Dear Dr. Paul A. Bartell,

We thank the reviewers for their constructive efforts in helping us to improve our manuscript. It is worth mentioning that the current manuscript comprises only a fragment of a broad research program that was conceived to understand the contribution of maternal nutrition on offspring 24 hour rhythmicity, including the expression of different physiological and behavioral variables throughout life, as well as changes in the mechanisms of generation and maintenance of circadian rhythmicity at neuroanatomical, biochemical and molecular levels. In this first report, we analyzed the diurnal pattern of gross locomotor activity and core body temperature, variables that in rabbits are known to be regulated by the circadian system from the first days of life. The methods used in the current report (automated recordings by biotelemetry) and the sampling frequency used (2-min bins, during 20 consecutive cycles), allow us the proper evaluation of different characteristics associated with circadian phenomena. Therefore, we consider it relevant to include updated information about the state of the art on circadian anatomical and molecular regulation in the introduction. Additionally, we have made changes in the introduction, and have responded to all the comments made by the reviewers as detailed below. In doing so, we hope that the paper is now suitable for publication in PlosOne. Nevertheless, whatever the outcome, we have profited substantially from this exchange.

Sincerely,

Ivette Caldelas, on behalf of all co-authors.
Response to Reviewer #1

Results:

I am not sure Table 1 needs to be reported or could be a supplement, but I will leave that to the editor and author to decide.

Table 1 was removed from results and included as supplement 1.

741: Access

the word “asses” was changed to “determine”.

Figures:

Resolution of some of the figures is very poor and impossible to read some of the smaller figures.

Resolution of images was improved.

Response to Reviewer #2

Abstract:

43- Please more clearly describe the ‘anticipatory component” of body temperature or use different terminology. Same for “energetic components of the rhythm”. The meaning of these phrases is non-obvious.

45 The sentence “…decrease in the duration and intensity of the anticipatory component, and changes in the most energetic components of the rhythms” was changed to “…decrease in the duration and intensity of the anticipatory rise to nursing, and changes in frequency of the rhythms”.

Introduction:

117 – Replace with “Women of reproductive age”
The sentence “…woman in reproductive age” was changed to “…woman of reproductive age”.

**120- Be more specific about what conditions these women exhibit**

The sentence “…exhibit some of these conditions” was changed to “…exhibit obesity and metabolic alterations”.

**123- Within the introduction there must be justification for why rabbits are used as a model species for maternal overnutrition, and specifically why they can be a good model for humans, since research in human subjects is directly described in the introduction**

A justification about the rabbit model was included: “The pathogenesis of human obesity and development of metabolic syndrome (MS) is not fully understood, in order to elucidate the mechanisms and develop new therapeutic strategies, it is essential to have an appropriate animal model that shares the most important aspects of the disease process with humans. Rabbits are a widely used experimental model in biomedical research, and have been proposed as an experimental alternative for the study of MS and its complications, such as atherosclerosis and coronary heart disease, which is the major cause of death in MS patients. Unlike rodents, rabbits have close similarities to human cardiovascular and lipoprotein profiles, with higher levels of apoB-containing low density lipoproteins, and abundant cholesteryl ester transfer protein in plasma, an important regulator of reverse cholesterol transport [Fan et al. 1999; Fan et al. 2015; Fan and Watanabe, 2003; Kawai et al 2006; Furukawa et al 2014; Yin et al. 2002; Such et al 2008; Noujaim et al 2010; Zarzoso et al 2012]. In addition, rabbits fed a high fat and sugar diet develop many characteristics of MS observed in humans [Carroll et al. 1996; Zhang et al 2008]. Furthermore, rabbits are an ideal model for the study of transgenerational effects of maternal overnutrition in newborn metabolic regulation, since the placental structure and
materno-fetal blood flow interrelationships are closer to the human, in comparison to other models, such as rodents. Humans have discoid and hemochorial type placentas, and the number of trophoblastic layers in the placental barrier, such as the border between fetal and maternal blood systems, differs between species. In humans it is hemomonochorial, with only one layer, while in rabbits it is hemodichorial and in rodents hemotrichorial [Leiser and Kauffman 1994; Review in Perry, 1981; Carter, 2007; Hafez and Tsutsumi, 1966; Furukawa et al.2011]”.

134 – There is a lot of information in the introduction describing the effects of maternal overnutrition on the circadian clock. However, there is no measurement of the circadian clock gene expression or circadian rhythms of energy metabolism within this paper. There should be a better connection between how the research presented in this paper relates to the circadian clock, or this information should be removed and the introduction should focus more on behavioral and body temperature outputs

161 Information associated to the molecular clockwork was reduced in the introduction.

143- A hypothesis must be provided

161-167 The hypothesis was modified.

144 – Objective does not include anything about UCP1 or CIDEA expression levels or blood metabolites, so why were these measured? Please add objectives related to these outcomes. Also, circadian rhythms in behavioral and physiologic markers weren’t measured. In order to characterize a true circadian rhythm, responses must be measured in consistent lighting (i.e. 24 dim light). Please refer to the rhythms measured (really only locomoter activity) as a diurnal or daily rhythm.

167-171 The objective was modified.

Materials and methods:

156 – Must state the experimental design in the methods

183-185 Additional information about the experimental design was included.
164 – Please provide the total kcal/g of each diet

The total amount Kcal/kg of each diet was included in the text.

166 – Replace elaborated with a more appropriate word. Could just say HFCD contained SD supplemented with…

The word “was elaborated” was changed to “contained”.

170 – Why where # of does unevenly distributed between treatments. Please provide justification for sample size with power calculation.

Due to the difficulties in obtaining the offspring of the HFCD does (important alterations during conception and spontaneous miscarriages) and greater number of dead offspring during the first weeks of life, we decided to increase the number of HFCD does in order to ensure enough animals for the protocol. In this manuscript we did not report any data about female rabbits, such information, in which the power calculation was provided, was submitted elsewhere.

173 – Please justify that human chorionic gonadotropin would not affect fetal metabolic programming

In our study we administered a single dose (30 UI) of human chorionic gonadotropin (hGC). It is well known that the half-life of this hormone is approximately 37 h [Feiman et al. 1968], therefore the transitory action of this hormone is short-lived to improve the conception rate. There are no reports in the literature about the effects of a single administration of hGC in fetal metabolic programming.

178 – Just to clarify- HFCD was really fed -8 to -2 week prior to mating, SD is fed for two weeks prior to mating, and HFCD was fed after mating? To me, this is OK, but it must be made much clearer during the description of the treatments.

Additional information about the feeding procedure was included.
Please mention explicitly that you there is a companion paper with the same experimental design that measured fertility, body weight, etc. Also, I hope that the body weight presented in Figure 1 is also not reported in the other paper.

The information was removed, since this is part of another manuscript submitted elsewhere.

Please define that litter is the experimental unit

With the term litter, we refer to “the offspring at one birth of a multiparous animal” [Webster's Dictionary of The English Language].

How were the subsample of pups chosen for measurement?

The word “randomly” was included in the sentence.

Replace “breastfeeding” with either “nursing” or “suckling” here and elsewhere.

The word “nursing” was included in the sentence.

Replace “produce” with “stimulate”

The word “stimulate” was included in the sentence.

Be specific about which behavior and physiologic recordings were made

In the sentence, the term “core body temperature and gross locomotor activity” was included.

Why was the lactation period only P12 to P31. Shouldn’t it be P1 to P31?

Due to the size of the newborn rabbit, the surgery for transponder implantation was made at postnatal day 8 and four days were considered for the complete surgery recovery of the pups. At this time, the telemetry recordings were initiated.
mRNA levels were not determined by extraction. RNA was first extracted and then mRNA expression of UCP1 and CIDEA were determined by RT-qPCR. Correct wording

The sentence was corrected.

Duration and intensity of the anticipatory component are never defined. Please discuss how this was determined because it is completely unclear

Information about the duration and intensity of the anticipatory component was included.

You report acrophase, but there is no description of how this is determined. Determination of acrophase must have been performed using cosinor rythmometry but there is no description of this in the methods. Cosinor rhythmometry should be performed on data where a diurnal response is measured – so for body temp. and for locomotor activity.

Originally, in the manuscript we indicate that “To evaluate the rhythmicity in the rabbits’ core body temperature and locomotor activity, we used a previously reported procedure [Montúfar-Chavezna R, et al. 2013; Trejo-Muñoz L, et al. 2012]”. This was replaced by a full description of the rhythmicity procedure (332-339).

It is well known that single Cosinor was developed to analyze short and sparse data series [Halberg et al., 1967]. In this work, as we have a long time series for body temperature and locomotor activity, we employed two different methods. The first method used was the Fourier theory, to determine the amplitude and phase (or acrophase) of specific sinusoids. In particular, we employed the Fast Fourier Transform for frequency decomposition considering we have discrete data; next we selected the frequencies (or periods) of interest (i.e. 24h) and recovered the information from the polar representation of Fourier coefficients. In the second method used, since the 24 h phase obtained with Fast Fourier
Transform does not always fit the data, we constructed a pulse sequence of a 24h period which was shifted along the data to find time pulse and data fits using a similarity metric.

292 – *Time course data should be analyzed as repeated measures analysis with day as a repeated factor. A two*

The data was analyzed as repeated measures, as mentioned.

302 – *GraphPad Prism*

365 The word “GraphaPad” was corrected to “GraphPad Prism”

*Results:*

336 – *Replace “close similarities” with “no difference”*

399 The term “close similarities” was replaced with “no difference”.

337 – *Replace “plasmatic level” with “plasma concentrations” here and elsewhere*

400 The term “plasmatic levels” was replaced with “plasma concentrations”.

*Figures:*

All the figures were modified according to comments.

*References:*

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