SOCIETÀ ITALIANA DI FARMACOLOGIA

RELAZIONE DI FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: Borsa per soggiorno all'estero

TIPOLOGIA DI RELAZIONE: Fine periodo

TITOLO DELLA RELAZIONE: Modulation of mitochondrial dynamics in ventromedial nucleus neurons: its pivotal role in glycemic metabolism

RELAZIONE:

Background

Type 2 diabetes mellitus (T2DM) is a disease characterized by insulin resistance, inappropriate insulin secretion and activity. Over the past three decades, this metabolic pathology has reached epidemic proportions worldwide and its incidence will increase, becoming the seventh leading cause of death in 2030 (1).

In the last years, overwhelming evidences suggest a strong link between brain and liver in the regulation of whole-body energy metabolism (2). Indeed, the hypothalamus, in particular its ventromedial nucleus (VMH), represents the primary site that manages biological information arising from peripheral organs to monitor the nutritional status of the organism (3). The development of the VMH architecture requires the presence and physiological activity of steroidogenic factor (SF)-1 neurons (4). This population of neurons plays a pivotal role in the regulation of metabolic features, such as body weight and energy homeostasis, and together with proopiomelanocortin (POMC) and neuropeptide-Y-agouti-related-protein (NPY-AgRP) neurons constitute the central regulatory circuit

of metabolism located within the hypothalamus (5). Nevertheless, molecular mechanisms of SF1 neurons involved in managing metabolic changes keep still unclear.

The metabolic role of mitochondria as source of cellular energy is well known (6-8). These organelles produce ATP by using the electro-proton gradient made by electron transport chain and uncoupling proteins (UCPs) are involved in uncoupling the oxidative phosphorylation from ATP production. Beyond their physiological function, these proteins, including UCP2, have been recognized as key factors in managing host homeostasis and their defects lead to impaired mechanisms related to several disorders, such as inflammation (9), cancer (10), and metabolic syndrome (11).

In physiological conditions, mitochondria continually adapt in response to external changes by means the mechanisms of fission and fusion. Fission has a main function to divide damaged mitochondria from healthy cellular components, a process that is enhanced by several proteins, including dynamin-1-like protein (Drp1); while fusion, enhanced by mitofusin 1 and 2 (MFN1 and MFN2), allows to compensate eventual defects of malfunctioning mitochondria. Alterations of fusion and fission proteins are involved in onset of metabolic disorders. Sebastian et al. (12) demonstrated that MFN2 deficiency is strongly linked to mitochondrial dysfunction and insulin resistance, caused by ER stress and the production of reactive oxygen species (ROS). Another *in vivo* study demonstrated the beneficial effects of metformin and resveratrol by protecting mitochondrial integrity through the inhibition of DRP1 activity in T2DM (13).

Despite the strong link between mitochondria dynamics and metabolic disorders, the role of mitochondrial proteins in VMH is still unclear. Toda et al. (14) have recently demonstrated that high concentrations of glucose increase mitochondrial fission and reduce ROS in VMH neurons by activating DRP1 and under UCP2 control.

Taken together, the aim of this project was to investigate central mechanisms involved in control of glycidic homeostasis and the role played by mitochondrial proteins in VMH.

Methods

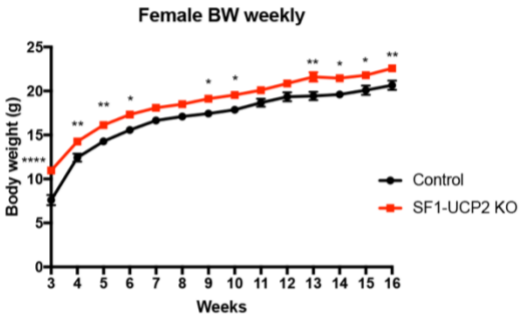
At the beginning of this project, we generated mice (UCP2KO$^{Sf1\text{-}Gfp}$ and MFN2KO$^{Sf1\text{-}Gfp}$) with selective deletion of UCP2 or MFN2 in VMH neurons by crossing Ucp2 floxed mice (UCP2$^{fl/fl}$) or MFN2 floxed mice (MFN2$^{fl/fl}$) with Sf1-cre-Gfp mice (already in our facility). As control mice, UCP2$^{fl/+}$-Sf1-cre negative or MFN2$^{fl/+}$-Sf1-cre negative and UCP2$^{-/-}$- Sf1-cre-Gfp or MFN2$^{-/-}$- Sf1-cre-Gfp littermates were used.

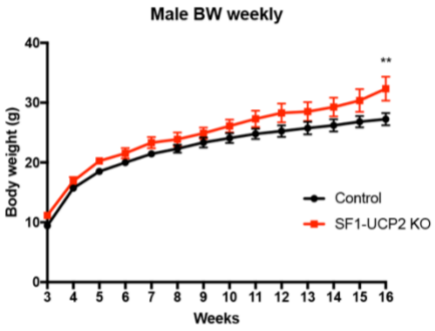
During all experimental period, body weight (weekly), while GTT, ITT, 2DG tests and body composition (1, 2, 3 and 4 months of age) of male and female mice were measured in vivo by MRI (EchoMRI; Echo Medical System). Glucose tolerance test was performed in 16- to 17-h-fasted animals. After the

determination of blood glucose level, fasted animals were injected intraperitoneally with 20% glucose (10 ml/kg; DeltaSelect) in saline. Blood glucose levels were then monitored at 15, 30, 60, and 120 min from the injection. Insulin tolerance test was performed with mice fed ad libitum. After determination of basal blood glucose level, each animal received an intraperitoneal injection of insulin, 0.75 U/kg (Actrapid; Novo Nordisk). Blood glucose levels were then measured at 15, 30, 60, and 120 min after insulin injection. The 2DG test was performed by i.p. injection of 200 mg/kg BW 2DG (Sigma-Aldrich, St. Louis, MO). Blood glucose levels were then monitored at 15, 30, 60, and 120 min after the injection. At 4th month of age, fasted mice were injected glucose (2g/kg, ip). After 1 hr they were perfused with 4% paraformaldehyde (PFA) transcardially. Serial sections of the entire VMH (150 μm) were collectec and incubated with the rabbit anti-cfos antibody (Santacruz, 1:2000), and the mouse anti-mCherry antibody (Life Technologies Corporation, 1:3000) in PB containing 4% normal goat serum, 0.1% glycine, and 0.2% Triton X-100 for 24 hr at room temperature. After several washes with phosphate buffer (PB), sections were incubated with the secondary antibodies (biotinylated goat anti-rabbit immunoglobulin G [IgG]; 1:250 in PB; Vector Laboratories and goat anti-mouse fluor 633; 1:250 in PB; Life Technologies) for 2 hr at room temperature, then rinsed in PB three times, 10 min each time, and incubated for 2 hr at room temperature with Alexa Fluor 594 streptavidin (Life Technologies, 1:2000 in PB). Sections were mounted on glass slides with vectashield (Vector Laboratories) and analyzed with fluorescent microscope.

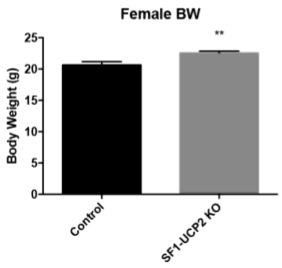
Results

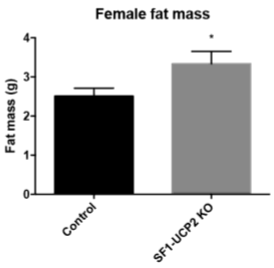
As shown below, the body weight of female SF1-UCP2 KO mice is significantly higher than control mice during all experimental period. This difference was related to an increase of fat mass, demonstrating that the deletion of this mitochondrial protein in VMH induces to lipid accumulation. At 4th month, this metabolic impairment is accompanied by glucose intolerance. Indeed, female SF1-UCP2 KO showed a higher peak of glycaemia than control group at 15 minutes from glucose injection. For ITT and 2DG experiments, no differences were evidenced between both female groups. The metabolic features of male SF1-UCP2 KO and control group did not significantly differ, even if at 4th month of age, we noticed an increase of body weight on SF1-UCP2 KO than control.

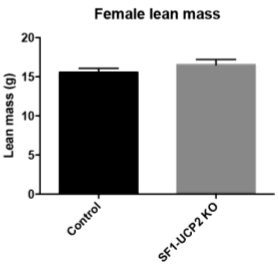




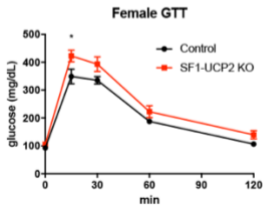
4th month

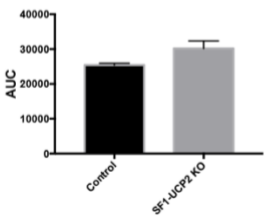


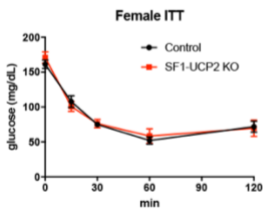


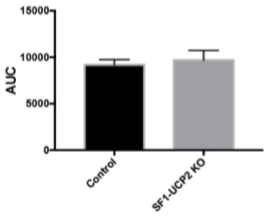


4th month

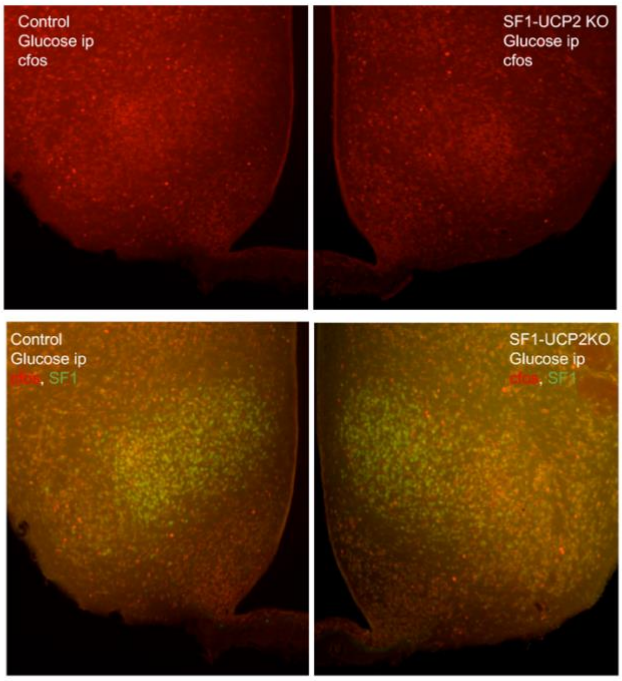


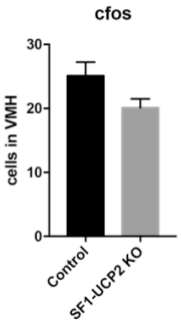




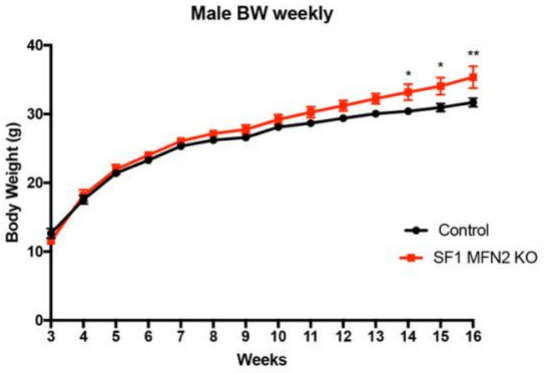


Furthermore, from a preliminary analysis, SF1-UCP2 KO showed a reduction of c-fos activity in VMH neurons than control group, suggesting an impairment in central control of glycemic homeostasis.

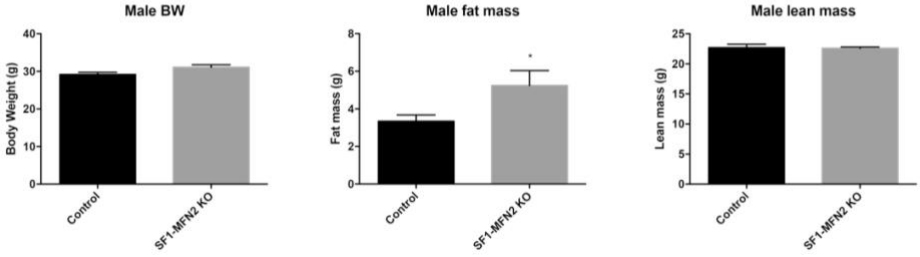




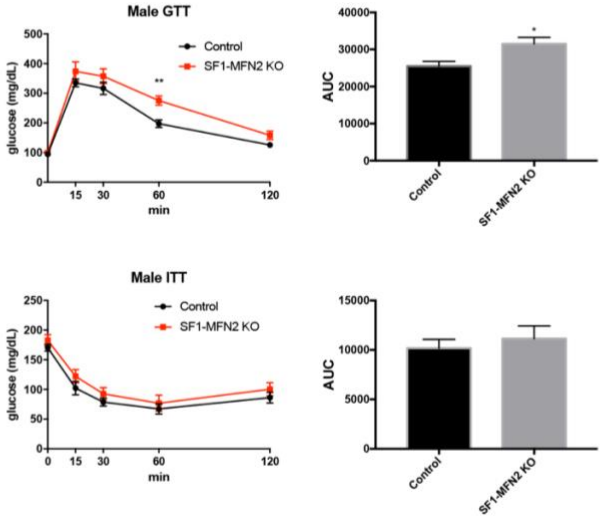
While in SF1-UCP2 colony we noticed metabolic differences mainly in female, the profile in SF1-MFN2 mice was opposite. Indeed, we demonstrated that in the last three weeks of the experimental period (14^{th}- 16^{th} week), male SF1-MFN2 KO mice showed a significant increase of body weight compared to control. Here, the higher body weight was related to an increase of fat mass. The glycidic profile of SF1-MFN2 KO was characterized by a reduced glucose and insulin response compared to control mice, both at 3^{rd} and 4^{th} months of age.

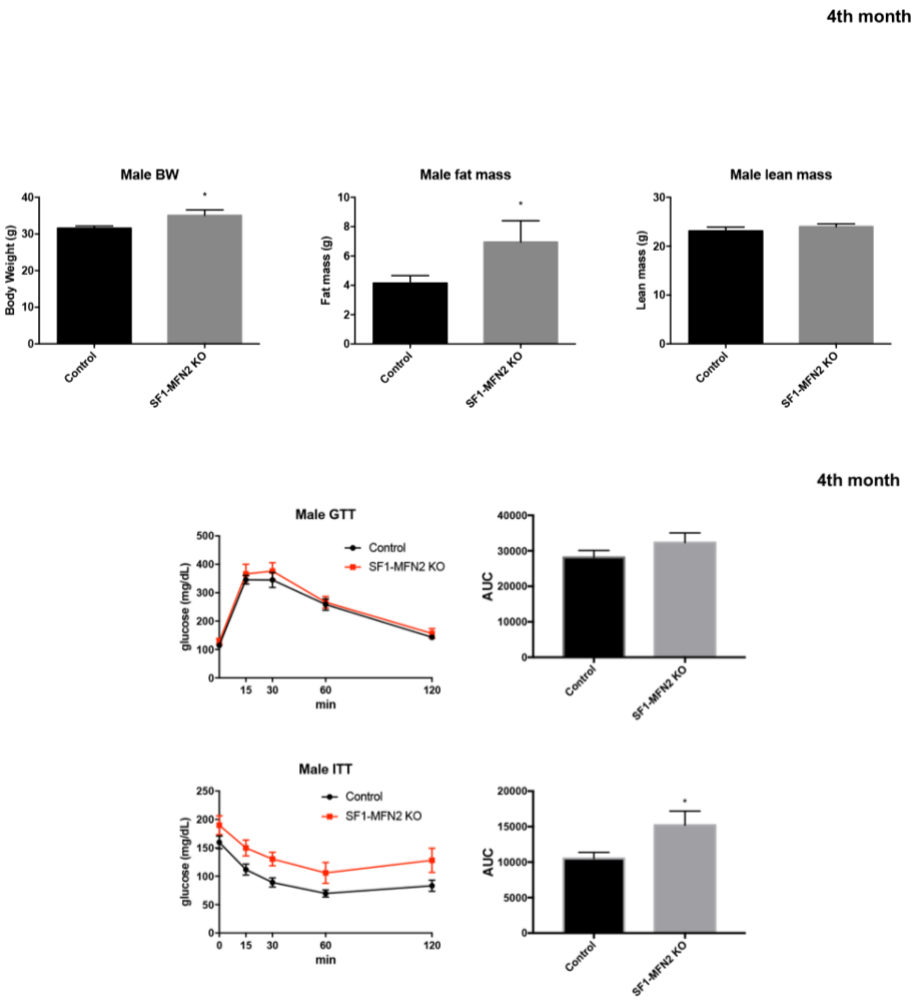


3rd month



3rd month





Conclusions

Although further investigations are needed, we demonstrated the key role of the proteins involved in mitochondrial dynamics in SF1-neuron in controlling glycemic homeostasis. In fact, the deletion of UCP2 and MFN2 in SF1 neurons of VMH leads to an impairment of glucose response and fat metabolism.

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