Plasma MOTS-c levels are associated with insulin sensitivity in lean but not in obese individuals

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ABSTRACT

Mitochondrial open reading frame of the 12S rRNA-c (MOTS-c) is a mitochondrial-derived peptide that attenuates weight gain and hyperinsulinemia when administered to high fat-fed mice. MOTS-c is therefore a potential regulator of metabolic homeostasis under conditions of high-energy supply. However, the effect of insulin resistance and obesity on plasma MOTS-c concentration in humans is unknown. To gain insight into MOTS-c regulation, we measured plasma MOTS-c concentration and analyzed its relationship with insulin sensitivity surrogates, in lean and obese humans (n=10 per group). Obese individuals had impaired insulin sensitivity as indicated by low Matsuda and high Homeostatic Model Assessment (HOMA) indexes. Although plasma MOTS-c concentration was similar in lean and obese individuals (0.48±0.16 and 0.52±0.15 ng/mL; p=0.60), it was correlated with HOMA (r=0.53; p<0.05) and Matsuda index (r=−0.46; p<0.05). Notably, when the groups were analyzed separately, the associations remained only in lean individuals. We conclude that plasma MOTS-c concentration is unaltered in human obesity. However, MOTS-c associates positively with insulin resistance mostly in lean individuals, indicating that plasma MOTS-c concentration depends on the metabolic status in this population. Such dependence seems altered when obesity settles. The implications of plasma MOTS-c for human metabolic homeostasis deserve future examination.

INTRODUCTION

Mitochondrial DNA has a wider genetic repertoire than previously envisaged. Recent studies have found short open reading frames (sORF) within specific mitochondrial genes.[1] Humanin was the first sORF-encoded peptide uncovered, whose coding sequence localizes within the 16S rRNA gene.[2 3] Recently, MOTS-c, another sORF-encoded peptide, was identified in the 12S rRNA gene.[4] These mitochondrial-derived peptides are released into circulation and may exert endocrine actions.[4 5] Thus, a signaling from mitochondria towards distant tissues could exist.

Findings in mice show that MOTS-c regulates in vivo metabolic homeostasis.[4] Exogenous MOTS-c administration for 7 days improved glucose tolerance and skeletal muscle insulin sensitivity. In mice fed with high-fat diet, MOTS-c treatment attenuated weight gain and prevented hyperinsulinemia. Those effects appear to be explained by increased thermogenesis (in kcal/h) in MOTS-c-treated mice, without changes in energy intake or physical activity.

Such evidence suggests that MOTS-c is part of a mechanism that handles excess energy supply by increasing energy flux and insulin sensitivity to preserve metabolic homeostasis. In this regard, fasted versus fed mice show lower plasma and skeletal muscle MOTS-c level.[4] Noteworthy, whether plasma MOTS-c concentration is altered in obese animals or humans has not been reported. Hypothetically, while obesity and insulin resistance develop, MOTS-c expression increases to preserve fuel homeostasis.

To gain insight into the regulation of circulating MOTS-c in humans, we compared plasma MOTS-c concentration in obese versus lean individuals and examined the relationship between plasma MOTS-c and some metabolic variables.

MATERIALS AND METHODS

Volunteers

Twenty healthy (by physical examination and routine laboratory tests), sex-matched and age-matched volunteers were separated into lean and obese groups (body mass index <25.0 and >30.0 kg/m^2, respectively; table 1). They had stable body weight (change <2 kg over the past 3 months), and none performed regular physical activity (<60 min/week) or took medications other than oral contraceptives. All individuals provided written informed consent before their participation.

Experimental design

From overnight-fasted individuals, a blood sample was drawn to determine circulating concentration of glucose, lactate, insulin, and MOTS-c. Then, a standard 75 g oral glucose load was supplied, and blood concentrations of glucose, lactate, and insulin were determined every 30 min for the next 3 hours (oral glucose tolerance test (OGTT)).

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Table 1 Characteristics of the individuals

	Lean		Obese		Two-way analysis of variance (p values)		
	Males	Females	Males	Females	Sex	Group	Sex*group
n	5	5	5	5			
Age (years)	35.2±8.5	31.1±5.0	37.8±3.1	34.0±10.6	0.25	0.42	0.97
Body weight (kg)	67.5±5.2	55.4±3.9	100.8±10.5	83.5±7.4	<0.001	<0.0001	0.43
BMI (kg/m^2)	22.4±1.0	21.7±1.2	32.5±1.5	32.3±1.6	0.46	<0.0001	0.76
Fasting							
Glucose (mg/dL)	89.5±5.7	88.8±3.0	94.2±2.4	90.8±2.9	0.23	0.06	0.44
Insulin (mU/L)	5.8±2.3	4.2±2.0	9.5±2.5	14.9±7.2	0.30	<0.01	0.08
Lactate (mmol/L)	1.16±0.27	0.84±0.33	1.54±0.14	1.21±0.41	<0.05	<0.05	0.97
Triglycerides (mg/dL)	112±52	67±50	113±56	97±39	0.20	0.49	0.52
HOMA	1.26±0.50	0.94±0.48	2.21±0.64	3.33±1.52	0.34	<0.001	0.09
OGTT							
2 hour glucose (mg/dL)	107.3±16.1	111.6±22.5	144.2±22.9	119.0±32.1	0.35	0.06	0.19
2 hour insulin (mU/L)	45.1±25.2	46.6±47.3	75.5±31.5	101.2±50.0	0.46	<0.05	0.51
Matsuda index	5.70±2.99	8.08±4.22	2.96±0.95	2.19±0.45	0.50	<0.01	0.20
OGIS-2h	430.7±49.4	428.9±72.3	340.9±40.6	351.1±43.3	0.87	<0.01	0.81
MOTS-c (ng/mL)	0.54±0.21	0.42±0.08	0.49±0.11	0.55±0.19	0.71	0.60	0.22

Data are means±SD.
BMI, body mass index; HOMA, homeostatic model assessment; OGTT, oral glucose tolerance test; OGIS, oral glucose insulin sensitivity.

Biochemical analyses

Plasma glucose and plasma lactate were measured by the glucose oxidase and lactate oxidase methods. Serum insulin was measured by direct chemiluminescence (Advia Centaur; Bayer Corporation, Newbury, UK). Plasma MOTS-c was measured by a non-commercial ELISA at the University of Southern California, where MOTS-c was first described.[4] Homeostatic model assessment (HOMA), Matsuda index,[6] and Oral Glucose Insulin Sensitivity (OGIS)-2h index[7] were calculated as insulin sensitivity surrogates.

Statistical analyses

Data are presented as mean±SD. Analyses were performed using SAS V.9.2. Two-way analysis of variance was used to determine the effects of sex (males vs females), group (lean vs obese), and sex*group interaction. Glass's effect size was determined as the difference between means divided by the SD of the lean group.[8] Spearman's test was used to assess associations between variables. The significance level was set at $p<0.05$.

RESULTS

Females versus males had lower body mass (69.4±15.8 vs 84.1±19.2 kg, respectively; $p<0.001$) and lower lactatemia (1.03±0.40 vs 1.35±0.28 mmol/L, respectively; $p<0.05$). Sex did not affect any other variable (table 1).

Fasting lean versus obese individuals showed lower insulinemia (5.0±2.2 vs 12.2±5.9 mU/L, respectively; $p<0.01$), lower lactatemia (1.00±0.33 vs 1.38±0.34 mmol/L, respectively; $p<0.05$) and a borderline lower glycemia (89±4 vs 93±3 mg/dL, respectively; $p=0.06$). Two hours after the glucose load, insulinemia was lower in lean versus obese individuals (45.9±35.7 vs 88.4±41.6 mU/L, respectively; $p<0.05$) and glycemia had a similar trend (109.5±18.6 vs 131.6±29.5 mg/dL, respectively; $p=0.06$). Also, similar postprandial mean lactatemia and mean glycemia were observed between groups (data not shown). Lean versus obese individuals had lower HOMA and higher Matsuda and OGIS-2h indexes (table 1).

Plasma MOTS-c concentration was similar in lean and obese groups (0.48±0.16 and 0.52±0.15 ng/mL, respectively; $p=0.60$, table 1 and figure 1A), with a Glass's effect size of 0.24. Considering all individuals, plasma MOTS-c concentration correlated positively with HOMA (figure 1B) and negatively with Matsuda index (figure 1C). In contrast, plasma MOTS-c concentration was not associated with the OGIS-2h index ($r=-0.33$, $p=0.17$), lactatemia ($r=0.27$, $p=0.25$), the incremental area under the curve (AUCi) for glucose during the OGTT ($r=0.03$, $p=0.89$), or the ratio between AUCi for insulin and AUCi for glucose during the OGTT as a surrogate of insulin secretion ($r=0.17$, $p=0.48$).

When lean individuals were analyzed separately, fasting lactatemia (figure 1D) and HOMA (figure 1E) correlated positively with plasma MOTS-c concentration, whereas Matsuda index (figure 1F) correlated negatively. Similar analyses in obese individuals showed no such associations (figure 1G–I).

DISCUSSION

We found that lean versus obese humans displayed similar plasma MOTS-c concentration. However, MOTS-c associated with insulin sensitivity surrogates in the overall group. Further analyses considering the groups separately indicated that lean individuals mostly explain the associations in the overall group. These results suggest that a differential regulation of MOTS-c occurs while obesity develops.

Lack of difference in plasma MOTS-c between lean and obese individuals may not be attributable to underpowered sample size, since a small Glass's effect size was detected. In fact, over 200 individuals per group will be needed to detect such a difference in plasma MOTS-c concentration (power of 80%). Thus, based on MOTS-c circulating concentration, one can challenge its relevance as a surrogate measure of its tissue action as well as its proposed role as mitokine.[9]

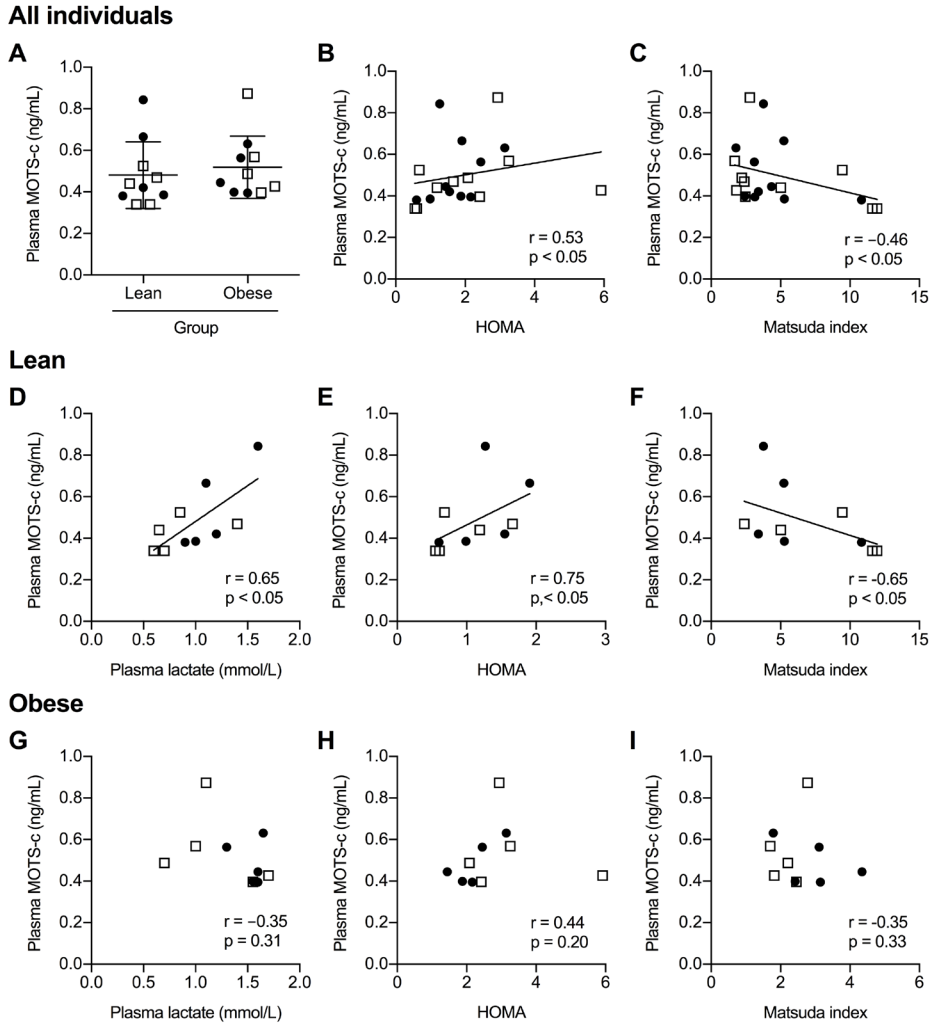

Figure 1 Plasma MOTS-c concentration, plasma lactate, and insulin sensitivity surrogates in lean and obese individuals. (A) Plasma MOTS-c concentration in lean and obese groups (n=10 per group). (B–C) Relationship between plasma MOTS-c concentration with HOMA and Matsuda index in the overall group (n=20). (D–I) Relationship between plasma MOTS-c concentration with lactatemia, HOMA, and Matsuda index in lean (D–F) and obese (G–I) individuals (n=10 per group). Circles represent the males and square represent the females. HOMA, homeostatic model assessment; MOTS-c, mitochondrial open reading frame of the 12S rRNA-c.

The notion that plasma MOTS-c concentration differ according to body size lies on earlier findings in mice, which showed that administration of MOTS-c attenuated dietary fat-induced weight gain.[4] Such finding was accompanied by similar glycemia and insulinemia when compared with lean animals fed with normal diet. Thus, the well-known influence of high-fat diets promoting obesity and insulin resistance was diminished when MOTS-c action was enhanced through chronic MOTS-c administration. Considering that fasting decreases circulating and skeletal muscle MOTS-c content in mice,[4] we hypothesized that obesity increases circulating MOTS-c concentration, which would lead to enhanced tissue action to preserve metabolic homoeostasis. Lack of difference in circulating MOTS-c concentration in lean versus obese individuals did not support such hypothesis. However, we cannot neglect that tissue MOTS-c content and its action may still differ in lean versus obese individuals.

An aspect to consider when comparing plasma MOTS-c concentration between groups relates with its role enhancing mitochondrial capacity to meet ATP demand under conditions of cellular stress.[10] We cannot ascertain the extent of the cellular stress at which obese and lean individuals were exposed. To judge from circulating triglyceride concentration, a marker of systemic metabolic control, both groups showed similar values. Thus, obese volunteers in this study, despite having insulin resistance, share some of the features found in metabolically 'healthy' obesity.[11] One can speculate that cellular stress in obese volunteers was insufficient to trigger a differential synthesis and/or release of MOTS-c versus lean volunteers.

Lack of difference in circulating MOTS-c levels in lean and obese groups might be because the synthesis of MOTS-c is impaired in obesity states as a consequence of mitochondrial dysfunction. In this regard, subcutaneous fat cells taken from heavier versus leaner discordant twin

donors show lower mitochondrial-encoded gene expression including the 12S rRNA gene.[12] Whether this finding has any implications for muscle MOTS-c expression and release is unknown.

Remarkably, we found that insulin sensitivity (under fasting and postprandial conditions) is related to lower plasma MOTS-c concentration in the overall group. The effect was also observed in lean individuals, but it was weaker and not significant in obese volunteers. These findings are interesting considering the small sample size of our study and suggest a mechanism to preserve insulin sensitivity at early stages of insulin resistance. In turn, the relevance of MOTS-c action (or its circulating levels) would decrease at later stages of insulin resistance. In the same line, circulating MOTS-c concentration is related to fasting lactate concentration in lean individuals. Such association may be concordant with earlier evidence showing higher lactate release from HEK293 cells having stable MOTS-c overexpression.[4] Such differential response in lean versus obese individuals may suggest that circulating MOTS-c concentration in lean, but not obese, individuals is reflective of its tissue action. Finally, the proposed endocrine role of MOTS-c can be challenged considering that most cell types have mitochondria. Thus, MOTS-c might have an auto/paracrine rather an endocrine action. Eventually, circulating MOTS-c might result of tissue spillover instead of an actual interorgan communication.

In conclusion, plasma MOTS-c concentration in lean and obese individuals was similar. Intriguingly, plasma MOTS-c concentration correlated inversely with insulin sensitivity surrogates, an effect mostly explained by lean individuals. This result suggests that circulating MOTS-c better reflects MOTS-c action in lean individuals. The role of MOTS-c as part of a mechanism to preserve metabolic homeostasis at early stages of insulin resistance deserves further assessment.

Contributors JEG, LRC and JLS conceived the study. All authors participated in data analysis and interpretation. JEG, LRC and RF-V wrote the manuscript, which was approved by all the co-authors.

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Competing interests None declared.

Patient consent Obtained.

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