Journal of Food and Nutrition Research, 2019, Vol. 7, No. 2, 96-104
Available online at http://pubs.sciepub.com/jfnr/7/2/1
Published by Science and Education Publishing
DOI:10.12691/jfnr-7-2-1

Methionine Restriction Diet Improves Metabolic Function in Obese C57BL/6 Female Mice via AMPK/SIRT1/PGC1α Pathway

Seba Harphoush[1,2,3], Guoqing Wu[1,3], Margaret Zaitoun[1,2,3], Yonghui Shi[1,3], Guowei Le[1,3,*]

[1]State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, PR China
[2]Faculty of Health Science, Al-baath University, Homs, Syria
[3]School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, PR China
*Corresponding author: lgw@jiangnan.edu.cn

Received December 15, 2018; Revised January 19, 2019; Accepted February 03, 2019

Abstract Methionine restriction has been proven to reverse age and obesity related metabolic diseases. Basically, MR regulates energy homeostasis in the whole body contributing to enhanced insulin sensitivity and improved redox status. There is no studies until now that comprehensively focus on the impact of MR on reproduction performance. The aim of this work was to investigate the reproductive system response of female obese model toward MR and clarify the possible mechanism. Females C57BL/6 were subjected to control diet (CON, 0.86% methionine + 4% fat), High Fat Diet (HFD, 0.86% methionine + 20% fat), High fat methionine Restricted diet (MR, 0.17% methionine + 20% fat). HFD showed impaired reproduction performance characteristic by impaired conception ability and lactation difficulties. MR improved oxidative stress, metabolic parameters, mammary and ovarian morphology. MR enhanced ovulation, lactation, and especially regulated metabolism efficiency via AMPK/SIRT1/PGC1. This study is the first to suggest that MR can ameliorate reproductive disorders induced by obesity.

Keywords: *methionine restriction, high fat diet, obesity, reproduction, metabolic dysfunction*

Cite This Article: Seba Harphoush, Guoqing Wu, Margaret Zaitoun, Yonghui Shi, and Guowei Le, "Methionine Restriction Diet Improves Metabolic Function in Obese C57BL/6 Female Mice via AMPK/SIRT1/PGC1α Pathway." *Journal of Food and Nutrition Research*, vol. 7, no. 2 (2019): 96-104. doi: 10.12691/jfnr-7-2-1.

1. Introduction

Obesity contributes to a disruption of women health and a complicated reproductive life [1,2]. Obesity defects several systemic metabolic pathways regulating reproduction performance. Studies have proven ovulation dysfunction, impaired conception ability, and disrupted lactation efficiency in obese individuals [3,4,5,6]. The prevalence of both obesity and infertility problems is quite increased among women, dietary management and weight loss can effectively reverse reproductive dysfunction induced by obesity [7,8].

Methionine restriction (MR) has been proved to ameliorate obesity negative effects in multiple organs, by reducing body fat accumulation, boost insulin sensitivity, improve metabolic flexibility, and relief of oxidative stress [9-13]. Although methionine is an essential sulphur- amino acids for fetal growth, lower methionine and homocysteine blood concentrations were observed during the progress of healthy pregnancies [14]. In addition, experimental evidence has shown that excessive dietary methionine contributes to impaired fetal health [15]. Moreover, few studies have assessed MR impacts on reproduction performance.

Reproduction is high-cost process demands several metabolic adaptations in multiple organs. Ovary and mammary gland are two vital organs in women reproduction system; both organs have been proved to be highly altered by high fat diet induced obesity. Disruption of mammary gland morphology and failure to establish early lactogenesis with insufficient volumes of milk were associated with obesity in different species [16,17]. Obesity disturbed ovarian function by increasing ovarian oxidative stress, triggering excessive apoptosis, impaired the quality and quantity of the oocytes, and accelerated follicle development and loss [18-21]. The aim of this study was to investigate the ability of MR to reverse metabolic dysfunction induced by obesity and impaired reproduction performance in obese models.

2. Materials and Methods

In this study, all experimental procedures and animal care were carried out in accordance with the ethical

standards laid down in the Guidelines for Laboratory Animals Care, Jiangsu Province (China) and in the 1964 Declaration of Helsinki and its later amendments.

2.1. Experiment 1

Female C57BL/6 mice 3 weeks of age and weighing 18 g were obtained from Shanghai Slack Laboratory Animals Co., Ltd. and divided to three experimental diet groups, 18 mice in each group: the control group fed with the normal diet (CON, 0.86% methionine + 4% fat), High Fat Diet (HFD, 0.86% methionine + 20% fat), High Fat Diet+ methionine Restricted diet (MR, 0.17% methionine + 20% fat). The mice maintained under standard laboratory conditions: a temperature of 25°C, a relative humidity of 60-70%, and a 12-hour light/dark cycle, at the Experimental Animal Centre of Jiangnan University (Wuxi, China). The mice fed a standard commercial pellet diet and water for one week before started the above mentioned diets for 11 weeks. After mating, the first day of gestation was determined by the presence of spermatozoids in the vaginal smears. Pregnant mice were housed individually in metal cages under the above- mentioned conditions and diets. After birth only lactating females with 4 or more pups were included in the study. C57BL/6' females tend to a nervous behavior and eating pups especially at first pregnancy because of lack experience, so pregnancy must be induced more than once to get enough lactating females. The mice were sacrificed using cervical dislocation on day 15 of lactation (LD 15), blood was collected from the carotid artery. Plasma was immediately separated by centrifugation (4,000g, 15 min, 4°C) and stored at -80°C until it was used in an assay. On LD15, bilateral abdominal mammary glands (the fourth mammary gland) were excised. The right gland was immediately removed and homogenized on ice with saline 0.9 percent, whereas the left gland was weighed then divided after removing the lymph node, a part was preserved at -80°C in Trizol for later RNA isolation and other part was in paraformaldehyde 4% in PBS for H & E staining. A subgroup of mice was sacrificed on day 18 of pregnancy, the right bilateral abdominal mammary gland was excised and spread on a glass slide for whole-mount staining.

2.2. Experiment 2

At the end of experiment 1, 6 mice each group from the previous diets, which failed in sustain lactation kept on the same diets until 28 weeks old. At the end of the experimental period, the mice were sacrificed using cervical dislocation and blood was collected from the carotid artery. Ovaries were excised, right ovary was preserved at -80°C in Trizol for later RNA isolation, and left ovary was in paraformaldehyde 4% in PBS for H&E staining.

2.3. Weigh-Suckle-Weigh

On LD 2, 5, and 10, lactating females with relative were subjected to a weigh-suckle-weigh experiment in which pups were removed from dams for 4 hr which allowed the milk supply to replenish and pups to grow hungry. Pups were then weighed, returned to dams and suckled for 30 minutes, then weighed again. The difference between the final and initial litter weight represents milk yield for one dam. Then milk yield was estimating per pup for one bout of nursing.

2.4. Whole-Mount Examination of Mammary Gland

Mammary glands were spread on glass slides, fixed in Carnoy's fixative overnight, washed with 70% ethanol, and rehydrated with decreasing concentrations of ethanol followed by distilled water. The gland was stained 3-4 hours in carmine alum, and dehydrated again through serial ethanol baths 70, 80, 90, and 100% then with xylene, according to the method previously described [22]. The gland was photographed with a digital camera under a dissecting microscope, and the image was analyzed using Image-J NIH software, quantitative assessment of mammary gland epithelial density area was performed according to the protocol described by John N McGinley and Henry J Thompson [23].

2.5. H & E Staining

The left ovary from experiment 2's females and a part of left mammary gland from experiment 1's females were fixed in paraformaldehyde 4% in PBS for 24 h, and embedded in paraffin. Hematoxylin and eosin (HE) staining was performed on 5 mm serials to analyze the histopathological changes of ovarian follicle cells and mammary alveolar lumens.

2.6. Measurement of Plasma Parameters

The bodyweights of the mice were measured every six days before and through pregnancy. The body weight of offspring was measured at birth, at one week, and at two weeks old. Plasma insulin, leptin, and prolactin concentrations were measured using the mice ELISA kits (Huijia Biotechnology, Xiamen, P. R. China), according to the manufacturer's instructions. Blood glucose was tested using One Touch Sure Step test strips (Lifescan, Milpitas, California, U.S.A.). TG (Triglycerides), HDL (High-density lipoprotein), and LDL (low-density lipoprotein) were measured by corresponding commercial kits (Jiancheng Bioengineering Institute, Nanjing, P. R. China) according to the manufacturer's instructions.

2.7. Oxidative Stress Biomarkers and Activities of Antioxidant Enzymes

The activity of superoxide dismutase (SOD), catalase (CAT), and the level of malondialdehyde (MDA), total antioxidants (T-AOC), and reduced glutathione (GSH), were assayed by corresponding kits (Jiancheng Bioengineering Institute, Nanjing, P. R. China). The levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) in the plasma, (TNF-α, and IL-6) in the tissue were measured using the mice ELISA kits (Huijia Biotechnology, Xiamen, P. R. China), according to the manufacturer's instructions.

Table 1. Primer's sequences of C57BL/6 mice for qRT-PCR

Gene	Forward (5' to 3')	Reverse(5' to 3')
AMPK	GAAGATCGGACACTACGTGC	AGTCCACGGCAGACAGAATC
AKT1	ATCCGCTGCCTGCAGTGGACC	CCTTGTCCAGCATGAGGTTCTCC
mTORC1	GCAGATTTGCCAACTACC	TCTCCGGCCCTCATTTCG
K18	CGAGGCACTCAAGGAAGAAC	CTTGGTGGTGACAACTGTGG
PGC-1α	ACAGCTTTCTGGGTGGATTGAAGTG	AGACTGTCCAGTGTCTCTGTGAGGA
SIRT1	CTGTTTCCTGTGGGATACCTGACT	ATCGAACATGGCTTGAGGATCT
UCP2	CACGCAGCCTCTACAATG	TCAGCACAGTTGACAATGG
β-actin	TGTCACCAACTGGGACGATA	GGGGTGTTGAAGGTCTCAAA

2.8. Total mRNA Isolation and Quantitative RT-PCR (qRT-PCR)

Total RNA of tissues was extracted with Trizol reagent according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). The concentration of total RNA in each sample was quantified by Nano Drop Spectrophotometer (ND2000, Thermo, and Waltham, MA, USA). A SYBR green based qRT-PCR kit was used according to the manufacturer's instructions in a 7900HT instrument (Applied Biosystems, Forster, CA, USA). The specificity of the product was assessed from melting curve analysis. Gene expressions were determined using the $2^{-\Delta\Delta Ct}$ method. The primer's sequences for the genes are shown in Table 1.

2.9. Statistics

All measurement values were expressed as means ± Standard deviation. Significant differences between groups were determined by one-way analysis of variance using SPSS 20.0 software (SPSS Inc. Chicago IL., U.S.A.) for windows. A difference was considered significant at the $p<0.05$ level.

3. Results

3.1. Dams and Offspring Weight

In experiment 1, at mating day after 11 weeks, the body weight in HFD groups was significantly higher than control and reduced in MR group ($p< 0.05$) (Figure 1.A), with noticeable increased in MR and HFD food intake (Figure 1.B). This increment in HFD group body weight was maintained throughout pregnancy (Figure 1.C). However, the body weight difference between control and fat diet groups was no longer present on LD 15 (Figure 1.D). In experiment 2, at 28 weeks old, the body weight was significant higher in the HFD group with a significant reduction in MR group ($p< 0.05$), (Figure 1.E). We investigated the effect of MR on offspring growth. There was no difference between HFD and MR fetus Crown-rump length measured at 18 day pregnancy (Figure 2.A), and no difference between HFD and MR offspring weight at birth, but offspring weight in MR group decreased markedly at 1 week old, whereas at 2 weeks old there was no significant difference between all the groups offspring weight (Figure 2.B).

3.2. Milk Yield

On LD 2, LD 5 and LD 10 milk yield was markedly less in HFD group and markedly higher in MR group (Figure 2.C), MR improved surviving offspring rate at 2 weeks old markedly ($p< 0.05$) (Figure 2.D).

3.3. Fasting Blood Sugar, Insulin, Leptin Secretion, Prolactin and Lipid Profile

HFD-induced obesity disturbed fasting blood sugar (FBS) levels of females of both experiments with increased insulin and leptin levels which indicated insulin resistance status. MR markedly decreased FBS and insulin levels in the two experiments ($p< 0.05$) (Figure 3. A,B). Moreover, MR decreased leptin blood level in experiment 2 consistently with the decreased body weight but not in experiment 1 as no difference was notable in the weight between the groups (Figure 3.C). MR elevated prolactin levels in experiment 1's females on LD15 ($p< 0.05$) (Figure 3.D). In experiment 2's females, MR decreased TG and LDL blood levels markedly and improved HDL levels in the blood (Figure 3.E).

3.4. Redox Status

HFD induced an oxidative stress status characterised by reduced GSH/GSSG ratio, elevated TNFα, IL6 in the blood and mammary tissue of the experiment 1's females and elevated TNFα and IL6 in the blood of experiment 2's females. As shown in Table 2, MR elevated GSH/GSSG ratio in the blood but not in the mammary of experiment 1's females, and decreased GSH in the mammary gland. Moreover, MR markedly enhanced antioxidant enzyme activities in the blood and mammary tissue of experiment 1's females. MR relieved the inflammation by reducing MDA, TNFα, and IL6 in the blood and mammary tissue of the experiment 1's females, in addition to TNFα and IL6 in the blood of experiment 2's females (Figure 3.F,G).

3.5. Histopathological Evaluation of Mammary Glands of Experiment 1's Females

Distortion in mammary gland morphology was detected by analyzing mammary gland whole-mount of experiment 1's HFD females on day 18 of pregnancy. Mammary epithelial density was diminished in HFD group but improved markedly in MR group. In the same manner, the prevalence of alveolar lumens area in HFD group was minimized with larger adipocytes. MR significantly increased the alveolar lumens prevalence in the parenchymal tissue (Figure 4).

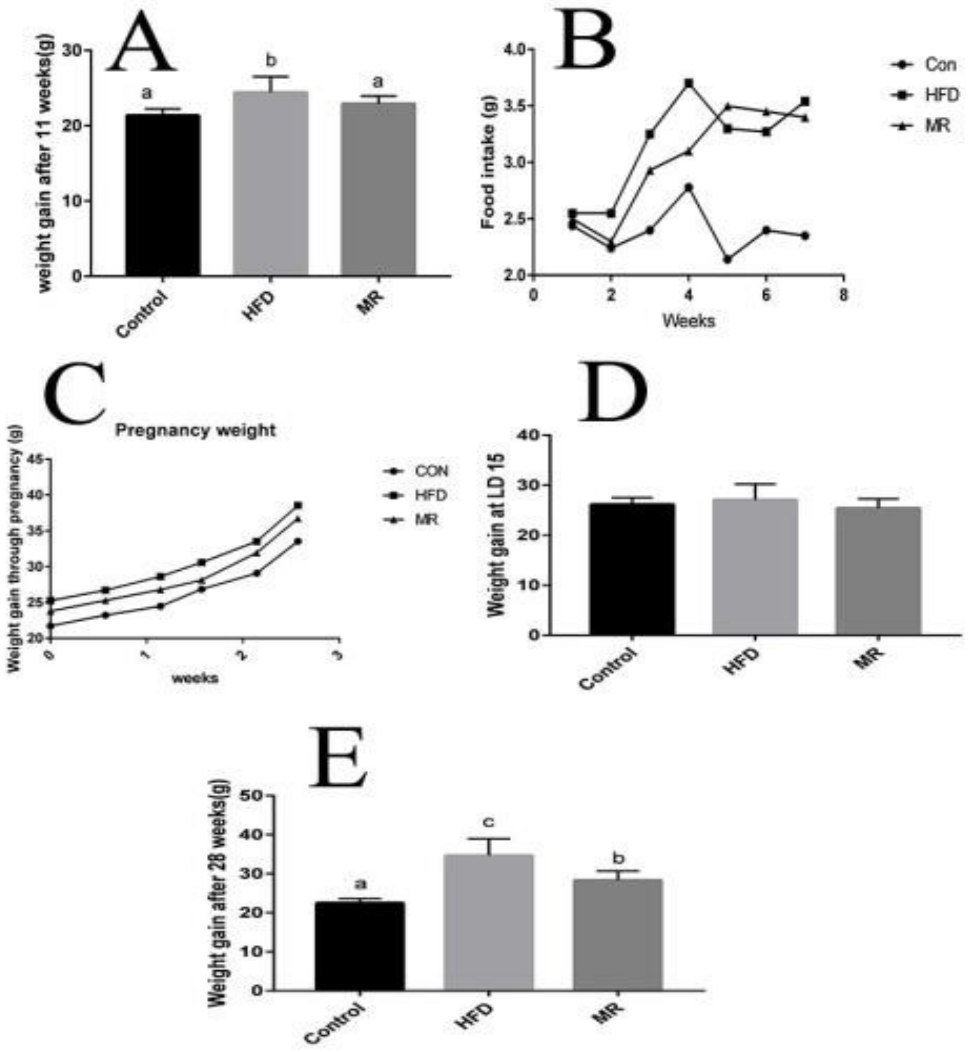

Figure 1. Effects of MR on body weight and food intake ($p < 0.05$) (Data were expressed as Means ± SDs. Data were expressed as Means ± SDs)

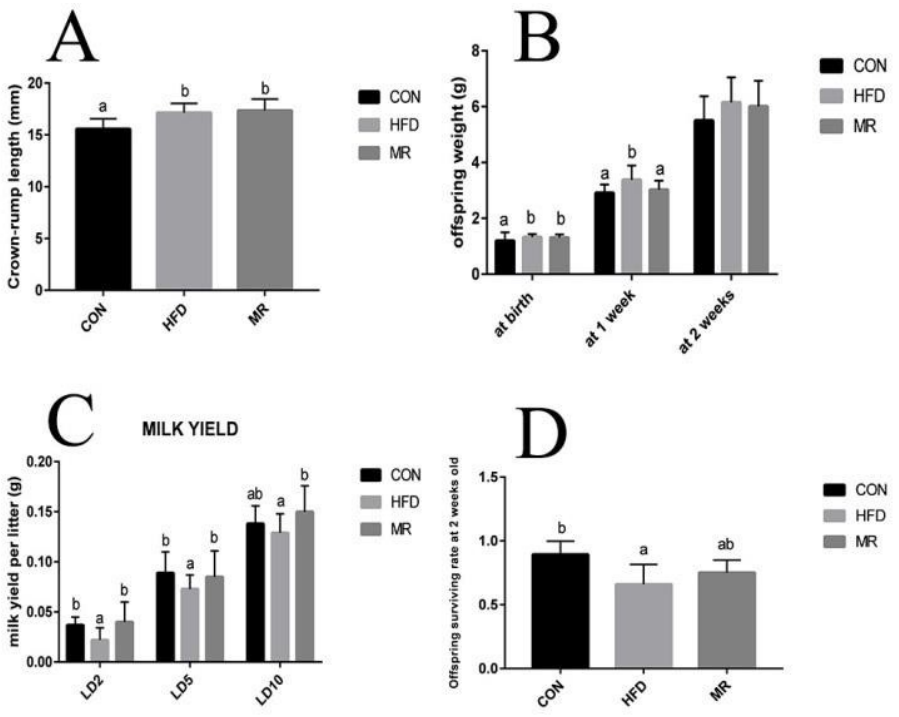

Figure 2. Effects of MR on offspring growth, offspring survive, and milk yield. (n=6 for each group, $p<0.05$). Data were expressed as Means± SDs

3.6. Histopathological Evaluation of Ovaries of Experiment 2's Females

Accelerating in the growth of follicle cells in the HFD ovaries was noticed as most of the follicles were stuck in the early stages of development (primary, secondary and antral follicles) or follicles were turned into atretic follicles. In return, the majority of follicles cells in CON and MR groups reached the later stages (Graafian follicles, ovulated oocytes, and corpus luteum), which indicates improved ovulation (Figure 5).

3.7. mRNA Expression of Genes Involved in Mitochondrial Function and Metabolism in Ovarian and Mammary Tissues

MR increased mRNA expressions of genes involved in stimulating metabolism AMPK, PGC1α, SIRT1 and decreased AKT1, mTORC1 in both mammary and ovarian tissue. MR increased K18 in mammary tissue. MR down- regulate the expressions UCP2 in mammary tissue but not in the ovary (Figure 6).

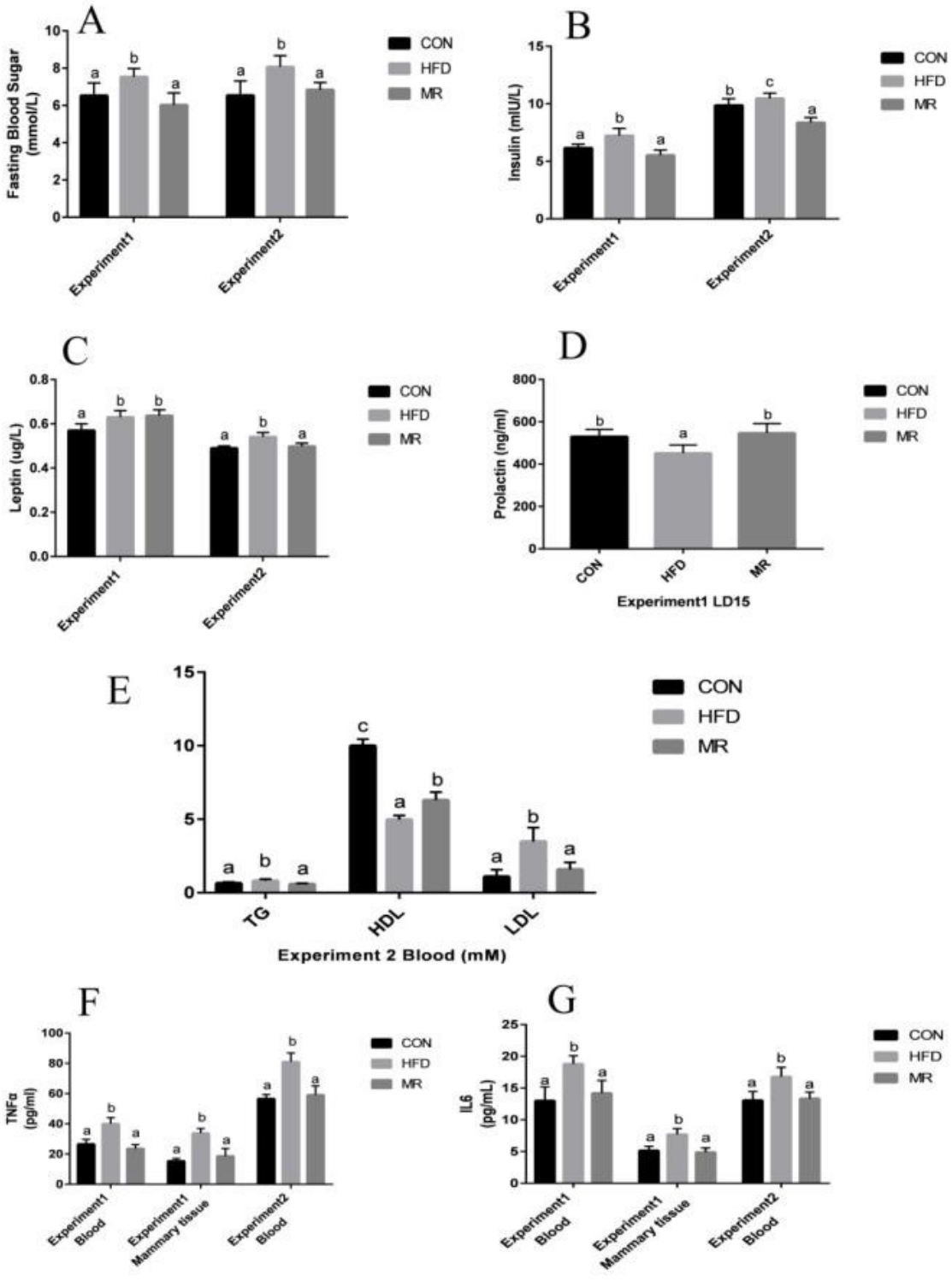

Figure 3. Effects of MR on metabolic parameters, prolactin, lipid profile, and inflammation markers

Table 2. Effects of MR on the redox satus in blood and mammary tissue of lactating females in Experiment 1 on LD15

Blood of Experiment 1'females on LD 15						
	T-AOC U/mg protein	MDA (nmol/mL)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (Ug/mL)	GSH/GSSG
CON	4.7±0.45	3.11±0.5^{a}	49.51±2.11^{b}	9.68±0.85^{c}	48.32±1.85^{b}	0.51±0.09^{b}
HFD	4.3±0.43	6.09±1.52^{b}	33.12±2.77^{a}	2.82±0.98^{a}	31.75±5.8^{a}	0.27±0.05^{a}
MR	4.5±0.37	2.05±0.65^{a}	46.9±3.3^{b}	7.85±0.97^{b}	76.97±4.4^{c}	0.75±0.07^{c}
Mammary tissue of Experiment 1'females on LD15						
	MDA (nmol/mL)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (Ug/mL)		GSH/GSSG
CON	0.25±0.04^{a}	2.41±0.32^{b}	22.3±1.46^{b}	2.91±0.58^{b}		1.35±0.15^{b}
HFD	1.48±0.39^{b}	1.08±0.4^{a}	19.16±1.94^{a}	1.43±0.3^{a}		1.03±0.24^{a}
MR	0.17±0.03^{a}	2.98±0.59^{b}	24.2±1.16^{b}	1.39±0.34^{a}		1.02±0.21^{a}
The data marked with different letters in the same row were statistically different at $p<0.05$ (n=6).						

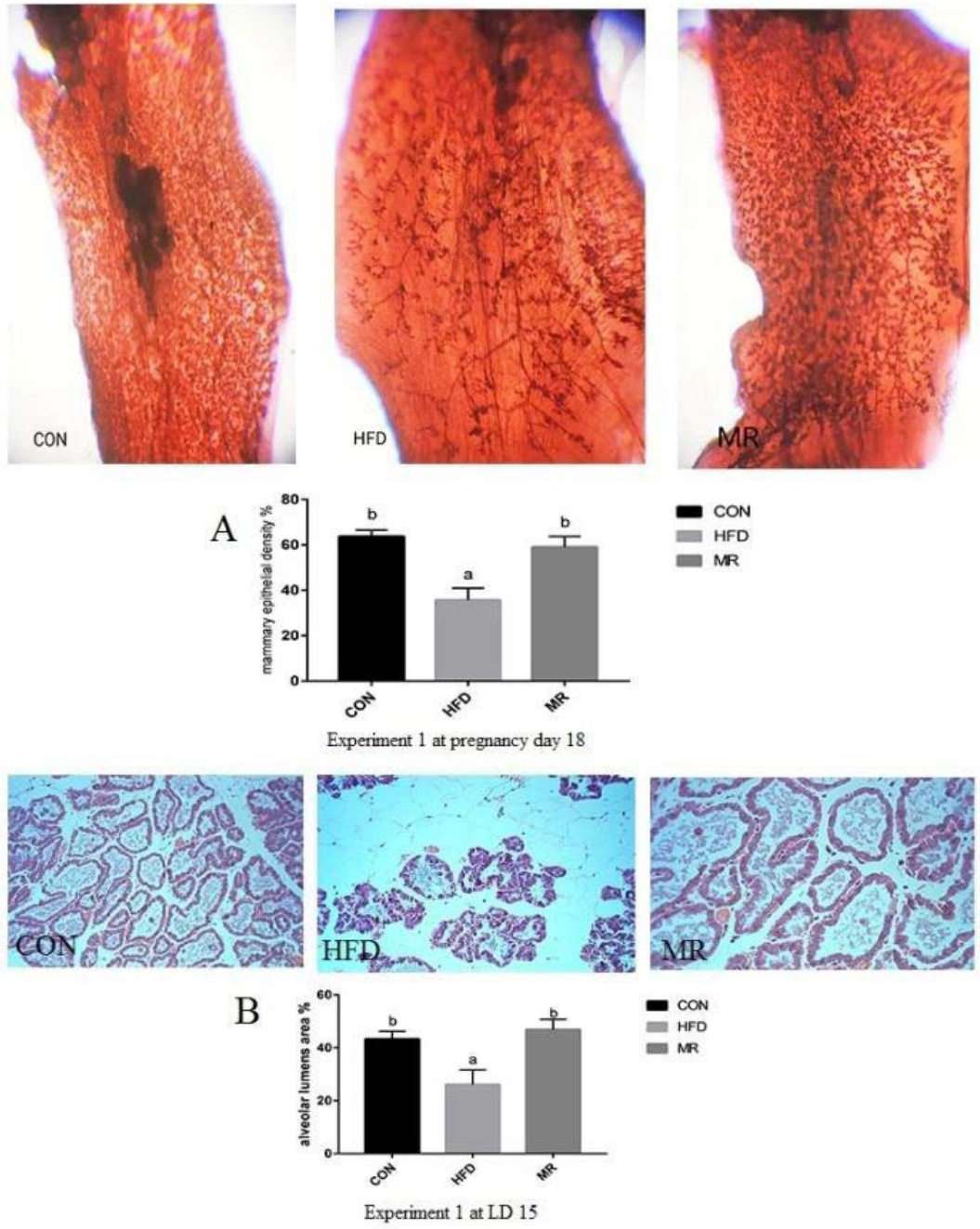

Figure 4. Representative whole mount gland on day 18 of pregnancy (10×) & Representative HE-stained sections of the mammary glands on LD 15 (20×). A: MR effect on mammary epithelial density(n=3, $p<0.05$), B: MR effect on alveolar Lumens area. (n=6, $p<0.05$)

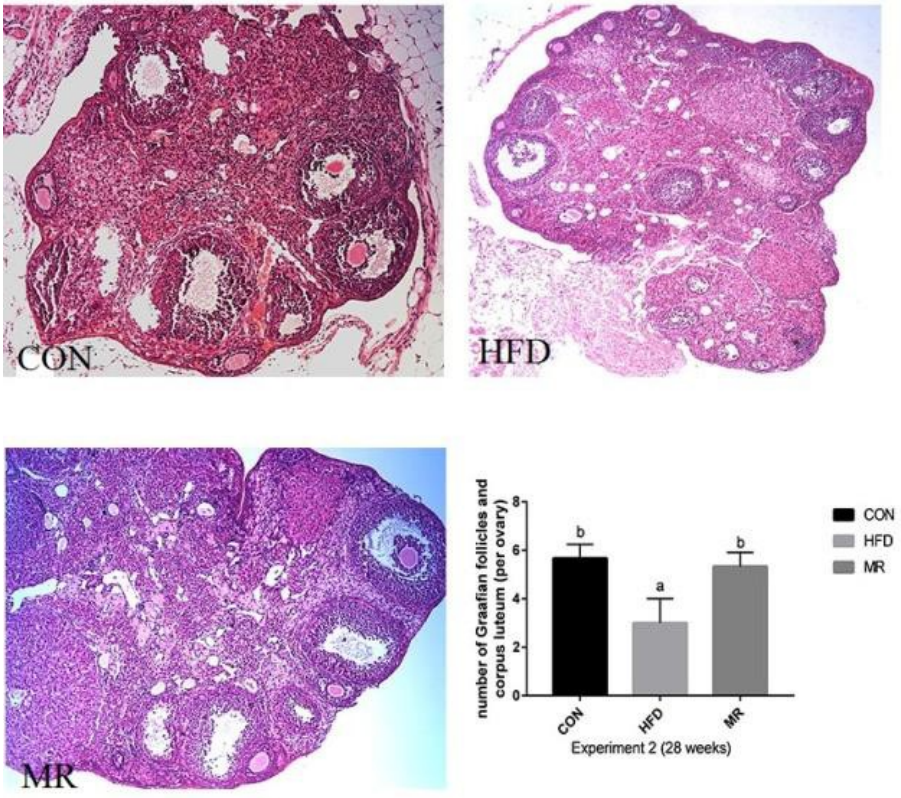

Figure 5. Representative HE of ovarian tissues. 10× (n=3 for each group, $p<0.05$)

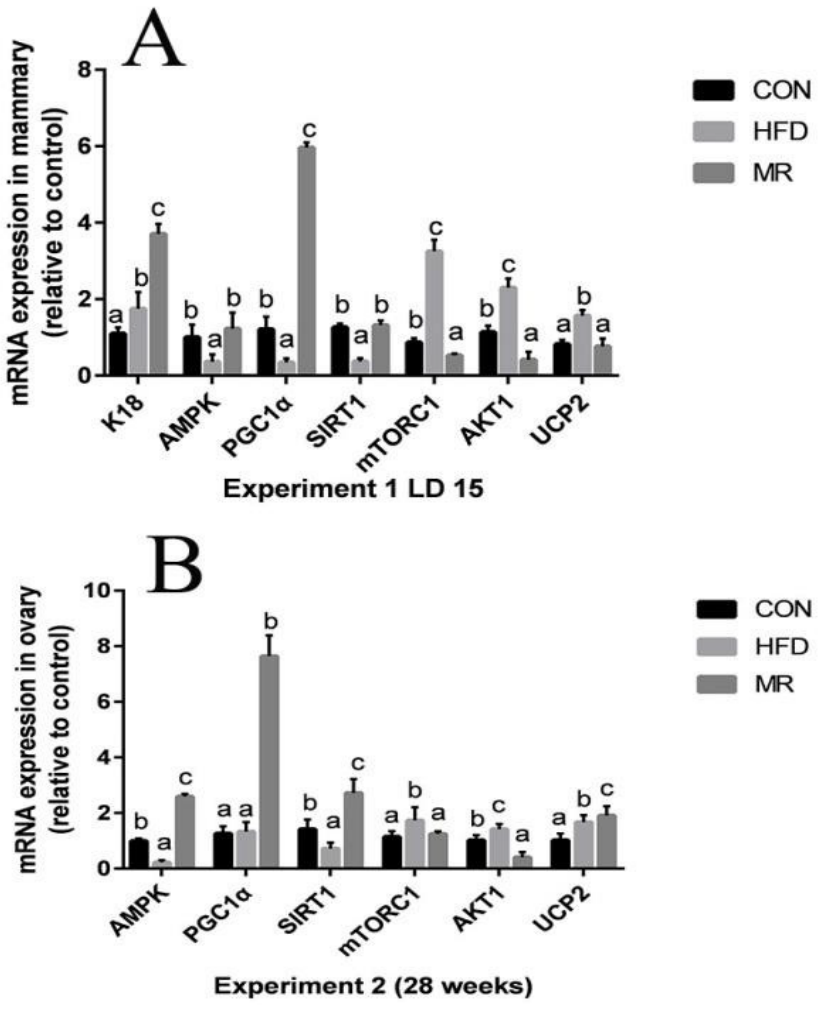

Figure 6. Effects of MR on mRNA genes expression of metabolic function, A: in mammary tissue, B: in ovarian tissue

4. Discussion

IN the present study, several methods were conducted to investigate the impact of MR on two important organs in female reproduction system, our results support the hypothesis that MR can improve reproductive disorders caused by obesity. In addition, metabolism mechanism is likely to be mediated through improved reproductive performance.

MR decreased mice weight after 11 weeks and increased food intake as previously described. In experiment 1, on LD 15 MR did not affect the mice weight and blood leptin level was also high but in experiment 2, MR decreased weight markedly and blood leptin level. Lactation is energy demanding process in the purpose of producing sufficient mounts milk, MR increases energy expenditure in different ages with no effects on growth requirements in small ages because of the higher energy intake [24]. MR did not impact the weight in lactation situation and that could be explained by the whole body adaptations associated with this physiological condition to provide enough energy for milk production [25]. Although, MR decreased female weight through pregnancy, MR did not affect fetus crown-rump length and birth weight, which could refers to enough methionine supply access the fetus blood through placenta. More investigations need to be done about the effects of MR on fetus nutrition. At one week age, MR showed its effects on offspring weight and that could be explained by low methionine level in the milk, which will need more investigations also. From another sight, MR increased milk yield through lactation and by promoting early lactogenesis. In addition, MR increased offspring survival rate comparing to HFD.

As previously described, MR ameliorated insulin resistance induced by obesity, decreased insulin and FBS in the females of both experiments and improved lipid profile in experiment 2 females [11,26-30]. In experiment 1, MR improved mammary morphology by increased epithelial density and enhanced alveolar lumens expansion, which was associated with up-regulated mRNA expression of K18 (a luminal marker). In addition, MR improved mammary glands oxidative stress caused by obesity through enhancing the activity of antioxidant enzymes and reducing inflammation markers as other studies have shown [30,31,32]. At the genes level, up-regulation of the genes evolved in energy production was noticed, MR enhanced mRNA genes expression of AMPK, PGC1, and SIRT1. The role of AMPK and SIRT1 in the reverse of metabolic syndrome symptoms is well reported [21,33]. This mechanism was previously described to mediate the longevity effects of MR [34,35,36], MR down-regulated mRNA genes expression of mTORC1 and AKT1. Taken together, MR improved metabolism function through a nutrient-sensing mechanism contributed to enhanced nutrients uptake and energy production in mammary glands, which finally resulted in increased milk yield. At the same manner, MR improved ovarian metabolic function in experiment 2, and MR increased mRNA genes expressions of AMPK/PGC1α/SIRT1 pathway in ovarian tissue, which was combined with developed follicle cells and improved ovulation [37,38]. MR slowed down the follicle overgrowth process by down-regulate AKT1, mTORC1 genes expressions. Obesity is well proven to alter mitochondrial function. Uncoupling protein (UCP2) has been linked to obesity, insulin resistance, and decreased ATP production [39]. UCP2 has been proved to play a role in lipid synthesis in mammary gland, and UCP2 mRNA has shown to be stimulated in mammary tissue by fatty acids administration [40]. MR markedly decreased UCP2 mRNA expression in lactating mammary glands. On the opposite, MR increased UCP2 mRNA expression in ovarian tissue, this is result comes along with previous result about the role of UCP2 in the regulation of follicle development and oocyte maturation and quality [41].

The present work implies that reducing methionine in the diet could be a successful treatment for female reproductive disorders associated with obesity. The possible mechanism involves improve metabolism efficiency and mitochondrial function through up-regulate AMPK/PGC1α/SIRT1 pathway in mammary and ovarian tissues.

Abbreviations

CON: control
HFD: High Fat Diet (HFD, 0.86% methionine + 20% fat)
MR: methionine Restricted diet (0.17% methionine + 20% fat)
LD: lactation day
TG: Triglycerides
HDL: High-density lipoprotein
LDL: low-density lipoprotein
SOD: superoxide dismutase
CAT: catalase
MDA: malondialdehyde
T-AOC: total antioxidants
GSH: reduced glutathione; GSSG:Glutathione disulfide
TNF-α: tumor necrosis factor-α; IL-6: interleukin-6
FBS: fasting blood sugar
AMPK: 5′ AMP-activated protein kinase
SIRT-1: Sirtuin 1
mTORC1: The mammalian target of rapamycin complex 1
AKT1: serine-threonine protein kinase
UCP2: Uncoupling Protein 2
K18: keratin 18.

Conflicts of Interest

There are no conflicts to declare.

Acknowledgements

This work was supported by State Key Laboratory of Food Science and Technology of Jiangnan University in China (No. SKLF-ZZB-201609), the National Natural Science Foundation of China (No. 31571841).

References

[1] Broughton, D.E. and K.H. Moley, Obesity and female infertility: potential mediators of obesity's impact. Fertil Steril, 2017. 107(4): p. 840-847.

[2] He, Y., et al., Association of childhood obesity with female infertility in adulthood: a 25-year follow-up study. Fertil Steril, 2018. 110(4): p. 596-604 e1.

[3] Moslehi, N., et al., Is ovarian reserve associated with body mass index and obesity in reproductive aged women? A meta-analysis. Menopause, 2018. 25(9): p. 1046-1055.

[4] Gesink Law, D., R.F. Maclehose, and M.P. Longnecker, Obesity and time to pregnancy. Human Reproduction, 2006. 22(2): p. 414-420.

[5] Rasmussen, K.M., J.A. Hilson, and C.L. Kjolhede, Obesity may impair lactogenesis II. The Journal of nutrition, 2001. 131(11): p. 3009S-3011S.

[6] Amir, L.H. and S. Donath, A systematic review of maternal obesity and breastfeeding intention, initiation and duration. BMC pregnancy and childbirth, 2007. 7(1): p. 9.

[7] Legro, R.S., Effects of obesity treatment on female reproduction: results do not match expectations. Fertil Steril, 2017. 107(4): p. 860-867.

[8] Stang, J. and L.G. Huffman, Position of the Academy of Nutrition and Dietetics: Obesity, Reproduction, and Pregnancy Outcomes. J Acad Nutr Diet, 2016. 116(4): p. 677- 91.

[9] Malloy, V.L., et al., Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer 344 rats independent of energy restriction. Aging cell, 2006. 5(4): p. 305-314.

[10] Stone, K.P., et al., Mechanisms of increased in vivo insulin sensitivity by dietary methionine restriction in mice. Diabetes, 2014: p. DB_140464.

[11] Hasek, B.E., et al., Dietary methionine restriction enhances metabolic flexibility and increases uncoupled respiration in both fed and fasted states. Am J Physiol Regul Integr Comp Physiol, 2010. 299(3): p. R728-39.

[12] Yang, Y., et al., Dietary methionine restriction reduces hepatic steatosis and oxidative stress in high-fat-fed mice by promoting H2S production. Food Funct, 2018.

[13] Yang, Y., et al., Dietary methionine restriction regulated energy and protein homeostasis by improving thyroid function in high fat diet mice. Food Funct, 2018. 9(7): p. 3718-3731.

[14] Kalhan, S.C. and S.E. Marczewski, Methionine, homocysteine, one carbon metabolism and fetal growth. Reviews in Endocrine and Metabolic Disorders, 2012. 13(2): p. 109-119.

[15] Rees, W.D., F.A. Wilson, and C.A. Maloney, Sulfur amino acid metabolism in pregnancy: the impact of methionine in the maternal diet. The Journal of nutrition, 2006. 136(6): p. 1701S-1705S.

[16] Kamikawa, A., et al., Diet-induced obesity disrupts ductal development in the mammary glands of nonpregnant mice. Developmental dynamics: an official publication of the American Association of Anatomists, 2009. 238(5): p. 1092-1099.

[17] Flint, D.J., et al., Diet-induced obesity impairs mammary development and lactogenesis in murine mammary gland. American Journal of Physiology-Endocrinology and Metabolism, 2005. 288(6): p. E1179-E1187.

[18] Xu, M., et al., Effect of high fat dietary intake during maternal gestation on offspring ovarian health in a pig model. Nutrients, 2016. 8(8): p. 498.

[19] Sohrabi, M., et al., Effect of a high fat diet on ovary morphology, in vitro development, in vitro fertilisation rate and oocyte quality in mice. Singapore medical journal, 2015. 56(10): p. 573.

[20] Wu, Y., et al., High fat diet triggers cell cycle arrest and excessive apoptosis of granulosa cells during the follicular development. Biochemical and biophysical research communications, 2015. 466(3): p. 599-605.

[21] Wang, N., et al., Obesity accelerates ovarian follicle development and follicle loss in rats. Metabolism, 2014. 63(1): p. 94-103.

[22] Plante, I., M.K. Stewart, and D.W. Laird, Evaluation of mammary gland development and function in mouse models. Journal of visualized experiments: JoVE, 2011(53).

[23] McGinley, J.N. and H.J. Thompson, Quantitative assessment of mammary gland density in rodents using digital image analysis. Biological procedures online, 2011. 13(1): p. 4.

[24] Wanders, D., et al., The Components of Age-Dependent Effects of Dietary Methionine Restriction on Energy Balance in Rats. Obesity (Silver Spring), 2018. 26(4): p. 740-746.

[25] Mowry, A.V., et al., Mitochondrial function and bioenergetic trade-offs during lactation in the house mouse (Mus musculus). Ecology and evolution, 2017. 7(9): p. 2994-3005.

[26] Stone, K.P., et al., Mechanisms of increased in vivo insulin sensitivity by dietary methionine restriction in mice. Diabetes, 2014. 63(11): p. 3721-33.

[27] Latimer, M.N., B.M. Cleveland, and P.R. Biga, Dietary methionine restriction: Effects on glucose tolerance, lipid content and micro-RNA composition in the muscle of rainbow trout. Comp Biochem Physiol C Toxicol Pharmacol, 2018. 208: p. 47-52.

[28] Ying, Z., et al., Effects of dietary methionine restriction on postnatal growth, insulin sensitivity, and glucose metabolism in intrauterine growth retardation pigs at 49 and 105 days of age. J Anim Sci, 2018.

[29] Lees, E.K., et al., Direct comparison of methionine restriction with leucine restriction on the metabolic health of C57BL/6J mice. Sci Rep, 2017. 7(1): p. 9977.

[30] Yin, J., et al., Metabolic Regulation of Methionine Restriction in Diabetes. Mol Nutr Food Res, 2018. 62(10): p. e1700951.

[31] Liu, G., et al., Methionine restriction on oxidative stress and immune response in dss-induced colitis mice. Oncotarget, 2017. 8(27): p. 44511-44520.

[32] Caro, P., et al., Forty percent and eighty percent methionine restriction decrease mitochondrial ROS generation and oxidative stress in rat liver. Biogerontology, 2008. 9(3): p. 183-96.

[33] Nteeba, J., et al., Progressive obesity alters ovarian insulin, phosphatidylinositol-3 kinase, and chemical metabolism signaling pathways and potentiates ovotoxicity induced by phosphoramide mustard in mice. Biol Reprod, 2017. 96(2): p. 478-490.

[34] Barcena, C., et al., Methionine Restriction Extends Lifespan in Progeroid Mice and Alters Lipid and Bile Acid Metabolism. Cell Rep, 2018. 24(9): p. 2392-2403.

[35] Brown-Borg, H.M., Reduced growth hormone signaling and methionine restriction: interventions that improve metabolic health and extend life span. Ann N Y Acad Sci, 2016. 1363: p.40-9.

[36] Perrone, C.E., et al., Methionine restriction effects on mitochondrial biogenesis and aerobic capacity in white adipose tissue, liver, and skeletal muscle of F344 rats. Metabolism, 2010. 59(7): p. 1000-11.

[37] Zhang, H.-H., et al., SIRT1 attenuates high glucose-induced insulin resistance via reducing mitochondrial dysfunction in skeletal muscle cells. Experimental Biology and Medicine, 2015. 240(5): p. 557-565.

[38] Zhou, X.-L., et al., SIRT1 activator (SRT1720) improves the follicle reserve and prolongs the ovarian lifespan of diet-induced obesity in female mice via activating SIRT1 and suppressing mTOR signaling. Journal of ovarian research, 2014. 7(1): p. 97.

[39] Langin, D., The role of uncoupling protein 2 in the development of type 2 diabetes. Drugs Today (Barc), 2003. 39(4): p. 287-295.

[40] Yonezawa, T., et al., Regulation of uncoupling protein 2 expression by long-chain fatty acids and hormones in bovine mammary epithelial cells. Biochemical and biophysical research communications, 2008. 375(2): p. 280-285.

[41] Ge, H., et al., Effects of mitochondrial uncoupling protein 2 inhibition by genipin in human cumulus cells. BioMed research international, 2015. 2015.