Abstract

Introduction: Obesity is rapidly becoming a worldwide problem affecting the reproductive system. Some studies have shown the relationship between obesity and infertility, but until now it remains controversial. The present study was undertaken to investigate the effects of a specific high-fat diet on the male rat reproductive performances.

Materials and Methods: Male Wistar rats were fed either with a 15% palm oil diet (POD) or a standard diet (SD) for 16 weeks. At the end of the feeding period, copulatory activity, electromyograms (EMG) of the bulbospongious muscles and intraseminal pressure, body and organ weights, Lee index, sperm characteristics were evaluated.

Results: Feeding of male rats for 16 weeks with POD brought out an obesity status characterized by a significant increase in the growth rate percentage (P<0.05), Lee index (P<0.001) and total cholesterol level (P<0.05) compared to SD-fed animals. Absolute weights of testis and epididymis were significantly (P<0.001) higher in POD animals compared to SD group. A significant increase (P<0.05) in M/L, IL and PEI was also observed. The pro-ejaculatory effect of dopamine was significantly lowered (P<0.001) in POD rats when compared to SD animals. Sperm motility, viability and normality were significantly decreased (P<0.001) in POD group. Significant abnormal head (P<0.05), abnormal tail (P<0.001) and cytoplasmic droplet (P<0.001) were also observed in POD rats. The sperm counts and the sperm with tailless head remained statistically unchanged between POD and SD groups.

Conclusion: POD seriously impairs sexual behavior, ejaculatory activities and sperm parameters of male rats.

INTRODUCTION

Obesity is the most common metabolic disease emerging as a global health problem in both developed and developing countries. It may be defined as an excess weight with a body mass index (BMI) greater than 30 kg/m² [1]. Excessive weight gain arises from the interactions among environmental factors, genetic predisposition and individual behaviors [2]. Whereas most studies of the effects of obesity on reproduction have focused on the female partner, a growing body of evidence suggests that obesity also has independent adverse effects on the male reproductive function [3]. Obesity thus contributes to peripheral vascular disease, including the vascular bed of the penis, with adverse effects on endothelial function and reduced circulating androgen levels. These processes result in reduced penile tissue compliance and diminished penile hemodynamics, and hence physiological response, leading to erectile dysfunction [4]. Altered semen parameters ascribed to obesity include decreased sperm concentration, abnormal morphology, compromised chromatin integrity and abnormal motility [5]. The induction of obesity may be performed in animals by genetic, neuroendocrine or dietary changes. The study of these models has shown that it is the central nervous system that regulates energy expenditure, food intake, and it has also identified interrelationships among adrenal glucocorticoids, autonomic nervous system and dietary behavior in the development of obesity [6]. Genetically, the gene of the obese rat (ob/ob) present a mutation in chromosome 6 and develop a syndrome together with hyperphagia, diabetes and obesity, whose origin is due to the absence of leptin or to the presence of non-functional leptin [7]. The administration of monosodium glutamate to newborn rats causes the destruction of the ventromedial hypothalamic and arcuate nuclei, leading the rats to develop obesity due to the lack of control between absorption and energy expenditure [8]. A hypercaloric diet is the simplest obesity-induction model, and possibly the one that most closely resembles the reality of obesity in humans [9]. There are several types of diets to induce obesity that have been proven effective [10]. A few diets attain hypercaloric values by adding carbohydrates and others by fats, and all of them are highly palatable and induce obesity.

Because inadequate nutrition impairs the reproductive function in many mammalian species, this study was undertaken to investigate the effect of a high fat diet on sexual behavior, fictive ejaculation and sperm parameters of the male rat. We used a 15%
palm oil diet model which has been proven by our research group to seriously disrupt the estrus cyclicity in female rat [11].

**MATERIALS AND METHODS**

**Animals**

Sexually experienced Wistar rats (> 90 days, 200-300 g. bw) used in this study were maintained at room temperature under a natural light-dark cycle in the animal house of the Faculty of Science, University of Dschang, Cameroon. Food and water were available ad libitum. The experiments were performed in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in [12].

**Drugs**

Urethane, dopamine, estradiol, progesterone (Sigma Chemicals, St Louis, USA), were used in this study. Estradiol and progesterone were dissolved in ethanol and administered in soya oil while other chemicals were freshly prepared in saline solution. Doses were selected from our previous studies [13-17].

**Experimental design: Induction of obesity**

Standard diet (SD; 7-10 fats, 68-70 CH, 18-20 proteins, 1-2 vitamins and minerals) and high fat diet (POD; 30 fats, 50-52 CH, 18-20 proteins, 1-2 vitamins and minerals) were used in the present study. The palm oil is characterized by a high amount of saturated fat (56%) (Table 1). The dietary regimen was adapted from our previous study [11]. Rats were fed with POD or SD for 16 weeks. At the end of the feeding period, increase in body weight (more than 15% of initial body weight prior to hyperlipidic diet), Lee index (above 0.30) and hypercholesterolemia (above 100 mg/dl) [18] were considered in order to validate the obesity status of each animal. The Lee index was calculated using the following formula: \( \text{LI} = \left( \frac{\text{tube root of the body weight (g)}}{\text{nasoanal length (mm)}} \right) \times 10 \).

Sexual behavior and fictive ejaculation were evaluated in SD rats and POD-induced obesity rats. SD rats (n=8) and POD rats (n=8) were further sacrificed and, body and organ weights, Lee index, sperm parameters (motility, viability, normality and count) and sperm abnormalities (abnormal head, abnormal tail, cytoplasmic droplet and tailless head) were evaluated. A known volume of the blood (5 ml) was obtained from the abdominal artery to prepare the plasma for total cholesterol assay as described by Friedeward [19]. Sexual behavior was performed as previously described [13].

**Fictive Ejaculation Study:** Rats were urethane-anesthetized (1.5 g/kg, ip) and the bulbospongious genital muscles were identified and exposed. Two electrodes (EL 452, 12 mm, BIOPAC) were inserted into the ejaculatory muscles and a catheter connected to a pressure transducer was introduced into the seminal vesicles to record electromyograms (EMG) and intra-seminal pressure which characterized the expulsion and emission phases of ejaculation respectively. The bulbar portion of the penis and its anatomical connections with the striated bulbospongious muscles was exposed and, the spinal cord was transected around T6 spinal level. Treatments were administered by infusing the selected drugs into a jugular vein. Activation and recording of the rhythmic genital motor pattern of ejaculation was performed as previously described [14, 16, 17].

**Study on sperm characteristics:** Immediately after euthanasia, the cauda epididymis was dissected out, chopped and placed in 5 ml physiological saline (0.9% NaCl) and incubated for 5 min at 37°C in water bath to allow sperm to leave the epididymal tubules.

**Sperm Count:** Sperm count was evaluated using Mallassez hemocytometer as described previously [20]. At the end of this experiment, results were summarized and expressed as number of sperm per ml of solution.

**Sperm Motility:** Sperm-progressive motility was evaluated microscopically within 2-4 min of their isolation from the cauda epididymis as described by [21]. For this purpose, fluid was obtained from the cauda epididymis with a pipette and diluted to 2 ml with Tris buffer solution. The percentage of motility was evaluated at X400 magnification. Sperm forward motility was expressed as percentage of motile sperm to total sperm counted.

**Sperm Viability:** The ratio of live to dead sperm was determined using 1% trypan blue staining following the method described by [22]. A total number of 200 sperms were counted per slide and the results were expressed as percentage of the live sperm.

**Sperm Morphological Abnormalities:** Percentages of abnormal head, abnormal tail, cytoplasmic droplet and tailless head sperm were determined from a total of 300 sperms per rat [23]. Sperm morphology was viewed under a light microscope (OLYMPUS, X400). Data were expressed as percentage of morphologically abnormal sperm to total sperm count.

**Statistical Analysis:** All results were expressed as mean plus or minus standard error of mean (M ± SEM). Statistical analyses were performed using StatSoft, Inc. (2008) STATISTICA (data analyses software system), version 9.0.www.statsoft.com. Statistical significance was determined by one-way ANOVA followed with post-hoc Tukey HSD test for multiple comparisons. A probability level of less than 0.05 \( (p < 0.05) \) was considered as statistically significant.

**RESULTS**

**Effects of POD on body weight, Lee index, total cholesterol level and organ weights**

Rats fed with POD and SD showed a net body weight gain which was time-dependent. However, the body weight gain

<table>
<thead>
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<th>Table 1: Composition of the palm oil.</th>
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<tr>
<td><strong>Ingredients</strong></td>
</tr>
<tr>
<td>Lauric acid</td>
</tr>
<tr>
<td>Myristic acid</td>
</tr>
<tr>
<td>Palmitic acid</td>
</tr>
<tr>
<td>Stearic acid</td>
</tr>
<tr>
<td>Oléic acid</td>
</tr>
<tr>
<td>Linoleic acid</td>
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<td>α-linoleic acid</td>
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increased significantly at all-time points in rats receiving the POD compared to those fed with SD (Figure 1A). This gain in the body weight finally rose to 38.53% in POD group compared to the control group (10.18%) at 16th week of feeding. In some cases, the growth rate was less than 15%, compared to the initial weight of each animal. Of the 60 rats subjected to POD for 16 weeks, 40 were declared obese (60.67%) and used for further experiments. At the end of the sixteen week of POD exposure, there was a significant increase in the Lee index (P<0.001) (from 0.29 to 0.36) and total cholesterol concentration (P<0.01) (from 75.87 ± 4.02 to 98.62 ± 5.19 mg/dl) (Figure 1B and 1C). Except the absolute weights of the testis (P<0.001) and epididymis (P<0.001) where there was an increase, no statistical change in the weights of other reproductive organs after 16 weeks of diet exposure was recorded (Figure 1D and 1E).

Effects of POD on sexual behaviour

The copulatory parameters recorded during this study were the mount, intromission and ejaculation. It was generally observed that POD male rats exhibited a sluggish sexual behavior towards proven receptive female as evidenced by the significant delay recorded in the latencies of mount, intromission and post-ejaculatory interval (Table 2) in one hand, and the significant decrease of the percentage of mount, intromission and ejaculation (Figure 2) in the other hand. Moreover, some POD animals (2 of 8 rats, 25%) did not show any sexual activity (mounting and intromission) up to 20 minutes after introduction of the receptive female rats.

Activation of the ejaculatory motor response by urethral, penile and dopamine stimulations in SD and POD rats

The detrimental effects of POD on ejaculation are indicated in (Figure 3, Table 3). It is clearly shown that the hyperlipidic condition almost prevented the sensory-induced (urethral and penile stimulations) and pharmacology-induced (dopamine) fictive ejaculation in spinal cord transected and urethane-anesthetized rats. For instance, after application of dopamine (0.1 μM/kg) (the best standard pro-ejaculatory drug) the intraseminal pressure and the number of contractions of the bulbospongious muscles were significantly lower in POD group compared to SD animals (1.85 ± 0.86 vs 9.80 ± 0.86 contractions and 2.65 ± 1.01 vs 6.45 ± 0.63 mmHg). An increase in the latency of ejaculation due to dopamine (POD, 61.86 ± 3.34s vs SD, 14.60 ± 3.34s) was also noticed. Despite these changes, the frequency

\[ \text{Table 2: Effects of SD and POD on male rat sexual behavior after 16 weeks of diet.} \]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mount Latency (s)</th>
<th>Intromission Latency (s)</th>
<th>Mount frequency</th>
<th>Intromission frequency</th>
<th>Ejaculation Latency (s)</th>
<th>Post-ejaculatory interval (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD rats</td>
<td>11.57 ± 2.09</td>
<td>17.29 ± 3.28</td>
<td>18.29 ± 2.88</td>
<td>15.00 ± 1.99</td>
<td>462.14 ± 71.17</td>
<td>5.31 ± 0.35</td>
</tr>
<tr>
<td>POD rats</td>
<td>98.44 ± 19.68*</td>
<td>129.00 ± 22.05*</td>
<td>18.08 ± 4.21</td>
<td>12.96 ± 2.69</td>
<td>1231.50 ± 395.77</td>
<td>6.98 ± 0.57*</td>
</tr>
</tbody>
</table>

Number of rats per group = 8. All values are expressed as mean ± SEM. *p < 0.05: significantly different compared to SD group.

\[ \text{Figure 1 Percentage of growth (A), Lee index (B), total plasmatic cholesterol (C) and absolute (D) and relative (E) weights of the reproductive organs in rats submitted to SD and POD for 16 weeks.} \]

Number of rats per group = 8. All values are expressed as Mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001: significantly different compared with SD group.
of contractions remained statistically unaffected. Urethral and penile stimulations almost provoked similar effects. It is noteworthy mentioning that the contraction of the ejaculatory muscles was always accompanied with the expression of pressure in the seminal vesicles (Figure 3).

Effects of POD on sperm parameters and morphological abnormalities

In all animals submitted to POD for 16 consecutive weeks, the sperm parameters were negatively affected with the most harmful effects recorded for sperm motility (SD: 39.25 ± 8.00 % vs POD: 7.28 ± 1.58 %), sperm viability (SD: 24.48 ± 6.95 % vs POD: 2.35 ± 0.75 %) and sperm normality (SD: 81.79 ± 2.85 % vs POD: 19.96 ± 2.72 %) in this order. In (Figure 4A), it is clearly shown that all sperm parameters (motility, viability and normality) were significantly (P < 0.001) decreased in POD rats, exception of sperm count when compared with SD values.

The rats in the POD group also showed significantly abnormal head (POD: 7.85 ± 2.21 % vs SD: 1.14 ± 0.81 %), abnormal tail (POD: 47.78 ± 3.54 % vs SD: 4.35 ± 1.20 %), and cytoplasmic droplet (POD: 29.72 ± 2.84 % vs SD: 2.62 ± 1.18 %). There was no significant difference in the sperm with normal head without tail or tailless head in POD rats when compared with control group (Figure 4B).

DISCUSSION

This study was undertaken to investigate the effect of POD-induced obesity on sexual behavior, fictive ejaculation and sperm...
characteristics in male rats. It is believed that with the increasing prevalence of sedentary lifestyles and dietary changes, obesity is emerging, in turn, as an important cause of adverse health outcomes, including male infertility [5]. In an attempt to achieve deeper knowledge about obesity, several animal models have been developed, among which rodent models of diet-induced obesity that may provide the best parallels in relation to human obesity [24]. In this study, an obesity model induced by high fat diet consumption was chosen. This hyperlipidic diet was essentially characterized by enrichment in palm oil (15 %) (POD) compared to the standard diet (SD). Data from the literature indicate that diet-enriched fat is suitable for inducing obesity in a variety of mammals including nonhuman primates, dogs, pigs, hamsters, squirrels, mice and rats [25]. The POD used in this study was effective in promoting obesity, as demonstrated by the significant increase in the growth rate (P<0.05), Lee index (P<0.001) and total cholesterol concentration (P<0.05). The hyperlipidic diet used in this study essentially comprised of palm oil (15 %) which was commonly available in local market. This palm oil contained high percentage of saturated fats (56%). In fact, HFD rich in saturated fat facilitates accumulation of body fat and are considered more deleterious for human health than those rich in unsaturated fats [26]. After 16 weeks of POD exposure, 60.67% of rats were declared obese while 33.33% failed to respond. We found difficult to explain this result; but, it could be proposed that this difference in the response of the animals issued from the same husbandry and submitted to the same stimulating factor could be related to the intraspecific response among those animals [27]. This success in POD-induced obesity matches the view of many researchers who early demonstrated that high fat diet is capable of inducing obesity after 6 [28] or 7 weeks [29].

It is well established that obesity seriously impairs the male reproductive function including sexual behavior, ejaculatory activities and sperm characteristics [3]. In line with that, the POD was tested in the present study on the reproductive system of male rats after 16 weeks of continuous feeding. It was observed that the relative sexual organ weights remained unchanged. In Wistar rats, obese animals show no difference in the reproductive organ weights excepting the relative weight of empty seminal after 45 weeks of diet exposure, compared with control rats [30]. Similarly, diet-induced obesity in male mice exhibited no changes in the average weight of the testis or epididymis [31]. These data are in accordance with the results obtained in the present study.
From the data recorded, total cholesterol level increased significantly (P<0.05) in POD rats compared to normal rats. In addition, it was clearly shown in this study that POD severely impaired sexual behavior. Thus, ML, IL, PEI as well as the percentages of mount, intromission and ejaculation were significantly (P<0.05-0.01) lowered. Several findings show that hypercholesterolemia induced erectile dysfunction mostly by increasing oxidative stress and impairing endothelial function in the penis [32]. Hypercholesterolemia impairs endothelium-dependent and endothelium-independent relaxations of the corpus cavernosum, decreases the cavernosal content of endothelial cells, alters the function of smooth muscle cells, increases collagen content and could lead to erectile dysfunction [33].

Further, the POD-induced obesity was evaluated on fictive ejaculation characterized by the rhythmic contraction of the bulbospongious muscles and the expression of intra-seminal pressure [14-17]. After injection of dopamine, the number of contractions of the bulbospongious muscles was significantly decreased (P< 0.001) in POD group compared to SD animals. Several studies have shown that the availability of dopamine receptors decreases in obese individuals proportionally to the increase of their BMI. Dopamine modulates sexual motivation and rewards circuits and hence, dopamine deficiency in obese individuals may disturb ejaculatory process [34].

An evaluation of sperm characteristics is useful when investigating the underlying cause of male infertility [35]. Obesity has been reported to affect fertility by decreasing the quantity and quality of spermatozoa [5]. In the present study, sperm motility, viability and normality were significantly decreased (P<0.001) in POD group compared to SD group. A recent study demonstrated that pasteurized oil palm sap (Elaeis guineensis) altered sperm characteristics by significantly (P<0.05) decreasing sperm count [36]. Moreover, no significant difference was observed in the sperm counts in POD rats when compared with their SD group. Some works showed a reduction in sperm concentration [5] and sperm quality and motility [30] while other found no alterations in sperm concentration related to obesity [37]. Another important sperm parameter for evaluating male fertility is sperm concentration related to obesity [37]. Some works showed a reduction in sperm concentration [5], sperm quality and motility [30] while other found no alterations in sperm concentration related to obesity [37].

CONCLUSION

Present results showed that HFD (15% POD) treatment induced obesity in rats after 16 weeks. POD-induced obesity impairs sexual behavior and ejaculatory process by decreasing the number of mount and intromission as well as the contraction of the bulbospongious muscles. The sperm motility, viability and normality were also significantly reduced (P<0.001) in POD rats. Overall findings clearly indicate that the 15% POD is strongly detrimental for the male reproductive system after 16 weeks. Since obesity is a growing health problem worldwide, additional studies are needed to elucidate the mechanism of altered reproductive parameters associated with obesity condition.

ACKNOWLEDGEMENTS

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