

Activation of insulin signaling in gastrocnemius after central leptin infusion is P1-D1-27 associated with an increase in proliferation and muscle fiber size

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Background

Skeletal muscle is the largest tissue involved in the insulin-stimulated disposal of glucose, with its size being controlled by hormonal status, among other factors. Leptin plays a primary role in the regulation of glucose homeostasis with a substantial degree of insulin and leptin cross-talk in muscle. However, the relationship between the leptin's central effects on insulin sensitivity in muscle and associated structural changes remain unclear.

Hypothesis and objective

We hypothesized that chronic central leptin infusion modifies muscle proliferation and fiber size through activation of insulin sensitivity. Thus, we analyzed whether the possible changes in insulin signaling and glucose uptake in the gastrocnemius are associated with structural modifications.

Animals and methods

<u>Animals</u>

Eighteen male Wistar rats:

- Controls (C, icv saline during 14 days)
- Pair-feds (PF, icv saline plus caloric restriction)
- Leptin (L, icv leptin, 12 µg/day during 14 days)

Methods

- Serum insulin and glucagon: ELISA
- Serum acylated and total ghrelin: RIA
- Muscle glucose and glycogen: colorimetric method
- Glut4, PEPCK and insulin receptor: Western blot
- Insulin signaling: multiplexed bead immunoassay
 Proliferating cell nuclear antigen: immunohistochemistry
- Size of fibers: hematoxylin-eosin staining. Area determined with the program Axiovision 4.6 (Zeiss)

Serum hormone levels A (|m/bu|) unsalin | D C (|m/bu|) | D (|m/bu|)

A. Serum insulin concentrations in control rats (C), pair-fed rats (PF) and rats treated with chronic icv leptin infusion (L). **B**. Serum glucagon concentrations. **C**. Serum acylated ghrelin levels. **D**. Serum total ghrelin levels. NS, non-significant. *p<0.05.

Leptin infusion increases glucose uptake A (anssit diplomit) C (alpitation of the property o

A. Hepatic free glucose levels in control rats (C), pair-fed rats (PF) and rats treated with chronic icv leptin infusion (L). **B.** Hepatic glycogen concentrations. **C.** Relative protein levels of glucose transporter 4 (Glut 4). **D.** Relative protein levels of phosphoenolpyruvate carboxykinase (PEPCK). DU, densitometry units, NS, non-significant. *p<0.05 **p<0.01.

Central leptin infusion promotes activation of muscle insulin signaling A (% control MFI) A (% control MFI) B (% control MFI) C (% control MFI) B (% control MFI) C (% control MFI) C (% control MFI) D (% control MFI

A. Relative protein levels of the insulin receptor beta chain (IRβ) in control rats (C), pair-fed rats (PF) and rats treated with chronic icv leptin infusion (L). **B**. Relative phosphorylated (p) IRS1 protein levels. **C**. Relative pAkt on threonine 308 (pThr308Akt) protein levels. **D**. Relative pAkt on serine 473 (pSer473Akt) protein levels. **E**. Relative phosphorylated phosphatase and tensin homolog on chromosome 10 (PTEN) on serine 380 (pSer380PTEN) protein levels. **F**. Relative phosphorylated mammalian target of rapamycin (mTOR) on serine 2448 (pSer2448mTOR) protein levels. DU, densitometry units, MFI, median fluorescence intensity. *p<0.05, **p<0.01.

Cell proliferation and area of fibers are augmented after chronic central leptin infusion A B B Cell proliferation and area of fibers are augmented after chronic central leptin infusion B Cell proliferation and area of fibers are augmented after chronic central leptin infusion

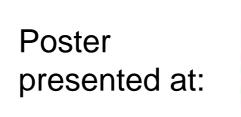
A-C. Proliferating cell nuclear antigen (PCNA)-positive nuclei in control rats (C), pair-fed rats (PF) and rats treated with chronic icv leptin infusion (L), respectively. **D.** PCNA-positive nuclei per 100 fibers in the same groups. **E-G.** Hematoxylin-eosin staining in the same groups. **H.** Area of fibers in the same groups. *p<0.05, **p<0.01.

Conclusion

Central leptin promotes an increase in muscle proliferation and size related to improved insulin sensitivity.



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