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Research report

# Saturated lipids decrease mitofusin 2 leading to endoplasmic reticulum stress activation and insulin resistance in hypothalamic cells

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ABSTRACT

Endoplasmic reticulum (ER) and mitochondria dysfunction contribute to insulin resistance generation during obesity and diabetes. ER and mitochondria interact through Mitofusin 2 (MTF2), which anchors in the outer mitochondrial and ER membranes regulating energy metabolism. Ablation of MTF2 leads to ER stress activation and insulin resistance. Here we determine whether lipotoxic insult induced by saturated lipids decreases MTF2 expression leading to ER stress response in hypothalamus and its effects on insulin sensitivity using *in vitro* and *in vivo* models. We found that lipotoxic stimulation induced by palmitic acid, but not the monounsaturated palmitoleic acid, decreases MTF2 protein levels in hypothalamic mHypoA-CLU192 cells. Also, palmitic acid incubation activates ER stress response evidenced by increase in the protein levels of GRP78/BIP marker at later stage than MTF2 downregulation. Additionally, we found that MTF2 alterations induced by palmitic, but not palmitoleic, stimulation exacerbate insulin resistance in hypothalamic cells. Insulin resistance induced by palmitic acid is prevented by pre-incubation of the anti-inflammatory and the ER stress release reagents, sodium salicylate and 4 phenylbutirate, respectively. Finally, we demonstrated that lipotoxic insult induced by high fat feeding to mice decreases MTF2 proteins levels in arcuate nucleus of hypothalamus. Our data indicate that saturated lipids modulate MTF2 expression in hypothalamus coordinating the ER stress response and the susceptibility to insulin resistance.



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## 1. Introduction

Excessive adiposity is a major risk factor for developing type 2 diabetes mellitus (T2DM) and several chronic metabolic disorders in part by disrupting insulin sensitivity and promoting increase in plasma glucose levels (Malik et al., 2013). Glucose homeostasis is regulated by the hypothalamic nucleus (arcuate, dorsomedial, lateral) involved in the control of energy balance through the integration of peripheral metabolic signals (Scarlett and Schwartz, 2015). Disruption of signaling integration in hypothalamic nucleus might result in insulin resistance states, characterized in obesity and diabetes (Scarlett and Schwartz, 2015).

The hypothalamus is an important target of lipids toxicity during obesity (Williams, 2012). High fat diet (HFD) intake in mice promotes lipotoxicity by activating ER stress and mitochondrial dysfunction resulting in insulin resistance and T2DM (Serra et al., 2013; Williams, 2012). Our investigation group as well as others have published that the quantity and the quality of lipids determines the toxic effects, being that accumulation of ceramides, acylcarnitines and diacylglycerols are deleterious in compare to triglycerides in liver, adipose tissue, muscle and also brain (Camacho et al., 2012, 2013; Medina-Gomez et al., 2007). In this context, in contrast to unsaturated fatty acids, saturated fats promote deregulation of food intake, increase body weight, obesity and are detrimental in the coordination of insulin sensitivity (Larsen and Tennagels, 2014). *In vitro* evidence has shown that stimulation with the saturated lipid, palmitic acid, promotes mitochondrial deregulation, oxidative stress and JNK and NF-kB activation in muscle cells resulting in insulin resistance (Nie et al., 2014). Also, palmitic promotes ER stress activation in different cell types including neurons (Kwon et al., 2014; Nie et al., 2014; Yuzefovych et al., 2012). These evidences suggest that ER and mitochondria are primary targets of lipids leading to insulin resistance states and the development of metabolic disorders including obesity and T2DM.

The ER and mitochondria are functionally and metabolically associated through a region known as mitochondria-associated membranes (MAMs) (Giorgi et al., 2015; van Vliet et al., 2014). Mitochondria and ER crosstalk into MAMs domains are maintained in part by mitofusin 2 (MTF2) protein. MTF2 is a membrane protein that regulates ER homeostasis coupling to mitochondrial activity coordinating body energy homeostasis (Giorgi et al., 2015). Disruption of MAMs integrity might result in ER stress activation and mitochondria dysfunction leading to metabolic failure (van Vliet et al., 2014). In fact, MTF2 ablation in skeletal muscle or liver of mice modifies mitochondria morphology and function resulting in the generation of ROS and insulin resistance (Sebastian et al., 2012). Also, MTF2 ablation in liver activates JNK and ER stress leading to insulin resistance (Sebastian et al., 2012). It is noteworthy that deletion of MTF2 in anorectic POMC neurons of the arcuate nucleus promotes disruption of ER-mitochondria contacts, ER stress activation, leptin resistance and obesity (Schneeberger et al., 2013). Conversely, Agrp-specific MTF2 knockout mice gained less weight when fed a high fat diet presumably due to decreased fat mass (Dietrich et al., 2013). These evidences suggest that MTF2 seems to play a fundamental role in the ER–mitochondria crosstalk in metabolic relevant organs coordinating metabolic homeostasis by insulin or leptin sensitivity.

MTF2 expression appears to be regulated by nutrition. HFD intake in rodents decreases gene expression and MTF2 protein levels in skeletal muscle which correlates with insulin resistance state (Zhang et al., 2013). Of importance, *in vitro* stimulation of muscle cells with the saturated lipid palmitic acid, decreased MTF2 levels, which correlates with downregulation in the expression of antioxidant enzymes, oxidative stress activation and JNK and NF-kB activation leading to insulin resistance (Nie et al., 2014). Whether saturated lipids alter MTF2 expression at hypothalamus nucleus resulting in ER stress generation leading to insulin sensitivity during obesity is unknown. Here we seek to determine whether saturated lipids coordinate hypothalamic MTF2 expression, ER stress and its effect on insulin sensitivity using *in vivo* and *in vitro* models.

## 2. Results

### 2.1. *Palmitic acid stimulation decreases MTF2 protein levels*

We addressed if palmitic acid stimulation modulates MTF2 expression. We found that dose response of palmitic acid incubation for 12 h in hypothalamic cell line (mHypoA-CLU192) decreases the protein levels of MTF2, showing significant effect at 250 μM (Fig. 1A). This result correlates to ER stress activation evidenced by upregulation of GRP78/BIP marker (Fig. 1B). We have shown that 250 μM stimulation for 12 h does not promote significant cell death (Delint-Ramirez et al., 2015), which suggest that palmitic acid might coordinate MTF2 expression and ER stress response previous to cell damage. Given that saturated lipids are detrimental to energy homeostasis, we seek to determine whether monounsaturated fatty acid prevents MTF2 decrease and ER stress activation. A time course of lipid stimulation showed that 250 μM saturated and monosaturated lipids induced significant decrease of MTF2 protein levels, starting at 12 h and 24 h for palmitic acid and palmitoleic acid, respectively, when compare to control values (Fig. 1C). However, in contrast to monounsaturated lipid, palmitic stimulation exacerbates MTF2 downregulation at 48 h. Additionally, we detected that 250 μM palmitic acid time course incubation, but not palmitoleic acid, promoted BIP increases at 12 (Fig. 1C).

It is known that decrease in MTF2 expression leads to ER stress activation and metabolic complications (Dietrich et al., 2013; Schneeberger et al., 2013; Sebastian et al., 2012). Given that palmitic stimulation decreases MTF2 and increases BIP protein levels at 12 h (Fig. 1B), we tested the hypothesis if MTF2 modulates ER stress response at earlier stages. To address this aim we identified if MTF2 downregulation during lipotoxic insult is previous to ER stress activation. Our results showed that in contrast to palmitoleic acid, 8 h stimulation of 250 μM palmitic acid decreases MTF2 protein levels with no changes in BIP ER stress marker (Fig. 2). Globally, these results suggest that saturated lipids efficiently decrease MTF2 protein levels at earlier stages than ER stress response.