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MOTS-c and its K14Q variant demonstrate significant lipid-lowering and weight-reducing effects in obese mice

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A 1382A>C substitution in the MOTS-c gene results in a K14Q amino acid replacement. Research on the MOTS-c K14Q variant is still limited, and its role in blood lipid metabolism remains unclear. In this study, we investigated the effects of MOTS-c and its K14Q variant on obesity. A diet-induced obesity mouse model was established and treated with injections of either MOTS-c or the K14Q variant. Subsequent analyses examined AMPK (*PRKAA1*) expression, changes in body weight, and serum lipid profiles, including total cholesterol (TC), triglycerides (TG), and free fatty acids (FFA). Both MOTS-c and the K14Q variant activated AMPK expression and reduced body weight as well as levels of TC, TG, and FFA. However, MOTS-c exhibited a stronger effect on reducing TC and TG, whereas the K14Q variant showed a greater impact on lowering FFA. In conclusion, MOTS-c and its K14Q variant both exert lipid-lowering effects, but their differential impacts on TC, TG, and FFA suggest distinct regulatory roles in blood lipid metabolism.

Keywords: MOTS-c, MOTS-c K14Q, diet-induced obesity mice, total cholesterol, triglycerides, free fatty acids

Introduction

MOTS-c is a 16-amino-acid mitochondrial-derived peptide encoded by the 12S rRNA region of the mitochondrial genome[1]. The K14Q variant of MOTS-c results from a mitochondrial polymorphism (m.1382A>C) that produces a lysine-to-glutamine substitution at position fourteen[2]. Reduced circulating MOTS-c levels have been reported in obese male children and are negatively correlated with insulin resistance[3]. Previous studies have demonstrated that MOTS-c suppresses lipid synthesis and alleviates obesity by activating AMP-activated protein kinase (AMPK), a central regulator of cellular energy homeostasis[4]. The K14Q polymorphism has been reported to be specific to Northeast Asian populations and has been associated with increased longevity[5]. Additional studies suggest that this variant may influence sprint and power performance by modulating skeletal muscle fiber composition[6], and it has also been linked to age-related sarcopenia in older Korean men[7]. Moreover, the m.1382A>C polymorphism has been associated with increased susceptibility to type 2 diabetes, particularly in men[8]. However, its potential relationship with obesity has not yet been investigated or reported.

Obesity is commonly accompanied by elevated blood lipid levels including total cholesterol (TC), triglycerides (TG), and free fatty acids (FFA), which serve as key biochemical indicators of metabolic dysfunction[9]. In clinical practice, TC, TG, and FFA are commonly used as key biomarkers for assessing obesity-related metabolic disturbances[10]. However, the effects of MOTS-c and its K14Q variant on these lipid parameters have not yet been elucidated. Therefore, evaluating the influence of MOTS-c and the K14Q variant on TC, TG, and FFA is essential for understanding their roles in obesity and related metabolic disorders. To address this gap, we established a diet-induced obesity (DIO) mouse model and administered MOTS-c or MOTS-c K14Q to assess their respective effects on serum lipid profiles, with the aim of exploring their therapeutic potential for obesity and metabolic disease.

Materials and methods

Synthesis of MOTS-c and MOTS-c K14Q

Freeze-dried peptide powders of MOTS-c and its K14Q variant were synthesized by Shanghai QYAOBIO (ChinaPeptides) Co., Ltd. The peptide sequences were MRWQEMGYIFYPRKLR (MOTS-c) and MRWQEMGYIFYPRQLR (MOTS-c K14Q). The amino acid substitution corresponding to the K14Q variant is presented in Table 1. Both peptides were verified to have >95% purity by high-performance liquid chromatography (HPLC) and correct molecular weight by mass spectrometry.

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Table 1 — The m.1382A>C polymorphism (highlighted in bold) in the 12S rRNA region causes a nonsynonymous change in the MOTS-c peptide

Nucleotide position (mtDNA)	Nucleotide sequence	Amino acid (3-letter code)	Amino acid position
1343-1345	ATG	Met (M) (Start codon)	1
1346-1348	AGG	Arg (R)	2
1349-1351	TGG	Trp (W)	3
1352-1354	CAA	Gln (Q)	4
1355-1357	GAA	Glu (E)	5
1358-1360	ATG	Met (M)	6
1361-1363	GGC	Gly (G)	7
1364-1366	TAC	Tyr (Y)	8
1367-1369	ATT	Ile (I)	9
1370-1372	TTC	Phe (F)	10
1373-1375	TAC	Tyr (Y)	11
1376-1378	CCC	Pro (P)	12
1379-1381	AGA	Arg (R)	13
1382-1384	[A>C]AA	Lys (K) > Glu (Q)	14
1385-1387	CTA	Leu (L)	15
1388-1390	CGA	Arg (R)	16
1391-1393	TAG	Stop	

Establishing a DIO mouse model

C57BL/6 male mice were used due to their stable genetic background and frequent application in metabolic disease research. Forty-eight mice were individually housed and acclimated for one week before baseline body weights were recorded. After weighing, mice were randomly assigned to either a normal diet (ND) group (n = 12) or a high-fat diet (HFD) group (n = 36). The ND group received standard chow, whereas the HFD group was fed a diet containing 45 kcal% fat. Body weights were measured weekly. Mice in the HFD group whose body weight exceeded the ND group average by at least 20% were considered successfully induced for obesity.

Peptide administration

Freeze-dried MOTS-c and MOTS-c K14Q powders were dissolved in saline to prepare peptide solutions at a concentration of 0.5 mg/mL. A dosage of 5 mg/kg/day was selected based on previous reports demonstrating significant metabolic effects in DIO mice without observed toxicity[11]. Successfully modeled obese mice were randomly divided into three groups: HFD + MOTS-c (n = 12), HFD + MOTS-c K14Q (n = 12), and HFD control (n = 12). Mice in the treatment groups received daily intraperitoneal injections of MOTS-c or MOTS-c K14Q at 5 mg/kg/day. The HFD control group received an equivalent volume of saline. Treatments were administered for 6 weeks. Every two weeks, four mice were randomly selected from each group, fasted for 12 hours, and euthanized by cervical dislocation under anesthesia. Blood samples were collected, centrifuged at 3,500 rpm for 5 minutes, and serum was stored at -20 °C. The overall experimental design is illustrated in Fig 1.

Blood lipid measurements

Serum total cholesterol (TC), triglycerides (TG), and free fatty acids (FFA) were measured using a Total Cholesterol Determination Kit (A111-1-1, Nanjing Jiancheng Bioengineering Institute), a Triglyceride Colorimetric Test Kit (E-BC-F033, Wuhan Yilairuite Biotechnology), and a Free Fatty Acid Test Kit (A042-1-1, Nanjing Jiancheng Bioengineering Institute), respectively, following the manufacturers' instructions.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from abdominal adipose tissue using TRIzol reagent (Sigma-Aldrich, China). Equal amounts of RNA were reverse transcribed into cDNA using the RevertAid Master Mix (Thermo Scientific, USA). qRT-PCR was performed using primers synthesized by RiBoBio (Guangzhou, China). Reactions were conducted in a 20 μL system containing 10 μL PowerUp SYBR Green Master Mix (Thermo Scientific, USA) on a StepOnePlus Real-

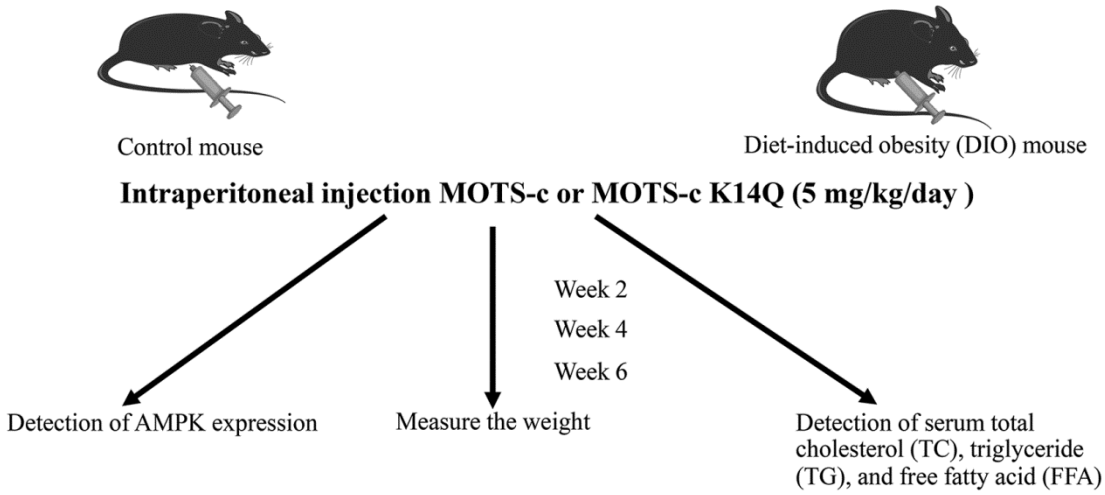

Fig. 1 — Schematic of the experimental design
Overview of diet-induced obesity (DIO) mouse model establishment, randomization into treatment groups, peptide administration (MOTS-c or MOTS-c K14Q, 5 mg/kg/day), and timing of sample collection for body weight, serum lipid, and AMPK analysis.

Time PCR System (Applied Biosystems, USA). Primer sequences were as follows: AMPK (*PRKAA1*): forward 5'-TACTCAACCGGCAGAAGATTCG-3', reverse 5'-AGACGGCGGCTTTCCTTTT-3'; *GAPDH*: forward 5'-AGGTCGGTGTGAACGGATTTG-3', reverse 5'-TGTAGACCATGTAGTTGAGGTCA-3'. Relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Data are presented as mean ± standard deviation (SD) from at least three independent experiments. Paired-sample *t*-tests were used to assess differences before and after treatment where applicable. One-way ANOVA was performed for comparisons among the ND, HFD, MOTS-c, and MOTS-c K14Q groups using SPSS 20.0 (SPSS Inc., Chicago, IL). A P value < 0.05 was considered statistically significant.

Results and Discussion

Both MOTS-c and MOTS-c K14Q activate AMPK expression

Our findings showed that both MOTS-c and its K14Q variant significantly upregulated AMPK expression in obese mice (Fig. 2). AMPK expression levels in the ND group were included as baseline reference values. MOTS-c induced a stronger increase in AMPK expression compared with MOTS-c K14Q, suggesting that the wild-type peptide retains greater metabolic efficacy. As a central energy sensor, AMPK regulates cellular homeostasis by promoting glucose uptake, enhancing lipid oxidation, and inhibiting fatty acid and cholesterol synthesis[12]. Thus, increased AMPK expression represents a key mechanism through which MOTS-c exerts its lipid-lowering and weight-reducing effects.

The K14Q variant originates from the mitochondrial DNA polymorphism m.1382A>C in the 12S rRNA region, causing a lysine-to-glutamine substitution at position 14. This amino acid change does not eliminate function but appears to attenuate AMPK activation, as reflected in our results. Epidemiological studies have reported that the K14Q polymorphism is associated with an elevated risk of type 2 diabetes in sedentary males[8], while the studies have shown impaired glucose tolerance and altered insulin sensitivity[8]. These reports align with our observations, indicating that the variant exhibits partially reduced metabolic benefits compared with wild-type MOTS-c.

Functional differences between MOTS-c and MOTS-c K14Q in AMPK activation may result from subtle structural changes that influence interactions with upstream regulators or downstream targets. Reduced AMPK activation could lead to weaker stimulation of glucose transporters, diminished suppression of lipogenesis, and less effective regulation of lipid accumulation in peripheral tissues[13]. Even modest declines in AMPK activity may substantially affect long-term energy homeostasis, given AMPK's pivotal regulatory role[14]. Collectively, these results indicate that although both peptides activate AMPK and promote metabolic regulation, the K14Q variant is less efficient,

offering a mechanistic explanation for its association with metabolic disorders.

Both MOTS-c and MOTS-c K14Q reduce body weight

Both peptides reduced body weight in obese mice, with MOTS-c producing a significantly greater effect, compared to HFD group (Fig. 3A). Weight reduction was evident as early as week 2, with progressively larger differences observed at weeks 4 and 6. These findings are consistent with previous research demonstrating that MOTS-c enhances metabolic homeostasis and alleviates obesity by increasing energy expenditure and regulating glucose and lipid utilization[15]. The attenuated weight-reducing effect of the K14Q variant may stem from its reduced ability to activate AMPK, resulting in less effective inhibition of lipogenesis and energy imbalance. Although no prior studies have directly linked the K14Q polymorphism to obesity, our results indicate that the variant retains partial anti-obesity activity. These observations further suggest that genetic variation in MOTS-c may influence individual susceptibility to obesity, underscoring the peptide's therapeutic potential.

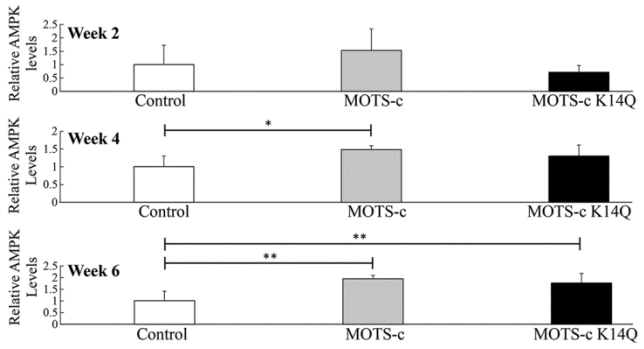

Fig. 2 — *PRKAA1* expression induced by MOTS-c and MOTS-c K14Q

PRKAA1 mRNA expression (AMPK activation) in abdominal adipose tissue was evaluated at weeks 2, 4, 6 after MOTS-c or MOTS-c K14Q administration in the normal-diet, high-fat diet (HFD), HFD+MOTS-c, and HFD+MOTS-c K14Q groups. The expression level in the normal diet (ND) was set to 1. Error bars represent standard deviation (SD) from three independent biological replicates. Statistical significance: $*P<0.05$, $**P<0.01$, $***P<0.001$.

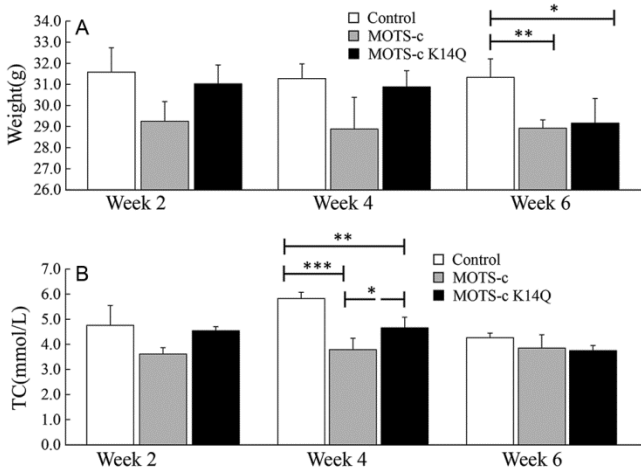

Fig. 3 — Effects of MOTS-c and MOTS-c K14Q on body weight and total cholesterol

Body weight (A) and total cholesterol levels (B) were measured at weeks 2, 4, and 6 after administration of MOTS-c or MOTS-c K14Q in the normal-diet, high-fat diet (HFD), HFD+MOTS-c, and HFD+MOTS-c K14Q groups. Error bars represent the standard deviation (SD) from three independent biological replicates. Statistical significance: $*P<0.05$, $**P<0.01$, $***P<0.001$.

Both MOTS-c and MOTS-c K14Q lower TC and TG

Serum lipid analyses showed that both peptides significantly reduced TC (Fig. 3B) and TG (Fig. 4A) levels, with MOTS-c exerting stronger effects. Hypercholesterolemia and hypertriglyceridemia are major risk factors for metabolic and cardiovascular diseases, reflecting excessive lipid synthesis or impaired lipid clearance[16,17]. Previous studies have shown that MOTS-c activates AMPK and downstream pathways such as acetyl-CoA carboxylase phosphorylation, which inhibits fatty acid and cholesterol synthesis[18]. The reduced efficacy of MOTS-c K14Q suggests that the

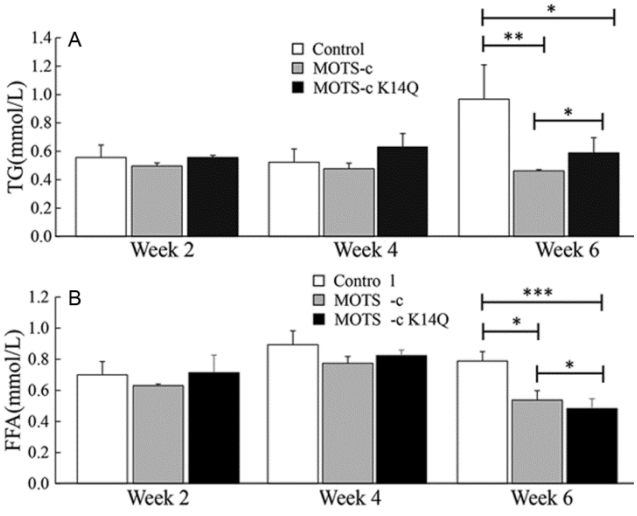

Fig. 4 — Effects of MOTS-c and MOTS-c K14Q on triglycerides and free fatty acids

Triglyceride (A) and free fatty acid (B) levels were assessed at weeks 2, 4, and 6 following treatment with MOTS-c or MOTS-c K14Q in the normal-diet, high-fat diet (HFD), HFD+MOTS-c, and HFD+MOTS-c K14Q groups. Error bars represent the standard deviation (SD) from three independent biological replicates. Statistical significance: $*P<0.05$, $**P<0.01$, $***P<0.001$.

polymorphism weakens its capacity to modulate lipid metabolism, leading to higher residual TC and TG levels. These functional differences may help explain epidemiological links between the K14Q variant and metabolic disorders. While both peptides improved lipid homeostasis, their differing potencies emphasize the clinical significance of MOTS-c genetic variability.

Both MOTS-c and MOTS-c K14Q lower FFA

Interestingly, both peptides also lowered free fatty acid (FFA) levels, but MOTS-c K14Q exhibited a stronger effect than wild-type MOTS-c (Fig. 4B). Elevated FFA levels contribute to insulin resistance and lipotoxicity by indicating dysregulated adipose tissue lipolysis[19,20]. The greater reduction in FFA observed in MOTS-c K14Q, treated mice may reflect enhanced suppression of lipolysis, potentially through insulin-sensitizing mechanisms or variant-specific effects on adipocyte metabolism[21,22]. This divergent response suggests that the K14Q variant may selectively modulate particular aspects of lipid metabolism, producing a metabolic profile distinct from that of wild-type MOTS-c. These findings highlight the complexity of MOTS-c biology and suggest that functional polymorphisms may shift the metabolic balance in nuanced and unexpected ways.

This mechanism may be related to the fact that the K14Q variant alters a key residue within the functional domain of the MOTS-c peptide, potentially modifying its interaction with metabolic signaling pathways and leading to enhanced regulation of lipid metabolism, particularly a stronger suppression of circulating FFAs. Because elevated FFA levels are closely associated with insulin resistance[23], one plausible explanation is that the K14Q variant improves insulin signaling more effectively than wild-type MOTS-c. This enhanced insulin sensitivity could strengthen insulin's anti-lipolytic effects in adipocytes, thereby reducing the release of FFAs into the bloodstream. Moreover, as MOTS-c is a mitochondria-encoded peptide, structural variation at position K14 could increase FFA uptake by peripheral tissues, or shift substrate utilization toward greater fatty acid catabolism, suggesting that the variant engages a partially distinct metabolic regulatory mechanism compared with the wild-type peptide.

Conclusion

This study demonstrates that both MOTS-c and its K14Q variant reduce body weight and improve lipid profiles in obese mice. However, notable differences were observed: MOTS-c was more effective in reducing TC and TG, whereas the K14Q variant showed a stronger impact on FFA reduction. These findings indicate that although the two peptides share core metabolic functions, the K14Q polymorphism modifies their relative efficiency, potentially engaging distinct pathways in lipid metabolism. Further research is needed to elucidate these mechanisms and explore their therapeutic implications.

Ethical statement

This study protocol was reviewed and approved by Ethics Committee of Guilin Medical University, approval number GYLL2022070.

Funding statement

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Conflict of interest

The authors declare that there are no conflicts of interest related to this article.

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