METABOLIC AND GONADOTROPIC IMPACT OF SEQUENTIAL OBESOGENIC INSULTS IN THE FEMALE: INFLUENCE OF THE LOSS OF OVARIAN SECRETION

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INTRODUCTION

Reproduction is an indispensable function for the perpetuation of the species and, as such, is under the control of a sophisticated network of regulatory signals, which is sensitive to an ample variety of endogenous and environmental modulators. In this sense, It is well known that body energy balance and metabolic status can alter fertility. While female fertility is known to be sensitive to conditions of low body fuel reserves, the reproductive consequences of persistent energy excess remain ill defined. Yet, the pandemic proportions of obesity, and its plausible impact on the female gonadotropic axis, call for a better understanding of this phenomenon. Alike, the influence of ovarian hormones on the patho-physiology of obesity and its complications merits further investigation.

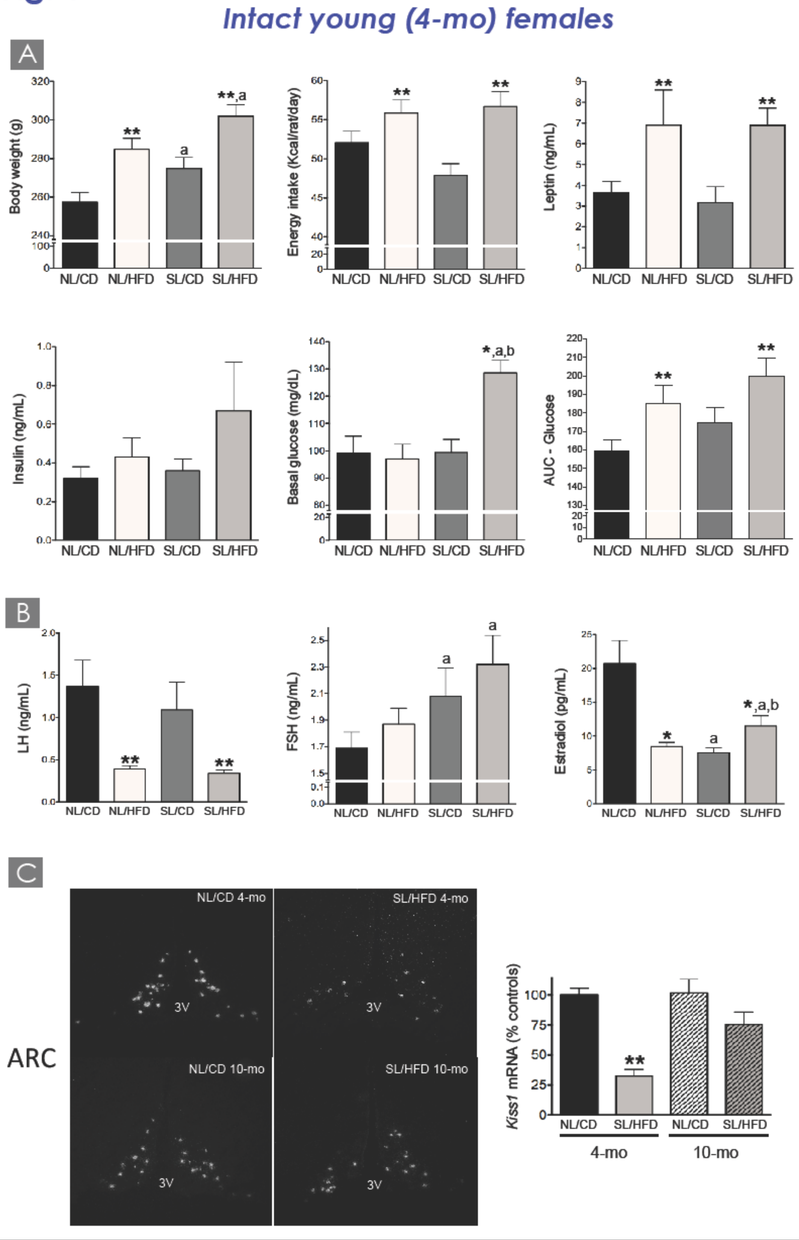
EXPERIMENTAL DESINGS & AIMS

The aim of this work was to evaluate the metabolic and gonadotropic impact of sequential obesogenic insults, namely, postnatal over-nutrition (by rearing in small litters; SL) and high fat diet (HFD) after weaning, in gonadal-intact and ovariectomized (OVX) female rats. To cover this goal, Wistar female rats were bred in normal (NL) or small litters (SL) during lactation in order to induce early postnatal normo- or over-nutrition, respectively, and fed a control diet (CD) or HFD after weaning. Thus, four experimental groups (NL/CD, NL/HFD, SL/CD & SL/HFD) were generated. At PND-90, subsets of animals from each group were subjected to OVX, as preclinical model of cessation of ovarian secretions to mimic human menopause. Analyses in intact rats were conducted at two age-points, 4-mo- and 10-mo-old, representative of young adult and middle-aged rats, whereas analyses in OVX rats were applied at 4-mo-old (PND-120), thirty days after OVX (see experimental design below). Such analyses included the study of phenotypic indices and serum biochemical/hormonal parameters, as well as expression studies in brain samples.

RESULTS

Fig. 1

Intact young (4-mo) females



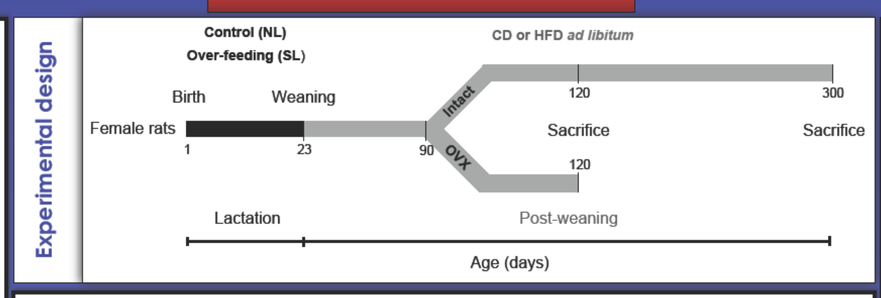


Fig. 2

Intact middle-aged (10-mo) females

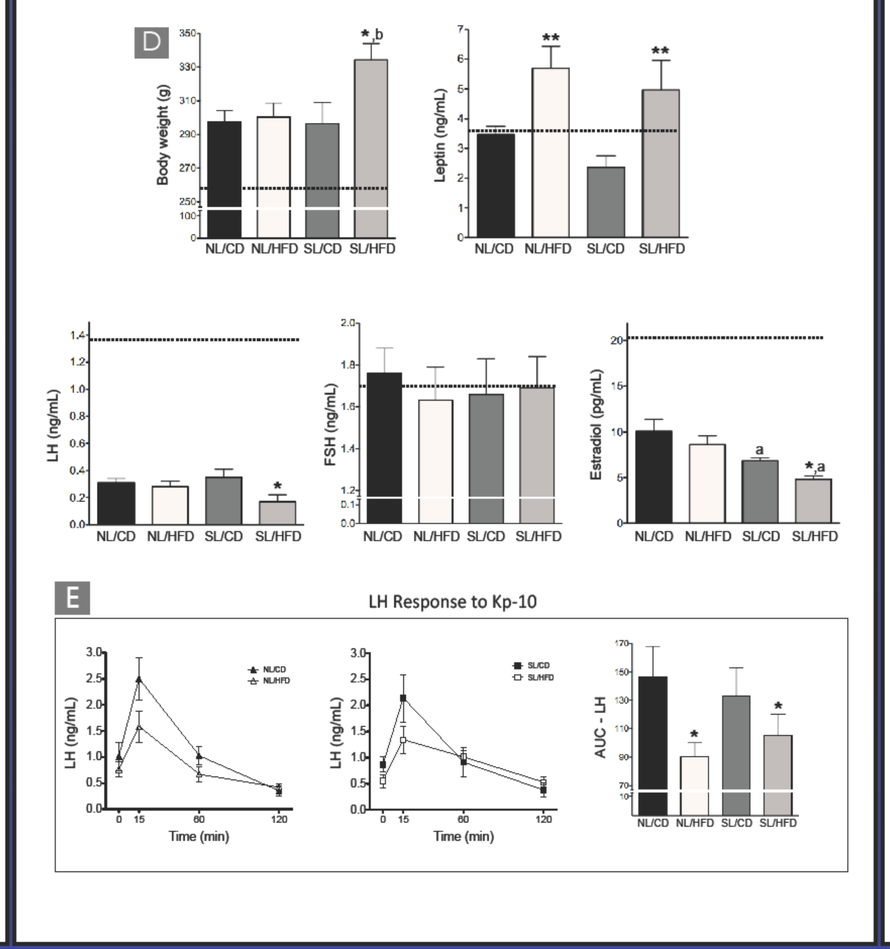


Fig. 3

OVX young (4-mo) females

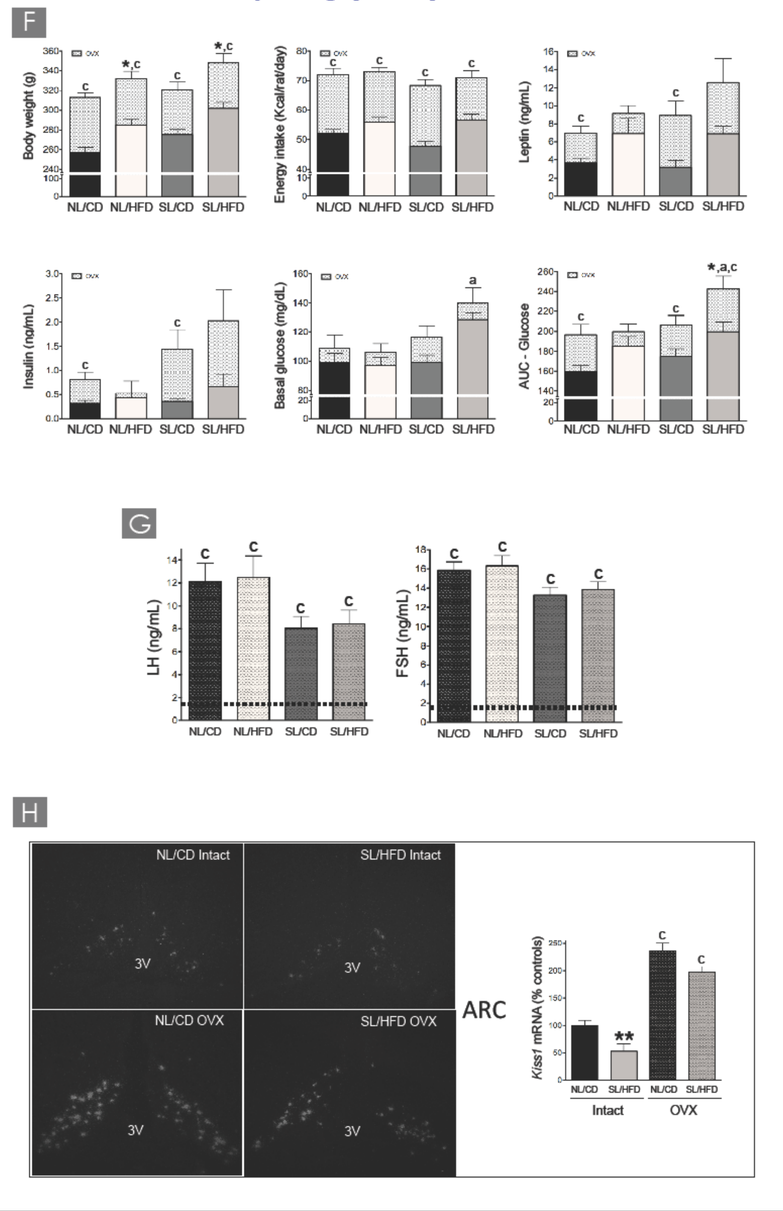


Fig. 1. **(A)** *Metabolic characterization of young adult (4-mo-old) intact female rats, subjected or not to postnatal overnutrition during lactation and/or HFD post-weaning.* **(B)** *Serum LH, FSH and estradiol levels in basal blood samples are presented from young adult female rats subjected to the same obesogenic insults.* **(C)** *Representative in situ hybridization photomicrographs of Kiss1 mRNA expressing-neurons and quantification of Kiss1 mRNA expression in the arcuate nucleus (ARC) of the hypothalamus from young adult and middle-aged female rats are presented. Analyses were specifically conducted in groups with extreme BW differences (NL/CD vs. SL/HFD) at two age-points, 4- and 10-mo. *, P<0.05 and **, P<0.01: effect of HFD;* ***a****, P<0.05: effect of litter size;* ***b****, P< 0.05: interaction of HFD/litter size (two-way ANOVA followed by Newman-Keuls tests).*

Fig. 2. **(D)** *Metabolic and gonadotropic characterization of middle aged (10-mo-old) intact female rats, subjected or not to postnatal overnutrition during lactation and/or HFD post-weaning. Note that control values from young adult (4-mo-old) NL/CD females are denoted for reference purposes, as dotted lines.* **(E)** *LH responses to an icv bolus of kisspeptin-10 are presented from middle-aged (10-mo-old) female rats. Animals were icv injected with a single dose of Kp-10 (50 pmol/rat), and blood samples were obtained at 0-min (before) and 20-, 60- and 120-min after Kp-10 administration. Integral LH levels, estimated as AUC are also shown. *, P<0.05 and **, P<0.01: effect of HFD;* ***a****, P<0.05: effect of litter size;* ***b****, P< 0.05: interaction of HFD/litter size (two-way ANOVA followed by Newman-Keuls tests).*

Fig. 3. **(F & G)** *Metabolic and gonadotropic changes induced by ovariectomy (OVX) in young adult female rats, subjected or not to postnatal overnutrition during lactation and/or HFD post-weaning. For comparative purposes, metabolic indices in the corresponding gonadal-intact groups are shown in the histograms using similar color codes as in Fig. 1 and 2, while the impact of OVX is shown in grey. Control values from intact young adult (4-mo-old; NL/CD) females are shown as dotted lines.* **(H)** *Representative in situ hybridization photomicrographs of Kiss1 mRNA expressing-neurons and quantification of Kiss1 mRNA expression in the arcuate nucleus (ARC) of the hypothalamus from young adult intact and OVX female rats are presented. Analyses were specifically conducted in groups with extreme BW differences, namely NL/CD vs. SL/HFD.* ***c****, P<0.05: effect of OVX vs. corresponding intact group.*

- **In young (4-mo) cyclic females, SL or HFD caused similar increases in body weight; yet, only HFD evoked additional metabolic perturbations, some of which were worsened by precedent SL (Fig. 1A).**
- **HFD caused a decrease in LH and estradiol levels (Fig. 1B), and suppressed *Kiss1* expression in the arcuate nucleus (ARC) of the hypothalamus in 4-mo females (Fig. 1C), while persistent HFD up to 10-mo induced also suppression of LH responses to kisspeptin-10 (Fig. 2E).**
- **OVX caused a severe and rapid deterioration of the metabolic profile, with overweight, increased energy intake and deregulation of leptin and insulin levels and glucose intolerance; effects whose magnitude was similar, if not higher than HFD (Fig. 3F).**
- **Summation of previous obesogenic insults maximally increased body weight, basal leptin, insulin and glucose levels, and glucose intolerance (Fig. 3F). Yet, OVX obliterated the inhibitory effects of overweight/HFD on gonadotropin levels and ARC *Kiss1* expression (Fig. 3G & 3H), which were heightened due to the loss of negative feedback.**

CONCLUSIONS

Our data document the deleterious consequences of overweight on the female gonadotropic axis, which involves the impairment of Kiss1 system, and substantiate the dramatic impact of OVX, as menopausal model, on the metabolic profile, especially when combined with preceding obesogenic insults (SL and HFD).

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