



## Glucose homeostasis in rats treated with 4-vinylcyclohexene diepoxide is not worsened by dexamethasone treatment



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### ABSTRACT

4-vinylcyclohexene diepoxide (4-VCD) causes premature ovarian failure and may result in estrogen deficiency, characterizing the transition to estropause in rodents (equivalent to menopause in women). Estropause/menopause is associated with metabolic derangements such as glucose intolerance and insulin resistance. Glucocorticoids (GCs) are known to exert diabetogenic effects. Thus, we aimed to investigate whether rats with premature ovarian failure are more prone to the diabetogenic effects of GC. For this, immature female rats received daily injections of 4-VCD [160 mg/kg body weight (*b.w.*), intraperitoneally (*i.p.*)] for 15 consecutive days, whereas control rats received vehicle. After 168 days of the completion of 4-VCD administration, rats were divided into 4 groups: CTL—received daily injections of saline (1 mL/kg, *b.w.*, *i.p.*) for 5 days; DEX—received daily injections of dexamethasone (1 mg/kg, *b.w.*, *i.p.*) for 5 days; VCD—treated as CTL group; VCD + DEX—treated as DEX group. Experiments and euthanasia occurred one day after the last dexamethasone injection.

4-VCD-treated rats exhibited ovary hypotrophy and reduced number of preantral follicles ( $p < 0.05$ ). Premature ovarian failure had no impact on the body weight gain or food intake, but both were reduced by the effects of dexamethasone. The increase in blood glucose, plasma insulin and triacylglycerol levels as well as the reduction in insulin sensitivity caused by dexamethasone treatment was not exacerbated in the VCD + DEX group of rats. Premature ovarian failure did change neither the hepatic content of glycogen and triacylglycerol nor the glycerol release from perigonadal adipose tissue. Glucose intolerance was observed in the VCD group after an ipGTT ( $p < 0.05$ ), but not after an oral glucose challenge. Glucose intolerance and compensatory pancreatic  $\beta$ -cell mass caused by GC were not modified by ovarian failure in the VCD + DEX group. We conclude that reduced ovarian function has no major implications on the diabetogenic effects promoted by GC treatment, indicating that other factors related to aging may make rats more vulnerable to GC side effects on glucose metabolism.

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**Abbreviations:** *b.w.*, body weight; CTL, rats treated with saline; DEX, rats treated with dexamethasone; ELISA, enzyme-linked immunosorbent assay; FSH, follicle-stimulating hormone; HOMA, homeostatic model assessment; *i.p.*, intraperitoneal; ipGTT, intraperitoneal glucose tolerance test; LDL, low density lipoprotein; GC, glucocorticoid; OVX, ovariectomy; RIA, radioimmunoassay; *s.c.*, subcutaneous; TyG, triacylglycerol and glucose index; 4-VCD, 4-vinylcyclohexene diepoxide; VCD, rats treated with 4-VCD; VCD + DEX, rats treated with 4-VCD and dexamethasone; VLDL, very-low density lipoprotein.

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## 1. Introduction

During natural aging many physiological functions may be impaired, *e.g.*, changes of neurologic, metabolic, and cardiovascular magnitudes. Almost all species have a lifespan that does not surpass their reproductive years [1]. This is not the case in women, who experience a reproductive senescence around the fifth decade of life as a consequence of a depletion of their finite pool of ovarian follicles, a process known as atresia [1]. This midlife transition state that culminates with reproductive senescence in women is termed perimenopause and can last 5–8 years on average until

menopause occurs (cessation of menstrual period) [2]. About one third of every woman's life is spent in the post-reproductive stage, since the average life expectancy at birth for both sexes worldwide is 71 years [3]. The impacts of perimenopause and menopause on health and diseases are unequivocally an important matter for investigation, which continuously supports the actions of the health-related professionals dealing with elderly patients.

Different animal models have been considered in scientific researches to mimic some aspects of women reproductive senescence, without the heterogeneous variations found in humans (e.g., genetic characteristics, diet, age, parity, socioeconomic status, and environmental exposures) [1]. Different from human females, rodents present an estrous cycle rather than a menstrual cycle and the estrous cycle lasts 4–5 days. Also, rodents do not exhibit menstruation, but have the uterine lining reabsorbed [4]. Rats and mice commonly experience irregular estrous cycle around 9–12 months of age, a condition termed estropause [1]. Three main laboratory models define the preclinical models for evaluation of reproductive aging: i) natural aging animals with intact ovaries (ovary-intact), ii) ovariectomized animals (OVX) and iii) a more recent model induced by the administration of 4-vinylcyclohexene diepoxide (4-VCD). Each model has its advantages and disadvantages (for a comprehensive review, read [1]).

4-VCD is a metabolite of 4-vinylcyclohexene, a compound formed from the dimerization of 1,3-butadiene in the manufacture of synthetic rubber, insecticides, plasticizers, flame retardants, and antioxidants [5]. It is also applied against pests, e.g., for reduction of rodent fecundity and proliferation [6]. 4-VCD selectively targets the small preantral ovarian follicles (mainly primordial and primary follicles), leading to an accelerated follicular depletion and different degrees of ovarian failure, depending on the dose and period of investigation after the completion of 4-VCD administration [7–10]. For instance, the complete interruption of cyclicity and the morphologic evidence for reproductive senescence (total absence of oocytes) occurs only around 350–360 days after 4-VCD treatment in rats [8]. Thus, 4-VCD-treated rodents become an interesting preclinical model for studying the transitional estropause. 4-VCD-treated rodents have the advantage of maintaining the ovarian follicles and circulating steroid hormones more similar to those found in women under menopause transition; aspects not mimicked by the ovary-intact or OVX rodent models [1].

The vast majority of studies conducted with 4-VCD model sought to elucidate its mechanisms of action and the comprehension of the profile of ovary-derived hormones (e.g., concentrations and time-course fluctuations) [7–15]. There are few studies exposing these animals to a physiological challenge [16–18]. For instance, 4-VCD-treated mice under menopause become more responsive to the angiotensin II hypertensive effects, which do not happen during perimenopause [18]. Similarly, mice become glucose intolerant 26 weeks after 4-VCD administration [17]. In both studies, 17 $\beta$ -estradiol replacement normalized the impairments. 17 $\beta$ -estradiol has important implications on the regulation of metabolism and there is a positive relationship between perimenopause/menopause and the development of metabolic-related derangements (for a comprehensive review, read Mauvais-Jarvis et al. [19]). These derangements include reduction of the energy expenditure, increased adiposity and altered adipocytokine secretory profile,  $\beta$ -cell dysfunction, reduced glucose disposal, which altogether may contribute to the development or exacerbation of obesity and/or type 2 diabetes mellitus [19]. Previous studies with 4-VCD-treated mice have not shown a significant alteration in the glucose metabolism (e.g., glycemia, insulinaemia and triacylglyceridemia) in normal animals [16,17]. However, the biological system may mask certain vulnerabilities while it is not

submitted to certain stressing or challenging conditions, e.g., exposure to diabetogenic hormones.

Glucocorticoids (GCs) are among the most effective diabetogenic hormones and there is substantial evidence with humans and rodents treated with synthetic GC (e.g., dexamethasone) in different contexts (acutely with high doses or chronically with low doses) demonstrating its impact on glucose and lipid metabolism such glucose intolerance, reduction of insulin sensitivity, elevation in blood glucose, plasma insulin and triacylglycerol levels, etc. (reviewed in [20–22]). We have a well-established model of rats treated with dexamethasone that exhibit prediabetes like phenotype (e.g., glucose intolerance, insulin resistance, elevation in blood glucose, plasma insulin, glucagon, triacylglycerol levels) [23–25], which serves as a good model to stress the glucose homeostasis.

Thus, based on the evidence that 4-VCD administration in immature rats leads to ovarian failure, which may turn these animals vulnerable to metabolic insults [17], we sought to explore whether 4-VCD-treated rats are more susceptible or not to the well-known adverse effects of dexamethasone on glucose homeostasis during a period considered as estropause (168–173 days after completion of 4-VCD treatment). By treating Wistar rats with 4-VCD early in life and exposing them to a short-term high-dexamethasone treatment as adults, we were able to show that reduced ovarian function has no major implications on the diabetogenic effects promoted by GC treatment, indicating that other factors related to aging may turn rats more vulnerable to GC side effects on glucose metabolism.

## 2. Materials and methods

### 2.1. Ethical approval

The experiments with rats were approved by the Federal University of Santa Catarina Committee for Ethics in Animal Experimentation (approval ID: PP00782 and PP00842) that is in accordance with the National Council for Animal Experimentation Control (CONCEA).

### 2.2. Materials

4-Vinylcyclohexene diepoxide was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dexamethasone phosphate (Decadron<sup>®</sup>) was purchased from Aché (Campinas, SP, Brazil). Regular human recombinant insulin (Humulin<sup>®</sup>) was acquired from Lilly (Indianapolis, IN, USA). The reagents used in the glucose tolerance test, hepatic glycogen and fat content protocols, fat lipolysis protocol, and histology and immunochemistry were acquired from LabSynth (Diadema, SP, Brazil) and Sigma. Plasma insulin was quantified by AlphaLISA<sup>®</sup> technology (PerkinElmer, Waltham, MA, USA—cat. no. AL204). Plasma 17 $\beta$ -estradiol was determined by ELISA (DRG International, Inc., Springfield, NJ, USA—cat. no. EIA-2693). Plasma progesterone was determined using specific MP kits (MP Biomedicals; Orangeburg-NY, USA, cat. no. 07DE9988) [15]. Enzymatic colorimetric assay for the quantification of triacylglycerol, total cholesterol and lactate were from Biotécnica (Varginha, MG, Brazil—cat. no. BT1001000). Primary antibodies for insulin (sc-9168) and the ABC staining system (ImmunoCruz<sup>™</sup>) (sc-2018) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

### 2.3. Animals

Experiments were performed on 3 groups of 40 female Wistar rats accounting for a total of 120 rats. The post-weaned rats were obtained from the Federal University of Santa Catarina Animal Breeding Center and were kept at 21  $\pm$  2 °C on a 12-h light–dark cycle (lights on at 0600, lights off at 1800). Rats had access to food

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