## Ghrelin antagonists reduce body weight gain in diet-induced obese mice

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Food intake is regulated by the interaction between neural and hormonal signals arising both from periphery and specific areas of the brain, and in particular the hypotalamus. Among peripheral mediators, ghrelin, an endogenous 28 aminoacid acylated peptide secreted mainly by the stomach, has an important role in the regulation of feeding. Ghrelin by binding to its receptor (GHS-R1a) effectively stimulates feeding behavior.

The purpose of this study was the pharmacological characterization *in vitro* and *in vivo* of new, synthetic peptidic and non peptidic molecules, analogues of ghrelin, named growth hormone secretagogues (GHS). The GHS have been screened *in vitro* for their ability to inhibit the GHS-R1a activation, by measuring the intracellular calcium mobilization using fluorescent indicator FLUO-4; *in vivo*, they have been evaluated on the capability to inhibit feeding behaviour and decrease body weight gain in a mouse model of obesity.

The *in vitro* studies were performed in CHO cells transiently transfected with the human GHS-R1a. In order to test the experimental conditions, CHO-GHS-R1a cells were treated with two effective agonists (ghrelin and hexarelin) alone or in combination with the GHS-R1a antagonist (D-Lys_GHRP6). As expected, acute ghrelin treatment induced a significant intracellular increase of calcium, superimposable to that induced by hexarelin, that was used in all the following experiments as a positive control. Co-incubation with D-Lys_GHRP6 blunted the calcium response induced by ghrelin or hexarelin. JMV 2959 and EP 80317 did not induce any calcium response, but both antagonized the calcium release induced by hexarelin, indicating that JMV 2959 and EP 80317 are antagonists of the GHS-R1a.

To induce obesity, the mice were fed for 9 weeks with a standard diet or an hypercaloric high fat (HFD) diet; the latter induced a significant increase of body weight, two-fold compared to control. The obese mice were treated with the antagonists JMV 2959 or EP 80317 or saline (ip, b.i.d.) for ten days; body weight and food intake were measured daily. EP80317 administration significantly inhibited body weight gain in obese mice in comparison to control mice, whereas JMV 2959 was ineffective.

These results were confirmed also by Sky-Scan analisys, that demonstrated a reduced fat mass in EP 80317 treated mice. In conclusion, the results obtained in this research show that EP 80317 is effective in inhibiting body weight gain in obese mice and could be a useful tool to counteract obesity.