Scand. J. Lab. Anim. Sci. No. 2. 2002. Vol. 29

Possible role of antibodies to leptin as growth promoters – preliminary study in mice

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Summary
The aim was to investigate the effect on normal mice of an intraperitoneal injection of antibodies to leptin. Eight weaned male mice (30-day-old, average weight 33 g), were divided into two groups (n = 4), C (Control) and T (Treated), and fed *ad libitum* standard milled mice chow. Treated animals were injected i.p. twice a day (12 h interval) with a 0.2 ml solution of Anti-mouse Leptin antibody (4 μg / 0.2 ml of sterile placebo); control animals were also injected twice a day with 0.2 ml of sterile placebo. At day 10 the mice were killed, carcasses dressed and frozen, and their characteristics determined. No significant differences were recorded possibly suggesting lack of effect of the leptin antibody.

Introduction
Leptin is a protein coded by the obese (ob) gene *(Houseknecht et al., 1998)*. In mammals, it is a 16 kD protein (167 amino acids) that belongs to the cytokine family (*Chemineau et al., 1999*). It is secreted by the adipocytes, but also in the gastric epithelium *(Bado et al., 1998)* and it acts mainly on the limbic lobe, higher cortical centers and brain stem, via the hypothalamus *(Friedman and Halaas, 1998)*. Leptin is a physiologically important regulator of food intake, body weight and also energy expenditure, affecting protein, fat and glucose metabolisms (*Friedman and Halaas, 1998)*.
Undernutrition poses a serious limitation to animal production in tropical areas. In fact, due to the poor quality of pastures in the dry season, animals may loose up to 40 % of their bodyweight *(Clariget et al., 1998)*, which significantly affects productive parameters of dairy, beef or mutton production *(Lusweti, 2000)*. In contrast in the rainy season pastures are often considered of good quality and available in adequate quantities *(Butterworth, 1984)*.
Leptin plasma concentrations are directly related to body fat deposits, higher in well fed and lower in underfed animals as demonstrated in both cattle *(Chilliard et al., 1998)* and sheep *(Bocquier et al., 1998)*. It can therefore be expected that leptin plasma concentrations are higher in the rainy season and lower in the dry season, although no references seem to be available that could confirm this theory. Therefore blocking the action of this hormone, could be used as a means of manipulating food intake during *ad libitum* feeding periods, in order to minimise subsequent severe weight loss. Several authors have attempted blocking leptin's action in mice, for instance through the use of antibodies to leptin *(Brunner et al., 1997)* and also by using leptin mutants, such as R128Q *(Verploegen et al., 1997)*, injected in the right lateral ventricle. In both these attempts, mice responded positively, increasing food intake as well as body weight, although the effects of R128Q are still questioned *(Brunner et al., 1999)*, hence suggesting a possible role for leptin antagonists as growth promoters that could be used as a mean of preventing severe seasonal undernutrition.
This study aimed to evaluate the effects of an intraperitoneal injection of antibodies to leptin on normal mice, namely on food intake and liveweight, as well as carcass composition, thus contributing to the definition of a possible role for antagonists to leptin as growth promoters.

Materials and Methods

Eight weaned male Charles River male mice (30-day-old, average weight 33 g), were obtained from the colony maintained at the Gulbenkian Science Institute (Oeiras, Portugal). They were divided into two weight matched groups (n = 4), C (Control) and T (Treated), fed *ad libitum* standard milled mice chow and kept in individual mice cages with wood shave bedding. The room was at 18 to 22°C and 60% humidity with a 10 h dark to 14 h light cycle. Treated animals were injected i.p. twice a day (12 h interval) with a 0.2 ml solution of Anti-mouse Leptin antibody (R&D Systems, Abingdon, UK) of 4 µg antibody per 0.2 ml of sterile placebo. This dilution was calculated based on serum leptin concentrations established by Escobar-Morreale and colleagues (1997) for the rat, and on blood and plasma quantities present in normal mice *(Withers 1992)*. Group C animals were also injected twice a day, with 0.2 ml of sterile placebo.

At day 10 of the experiment, the animals were anaesthetised (by ether inhalation) and sacrificed. Carcasses were dressed and frozen. Frozen carcasses were dehydrated and Gross Energy and Protein determined.

Results and Discussion

Data concerning the development of liveweight, food intake and the relation food intake / liveweight for days 0 to 10 are presented in Table 1. Carcass characteristics are presented in Table 2. No significant difference was found between the two experimental groups for any of the above mentioned variables. The relation Food intake / Liveweight, with the exception of day 4, tended to be higher in treated mice, although no significant differences occurred. These results may indicate that treatment with anti leptin antibody caused an increase in food intake. Despite the fact that no significant differences were registered, these results seem to be in accordance with those of Brunner et al. (1997) and Verploegen et al. (1997). No significant differences were registered between groups concerning carcass dry matter, fat, protein and gross energy, indicating that the injection of antibodies did not influence carcass characteristics.

Table 1. Growth and food intake in both experimental groups

	Liveweight (g)		Food Intake (g)		FI / LW [a]	
	C [b]	T [c]	C [b]	T [c]	C [b]	T [c]
Day 0	33.70	32.80	6.91	7.03	0.21	0.21
	(.630)	(.460)	(.230)	(.250)	(.003)	(.008)
Day 2	34.20	33.00	7.40	7.60	0.21	0.22
	(.790)	(.550)	(.940)	(.260)	(.023)	(.005)
Day 4	35.05	33.50	7.40	6.98	0.21	0.21
	(1.650)	(.610)	(.450)	(.310)	(.009)	(.008)
Day 6	35.18	34.20	6.77	6.98	0.20	0.21
	(1.540)	(1.630)	(.170)	(.310)	(.011)	(.017)
Day 8	35.73	33.95	6.50	6.48	0.18	0.19
	(1.210)	(.610)	(.220)	(.400)	(.010)	(.009)
Day 10	35.20	32.60	6.50	6.53	0.19	0.20
	(1.230)	(.650)	(.520)	(.260)	(.018)	(.006)

Values in means (SEM=4); FI/LW [a] = (Food intake) / (Liveweight) C [b] – Control animals; T [c] – Treated animals; No significant difference was observed between control and treated animal results (ANOVA single factor)

Table 2. Carcass Characteristics in both experimental groups

	CW [a]	DP [b]	% DM [c]	% Humidity	% Protein [d]	% Fat [e]	GE [f]
Group C	13.75	39.24	26.11	73.89	54.25	45.75	22172
	(.539)	(.015)	(2.827)	(2.827)	(2.502)	(2.502)	(436.000)
Group T	13.18	30.86	29.59	70.41	51.41	48.59	23152
	(.187)	(.006)	(1.474)	(1.474)	(2.358)	(2.358)	(556.000)

[a] CW – Carcass Weight (g); [b]DP – Dressing Percentage (%);[c] DM – Dry Matter; [e] (in percentage of Dry Matter); [f] Gross Energy (J/g); Values in means (SEM=4); No significant difference was observed between Control and treated animals results (ANOVA single factor)

Such results indicate that the influence of antibodies against leptin in promoting food intake and weight gain, under the experimental conditions used, is apparently negligible. Nevertheless, this trial was limited due to the fact that groups were very small, consisting of only four mice, which caused very high variances and, as a consequence, the lack of significance. Therefore, it seems that the role of antibodies against leptin as growth promoters needs further clarification, through a similar trial, with larger experimental groups, and higher concentrations of injected antibodies.

Acknowledgements

The authors would like to thank the Science and Technology Foundation of Portugal (FCT - PRAXIS XXI/BM/17921/98) for financial support.

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