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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 in 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, the miR-133a might play a role in tissue response to MR.

Keywords

Muscle; Rainbow Trout; Micro-RNAs; Methionine Restriction

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

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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., 2017b).

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., 2017b). 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.

Materials and Methods

Dietary Methionine Restriction

Excess juvenile rainbow trout (~50g, 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).

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).

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 (Fowler et al., 2016). Briefly, livers were freeze dried for 48h until attainment of stable weight before being minced and weighed for triplicate samples (~150mg) placed into individual 40ml glass vials. To each sample 25ml of a solution of 2:1 v:v chloroform:methanol was added before being heated to 60°C for a period of 30 minutes. Following heating vials were left to stand for 10 minutes before the addition of solvent to return vials to 25ml total volume. Samples were then filtered and 4ml 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 48h before final weight of lipid was determined.

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 RNeasy® (Qiagen) 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., 2017b, 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 5µl cDNA, 5µl SYBR® Green Master Mix, and 0.5µl primer mix (300nM). PCR (Applied Biosystems, StepOne Plus) was performed with cycling protocol 20s at 94°C , 40 cycles of 3s 94°C and 30s 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., 2017a).

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 (Figueiredo-Silva et al., 2012) with modifications for glucose load.

Briefly eight trout from each treatment were fasted for 24h 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 12h post injection. Glucose load was measured using the Accu-Check glucometer (Cooke, 2008, Figueiredo-Silva et al., 2012, Galt et al., 2014).

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).

Results

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 (Figure 1). Lipid analysis revealed that liver lipid content was significantly increased ($p=0.028$) at 8 weeks in fish receiving the Met- feed (Figure 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 ($p=0.0151$, Figure 2D) with no difference at 4 weeks.

miR Abundance and Glucose Tolerance

Following 4 weeks of MR, muscle miR-133a expression was significantly decreased (Figure 3; $p=0.0163$) compared to fish receiving MS diets. miR-210 and miR-206 were not affected by 4 or 8-weeks of MR (Figure 3). Glucose area under the curve was significantly lower in those fish receiving MR diets ($p=0.0005$; Figure 4).

Discussion

Methionine Restriction (MR) alters metabolism through mechanisms not yet well understood. It was previously published that MR's metabolic effects were largely a result of the accumulation of tRNAs leading to activation of the GCN2 kinase (EP Plaisance, 2012, Gallinetti et al., 2013). While this may still account for some of the metabolic effects it was recently shown that MR could also be sensed through a non-canonical PERK/NRF2 pathway (Wanders et al., 2016). Metabolic changes could also be partially explained by changes in miR abundance due to their dynamic nature and ability to target multiple mRNA transcripts.

Previously in cultured rainbow trout myocytes it was found that miR-133a was significantly reduced during MR (Latimer et al., 2017b). In this study, methionine restriction (MR) *in vivo* also decreased muscle miR-133a, mirroring the *in vitro* results. In addition juvenile rainbow trout on MR diets exhibited decreased index of glucose tolerance, increased liver lipid content, and decreased muscle lipid over 8 weeks.

miR-133a is known to be involved in the process of satellite cell differentiation in mammals (Chen et al., 2006) and is strongly regulated by the myogenic factors Myogenin and MyoD1 (Rao et al., 2006). miR-133a increases proliferation through repression of serum response factor (SRF) (Chen et al., 2006) and is induced during normal differentiation through its upstream binding sites for Myogenin and MyoD1 (Rao et al., 2006). Previous *in vitro* work in rainbow trout myocytes showed increases in Myogenin and MyoD1 during normal differentiation *in vitro* and showed an associated reduction in Myogenin and MyoD1 during MR (Latimer et al., 2017b), based on this evidence we suggest this miR has the same conserved functions in rainbow trout. The reduction in miR-133a could also be correlated with a decrease in growth seen in previous studies where MR diets were fed for prolonged periods of time (Craig and Moon, 2013, Alami-Durante et al., 2010) although a growth reduction was not seen here likely due to the relatively short duration of feeding. The suggested functional conservation of miR-133a could also explain the change in glucose tolerance observed through its strong regulation by insulin via action by SREBP-1c and MEF2C (Granjon et al., 2009) although this has only been shown in humans.

The observed reductions in miR-133a in response to MR suggest a potential role of miR-133a during methionine deficiency-related reductions in overall growth. Even though growth reductions were not detected in this study, it is likely that prolonged feeding of MR diets would elicit growth differences as observed elsewhere (Craig and Moon, 2013, Alami-Durante et al., 2010).

It is well demonstrated that glucose uptake and utilization is poor in multiple fish species when compared to mammalian values (Garin et al., 1987, Hama and Weber, 1996). Glucose uptake, especially during exercise, is primarily handled by the muscle tissue and ninety percent of rainbow trout muscle is characterized as white muscle (Johnston and Moon, 1981). While MR is known to increase glucose uptake at the muscle (Stone et al., 2014) a complete mechanism is not understood. The changes in miR-133a demonstrated here may play a role in increasing glucose uptake via its direct regulation by insulin concentrations (Granjon et al., 2009). Though this result must be interpreted cautiously as the decrease in miR-133a was only seen at 4 weeks with other hallmarks (decreased muscle lipid, increased glucose tolerance) seen at 8 weeks. It may suggest that early decreases in miR-133a signal the initial response of the organism to MR mediated by GCN2/EIF2 α or PERK/NRF2 pathways. The activation of these stress pathways has previously been demonstrated in rainbow trout liver during MR (Skiba-Cassy et al., 2016). This phenotypic response to MR is further demonstrated by the decrease in glucose area under the curve following a glucose challenge at 8 weeks.

Methionine/Choline deficient (MCD) diets have been used extensively to induce hepatic steatosis in mammals (Rinella et al., 2008, Itagaki et al., 2013). MCD diets have not been extensively investigated in fish species but our results indicate that methionine deficiency induces liver fat accumulation over a period of 8 weeks clarifying the increase in liver lipid observed at 8 weeks in fish on MR diets. While liver lipid increased, muscle lipid was significantly decreased at 8 weeks in fish receiving MR diets. It is well documented that intramyocellular lipid content is inversely correlated with insulin stimulated glucose uptake at the muscle in mammals and humans (Storlien et al., 1991, Pan et al., 1997, Phillips et al.,

1996, Krssak et al., 1999). Our results show that animals receiving MR diets exhibited increased glucose tolerance and decreased lipid content in the muscle. Therefore, the MR phenotype of improved metabolic regulation appears highly conserved across taxa.

Conclusion

Rainbow trout fed MR diets for 8 weeks exhibit metabolic hallmarks of MR in increased glucose tolerance following a challenge. This may be due to decreases in miR-133a signaling at the level of the muscle, inducing decreased muscle lipid accumulation and enhanced insulin sensitivity. While the mechanism of action is not yet understood, miRs in the muscle may play a role in mediating the response of rainbow trout to dietary MR.

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Abbreviations

miR	Micro-RNA
MR	Methionine Restricted
MS	Methionine Sufficient

References Cited

- ALAMI-DURANTE H, WRUTNIAK-CABELLO C, KAUSHIK SJ, MEDALE F. Skeletal muscle cellularity and expression of myogenic regulatory factors and myosin heavy chains in rainbow trout (*Oncorhynchus mykiss*): effects of changes in dietary plant protein sources and amino acid profiles. *Comp Biochem Physiol A Mol Integr Physiol*. 2010; 156:561–8. [PubMed: 20434580]
- ALLISON DB, ANTOINE LH, BALLINGER SW, BAMMAN MM, BIGA P, DARLEY-USMAR VM, FISHER G, GOHLKE JM, HALADE GV, HARTMAN JL, HUNTER GR, MESSINA JL, NAGY TR, PLAISANCE EP, POWELL ML, ROTH KA, SANDEL MW, SCHWARTZ TS, SMITH DL, SWEATT JD, TOLLEFSBOL TO, WATTS SA, YANG Y, ZHANG J, AUSTAD SN. Aging and energetics' 'Top 40' future research opportunities 2010–2013. *F1000Res*. 2014; 3:219. [PubMed: 25324965]
- AMBROS V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell*. 2003; 113:673–6. [PubMed: 12809598]
- BERGOT F. Specific problems posed by carbohydrate utilization in the rainbow trout. *Ann Nutr Aliment*. 1979; 33:247–57. [PubMed: 496176]
- BRAUGE C, CORRAZE G, MEDALE F. Effect of dietary levels of lipid and carbohydrate on growth performance, body composition, nitrogen excretion and plasma glucose levels in rainbow trout reared at 8 or 18 degrees C. *Reprod Nutr Dev*. 1995; 35:277–90. [PubMed: 7612167]
- CARO P, GOMEZ J, SANCHEZ I, NAUDI A, AYALA V, LOPEZ-TORRES M, PAMPLONA R, BARRIA G. Forty percent methionine restriction decreases mitochondrial oxygen radical production and leak at complex I during forward electron flow and lowers oxidative damage to proteins and

- mitochondrial DNA in rat kidney and brain mitochondria. *Rejuvenation Res.* 2009; 12:421–34. [PubMed: 20041736]
- CARTHEW RW, SONTHEIMER EJ. Origins and Mechanisms of miRNAs and siRNAs. *Cell.* 2009; 136:642–55. [PubMed: 19239886]
- CHEN JF, MANDEL EM, THOMSON JM, WU Q, CALLIS TE, HAMMOND SM, CONLON FL, WANG DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet.* 2006; 38:228–33. [PubMed: 16380711]
- COOKE SJ, SUSKI CD, DANYLCHUK SE, DANYLCHUK AJ, DONALDSON MR, PULLEN C, BULTE G, O'TOOLE A, MURCHIE KJ, KOPPELMAN JB, SCHULTZ AD, BROOKS E, GOLDBERG TL. Effects of different capture techniques on the physiological condition of bonefish *Albula vulpes* evaluated using field diagnostic tools. *Journal of Fish Biology.* 2008:1351–1375.
- CRAIG PM, MOON TW. Methionine restriction affects the phenotypic and transcriptional response of rainbow trout (*Oncorhynchus mykiss*) to carbohydrate-enriched diets. *Br J Nutr.* 2013; 109:402–12. [PubMed: 22583536]
- PLAISANCE EP, NV, ORGERON M, MCDANIEL AN, BEHRENS PH, GETTYS TW, ANTHONY TG. Role of General Control Nonderepressible 2 (GCN2) kinase in mediating responses to dietary methionine restriction. *FASEB J.* 2012:26.
- ESQUELA-KERSCHER A, SLACK FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer.* 2006; 6:259–69. [PubMed: 16557279]
- FIGUEIREDO-SILVA AC, PANSEERAT S, KAUSHIK S, GEURDEN I, POLAKOF S. High levels of dietary fat impair glucose homeostasis in rainbow trout. *J Exp Biol.* 2012; 215:169–78. [PubMed: 22162865]
- FLORES-JASSO CF, ARENAS-HUERTERO C, REYES JL, CONTRERAS-CUBAS C, COVARRUBIAS A, VACA L. First step in pre-miRNAs processing by human Dicer. *Acta Pharmacol Sin.* 2009; 30:1177–85. [PubMed: 19654583]
- FOLCH J, LEES M, SLOANE STANLEY GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* 1957; 226:497–509. [PubMed: 13428781]
- FOWLER LA, DENNIS LN, BARRY RJ, POWELL ML, WATTS SA, SMITH DL JR. In Vivo Determination of Body Composition in Zebrafish (*Danio rerio*) by Quantitative Magnetic Resonance. *Zebrafish.* 2016; 13:170–6. [PubMed: 26974510]
- GALLINETTI J, HARPUTLUGIL E, MITCHELL JR. Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. *Biochem J.* 2013; 449:1–10. [PubMed: 23216249]
- GALT NJ, FROEHLICH JM, MEYER BM, BARROWS FT, BIGA PR. High-fat diet reduces local myostatin-1 paralog expression and alters skeletal muscle lipid content in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiol Biochem.* 2014; 40:875–86. [PubMed: 24264425]
- GARIN D, ROMBAUT A, FREMINET A. Determination of glucose turnover in sea bass *Dicentrarchus labrax*. Comparative aspects of glucose utilization. *Comp Biochem Physiol B.* 1987; 87:981–8. [PubMed: 3665443]
- GRANJON A, GUSTIN MP, RIEUSSET J, LEFAI E, MEUGNIER E, GULLER I, CERUTTI C, PAULTRE C, DISSE E, RABASA-LHORET R, LAVILLE M, VIDAL H, ROME S. The microRNA signature in response to insulin reveals its implication in the transcriptional action of insulin in human skeletal muscle and the role of a sterol regulatory element-binding protein-1c/myocyte enhancer factor 2C pathway. *Diabetes.* 2009; 58:2555–64. [PubMed: 19720801]
- HA M, KIM VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol.* 2014; 15:509–24. [PubMed: 25027649]
- HAMAN F, WEBER JM. Continuous tracer infusion to measure in vivo metabolite turnover rates in trout. *J Exp Biol.* 1996; 199:1157–62. [PubMed: 9318994]
- ITAGAKI H, SHIMIZU K, MORIKAWA S, OGAWA K, EZAKI T. Morphological and functional characterization of non-alcoholic fatty liver disease induced by a methionine-choline-deficient diet in C57BL/6 mice. *Int J Clin Exp Pathol.* 2013; 6:2683–96. [PubMed: 24294355]
- JOHNSTON IA, MOON TW. Fine structure and metabolism of multiply innervated fast muscle fibres in teleost fish. *Cell Tissue Res.* 1981; 219:93–109. [PubMed: 7285101]

- JUANCHICH A, BARDOU P, RUE O, GABILLARD JC, GASPIN C, BOBE J, GUIGUEN Y. Characterization of an extensive rainbow trout miRNA transcriptome by next generation sequencing. *BMC Genomics*. 2016; 17:164. [PubMed: 26931235]
- KIM HK, LEE YS, SIVAPRASAD U, MALHOTRA A, DUTTA A. Muscle-specific microRNA miR-206 promotes muscle differentiation. *J Cell Biol*. 2006; 174:677–87. [PubMed: 16923828]
- KRSSAK M, FALK PETERSEN K, DRESNER A, DIPIETRO L, VOGEL SM, ROTHMAN DL, RODEN M, SHULMAN GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ¹H NMR spectroscopy study. *Diabetologia*. 1999; 42:113–6. [PubMed: 10027589]
- LAGOS-QUINTANA M, RAUHUT R, LENDECKEL W, TUSCHL T. Identification of novel genes coding for small expressed RNAs. *Science*. 2001; 294:853–8. [PubMed: 11679670]
- LATIMER M, SABIN N, LE CAM A, SEILIEZ I, BIGA P, GABILLARD JC. miR-210 expression is associated with methionine-induced differentiation of trout satellite cells. *J Exp Biol*. 2017a; 220:2932–2938. [PubMed: 28576820]
- LATIMER M, SABIN N, LE CAM A, SEILIEZ I, BIGA P, GABILLARD JC. miR-210 expression is associated with methionine-induced differentiation of trout satellite cells. *J Exp Biol*. 2017b
- LAU NC, LIM LP, WEINSTEIN EG, BARTEL DP. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science*. 2001; 294:858–62. [PubMed: 11679671]
- LEGATE NJ, BONEN A, MOON TW. Glucose tolerance and peripheral glucose utilization in rainbow trout (*Oncorhynchus mykiss*), American eel (*Anguilla rostrata*), and black bullhead catfish (*Ameiurus melas*). *Gen Comp Endocrinol*. 2001; 122:48–59. [PubMed: 11352553]
- MALLOY VL, KRAJCIK RA, BAILEY SJ, HRISTOPOULOS G, PLUMMER JD, ORENTREICH N. Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer 344 rats independent of energy restriction. *Aging Cell*. 2006; 5:305–14. [PubMed: 16800846]
- ORENTREICH N, MATIAS JR, DEFELICE A, ZIMMERMAN JA. Low methionine ingestion by rats extends life span. *J Nutr*. 1993; 123:269–74. [PubMed: 8429371]
- PAN DA, LILLIOJA S, KRIKETOS AD, MILNER MR, BAUR LA, BOGARDUS C, JENKINS AB, STORLIEN LH. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes*. 1997; 46:983–8. [PubMed: 9166669]
- PFÄFFL MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res*. 2001; 29:e45. [PubMed: 11328886]
- PHILLIPS DI, CADDY S, ILIC V, FIELDING BA, FRAYN KN, BORTHWICK AC, TAYLOR R. Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism*. 1996; 45:947–50. [PubMed: 8769349]
- PLUMMER J, PARK M, PERODIN F, HOROWITZ MC, HENS JR. Methionine-Restricted Diet Increases miRNAs That Can Target RUNX2 Expression and Alters Bone Structure in Young Mice. *J Cell Biochem*. 2017; 118:31–42. [PubMed: 27191548]
- RAO PK, KUMAR RM, FARKHONDEH M, BASKERVILLE S, LODISH HF. Myogenic factors that regulate expression of muscle-specific microRNAs. *Proc Natl Acad Sci U S A*. 2006; 103:8721–6. [PubMed: 16731620]
- RICHIE JP JR, LEUTZINGER Y, PARTHASARATHY S, MALLOY V, ORENTREICH N, ZIMMERMAN JA. Methionine restriction increases blood glutathione and longevity in F344 rats. *FASEB J*. 1994; 8:1302–7. [PubMed: 8001743]
- RINELLA ME, ELIAS MS, SMOLAK RR, FU T, BORENSZTAJN J, GREEN RM. Mechanisms of hepatic steatosis in mice fed a lipogenic methionine choline-deficient diet. *J Lipid Res*. 2008; 49:1068–76. [PubMed: 18227531]
- SKIBA-CASSY S, GEURDEN I, PANSEERAT S, SEILIEZ I. Dietary methionine imbalance alters the transcriptional regulation of genes involved in glucose, lipid, and amino acid metabolism in the liver of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 2016; 454:56–65.
- STONE KP, WANDERS D, ORGERON M, CORTEZ CC, GETTYS TW. Mechanisms of increased in vivo insulin sensitivity by dietary methionine restriction in mice. *Diabetes*. 2014; 63:3721–33. [PubMed: 24947368]

- STORLIEN LH, JENKINS AB, CHISHOLM DJ, PASCOE WS, KHOURI S, KRAEGEN EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes*. 1991; 40:280–9. [PubMed: 1991575]
- WANDERS D, STONE KP, FORNEY LA, CORTEZ CC, DILLE KN, SIMON J, XU M, HOTARD EC, NIKONOROVA IA, PETTIT AP, ANTHONY TG, GETTYS TW. Role of GCN2-Independent Signaling Through a Noncanonical PERK/NRF2 Pathway in the Physiological Responses to Dietary Methionine Restriction. *Diabetes*. 2016; 65:1499–510. [PubMed: 26936965]

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Figure 1. Hepatosomatic (A,B) and Viscerosomatic (C,D) indices at 4 weeks and 8 weeks following MR or MS feeding

Analysis of Hepatosomatic index (Liver Weight / Total Weight) and Viscerosomatic Index (Viscera Weight/ Total Weight) revealed no difference in body condition at 4 weeks or 8 weeks between MR (black bars) and MS (white bars) animals as indicated by Two-Way ANOVA (N=15). Error bars indicate standard deviation.

Figure 2. Liver (A,B) and Muscle (C,D) lipid content after 4 weeks and 8 weeks of MR or MS feeding

Analysis of total muscle and liver lipid by chemical carcass analysis revealed a significant ($p=0.0041$, $N=15$) increase in liver lipid at 8 weeks (B) and significant ($p=0.0187$, $N=15$) decrease in muscle lipid at 8 weeks (D) between MR (black bars) and MS (white bars) animals as indicated by Two-Way ANOVA. Errors bars indicate standard deviation.

Figure 3. Expression of miR-133a, miR-206, and miR-210 during 8 weeks of MR or MS feeding qPCR analysis of miRNA abundance. Asterisks indicate a significant difference ($p < 0.0163$, $N=15$) as indicated by Two-Way ANOVA. Error bars indicate standard deviation.

Figure 4. Analysis of Glucose Area under the curve following a Glucose Challenge at 8 weeks in fish fed MR or MS diets

Asterisks signify a significant difference in area under the curve ($p < 0.0005$, $N=9$) as indicated by Unpaired T-test.

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

Table 2

Rainbow trout growth presented as individual fish weight change calculated (Batch Weight / # of fish), all values in grams.

	Initial (wt g)	4 weeks (wt g)	8 weeks (wt g)	Total Change
Tank A (Met +)	46.4	87.8	142.4	96.0
Tank B (Met -)	50.6	91.8	162.1	111.5
Tank C (Met +)	47.6	91.2	154.8	107.2
Tank D (Met -)	45.1	91.4	147.2	102.1
Tank E (Met +)	48.1	95.6	148.2	100.0
Tank F (Met -)	42.8	98.1	141.4	98.6