RBM ONLINE

ELSEVIER

Article

Hyperhomocysteinemia in polycystic ovary syndrome: decreased betaine-homocysteine methyltransferase and cystathionine β-synthase-mediated homocysteine metabolism

Da Li [a], ***Hong-Xiang Liu*** [b], ***Yuan-Yuan Fang*** [a], ***Jia-Ning Huo*** [b], ***Qi-Jun Wu*** [c], ***Tian-Ren Wang*** [d], ***Yi-Ming Zhou*** [e], ***Xiu-Xia Wang*** [a,*], ***Xiao-Xin Ma*** [b,*]

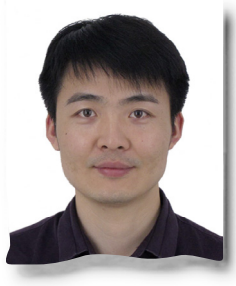
[a] *Centre of Reproductive Medicine, ShengJing Hospital of China Medical University, Shenyang 110004, China*

[b] *Department of Obstetrics and Gynecology, ShengJing Hospital of China Medical University, Shenyang 110004, China*

[c] *Department of Clinical Epidemiology, ShengJing Hospital of China Medical University, Shenyang 110004, China*

[d] *Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale School of Medicine, New Haven, CT 06520, USA*

[e] *Department of Medicine, Brigham and Women's Hospital, Harvard Institutes of Medicine, Harvard Medical School, Boston, MA 02115, USA*



Da Li is an Associate Professor at the Center for Reproductive Medicine, ShengJing Hospital of China Medical University. His main research is on PCOS and infertility. He has published over 40 peer-reviewed articles, served on the editorial board of five SCI journals, and as a reviewer for 23 peer-reviewed journals.

KEY MESSAGE

Serum homocysteine concentrations are significantly increased in obese PCOS patients. Decreased *Bhmt* and *Cbs*-mediated homocysteine metabolism in the liver may be responsible for hyperhomocysteinemia in PCOS.

A B S T R A C T

Research question: What are the metabolic characteristics of homocysteine in polycystic ovary syndrome (PCOS)?

Design: Homocysteine concentrations were determined in serum samples from non-obese and obese control subjects and PCOS patients. Homocysteine metabolism was studied in a rat model of PCOS established using dehydroepiandrosterone (DHEA) or DHEA in combination with a high-fat diet (HFD).

Results: It was shown that (i) serum homocysteine concentrations were greater in PCOS patients than in control subjects in the obese group ($P < 0.05$) and serum homocysteine concentrations were significantly higher in the obese group than in the non-obese group, regardless of PCOS status (both

* Corresponding authors.

E-mail addresses: wangxxsj@sina.cn (X-X Wang); maxiaoxin666@aliyun.com (X-X Ma).

https://doi.org/10.1016/j.rbmo.2018.05.008

Please cite this article in press as: Da Li, Hong-Xiang Liu, Yuan-Yuan Fang, Jia-Ning Huo, Qi-Jun Wu, Tian-Ren Wang, Yi-Ming Zhou, Xiu-Xia Wang, Xiao-Xin Ma, Hyperhomocysteinemia in polycystic ovary syndrome: decreased betaine-homocysteine methyltransferase and cystathionine β-synthase-mediated homocysteine metabolism, Reproductive BioMedicine Online (2018), doi: 10.1016/j.rbmo.2018.05.008

$P < 0.05$); (ii) serum homocysteine concentrations were significantly increased in DHEA + HFD-induced rats compared with controls ($P < 0.05$); (iii) when compared with the control group, mRNA concentrations of homocysteine metabolic enzymes *Bhmt* and *Cbs* were significantly reduced in the liver tissues of DHEA + HFD-induced rats (both $P < 0.0001$); (iv) when compared with the control group, there was a significant decrease in the methylation concentrations of the *Cbs* ($P < 0.05$) and *Bhmt* ($P < 0.05$ and $P < 0.0001$) promoter in the DHEA + HFD group. The methylation patterns, together with previous data, indicate that hypomethylated promoter-mediated transcriptional activation of *Bhmt* and *Cbs* might be a defence mechanism against PCOS-related hyperhomocysteinemia.

Conclusions: These findings indicate that decreased liver *Bhmt* and *Cbs*-mediated homocysteine metabolism might have a role in hyperhomocysteinemia in PCOS and provides further evidence for a potential role of decreased liver function in PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting approximately 10% of the female population of reproductive age (Goodarzi et al., 2011), with significant and diverse reproductive, metabolic and psychological features (Li et al., 2015; Teede et al., 2010). In addition to a genetic predisposition, environmental and lifestyle factors contribute to the pathogenesis of PCOS (Insenser et al., 2013).

Homocysteine is a toxic sulphur-containing amino acid formed during the methionine cycle (Lehotsky et al., 2016). Recently, emerging evidence has suggested an important link between elevated homocysteine concentrations and PCOS (Qi et al., 2017). Elevated concentrations of homocysteine are a recognized risk factor for reproductive dysfunction (Aitken et al., 2016), metabolic disorders (Schalinske and Smazal, 2012), reduced prenatal brain growth and neurodevelopmental delays (Ars et al., 2016). Furthermore, genetic, dietary and other lifestyle factors are independent factors affecting homocysteine metabolism (Saw et al., 2001), and elevated homocysteine concentrations are associated with almost all of the symptoms of PCOS. For example, hyperhomocysteinemia is a risk factor for obesity (Tabassum et al., 2012), and homocysteine concentrations are positively correlated with plasma triglyceride content (Fulghesu et al., 2010). Women with PCOS display significantly elevated homocysteine concentrations (Toulis et al., 2011), and this elevation is associated with their serum insulin concentration (Yilmaz et al., 2008). Notably, homocysteine concentration is worsened by increasing insulin resistance, dyslipidaemia and poor glucose control (Ala et al., 2017). However, to date, little is known about the regulatory mechanism for homocysteine accumulation in PCOS.

Homocysteine may be remethylated to methionine via the betaine-dependent pathway utilizing betaine-homocysteine methyltransferase (BHMT), or the folate-dependent pathway utilizing methionine synthase (MTR), or converted to cystathionine by cystathionine β-synthase (CBS) in the liver (Li et al., 2013; Zhou et al., 2011). To further elucidate the relationship between homocysteine metabolism and PCOS, this study examined homocysteine concentrations in serum samples from non-obese and obese control subjects and PCOS patients; and investigated *Mtr*, *Bhmt* and *Cbs*-mediated homocysteine metabolism in a dehydroepiandrosterone (DHEA) and DHEA + high-fat diet (HFD)-induced rat model of PCOS. DHEA can induce PCOS phenotypes through the hypothalamus–pituitary–ovarian axis, whereas the addition of a HFD exaggerates endocrine and metabolic dysfunction of the PCOS model (Zhang et al., 2016).

Materials and methods

Ethical statement

The study was conducted in accordance with ethical standards and the Helsinki Declaration of 1975.

Participants

This study was approved by the Institutional Review Board at the China Medical University on 28 February 2015 (reference number 2015PS108K). All participants provided written informed consent. A total of 201 control subjects and 348 PCOS patients were recruited. A female body mass index (BMI) ≥ 23 kg/m^2 was used as the diagnostic criterion for overweight and obesity in Asians (World Health Organization, International Obesity Task Force, 2000). Control non-obese (age 29.62 ± 3.47, n = 103), PCOS non-obese (age 28.87 ± 2.81, n = 154), control obese (age 29.74 ± 3.53, n = 98) and PCOS obese (age 28.57 ± 2.90, n = 194) subgroups were created according to BMI ≥ 23 or <23. PCOS was defined according to the Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). The exclusion criteria were: < 3 years since menarche; tobacco smoking, hormonal medication, pregnancy or lactation; medications (insulin-sensitizing drugs, oral contraceptives, antiandrogens, statins, aspirin, nicotinic acid, corticosteroids, and GnRH agonists and antagonists) taken within the preceding 6 months; endocrine abnormality such as diabetes mellitus, hyperprolactinemia, congenital adrenal hyperplasia, androgen-secreting tumour, Cushing's syndrome; a history of any known neoplasm, infectious, or inflammatory diseases. All control women had regular menstrual cycles (24–38 days), and none of the control women fulfilled any of the Rotterdam criteria for PCOS. BMI, serum triglyceride, homocysteine and testosterone concentrations were assessed. BMI was calculated by dividing weight by height squared (kg/m^2).

Animal model and sample collection

The PCOS rat model was established using DHEA injection and DHEA + HFD (Li et al., 2018; Zhang et al., 2016). All experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and all experimental procedures involving animals were approved by the Animal Care and Use Committee of the China Medical University on 28 February 2015 (reference number 2015PS108K). Female Sprague-Dawley rats (3 weeks old) were purchased from

Please cite this article in press as: Da Li, Hong-Xiang Liu, Yuan-Yuan Fang, Jia-Ning Huo, Qi-Jun Wu, Tian-Ren Wang, Yi-Ming Zhou, Xiu-Xia Wang, Xiao-Xin Ma, Hyperhomocysteinemia in polycystic ovary syndrome: decreased betaine-homocysteine methyltransferase and cystathionine β-synthase-mediated homocysteine metabolism, Reproductive BioMedicine Online (2018), doi: 10.1016/j.rbmo.2018.05.008