Effect of Food Restriction and Intense Physical Training on Estrous Cyclicity and Plasma Leptin Concentrations in Rats

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Summary Intense physical training and dietary energy restriction have been associated with consequences such as nutritional amenorrhea. We investigated the effects of intense physical training, food restriction or the combination of both strategies on estrous cyclicity in female rats, and the relationship between leptin ad these effects. Twenty-seven female Wistar rats were distributed into four groups: SF: sedentary, fed ad libitum; SR: sedentary subjected to 50% food restriction (based on the food intake of their fed counterparts); TF: trained (physical training on a motor treadmill with a gradual increase in speed and time), fed ad libitum; TR: trained with 50% food restriction. We analysed estrous cyclicity, plasma leptin and estradiol as well as chemical composition of the carcass, body weight variation, and weight of ovaries and perirenal adipose tissue. Data demonstrate that physical training alone was not responsible for significant modifications in either carcass chemical composition or reproductive function. Food restriction reduced leptin levels in all animals and interrupted the estrous cyclicity in some animals, but only the combination of food restriction and physical training was capable of interrupting the estrous cyclicity in all animals. Leptin was not directly related to estrous cyclicity. From our findings, it may be concluded that there is an additive or synergistic effect of energy intake restriction and energy expenditure by intense physical training on estrous cyclicity. Leptin appears to be one among others factors related to estrous cycle, but it probably acts indirectly.

Key Words energy intake, body composition, estrous cycle, leptin, physical exercise

Some female athletes are constantly under pressure to achieve and/or to maintain low body weight, leading to potentially harmful patterns of restricted dieting and over exercising. Some of the health consequences of long-term energy restriction include poor nutritional status and risk of exercise-induced amenorrhea (1, 2). In humans, this condition is characterized by the absence of menstruation for 90 d or more.

Based on epidemiological data, Frisch (3) reported that, in women and girls, body fat content and ovulatory cyclicity were positively associated. However, one or more main signals that centrally indicates the availability of energy is still unknown (4). Since physical activity may reduce leptin mRNA expression (5, 6) and athletes that train intensively, under restricted calorie intake, present a higher percentage of amenorrhea than sedentary women (7), it may be speculated that leptin is involved in the transduction of the energy availability signals.

Studies on the effects of exercise, reproductive function and leptin response have been hindered by the inability to disassociate the effects of exercise itself from other confusing factors. These factors could be related to energy intake below the requirements or different hormones involved (8, 9). It is not clear whether the reproductive functions are interrupted due to exercise itself, or due to a low energy intake from diet (10–13).

As such, it is important to separately investigate the possible effects of exercise per se and energy restriction on leptin and its consequences on reproductive function. With this in mind, this text aims to investigate the effects of intense physical exercise, food restriction or the combination of both strategies on estrous cyclicity in female rats, and the relationship between leptin and these effects.

METHODS

Animals. Female Wistar rats were provided by the animal house of São Judas Tadeu University, São Paulo, Brazil. Animals were 3 mo old and were all in the reproductive phase of life, with a mean body weight of 227.4±20.0 g (200–260 g range). The animals were placed in individual stainless steel cages in the animal house at a constant temperature of 23–25°C and on a 12 h light–12 h dark cycle. Animals had water ad libitum throughout the experiment and food intake was controlled as described below. The animals were cared

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for in accordance with Guide for the Care and Use of Laboratory Animals (14); animal procedures were approved by the ethics committee of São Judas Tadeu University, São Paulo, Brazil, protocol number 074/2004. All animals were weighed daily at 0800 h, before the training period. At that time, the water and the non-ingested food was weighed and withdrawn from the cages. After the training period of trained animals, the new food was weighed and offered to the specific recipients inside the cages. During the rest of the time, the animals were allowed to have food and water ad libitum. Therefore, this schedule ensured that animals of all groups (whether training or not) had the same feeding time.

From the body weight and food consumption data, we calculated the food efficacy coefficient, as follows: FEC = [(mean food consumption)/(daily body weight gain)] (15).

**Experimental design.** Figure 1 describes the experimental design. The experiment was carried out over 8 wk, as follows:

1st week: The animals were submitted to an adaptation period in individual cages.

2nd week: The animals were put on an adapted treadmill on two consecutive days for 15 min to familiarize them with the appliance. The animals were then assigned to a maximal test, according to Irigoyen et al. (16).

3rd to 6th week: The experiment was initiated. Animals were assigned to groups. Four experimental groups were constituted: SF: sedentary, fed ad libitum; TF: trained, fed ad libitum; SR: sedentary, food restricted; TR: trained, food restricted. Both SR and TR had their food intake calculated from 50% of their ad libitum counterpart group (average value of food intake by SF and TF, divided by two). The 50% food restriction and exercise protocol were chosen based on studies with humans, which suggested that, in some athletes, this may constitute an extreme dietary restraint and/or pronounced over-exercising, related to so-called athletic anorexia (1, 2). The diets were elaborated according to the rules of the Committee of Laboratory Animal Diets (17), with calorie levels of approximately 386 kcal·100 g⁻¹ ration.

The physical training program was developed on a motor treadmill with a gradual increase in speed (from 0.4 to 1.2 km·h⁻¹) and time (from 40 to 60 min). On the 4th week of training, this program reached 1 h of training and 85% of the speed obtained by the maximal speed at an exercise test (16). The animals trained for 5 d per week, for 1 h, twice a day, with an interval of at least 5 h between the training sessions. Note that the maximal test was repeated at the beginning of the fifth week of training in order to guarantee that the animals maintained an exercise level at 85% of the maximal speed test result.

**Estrous cycle analysis.** The rat estrous cycle consists of four phases: diestrus, proestrus, estrus and metestrus. Diestrus and metestrus are anovulatory phases and these are not fertile periods. Estrous and proestrus are phases in which ovulation occurs. Characteristic features of specific phases are: a smear consisting almost exclusively of leucocytes depicts diestrus; a thin smear of equal numbers of leucocytes and elongated nucleated epithelium indicates proestrus; large cornified epithelial cells are exclusively found in estrus; and metestrus is marked by a thick smear composed of equal numbers of nucleated epithelial cells and leucocytes (18). From the first week of the experiment (adaptation period) onwards, the estrous cycle was monitored by vaginal smear between 0700 h and 0900 h. The proportion of cell types in the smear was observed with an optical microscope (Olympus, CH20 BIMF110–110/115-120 v-40 X) (19).
Euthanasia. Two days after the last day of training, after 4 h of fasting (generally associated with postabsorptive state; 20), the animals were euthanatized by decapitation without anesthesia. Blood was collected by animal inversion and centrifuged and the plasma stored in a freezer at −80 °C. The ovaries and perirenal adipose tissues (PAT), liver, gastrocnemius and soleus muscle were removed and weighed (scale with 0.1000 precision). The gastrointestinal tract was completely emptied and washed with a physiologic saline. Liver, gastrocnemius muscle and soleus muscle were stored in a −80°C freezer for posterior analyses. Ovaries, PAT and gastrointestinal tract were returned to the remaining carcass for subsequent determination of chemical composition.

Chemical analysis of the carcass. Chemical analysis of the carcass was performed according to a protocol described by Donato et al. (15). The carcass comprised the whole body of the animal, except for the blood sample (approximately 5 mL), and the tissues that were removed for other analyses (liver, gastrocnemius and soleus muscle).

Initially, moisture content was determined by drying the whole carcass in a ventilated oven (~70°C) for 7 d. Carcasses were weighed before being placed in the oven and after being dried, and the difference between measurements was considered to be the absolute moisture content. The whole dry carcass was then chopped up and wrapped in gauze and filter paper for determination of body fat by the solvent extraction technique using a Soxhlet apparatus and ethyl ether as solvent to obtain the lipid content (lipid content of the carcass: LCC). The remaining carcass without moisture and fat was completely ground (IKA M20 grinder, Labortechnik, Wassenburg, Germany) and sifted for the removal of hair, which could decrease the homogeneity of the sample. This process resulted in a highly homogeneous powder that was used to determine carcass protein by the micro-Kjeldahl method (21) and ash content. Two grams of powder were placed in a muffle furnace for 12 h at 550°C and then cooled, and the ash weight of the sample was determined. The amount of lean mass (LM) was calculated by subtracting absolute fat mass from the total carcass mass. Fat, protein, moisture and ash were calculated in absolute values (15).

Liver and muscle glycogen content. Muscle (soleus) and liver glycogen concentrations were determined according to Hassid and Abraham (22). Tissues were treated with 6 N KOH for 30 min in a 37°C water bath for previous extraction, and 70% ethanol was added to precipitate glycogen. Next, the solution was centrifuged for 20 min at 3,000 rpm and the pellet was diluted in hot deionized water. Anthrone diluted in sulfur acid was then added to turn the solution green, and the reaction was analyzed with a spectrophotometer at 650 nm.

Hormone assay. Plasma leptin and estradiol levels were measured by radioimmunoassay (RIA) using a standard commercial kit (Linco RL 83 K, Missouri, USA and DSL-4800, Webster, Texas respectively). The limit of sensitivity for leptin was 0.5 ng/dL, intra-assay CV was 9.5% and inter-assay CV was 9.2%. The limit of sensitivity for estradiol was 0.5 ng/dL and intra-assay CV and inter-assay CV were 9% and 11% respectively. Assays were performed in duplicate.

Statistical analysis. Experimental data were first tested for normality by the Komarov-Smirinov test. Two-way ANOVA was used for comparison among groups and the Tukey test was used post-hoc. In addition, to compare the evolution of an animal’s body weight, we adopted ANOVA for repeated measures followed by a Bonferroni test post-hoc. Partial correlation (Pearson’s and Spearmen’s correlation), simple or multiple (stepwise) linear regressions and non-parametric association analysis (chi-squared and multiple comparisons) were determined and p value ≤0.05 was considered as significant in all analyses. Data were analyzed with Statistica version 7.0, Statsoft, Inc, USA.

RESULTS

Body weight variation (ΔBW), food intake (FI) and food efficacy coefficient (FEC) are presented in Table 1. In addition, Fig. 2 shows the body weight variation throughout the experiment. It can be observed that food restriction, but not physical training, corresponded to body weight loss and negative FEC.

Table 1. Body weight variation (ΔBW), food intake (FI) and food efficacy coefficient (FEC). 1

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>ΔBW (g)</th>
<th>FI (g·d⁻¹)</th>
<th>FEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR (n=5)</td>
<td>−45.7±18.4a</td>
<td>11.6±0.0a</td>
<td>−14.9±8.1a</td>
</tr>
<tr>
<td>TF (n=7)</td>
<td>45.6±13.0b</td>
<td>23.8±1.5b</td>
<td>26.5±9.3b</td>
</tr>
<tr>
<td>SR (n=7)</td>
<td>−43.7±16.4a</td>
<td>10.9±0.1a</td>
<td>−13.8±5.2a</td>
</tr>
<tr>
<td>SF (n=8)</td>
<td>43.5±9.7c</td>
<td>21.8±1.3c</td>
<td>25.5±5.4c</td>
</tr>
</tbody>
</table>

1 Mean and SD; different letter means statistical difference (p<0.05).
TR: trained and restricted group; TF: trained group fed ad libitum; SR: sedentary and food restricted group; SF: sedentary group fed ad libitum.

Body weight variation (ΔBW), food intake (FI) and food efficacy coefficient (FEC) were significantly lower in the SF group compared to the TR group. The SF group had a higher FI and lower FEC than the TR group. The TF group had a similar ΔBW and FI to the SF group, but a higher FEC. The SR group had the highest ΔBW and FI, and the lowest FEC.

Table 2 shows the chemical composition of the carcass, fat tissue weight, liver and muscle glycogen content. Ad libitum fed animals had higher values of moisture, protein, carcass fat and perirenal fat. Fed animals presented higher liver glycogen content. In contrast, a lower energy balance (energy restriction plus intense physical training) was associated with higher muscle glycogen content.

Figure 3 shows leptin and estradiol levels in the experimental groups. Food restriction, but not physical training, was observed to be responsible for a decrease in leptin levels. Although there was no significant difference in estradiol level, a tendency towards a higher concentration in the fed groups can be observed (p=0.07).

Ovary weight differed among the groups in absolute values (ovary weight in grams (g)): TR=0.04±0.00;
TF = 0.07 ± 0.02; SR = 0.07 ± 0.04; SF = 0.08 ± 0.01; p = 0.05), but not when corrected by body weight [% ovary weight: TR = 0.02 ± 0.00; TF = 0.02 ± 0.01; SR = 0.03 ± 0.02; SF = 0.03 ± 0.00; p = 0.15]. Estradiol level did not significantly correlate with ovary weight, either in absolute or in percentage values (r = 0.15; p = 0.45 and r = -0.05; p = 0.79, respectively).

Figure 4 shows the estrous cycle phases in the last 7 d before euthanasia. Significant differences among the groups were found at: D7 (seven d before euthanasia; p = 0.04); D6 (p = 0.00); D5 (p = 0.00); D4 (p = 0.01) and D1 (p = 0.03). D2 and the day of euthanasia presented a statistical difference that approached significance (p = 0.06). The only day that the estrous cycle did not present significance among groups was D3 (p = 0.18).

Irrespective of the statistical analysis, we may note that TR animals presented only diestrus and metestrus in the last 7 d before euthanasia. In contrast, six of the seven animals in the SR group interrupted their cyclic-ity during the 4 d before euthanasia. Among the fed and exercised animals (TF), only two of the seven animals interrupted their cyclic-ity during the last 4 d of the experiment. All animals in the fed and sedentary group (SF) demonstrated a dynamic cyclic-ity during their estrous cycle, which could mean fertile activity for these animals.

Table 2. Chemical composition of the carcass and tissue glycogen content.

<table>
<thead>
<tr>
<th></th>
<th>TR (n = 5)</th>
<th>TF (n = 7)</th>
<th>SR (n = 7)</th>
<th>SF (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-free mass (g)</td>
<td>161.9 ± 8.7a</td>
<td>226.3 ± 14.9b</td>
<td>160.1 ± 9.4a</td>
<td>210.5 ± 21.8b</td>
</tr>
<tr>
<td>Moisture (g)</td>
<td>115.7 ± 5.7a</td>
<td>161.2 ± 11.7b</td>
<td>114.4 ± 6.3a</td>
<td>151.1 ± 15.8b</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>15.5 ± 2.6a</td>
<td>26.4 ± 6.8b</td>
<td>16.7 ± 2.3a</td>
<td>26.8 ± 6.0b</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>10.0 ± 6.5a</td>
<td>15.0 ± 2.0b</td>
<td>10.3 ± 1.5a</td>
<td>13.2 ± 2.2a</td>
</tr>
<tr>
<td>LCC (g)</td>
<td>4.3 ± 2.0a</td>
<td>27.9 ± 5.6b</td>
<td>6.6 ± 2.6a</td>
<td>29.5 ± 6.3b</td>
</tr>
<tr>
<td>PAT (g)</td>
<td>0.2 ± 0.2a</td>
<td>11.8 ± 3.6b</td>
<td>0.4 ± 0.3a</td>
<td>12.2 ± 1.4b</td>
</tr>
<tr>
<td>Fat-free mass (%)</td>
<td>97.4 ± 13a</td>
<td>89.0 ± 2.4b</td>
<td>96.0 ± 1.6a</td>
<td>87.8 ± 1.9b</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>69.6 ± 0.8a</td>
<td>63.4 ± 2.1b</td>
<td>68.6 ± 1.4a</td>
<td>64.0 ± 1.4b</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>9.6 ± 1.7a</td>
<td>11.7 ± 3.2a</td>
<td>10.4 ± 1.0a</td>
<td>12.7 ± 2.2a</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>2.6 ± 1.3a</td>
<td>11.0 ± 2.4b</td>
<td>4.0 ± 1.6a</td>
<td>12.2 ± 1.9b</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>7.6 ± 1.9a</td>
<td>6.7 ± 1.0a</td>
<td>6.4 ± 0.8a</td>
<td>6.3 ± 1.2a</td>
</tr>
<tr>
<td>Muscle glycogen content (mg% tissue weight)</td>
<td>0.06 ± 0.01a</td>
<td>0.05 ± 0.01ab</td>
<td>0.06 ± 0.01a</td>
<td>0.04 ± 0.00b</td>
</tr>
<tr>
<td>Liver glycogen content (mg% tissue weight)</td>
<td>2.63 ± 1.11a</td>
<td>3.10 ± 0.25b</td>
<td>2.61 ± 0.21a</td>
<td>3.00 ± 0.28b</td>
</tr>
</tbody>
</table>

1 Presented by mean and SD; different letters mean statistical differences (p<0.05) by ANOVA two-way.
2 Soleus muscle.
LCC: lipid content of the carcass; PAT: perirenal adipose tissue weight. TR: trained and restricted group; TF: trained group fed ad libitum; SR: sedentary and food restricted group; SF: sedentary group fed ad libitum.
Some variables were submitted to a multiple forward stepwise regression analysis. ΔBW accounted for 77.4% of leptin \( (p=0.02) \). In addition, LCC accounted for 97.5% of leptin \( (p=0.00) \) and PAT accounted for 94.0% of this hormone \( (p=0.00) \); excluding colinearity between LCC and PAT, leptin is predominantly under the control of LCC. LCC and PAT were not significantly correlated with estradiol level \( (r=0.29; \ p=0.13 \) and \( r=0.23; \ p=0.24, \) respectively). Correlation analysis between leptin and estradiol was not significant \( (r=0.18; \ p=0.38) \). Estradiol \( (p=0.00) \) and ΔBW \( (p=0.02) \), but not leptin \( (p=0.57) \), were significantly associated with estrous cycle variation.
DISCUSSION

The main finding of the present work is that physical training alone was not responsible for significant modifications in either body composition or in reproductive function. Additionally, food restriction reduced leptin levels and interrupted the estrous cyclicity in some animals, but only the combination of food restriction and physical training was capable of interrupting the estrous cyclicity in all animals.

Some studies pointed out that the higher the negative energy balance, the higher the probability of reproductive alterations (23–25), and our results agreed with this statement. When food is scarce, animals adjust their energetic priorities. Given that fat storage and reproduction have similar low priorities for energy partitioning, it should not be surprising that they are highly correlated with one another, since both are reduced as a consequence of negative energy balance. In food-restricted animals, we observed that the body weight underwent a profound reduction throughout the first 4 wk of the experiment and, after this, body weight tended to stabilize. Coincidentally, from the 4th week onwards, TR animals did not achieve ovulatory phases, which can indicate a body strategy for survival. In addition, SR animals lost body weight in the last week of the experiment and, after this period, many of them interrupted their estrous cyclicity. This clearly shows an additive or synergistic effect of energy restriction and energy expenditure (exercise). Energy balance was less negative in SR than in TR, and from these data we can explain why SR maintained their estrous cyclicity longer than TR. However, our study did not allow us to find any definite explanation for the fact that the SR and TR groups showed a decline in their body weight during the last week of the experiment. We may speculate on the existence of a “time limit” to sustain the stress imposed (energy restriction and/or intense physical exercise). It is probable that, even by interrupting the estrous cycle in order to adjust the energy needs, these animals would not survive any longer.

With regard to leptin, it is noteworthy that in both restricted groups, this hormone decreased, probably as a result of the massive decrease in adipose tissue. In turn, under the experimental conditions of our study, there was no difference between the leptin levels of TF (trained and fed) and SF (sedentary fed), signifying that intense physical training was not enough to promote leptin reduction. Therefore, body fat reduction seems to be an important factor responsible for leptin reduction. In contrast, Pasman et al. (26) showed, in a physical activity program with obese men, that the leptin level was reduced independently of body fat.

Estrous cyclicity was significantly associated with estradiol and ΔBW, but not with leptin. Notably, a higher standard deviation may be observed in groups less subject to estrous interruption (fed groups). This variability was expected, and can be explained by the fact that we did not analyse the estradiol levels according the estrous cycle. Thus, the effect of leptin on estrous cycle variability may be indirect. Many studies have pointed out that leptin could be an important, but not the sole, metabolic factor in reproductive axis regulation. Matsuyama et al. (27) analyzed the gonadotropin-releasing hormone (GnRH) pulses in fasted and refed animals and evaluated a number of plasma molecules, including insulin. These authors suggested that plasma glucose is the favorite substrate of nervous tissue; thus, insulin and glucose transporter-4 (GLUT-4) are activators of GnRH secretion for the stimulation of neurons’ glucose intake. As such, the lower glucose supply to the central nervous system impairs ovulation, because insulin signals GnRH pulsatility and gonadal lutenizing hormone (LH) activity (28–30).

In our experiment, food restriction was responsible for a lowering in liver glycogen, consequently decreasing the amount of oxidable energy substrate (glucose). We observed that higher liver glycogen levels were associated with higher estrous cyclicity. However, physical training was capable of improving glycogen deposits in muscle, and physical training combined with food restriction, tended to improve these reserves further. This observation indicates that liver glycogen is a biochemical marker of energy reserves of the body. The liver has been suggested as a site of metabolic fuel detectors and it communicates with the brain via autonomic and somatic afferents (31, 32).

Leptin was probably indirectly responsible for the estrous cycle interruption, since in both groups submitted to energy restriction, the lipid and glycogen reserves (oxidable nutrients) were significantly reduced. Fishman and Dark (33) compared Syrian hamster cyclicity after treatment with leptin in comparison with animals with inhibited peripheral fat and glucose oxidation. The authors observed that cyclicity of estrous and reproductive behavior was reversed only when the peripheral metabolic oxidation was not inhibited. As such, the presence of enough oxidable fuel is an important additional factor, and leptin has an important peripheral effect on substrate oxidation. Adipose tissue is an important factor that is highly dependent on fuel oxidation, and this information can be transmitted to the brain in at least two ways. The brain could monitor adipocyte function via sensory innervation of adipose tissue (34). Adipose tissue can also communicate with the brain through changes in circulating leptin levels because adipocyte leptin production responds to metabolic states. This observation indicates that liver glycogen is a biochemical marker of energy reserves of the body. The liver has been suggested as a site of metabolic fuel detectors and it communicates with the brain via autonomic and somatic afferents (31, 32).

Leptin was probably indirectly responsible for the estrous cycle interruption, since in both groups submitted to energy restriction, the lipid and glycogen reserves (oxidable nutrients) were significantly reduced. Fishman and Dark (33) compared Syrian hamster cyclicity after treatment with leptin in comparison with animals with inhibited peripheral fat and glucose oxidation. The authors observed that cyclicity of estrous and reproductive behavior was reversed only when the peripheral metabolic oxidation was not inhibited. As such, the presence of enough oxidable fuel is an important additional factor, and leptin has an important peripheral effect on substrate oxidation. Adipose tissue is an important factor that is highly dependent on fuel oxidation, and this information can be transmitted to the brain in at least two ways. The brain could monitor adipocyte function via sensory innervation of adipose tissue (34). Adipose tissue can also communicate with the brain through changes in circulating leptin levels because adipocyte leptin production responds to changes in substrate oxidation (35–37).

In our study, ovary weight was used to indirectly reflect the reproductive activity. The SF animals, the only group that was not submitted to any kind of stress, presented a higher ovary weight. Monget and Martin (38) showed that changes in body weight, due to malnutrition in male rats, leads to a low testis weight. These results can be compared with our results, even considering difference in gender.

A tendency towards lower values of estradiol in food-restricted animals can be noticed. Undernourishment halts ovulatory cycles by inhibiting hypothalamic secretion of GnRH. This, in turn, slows the pulsatile release
of LH and damps the preovulatory LH surge (39–41). This may be due, in part, to a disruption in the pattern of the release of ovarian steroids, although this effect is not strictly dependent on the changes in ovarian hormone secretion (41–43).

Therefore, many possible explanations exist regarding estrous cyclicity and sexual behavior. In an elegant literature review, Wade and Jones (25) enumerated the metabolic cues related to nutritional amenorrhhea: a) peripheral detectors with neural projection to the brain; b) peripheral detectors with humoral signaling to the brain; c) central nervous system detectors with central transmission to effector circuits. We can enumerate many other studies investigating markers and/or regulators of reproduction, such as kisspeptin (44), ghrelin (45), and cholecystokinin (46), among others (47).

Finally, it is also important to enumerate some drawbacks to our methodological procedure. For example, the interpretation of the phase of estral cycle is subjective, which increases the risk of mistakes. A higher number of hormones could be analyzed, such as insulin and GnRH, among others.

From our findings, it was possible to conclude that there is an additive or synergistic effect of energy intake restriction and energy expenditure by intense physical training on estrous cyclicity. Leptin appears to be one among several factors related to the estrous cycle, but it probably acts indirectly.

REFERENCES


