micro-RNAs (miRs) in regulating the expression of muscle specific transcription factors myoD and myogenin as well as signaling molecules involved in skeletal muscle differentiation has been reported *in vitro*. We performed an 8 week feeding trial to determine if MR *in vivo* could alter miR abundance as well as change metabolic markers. Results show changes in muscle miR abundance for miR-133a at 4 weeks with no significant difference seen in miR-210 or miR-206. After 8 weeks of MR feeding fish demonstrated increased clearance of glucose, increased fat accumulation in the liver, and decreased fat accumulation in the muscle. These data demonstrate conservation of MR effects on fish metabolism, and suggest, for the first time, that miR-133a might play a role in tissue response to MR.

1. Introduction

Methionine restriction (MR) alters metabolic function producing a desirable phenotype of increased energy expenditure (Malloy et al., 2006; Caro et al., 2009; Orentreich et al., 1993), limited fat deposition, and enhanced insulin sensitivity (Stone et al., 2014; Richie et al., 1994). While the beneficial effects of MR on metabolism have classically been attributed to decreases in visceral fat deposition, insulin, glucose, and leptin, a definitive mechanism of action remains elusive. To better understand the pathways involved in MR this paper utilizes the rainbow trout (*Oncorhynchus mykiss*) as a fish model of glucose intolerance. Rainbow trout are glucose intolerant as they exhibit poor utilization of glucose following both oral glucose administration and high carbohydrate meals (Bergot, 1979; Brauge et al., 1995; Legate et al., 2001). Although the beneficial phenotype of MR is multifactorial, recent articles have begun exploring the role microRNA's (miR's) play in the overall presentation of MR effect (Plummer et al., 2017; Latimer et al., 2017).

MiR's are small 18–22 nucleotide sequences produced from double stranded precursors (Carthew and Sontheimer, 2009; Flores-Jasso et al., 2009). These regulatory sequences undergo extensive post-transcriptional modification before targeting multiple mRNA transcripts for regulation (Ha and Kim, 2014; Allison et al., 2014). MiRs can suppress both transcription as well as translation of target transcription factors and mRNA transcripts by complementary base-pairing. Having been studied considerably since their discovery in *C. elegans* and *D. melanogaster* over 15 years ago (Lagos-Quintana et al., 2001; Lau et al., 2001), roles in pathological processes (*i.e.* cancer), developmental timing, apoptosis, proliferation, differentiation, and organ development (Ambros, 2003; Esquela-Kerscher and Slack, 2006) have been identified.

In the skeletal muscle miRs involved in proliferation and differentiation have been identified as muscle specific miRs (myo-miRs); including miR-133a, miR-1, and miR-206 (Chen et al., 2006; Kim et al., 2006). Changes in miRNA composition during MR have recently been investigated *in vivo* in mice (Plummer et al., 2017) as well as *in vitro* in rainbow trout myocytes (Latimer et al., 2017). The present study examines myo-miRs changes during MR *in vivo* in skeletal muscle. This study presents the first evidence suggesting short-term MR leads to decreases in miR-133a abundance and overall improvement in glucose tolerance following a challenge.

Abbreviations: miR, micro-RNA; MR, methionine restricted; MS, methionine sufficient

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Table 1
Breakdown of methionine restricted (MR, Met −) and methionine sufficient (MS, Met +) diets.

Ingredient (g per 100 g)	Met +	Met −
Soy protein isolate	32.5	32.5
Fish protein hydrolysate	10	10
Casein - vitafree	7.5	7.5
Wheat starch	13.28	13.50
Dextrin	6.39	6.50
Menhaden fish oil	10	10
Alginate (TIC Algin 400)	5.38	5.38
Soy lecithin	4	4
Vitamin mix (MP-VDFM)	4	4
Mineral mix BTm	3	3
Canthaxanthin (10%)	2.31	2.31
Potassium phosphate monobasic	1.15	1.15
DL methionine	0.33	0
Cholesterol	0.12	0.12
Ascorbypalmitate	0.04	0.04
Total	100	100

2. Materials and methods

2.1. Dietary methionine restriction

Excess juvenile rainbow trout (~50 g, all female) produced from the breeding program at the National Center for Cool and Cold Water Aquaculture were placed into six tanks (28/tank, 150 L volume, NCCCWA IACUC protocol #098). Body weights were recorded on individual fish at the beginning of the study and after 8 weeks of feeding experimental diets. Average body weight was recorded by weight tank biomass at the experimental mid-point (4 weeks). Three of the six tanks were fed a methionine restricted feed "Met −" while the remaining three were fed a methionine sufficient feed "Met +" (Table 1).

2.2. Diet formulation

Diets designed by NORC Aquatic Animal Research Core at UAB included three protein sources (soy protein isolate, fish protein hydrolysate, casein) and were adjusted for methionine content using crystalline methionine (Met + = 1.10%, Met − = 0.775% methionine). Diets were adjusted for methionine content by replacing the amino acid dry matter with wheat starch and dextrin to remain iso-caloric. Diets were extruded, dried to approximately a 9% moisture content, and ground to a crumble for feeding. Fish were hand fed twice daily at approximately 2% of tank biomass for 8 weeks. Fish were harvested at 4 and 8 weeks using a lethal dose of MS222 (tricaine methanesulfonate, 250 mg/L).

2.3. Chemical carcass analysis

To determine total hepatic lipid content by Chemical Carcass Analysis (modified from Folch et al., 1957) fish were euthanized by submersion in (MS-222) and whole viscera frozen at −80 °C until analysis. At time of analysis total viscera was weighed for calculation of viscerosomatic indices (VSI) as well as individual liver for calculation of hepatosomatic indices (HSI). Individual liver and muscle samples were analyzed for total lipids using an adaptation of the protocol described by Fowler et al. (2016). Briefly, livers were freeze dried for 48 h until attainment of stable weight before being minced and weighed for triplicate samples (~150 mg) placed into individual 40 mL glass vials. To each sample 25 mL of a solution of 2:1 v:v chloroform:methanol was added before being heated to 60 °C for a period of 30 min. Following heating vials were left to stand for 10 min before the addition of solvent to return vials to 25 mL total volume. Samples were then filtered and 4 mL of Nano-pure water was added before agitation and centrifugation. The upper phase was then removed and the lower phase evaporated under air in a 50 °C water bath. Lipid was transferred to pre-weighed shell vials using three separate chloroform rinses evaporated under air. Dry vials were then placed in a vacuum desiccator for 48 h before final weight of lipid was determined.

2.4. RNA extraction and miR quantification

Immediately after euthanasia, muscle samples were taken from the epaxial muscle, snap frozen in liquid nitrogen, and stored at −80 °C until analysis. RNA was extracted using RNAzol® (SigmaAldrich) following manufacturer's instructions for isolation of total RNA. miRs were reverse transcribed using the Mysti Cq® microRNA cDNA Synthesis mix (Sigma Aldrich) following manufacturer's instructions. miR quantification in the muscle was done following previously-described methods (Latimer et al., 2017; Pfaffl, 2001). For miR quantification forward primer sequences (Eurofins; Huntsville, AL) for miR-133a: TTGGTCCCCTTCAACCAGCTG, miR-210: AGCCACTGACTAACGTACATTG, and miR-375: TTTGTTCGTTCGGCTCGCGTTA were taken directly from (Juanchich et al., 2016) with universal reverse primers. RT products were diluted 1:20 and reaction mix was 5 µL cDNA, 5 µL SYBR® Green Master Mix, and 0.5 µL primer mix (300 nM). PCR (Applied Biosystems, StepOne Plus) was performed with cycling protocol 20 s at 94 °C, 40 cycles of 3 s 94 °C and 30 s 60 °C, miR-133a and miR-210, 62 °C miR-375. Relative abundance was calculated from a serially diluted 1:1–1:256 pool of cDNA using the Applied Biosystems software. All miR's were normalized by comparison to a reference miR (miR-375) found to be stable in muscle samples following methionine restriction (Latimer et al., 2017).

2.5. Glucose tolerance

In order to determine the effect of dietary methionine on glucose tolerance, fish were subjected to a glucose tolerance test at the cessation of feeding. Methods used followed Figueiredo-Silva et al. (2012) with modifications for glucose load. Briefly eight trout from each treatment were fasted for 24 h following the final 8 week feeding and injected intraperitoneally with glucose (250 mg/kg body weight) suspended in sterile 0.6% saline. Blood samples were drawn from caudal vasculature at 0 (baseline), 3, 6, and 12 h post injection. Glucose load was measured using the Accu-Check™ glucometer (Cooke et al., 2008; Figueiredo-Silva et al., 2012; Galt et al., 2014).

2.6. Statistical methods

Glucose area under the curve was calculated using the trapezoidal rule, differences in area under the curve were analyzed using an unpaired *t*-test. Hepatosomatic and viscerosomatic indices, muscle and hepatic lipid content, as well as miRNA composition were analyzed using a Two-Way ANOVA. All analyses done in Prism v7 (Graph Pad).

3. Results

3.1. Body condition/chemical carcass analysis

Overall fish weight at 8 weeks was similar for methionine restricted (MR, Met −) and methionine sufficient (MS, Met +) groups (Table 2). To determine overall body condition both the hepatosomatic index and viscerosomatic index were calculated at 4 weeks and 8 weeks. These two values did not differ between the groups (Fig. 1). Lipid analysis revealed that liver lipid content was significantly increased ($p = 0.028$) at 8 weeks in fish receiving the Met − feed (Fig. 2B), although no difference ($p = 0.528$) was observed at 4 weeks. Muscle analysis also revealed decreased muscle lipid accumulation at 8 weeks in MR fish ($p = 0.0151$, Fig. 2D) with no difference at 4 weeks.

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