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Induction of uterine hyperplasia after cafeteria diet exposure

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ABSTRACT

Our aim was to evaluate whether chronic administration of CAF affects the uterus and induces the morphological and molecular changes associated with endometrial hyperplasia. Female Wistar rats exposed to CAF from weaning for 20 weeks displayed increased energy intake, body weight and fat depots, but did not develop metabolic syndrome. The adult uteri showed an increase in glandular volume fraction and stromal area. The epithelial proliferation rate and protein expression of oestrogen receptor alpha (ERα) were also increased. The CAF diet enhanced leptin serum levels and the long form of leptin receptor (Ob-Rb) mRNA expression in the uterus. No changes were detected in either insulin serum levels or those of insulin growth factor I (IGF-I) mRNA expression. However the levels of IGF-I receptor (IGF-IR) mRNA were lower in CAF-fed animals. Overall, the results indicate that our rat model of the CAF diet produces morphological and molecular changes associated with uterine hyperplasia and could predispose to endometrial carcinogenesis.

1. Introduction

Many risk factors of endometrial cancer have been described such as, early age at menarche, nulliparity, late-onset menopause, exposure to exogenous oestrogens (without a progesterone component), diabetes and obesity (Parazzini et al., 1991; Amant et al., 2005). Currently, many groups have demonstrated that obesity increases the risk of endometrial cancer (Thompson, 2010) but the role of each diet component is less clear. It has been speculated that Asian diets, typically characterized by a lower intake of fat and higher intake of fish, soy products, and cruciferous vegetables, compared with Western diets, may contribute to lower endometrial cancer risk (Messina et al., 2006).

Endometrial cancer is often preceded by the occurrence of precursor lesions and is accompanied by incessant oestrogen stimulation usually referred to as oestrogen dominance (Lacey and Chia, 2009; Kirschner et al., 1982). In normal conditions, the estrogenic effects initiate a succession of biochemical reactions in uterine cells in anticipation of the possible pregnancy, involving cell hypertrophy and hyperplasia (Nephew et al., 2000).

Oestrogens act mainly through oestrogen receptor alpha (ERα) to promote cell proliferation, differentiation and growth. ERα is expressed in all uterine cells, including glandular and luminal epithelium and mesenchyma (stromal and myometrial cells) (Wang et al., 2000). The oestrogen receptor beta (ERβ) plays a less dominant role in the mature uterus and only modifies the effects of ERα (Koehler et al., 2005). Together with oestradiol (E2), insulin-like growth factors I (IGF-I) and II (IGF-II) and their signalling pathways also play significant roles in the regulation of uterine growth and differentiation during the oestrous cycle. Estrogen boosts uterine IGF-I gene expression and provokes endometrial proliferation. The results of the actions of IGF-I and IGF-II are facilitated mainly by activation of the IGF-I receptor (IGF-IR) (McCampbell et al., 2008).

In addition, other circulating hormones regulate uterine functional differentiation. Fat tissue has been proved to be an endocrine organ that synthesizes and secretes polypeptide hormones and adipokines having the capacity to produce effects on the function of many tissues, among them the uterus. Leptin exerts direct effects on proliferation and invasion, as well as the production of angiogenic proteins in tumourous endometrial cells through the long form of leptin receptor (Ob-Rb) activation (Tartaglia et al., 1995; Carino et al., 2008; Gao et al., 2009).

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Abbreviations

CAF diet	cafeteria diet
E2	estradiol
ERα	estrogen receptor alpha
ERβ	estrogen receptor beta
ERK	extracellular signal-regulated kinase
GE	glandular epithelium
IGF-I	insulin growth factor I
IGF-II	insulin growth factor II
IGF-IR	insulin growth factor I receptor
IL-6	interleukin 6
IOD	integrated optical density
IR	insulin receptor
JAK	januskinases 2
LE	luminal epithelium
Ob-Rb	long form of leptin receptor
PS	periglandular stroma
RIA	radioimmunoassay
SS	subepithelial stroma
STAT3	ignal transducers and activators of transcription 3
Vim	vimentin

Besides, hyperleptinaemia is a frequent characteristic of obese women, who are more likely to develop endometrial cancer than women with normal weight, suggesting that the adipose tissue plays a direct role through the hormone leptin (Petridou et al., 2002).

It is well known that obesity could predispose to endometrial cancer, but the effect of chronic administration of a westernized diet was not determined. In our experiment, western diet habits are reflected by the cafeteria diet (CAF), an experimental rodent diet model composed by a variety of highly savoury and energy-dense foods with a prevalence in Western society (a life style linked to the current obesity pandemic) (Sampey et al., 2011). Recently, in our laboratory, Lazzarino et al. found that the CAF diet differentially modifies the expression of feeding-related genes by affecting the DNA methylation mechanisms in individual hypothalamic nuclei (Lazzarino et al., 2017). In another work, the CAF diet was linked with increased weight gain, abdominal fat, and serum interleukin 6 (IL-6) levels, in addition to more damage in the kidney (chronic interstitial inflammation and glomerular sclerosis), heart (coronary perivascular fibrosis and steatosis), and liver (liver weight, portal fibrosis, apoptosis, and steatosis) in comparison with a high fat diet (Zeeni et al., 2015).

In the present work, we speculate that chronic administration of the CAF diet might induce uterine changes associated with endometrial hyperplasia. According to this, we assessed possible changes caused by CAF diet on the uterine morphology, the expression of oestrogen-sensitive genes and epithelial cell proliferation. In addition, we determined the expression of IGF-I, IGF-IR and Ob-Rb as molecular regulators of endometrial proliferation.

2. Materials and methods

2.1. Animals

All procedures in this study were approved by the Institutional Ethic Committee of the School of Biochemistry and Biological Sciences (Universidad Nacional del Litoral, Santa Fe, Argentina) and were performed in accordance with the principles and procedures outlined in the Guide of the Care and Use of Laboratory Animals issued by the U.S. National Academy of Sciences (Commission on Life Sciences, National Research Council, Institute of Laboratory Animal Resources, 1996).

2.2. Experimental design

Female Wistar rats were obtained at the Department of Human Physiology of the School of Biochemistry and Biological Sciences (UNL) where they were bred, weaned at 21 days of age, and randomly divided into two groups: control diet group (CON) (n = 6) and cafeteria diet group (CAF) (n = 6). The animals of the CON diet group were fed with a standard chow diet and the animals of the CAF diet group were fed with the diet described below. The diets were administered from weaning and for 20 weeks and water was administered *ad libitum*. Rats were housed two per cage and maintained in controlled conditions (22 ± 2 °C and 12-h light-dark cycle). The standard chow (Cooperación, ACA Nutrición Animal, Buenos Aires, Argentina) provided 3 kcal/g, 5% energy as fat, 23% protein and 72% carbohydrate. The CAF diet was composed of food items selected to reproduce the diversity, palatability, and energy density of the modern Western diet. The CAF diet incorporated standard chow, aside from french fries, parmesan cheese, cheese-flavored snacks, crackers, sweet biscuits, cookies, pudding, peanut butter, and chocolate. This diet supplied an average of 4.85 kcal/g, 49% of energy as fat, 7% as protein, and 44% as carbohydrate, in addition to that provide by standard chow. Three of the CAF foods were offered in excess quantities and were changed every day, by supplanting all the food with new items for more than two consecutive days. During the experimental time, body weights were registered once a week and food intake every day. Food intake was measured by the weight difference between the accessible and the remaining food, adjusted to the waste by collecting food spillage. Energy intake was calculated using the energy contents of each food (kcal/g) and the average intake.

The oestrous cycle was monitored by vaginal smears during two weeks to determine if the CAF diet alters the duration of particular phases of the oestrous cycle. Vaginal smears were obtained daily from lavage fluid collected by flushing the female's vagina with phosphate-buffered saline and were examined under a light microscope. The stage of the oestrous cycle was determined based upon vaginal cytology as described by Montes & Luque (Montes and Luque, 1988).

All animals were weighed and sacrificed on the dioestrus stage of the oestrous cycle after 20 weeks of treatment. Trunk blood was collected, samples were centrifuged, and serum was immediately used or frozen and stored at −80 °C until further use. Perigonadal and retroperitoneal fat pads were isolated and weighed. The uteri were sampled, and one uterine horn from each rat was placed immediately in liquid nitrogen and stored at −80 °C for RNA extraction. The other uterine horn was fixed by immersion in 4% paraformaldehyde buffer for 6 h at 4 °C and processed for histological studies (morphometric and immunohistochemical analysis).

2.3. Serum assessments

Fasting serum metabolites (glucose, triglycerides, and cholesterol) were assessed by a commercially available assay (Wiener Laboratorios, Argentina). Serum insulin levels were estimated by radioimmunoassay (RIA) using an anti-rat insulin antibody (Sigma, St. Louis, Missouri, USA) and standard rat insulin provided by Laboratorios Beta (Buenos Aires, Argentina). The circulating levels of leptin were determined by specific RIA (Giovambattista et al., 2006). Total E2 levels were measured using competitive RIA kits (Immunotech, Marseille, France) (Matthews et al., 1985).

2.4. Immunohistochemistry

A standard immunohistochemical technique was performed, following protocols previously described by our laboratory (Muñoz-de-Toro et al., 1998). Briefly, uterine longitudinal sections (5 μm thick)

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