High-fat-high-sugar diet alters hypothalamic mitochondrial function associated with changes in Mecp2 expression before weight gain

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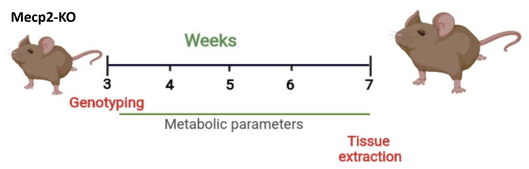
Introduction. The hypothalamus is the major brain area controlling energy homeostasis, and to that, it requires a large amount of energy to adequately integrate and respond to peripheral signals that account for the body's energy balance. This energy is mainly provided by the mitochondria, the major organelle producing ATP in neurons. Therefore, neuronal mitochondria play a fundamental role in maintaining synaptic functions in the hypothalamus. MeCP2 is a molecular bridge that binds to methylated CpG dinucleotides to orchestrate gene expression in response to environmental factors. MeCP2 loss-of-function mutations in the hypothalamus of mice cause obesity and metabolic disorders. Interestingly, Mecp2 could also be involved in mitochondrial function, since the cortex of mice lacking the expression Mecp2 has elongated mitochondria with less ATP production.
However, the role of Mecp2 in hypothalamic mitochondrial function, and how obesogenic factors affect its function is still unknown. Considering that hypothalamic mitochondrial dysfunction induced by an obesogenic diet could represent the cellular basis of metabolic diseases, it is crucial to understand the gene-environment interaction underlying the alteration in diet-induced energy disbalance for designing new therapeutic approaches to prevent overweight and obesity.

OBJECTIVE

1. To evaluate the role of Mecp2 on hypothalamic mitochondrial function.
2. To determine the effect of a high fat/high sugar diet (HFHS) on Mecp2 expression and mitochondrial function in the hypothalamus.

METHODOLOGY



RESULTS

MICE LACKING THE EXPRESSION OF MECP2 SHOW REDUCED LOCOMOTOR ACTIVITY AND ALTERED RESPIRATORY EXCHANGE RATIO (RER) WITHOUT CHANGES IN FOOD INTAKE OR BODY WEIGHT

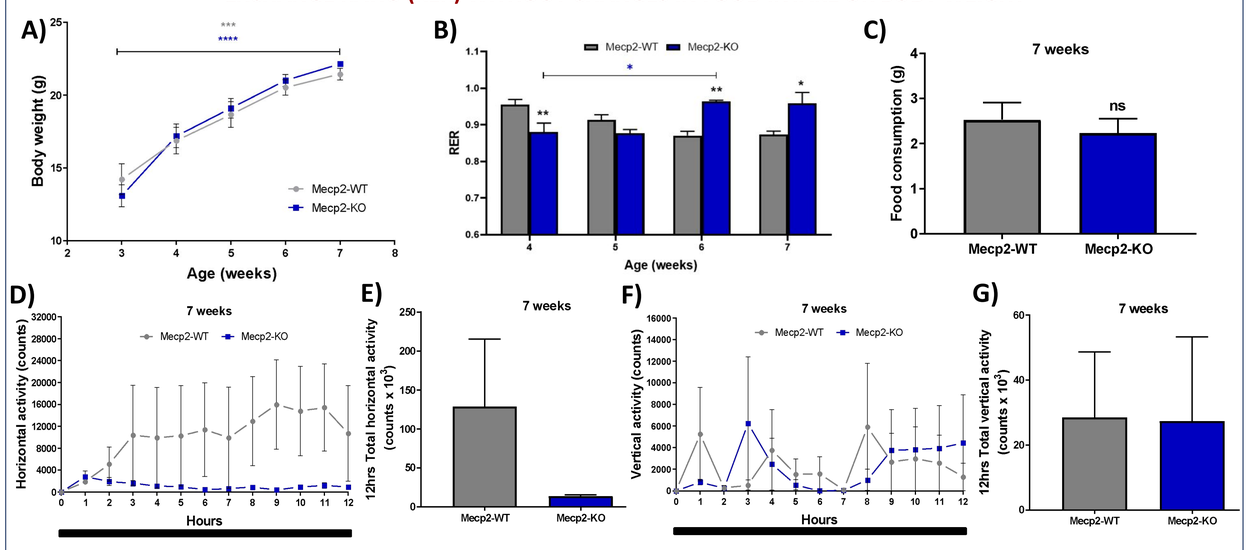


Figure 1. Metabolic alterations in mice lacking the expression of Mecp2: To determine the temporal progression of the metabolic changes observed in the absence of Mecp2, Mecp2-KO mice and their wild-type littermates (WT) were evaluated since weaning. **A.** Body weight gain was recorded once a week since weaning. Data represent the mean ± SEM of WT (n=10) and Mecp2-KO (n=10). Data were compared by repeated measures ANOVA ***p<0.001 (WT), ****p<0.00001 (Mecp2-KO). There was no difference in body weight gain between genotypes. **B.** Respiratory exchange ratio (RER) was recorded once a week since weaning. Data represent the mean ± SEM of WT (n=3) and Mecp2-KO (n=3) and were compared by two-way ANOVA with Sidak's multiple comparisons *p<0.05, **p<0.01. **C.** Food intake was recorded in 7-week-old WT and Mecp2-KO mice in metabolic cages during the dark phase. Data represent the mean ± SEM of WT (n=5) and Mecp2-KO (n=5). Data were compared by two-tailed t-test. **(D-G)** The horizontal (D-E) and vertical (F-G) locomotor activity exhibited by 7-week-old WT and Mecp2-KO mice in metabolic cages during the dark phase. Data represent the mean ± SEM of real-time recorded locomotor activity (D, F) and the total locomotor activity recorded during 12 h (E, G) of WT (n=5) and Mecp2-KO (n=5) mice. Data were compared by two-tailed t-test *p<0.05.

NORMAL RESPONSE TO CALCIUM OVERLOAD AND REDUCED MITOCHONDRIAL CALCIUM LEVELS IN MICE LACKING THE EXPRESSION OF MECP2

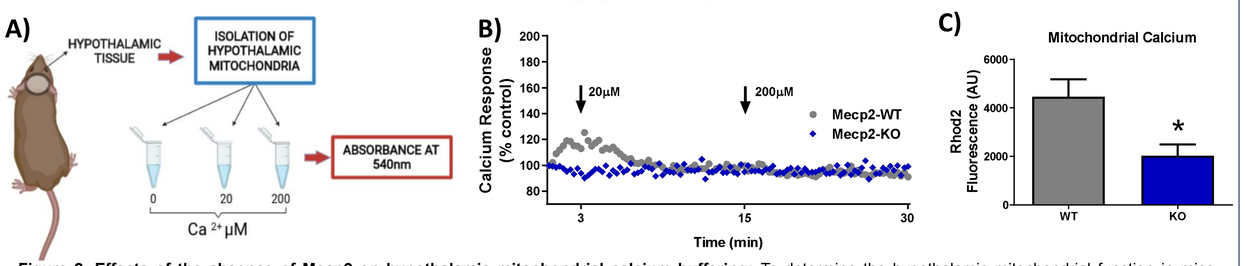
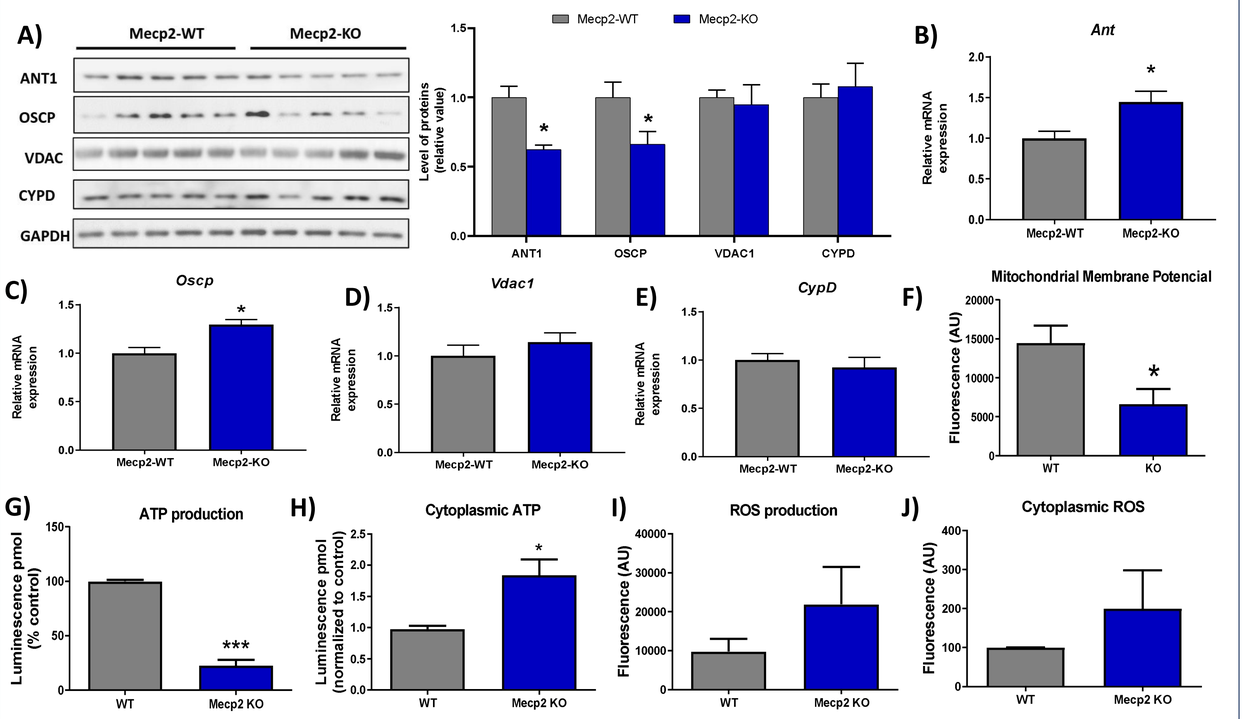


Figure 2. Effects of the absence of Mecp2 on hypothalamic mitochondrial calcium buffering: To determine the hypothalamic mitochondrial function in mice lacking the expression of Mecp2, the calcium buffering capacity was evaluated in isolated mitochondria **A.** Representation of mitochondrial calcium overload assay. **B.** The response to calcium overload was evaluated in WT (n=4) and Mecp2-KO (n=4) mice. Data represent the absorbance at 540 nm, which indicates the density exhibited by mitochondria. **C.** Mitochondrial calcium levels measured by fluorescent dye Rhod-2 probe in isolated hypothalamic mitochondria from WT and Mecp2-KO mice. Data represent means ± SEM of WT (n=4) and Mecp2-KO (n=4). Data were compared by two-tailed t-test *p<0.05.

THE LACKING OF MECP2 ALTERS MITOCHONDRIAL STRUCTURAL PROTEIN EXPRESSION AND REDUCED MITOCHONDRIAL BIOENERGETICS



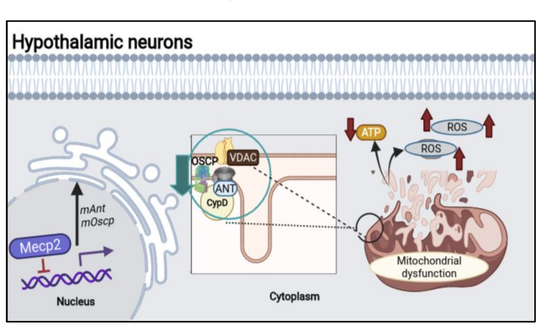


Figure 3. Effects of the absence of Mecp2 on hypothalamic mitochondrial structure and bioenergetic function: To determine the mitochondrial function in the hypothalamus of mice lacking the expression of Mecp2, the expression of mitochondrial structure-related proteins and bioenergetic function were evaluated. **A.** Western blot of hypothalamic lysates and densitometric analysis of mPTP-forming protein was evaluated in the hypothalamus from in 7-week-old WT and Mecp2-KO mice. Data represent the mean ± SEM of WT (n=5) and Mecp2-KO (n=5) mice. Data were compared by two-tailed t-test *p<0.05. **(B-E)** Relative mRNA expression of genes encoding for mPTP-forming proteins: **(B)** mAnt1, **(C)** mOscp, **(D)** mVdac1, and **(E)** mCypD. Gene expression was evaluated by RT-qPCR from reverse-transcribed RNA from the hypothalamus of 7-week-old WT and Mecp2-KO mice. Data represent the mean ± SEM of WT (n=8) and Mecp2-KO (n=8). Data were compared by two-tailed t-test *p<0.05. **(F)** Mitochondrial membrane potential measured by fluorescent probe in isolated mitochondria from the hypothalamus. Data represent means ± SEM of WT (n=4) and Mecp2-KO (n=4) and were compared by two-tailed t-test *p<0.05. **(G-H)** ATP concentrations were measured in isolated mitochondria from the hypothalamus by an ATP bioluminescence assay kit.

Data represent the mean ± SEM of (G) ATP production and (H) cytoplasmic ATP of WT (n=3) and Mecp2-KO (n=5) mice. Data were compared by two-tailed t-test *p<0.05. **(I-J)** ROS concentrations were measured in isolated hypothalamic mitochondria by using 25 µM of DCF probe. Data represent the mean ± SEM of (I) ROS production and (J) cytoplasmic ROS of WT (n=3) and Mecp2-KO (n=5) mice.

MICE LACKING THE EXPRESSION OF MECP2 SHOW INCREASED A MITOCHONDRIAL FISSION PROTEIN AND ITS mRNA LEVELS

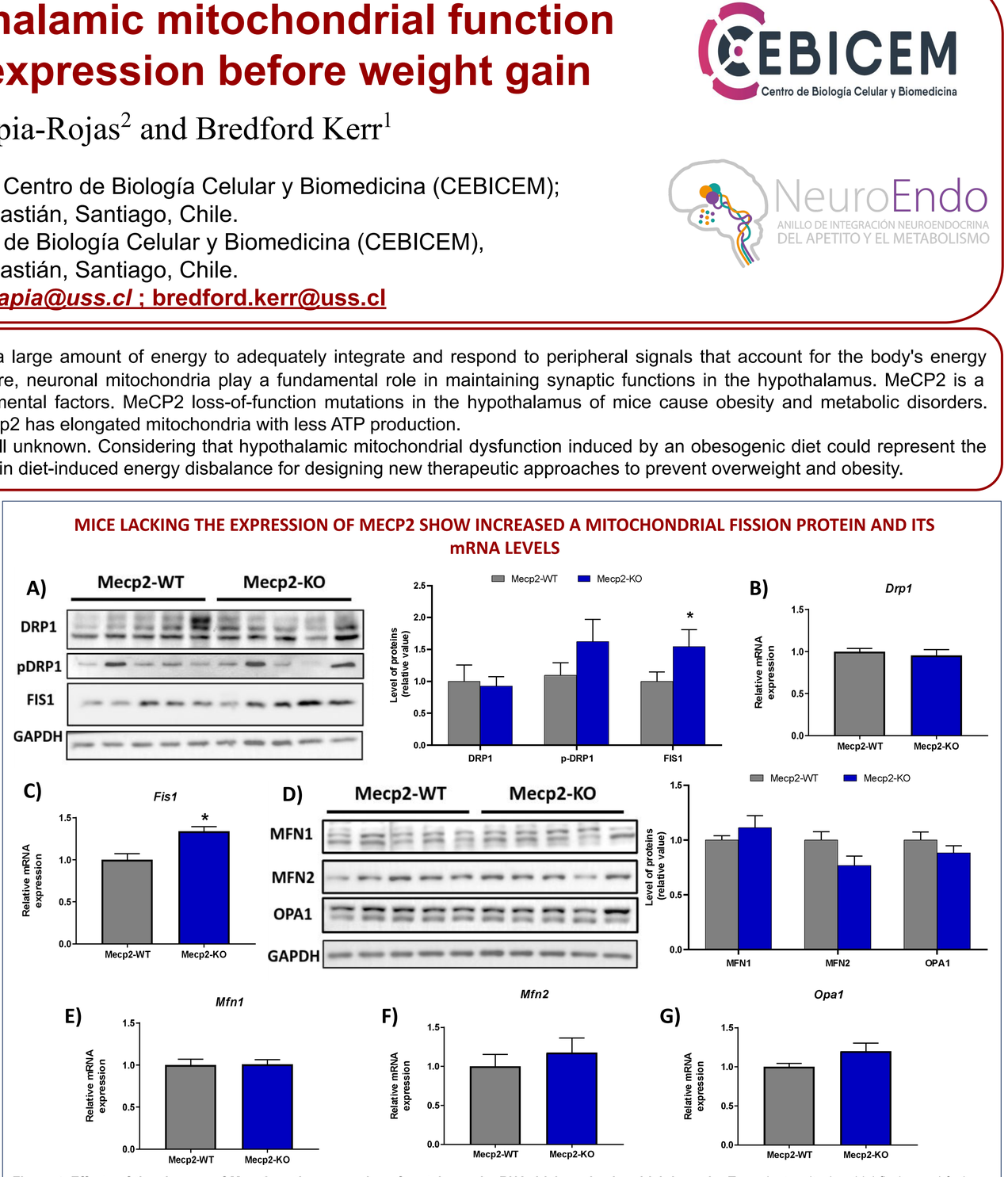


Figure 4. Effects of the absence of Mecp2 on the expression of proteins and mRNA driving mitochondrial dynamic: To evaluate mitochondrial fission and fusion events in the hypothalamus of mice lacking the expression of Mecp2-KO, the expression of proteins and mRNA encoding for mitochondrial fission and fusion were evaluated. **A.** Western blot of hypothalamic lysates and densitometric analysis of proteins involved in mitochondrial fission: phospho-DRP1, total DRP1 and FIS1 were measured in the hypothalamus from 7-week-old WT and Mecp2-KO mice. Data represent the mean ± SEM of WT (n=5) and Mecp2-KO (n=5). Data were compared by two-tailed t-test *p<0.05. **(B-C)** Relative mRNA expression of genes encoding for mitochondrial fission proteins **B.** *mDrp1*, **C.** *mFis1* were measured in the hypothalamus of 7-week-old WT and Mecp2-KO mice. Data represent the mean ± SEM of WT (n=8) and Mecp2-KO (n=8). Data were compared by two-tailed t-test *p<0.05. **D.** Western blot of hypothalamic lysates and densitometric analysis of the proteins involved in mitochondrial fusion: MFN1, MFN2 and OPA1 were evaluated in the hypothalamus of 7-week-old WT and Mecp2-KO mice. Data represent the mean ± SEM of WT (n=5) and Mecp2-KO (n=5). Data were compared by two-tailed t-test. **(E-G)** Relative mRNA expression of genes encoding mitochondrial fusion proteins **E.** *mMfn1,* **F.** *mMfn2* and **G.** *mOpa1* were evaluated in the hypothalamus of 7-week-old WT and Mecp2-KO mice. Data represent the mean ± SEM of WT (n=8) and Mecp2-KO (n=8). Data were compared by two-tailed t-test.

HFHS FEEDING DECREASES HYPOTHALAMIC MECP2 EXPRESSION AND REDUCES MITOCHONDRIAL FUNCTION

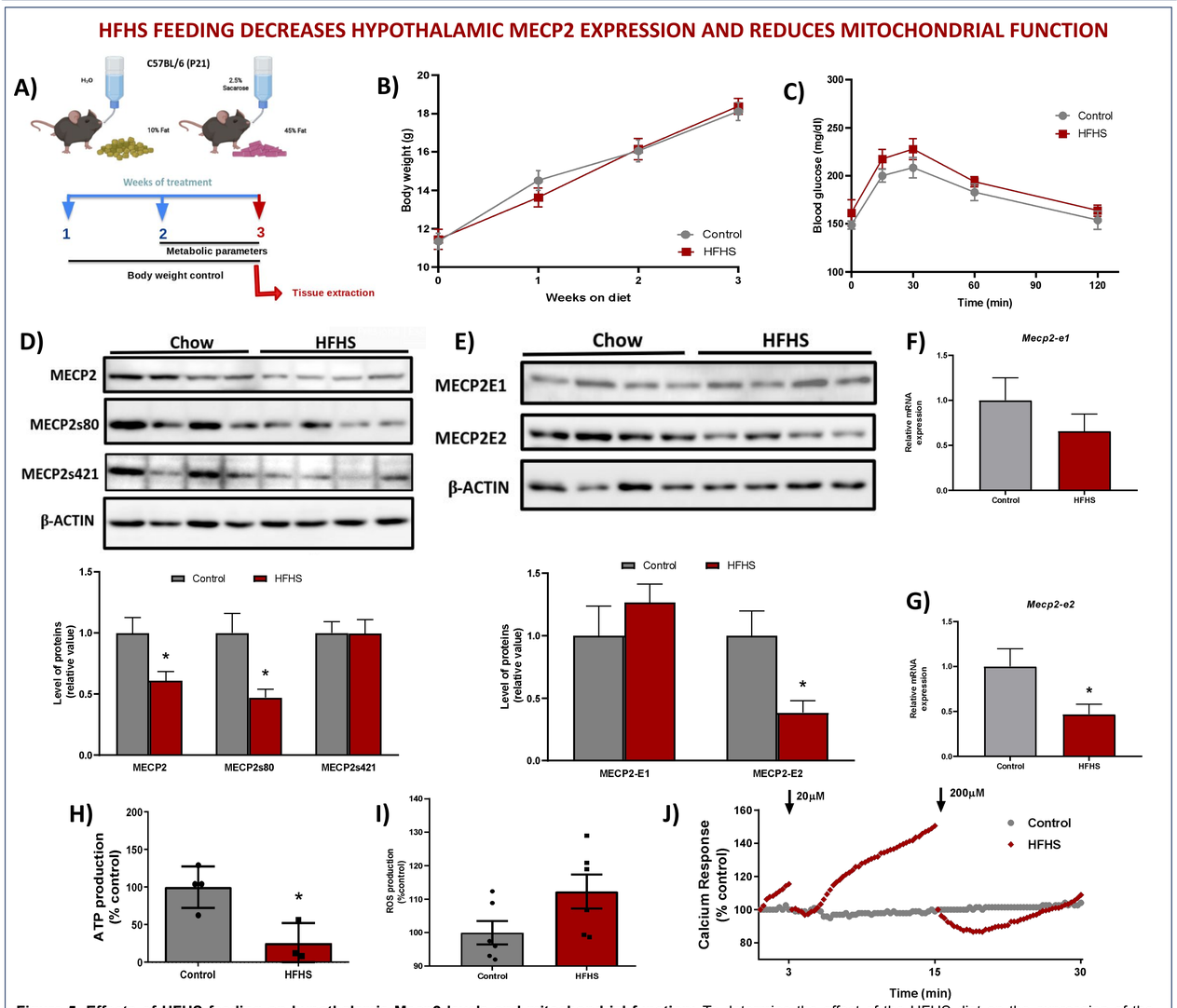


Figure 5. Effects of HFHS feeding on hypothalamic Mecp2 levels and mitochondrial function: To determine the effect of the HFHS diet on the expression of the gene-environment interaction-related protein Mecp2, its hypothalamic expression, phosphorylation, and mRNA splicing were determined by western blot and RT-qPCR. Besides, mitochondrial function parameters were evaluated. **A.** Diet-induced obesity mouse model: 3-week-old C57/BL6 mice were fed with control or HFHS diet for 3 weeks. **B.** Body weight gain was recorded once a week for 3 weeks since weaning**.** Data represent the mean ± SEM of control diet (n=18) and HFHS diet (n=20) fed mice and were compared by repeated measures ANOVA. **C.** Fasting glucose levels and the response to an ip injection of 2 mg/kg of glucose. Data represent the mean ± SEM of control diet (n=6) and HFHS diet (n=6) fed mice. **D.** Western blot for MECP2 from hypothalamic lysates of 6-week-old mice to evaluate the expression of total MECP2, MECP2 phosphorylated at serine 80 and 421. Data represent the mean ± SEM of control diet (n=4) and HFHS diet (n=4) fed mice and were compared by two-tailed t-test *p<0.05. **E.** Western blot for Mecp2 isoforms from hypothalamic lysates of 6-week-old mice to evaluate the expression of MECP2-E1 and MECP2-E2. Data represent the mean ± SEM of control diet (n=4) and HFHS diet (n=4) fed mice and were compared by two-tailed t-test *p<0.05. **(F-G)**. Relative mRNA expression of gene encoding for MECP2 mRNA isoforms F. *mMecp2-e1*, G. *mMecp2-e2* were evaluated in hypothalamic lysate from 6-week-old mice. Data represent the mean ± SEM of control diet (n=5) and HFHS diet (n=5) fed mice and were compared by two-tailed t-test *p<0.05. To determine the mitochondrial function in the hypothalamus of mice fed with control or HFHS diet, mitochondrial function parameters were evaluated **H.** ATP concentrations were measured in hypothalamic isolated mitochondria by using an ATP bioluminescence assay kit. Data represent the mean ± SEM of mitochondria from 6-week-old mice fed with control diet (n=4)or HFHS diet (n=3). Data were compared by two-tailed t-test *p<0.05. **I.** ROS concentrations were measured in isolated mitochondria from the hypothalamus using 25 µM of DCF probe. Data represent the mean ± SEM of ROS mitochondrial production from 6-week-old mice fed with control diet (n=6) or HFHS diet (n=6). Data were compared by two-tailed t-test. **J.** The response to a calcium overload was evaluated in hypothalamic mitochondria from 6-week-old mice fed with control diet(n=4) or HFHS diet(n=4). Data represent the absorbance at 540nm, which indicates the density exhibited by mitochondria.

Conclusion: Our results suggest that mice lacking Mecp2 expression have hypothalamic mitochondrial dysfunction, which is mainly due to a failure in mitochondrial bioenergetics, but not in calcium buffering. This was associated with defects in the expression of proteins involved in mitochondrial structure and dynamics. In addition, an obesogenic diet feeding induces changes in Mecp2 expression and post-translational modification related to its function previous to inducing an increase in body weight. These metabolic and transcriptional/translational changes induced by HFHS feeding are accompanied by hypothalamic mitochondrial dysfunction

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