Methionine restriction improve insulin resistance by reducing mitochondria oxidative stress

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Abstract

Type 2 diabetes is a chronic metabolic disorder whose prevalence has been increasing steadily worldwide and is closely linked to the epidemic of obesity. The most common features of Type 2 Diabetes are hyperglycemia, insulin resistance, and relative insulin deficiency. Insulin resistance refers to incorrect responses to insulin among cells whose physiology depends on insulin, such as adipocytes and cardiomyocytes. Methionine is an essential amino acid, so it must be consumed in the diet to avoid neurological impairment and visual symptoms caused by methionine deficiency. This provides a potential explanation that methionine restriction can alleviate insulin resistance by regulating mTORC1. This study aims to investigate the effect of methionine restriction on insulin resistance and mitochondria oxidative stress.

Keywords: insulin, Skeletal muscle, test

1 Introduction

Type 2 diabetes (T2D) is a chronic metabolic disorder, whose prevalence has been increasing steadily all over the world and is closely linked to the epidemic of obesity [1]. The most common features of Type 2 Diabetes are hyperglycaemia, insulin resistance, and relative insulin deficiency [2]. Insulin resistance refers to incorrect respond to insulin among cells whose physiology depends on insulin, such as adipocytes and cardiomyocytes [3].

Methionine is an essential amino acid, so it must be consumed in the diet to avoid neurological impairment and visual symptoms caused by methionine deficiency [4]. As cells age, enhanced oxidative stress renders cells unable to maintain homeostasis while increasing the risk of aging and metabolic-related diseases. By reducing oxidative stress, methionine restriction (MetR) can induce beneficial lifespan extension and metabolic health [5]. This has been confirmed in rat experiments, furthermore, lower methionine concentration in cells is positively correlated with longevity of yeast cells [6]. Conversely, increasing the concentration of methionine also enhanced mitochondria-related respiration and oxidative stress [7].

The mammalian target of rapamycin complex 1 (mTORC1) regulates metabolism and promotes cell growth when energy is abundant [8]. In the context of methionine, mTORC1 can be activated to reduce autophagy and limit lifespan [9]. Short-term rapid increases in insulin also acutely activate mTOR, whereas overactivation of mTORC1 leads to insulin resistance [10]. Furthermore, studies have shown that limiting methionine in the diet of animals or in cell culture media provides metabolic benefits such as increasing insulin sensitivity [11]. This seems to provide a potential explanation that methionine restriction can alleviate insulin resistance by regulating mTORC1. The aim of this study is to investigate the effect of methionine restriction on insulin resistance and mitochondria oxidative stress.

2 Method

2.1 Prepare

Four groups of 6-week-old healthy mouse models with three males and three females in each group. Two groups of mice were injected with STZ for 5 days to induce type 2 diabetes at a dose of 55 mg/kg BW of STZ for male mouse and 75 mg/kg BW of STZ for female mouse [12].

2.2 Methionine Managed Feeding and Living Environment

Single-housed in a 12-h light/dark cycle (lights on at 6:00 am) at a temperature of 21 ± 1°C with ad libitum access to food and water [13]. 1 group of healthy mouse model and 1 group of T2D mouse model were placed in a normal diet, which provided methionine at the control (Con) level, whereas 1 group of healthy mouse model and 1 group of T2D mouse model were placed in a diet contain low level of methionine. Two different diet groups experimented simultaneously.

2.3 Test

After 6 weeks of feeding, the fasting blood glucose and postprandial blood glucose of the mouse models in each group were measured. And dissection to observe the effect of liver, skeletal muscle, and cardiac muscle.

2.3.1 *Insulin resistance & tolerance test*

Although the Euglycemic hyper-insulinemic clamp (EHC)

is the gold standard for the measurement of IR in muscle, liver, and adipose tissue, many devices are not available due to the small size of the experimental mice, and the blood volume of the mice cannot support the mice The experiment was completed safely, therefore, the Avignon index which about oral glucose tolerance test (OGTT) was used in this experiment instead of EHC detection.

After the OGTT, Sib and Si2h data were measured, which were derived from fasting plasma insulin and glucose concentrations and derived from plasma insulin and glucose concentrations in the 120th min of OGTT, respectively.

SiM values are calculated from the previous results, which is derived by averaging Sib and Si2h after balancing Sib by a coefficient of 0.137 to give the same weight to both indices. From this, the value of Avignon index, an alternative to EHC, is obtained

3 Result

3.1 Insulin resistance and insulin tolerance

In this experiment, the result clearly indicates that insulin changes between the control group and methionine restriction groups, which is a significant change between the control group and the methionine restriction group in terms of insulin level and glucose level. As shown in Table 1, the insulin level in the experimental group decreased to 0.43ng/ml, and it is nearly fourfold in the control group. It is worth noting that although in this experiment, the glucose level between the control group and the methionine group also showed 186 ± 8 and 161 ± 3, respectively, which fluctuated greatly, the tendency of decreased blood sugar level by restriction methionine in the diet is unchangeable [14]. The possible reason for this change because of reducing Reactive Oxygen Species (ROS). Ros production is highly correlated with mitochondrial exposure. Koziel pointed out that twofold ROS would be produced under high glucose concentrations, and increased ROS would damage the mitochondrial DNA [15]. In addition, this paper has proved that damaged mitochondrial DNA, would damage the main transcription site of the mtDNA, which responsible for electron transport is decreased, and then superoxide radicals would also increase. Therefore, reducing ROS production through dietary restriction is critical for delaying or preventing diabetes. Furthermore, another possible reason is that the oxidation of methionine is related to the alternation functions of protein and many diseases [16]. ROS is a well-known oxidant which oxidizes sulphur-containing side chains of methionine. Methionine is oxidized to methionine sulfoxide by the addition of an extra oxygen atom, which can cause abnormal protein synthesis. Therefore, methionine oxidation is also a possible reason to cause insulin resistance. Reducing methionine intake could decrease the production of dysfunctional protein, which might explain why insulin levels and glucose levels are decreased after methionine restriction.

Table 1. The result of Insulin resistance and insulin tolerance

Parameter	CF	MR
Body Weight (g)	350±12	191±3
Glucose (mg/dL)	186±8	161±3
Hormones		
Insulin (ng/mL)	1.14±0.09	0.43±0.04

3.2 Insulin tolerance

Insulin tolerance is to tolerant insulin and makes hormones less effective impaired insulin sensitivity requires more insulin to produce to respond to glucose. As shown in Figure 1, significant differences have been shown between the control group and the methionine restriction group. With time changes, the glucose tolerance level of the experimental group is lower than the control group. Reducing insulin tolerance is important to delay the development of diabetes.

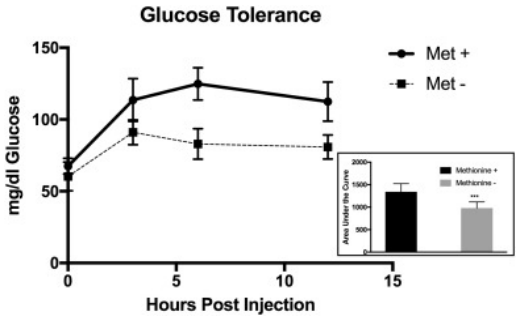

Fig. 1. Difference of glucose tolerance between Met+ diet group and Met- diet group [16].

3.3 Liver

Hagopian et al point out that the liver has relatively rich mitochondria, which is for better liver metabolism, and H2O2 is the main ROS which is produced by mitochondria. After restricting methionine in diet, Antioxidant Glutathione, GSH in liver tissue, was of methionine restriction group were markedly lower, as compared with the controls, and These results mentioned that reduced oxidative damage in hepatic mitochondria was positively related to diminished ROS generation instead of enhanced anti-oxidative capacity [17]. In addition, this paper also indicated the possible molecular mechanism to decrease ROS generation and protect mitochondria is decreasing the Apoptosis-inducing factor

(AIF) protein level in the liver.

3.4 Skeletal muscle

As shown in Figure 2, glycogen levels were significantly decreased in control mice and increased in methionine restriction mice at 16 weeks and 24 weeks [18]. In this paper, the authors prove that methionine restriction can normalize insulin resistance by promoting glucose utilization in skeletal muscle, which is a possible molecular mechanism to explain this result.

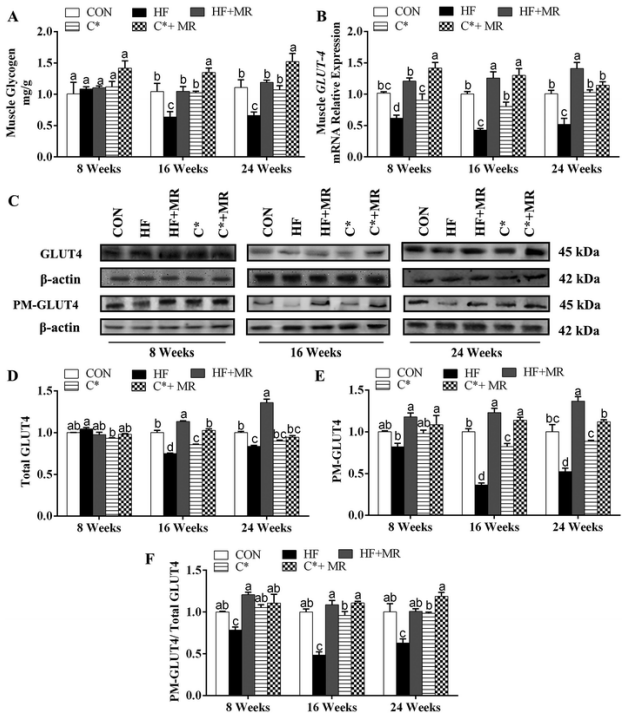

Fig. 2. Glycogen levels were compared between control and experimental mice at 16 and 24 weeks of a restricted diet. The glycogen level of the control group mice is significantly reduced, while the glycogen level of the experimental group (methionine-restricted) mice is increased [19].

4 Conclusion

Methionine is an essential amino acid, so it must be consumed in the diet to avoid nerve damage and visual symptoms caused by methionine deficiency. As cells age, increased oxidative stress prevents them from maintaining homeostasis and increases the risk of aging and metabolism-related diseases [20]. By reducing oxidative stress, methionine restriction (MetR) can induce beneficial longevity extension and metabolic health. This has been demonstrated in rat experiments, and lower methionine concentrations in cells are positively correlated with yeast cell lifespan. Conversely, increasing methionine concentrations also enhanced mitochondria-related respiratory and oxidative stress. Here we demonstrate that methionine restriction normalizes insulin resistance by promoting skeletal muscle glucose utilization, which may be a molecular mechanism explaining this result [21-25].

Reference

[1] DEFRONZO, R. A., FERRANNINI, E., GROOP, L., HENRY, R. R., HERMAN, W. H., HOLST, J. J., HU, F. B., KAHN, C. R., RAZ, I., SHULMAN, G. I., SIMONSON, D. C., TESTA, M. A. & WEISS, R. 2015. Type 2 diabetes mellitus. *Nat Rev Dis Primers,* 1**,** 15019.

[2] KOZIEL, A., WOYDA-PLOSZCZYCA, A., KICINSKA, A. & JARMUSZKIEWICZ, W. 2012. The influence of high glucose on the aerobic metabolism of endothelial EA.hy926 cells. *Pflugers Arch,* 464**,** 657-69.

[3] SAADANE, A., LESSIEUR, E. M., DU, Y., LIU, H. & KERN, T. S. 2020. Successful induction of diabetes in mice demonstrates no gender difference in development of early diabetic retinopathy. *PLoS One,* 15**,** e0238727.

[4] LUO, T., YANG, Y., XU, Y., GAO, Q., WU, G., JIANG, Y., SUN, J., SHI, Y. & LE, G. 2019. Dietary methionine restriction improves glucose metabolism in the skeletal muscle of obese mice. *Food Funct,* 10**,** 2676-2690.

[5] HOSHI, T. & HEINEMANN, S. 2001. Regulation of cell function by methionine oxidation and reduction. *J Physiol,* 531**,** 1-11.

[6] YOON, M. S. 2017. The Role of Mammalian Target of Rapamycin (mTOR) in Insulin Signaling. *Nutrients,* 9.

[7] TIWARI, B. S., BELENGHI, B. & LEVINE, A. 2002. Oxidative stress increased respiration and generation of reactive oxygen species, resulting in ATP depletion, opening of mitochondrial permeability transition, and programmed cell death. *Plant Physiol,* 128**,** 1271-81.

[8] ZOU, K., ROUSKIN, S., DERVISHI, K., MCCORMICK, M. A., SASIKUMAR, A., DENG, C., CHEN, Z., KAEBERLEIN, M., BREM, R. B., POLYMENIS, M., KENNEDY, B. K., WEISSMAN, J. S., ZHENG, J., OUYANG, Q. & LI, H. 2020a. Life span extension by glucose restriction is abrogated by methionine supplementation: Cross-talk between glucose and methionine and implication of methionine as a key regulator of life span. *Sci Adv,* 6**,** eaba1306.

[9] KITADA, M., OGURA, Y., MONNO, I., XU, J. & KOYA, D. 2021. Effect of Methionine Restriction on Aging: Its Relationship to Oxidative Stress. *Biomedicines,* 9.

[10] TARDY, A. L., POUTEAU, E., MARQUEZ, D., YILMAZ, C. & SCHOLEY, A. 2020. Vitamins and Minerals for Energy, Fatigue and Cognition: A Narrative Review of the Biochemical and Clinical Evidence. *Nutrients,* 12.

[11] TRIPODI, F., CASTOLDI, A., NICASTRO, R., REGHELLIN, V., LOMBARDI, L., AIROLDI, C., FALLETTA, E., MAFFIOLI, E., SCARCIA, P., PALMIERI,

L., ALBERGHINA, L., AGRIMI, G., TEDESCHI, G. & COCCETTI, P. 2018. Methionine supplementation stimulates mitochondrial respiration. *Biochim Biophys Acta Mol Cell Res,* 1865**,** 1901-1913.

[12] PERRONE, C. E., MATTOCKS, D. A., JARVIS-MORAR, M., PLUMMER, J. D. & ORENTREICH, N. 2010. Methionine restriction effects on mitochondrial biogenesis and aerobic capacity in white adipose tissue, liver, and skeletal muscle of F344 rats. *Metabolism,* 59**,** 1000-11.

[13] CASTANO-MARTINEZ, T., SCHUMACHER, F., SCHUMACHER, S., KOCHLIK, B., WEBER, D., GRUNE, T., BIEMANN, R., MCCANN, A., ABRAHAM, K., WEIKERT, C., KLEUSER, B., SCHURMANN, A. & LAEGER, T. 2019. Methionine restriction prevents onset of type 2 diabetes in NZO mice. *FASEB J,* 33**,** 7092-7102.

[14] ORENTREICH, N., MATIAS, J. R., DEFELICE, A. & ZIMMERMAN, J. A. 1993. Low methionine ingestion by rats extends life span. *J Nutr,* 123**,** 269-74.

[15] KITADA, M., XU, J., OGURA, Y., MONNO, I. & KOYA, D. 2020. Mechanism of Activation of Mechanistic Target of Rapamycin Complex 1 by Methionine. *Front Cell Dev Biol,* 8**,** 715.

[16]. HAGOPIAN, K., HARPER, M. E., RAM, J. J., HUMBLE, S. J., WEINDRUCH, R. & RAMSEY, J. J. 2005. Long-term calorie restriction reduces proton leak and hydrogen peroxide production in liver mitochondria. *Am J Physiol Endocrinol Metab,* 288**,** E674-84.

[17] WANDERS, D., STONE, K. P., FORNEY, L. A., CORTEZ, C. C., DILLE, K. N., SIMON, J., XU, M., HOTARD, E. C., NIKONOROVA, I. A., PETTIT, A. P., ANTHONY, T. G. & GETTYS, T. W. 2016. Role of GCN2-Independent Signaling Through a Noncanonical PERK/NRF2 Pathway in the Physiological Responses to Dietary Methionine Restriction. *Diabetes,* 65**,** 1499-510.

[18] NAVIK, U., SHETH, V. G., KABEER, S. W. & TIKOO, K. 2019. Dietary Supplementation of Methyl Donor l-Methionine Alters Epigenetic Modification in Type 2 Diabetes. *Mol Nutr Food Res,* 63**,** e1801401.

[19] LIANG, Y. Z., DONG, J., ZHANG, J., WANG, S., HE, Y. & YAN, Y. X. (2018). Identification of Neuroendocrine Stress Response-Related Circulating MicroRNAs as Biomarkers for Type 2 Diabetes Mellitus and Insulin Resistance. *Front Endocrinol (Lausanne),* 9**,** 132.

[20] DEFRONZO, R. A., FERRANNINI, E., GROOP, L., HENRY, R. R., HERMAN, W. H., HOLST, J. J., HU, F. B., KAHN, C. R., RAZ, I., SHULMAN, G. I., SIMONSON, D. C., TESTA, M. A. & WEISS, R. 2015. Type 2 diabetes mellitus. *Nat Rev Dis Primers,* 1**,** 15019.

[21] SAMUEL, V. T. & SHULMAN, G. I. 2016. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest,* 126**,** 12-22.

[22] YING, Y., YUN, J., GUOYAO, W., KAIJI, S., ZHAOLAI, D. & ZHENLONG, W. 2019. Dietary L-methionine restriction decreases oxidative stress in porcine liver mitochondria. *Exp Gerontol,* 65**,** 35-41.

[23] ONG, P. S., WANG, L. Z., DAI, X., TSENG, S. H., LOO, S. J. & SETHI, G. 2016. Judicious Toggling of mTOR Activity to Combat Insulin Resistance and Cancer: Current Evidence and Perspectives. *Front Pharmacol,* 7**,** 395.

[24] KOWLURU, R. A. & MISHRA, M. 2015. Oxidative stress, mitochondrial damage and diabetic retinopathy. *Biochim Biophys Acta,* 1852**,** 2474-83.

[25] ZOU, Z., TAO, T., LI, H. & ZHU, X. 2020b. mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. *Cell Biosci,* 10**,** 31.