Reproductive BioMedicine Online (2015) 31, 655–667

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ARTICLE

Obesity induced by cafeteria diet disrupts fertility in the rat by affecting multiple ovarian targets

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Abstract Obesity constitutes a health problem of increasing worldwide prevalence. Among the health detriments caused by obesity, reproduction is disrupted. However, the mechanisms involved in this disruption are not fully understood. Animals fed a cafeteria diet constitute the model for the study of obesity that most closely reflects Western diet habits. The aims of this study were to evaluate whether a cafeteria diet affects ovarian function and to contribute to the understanding of the mechanisms involved. For that purpose, 22-day-old female Wistar rats were fed *ad libitum* with a standard diet (control group; $n = 20$) or cafeteria diet (CAF group; $n = 20$). The cafeteria diet induced obesity and hyperglycaemia, without altering serum triglycerides, cholesterol or C-reactive protein concentrations. This diet also altered ovarian function: the rats showed prolonged dioestrous phases, decreased serum oestradiol concentrations and increased number of antral atretic follicles. Moreover, follicular cysts were detected in the CAF group, concomitantly with a decrease in the number of anti-Müllerian hormone immunoreactive pre-antral follicles and COX-2-positive antral and pre-ovulatory follicles. The authors conclude that a cafeteria diet reduces ovarian reserve, induces the presence of follicular cysts and disturbs the ovulatory process, leading to the delayed pregnancy observed in these animals.

KEYWORDS: anti-Müllerian hormone, fertility, follicular cyst, folliculogenesis

http://dx.doi.org/10.1016/j.rbmo.2015.08.004

Introduction

Obesity presents a major public health concern since it constitutes a health problem of increasing worldwide prevalence. Obesity is a disorder of energy imbalance that develops when energy intake exceeds energy expenditure (Spiegelman and Flier, 2001). Reproduction is one of the health detriments caused by obesity (Hall and Neubert, 2005). Although most obese women are not infertile, obesity impacts negatively upon fecundity and fertility (Brewer and Balen, 2010). Obese women are three times more likely to suffer infertility than women with a normal body mass index (BMI) (Rich-Edwards et al., 1994) and to experience impaired fecundity both in natural and assisted conception cycles (Zaadstra et al., 1993). In humans, obesity induces anovulatory cycles and irregular menses (Douchi et al., 2002), reduces implantation and pregnancy rates (Hall and Neubert, 2005) and can be associated with polycystic ovarian syndrome (Rittmaster et al., 1993). However, the mechanisms by which excess body fat interferes with reproductive function are still not fully understood. Significant evidence suggests that excess body fat negatively affects female reproductive functions not only in humans but also in many models of obesity (Tortoriello et al., 2004).

Several animal models for studying obesity have been described, especially rodents fed on the 'cafeteria diet', which most closely reflects Western diet habits. In this model obesity is induced by feeding rats an assortment of highly palatable supermarket foods in addition to standard laboratory chow (Sclafani and Springer, 1976). Since it is considered a more palatable diet, the cafeteria diet has been associated with increased adiposity and insulin resistance (Akyol et al., 2012). Cafeteria feeding provides a useful alternative to feeding on conventional purified high-fat diets to induce obesity. It avoids the use of very high intakes of a particular type or source of fat while inducing persistent hyperphagia and increased energy intake (Rothwell and Stock, 1979; Shafat et al., 2009) thereby resembling more closely human dietary patterns. It has been shown that the cafeteria diet can impact on metabolic function, with changes in glucose homeostasis (Akyol et al., 2012; Higa et al., 2014; Sampey et al., 2011), but little is specifically known about ovarian glucose metabolism, as few researchers have studied its effects on the ovaries in animal models. It has been described that a high-fat diet can induce obesity and accelerate the development of ovarian follicles and the rate of follicle loss, leading to premature ovarian failure (Wang et al., 2014). Sagae *et al*. found that the cafeteria diet negatively affects female reproduction by reducing the number of oocytes and the thickness of the follicular layer. These authors also found that obese female rats showed no preovulatory progesterone or LH surges but that sexual receptiveness was not altered (Sagae et al., 2011). The aims of the present study were to evaluate whether obesity induced by the cafeteria diet affects ovarian function in female rats and to contribute to an understanding of the mechanisms involved.

Materials and methods

Animals and study protocol

Twenty-two-day-old female Wistar rats (*Rattus norvegicus*) weighing 120–130 g were obtained from the animal facilities of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. All research animals were treated in compliance with the guidelines for the care and use of animals approved by the Comité Institucional de Cuidado y Uso de Animales de Experimentación (CICUAL, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires) in accordance with the principles of laboratory animal care (NIH Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, Washington, D.C., USA).

Rats were divided randomly into two groups: (i) control group ($n = 20$) fed a standard rodent chow diet (ACA Nutrición Animal, Argentina) containing 11% fat, 23% protein and 66% carbohydrate; energy value 3.3 kcal/g; and (ii) cafeteria diet (CAF) group ($n = 20$), fed a varying menu of highly palatable human food comprising sausages, cheese, snacks, peanuts, biscuits and chocolate biscuits, adapted from previous studies (Akyol et al., 2009). In this group, the animals also had access to standard rodent chow. The highly palatable foods provided were altered daily to maintain variety, and the rats ate relatively large amounts of it.

Both the standard chow and cafeteria diet foods were individually weighed in and out of the cage between 10:00 h and 11:00 h daily. Daily intakes of energy, macronutrients and micronutrients in the CAF group were calculated from the manufacturers' data. All rats had ad-libitum access to all diet components as well as to water and were kept on a 12:12 h light:dark cycle at 22 °C. Food consumption was monitored daily for 60 days and weight gain was monitored twice a week.

Oestrous cycle staging

Beginning on day 50, vaginal smears were collected by lavage with 0.9% saline between 09:00 h and 11:00 h from each animal. The fluid was spotted thinly on a microscope slide, and the dried slides were stained with 0.1% Trypan Blue in deionized water. The oestrous cycle stage was determined by microscopic examination (Westwood, 2008). Vaginal cytology was examined until every rat passed through the oestrous phase after day 60. At that moment, when rats were 82–86 days old (to assure that animals had reached reproductive maturity), they were killed. Results are expressed as the time that animals spent in each phase of the oestrous cycle.

Anaesthesia and tissue collection

Twenty animals (10 from the control group and 10 from the CAF group) were killed in the first oestrous phase after day 60. After weighing animals, anaesthesia was performed with a 50 mg/kg solution of ketamine (Brouwer, Argentina) associated with 10 mg/kg xylazine (Alfasan, Holland) injected intramuscularly into the inner side of one of the hind legs.

Blood was obtained by cardiac puncture after anaesthesia and drawn into tubes with no anticoagulant. Blood glucose concentrations were determined immediately and blood was then centrifuged at 2000*g* to obtain serum for C-reactive protein (CRP), cholesterol, triglyceride determinations and hormone assays. All serum samples were frozen at −70 °C and