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Review

Sexual dimorphism in the glucose homeostasis phenotype of the Aromatase Knockout (ArKO) mice

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ABSTRACT

We investigated the effects of estrogens on glucose homeostasis using the Aromatase Knockout (ArKO) mouse, which is unable to convert androgens into estrogens. The ArKO mouse is a model of total estrogen ablation which develops symptoms of metabolic syndrome.

To determine the development and progression of whole body state of insulin resistance of ArKO mice, comprehensive whole body tolerance tests were performed on WT, ArKO and estrogen administrated mice at 3 and 12 months of age. The absence of estrogens in the male ArKO mice leads to hepatic insulin resistance, glucose and pyruvate intolerance from 3 to 12 months with consistent improvement upon estrogen treatment. Estrogen absence in the female ArKO mice leads to glucose intolerance without pyruvate intolerance or insulin resistance. The replacement of estrogens in the female WT and ArKO mice exhibited both insulin sensitizing and resistance effects depending on age and dosage.

In conclusion, this study presents information on the sexually dimorphic roles of estrogens on glucose homeostasis regulation.

1. Introduction

Sex hormones have been implicated in both insulin resistance and sensitivity. The involvement of estrogens in insulin resistance is evident clinically and experimentally although the relationship is not linear. States of high estrogen such as in male-to-female *trans*-sexuality, pregnancy and menstrual cycling are known to contribute to insulin resistance [1,2]. In contrast, in cases of estrogen insufficiency such as menopause, ovariectomy and aromatase deficiency, insulin resistance is also observed which can in many cases be improved upon estrogen treatment [3,4]. This is also observed for androgens – hyperandrogenism in women resulting in insulin resistance whereas insulin resistance in aging men with low androgen levels can be rectified by testosterone treatment. These observations suggest a crucial role of hormone imbalance in insulin resistance.

Estrogens modulate their effects through two estrogen receptor isoforms, α and β (ERα and ERβ). ERα is the predominant isoform expressed in insulin responsive tissues such as adipose tissue, muscle and liver [5,6]. Incidentally, estrogen receptor expression has been linked with metabolic syndrome in humans, with adipose tissue from obese patients expressing reduced levels of ERα [7]. Furthermore, ERα-deficient (but not ERβ deficient) mice display impaired glucose homeostasis and insulin resistance [8,9].

Insulin resistance is defined as decreased glucose uptake by insulin sensitive peripheral tissues. In an effort to maintain glucose homeostasis; the pancreas releases insulin which stimulates glucose uptake into peripheral tissues and inhibits endogenous glucose production from the liver. Insulin interacts with its

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receptor, whereby a signalling cascade is initiated. A proposed link between insulin resistance and obesity is the process of ectopic lipid accumulation in peripheral tissues. When the adipose tissue exceeds its storage capacity, excessive serum free fatty acids (FFA) begin to increase storage in other peripheral tissues [10].

A key element in maintaining glucose homeostasis is the insulin regulated production of glucose by the liver called gluconeogenesis [11]. Ectopic accumulation of lipids in the liver (hepatic steatosis) is also associated with hepatic insulin resistance [12]. Sexual dimorphic differences in the prevalence of hepatic steatosis occur with males displaying significantly higher (51.2%) rates than age matched females (12.2%) [13] thereby suggesting that perhaps these enzymes are regulated by estrogens.

In agreement with the human data, we demonstrated that the male but not female ArKO mice suffer from hepatic steatosis which is rectified upon estrogen treatment [14]. Furthermore, hyperinsulinemia but not hyperglycemia has been identified in our 12 month-old male ArKO mice [15]. We also reported that in 6 month-old mice, omental adipose tissue in both sexes increased in the ArKO compared to WT counterparts and was reduced upon E2 treatment [16,17]. Serum leptin and adipose leptin transcript levels were increased in both sexes of 6 month-old ArKO mice which were reduced upon E2 treatment. Conversely, adiponectin was decreased in the male ArKO mice and restored with E2 treatment. However, female ArKO mice while showing no changes in adiponectin as compared to WT, their adiponectin levels significant decreases after E2 treatment, which was also associated with significant increases in TNFα and IL6 serum and adipose transcript levels.

The ArKO mouse has no aromatase and hence no endogenous estrogen synthesis. This allows us to investigate the effects of sex hormones on the progression and development of insulin resistance. It also provides a model to test specific exogenous estrogen dosage without the interference of uncontrolled endogenous production from extra-gonadal tissues. In this paper, we compare the sexual- and age-related differences (3 verses 12 month-old) between in glucose homeostasis in the wild type and aromatase deficient mice.

2. Materials and methods

All efforts were made to minimize animal suffering and procedures were approved by the Monash Medical Centre Animal Ethics Committee (Permit Number: MMCB2008/08)

2.1. Mice

Aromatase Knockout (ArKO) mice (C57Black6 X J129) were generated by disruption of the *Cyp19A1* gene. Homozygous null or wild-type (WT) offspring were bred by crossing heterozygous ArKO mice, and genotyped by PCR. Mice were housed in groups under pathogenic free conditions, fed a soy-free mouse chow (Glen Forest Stock feeders, Perth, Australia) and water *ad libitum* as previously described [14]. Male and female, WT and ArKO mice at 3 and 12 months of age were used in these studies (n = 7–17 as stated in figure legends).

2.2. Treatments

2.2.1. Estrogen and sham treatments

ArKO mice were implanted with a 17β-estradiol pellet (E2; 0.15 mg in 60 days i.e. 2.5 μg/day; Innovative Research of America, Toledo, OH, USA) for 3 or 6-weeks in 3 or 12 month old animals respectively. We reported previously that no differences in body mass, organ weights or glucose tolerance were detected between untreated ArKO and littermates implanted with a placebo pellet (saline 60 day slow release; Innovative Research of America, Toledo, OH, USA) [16], hence untreated ArKO mice were used in this study. After treatment, mice were killed using a lethal dose of anesthetic (100 mg/ml Ketamine and 20 mg/ml Xylazine in PBS). Blood was collected by cardiac puncture and serum was separated, and stored at −20 °C. Adipose, liver and muscle tissues were removed, weighed and snap frozen in liquid nitrogen and stored at −80 °C.

2.3. Insulin assay

Insulin was measured by ELISA (#EZRMI–13 K, Linco Research, St. Charles, MO, USA) following manufacturer's protocol. Briefly, a 96-well microtitre plate pre-coated with monoclonal mouse anti-rat insulin antibodies was washed thrice with kit wash buffer before incubation with 10 μl of control, standards or sample serum, plus a biotinylated anti-insulin antibody at room temperature. Unbound material was rinsed off and horseradish peroxidase was added to wells. Free enzyme conjugates were rinsed off and a light sensitive 3,3′,5,5′ tetramethylbenzidine substrate added. Enzyme activity was measured spectrophotometrically at 450 nm on the Envision Plate reader v 1.09 (Perkin Elmer, Waltham, MA, USA).

2.4. Tolerance tests

All mouse cohorts were subjected to glucose tolerance test (GTT – 1 g glucose/kg of body weight i.p, after 8 h of fasting; Sigma, St Louis USA), insulin tolerance test (ITT – i.p. 0.5U insulin/kg of body weight for male mice and 0.25U or i.p. 0.5U insulin/kg of body weight female mice, after 8 h of fasting; Actrapid; Novo Nordisk, Bagsvaerd, Denmark) and pyruvate tolerance test (PTT – i.p 1 g pyruvate/kg of body weight, after ∼16–20 h of fasting; Sigma, St Louis USA). At least five days of recovery were allowed between each test. Tail bleeding at specific time point was used to obtain blood samples which were analyzed for glucose content (AccuChek Performer, Roche, Mannheim, Germany) immediately before, and at 20, 40, 60, 90 and 120 min after an intraperitoneal injection. Tolerance test results are presented as a time course of absolute blood glucose measurements and area under curve (AUC calculations completed on 120mins for GTT and ITT and 40mins for ITT), and not adjusted for lean mass.

2.5. Statistical analysis

All statistical analyses were performed using GraphPad Prism® version 6 for Windows (GraphPad Software Inc). Data were analyzed using one way ANOVA with a tukey post-hoc test or student *t*-tests. Data are expressed as mean ± standard deviation (SD). Differences between groups are considered statistically significant if *p*-value is lower than or equal to 0.05.

3. Results

3.1. Body and adipose tissue weights;

3.1.1. Effects of estrogens

The effects of 17β-estradiol (E2, the most potent form of estrogens) on body and omental adipose tissue weights were assessed in the WT, ArKO and ArKO mice treated with E2 at 3 and 12 months of age.

3.1.1.1. Three month-old animals. Female 3 month-old ArKO mice were significantly heavier and had increased omental adipose tissue compared to WT ($p < 0.01$ and $p < 0.01$ respectively). E2 treatment further increased body weight of the 3 month-old