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High-fat diet and glucose and albumin circadian rhythms' chronodisruption in rats

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Abstract: Obesity is one of the most widespread nutritional diseases in developed societies and it is considered a cardiovascular disease risk factor. The aim of the present work was to evaluate how a high-fat diet may influence the chronobiology of glucose and albumin circadian rhythms. Eighty-four male common Wistar rats were separated into two groups: a control group (n = 42) and a group fed a high-fat diet (n = 42); both for the control and the obesity-induced group we stablished 7 subgroups (6 rats per subgroup) to take blood samples at 0000, 0200, 0400, 1000, 1400, 1800, and 2200 hours. Glucose and albumin plasma levels were analyzed in blood samples and their circadian rhythms were evaluated through the cosinor method. Our results showed clear chronodisruption symptoms in both glucose and albumin oscillations, although these circadian disorders were more evident in glucose rhythms.

Key words: Chronobiology, chronodisruption, obesity, high-fat diet, rats, glucose, albumin

1. Introduction

Obesity and overweight are problems that are continually growing from a public health point of view and they are considered as main causes of pathologic disorders such as metabolic syndrome or cardiovascular diseases (Bravo et al., 2014). Moreover, the most recent data from the World Health Organization show that this epidemic not only affects developed countries, but are also spreading in emerging countries (http://www.who.int/dietphysicalactivity/childhood/en/; Peralta et al., 2015).

The lifestyle of any person is a well-defined rhythmic phenomenon and it is controlled by the circadian system (Corbalán-Tutau et al., 2011). This system coordinates living organisms with their changing environments and allows them to perform those biochemical, physiological, or behavioral functions that ensure the survival of all species at the proper times. Most of these functions are orchestrated by a biological clock system comprising a master clock, the suprachiasmatic nucleus of the hypothalamus, and peripheral clocks (also called oscillators), located in most tissues in the body (Serón-Ferré et al., 2013). The suprachiasmatic nucleus receives information from the retina through the retino-hypothalamic tract about environmental light/dark conditions; this information is transmitted through neurohormonal signals to the rest of the tissues to set the body's internal clock (Bravo et al., 2013).

Chronodisruption can be defined as the alteration of circadian rhythms in living organisms. It can provoke negative effects in the body and highlight the temporal reorganization of physiological systems (Fu et al., 2015). A classical chronodisruption example is that produced during aging. The elderly stage usually shows an advanced phase, a reduced amplitude, a higher fragmentation, and a lower consistency in circadian rhythms (Witting et al., 1990; van Someren, 2000). Recently, our research group has showed that an obesity-induced study carried out in rats resulted in circadian alterations very similar to those observed in old animals (Bravo et al., 2014, 2016).

However, there is limited information about the effects of obesity on circadian rhythms and therefore more studies are required that focus on this pathology from a chronobiological perspective with the aim of obtaining better knowledge about what happens in circadian rhythms. Our research group decided to investigate what consequences a high-fat diet may have on glucose and albumin circadian rhythms in rats, due to the fact that these metabolites are essential for the maintenance of physiological functions in living organisms.

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2. Materials and Methods

2.1. Animals

To perform this research, 84 four-month-old adult male Wistar rats were used in this assay. Our research group decided to choose this age in rats in order to avoid circadian changes that may be common in younger animals. Animals were separated into two groups: a control group (n = 42; body weight [mean ± SEM]: 488 ± 9.26 g), which was fed a standard food, and a group fed a high-fat diet (n = 42; body weight: 490 ± 3.68 g). The animals were individually housed under controlled environmental conditions at 24 ± 1 °C, with a 12 h/12 h light/dark period.

The study was approved by the Bioethical Committee of the University of Extremadura (Badajoz, Spain) in accordance with the National Institute of Health's Guidance for the Care and Use of Laboratory Animals and the European Community's Council Directives (86/609/EEC).

2.2. Animal diets

The control group was fed maintenance food for rodents (A04, SAFE: Scientific Animal Food & Engineering; Table 1). The high-fat diet group was fed a 60% lipids high-fat diet whose composition is presented in Table 2. Both groups were allowed food and water ad libitum throughout the trial. From weaning until the experiment started rodents were fed a common food for growing.

2.3. Blood samples

Both the control group and the high-fat group were separated randomly into seven subgroups (n = 6 per group) to extract blood samples at 0000, 0200, 0400, 1000, 1400, 1800, and 2200 hours in order to gain reliable information about circadian rhythms in glucose and albumin levels.

Blood extractions were carried out by making an incision in the tail and collecting the blood (0.75 mL) in vials containing 50 µL of 10% ethylenediaminetetraacetic acid. The vials were centrifuged at 300 × *g* for 15 min. Aliquots of the resulting plasma were frozen at –20 °C and assayed at the end of the trial with commercial enzymatic kits provided by Spinreact to measure glucose and albumin.

2.4. Statistical analysis

Rhythmicity for metabolite concentrations was determined using the Ritme software package. This software performs cosinor analysis by fitting a sinusoidal function to the experimental variables. The mathematical expression used is

Table 1. The control diet (SAFE A04) administered to 42 control rats for 11 weeks. Nonspecified percentages are due to nonenergetic fractions such as moisture or fiber.

Nutrients	% in diet	Energetic value (kcal)
Proteins	16	64
Carbohydrates	47	176.2
Lipids	3	27
Vitamin supplement	1.7	---
Mineral supplement	5	---
Fatty acids	% in lipid fraction	% in total diet
Lauric acid (C12:0)	0.100	0.003
Myristic acid (C14:0)	1.400	0.042
Palmitic acid (C16:0)	24.400	0.732
Palmitoleic acid (C16:1)	2.800	0.084
Margaric acid (C17:0)	0.300	0.009
Margaroleic acid (C17:1)	0.300	0.009
Stearic acid (C18:0)	12.800	0.360
Oleic acid (C18:1)	43.700	1.311
Linoleic acid (C18:2)	10.800	0.324
Linolenic acid (C18:3)	0.900	0.027
Arachidic acid (C20:0)	0.200	0.006
Gadoleic acid (C20:1)	0.900	0.027

Table 2. High-fat diet administered for 11 weeks to 42 rats.

Nutrients	% in diet	Energetic value (kcal)
Proteins (casein)	17	68
Carbohydrates (sucrose)	18.5	69.4
Lipids (pork lard)	60	540
Vitamin supplement AIN-93-MX	1	---
Mineral supplement	3.5	---
Fatty acids	% in lipid fraction	% in total diet
Lauric acid (C12:0)	0.200	0.120
Myristic acid (C14:0)	1.680	1.008
Palmitic acid (C16:0)	24.860	14.916
Palmitoleic acid (C16:1)	2.500	1.500
Margaric acid (C17:0)	0.460	0.276
Margaroleic acid (C17:1)	0.360	0.216
Stearic acid (C18:0)	14.280	8.568
Oleic acid (C18:1)	41.560	24.936
Linoleic acid (C18:2)	12.480	7.488
Linolenic acid (C18:3)	0.610	0.366
Arachidic acid (C20:0)	0.280	0.168
Gadoleic acid (C20:1)	0.740	0.444

$$Y = M + A \cos(\omega - \varphi),$$

where M is the MESOR (midline estimating statistic of rhythm); A is the amplitude of the function, defined as the distance between the peak value and the MESOR; ω is the angular frequency equal to 2π/T, with T being the period of the rhythm (in our case T = 24 h); and φ is the acrophase, which indicates the hour of the maximal value during the circadian fluctuation (Franco et al., 2012).

Statistical analysis of our data was conducted using the GraphPad Prism v.6 software package. Three types of study were carried out:

- Descriptive analysis, calculating the arithmetic mean ± standard error of the mean as representative values.
- Correlations and linear regressions. For the correlation analysis, Spearman's test was used due to the non-Gaussian distribution of the data, with the significance level taken to be $P < 0.05$. Linear regressions were performed on both the correlative and the noncorrelative data to evaluate any differences occurring during the experiment.

3. Results

During the 11 weeks of the study, an increase in body weight was observed both in the control group and the high-fat group. However, the obesity-induced group had a higher increase compared with the control group from the second week of the assay ($P < 0.001$) and the difference in weight remained the same during the assay (Figure 1).

During the time in which we administered the high-fat diet, no correlation between body weight and the chronobiological parameters that we studied was observed in either the control group or the obesity-induced group (Figure 2). Nevertheless, we found a trend close to significance ($P = 0.058$) between rat body weight and glucose acrophase in the control group. The glucose circadian rhythm was present in every week in the control group (Table 3). On the other hand, rats fed the high-fat diet only showed a circadian glucose rhythm in the 7th experimental week.

Albumin assay showed that control rats fed standard food had a circadian rhythm during all the experimental weeks, while the high-fat group lost its albumin rhythmicity in weeks 3, 5, and 9 (Table 4). Moreover, there

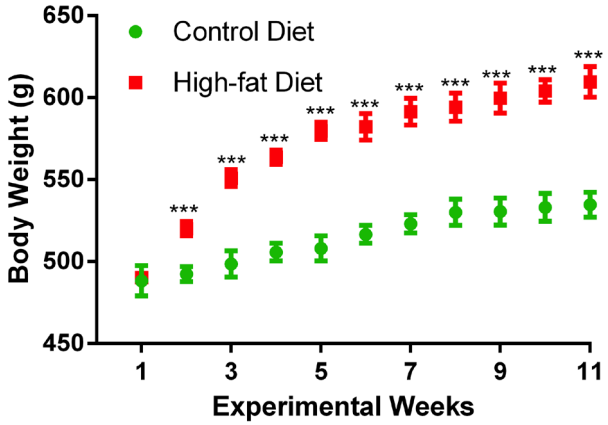

Figure 1. Body weight evolution during 11 weeks in a control group (n = 42) fed control chow and an induced-obesity group (n = 42). ***P < 0.001.

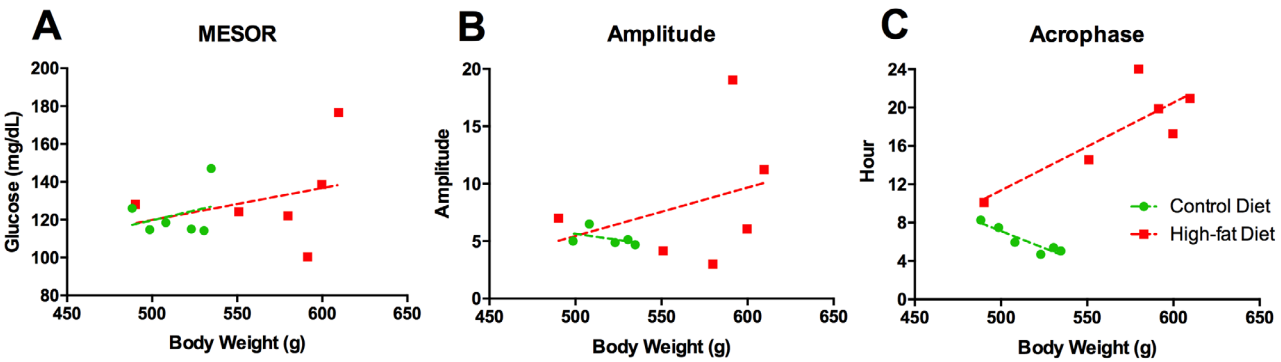

Figure 2. Glucose circadian rhythms in a control group (n = 42) fed control food and an induced-obesity group (n = 42): A) MESOR, B) amplitude, C) acrophase. Noncontinuous lines represent nonsignificant correlation.

Table 3. Glucose circadian rhythms in the control group (n = 42) fed control food and the obesity-induced group (n = 42). Blood extractions were carried out at 0000, 0200, 0400, 1000, 1400, 1800 and 2200 hours (6 rats per extraction group). Cosinor analysis was performed with Ritme software with a significance level of P < 0.05 (*) to establish circadian rhythms. Glucose concentration (MESOR) is expressed as mg/dL.

Week	Control diet				High-fat diet			
	MESOR	Amplitude	Acrophase	Sig.	MESOR	Amplitude	Acrophase	Sig.
1	126.04	29.54	8:16	0.00*	128.20	7	10:06	0.05
3	114.73	5.00	7:29	0.00*	124.24	4.15	14:33	0.34
5	118.44	6.49	5:57	0.02*	122.07	3	0:12	0.53
7	115.01	4.87	4:42	0.04*	100.48	19.04	19:53	0.00*
9	114.27	5.14	5:25	0.01*	138.69	6.06	17:16	0.28
11	147.02	4.69	5:03	0.04*	176.64	11.24	20:58	0.21

Table 4. Albumin circadian rhythms in the control group (n = 42) fed control food and the induced-obesity group (n = 42). Blood extractions were carried out at 0000, 0200, 0400, 1000, 1400, 1800, and 2200 hours (6 rats per extraction group). Cosinor analysis was performed with Ritme software with a significance level of $P < 0.05$ (*) to establish circadian rhythm. Albumin concentration (MESOR) is expressed as g/dL.

Week	Control diet				High-fat diet			
	MESOR	Amplitude	Acrophase	Sig.	MESOR	Amplitude	Acrophase	Sig.
1	4.09	0.11	8:40	0.02*	4.33	0.50	4:36	0.00*
3	5.01	0.33	5:28	0.01*	3.65	0.22	23:00	0.09
5	3.61	0.61	8:12	0.00*	3.16	0.19	19.26	0.23
7	3.65	0.57	6:17	0.00*	3.78	0.38	7:57	0.00*
9	3.85	0.28	5:20	0.03*	3.33	0.09	2:17	0.71
11	3.72	0.32	5:38	0.02*	3.87	0.48	4:42	0.00*

were not clear trends in the chronobiological variables that we studied (Figure 3).

4. Discussion

Obesity is a physiopathologically complex condition with genetic, psychological, and/or environmental causes, which is growing among the world's population. Both overweight and obesity show many characteristics such as high body mass index, endothelial dysfunction, atherosclerosis, hypertension, insulin resistance, and consequently diabetes mellitus type II, among other physiological disorders (Aranceta et al., 2006; Bravo et al., 2014).

Several studies focused on laboratory animals have used cafeteria diets. However, these diets have as their main problem the fact that they do not provide animals with all the nutrients needed for correct development (Moore, 1987; Reeves et al., 1993). For this reason, in the present research, we used a high-fat diet designed to induce animals to obesity with every requirement (Hariri and Thibault, 2010). Previous works about obesity-induced diets established study periods longer than ours (11 weeks), which varied between 15 and 16 weeks (Laposky et al., 2006; Ríos Lugo et al., 2010; Nagatomo et al., 2012; Bravo et al., 2014). Moreover, in this assay, a diet with a lipid percentage of 60% and a carbohydrate percentage of 18.5% was used, while the previously mentioned articles had a lipid composition of 34.9%–35.8% and a carbohydrate composition of 25.9%–35%. With our own formula, we were able to create a new experimental way to induce obesity in rodents in less time.

The glucose circadian rhythm study showed higher glucose concentrations in most of the experimental weeks in the high-fat group, which may be linked to a status of prediabetes in these rats. Previous studies associated this kind of alterations with obesity and sleep/wake circadian rhythm disorders (Bravo et al., 2014; Kalsbeek et al., 2014). Glucose circadian data showed clear symptoms of chronodisruption as can be observed in MESOR, amplitude, and acrophase, which showed different trends regarding the control group, the acrophase of glucose being the most evident parameter. Albumin chronobiological data showed a circadian rhythm during the assay in control rats, with

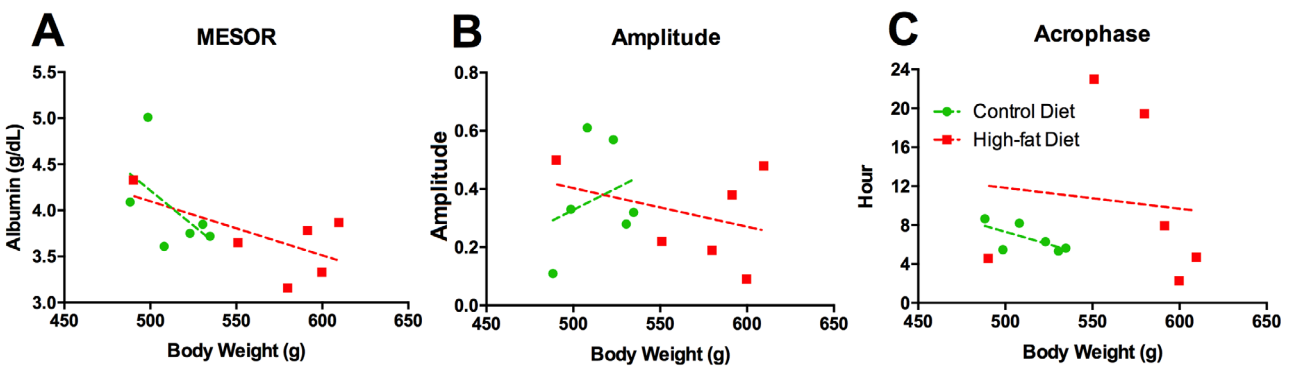

Figure 3. Glucose circadian rhythms in a control group (n = 42) fed control food and an induced-obesity group (n = 42): A) MESOR, B) amplitude, C) acrophase. Noncontinuous lines represent nonsignificant correlation.

an acrophase during the activity phase as has been shown in humans. This was found previously for other molecules that participate in protein metabolism and show their circadian peak during the activity phase (Buzio et al., 1989). Notwithstanding, our finding seems to be the first one that shows chronodisruption with the albumin circadian rhythm losing its rhythmicity in 3 weeks during our study; this alteration may affect arterial function, producing cardiovascular risks associated with metabolic syndrome such as hypertension (Cho et al., 2012).

As has been mentioned, molecular clocks modulate the circadian rhythm, present in most tissues and organs. The first evidence of this was the finding of the influence of the pancreas clock in insulin release with a key role in diabetes mellitus type II development and the role of the liver in glucose tolerance control through liver gluconeogenesis. Otherwise, clock genes in adipocytes do not seem to affect glucose homeostasis, but they have an indirect effect on the appetite-controlling nucleus in the hypothalamus (Garaulet and Ordovás, 2013). These data, together with results obtained in the present work, show how desynchronization in different clocks in the body due to a physiopathology like obesity affects both glucose and albumin homeostasis and produces a loss of rhythmicity. Finally, we want to highlight that due to the limited data on this topic, it is difficult to understand the complexity involved in it, and more studies are required for its complete comprehension.

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