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Compromised responses to dietary methionine restriction in adipose tissue but not liver of *ob/ob* mice

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Abstract

Objective—Dietary methionine restriction (MR) reduces adiposity, hepatic lipids, and increases overall insulin sensitivity in part by reducing lipogenic gene expression in liver, inducing browning of white adipose tissue (WAT), and enhancing the lipogenic and oxidative capacity of the remodeled WAT.

Methods—*Ob/ob* mice have compromised β -adrenergic receptor expression in adipose tissue, and were used to test whether MR could ameliorate obesity, insulin resistance, and disordered lipid metabolism.

Results—In contrast to responses in wild type-mice, MR failed to slow accumulation of adiposity, increase lipogenic and thermogenic gene expression in adipose tissue, reduce serum insulin, or increase serum adiponectin in *ob/ob* mice. However, MR produced comparable reductions in hepatic lipids and lipogenic gene expression in both genotypes. In addition, MR was fully effective in increasing insulin sensitivity in *adiponectin*^{-/-} mice.

Conclusions—These findings show that diet-induced changes in hepatic lipid metabolism are independent of weight loss and remodeling of WAT, and are not required for insulin sensitization. In contrast, the failure of *ob/ob* mice to mount a normal thermogenic response to MR suggests that the compromised responsiveness of adipose tissue to SNS input is an important component of the inability of the diet to correct their obesity and insulin resistance.

Keywords

amino acid; obesity; β -adrenergic receptors; leptin; lipid; adiponectin

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Introduction

Dietary methionine restriction (MR) produces a highly beneficial metabolic phenotype by increasing energy expenditure (EE), limiting fat deposition, and enhancing insulin sensitivity (1). The sensing and signaling systems that detect and coordinate the developmental progression of these responses are poorly defined, but it has become clear that MR provides regulatory input to multiple tissue-specific transcriptional programs. Recent work makes a compelling case that liver and adipose tissue are key targets of the diet, where the transcriptional responses effectively remodel the integration of lipid metabolism between the two tissues (2–5). For example, the MR diet diminishes the capacity of the liver to synthesize and export lipid, while simultaneously increasing these functions in white adipose tissue (WAT) (2). The respective transcriptional programs were identified by screening differentially expressed genes in each tissue against annotated databases to identify gene set enrichment within canonical pathways. Then an upstream regulator algorithm identified the transcription factors consistent with the observed programmatic changes in gene expression. In liver, the algorithm predicted that sterol regulatory element-binding proteins (SREBP)-1 and SREBP-2 (*Srebf1*, *Srebf2*), and carbohydrate response element binding protein (*Mlxipl*) were functioning as an inhibitory network to down-regulate 28 genes involved in lipid metabolism in response to the diet (2). In inguinal WAT, the algorithm predicted that SREBP-1 and PPAR α were activated by the diet and functioning as a network to increase expression of 23 genes involved in lipid metabolism (2). Although the underlying mechanisms are unclear, the observed decreases in hepatic *Srebf1*, *Srebf2*, and *Mlxipl* mRNA expression and reduction in nuclear SREBP-1c expression confirm the prediction of the algorithm for the observed decline in lipogenic capacity (2). In IWAT, the predicted mechanisms are somewhat less ambiguous since several lines of evidence indicate that dietary MR produces a chronic increase in SNS-dependent activation of β -adrenergic receptors in this tissue (6, 7). The morphological remodeling of the depot and increase in catabolic gene expression are characteristic of the observed browning produced by MR in this tissue (2, 6, 7). Although it is well known that cAMP-dependent activation of PKA increases expression of genes such as *Ucp1* and *Ppargc1a* through cAMP response elements in their promoters, it remains unclear how MR produced the observed up-regulation of SREBP1 and PPAR α -sensitive genes in IWAT. Recent work has shown that PKA-dependent activation of adipose triglyceride lipase (ATGL) serves the dual purpose of releasing free fatty acids for oxidation but also generating ligands for PPAR α and PPAR δ (8, 9). The resulting ATGL-mediated transactivation of PPARs is essential to the remodeling of WAT that occurs after chronic activation of β -adrenergic receptors (9). More interestingly, chronic adrenergic activation of adipose tissue produces a simultaneous up-regulation of both lipogenic and oxidative capacity across brown and white adipose depots (9). Therefore, the observed upregulation of PPAR-sensitive genes in IWAT after MR supports the view that the chronic adrenergic stimulation produced by the diet is regulating lipid metabolism through this mechanism (2). In contrast, cross-talk with SREBP1, if it is occurring, remains poorly understood.

The *ob/ob* mouse is a genetic model of obesity that develops hyperphagia, insulin resistance and disordered lipid metabolism after weaning (10). The mice become obese even when

pair-fed to lean littermates, due in part to more frequent entry and defective exit from torpor (11–13). In addition to a nonfunctional leptin gene (14), adipose tissue of *ob/ob* mice is resistant to mobilization of FFAs by adrenergic stimulation (15, 16). The molecular defect is compromised β_3 - and β_1 -adrenergic receptor expression in WAT (17, 18), resulting in minimal cAMP production and PKA activation when adipocytes are stimulated with sympathomimetics (19). Therefore, *ob/ob* mice were used to test whether this signaling defect would compromise the normal metabolic and transcriptional responses to MR. A broader goal was to test the ability of dietary MR to reverse the development of obesity in these mice. Using *ob/ob* mice fed the MR diet for 3 months, we show that many but not all of the metabolic and transcriptional responses to MR are compromised in *ob/ob* mice.

Methods

Animal Studies

Experiment 1—These experiments were reviewed and approved by the Pennington Institutional Animal Care and Use Committee based on guidelines established for the humane care and use of laboratory animals. The study was conducted using singly housed *ob/ob* mice (B6.Cg-Lepob/J #000632, Jackson Labs, Bar Harbor, ME) and +/? (WT) littermates obtained at ~28 days of age and adapted to the Control diet for one week. Thereafter, 8 animals of each genotype were randomly assigned to receive either the Control diet (0.86% methionine) or a methionine-restricted diet (0.17% methionine) for the subsequent 12 wks. The diets were from Dyets Inc. (Bethlehem, PA) as described previously (6) and were provided ad libitum. At 2 week intervals throughout the study, food consumption was measured over a 48h period. At the beginning and at 7 day intervals, body composition was determined by NMR using a Bruker Mouse Minispec (Bruker Optics, Billerica, MA). The instrument was calibrated to an external standard before each use, and body composition was determined per manufacturer's instructions. At experiment's end, animals were fasted for 4h and then sacrificed by decapitation following CO₂-induced narcosis. Trunk blood, liver, IWAT, and brown adipose tissue (BAT) were collected and the tissues were snap frozen in liquid nitrogen.

Experiment 2—These experiments were approved by the UT Southwestern Medical Center IACUC. Adiponectin knockout (*AdipoQ*^{-/-}) mice were generated as previously described (20). Beginning at 6 weeks of age, male *AdipoQ*^{-/-} mice and WT littermates were fed the control or MR diets as previously described for 8 weeks. Glucose tolerance tests were conducted after a 6h fast as before (20). Mice were fasted for 6h prior to harvesting blood.

Serum Analysis

Insulin and adiponectin were measured using ELISA kits (Millipore; Billerica, MA) in serum obtained from trunk blood postmortem.

Hepatic Triglyceride Analysis

Livers (~50 mg) were homogenized in 10 volumes of 0.5% Triton-X 100 using a Polytron homogenizer, followed by centrifugation for 5min at maximum speed. Triglyceride content was determined using a glycerol assay (21) after alkaline saponification of the supernatant.

Western Blot Analysis

Pulverized livers of mice, were lysed in RIPA buffer [50mM Tris, pH 7.4, 150mM NaCl, 1mM EDTA, 1% NP40, protease and phosphatase inhibitor mixes (Pierce, Thermo Fisher Scientific Inc., Rockford, IL)], aspirated 3 times with a 20G needle and centrifuged at 10,000 rpm for 10min. Protein concentration was determined using Lowry assay. For western blot analysis, denatured tissue lysates (15µg) were separated on a 12.5% criterion gel (Biorad, Hercules, CA), transferred onto a PVDF membrane (Biorad) (12V, overnight), and then probed with a FAS antibody (Santa Cruz, sc-48357; Santa Cruz, CA). To normalize FAS expression, membranes were re-probed with β-actin antibody (Sigma, St. Louis, MO). After incubations with anti-mouse secondary antibody, signals were detected using ECL (GE). Image J was used for densitometry measurements.

RNA Isolation and Real-time PCR

Total RNA was isolated from frozen mouse livers, IWAT, and RPWAT using an RNeasy Mini Kit (QIAGEN Inc., Valencia, CA). Two micrograms of total RNA were reverse transcribed to produce complementary DNA. Gene expression was measured by RT-PCR in an ABI Prism 7900HT (Applied Biosystems, Foster City, CA). 2^{-CT} method was used to determine relative change of expression between livers of control- and MR-fed mice. Primer sequences are listed in Table 1.

Statistics

Data were obtained from 16 *ob/ob* and 16 lean littermates, equally divided between the diets. Changes in body weight, adiposity and food consumption over time were analyzed within genotype using multiple t tests with the Holm-Sidak correction for multiple comparisons, and glucose tolerance tests were compared based on the area under the curves. Protection against type I errors was set at 5% ($\alpha=0.05$).

Results

The inhibitory effect of MR on weight gain was detected in WT mice after 18 days on the MR diet, and the differences expanded over the subsequent 9 weeks of the study (Fig. 1A). The body composition of WT mice on the respective diets was indistinguishable for the first 3 weeks, but at 4 weeks the adiposity of the two groups began to diverge (Fig. 1B). While adiposity of mice on the control diet increased steadily from 14% to 34% over the 12 week study, the increase in adiposity of WT mice on the MR diet was limited to 4% over the same period (Fig. 1B). WT mice on the MR diet also consumed more food than mice on the control diet. After 2 weeks and at all subsequent times, weight-adjusted food consumption was significantly higher in WT mice on the MR compared to the control diet (Fig. 1C). The difference was ~40% at 2 weeks and at all subsequent time point. In contrast to WT mice, body weights of *ob/ob* mice on each diet did not differ at the beginning or at any subsequent

time point for the duration of the study (Fig. 1A). Adiposity of the *ob/ob* mice at the beginning of the study (e.g., 32 days) was ~49% and increased to ~58% in both treatment groups over the subsequent 4 weeks (Fig. 1B). And although body weight continued to increase in both groups over the final 8 weeks of the study, adiposity stayed nearly constant around 58% in both groups (Fig. 1B). Food consumption was significantly higher in *ob/ob* mice on the MR compared to the control diet at the 2 week time point, but at each subsequent interval food consumption was indistinguishable between the groups (Fig. 1C). Together, these data show that dietary MR was unable to reverse or slow the development of post weaning obesity in *ob/ob* mice. In contrast, the MR diet significantly limited the normal post weaning increases in adiposity in WT mice despite the significant increase in food consumption in the MR group during the entire study.

To evaluate the impact of altered signaling through β -adrenergic receptors in adipose tissue, real time PCR was used to examine a number of transcriptional responses that are known to be regulated by MR in inguinal WAT. Dietary MR significantly increased mRNA expression of *Scd1* (Fig. 2A), *Fasn* (Fig. 2B), *Acaca* (Fig. 2C), *Ucp1* (Fig. 2D), *Ppargc1a* (Fig. 2F), and *Adrb3* (Fig. 2G) in IWAT of WT mice, but in each instance MR failed to change mRNA levels for any of these genes in *ob/ob* mice. The first three genes code enzymes for the rate limiting steps in fatty acid synthesis and triglyceride assembly, and were increased by 2- to 3-fold in WT mice. The expression levels of these three genes were comparable in WT and *ob/ob* mice on the control diet. Expression of the thermogenic gene, *Ucp1* was also comparable between the genotypes in mice on the control diet, but in this case MR increased mRNA levels by 10-fold in IWAT of WT mice (Fig. 2D). *Ppargc1a*, a transcriptional co-activator and marker of browning in WAT, was increased 2-fold by MR in WT but not in *ob/ob* mice (Fig. 2E). *Adrb3* mRNA, which codes for the β_3 -adrenergic receptor, was increased by 2-fold in the WT group. In contrast, *Adrb3* mRNA was nearly undetectable in *ob/ob* mice on the control diet and was unaffected by MR (Fig. 2F). In BAT, MR produced a significant increase in *Ucp1* mRNA in the WT group but no detectable change was attributable to the diet in *ob/ob* mice (Fig 2G). Collectively, these data show that neither of the transcriptional programs (e.g., lipogenesis, thermogenesis) that are normally induced by dietary MR in IWAT was responsive to the diet in *ob/ob* mice. Induction of the thermogenic gene, *Ucp1* was also compromised in BAT of *ob/ob* mice.

The MR diet reduced liver triglyceride to comparable levels in both genotypes (Fig. 3A) despite the fact that baseline levels were 3-fold higher in *ob/ob* mice on the control diet. The net reduction (3-fold) was larger in *ob/ob* mice than WT mice (2-fold), but the difference is mainly attributable to the higher basal triglyceride levels in *ob/ob* mice (Fig. 3A). Examination of key genes involved in *de novo* lipogenesis and triglyceride synthesis showed that dietary MR produced comparable reductions in *Scd1*, *Acaca*, and *Fasn* mRNA in both genotypes (Figs. 3B, 3C, 3D). Fig. 3E shows that the diet-induced decrease in *Fasn* mRNA was paralleled by comparable decreases ($P<0.05$) in FASN protein expression by the diet in both genotypes. As noted previously (1), MR produced an increase in hepatic *Fgf21* mRNA in WT mice, but the effect of MR in *ob/ob* mice did not reach significance (Fig. 3F). This was primarily explained by the elevated expression of *Fgf21* mRNA in *ob/ob* mice on the control diet (Fig. 3F). Together, these findings show that dietary MR was equally effective

in livers of WT and *ob/ob* mice, producing comparable reductions in expression of the 3 key determinants of lipogenic capacity within the liver. Consequently, dietary MR reduced hepatic triglyceride levels in both genotypes, fully reversing the hepatic steatosis in *ob/ob* mice.

Two endocrine responses normally produced by dietary MR include increased plasma adiponectin and reductions in fasting insulin (6, 22). In WT mice, dietary MR increased plasma adiponectin by 2.5-fold (Fig. 4A) and reduced fasting insulin by 3-fold (Fig. 4B). Plasma adiponectin in WT and *ob/ob* mice on the control diet did not differ, but in contrast to WT mice, MR failed to increase adiponectin in the *ob/ob* group. In animals on the control diet, fasting insulin was 5-fold higher in *ob/ob* mice than WT mice, indicative of the significant insulin resistance that develops in *ob/ob* mice after weaning (23). And in contrast to WT mice, dietary MR had no discernible effect on fasting insulin in the *ob/ob* group (Fig. 4B). These findings indicate that in *ob/ob* mice, MR was unable to increase circulating levels of the insulin-sensitizing hormone, adiponectin, or reduce the elevated fasting insulin levels.

To explore the role of adiponectin as a potential mediator of the insulin-sensitizing effects of dietary MR, surrogate measures of insulin sensitivity were evaluated in mice lacking the gene for adiponectin. MR inhibited weight gain in both WT and *AdipoQ*^{-/-} mice (Fig. 5A). Fig. 5B shows that the ability of dietary MR to reduce fasting insulin was not compromised in *AdipoQ*^{-/-} mice. If anything, the reduction in fasting insulin by MR was somewhat larger in *AdipoQ*^{-/-} mice than WT mice. There were slight differences in glucose tolerance between WT and *AdipoQ*^{-/-} mice on the control diet as previously reported (20), but in both genotypes, dietary MR produced a significant and comparable increase in glucose tolerance (Fig. 5C). Considered together, the data make a compelling case that adiponectin is not required and is not a mediator of the increase in insulin sensitivity produced by dietary MR.

Discussion

Dietary MR induces a coordinated series of transcriptional programs in peripheral tissues that are ultimately translated into effects on energy balance and improved overall insulin sensitivity (1, 2, 6, 7). In the present study, MR-induced changes in food intake, adiposity, insulin, adiponectin, and transcriptional effects in WAT were absent in *ob/ob* mice, whereas changes in the expression of hepatic lipogenic genes were preserved. Thus the goal of the present work is to gain a better understanding of how MR produces its effects by understanding why some of its effects were compromised in these mice. *Ob/ob* mice become hyperphagic and obese after weaning, but they also develop a number of endocrine and signaling defects that are secondary to the absence of leptin (14, 24, 25). The higher metabolic efficiency, defective thermoregulatory thermogenesis, and compromised induction of *Ucp1* in BAT of *ob/ob* mice (11, 26) was originally attributed to lower norepinephrine turnover in BAT (27). However, more recent studies found normal norepinephrine turnover in BAT of *ob/ob* mice (28, 29), suggesting instead that compromised expression of β -adrenergic receptors (17, 18, 30) would limit the efficacy of SNS input in both BAT and WAT. Given that dietary MR regulates thermogenic gene expression by activating the SNS (6, 7), it is not surprising that induction of *Ucp1* in adipose

tissue from *ob/ob* mice was muted. The significance of this finding should not be underestimated, as recent work from our lab has shown that dietary MR is unable to increase energy expenditure and limit fat deposition in mice lacking *Ucp1* (31). Together, we interpret the inability of dietary MR to increase *Ucp1* in BAT and WAT of *ob/ob* mice as being a significant contributor to the inability of the diet to limit adipose tissue expansion in these mice.

An important unresolved question is whether refractoriness to SNS input is the only cause for failure of MR to increase *Ucp1* expression. Perhaps activation of the SNS by MR was also muted. The mechanisms underlying SNS activation by MR are unknown at present, but recent work showing that FGF21 acts centrally to increase SNS outflow (32) suggests that MR-dependent activation of hepatic transcription and release of FGF21 is a likely mechanism (1, 33). Together these findings support a mechanism where MR-dependent increases in hepatic FGF21 drive SNS stimulation of adipose tissue. FGF21 also acts directly on white adipocytes to induce browning (34) and enhance insulin-dependent glucose uptake (1). Thus, although MR failed to significantly increase hepatic *Fgf21* mRNA in *ob/ob* mice in the current study, their high basal expression suggests that FGF21 was not a limiting factor. Together, our data support a model in which low expression of β -adrenergic receptors in adipose tissue is important for the failure of MR to increase thermogenic genes in *ob/ob* mice.

Compromised activation of β -adrenergic receptors and downstream PKA-dependent targets also has the potential to affect genes in addition to those with cAMP response elements in their promoters. For example, work from Granneman's lab (8, 9, 35) has shown that PKA-dependent activation of hormone-sensitive lipase and ATGL serve the dual function of mobilizing fatty acids for oxidation and generating ligands that activate PPAR α and PPAR δ . The activated PPARs transactivate additional programs that remodel the capacity of adipocytes to both synthesize and oxidize lipids (9). Analysis of the transcriptional responses to MR in IWAT identified a coordinately upregulated set of genes linked to activation of PPAR α (2). Therefore, we suggest that the failure of MR to upregulate a subset of these lipogenic genes in IWAT of *ob/ob* mice may also be tied back to defective processing of β -adrenergic signals in these cells.

The major transcriptional effect of MR in liver is to coordinately downregulate the lipogenic capacity of the tissue (2, 4, 36). Using an upstream regulator algorithm designed to identify transcription factor involvement in observed patterns of gene expression, the algorithm identified an inhibitory network in the liver (2). The biological processes being down-regulated by this network were fatty acid and triglyceride synthesis, and surprisingly, despite the elevated hepatic triglyceride levels in *ob/ob* mice, dietary MR corrected their hepatic steatosis and significantly reduced the expression of *Scd1*, *Acaca*, and *Fasn* mRNA. Our previous work identified *Sreb1*, *Sreb2*, and *Mlx1* as the transcription factors being negatively regulated by MR (2), but did not address the mechanism of their regulation. A likely possibility is essential amino acid signaling, identified in HepG2 cells where culturing in media lacking any single essential amino acid resulted in decreased expression of *Fasn* (37). Restricting the availability of essential amino acids limits charging of tRNA with its cognate amino acid and activates signaling through general control nondepressible 2

(GCN2) (38). It remains unclear whether GCN2 is the nutrient sensor of MR that is responsible for down regulating lipogenic genes in the liver, but whatever the mechanism, our current data establish that the mechanism is fully intact in *ob/ob* mice.

Our findings that restricting dietary methionine to 0.17% in the diet modulates expression of hepatic lipogenic genes in *ob/ob* mice without affecting body weight are novel. Recent work (39) employed a more severe restriction of methionine (e.g., 0.12% methionine) in *ob/ob* mice and found that it reduced body weight, reversed the severity of hepatic steatosis, and reduced the expression of hepatic lipogenic genes. Adipose tissue accretion in *ob/ob* mice on the 0.12% methionine diet appears to have continued unimpaired, with the loss of body weight occurring at the expense of lean tissue (39). A second difference from our study is the 50% reduction in serum insulin produced by the 0.12% methionine diet (39) where we saw no reduction in insulin with the lesser degree of MR (0.17% methionine). This difference suggests that the weight loss obtained with the 0.12% methionine diet, despite no accompanying reduction in adiposity, afforded some improvement in overall insulin sensitivity. Since we saw equivalent reduction in hepatic lipids as Malloy et al. (39) without a reduction in fasting insulin, we conclude that the reduction in hepatic lipids obtained with both diets had little effect on overall insulin sensitivity.

Given the established insulin-sensitizing properties of adiponectin (20), the failure of dietary MR to increase serum adiponectin in *ob/ob* mice suggests the possibility that adiponectin may mediate the insulin-sensitizing effects of dietary MR. However, in mice lacking adiponectin, dietary MR produced an equivalent reduction in fasting insulin and improvement in glucose tolerance. Together, these findings establish that adiponectin does not mediate the improvements in insulin sensitivity produced by MR.

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What is already known about this subject?

- Methionine restriction improves insulin sensitivity and hepatic lipid profile, and reduces adiposity.
- Methionine restriction results in weight maintenance despite increased food intake.
- The methionine restriction-induced increase in energy expenditure may be the result of increased β -adrenergic input into adipose tissue.

What does your study add?

- Methionine restriction improves the hepatic lipid profile independent of insulin sensitivity, weight, and adiposity in *ob/ob* mice.
- Impaired β -adrenergic input in *ob/ob* mice correlates with a lack of adipose tissue response to methionine restriction.
- Adiponectin does not play a role in mediating the insulin-sensitizing effects of methionine restriction.

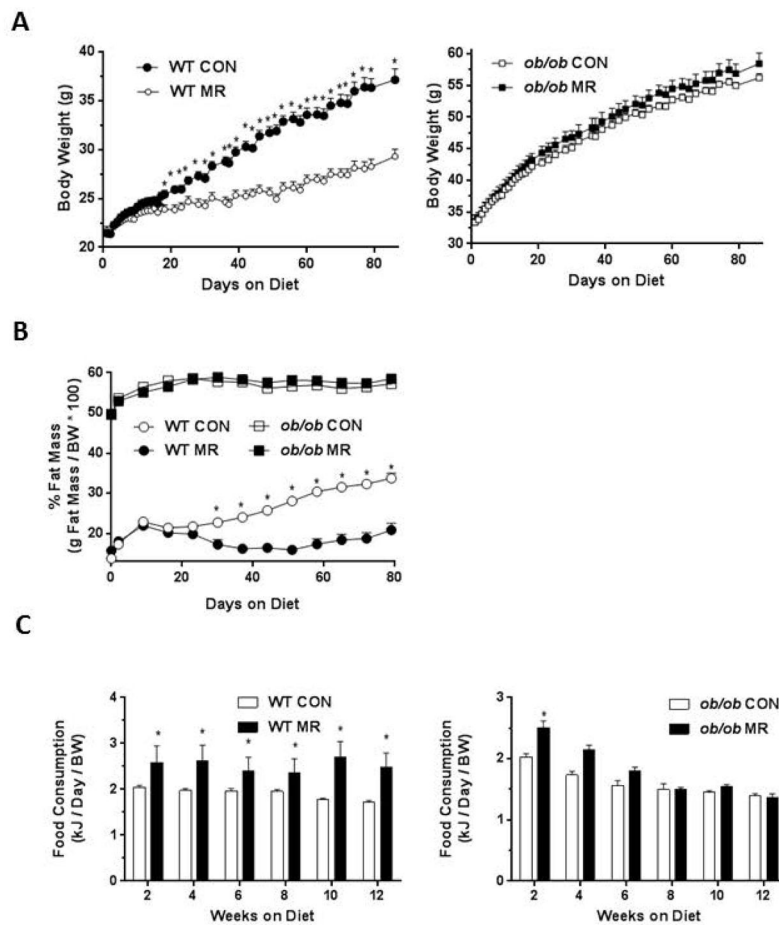


Figure 1.

MR does not affect body weight and adiposity in *ob/ob* mice. WT and *ob/ob* mice were fed either control (CON) or methionine-restricted (MR) diet for 12 weeks. Body weights (A), adiposity (B), and food intake (C) in WT and *ob/ob* mice were measured over the course of 12 weeks. Data are presented as mean \pm SEM (N=8) for each group and means annotated with asterisks differ at $P < 0.05$ within genotype.

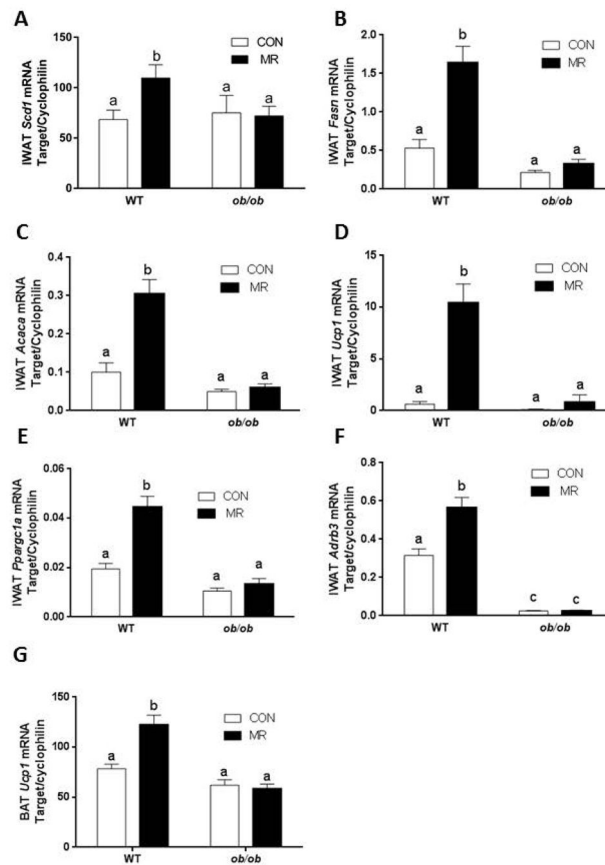


Figure 2.

MR has no effect on expression of target genes in adipose tissue of *ob/ob* mice. Expression of lipogenic genes [*Scd1*(A), *Fasn* (B), *Acaca* (C)], *UCP1* (D), *Ppargc1a* (E), and *Adrb3* (F) in IWAT, and *UCP1* in BAT (G) was compared between WT and *ob/ob* mice fed either control or MR diet for 12 weeks. Data are presented as mean \pm SEM (N=8), and means annotated with different letters differ at $P < 0.05$.

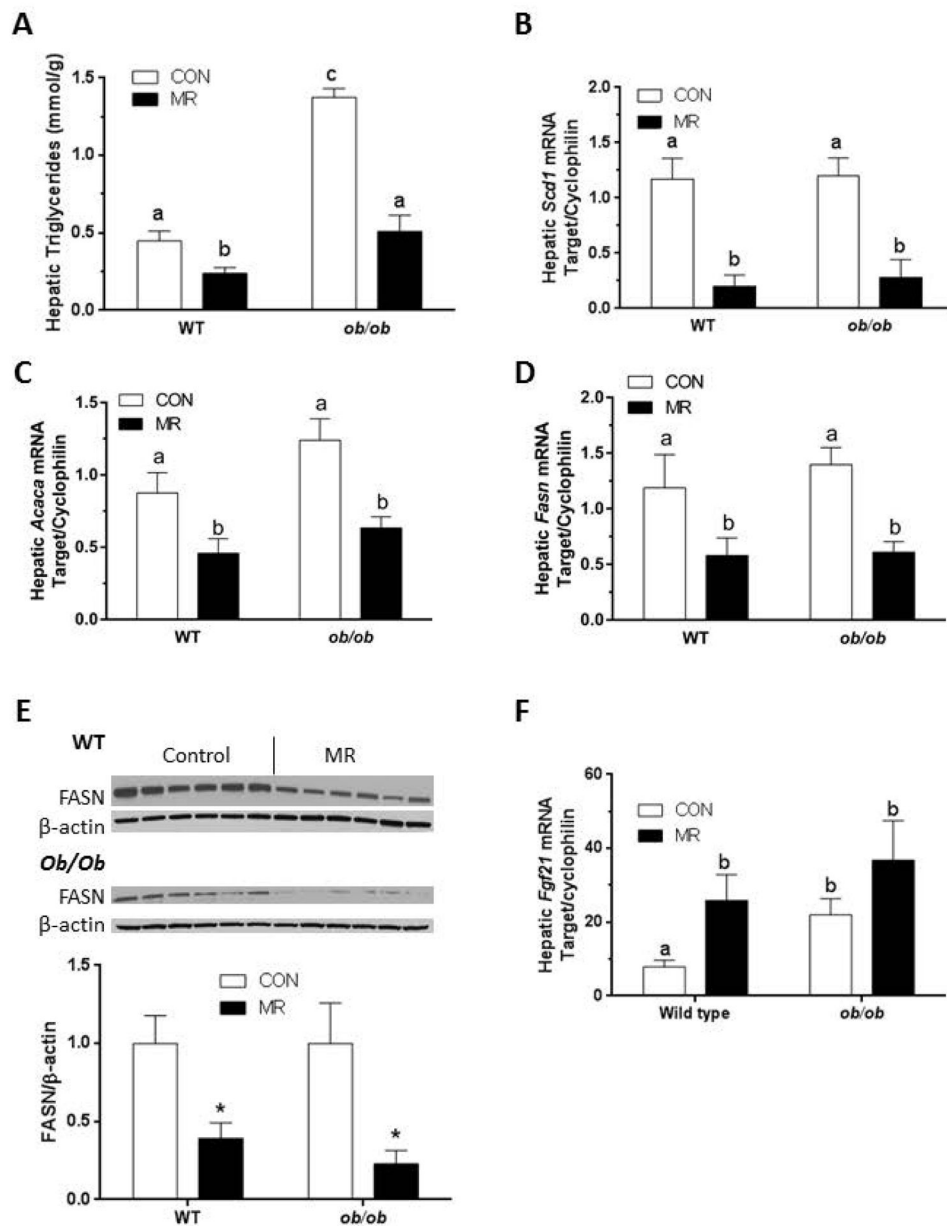


Figure 3. The effect of MR on hepatic triglyceride levels, lipogenic genes and FGF21 in WT and *ob/ob* mice. Hepatic triglyceride concentration (A), *Scd1* mRNA (B), *Acaca* mRNA (C), *Fasn* mRNA (D) FASN protein (E) and *FGF21* mRNA (F) were measured as described in Materials and Methods. Data are presented as mean \pm SEM (n=8), and means annotated with different letters differ at $P < 0.05$.

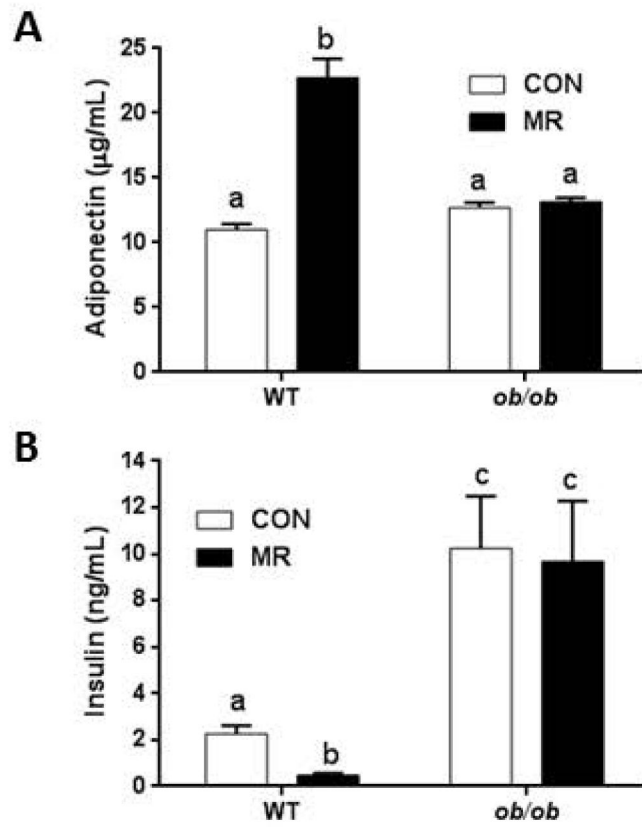
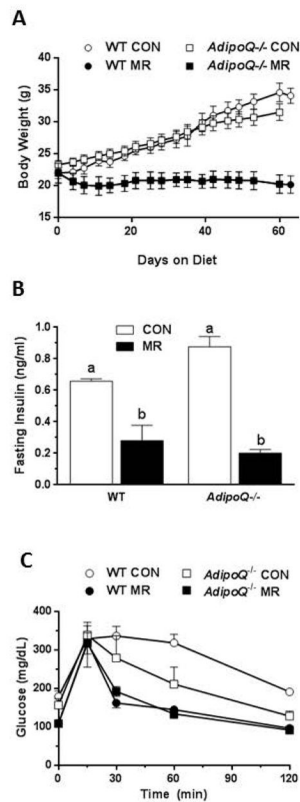


Figure 4. MR modulates serum adiponectin (A) and insulin (B) concentrations in WT but not in *ob/ob* mice. Data are presented as mean \pm SEM (N=8), and means annotated with different letters differ at $P < 0.05$.

**Figure 5.**

Adiponectin does not play a role in MR's effect on body weight and serum insulin. WT mice and mice lacking adiponectin (*AdipoQ*^{-/-} mice) were fed MR diet for 8 weeks. Body weights (A), serum insulin (B), and glucose tolerance (C) were measured in WT and *AdipoQ*^{-/-} mice on either control or MR diet. Data are presented as mean \pm SEM, and means annotated with different letters differ at $P < 0.05$.

Table 1

primer sequences for qPCR

Symbol	Name	Primer sequences
<i>Scd1</i>	Stearoyl-CoA desaturase-1	Forward: 5'-GTGCCGTGGGCGAGGGCTTC-3' Reverse: 5'-AGCCCAAAGCTCAGCTACTCTT-3'
<i>Fasn</i>	Fatty Acid synthase	Forward: 5'-TCCTGGAACGAGAACACGATCT-3' Reverse: 5'-GAGACGTGTCACCTCTGGACTTG-3'
<i>Acaca</i>	Acetyl-CoA carboxylase-1	Forward: 5'-CTCACCCAACCCAGAAAGGCCAA-3' Reverse: 5'-CAGGATCAGCTGGGATACTGAGT-3'
<i>Ucp1</i>	Uncoupling protein 1	Forward: 5'-GAT CCA AGG TGA AGG CCA GG-3' Reverse: 5'-GTT GAC AAG CTT TCT GTG GTG G-3'
<i>Adrb3</i>	β 3-adrenergic receptor	Forward: 5'-CCAGCCAGCCCTGTTGA-3' Reverse: 5'-GGACGCGCACCTTCATAGC-3'
<i>Ppargc1a</i>	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	Forward: 5'-TGC CAT TGT TAA GAC CGA G-3' Reverse: 5'-GGT CAT TTG GTG ACT CTG G-3'
<i>Fgf21</i>	Fibroblast growth factor 21	Forward: 5'-TGACCCCAGGATTGAATGAC-3' Reverse: 5'-GCAGCCAATGATGTGTGCTTAC-3'
<i>Ppia</i>	cyclophilin	Forward: 5'-CTT CGA GCT GTT TGC AGA CAA AGT-3' Reverse: 5'-AGA TGC CAG GAC CTG TAT GCT-3'