Nutritional Neuroscience

# Exposure to increased levels of estradiol during development can have long-term effects on the response to undernutrition in female rats

--Manuscript Draft--

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| **Manuscript Number:** | NNS447R1 |
| **Full Title:** | Exposure to increased levels of estradiol during development can have long-term effects on the response to undernutrition in female rats |
| **Article Type:** | Original Research Paper |
| **Keywords:** | Protein restriction; Estradiol; Orexinergic and anorexinergic neuropeptides, Metabolic factors, PCR, Rat. |
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| **Abstract:** | Objectives: Undernutrition during development alters the expression of peptides that control energy expenditure and feeding behavior. Estrogens can also modulate these peptides. Here we analyzed whether early postnatal administration of estradiol modulates the effects of undernutrition on neuroendocrine parameters in adult female Wistar rats.<br>Methods: Control rats were fed a control diet. Undernourished pups were submitted to a restricted diet with half of the undernourished rats receiving 0.4 mg/kg s.c. of estradiol benzoate (EB) from postnatal day (P) 6 until P13. Quantitative real-time PCR was performed to determine expression in the hypothalamus of Agouti-related peptide (AgRP), proopiomelanocortin (POMC), neuropeptide Y (NPY) and cocaine- and amphetamine-regulated transcript (CART). Plasma estradiol, testosterone and adiponectin levels were measured by ELISA. Total and acylated ghrelin levels were measured in plasma by RIA.<br>Results: Undernourishment decreased body weight, fat mass, plasma leptin and insulin levels and hypothalamic POMC mRNA levels. An increase in orexigenic signals AgRP and NPY mRNA levels, and in plasma adiponectin levels were found in undernourished animals. Early postnatal treatment with EB to undernourished female rats reversed the effects of undernutrition on adult hypothalamic POMC mRNA levels. In addition, neonatal EB treatment to undernourished females significantly decreased adult plasma testosterone, estradiol and acylated ghrelin levels.<br>Discussion: Our results suggest that increased estradiol during a critical period of development has the capacity to modulate the alterations that undernutrition produces on energy metabolism. |

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Abstract

Objectives: Undernutrition during development alters the expression of peptides that control energy expenditure and feeding behavior. Estrogens can also modulate these peptides. Here we analyzed whether early postnatal administration of estradiol modulates the effects of undernutrition on neuroendocrine parameters in adult female Wistar rats.

Methods: Control rats were fed a control diet. Undernourished pups were submitted to a restricted diet with half of the undernourished rats receiving 0.4 mg/kg s.c. of estradiol benzoate (EB) from postnatal day (P) 6 until P13. Quantitative real-time PCR was performed to determine expression in the hypothalamus of Agouti-related peptide (AgRP), proopiomelanocortin (POMC), neuropeptide Y (NPY) and cocaine- and amphetamine-regulated transcript (CART). Plasma estradiol, testosterone and adiponectin levels were measured by ELISA. Total and acylated ghrelin levels were measured in plasma by RIA.

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Keywords: Protein restriction; Estradiol; Orexinergic and anorexinergic neuropeptides, Metabolic factors, PCR, Rat.

## Introduction

It is well-known that an adequate nutritional status is crucial for normal development of the neural substrate of feeding behavior and suitable functioning of this system in adulthood.[1] Recent studies have shown that both pre- and postnatal food restriction can have severe effects on the morphology and peptide expression of structures controlling appetite regulation. Specifically, a caloric and/or protein restricted diet during development alters the morphology of cerebral structures and the expression of orexigenic and anorexigenic peptides in both male and female rats, as well as circulating levels of metabolic factors and sex steroids in adult rats. [2-9] These studies have demonstrated that significant changes in diet during development produce long-term effects, some of which only become manifest when the animals are adults. Some authors attribute these effects to an alteration in the development of the connectivity of neural circuits that control feeding behavior and others point to metabolic and/or physiological imbalances.[1,10-12]

Control of feeding involves several hypothalamic structures with the arcuate nucleus (ARC) being the first target for numerous peptides that convey metabolic status signals from the digestive system and adipocytes to the brain. Leptin is one of the most important metabolic signals produced in adipocytes and it also plays a relevant role in the programming of hypothalamic circuits that control feeding behavior.[1] In the regulation of energy homeostasis, leptin signals at the level of the ARC increase the synthesis of anorexigenic peptides such as POMC and CART, that together with other peptides promote an anorexigenic response and the inhibition of food intake. On the contrary, during fasting when plasma leptin levels are low, NPY and AgRP expression rise in the ARC, leading to an increase in food intake. The balance between anorexigenic and orexigenic peptide expression is a relevant regulator of feeding behavior and body weight.[13,14]

Numerous studies have demonstrated that in addition to its involvement in an array of physiological aspects of reproductive behaviors, estradiol also plays a role in the modulation of food intake, as changes occur during the ovarian cycle in women and other primates and

also in rats and mice.[15,16] There is an inhibitory effect of endogenous estradiol on eating during estrus, although the effects of this hormone are probably produced during proestrus when estradiol levels are higher.[15] Moreover, effects on body weight and metabolism have been reported when estrogenic compounds, such as phytoestrogens, are administered in the diet or estradiol benzoate is administered during pre and postnatal period.[17,18]. These results reinforce the involvement of estradiol on feeding regulation.[15] Hypothalamic STAT3 is a target of estrogen signaling in the regulation of food intake and body weight and this is the same pathway used by leptin to inhibit food intake. As with leptin, estradiol treatment increases the number of excitatory inputs on POMC neurons in the arcuate nucleus.[14,19] Indeed, it appears that STAT3 might be the common final pathway for the actions of leptin and estradiol in their inhibitory role on feeding behavior.[20]

Since estradiol is involved in the regulation of food intake and this hormone, either from endogenous or exogenous precedence, exerts a protective action on nervous tissue in different circumstances[21,22] it is of interest to determine whether exogenous administration of high doses of estradiol during development has an effect on hypothalamic feeding circuits and/or on metabolic parameters.

Previously we have shown the effects of severe pre and postnatal diet restriction on several morphological brain parameters, including the volume and number of neurons of the locus coeruleus and expression of hypothalamic orexin. Undernutrition-induced alterations in these parameters become evident when animals reach adulthood; thus, this appears to be a good model in which to test the vulnerability of hypothalamic feeding circuits and the possible agents involved in these alterations. Taking into account that undernutrition during development alters the expression of some hypothalamic peptides that control food intake, and that there is a possible interaction between estrogen and these peptides to control energy expenditure and food intake, our aim was to determine if early postnatal administration of estradiol can modulate the effects that undernutrition produces in several parameters related to the physiology of feeding.

## Methods

*Animals*

Wistar rats were maintained in an automatically controlled room programmed at a temperature of 22°C ± 2°C under a 12 h light/12 h dark cycle (lights on at 08:00) with water *ad libitum*. Throughout the study, animal care and handling practices were approved by the Local Ethics Committees and were in accordance with the European Union Directive, (2010/63/UE) and Spanish Government Directive (R.D. 53/2013). For mating, a male was placed in a cage with two females. Sperm-positive females were individually placed in plastic maternity cages with wood shavings as nesting material. On gestational day 6 (G6), pregnant rats were divided into two groups. Five pregnant rats were fed *ad libitum* with a control diet (20% casein, Panlab, Barcelona, Spain) and eleven pregnant rats were fed *ad libitum* with a low protein (8% casein) and 30% caloric restricted diet (Panlab, Barcelona, Spain). The composition of the diets is shown in Table 1.

The day after birth, pups born on the same day were weighed, sexed and randomly distributed (4 females and 4 males/dam, only females were used in this study) among different lactating females. The lactating mothers continued with the same control diet or the restricted diet defined above and all pups continued with the same diet as the dam that raised them. This resulted in the following experimental groups of pups: Control group (C; n= 11 females) where pups were submitted prenatally and postnatally to a control diet and injected with the estradiol benzoate (EB) vehicle (sesame oil) from postnatal day 6 (P6) until P13; Undernourished group (U; n=9 females) where pups were submitted prenatally and postnatally to the restricted diet and injected with the EB vehicle (sesame oil) from P6 until P13 and; Undernourished treated with estradiol benzoate group (UEB; n=10 females) where pups were submitted prenatally and postnatally to the restricted diet and treated with 0.4mg/kg s.c. of estradiol benzoate from P6 until P13.

In all cases, the body weight of the animals was measured every 7 days from P25 (the weaning day) to P45. Rats were weighed and decapitated on P60 between 9:00 and 11:00 a.m. Females were sacrificed in the diestrous phase of their estrous cycle. Trunk blood was collected in ethylenediamine-tetraacetic acid (EDTA) containing tubes and immediately placed on ice. The blood was centrifuged for 15 minutes at 2000 g and the plasma collected and stored al -80ºC. The hypothalami and subcutaneous and visceral fat pads were rapidly removed and frozen at -80ºC.

*Quantitative real-time polymerase chain reaction (PCR)*

Total RNA was extracted from the hypothalami according to the Tri-Reagent (Invitrogen, Carlsbad, CA, USA) protocol. [23]cDNA was synthesized from 2 μg of total RNA by using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA).

For quantitative real-time PCR assay-on-demand kits (Applied Biosystems) were used and corresponded to the following: Agouti-related peptide (AgRP: RN014311703_g1), POMC (Rn00595020_m1), NPY (Rn01410145_m1), cocaine- and amphetamine-regulated transcript (CART; Rn00567382_m1), and phosphoglycerate kinase (pgk1; Rn00569117_m1) TaqMan Universal PCR Master Mix (Applied Biosystems) was used for amplification in accordance with the manufacturer's instructions in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). All samples were amplified in duplication. Values were normalized to the housekeeping gene pgk1 [24] that did not vary among groups. According to the manufacturer's guidelines, the ΔΔCT method was used to determine relative expression levels. Statistics were performed using ΔΔCT values.

*Plasma sex steroid and metabolic factor levels*

Plasma estradiol, testosterone and adiponectin levels were measurement in duplicate and within the same assay by ELISA following the manufacture's instructions (CSB-E05110r for estradiol; CSB-E05100r for testosterone, Cusabio and EZRADP-62K, Millipore for adiponectin). Absorbance in each well was measured with a Tecan Infinite M2000 (Grödig, Austria). The sensitivity of the method was 23.1 pg/ml for estradiol, 0.06ng/ml for testosterone and 0.4 ng/ml for adiponectin. Plasma insulin, leptin and IL-6 concentrations were simultaneously quantification using a multiplex bead immunoassay (RADPK-81 K rat adipokine kit, Milliplex, EDM Millipore). A minimum of 50 beads/parameter were analyzed in the Bio-plex suspension array system 200 (BioRad Laboratories, Madrid, Spain). Raw data (mean fluorescence intensity) were analyzed using the Bio-Plex Manager Software 4.1 (Bio-Rad Laboratories). The sensitivity of the method was 52.5 pg/ml for insulin, 9.7 pg/ml for leptin and 8.8 pg/ml for IL-6. The intra-assay variation was 1.67-4.2%.

Total and acylated ghrelin levels were measured in plasma by RIA following the manufacturer's instructions (GHRT-89HK and GHRA-88HK,

RIA Kit, Millipore). The sensitivity of the method was 93 pg/ml and 7.8 pg/ml for total and acylated ghrelin, respectively.

## Statistical Analysis

Data were submitted to a one way ANOVA. Differences between groups were determined by post hoc comparisons based on the Student-Newman-Keuls test. Body weight data were analyzed using a repeated measure ANOVA (3x6) with experimental group as the within-subject factor and weight as the between-subject factor. Post hoc comparisons were made by using Turkey's honest significant difference. The significance level was set at least at $p<0.05$

## Results

*Body weight*

A main effect on weight ($F(5,27)=384,7$; $p<0.0001$) was observed showing an increase in this parameter with age in all groups studied. The control group had a greater body weight with respect to both undernourished groups at all ages studied ($p<0.0001$ in all cases). In the undernourished groups at P46 UEB had a greater body weight than U, although this did not reach significance ($p<0.05$). At P53 significant differences between these two groups were established with the UEB group having a greater body weight than U female rats [P53, ($p<0.02$); P60 ($p<0.005$)] (Fig. 1 A, B).

*Fat mass*

Subcutaneous and visceral fat pads were weighed and normalized to body weight. A similar pattern was found in both adipose depots, with significant differences between groups [subcutaneous fat: $F(2,29)=6.90$; $p<0.004$; visceral fat: $F(2,29)=17.69$; $p<0.0001$]. Undernutrition during development decreased the weight of both types of fat pads with no effect of EB (Fig.2 A,B).

*Hypothalamic mRNA levels*

Hypothalamic AgRP mRNA levels showed significant differences between groups [$F(2,15)=16.31$; $p<0.0001$] with diet restriction during development increasing hypothalamic AgRP mRNA levels. No effect of EB was observed (Fig. 3A.).

Hypothalamic POMC mRNA levels were affected by both undernutrition and EB treatment [$F(2,17)=12.82$; $p<0.001$; Fig. 3B]. Hypothalamic mRNA levels of POMC were reduced in undernourished rats, but neonatal administration of EB returned them to control levels ($p<0.05$).

Hypothalamic NPY mRNA levels showed significant differences among the groups [$F(2,15)=8.73$; $p<0.004$), with undernutrition increasing them, but with no effect of EB (Fig. 3C).

There were no significant differences among the groups in CART mRNA hypothalamic levels [$F(2,17)= 0.03$; $p>0.05$] (Fig. 3D).

*Plasma metabolic factors*

Plasma adiponectin levels were different between groups [$F(2,26)=7.49$; $p<0.003$] with undernutrition increasing them and EB having no effect (Fig. 4A).

Serum levels of leptin and insulin had similar responses to undernourishment and neonatal administration of EB. Both hormones were significantly affected [for leptin: $F(2,25)=15.65$; $p<0.0001$; for insulin: $F(2,25)=13.22$; $p<0.0001$)], with undernutrition decreasing them and EB having no effect (Fig. 4B, C).

Plasma acylated ghrelin levels were different amongst the experimental groups [$F(2,25)=6.01$; $p<0.007$] with undernutrition having no effect alone, but in combination with EB treatment there was a decline in the levels of this hormone (Fig. 4D).

*Plasma sexsteroid levels*

There was an interaction between experimental factors on sex steroid levels [for estradiol: $F(2,26)=6.43$; $p<0.006$; for testosterone: $F(2,21)=15.97$; $p<0.0001$]. There was no effect of diet restriction on circulating levels of estradiol or testosterone. However, treatment with EB in early postnatal days decreased levels of both hormones (Fig. 5 A,B).
In table 2 a summary of the results obtained in all parameters studied are shown.

## Discussion

Undernourishment, especially during the early developmental period, is a stressful situation that leads to certain changes and compensations in order to adapt the function and the physiology of energy metabolism to the internal and external environmental circumstances.[1] Decreased body weight is the first effect observed when a restriction in diet is implemented.[6,25] The present results confirm that undernourishment produces a decrease in body weight. In addition, here we show that early estradiol treatment in undernourished female rats increases body weight. Taking into account that estradiol administration was performed from P6 until P13, the period during which the ovary beings to become functional, it is possible that this represents a critical period during which estradiol is able to exert a modulatory/protective effect on body weight when the nutritional environment is adverse. It is possible that estradiol is acting through similar mechanisms as those that leptin employs to modify metabolic circuits in order to overcome the decrease in leptin induced by undernutrition.

A long-term decrease in fat mass due to undernutrition has been reported in rats.[11] In line with these results, in our study undernourishment significantly decreased subcutaneous and visceral fat mass in adult rats. However, neonatal administration of estradiol to undernourished rats had no effect on the weights of subcutaneous and visceral fat deposits, indicating that the increase in body weight in UEB females could be due to an increase in lean mass.

No effects of undernourishment were detected on either plasma estradiol or testosterone levels, as previously reported.[7,26] An important result is the significant decrease in plasma gonadal steroid levels that occurred in undernourished females treated with estradiol from P6 to P13. This is a critical period in development in which estradiol secreted from the ovary is involved in the physiology and behavioral organization of

important neural circuits that are activated in adulthood. Administration of EB during development to undernourished females appears to down-regulate the liberation of estradiol and testosterone in adulthood, as undernutrition alone had no effect on plasma levels of these two sex steroids. It could be suggested that elevated levels of estradiol in the critical developmental period programs a low release of ovarian estradiol in response to the early excess of plasma estradiol. This probably has a consequence in the observed down-regulation of testosterone in order to maintain the balance of this system, as inferred from the fact that these females have normal estrous cycle.

Although food intake was not measured, metabolic signals involved in the signaling pathway of eating such as NPY, AgPR or ghrelin, or in the signaling pathway of satiety such as POMC, CART, leptin and insulin were analyzed. Expression of the orexigenic peptides NPY and AgRP is increased by protein and caloric dietary restriction. Our results are in agreement with previous studies reporting an increase in NPY after chronic dietary restriction in male rats.[2,4,27] However, results referring to AgRP expression in response to undernutrition are not consistent, as AgRP is reported to increase in situations of acute food deprivation[2] but when chronic nutritional deprivation is implemented different result have been demonstrated. These outcomes depend on the protocol used to underfeed animals and also if a particular hypothalamic region or the whole hypothalamus were analyzed.[4,27] The NPY/AgRP orexigenic pathway is considered one of the strongest signals to induce food intake.[28] The present study demonstrates that undernourished animals submitted to a restricted protein diet, but with *ad libitum* access to the food present similar increments in hypothalamic NPY and AgRP expression to that observed in animals in a fasting situation.

Ghrelin, a peptide secreted mainly by the stomach, is an orexigenic signal that has an important role in the onset of eating.[29] Pre and postnatal undernourishment had no effect on plasma acylated ghrelin levels. These results are not in accordance with those from other studies where a decrease in serum ghrelin levels in pups of mothers submitted to a caloric restricted diet during gestation and lactation period has been described, although ghrelin production capacity was not affected.[3,9] The differences between our data and those reported by other authors could be due to the differences in the undernutrition protocol used. Thus, in our studies although NPY and AgPR increase in response to a restrictive diet

during development, ghrelin does not. Ghrelin not only stimulates food intake, but it is also involved in numerous other functions such as glucose homeostasis, fat deposition, growth hormone release and bone and muscle formation.[30] It is possible that no change in acylated ghrelin represents normal function of these other physiological processes.

Estradiol did not modulate the long-term adverse effects that diet restriction during development produced on the expression of the orexigenic peptides NPY and AgRP. However, plasma levels of acylated ghrelin were modulated by the administration of EB from P6 to P13 to undernourished rats. Indeed, neonatal EB treatment significantly decreased circulating levels of acylated ghrelin in adulthood. Estradiol has been shown to directly modulate AgRP and NPY and ghrelin in the adult animal[31,32] however, the results reported here are most likely not due to changes in sex steroid levels as these were only modulated in EB treated animals. Thus, the observed modifications in NPY and AgRP are probably a result of their diet and reduced weight and leptin levels. Estradiol is reported to have an inhibitory effect on ghrelin[33,34] however, both estradiol and ghrelin levels were reduced in EB treated rats. Taken together these data suggest a long term effect of estradiol on the expression of ghrelin, possibly due to the establishment of a compensatory mechanism required by the organism in the adverse condition of undernourishment, rather than a direct effect of this sex steroid in adulthood.

Expression of the anorexigenic neuropeptide POMC decreased in the hypothalamus of underfed female rats. These data are in line with previous studies showing a decrease in POMC expression under restricted diet conditions.[2,4,27,35] In contrast, CART expression is reported to change in response to chronic food restriction in some studies, but not in others.[4,27] Our data are in agreement with those of Johansson et al., (2008)[4] where chronic low protein restricted diet had no effect on the hypothalamic expression of this neuropeptide. The lack of effect of undernutrition on the hypothalamic expression of CART might be due to differential regulation of this neuropeptide by specific nutritional factors. Administration of estradiol to undernourished female rats during early development returned the expression of POMC to control levels in adult rats, which is in contrast to that found with anorexic neuropeptides. This could indicate that early exposure to estradiol has long-term effects on undernourished female rats that may be beneficial in the adaptation and compensation of the metabolic systems to reduced energy intake.

Circulating levels of both leptin and insulin levels are decreased in undernourished animals. It is well established that adipose depots and leptin levels are lower in undernourished animals.[36] On the other hand, data concerning insulin levels are inconsistent as authors have reported both increases and decreases due to undernutrition depending on the timing of deprivation, the type of dietary restriction implemented or even the sex of the animal studied.[9,37] Although a protective effect of estrogen mediated through ER alpha against adipose accumulation has been reported [38],conclusive data of a direct effect of estradiol on leptin or insulin levels has not been shown. Neither perinatal administration of EB nor a decrease of estradiol levels in adulthood was associated with changes in plasma insulin and leptin levels in undernourished female rats.

Adiponectin is suggested to play a protective role in the negative consequences that excessive accumulation of fat produces in the organism.[39] Levels of this adipokine negatively correlate with body fat, increasing in response to weight loss[40], which is in line with the data presented here. Early administration of EB or a decrease of estradiol levels in adulthood had no effect on adiponectin levels, suggesting that body fat mass directly controls adiponectin levels not only in obesity, but also in undernourishment conditions.

In conclusion our results reveal that undernutrition produces a variety of alterations in energy metabolism physiology even when animals have sufficient food availability, but the diet is restricted in protein and caloric content. The response of eating and satiety signaling pathways, as well as other parameters such as gonadal steroids or adipokines, appear to be adapted to the metabolic situation in the undernourished groups. The main aim of this study was to determine whether increased estradiol during a critical period of development has the capacity to modulate/adapt the alterations that undernutrition produces on energy metabolism. Indeed estradiol administration early in development up-regulated POMC mRNA levels and down-regulated plasma acylated ghrelin, estradiol and testosterone levels in undernourished female rats in adulthood, reversing the effect that undernutrition produces in these animals.

The mechanism through which estradiol during early development modulates alterations produced by undernourishment cannot be deduced

from our results, but one might speculate that this sex steroid affects the response of the developing brain to the undernutrition status. How the number of neurons in metabolic systems and their connectivity could be affected deserve further investigation.

We believe that it is worth highlighting the modulatory capacity of estradiol during development for two reasons. First, the potential benefits/risks of exogenous estrogenic compounds included in the diet are actually a great concern in our society because in the last decades in western countries there has been a great increase in soy consumption and a significant number of infants are fed with a soy-based formula. Soy is a natural source of estrogenic isoflavones and although some health benefits associated to soy consumption have been recognized, concerns about possible risks from estrogenic activity of isoflavones, mainly during development, must be taking into account.[41,42] Second, estrogen modulates brain development of systems involved in reproductive behaviors. [43] As estradiol modulates the effects that undernourishment produces on factors involved in food intake, it could be suggested that the physiological levels of estradiol during development might be involved in the establishment of neural metabolic circuits in adulthood. Since leptin is involved in the programming of neural metabolic circuits and the STAT3 pathway is common for both leptin and estradiol for the regulation of food intake, a mechanism that includes the interaction between leptin and estradiol through the STAT3 pathway could be determinant in the programming of hypothalamic metabolic circuits and as consequence in the degree of susceptibility for developing metabolic diseases in adult life. Further studies are necessary in order to clarify the role of estradiol in the development of the circuits that control food intake and the potential long term benefits/risks that intake of high doses of estrogenic compounds during this stage could produce on the physiology and behavior in adulthood.

**Acknowledgements:** The present work was supported by grant PSI2011-24943 (PC) and BFU2011-27492 and CIBERobn (JAC). We are grateful to Mr. L. Carrillo, Ms. A. Cámara, and Mr. G. Moreno for their technical assistance.

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**Figure Legends**

**Table 1**: Composition of the diets used in the experiment.

**Table 2**: Summary of the results.

**Figure 1**: A) Mean body weights at P60, B) body weight evolution in all groups from P25 to P60. A significant increase in body weight was observed from P25 to P60 in all groups ($p < 0.05$). U and UEB are significantly different from C in all ages ($p<0.05$). U and UEB differs significantly from P53 onwards ($p<0.05$). **C**: Control female; **U**: Undernourished female; **UEB**: Undernourished+Estradiol Benzoate female. ★= significant differences with respect to C. ●= significant differences with respect to U. All values are expressed as means ± S.E.M.

**Figure 2.** A) Subcutaneous and visceral, B) fat mass. ★= significant differences with respect to C ($p<0.05$). **C**: Control female; **U**: Undernourished female; **UEB**: Undernourished+Estradiol Benzoate female. All values are expressed as means ± S.E.M.

**Figure 3**: Hypothalamic mRNA levels of A) AgRP, B) POMC, C) NPY, D) CART**.** ★= significant differences with respect to C ($p<0.05$). ●= significant differences with respect to U ($p<0.05$). **C**: Control female; **U**: Undernourished female; **UEB**: Undernourished+Estradiol Benzoate female. All values are expressed as means ± S.E.M.

**Figure 4**: Plasmatic levels of A) adiponectin, B) leptin, C) insulin, D) acylated ghrelin. ★= significant differences with respect to C ($p<0.05$). ●= significant differences with respect to U ($p<0.05$). **C**: Control female; **U**: Undernourished female; **UEB**: Undernourished+Estradiol Benzoate female. All values are expressed as means ± S.E.M.

**Figure 5**: Plasmatic levels of A) estradiol, B) testosterone**.** ★= significant differences with respect to C ($p<0.05$). ●= significant differences with respect to U ($p<0.05$). **C**: Control female; **U**: Undernourished female; **UEB**: Undernourished+Estradiol Benzoate female. All values are expressed as means ± S.E.M.

**Title: Exposure to increased levels of estradiol during development can have long-term effects on the response to undernutrition in female rats**

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**Acknowledgements:** The present work was supported by grant PSI2011-24943 (PC) and BFU2011-27492 and CIBERobn (JAC). We are grateful to Mr. L. Carrillo, Ms. A. Cámara, and Mr. G. Moreno for their technical assistance.

All authors listed have contributed to the work and all authors have agreed to submit the manuscript. All authors read and approved the final manuscript and no portion of the work has been or are currently under consideration for publication elsewhere. Animal studies have been reviewed by the appropriate ethics committees, Local Committees and were in accordance with the European Community Council Directive, 1986 (86/609/EEC) and Spanish Government Directive (R.D. 1201/2005).

Table 1
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Table 1 Composition of diets

| | Control Diet | Low Protein Diet |
|---|---|---|
| Proteins (%) | 19.55 | 8.1 |
| Fat (%) | 5 | 5 |
| Carbohydrates (%) | 55.1 | 38 |
| Minerals and vitamins (%) | 8 | 8 |
| Cellulose (%) | 6 | 37.5 |
| Energy Content (Kcal/kg) | 3438 | 2294 |
| Energy from protein (Kcal/kg) | 22.8 | 14.10 |
| Energy from fat (Kcal/kg) | 13.1 | 19.60 |
| Energy from carbohydrates (Kcal/kg) | 54.1 | 66.30 |

Table 2

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# Table 2

| | U | UEB (with respect to U group) |
|---|---|---|
| Body Weight | ↓ | ↑ |
| Sub Fat Weight | ↓ | |
| Visceral Fat Weight | ↓ | |
| AgRP mRNA | ↑ | |
| POMC mRNA | ↓ | ↑ |
| NPY mRNA | ↑ | |
| CART mRNA | | |
| Adiponectin | ↑ | |
| Leptin | ↓ | |
| Insulin | ↓ | |
| Ghrelin | | ↓ |
| Estradiol | | ↓ |
| Testosterone | | ↓ |

Figure 1
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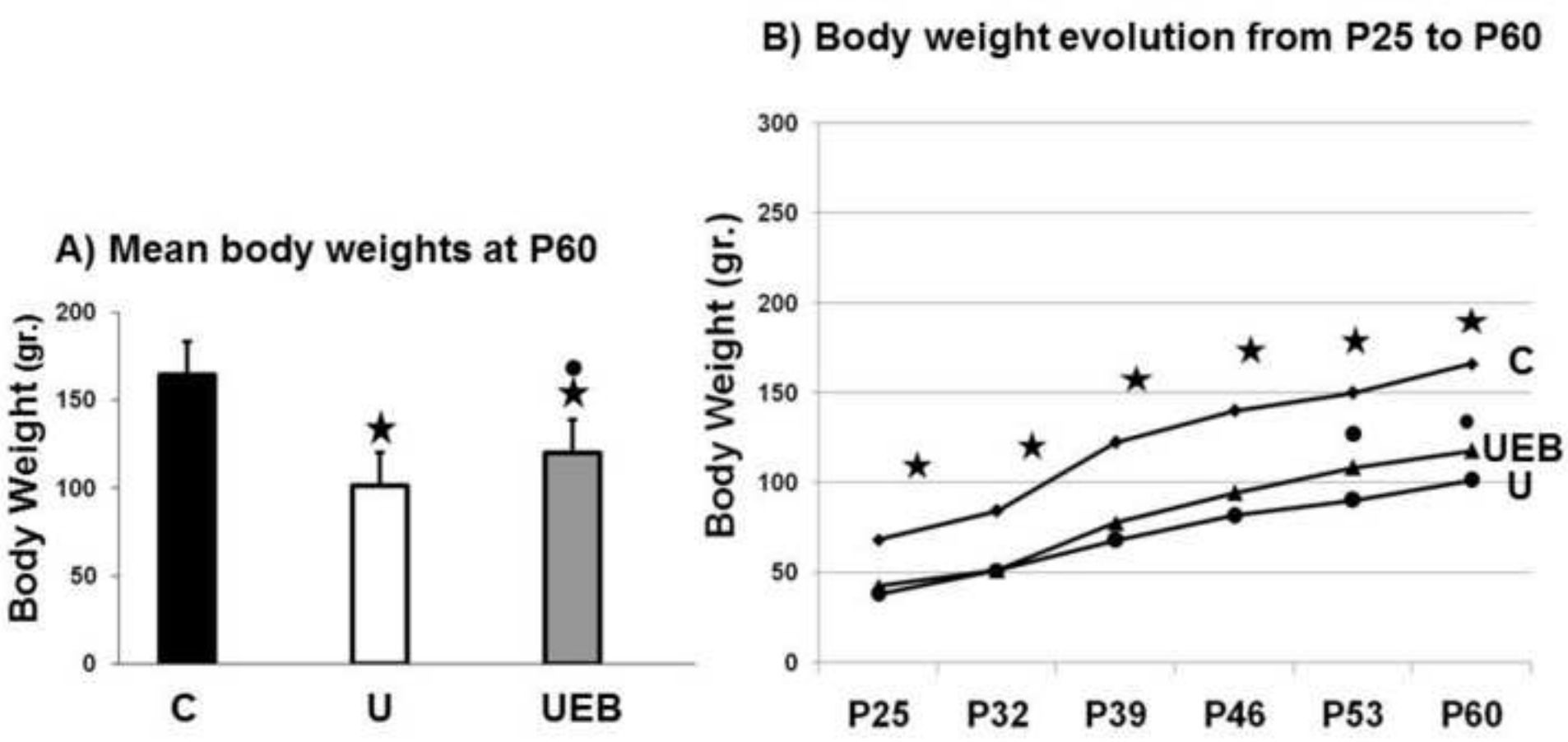

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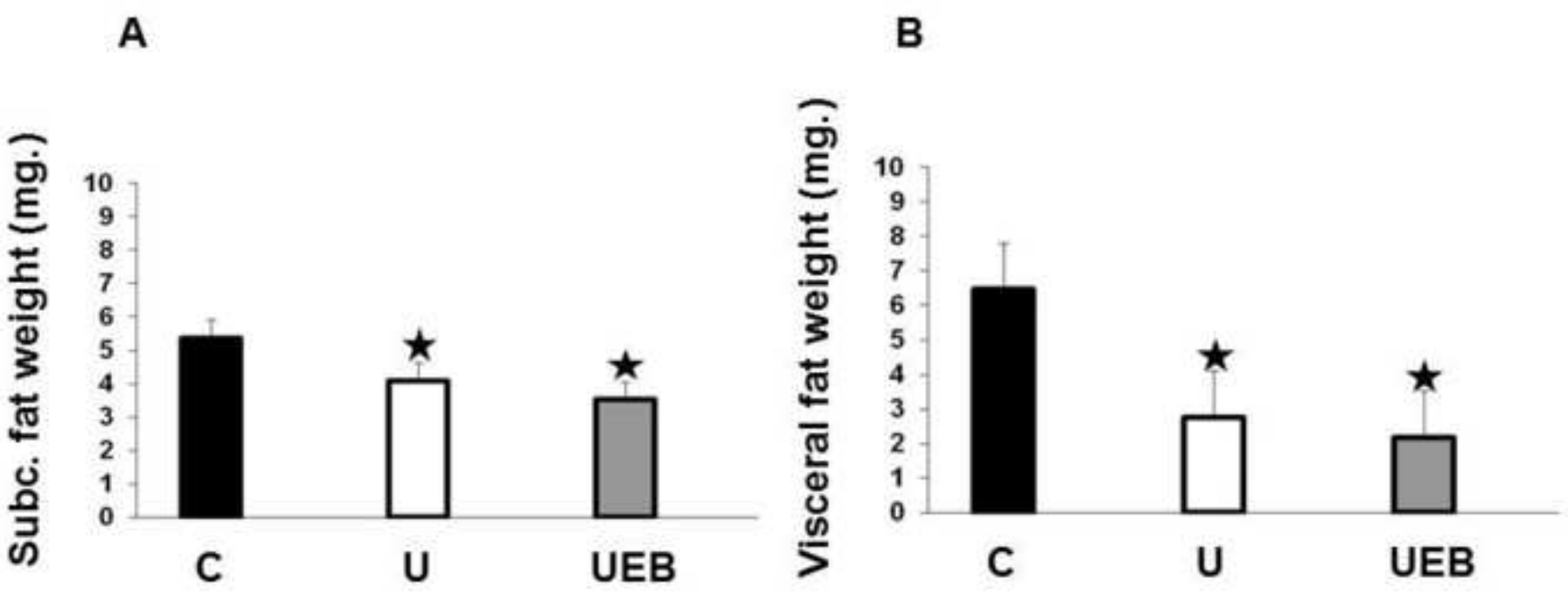

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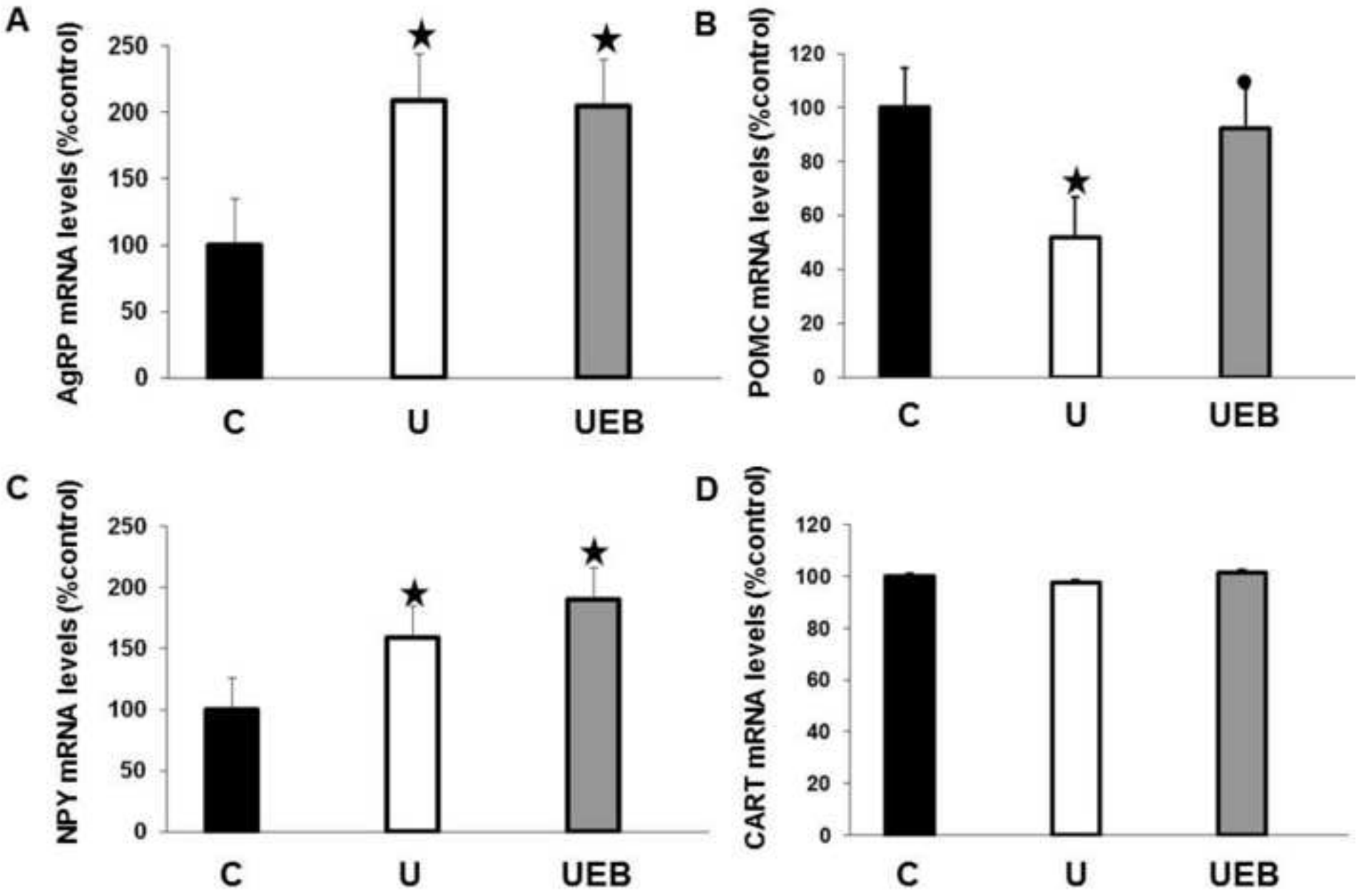

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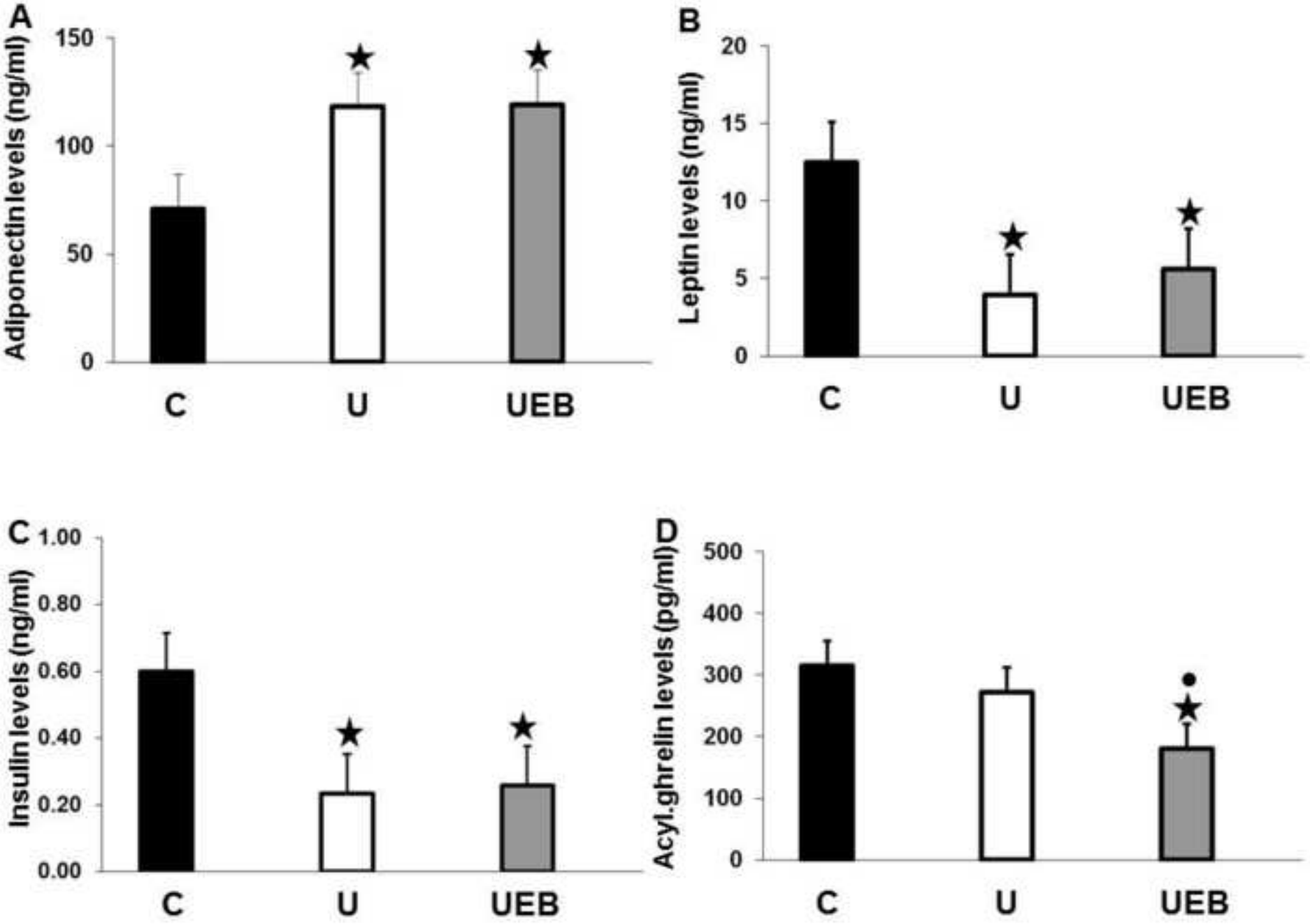

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