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Fluorine may intensify the mechanisms of polycystic ovary syndrome (PCOS) development via increased insulin resistance and disturbed thyroid-stimulating hormone (TSH) synthesis even at reference levels

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ABSTRACT

We were interested whether fluorine, at the concentrations regarded as normal, can play a role in PCOS pathogenesis. The effect was not described in PCOS.

Women with PCOS were diagnosed according to Rotterdam's criteria. The average age of 40 examined women with PCOS was 26.3 ± 5.5 years, BMI-29.16 ± 0.8, WHR-0.91 ± 0.08.

Main Outcome Measures: ECLIA was used to analyse testosterone, FSH, LH, oestradiol, TSH, prolactin, insulin and SHBG. Fluorine content was analysed by potentiometry using ion selective electrode.

Fluorine content in serum of women with PCOS did not statistically significantly differ from that of the control group and amounted to 0.224 ± 0.043 and 0.228 ± 0.023 ppm, respectively. There were significant differences in the levels of TSH and HOMA-IR between the groups.

Based on the correlation matrix, a negative correlation with the level of SHBG protein and the level of glucose on fasting was showed for the group with a lower of fluorine, and a positive correlation with HDL level was observed in the group with higher concentration of fluorine. In the phenotype with a higher level of androgens, there was a negative correlation with triglycerides level and a positive correlation with HDL.

Fluorine, even in concentrations regarded as proper, takes part in PCOS pathogenesis. It increases the synthesis of TSH and increases insulin resistance. Higher insulin resistance leads to the reduced synthesis of SHBG transport protein. Therefore, the key factor in PCOS pathogenesis is testosterone, but fluorine facilitates disruptions in carbohydrates and lipids metabolism leading to increased levels of androgens in blood.

Hypotheses

Fluorine, even in concentrations regarded as normal, affects metabolic pathways and is responsible for insulin resistance in the group of women with PCOS. Insulin resistance and the increase of TSH caused by increased concentration of fluorine in blood is one of the causes of PCOS.

Background

The concentration of fluorides in plasma does not undergo homeostatic regulation and the level of fluorides in human plasma is more or less equal to that in potable water, which is the main source of fluorine. Seawater contains 1.2–1.5 ppm of fluoride. Freshwater concentrations are usually Lower, ranging from 0.01 to 0.3 ppm [1]. Because the bioavailability of fluoride is generally reduced in humans when consumed with milk or a calcium-rich diet, it is highly recommended that the inhabitants of fluoride-contaminated areas should incorporate calcium-rich foods in their routine diet [2]. Concentration of fluorides can also be dependent on a diet (teas are especially rich in fluorine) and on individual differences in the speed of fluorine removal by kidneys. It seems obvious that fluorine included in toothpastes and fluorinated pesticides, such as fluoride insecticides (cryolite, sodium fluorosilicate, sulfuryl fluoride), may affect its concentration in our organisms [3,4].

Hydrogen fluoride (HF) is the substance which permeates the gastric mucosa. In the stomach, 40–50% of consumed fluorine compounds

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become absorbed. Fluorides in HF form increase the permeability of cell membranes ten-fold. HF in high concentrations strongly irritates gastric mucosa. The absorption of fluorides on fasting is almost complete (100%), whereas the presence of food decreases the effectiveness of absorption to 50–80%. Fluorine has a lipophilic character, therefore the presence of fat in food increases the amount of absorbed fluorides. Perhaps it is also correlated with slower emptying of the stomach, which results in longer contact between the food and gastric mucosa. Fluorides can stimulate the secretion of hydrochloric acid in stomach, decreasing the inflow of blood to gastric mucosa, thus leading to the necrosis of the epithelium [5].

Excessive concentration of fluorides has a negative effect on bone tissue and cartilage [6]. Due to the increased accumulation of glycogen in chondrocytes, which stiffens the cytoplasm, it causes the deformation of chondrocytes and even their necrosis [7]. There are also some remarks on unfavourable effects of fluorine compounds on the nervous system and neurotoxins' production, as well as on inducing neurons' apoptosis. This can lead to learning disabilities, memory disorders, lower intelligence quotient (IQ), and also to a higher risk of neurodegenerative diseases, such as dementia, Alzheimer's disease, Parkinson's disease and multiple sclerosis. It is the consequence of higher oxidative stress caused by fluorides. They stimulate lipids oxidation and the production of free radicals, simultaneously inhibiting the activity of antioxidative enzymes [8]. Fluorine ions interact with aluminium forming a highly neurotoxic complex leading to neurons' degradation. Neuroglia take part in this degradation [9]. The cytotoxicity of several fluorinated alternatives is also postulated by Sheng et al [10]. The probable toxic dose (PTD) was defined at 5 mg/kg of body mass. PTD in a 20 kg child would be achieved at ingesting 100 g (75 ml) of toothpaste which contains 1000–1500 ppm of fluorides [11].

Fluorine can also affect lower fertility in men and women causing menstrual disorders and other female reproductive system disorders [5,12]. Fluorides increase the activity of calcitonin, reduce glucose tolerance and cause secondary hyperparathyroidism. There are some reports that fluorine can stimulate the synthesis of androgens, or their fluoride derivatives, and increase their anabolic activity. Fluorine also limits iodine intake and thyroxine synthesis, disrupting thyroid function [13].

Fluorides inhibit numerous enzymes of cellular respiration through binding to metals: $Mg2+$, $Fe2+$, $Fe3+$, $Zn2+$, $Mn2+$, $Cu2+$ and $Mo2+$, which are present in active centres of metalloenzymes. Fluorides can also activate some of the enzymes, such as adenylyl cyclase and phosphoglucomutase [14]. They modify the metabolism of carbohydrates, lipids, and, to a lesser extent, proteins, as well as some biological functions of living organisms [15]. Higher exposition to fluorine can thus contribute to impaired glucose tolerance leading to reduced insulin secretion, inhibition of glycolysis, depletion of glycogen, hyperglycaemia and insulin resistance. The latter two are usually observed in women with PCOS. In the study on rats, a significant increase (by 30%) of triacylglycerols was observed in blood plasma after prolonged administration of sodium fluoride at the dose of 100 mg F-/l of potable water [16]. However, the concentration of cholesterol was not significantly changed. It is also correlated with fluorine induced hyperglycaemia, which can result from increased glycogenolysis in the liver. Fluoride increases the level of cAMP, which activates cAMP-dependent protein kinase, which is responsible for the activation of glycogen phosphorylase in the liver and triacylglycerol lipase in adipose tissue. The process of glycogenolysis starts and leads to glycogen breakdown, causing hyperglycaemia due to the release of glucose to blood. Therefore, the higher rate of triacylglycerol synthesis due to hypertriacylglycerolemia may result from increased lipolysis and fatty acids release from adipose tissue. Thus, hyperglycaemia induced with fluorine compounds may be related to leptin. Its deficiency or the presence of leptin resistance contributes to an increased synthesis of triacylglycerols. It is also possible that sodium fluoride inhibits the activity of leptin receptor through the inhibition of insulin receptor because the expression of the gene encoding the leptin receptor is most prominent in pancreatic β cells.

No reports suggesting the influence of fluorine on the development of polycystic ovary syndrome (PCOS) have been found and thus we decided to examine this problem. The typical biochemical characteristics of PCOS are: an increased level of testosterone and androstenedione in plasma, and a higher level of luteinizing hormone (LH), especially in women without ovulation. It is accompanied by increased response of LH to gonadotropin releasing hormone (GnRH). The diagnosis is based mainly on clinical criteria. Determination of an elevated level of testosterone in plasma and/or LH complements the clinical diagnosis. In obese women, the oral glucose tolerance test (OGTT) aims to determine impaired glucose tolerance, quite common in this condition [17]. PCOS aetiology is still unclear. It is supposed that the development of this disease is affected by both genetic and environmental factors. It is obvious that the excess of androgens results from primary disorders originating in the ovaries. Currently, it is believed that PCOS symptoms relate to both women with the symptoms of hyperandrogenism and regular menstrual cycles and those without elevated androgens and without ovulation. It is also known that caloric restrictions improve the sensitivity of tissues to insulin and glucose tolerance, and in many cases they contribute to the return of menstrual cycles [18].

Materials and methods

Test group

The test group comprised of 40 women at the age of 18–38 (26.76 ± 5.08) with polycystic ovary syndrome (PCOS) diagnosed according to Rotterdam's criteria [19]. Most women were overweight or obese, BMI – 29.16 ± 5.8, WHR – 0.91 ± 0.08. The percentage of adipose tissue was 38.79 ± 8.6%, the content of muscle mass − 44.58 ± 13.6%, and total body water − 45.04 ± 4.7%. The analyses of body composition were performed using electric bioimpedance with BIA 101 (Akern, Italy).

The criteria for exclusion were:

- hormonal disorders, such as hyperprolactinaemia, congenital adrenal hyperplasia, Cushing's syndrome, acromegaly,
- pharmacotherapy: hormonal contraception, hypoglycaemic drugs such as metformin,
- pregnancy,
- age above 40.

The control group comprised of 14 potentially healthy women with proper BMI, at the age of 30.23 ± 6.3, in whom PCOS was excluded. In this group, the percentage of adipose tissue was 24.36 ± 4.08%, the content of muscle mass – 46.76 ± 4.52%, and total body water – 46.98 ± 9.45%.

In the division of the groups based on the concentration of fluorine, the threshold level used was 0.200 ppm fluorine in plasma. In the division of groups differing in the levels of androgens, the level of testosterone and/or FAI index (Free Androgen Index) were used. FAI was calculated from the formula: FAI = 100 × (total testosterone nmol/l/SHBG nmol/l) [20]. The first group (n = 14) included women who had higher levels of androgens (testosterone > 0.82 ng/ml) and/or high FAI (FAI > 10). The second group (n = 26) comprised of women with an appropriate level of androgens and FAI.

Biochemical analyses

The analyses of FSH, LH, oestradiol, testosterone, TSH, prolactin, insulin and SHBG were performed using electro-chemiluminescence immunoassay – ECLIA (Kobas Rosche E411). Androstenedione concentration was measured using ELISA, DHEAS – with

immunoenzymatic assay, cholesterol – using the enzymatic method with esterase and cholesterol oxidase, and glucose – using the enzymatic technique with hexokinase. All the analyses were performed in an accredited hospital laboratory.

Determination of fluorine with an ion selective electrode

Fluoride concentrations were measured by potentiometric method with a fluoride ion-selective electrode (Orion 9409 BN, Thermo Scientific, USA). The electrode has been calibrated using standard solutions. A 0.5 ml sample of tested plasma was collected to plastic cups and 0.5 ml of buffer TISAB III was added. Next, a magnetic stirrer and ion-selective electrode were introduced to the container. The electrode was connected to the voltage tester (Beckmann type Φ 50). The electrode potential was read after 5 min. Next, 0.1 ml of appropriate standard was added and the measurement was repeated. The electrode potential was read after additional 5 min. The fluoride content in samples was calculated based on the difference of potentials measured in each sample and the concentration of the added standard. The correctness of the analytical procedure was controlled by determining the concentration of F− in NaF solutions with known concentrations: 0.1, 1.0 and 10.0 mg/L (Orion Company, USA).

Statistical analysis

Statistical analyses were carried out using Statistica 12 (Statsoft, Tulsa, Oklahoma, USA). Statistical analysis was based on the comparison between the group of women with PCOS considering two phenotypes – with a normal level of androgens (PCOS – NT) and a higher level of androgens (PCOS-HT), and with the control group (CG) of healthy women (n = 14). *T* test for independent samples was used to assess the significance of differences with $p > 0.05$. Additionally, a correlation matrix was used with relation to biochemical parameters, which is an advanced tool to assess large number of linear correlations between the data.

Results

Fluorine concentration in plasma of women with PCOS was compared with that of the group of healthy women (the control group). The concentration in both groups did not differ statistically significantly and amounted to 0.223 ± 0.037 and 0.228 ± 0.023 [ppm], respectively (Table 1). There were also no significant differences between the phenotypes of women with PCOS differing in androgens levels (Table 1).

Correlation matrix for all the tested parameters in the PCOS group in total, without division into groups with lower (LF) and higher (HF) level of fluorine, did not show any correlations. Only the division of the women with PCOS into two groups differing in fluorine levels (threshold of 0.200 ppm) made it possible to observe the effect of fluorine on particular biochemical parameters (Table 2). It was noted that women with PCOS with higher concentration of fluorine in plasma have a significantly higher level of TSH, still being within the range regarded as normal. What was worrying was that the insulin resistance index (HOMA-IR) was not only statistically significantly higher in the group with a higher level of fluorine but it also indicated advanced insulin resistance (Table 2).

Table 2
Significance of differences between the tested parameters in groups differing in terms of the fluorine level (LF, HF).

Parameter	LF group n-21	HF group n = 19	Statistical significance
Fluorine ppm	0.191 ± 0.008	0. 247 ± 0.04	0.024
BMI m/kg^2	28.7 ± 6.07	29.6 ± 6.03	NS
DHEA-SO_4 μg/dl	218.69 ± 91.75	262.58 ± 115.56	NS
Androstenedione ng/ml	3.94 ± 2.04	3.40 ± 1.18	NS
TSH mIU/ml	1.684 ± 0.81	2.182 ± 0.60	0.034
LH mIU/ml	9.16 ± 3.91	7.29 ± 2.92	NS
FSH mIU/ml	5.42 ± 1.32	4.914 ± 1.04	NS
Oestradiol pg/ml	65.87 ± 88.31	47.38 ± 29.05	NS
SHBG nmol/L	36.73 ± 13.85	34.37 ± 17.55	NS
Testosterone ng/ml	0.865 ± 1.357	0.571 ± 0.185	0.048
Prolactin ng/ml	16.09 ± 5.55	17.51 ± 5.77	NS
Insulin sample 0 mU/l	10.76 ± 5.97	16.06 ± 12.44	NS
Insulin after 2 h mU/l	79.88 ± 96.38	80.37 ± 45.34	NS
Glucose mg/dl	89.76 ± 9.04	93.82 ± 11.70	NS
Glucose after 2 h mg/dl	108.34 ± 20.77	114.67 ± 30.87	NS
HOMA-IR	2.38 ± 1.174	3.718 ± 2.83	0.043
Cholesterol mg/dl	183.07 ± 24.9	176.27 ± 31.41	NS
LDL mg/dl	106.43 ± 22.97	110.70 ± 29.71	NS
TG mg/dl	107.93 ± 52.85	99.8 ± 34.2	NS
HDL mg/dl	58.81 ± 18.34	59.16 ± 18.65	NS

LF – average value for a lower level of fluorine; HF – average value for a higher level of fluorine. BMI – Body Mass Index, TSH – Thyroid-stimulating hormone, LH – Luteinising hormone, HOMA-IR – Homeostatic model assessment – insulin resistance, DHSO4- , SHBG – Sex hormone binding globulin, FSH – Follicle-stimulating hormone, HDL – High-density lipoprotein, LDL – Low-density lipoprotein, TG – Triglycerides.

The next stage of our investigation was to analyse the correlation matrix for both groups differing in the level of fluorine in plasma. In the group with a higher level of fluorine (HF) there was a correlation with HDL at the level of 0.7759 (Table 3). In the group with a lower level of fluorine (LF) we observed negative correlations with the level of androgens transporting protein – SHBG (−0.605) and with the level of glucose on fasting (−0.615) (Table 3).

Finally, we checked the correlations between the two phenotypes of women with PCOS (differing in terms of the levels of androgens). There was a relation between higher concentration of androgens and a higher level of HDL and a lower level of triglycerides (Table 4). Such relations were not observed in women with PCOS with a normal level of androgens (Table 4).

Consequences of the hypothesis and discussion

The basic symptoms of PCOS are non-ovulating cycles or rare ovulations, clinically and/or biochemically confirmed androgenisation and a characteristic image of polycystic ovaries in ultrasound examination [21]. The lack of differences in fluorine levels between healthy women and those with PCOS and in two phenotypes of women with PCOS confirms our assumption that fluorine is not the main cause of this

Table 1
Comparison of fluorine concentration between the group of women with PCOS, in both phenotypes (PCOS-HT, PCOS-NT) and the control group (CG).

Parameter	CG	PCOS total	PCOS -HT	PCOS-NT
Fluorine level in plasma [ppm]	0.228 ± 0.023*	0.223 ± 0.037*	0.224 ± 0.048*	0.220 ± 0.028*

PCOS-HT – women with PCOS and a high testosterone level, PCOS-NT – women with PCOS and a normal testosterone level. BMI – Body Mass Index, TSH – Thyroid-stimulating hormone, LH – Luteinising hormone, HOMA-IR – Homeostatic model assessment – insulin resistance, DHSO4- , SHBG – Sex hormone binding globulin, FSH – Follicle-stimulating hormone, HDL – High-density lipoprotein, LDL – Low-density lipoprotein, TG – Triglycerides.

* Lack of significant differences between the groups.

Table 3
Correlation matrix in groups of women with PCOS with higher and lower levels of fluorine (HF, LF).

Parameter	Avg-LF n=21	Correlation	AVG-HF n=19	Correlation
BMI m/kg^2	29.572 ± 6.027	0.045	28.732± 6.068	-0.341
DHEA-SO4 µg/dl	262.582±115.559	0.492	218.693±91.754	-0.120
Androstenedione ng/ml	3.401±1.183	0.234	3.942±2.0443	0.393
TSH mIU/ml	1.694±0.818	0.507	2.182±0.603	-0.265
LH mIU/ml	7.288±2.924	-0.076	9.1579±3.912	-0.015
FSH mIU/ml	4.914±1.039	0.419	5.42±1.322	0.288
Oestradiol pg/ml	47.378±29.055	-0.147	65.875±88.306	-0.452
SHBG nmol/L	34.374±17.557	-0.605	36.735±13.851	0.363
Testosterone ng/ml	0.571±0.255	0.214	0.865±1.357	-0.200
Prolactin ng/ml	17.514±5.775	-0.040	16.092±5.55	0.493
Insulin sample 0 mU/l	16.063±12.441	-0.011	10.757±5.974	-0.501
Insulin after 2h mU/l	80.368±45.342	-0.005	79.886±96.385	-0.410
Glucose mg/dl	93.817±11.705	-0.615	89.763±9.043	-0.229
Glucose after 2h mg/dl	114.673±30.875	-0.047	108.341±20.769	-0.492
Cholesterol mg/dl	176.271±31.409	-0.411	183.074±24.899	0.422
LDL mg/dl	110.705±29.712	-0.340	106.428±22.975	-0.158
TG mg/dl	99.799±34.197	0.436	107.933±52.846	-0.511
HDL mg/dl	58.066±18.34	-0.297	59.16±18.647	0.776
Fluorine ppm	0.191±0.008	1.000	0.247±0.059	1.000

Avg-LF – average value for a lower level of fluorine; Avg-HF – average value for a higher level of fluorine; Values in red indicate a statistically significant correlation. BMI – Body Mass Index, TSH – Thyroid-stimulating hormone, LH – Luteinising hormone, HOMA-IR – Homeostatic model assessment – insulin resistance, DHSO4- , SHBG – Sex hormone binding globulin, FSH – Follicle-stimulating hormone, HDL – High-density lipoprotein, LDL – Low-density lipoprotein, TG – Triglycerides.

disease. Among the main causes of the syndrome the following are listed:

- genetic – increased activity of P450c17α cytochrome [22],
- endocrinological – increased ratio of LH/FSH, increased concentration of insulin and androgens [23],
- metabolic – insulin resistance, a lower level of SHBG [24],
- environmental (anabolic steroids).

The presence of statistically significant differences in TSH level in the group with a higher fluorine level in plasma indicates that fluorine competes with TSH for the receptor on thyroid cells, which limits the stimulating effect of this hormone on endocrine function of the thyroid gland [7]. Additionally, fluorine as a more electronegative and reactive element may be absorbed instead of iodine, which impairs the function of deiodinase (D1, D2, D3) transforming FT4 into FT3 [25]. Due to the formed hypothyroidism, an increased secretion of thyroliberin (TRH)

Table 4
Correlation matrix for the group of women with PCOS with a higher and normal level of androgens (PCOS-HT, PCOS-NT).

Parameter	Avg-NT n=26	Correlation	Avg-HT n=14	Correlation
BMI m/kg^2	28.042±6.27	-0.066	30.550±7.39	-0.248
DHEA-SO4 µg/dl	163.500±67.52	0.207	305.100±62.22	-0.501
Androstenedione ng/ml	2.667±1.2	0.772	4.218±1.86	-0.010
TSH mIU/ml	1.477±0.38	-0.697	2.158±0.95	-0.381
LH mIU/ml	7.083±1.89	-0.306	7.297±2.65	-0.137
FSH mIU/ml	5.658±0.56	-0.314	5.047±1.42	0.073
Oestradiol pg/ml	33.570±20.37	-0.293	45.846±22.15	-0.519
SHBG nmol/L	43.772±19.05	0.306	35.414±17.18	0.416
Testosterone ng/ml	0.402±0.14	0.453	0.722±0.24	-0.285
Prolactin ng/ml	16.420±3.68	0.679	14.796±4.10	0.464
Insulin sample 0 mU/l	9.900±6.24	-0.145	19.190±17.26	-0.245
Insulin after 2h mU/l	57.333±21.70	-0.381	111.600±108.39	-0.118
Glucose mg/dl	90.608±7.22	-0.149	89.756±16.90	-0.076
Glucose after 2h mg/dl	105.322±18.61	-0.527	120.973±34.95	-0.353
Cholesterol mg/dl	191.673±18.68	0.521	166.803±32.31	0.443
LDL mg/dl	109.348±24.13	0.016	104.863±35.01	-0.087
TG mg/dl	77.338±38.49	-0.255	89.986±25.02	-0.644
HDL mg/dl	76.773±23.77	0.405	56.478±21.25	0.737
Fluorine ppm	0.224±0.079	1.000	0.22 ±0.059	1.000

Avg-NT – average value for a normal level of androgens; Avg-HT – average value for a high level of androgens; Values in red indicate a statistically significant correlation. BMI – Body Mass Index, TSH – Thyroid-stimulating hormone, LH – Luteinising hormone, HOMA-IR – Homeostatic model assessment – insulin resistance, DHSO4- , SHBG – Sex hormone binding globulin, FSH – Follicle-stimulating hormone, HDL – High-density lipoprotein, LDL – Low-density lipoprotein, TG – Triglycerides.

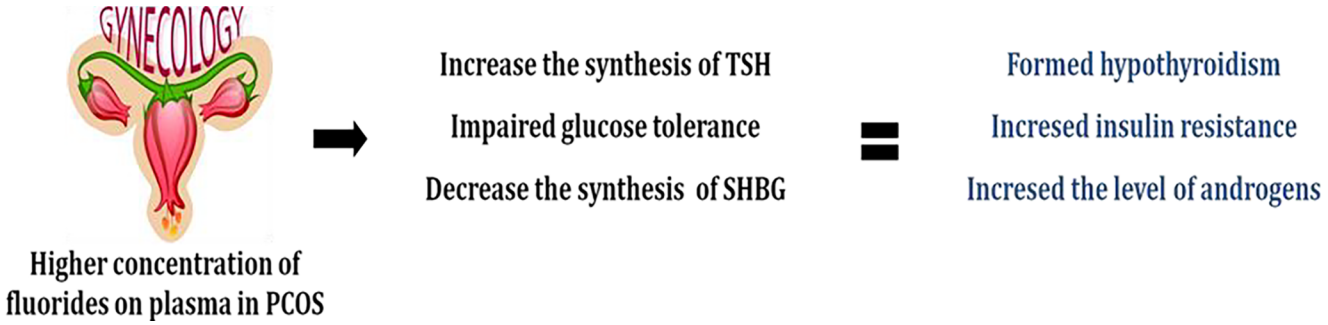

Fig. 1. The consequences of higher plasma concentrations fluoride on PCOS pathogenesis.

by hypothalamus occurs, which leads to increased concentration of thyrotropin (TSH) and prolactin, which in turn contributes to the disrupted function of ovaries and inhibited ovulation. The factor which stimulates this mechanism is the change of the ratio of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in favour of FSH, and specifically – decreasing their concentration and increasing the synthesis of dehydroepiandrosterone (DHEA) in the adrenal glands. It is also suggested that an elevated level of TSH influences the accumulation of collagen in ovaries [26].

Because TSH level (average 2.182 ± 0.603 mlU/l) is still within the reference level (from 0.4 to 4.0 mlU/l) for an adult person, this mechanism should be regarded as important but less significant in PCOS development. However, it is a mechanism which can contribute to excessive body weight and metabolic pathways' dysfunctions [27]. Thyroid gland dysfunctions will, in consequence, lead to the development of obesity, insulin resistance and cardiovascular diseases. Our studies evidently show that fluorine, even at the levels regarded as normal in blood plasma (> 0.2 ppm with the average in group 0.247 ppm), is responsible for the presence of insulin resistance. It reduces the activity of tyrosine kinase in muscles and white adipose tissue and contributes to increased activity of IRS-1 serine phosphorylase in white adipose tissue [28]. Next, serine phosphorylase facilitates the reduction of insulin signal by impairing the ability of insulin receptor to phosphorylate tyrosines, and thus it stops the signal from insulin receptor to the inside of a cell. Inhibition of tyrosine phosphorylase results in negative responses in the insulin pathway and leads to insulin resistance. Developing insulin resistance is not only accompanied by metabolic disorders but also by stroke, non-alcoholic fatty liver disease (NAFLD), asthma, some cancers, polycystic ovary syndrome (PCOS), and Alzheimer's disease [29]. An inherent problem of insulin resistance is hyperinsulinemia. It is diagnosed even in 50% of patients with PCOS. Hyperinsulinemia is the cause of a higher activity of the axis hypothalamus-pituitary gland-adrenal glands, which leads to increased secretion of adrenal androgens. Hyperinsulinemia also influences the growth of LH and the number of LH and IGF-1 receptors, which in turn causes an increased response to gonadotropins through the increased production of androgens, higher proliferation of theca cells, an increased activity of 17-hydroxylase and 17–20 lyase, and higher expression of 3β-hydroxysteroid dehydrogenase in ovarian granulosa cells [30]. Thus, hyperinsulinemia may negatively affect the formation of ovarian follicles and ovulation by, among others, increasing ovular production of androgens [31,32].

Hyperinsulinemia strengthens hyperandrogenism also by inhibiting liver synthesis of sex hormone binding globulin – SHBG and insulin-like growth factor binding protein 1 – IGFBP-1 [33]. In the group with a lower fluorine level, we observed the relation with lower concentration of SHBG and a lower level of glucose on fasting, which is probably caused by the fact that the impairing effect of fluorides on glucose tolerance does not exist. Moreover, a low level of SHBG seems to be a marker of metabolic syndrome in men, and especially in women [34]. Therefore, we think that the effect of a high level of androgens, which is observed in PCOS due to their increased synthesis or due to a reduced level of SHBG binding protein (higher concentration of free/active testosterone), masks in this particular case the effect of fluorine on reduced secretion of insulin and inhibited glycolysis pathway. Thus, the effect of fluorine in this aspect is modified.

Literature shows that the level of HDL is inversely proportional to the concentration of triglycerides in blood and directly proportional to the activity of lipoprotein lipase. The activity of microsomal liver enzymes, which synthetize HDL, is dependent on the concentration of androgens (it increases with higher level of testosterone and decreases under the influence of oestrogens). Therefore, we noted such a correlation in PCOS.

The studies of the effect of testosterone on HDL concentration in blood are not consistent. In the study on the group of patients with type II diabetes, a positive correlation between the concentration of testosterone and HDL was observed [35].

Testosterone, as well as high concentration of glucose, has a positive effect on the activity of lipoprotein lipase. It is an enzyme found in the walls of capillary vessels, anchored in their endothelium. Lipase is responsible for the metabolism of HDL and residual chylomicrons, it decomposes triacylglycerols into UFA and glycerol, which can permeate to cells and remain stored there in form of triacylglycerols. Therefore, in our opinion, the relation with HDL showed for the group with higher concentration of fluorine (Table 3) is related to the coexistent higher level of androgens in this group. This can be confirmed by the correlation matrix for the groups with high and low levels of androgens (Table 4).

However, it should be noted that the matrix does not to show accurate values for the series or single pieces of information, but only aims to indicate whether there are any correlations between the data.

Conclusions

Fluorine, even in concentrations regarded as normal, affects metabolic pathways and is responsible for insulin resistance in the group of women with PCOS. Insulin resistance and the increase of TSH caused by increased concentration of fluorine in blood is one of the causes of PCOS (Fig. 1).

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Ethics approval

The study was approved by the Ethics Committee of the Bioethical Commission of the Pomeranian Medical University in Szczecin. All patients gave written informed consent and their confidentiality and anonymity were protected.

Authors' contributions

M. Szczuko – research concept, collecting data, biochemical analyses, analysed and interpreted the patient data, writing the article.

J. Splinter – biochemical analyses, analysed and interpreted the patient data.

M. Zapałowska-Chwyć – collecting data.

M. Ziętek – interpreted the patient data.

D. Maciejewska – analysed and interpreted the patient data.

All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors have not reported any conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.05.007.

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