Endocrine (2012) 42:694–699
DOI 10.1007/s12020-012-9638-7

ORIGINAL ARTICLE

Nesfatin-1 and other hormone alterations in polycystic ovary syndrome

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Received: 6 January 2012 / Accepted: 15 February 2012 / Published online: 25 February 2012

Abstract Polycystic ovary syndrome (PCOS) is commonly characterised by obesity, insulin resistance (IR), hyperandrogenemia and hirsutism. Nesfatin-1 a recently discovered hormone, acts upon energy balance, glucose metabolism, obesity and probably gonadal functions. This study was to evaluate the circulating levels of nesfatin-1 in patients with PCOS ($n = 30$) and in age and body mass index (BMI)-matched controls ($n = 30$). PCOS patients had significantly lower levels of nesfatin-1 (0.88 ± 0.36 ng/mL) than healthy controls (2.22 ± 1.14 ng/mL). PCOS patients also had higher gonadotropin and androgen plasma concentrations, Ferriman–Gallwey scores, blood glucose levels and a homeostasis model of assessment-IR index (HOMA-IR) index than in healthy women. Correlation tests in PCOS subjects detected a negative correlation between nesfatin-1 levels and BMI, fasting blood glucose, insulin levels and a HOMA-IR index. Lower nesfatin-1 concentration may plays a very important role in the development of PCOS.

Keywords PCOS · Nesfatin-1 · Ferriman–Gallwey scores

Abbreviations

17-Ahp	17 Alpha hydroxyprogesterone
AS	Androstenedione
BMI	Body mass index
CSF	Cerebrospinal fluid
CV	Coefficient of variance
DHEA-S	Dehydroepiandrosterone sulphate
DM	Diabetes mellitus
E_2	Estradiol
EDTA	Ethylenediaminetetraacetic acid
FABP4	Fatty acid binding protein
FBG	Fasting blood glucose
FG	Ferriman–Gallwey
FSH	Follicle-stimulating hormone
FSI	Fasting serum insulin
HDL	High density lipoprotein
HOMA-IR	Homeostasis model of assessment-insulin resistance index
IGF-1	Insulin-like growth factor
IR	Insulin resistance
KIU	Kallikrein inactivation unit
LDL	Low density lipoprotein
LH	Luteinizing hormone
LMD	Last menstrual date
PCOS	Polycystic ovary syndrome
PRL	Prolactin
SHBG	Sex-hormone binding globulin
TSH	Thyroid-stimulating hormone
TT	Total testosterone

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VLDL Very low density lipoprotein
WHR Waist-hip circumference ratio

Introduction

Polycystic ovary syndrome (PCOS), a metabolic disease characterised by chronic anovulation and hyperandrogenism, affects ~7% of women of reproductive age [1–4]. It is a multi-factorial disease resulting from the synergistic effect of the dysfunction of several systems in those sufferers with PCOS phenotypes having a different hormonal, metabolic pattern [5–7] and adiponectin gene polymorphisms [8]. Patients with PCOS with polycystic ovaries-anovulation and hyperandrogenemia phenotype tend to have a low susceptibility for cardiovascular disease [9]. Increased expression of adipocyte fatty acid binding protein (FABP4) was also associated with the clinical characteristics of PCOS [10]. Insulin resistance (IR) and hyperinsulinemia are common in PCOS patients, both in obese and non-obese women. Reports on the prevalence of IR vary depending on the tests used and the heterogeneity of PCOS [7, 11]. Hyperinsulinemia and hyperandrogenemia typically decreases with weight loss [1, 4, 7].

The findings of hypothalamic satiety peptides acting upon energy balance, obesity, glucose metabolism and gonadal functions have led to the idea that they are potentially regulatory in normal and pathological ovarian conditions. Nesfatin-1, a peptide recently described by Oh et al. [12], is produced in the hypothalamus and other brain regions, as well as by the pancreas and stomach. It has potent anorexigenic actions when injected into the brains of rodents. A role in the activation of puberty, a permissive action on gonadotropin control and lower levels of nesfatin-1 being related to delayed puberty in rats has been suggested by Garcia-Galiano et al. [13]. Nesfatin-1 also shows an anti-hyperglycaemic action through peripheral effects [14]. The concentration of nesfatin-1 declines in Type 2 diabetes mellitus (DM) patients with IR [15]. A lower concentration of nesfatin-1 has been reported in human milk and is also lower in patients with gestational DM [16]. Nesfatin-1 levels were also found lower in patients with gestational DM compared with control pregnant women [17].

It appears that the nesfatin-1 hormone has not been studied in PCOS patients, who commonly suffer from co-present obesity and IR. The present study compared the difference of nesfatin-1 level between PCOS patient and controls, and examined the simple correlation using Pearson method.

Materials and methods

This study was conducted in the Obstetrics and Gynaecology Department of Firat University Medical School Hospital, with the approval of the ethics committee dated (no. 2008–2009/32). A total of 60 voluntary participants (30 healthy controls and 30 patients with PCOS) who met the study criteria between June 2009 and March 2010 were recruited and registered, with written consent being obtained. The control group was composed of healthy female volunteers who were tested negative for pregnancy at the Gynecology Obstetrics and Reproductive Medicine Clinics. Diagnosis of PCOS was based on the 2003 ESHRE/ASRM diagnostic criteria, according to which patients who had at least two of the following conditions were accepted as having PCOS: oligo or anovulation, clinical and/or biochemical hyperandrogenism signs [testosterone greater than 60 ng/dL (greater than 2.08 nmol/L), dehydroepiandrosterone sulphate (DHEA-S) 3 mg/L or greater (7.8 mmol/L or greater), or both] [18] or PCOS morphology, together with the exclusion of other causes. PCOS manifestation was defined as the presence of ≥12 unilateral follicles 2–9 mm in size on the ovary or having the least unilateral ovary volume of 10 cm^3 by ultrasonography (the measurement was performed when there was no follicle >10 mm) [19, 20]. Ovarian volume was calculated by the formula [0.5 × ovarian length × thickness × width]. In the case of transabdominal ultrasonography, the presence of at least 10 unilateral antral follicles was required. Cases in the age range 18–35 were included in the study.

The first evaluation consisted of gathering demographic information, which included the complaint at presentation, age (years), age of menarche, last menstrual date (LMD), gravid (number), parity (number), abortus (number), number of living children, menstrual cycle regularity (number of days between cycles/number of days of menstrual bleeding/total amount of bleeding in a cycle in pads), age of the first and last pregnancy, previous use of oral contraceptives and its duration, history of infertility, smoking and use of alcohol. Following a general physical and gynaecological examination, Ferriman–Gallwey (FG) scores (points), height (cm), weight (kg), waist circumference (cm), hip circumference (cm), blood pressure and waist-hip circumference ratio (WHR, as a percentage) were measured. Body mass index (BMI) was calculated [BMI: body weight (kg)/square height (m^2)]. FG scoring was used to assess hair growth in 11 areas of the body: upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, upper arms, forearms, thighs and legs, a score being established for each area. Absence of terminal hair growth was scored as 0 and maximal growth as 4+. A total score of 8 or higher was defined as hirsutism. WHR was calculated using the formula [WHR = waist circumference

(cm)/hip circumference (cm)]. This was accepted as the minimum diameter between the arcus costarum and the processus spina iliaca anterior superior. Hip circumference was accepted as the maximum diameter around the most prominent part of gluteus maximus posteriorly and pubic symphysis anteriorly. WHR was measured using an inelastic measuring tape when the subjects had an empty stomach, in a standing position dressed in indoor clothes and without shoes after a normal expirium.

Menstrual cycle days were determined by its length based on anamnesis. Transvaginal and/or transabdominal ultrasonography of all cases were performed at the same time to determine uterus size (mm), myometrial structure, endometrial thickness (mm), size of ovaries (mm), number of follicles (number) and their diameters as measured across the inside (mm). Blood sampling was done between 3 and 5 days of menstruation. Cases with ultrasonographic images consistent with ovarian cyst, endometrioma, myoma or polyp, congenital cavity disorders (e.g. septum uteri or acquired disorders such as Asherman's syndrome), malignity suspicion, Turner's syndrome, obstructive sleep apnea, epilepsy, chronic renal failure, hypertension, functional dyspepsia, DM or history of gestational DM, history of gastric or intestinal surgery, hepatic or haematologic disease, any endocrine disorder like Cushing's syndrome, 21-hydroxylase deficiency, congenital adrenal hyperplasia, thyroid dysfunction, hyperprolactinemia, those who had received medical treatment for any reason in the last 3 months and those who smoked or used alcohol were excluded from the study. All the diseases mentioned above have been evaluated and excluded by standard testing methods by specialised staff members. After being enrolled, the subject's medical examination and required tests were completed.

Collection and storage of blood samples

Venous blood samples of 5 mL were drawn simultaneously from the cases after one night fasting on 3–5 days of the follicular phase between 09:00 and 10:00 a.m. The blood samples were placed in ethylenediaminetetraacetic acid (EDTA) tubes containing 500 Kallikrein inactivation unit (KIU) aprotinin to prevent digestion of peptides by proteases [21]. These samples were stored at −20°C until the time of analysis.

Hormonal and biochemical measurements

Levels of estradiol (E_2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, prolactin (PRL), thyroid-stimulating hormone (TSH), free T4, total testosterone (TT), androstenedione (AS), DHEA-S, 17 alpha hydroxyprogesterone (17-Ahp), fasting serum insulin (FSI) and fasting blood glucose (FBG), sex-hormone binding globulin (SHBG), cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and very LDL (VLDL) were determined in the fasting venous blood samples. Nesfatin-1 was measured in blood samples using a Human Nesfatin-1 ELISA kit (Phoenix Pharmaceuticals Inc. Burlingame, CA; USA), with a measurement interval of 0.78–50 ng/mL. The intra-assay and the inter-assay coefficient of variance (CV) values were not supplied by the kit manufacturing company. Our laboratory showed that intra-assay and inter-assay CV for this kit ranged from <7.3 and from <9.8% respectively.

FSH, LH, E_2, AS, DHEA-S, TT, 17-Ahp, SHBG and insulin levels were measured in an Immulite 2006 (IEMA; Diagnostic Products Corporation, Loas Angeles, USA) autoanalyzer, and lipids in an Olympus AU2700 (Optical Co., Ltd., Tokyo-Japan) clinical chemistry analyzer, using the kits recommended by the manufacturers.

Homeostasis model of assessment-IR index (HOMA-IR) was calculated for each patient using the formula [fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5]. Nesfatin-1 levels in the venous blood samples of all participants were compared and correlated with other biochemical parameters.

Statistical analysis

SPSS 12.0 package software was used for statistical analyses. Continuous variables were expressed as mean ± standard deviation. *T* tests were used for comparison between patients and control. The correlation between the parameters was analysed by the Pearson method. Differences were considered significant at $p < 0.05$.

Results

Demographical characteristics and biochemical values of PCOS patients and healthy controls are shown in Table 1. The patients with PCOS and controls were similar in terms of mean age and BMI. WHR, HOMA-IR index and FG score, however, were significantly higher in patients with PCOS. LH, PRL, TT, SHBG, progesterone, AS and DHEA-S levels in patients with PCOS were significantly elevated compared with the controls ($p < 0.05$), but FSH and E_2 levels were not significantly different (Table 2). FBG and total cholesterol levels were statistically higher in patients with PCOS. Plasma nesfatin-1 levels ($p = 0.01$) in women with PCOS were significantly lower than those in the controls (Table 2). Multiple correlation tests gave negative correlation between nesfatin-1 levels and BMI, FBG, insulin levels and HOMA-IR index in PCOS patients (Table 3) whilst no correlation was found in the control group.

Table 1 Demographical characteristics and biochemical values of controls and PCOS patients

Parameters	Controls ($n = 30$)	Patients with PCOS ($n = 30$)	p Value
Age (year)	23.16 ± 3.66	23.56 ± 4.80	0.718
BMI (kg/cm^2)	24.43 ± 0.50	25.03 ± 0.86	0.127
Waist/hip ratio	75.76 ± 2.38	82.76 ± 4.86	0.001*
FG score	5.16 ± 1.14	9.30 ± 2.10	0.001*
FBG (mg/dL)	74.90 ± 10.72	94.26 ± 14.53	0.001*
HDL (mg/dL)	51.13 ± 9.08	50.25 ± 12.06	0.750
LDL (mg/dL)	116.66 ± 15.66	119.90 ± 27.53	0.579
VLDL (mg/dL)	28.5 ± 4.76	23.8 ± 14.33	0.097
Total cholesterol (mg/dL)	144.63 ± 18.73	169.80 ± 34.10	0.001*
TG (mg/dL)	125.96 ± 49.00	116.96 ± 72.59	0.311

BMI body mass index, *FBG* fasting blood glucose, *FG* Ferriman–Gallwey, *HDL* high density lipoprotein, *LDL* low density lipoprotein, *PCOS* polycystic ovary syndrome, *TG* triglyceride, *VLDL* very low density lipoprotein

Mean ± standard deviation. Statistical significance * $p < 0.05$

Table 2 Hormonal values of controls and with PCOS patients

Parameters	Controls ($n = 30$)	Patients with PCOS ($n = 30$)	p Value
Nesfatin-1	2.22 ± 1.14	0.88 ± 0 36	0.001*
AS (ng/mL)	2.55 ± 1.35	5.27 ± 2.74	0.001*
DHEAS (μg/dL)	84.93 ± 32.01	199.63 ± 102.13	0.001*
E_2 (pg/mL)	48.23 ± 9.43	59.47 ± 30.98	0.066
FSH (mIU/mL)	5.43 ± 2.73	5.26 ± 2.94	0.822
FSI (μU/mL)	8.0 ± 2.33	20.77 ± 9.28	0.001*
HOMA-IR	1.29 ± 0.39	4.40 ± 2.53	0.001*
LH (mIU/mL)	5.16 ± 2.05	7.81 ± 4.01	0.002*
Progesteron (ng/mL)	0.63 ± 0.34	1.80 ± 0.52	0.018*
PRL (ng/mL)	9.16 ± 3,75	14.90 ± 10.57	0.008*
SHBG (nmol/L)	44.83 ± 15.50	13.39 ± 5.57	0.001*
TT (ng/dL)	22.23 ± 6.63	40.50 ± 15.43	0.001*

AS androstenedione, *DHEAS* dehydroepiandrosterone sulphate, E_2 estradiol, *FSH* follicle-stimulating hormone, *FSI* fasting serum insulin, *HOMA-IR* homeostasis model of assessment-insulin resistance, *LH* luteinizing hormone, *PCOS* polycystic ovary syndrome, *PRL* prolactin, *SHBG* sex-hormone binding globulin, *TT* total testosterone

Mean ± standard deviation. Statistical significance * $p < 0.05$

Discussion

Although PCOS is a very common syndrome in women of reproductive ages, there is no definitive data on its development. A significant proportion of women with PCOS suffer from IR, and this appears to play a role in the etiology of PCOS [4, 7, 11, 22–26]. In this study, nesfatin-1 levels in women with PCOS were significantly lower than in the healthy controls. We do not know whether the decrease in nesfatin-1 levels seen in PCOS is mediated through IR or is the result of other metabolic factors. Qing-Chun Li et al. [15] found that nesfatin-1 levels in Type 2 DM patients were lower than those in Type 1 DM patients and healthy individuals. Nesfatin-1 levels were also found lower in patients with gestational DM compared with control pregnant women [17]. Significantly higher FSI levels and HOMA-IR index in this study confirm the presence of IR in patients with PCOS. Previous studies have demonstrated [25] that under normal conditions, the signal transmitted into the cell when insulin binds to the alpha subunit of the receptor initiates protein phosphorylation. However, in IR, serine rather than tyrosine is phosphorylated, which results in an interruption of signal transmission in the cell, inhibition of the post-receptor effect and the failure of GLUT-4 to transport glucose [24, 27].

Table 3 Spearman correlation coefficients (r) between nesfatin-1 levels and measured parameters in PCOS subjects

Parameters	r Value	p Value
Body mass index	−0.474	0.01
FBG	−0.272	0.05
FSI	−0.385	0.01
HOMA-IR	−0.352	0.01

FBG fasting blood glucose, *FSI* fasting serum insulin, *HOMA-IR* homeostasis model of assessment-insulin resistance

This significantly higher hyperinsulinemia found in PCOS cases may be another reason of reduced nesfatin-1 levels. A rat study [28] showed that pro-nesfatin-1 and insulin-secreting β cells were in the same location, and that pro-nesfatin-1 might play a potential role in insulin secretion and glucose metabolism. Nesfatin-1 enhances glucose-induced insulin secretion by promoting Ca^{++} influx through L-type channels in mouse islet beta-cells [29]. Despite peripheral IR, insulin causes hyperandrogenemia by increasing androgen synthesis through its action on ovarian theca cells via an insulin-like growth factor (IGF-1). This is one of the critical mechanisms contributing to the development of PCOS [30, 31]. The fact that insulin produces ovarian effects in spite of the peripheral IR in PCOS suggests that insulin may be acting through other receptors or via secondary precursors in different organs. Considering that both ligand and receptor components of the nesfatin-1 signal system may be present in the ovarian tissue, nesfatin-1 may have a regulatory role (or roles) in both normal and pathologic conditions of the ovary. Low nesfatin-1 levels in women with PCOS may be involved in the development of the syndrome through their effect on

the highly sensitive hypothalamic–pituitary–gonadal axis. These hypotheses need further investigations to support them.

Statistically higher glucose levels found in PCOS may also have inhibited nesfatin-1. Yijing Su et al. [14] showed in rats that iv injections of nesfatin-1 significantly reduced hyperglycaemic blood glucose levels, producing an anti-hyperglycaemic effect that arose through peripheral action, which was time-, dose- and insulin-dependent. It should be noted that it is only presumed that the anti-hyperglycaemic effect of nesfatin-1 arises through insulin signal pathways, the mechanism being uncertain [14]. Nesfatin-1 levels proved to be lower in type 2 diabetes compared to healthy controls [15].

Another reason for nesfatin-1 being lower in PCOS may be the increase in the BMI in these cases, although the increase is not statistically significant. Previous studies have revealed that intravenous, subcutaneous, intraperitoneal, intracerebrovascular and intranasal use of nesfatin-1 inhibits short and long-term food intake in a dose- and time-dependent manner, which results in a decrease in body weight [12, 32]. On the basis of the findings, peripheral nesfatin-1 administration might provide a novel alternative in the treatment of obesity. Obesity is co-present in $\sim$50% of the patients, and in accord with this $\sim$50% of the patients also reported suffering from IR [11, 27]. PCOS patients suffer particularly from android-type obesity; this distribution of fat tissue is accompanied by hyperinsulinemia, glucose intolerance, DM and an increased rate of androgen production. Obesity and IR are also strongly correlated. IR in this context is moderate, and weight loss is used as a treatment method for reducing IR [33, 34]. There was a significant linear relation between cerebrospinal fluid (CSF) and plasma nesfatin-1/NUCB-2 in lean (BMI < 25 kg/m^2 and obese (BMI ≥ 30 kg/m^2) subjects [35]. Correlation tests in PCOS subjects detected a negative correlation between nesfatin-1 levels and BMI even though some reports found no correlation [17] and some reports showed positive correlation with BMI [36–38] in humans. This negative correlation might be due to a reduction in nesfatin-1 levels in PCOS patients.

Conclusion

Results indicate that nesfatin-1 might be inhibited in conditions of hyperglycaemia, hyperinsulinemia and IR. Lowered nesfatin-1 in Type 2 DM patients known to suffer from IR, i.e. relative to Type 1 DM patients and healthy individuals, and the fact that nesfatin-1 administration to the obese (a majority of whom have IR) results in reduced food intake and body weight [12, 32]. The anti-hyperglycaemic effects of nesfatin-1 [14] suggest that nesfatin-1 is closely associated with glucose and insulin metabolism, and notably IR. It can be therefore postulated that the existence of IR may play a role in determining level of nesfatin-1 in PCOS patients.

Acknowledgments The authors like to extend their thanks to FUBAP for their financial support (project number 1889). And also their heartfelt thanks and never ending admiration to Harry Miller for his editing this manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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