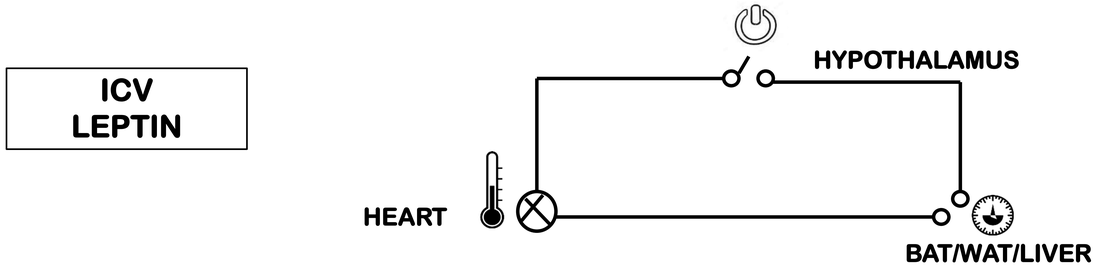
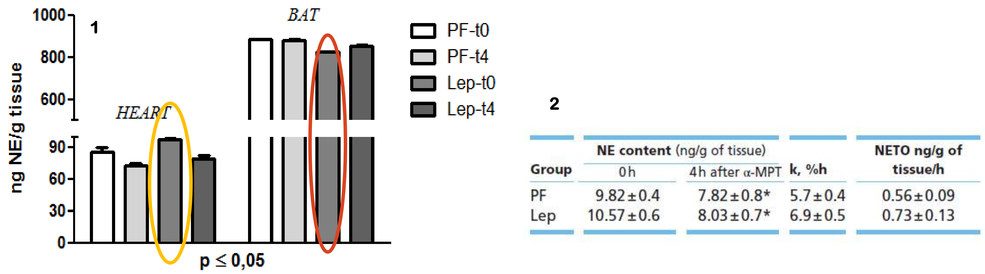
HAS CENTRAL LEPTIN THE ABILITY OF CONTROLLING THERMOREGULATION?





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Group	NE content (ng/g of tissue) 0h	4h after α-MPT	k, %h	NETO ng/g of tissue/h
PF	9.82±0.4	7.82±0.8*	5.7±0.4	0.56±0.09
Lep	10.57±0.6	8.03±0.7*	6.9±0.5	0.73±0.13

FIGURE A. Stimulation of the sympathetic nervous system : (1)Cardiac and BAT endogenous NE content; (2) NE turnover in heart.

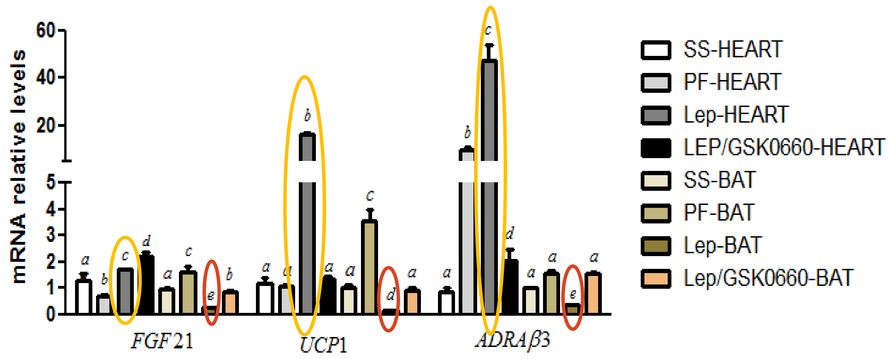

FIGURE B. mRNA levels. The PPARβ/δ specific antagonist GSK0660 reduces the mRNA levels of UCP1 and ADRAβ3 and Plin5 in central leptin treated rats.

Central leptin promotes the stimulation of the sympathetic nervous system as well as the turnover of NE (**Figure A**) in the heart, encouraging the expression of the ADRAβ3 receptor, UCP1 or FGF21 to the detriment of the expression in BAT (**Figure B**). In this case, there is a very significant decrease (approximately 50%) in the content of lipids in the heart (**Figure C**), a fact favored by the increase in peroxisomal β-oxidation (independent of CPT1) (**Figure D**). The expression of ATGL as a control of the study of the NST in heart shows us how the stimulation of the sympathetic system is fundamental by central leptin infusion (**Figure E**). Similarly, the increase in lipolysis in the heart, stimulation of thermoregulatory genes and peroxisomal β-oxidation is favored through the PPARβδ as a mediator of central leptin.

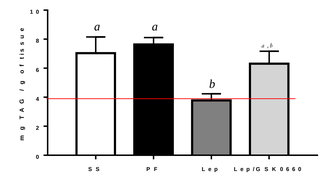

FIGURE C. TAG content decreases in the heart after central leptin infusion.

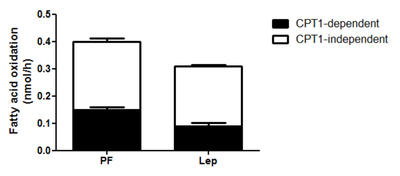

FIGURE D. Palmitate oxidation (CPT1-dependent) or etomoxir-independent (CPT1-independent) palmitate oxidation in saline-infused pair-fed and leptin-infused rats.

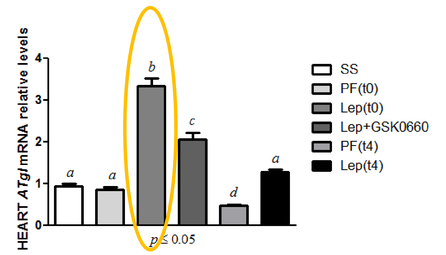

FIGURE E. mRNA levels of ATGL. GSK0660 reduces the mRNA level, as seen in time 4 after the injection by –MPT in saline-infused pair-fed and leptin-infused rats.

AUTHORS

Blanca María Rubio Muñoz[1], Lorena Mazuecos[1], Cristina Pintado[2], Carmen Arribas[2], Nilda Gallardo[1], Antonio Andrés[1].

[1]Biochemistry Section, Faculty of Science and Chemical Technologies (UCLM) and Regional Centre for Biomedical Research , (CRIB). *Ciudad Real, Spain.* [2]Biochemistry Section, Faculty of Environmental Sciences and Biochemistry (UCLM) and CRIB. *Toledo, Spain.*

Blanca María Rubio Muñoz.
blanca.rubio@uclm.es
http://crib.uclm.es/investigacion/diabetes-y-obesidad-con-el-envejecimiento/

Of late it has been described that the thermogenic capacity is not exclusively dependent on the brown adipose tissue (BAT). To know the effect of central leptin infusion in this alternative mechanism we studied the expression of genes related to this thermoregulation process such as adipose triglyceride lipase (ATGL), uncoupling protein 1 (UCP1), adrenoceptor beta 3 (ADRAβ3) and fibroblast growth factor 21 (FGF21), in heart and BAT. The mediation through the sympathetic nervous system leads us to see an increase in peroxisomal β-oxidation and a decrease in the content of triglycerides (TAG) in the heart; this facilitation of lipolysis by leptin allows us to relate these data to the action of the heart as a thermoregulatory organ.
In addition, the pharmacological inhibition of PPARβ/δ with the specific antagonist GSK0660 in leptin-infused rats leads us to believe that this action in the decoupling of oxidative phosphorylation and the generation of heat in the heart is mediated by PPARβ/δ.

Historically, BAT has been described as the only tissue with thermogenic capacity by the activation of the hypothalamus against cold. However, the thermoneutrality causes BAT to accumulate lipid droplets, resulting in whitening of the tissue, so the expression of genes intimately related to thermogenesis in this tissue such as UCP1 or ADRAβ3 is clearly diminished (Susan M. van de Berg et al., 2017). Recently have been shown that the release of fatty acids from intracellular fat stores by adipose triglyceride lipase (ATGL) is considered a key step in non-shivering thermogenesis (NST) (Renate Schreiber et al., 2017). Based on previous data from the group (Mora et al., 2018) we hypothesized that central leptin favours the thermoregulatory action of the heart guided by PPARβ/δ.

To answer this question, we performed an experiment based on ICV administration technique of rat leptin (0.2 μg / day) or vehicle (PBS) for 7 days in Wistar rats for 3 months as described (Gallardo et al., 2007):

	TREATMENT	PATTERNS OF FOOD INTAKE
SS	ICV saline (PBS)	Ad libitum
PF	ICV saline (PBS)	=Leptin
Lep	ICV Leptin (0.2 μg/day)	Ad libitum
Lep/GSK0660	ICV Leptin and ip GSK0660 (1mg/kg/day)	Ad libitum

At the same time, sympathetic nervous system activity was determined by measuring norepinephrine turnover (NE) or turnover rate of NE (NET) as a function of the rate of decrease in NE content in the tissue after inhibition of tyrosine hydroxylase with injection of methyl-p-tyrosine (-MPT), a competitive inhibitor of the speed-limiting enzyme in NE biosynthesis.

Gene expression of factor involved in thermoregulation was performed by real time RT-PCR. Cardiac TAG was also determined. Complete palmitate oxidation (as ^3H_2O) rates were measured in the absence/presence of 100 μM etomixir, in heart explants. Figure (A-E) shows the mean ± SEM of 6-8 rats per group. Different letters on top of error bars indicate significant differences among treatments ($p \leq 0.05$, one-way ANOVA followed by Tukey test).

The use of GSK0660, a selective inhibitor of PPARβ/δ, shows that in conditions of thermoneutrality, central leptin favours thermoregulation in the heart with the intervention of PPARβ/δ.

•Gallardo et al. 2007. Tissue-specific effects of central leptin on the expression of genes involved in lipid metabolism in liver and white adipose tissue. *Endocrinology. 148 5604-5610.*
•Renate Schreiber et al. 2017. Cold-Induced Thermogenesis Depends on ATGL Mediated Lipolysis in Cardiac Muscle, but Not Brown Adipose Tissue. *Cell Metabolism.* 26 753–763.
•Mora et al. 2018. Central leptin regulates heart lipid content by selectively increasing PPAR β/δ expression. *Journal of Endocrinology.* 236 43-56.
•Susan M. van de Berg et al. 2017. Immune Modulation of Brown(ing) Adipose Tissue in Obesity. *Endocrine Reviews.* 38 46-68.