Progress in Nutrition 2021; Vol. 23, N. 1: e2021015 DOI: 10.23751/pn.v23i1.9163

Original article

Effects of nutritional intervention with or without metformin on insulin resistance in adolescents with polycystic ovary syndrome: a preliminary study

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Abstract. The aim of the study is show whether, and to what extent, nutritional interventions with or without metformin that effectively result in weight reduction in obese adolescents with Polycystic Ovary Syndrome (PCOS) can help to alleviate insulin resistance. 35 obese adolescents with PCOS and insulin resistance were recruited to the study and they were randomly assigned to one of two subgroups: sixteen to a group given a nutritional intervention and metformin treatment (NM) and nineteen to a group given only nutritional intervention (NI). Body weight, body composition, energy and nutrients intake as well insulin and glucose levels were measured at the beginning of the study and after 8 weeks of the nutritional intervention. In both groups after 8 weeks of nutritional intervention the reduction in body weight and fat mass were observed. Fasting insulin concentrations measured after 8 weeks of NI were found to be significantly lower in the MN group. In group NI, the 8 weeks of intervention proved insufficient for the changes in fasting insulin concentrations. Longer term studies are needed to see if this therapy can result in sustained decreases in body weight and improvements in insulin resistance.

Key words: polycystic ovary syndrome, adolescents, obesity, nutrition,

Introduction

The increase in overweight and obesity in children and adolescents has resulted in a greater incidence of biochemical disorders that can precede type-2 diabetes, such as insulin resistance associated with hyperinsulinism and an abnormal glycemic curve, revealed by the oral glucose tolerance test (OGTT) (1). Presently, 20% of diabetic adolescents suffer from type-2 diabetes that develops as a consequence of excess body mass. It should be stressed that there are numerous factors contributing to the development of this disease, but that excess body mass is believed to be the most significant. Studies also indicate that girls with excess body mass enter puberty much earlier, and are more at risk of menstrual cycle disorders,than normal body weight subjects. The features of hyperandrogenism are common in obese adolescent girls; this is related to insulin resistance and hyperinsulinemia (2, 3). It has been demonstrated that untreated hyperandrogenism in young obese women, especially with concomitant insulin resistance, can even result in the development of polycystic ovary syndrome (PCOS), with all its consequences for metabolism and fertility. It is estimated that 11%–26% adolescents develop PCOS and at that least 50% of these are obese (2).

The recommended primary method of treating women diagnosed with insulin resistance and hyperandrogenism is normalization of the body weight through increased physical activity and a weight-reduction diet with low glycemic index (4). Garnet et

al. (5) demonstrated that reduced energy intake, combined with physical activity and assisted by metformin, is likely to be the mainstay for improving insulin sensitivity completed in a challenging developmental stage. This finding and the improvement in acanthosis nigricans, as a clinical indicator of insulin resistance suggest that a prescribed reduced energy diet is the important intervention message for overweight and obese adolescents at risk of type-2 diabetes.

Marsh et al. (4) suggested that low-GI diet, with or without metformin therapy, may provide an additional advantage over and above that of a conventional healthy diet in managing the underlying insulin resistance, cardiovascular risk, and irregular menstrual patterns in women with PCOS who are overweight but not morbidly obese. As we know, the normalization of body weight in patients with hyperandrogenism and insulin resistance is necessary, as a 4%–5% reduction can improve insulin sensitivity by a factor of up to three. Reducing visceral fat can also reduce the synthesis of testosterone and increase insulin sensitivity (4).

However, very few studies have attempted to implement nutritional interventions for weight reduction in obese adolescents suffering from insulin resistance and hyperandrogenism. We here show the results of the first step in a long-term cooperation with obese adolescents suffering from insulin resistance and hyperandrogenism, diagnosed with PCOS. Our aim is to assess the effectiveness of nutritional intervention in terms of achieving normal body weight and normal insulin resistance and hormonal parameters. The aim of the study is show whether, and to what extent, nonpharmacological controlled nutritional interventions that effectively result in weight reduction in obese adolescents can help to alleviate insulin resistance; and whether simultaneous pharmacotherapy with metformin in this group brings any additional benefits.

Material and Methods

Adolescents were recruited from University Hospital of Obstetrics and Gynecology, Poznań University of Medical Sciences. All subjects were postmenarchal adolescent females aged 16.8 ± 1.3 y with body mass index (BMI) above the 95th percentile.

The thirty five adolescent were randomly assigned to one of the two subgroups: sixteen to a group given a nutritional intervention and metformin treatment (NM) and nineteen to a group given only nutritional intervention (NI).

The inclusion criteria were: no use of pharmacological agents or hormones that may affect the course of the menstrual cycle or metabolic rate within the last three months, no use of medicament that could affect carbohydrate metabolism in the last four weeks, no use of weight loss supplements during the study, no clinical diagnosis of eating disorders, no clinical diagnosis of digestive disease (such as irritable bowel syndrome, ulcerative colitis, Crohn's disease, or celiac disease).

All subjects met the criteria for PCOS based on menstrual irregularity and androgen excess, according to the Rotterdam criteria. The diagnosis of PCOS was based on ultrasound examination of the ovaries and assessment of hormonal parameter level (6).

The study was approved by the Poznań Medical Ethics Committee (no. 868/15) and all subjects and their parents provided signed consent.

Each subject completed a medical questionnaire. The questions concerned menstruation and asked for age at menarche, length of menstrual cycles, and history of menstrual disorders.

Primary amenorrhea was diagnosed where there had been no onset of menses by 15 years, while secondary amenorrhea was diagnosed when there had been no menstruation for six months or for more than three times the previous cycle length. Menstrual periods that occurred more than 35 days apart were described as oligomenorrhea (7, 8).

Hirsutism was graded according to the standard Ferriman–Gallwey score, by which the density of terminal hairs is scored at nine different body sites. In each of these areas, a score of 0–4 was assigned. A total score of >8 was interpreted to mean that hirsutism was present. Acne was graded according to the Pillsbury method, which counts acne lesions, ranking their severity as grade I (absent or minor, 1–9 comedones), grade II (mild, 10–19 comedones), grade III (moderate, R20 comedones, inflammation), and grade IV (severe) (9).

Fasting blood was assayed for, total testosterone (T), sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), *thyroid-stimulating*

hormone (TSH), prolactin (PRL),luteinizing hormone (LH), foliculotropine hormone (FSH), estrogen (E), and lipid profile (cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides). Two-hour plasma glucose and insulin in a standard oral glucose tolerance test (OGTT) was also measured. The homeostatic model assessment of insulin resistance (HOMA-IR), calculated as fasting glucose levels in mmol/L × fasting insulin levels in mIU/L/22.5), was used to determine insulin resistance (10).

Blood samples were from menstruating subjects between days 2 and 5 of the menstrual cycle (in the early follicular phase, days 1–5), and at random in amenorrheic subjects. Blood samples were taken between 6.00 a.m. and 9.00 a.m. following overnight fasting and rest. The women were instructed to abstain from caffeine and alcohol for 24 hours prior to blood sampling, and to refrain from strenuous exercise on the day of sampling. All parameters were measured by immunochemical methods using the chemiluminescent microparticle immunoassay (CMIA) and microparticle chemiflex flexible assay protocols, with diagnostic sets and an Architect automatic analyzer. All parameters were determined in a duplicate.

In order to evaluate nutritional status and anthropometrical indices, the height and weight were measured using an anthropometer coupled with a WPT 200 OC verified medical scale (Rad Wag). The participants were dressed in minimal clothing during the measurements, which were rounded to the nearest 0.5 kg and 0.5 cm. Estimation of body fat mass (FM) and fat-free mass (FFM) was performed in the morning following an overnight fast with subjects lying in a supine position, using a BodPod analyzer (11).

Seven consecutive days of dietary records were obtained under the supervision of dieticians. Subjects were in regular contact with a registered dietitian who showed them how to record nutrition intake and monitored their progress. The daily diets were analyzed for their energy and nutrient levels (fat, protein, carbohydrate, dietary fiber, calcium, magnesium, vitamins D, B12, foliate and vitamin C) using the Dietician computer software package, based on Polish food composition tables (12).

After evaluation of the adolescents' nutritional habits, all the subjects were informed of the nutritional problems found in their current diets and of the health consequences of dietary deficiencies. We then prepared a low glycemic index weight-reduction dietfor participants qualified to the study. The weight-reduction diets for each participant were prepared using the five-meal system by a registered dietitian and indicate precise meals for each day of the week, as well as information on the selection of food, seasonal meals, food preparation, and serving sizes (in both grams and household measurements). All diets were be hypocaloric (i.e., they will result in a 400–600 kcal daily deficit). The level of carbohydrates and fat intake was determined, which respectively amounted to 40%–50% of total energy intake (with minimum sugar intake), especially low glycemic index (< 50 Glycemic Index) of the daily energy intake. Adequate daily intake for protein and fat were 15%–20% of total energy intake (50% vegetable protein) and 30%–35% of total energy intake (saturated fatty acids < 10% of total energy intake). The recommended intake of other vitamins and minerals was established in accordance with Recommended Dietary Allowances for girls aged 16–18 years and women over 19 years, in accordance with Jarosz et al. (13).

To reduce drop-outs and to increase adherence to the intervention, we relatively frequently contacted the subjects, through check-up visits and phone calls after the first, second, and third weeks of the nutritional intervention. To ensure compliance with the diet, each patient was also obliged to write down all food and beverages consumed in the last seven days before each dietary consultation.

The means and standard deviations of the quantitative variables were calculated and the normality of the distribution was checked. Comparisons between data from before and after the eight weeks of nutritional intervention were carried out using a t-test for independent variables. Statistical analysiswas performed using Statistica 13.0 software (StatSoft, 2018). P-values of less than 0.05 were considered statistically significant.

Results

The characteristics of subjects who completed the study are shown in Table 1. The hormonal parameters

Table 1. Baseline group characteristics M ± SD

Hormonal parameters and lipid profile			
	All (n = 35)	**MN (n = 16)**	**NI (n = 19)**
TSH (μIU/ml)	3.46 ± 1.81	3.89 ± 1.68	3.02 ± 1.93
PRL (ng/ml)	22.01 ± 5.42	23.37 ± 5.73	41.4 ± 4.82
T (ng/ml)	1.09 ± 0.80	1.07 ± 0.78	1.08 ± 0.99
DHEAS (μmol/L)	13.88 ± 4.11	10.71 ± 9.59	17.05 ± 29.36
SHBG (nmol/l)	31.00 ± 8.05	24.95 ± 9.59	37.04 ± 19.82
LH (mlU/ml)	17.78 ± 6.14	10.39 ± 5.75	13.17 ± 7.22
FSH (mlU/ml)	4.97 ± 1.18	4.38 ± 1.21	5.56 ± 1.62
E2 (pg/ml)	58.79 ± 61.14	79.28 ± 55.07	38.30 ± 17.71
Cholesterol (mg/dL)	180.08 ± 17.19	176.52 ± 18.91	185.13 ± 12.36
LDL (mg/dL)	106.47 ± 16.17	104.23 ± 23.11	108.71 ± 10.52
HDL (mg/dL)	48.36 ± 8.17	45.83 ± 8.15	50.89 ± 8.19
Triglycerides (mg/dL)	117.8 ± 27.5	124.22 ± 32.89	111.32 ± 22.18
Hirsutism (scores)	20.6 ± 2.9	19.4 ± 3.3	21.8 ± 2.3
Menstrual cycle			
Eumenorrhea (n; %)	0	0	0
Oligomenorrhea (n; %)	20; 53	9; 56	11; 58
Secondary amenorrhea (n; %)	15; 47	7; 44	8; 42
Primary amenorrhea (n; %)	0	0	0

M± SD: mean ± standard deviation

and lipid profile did not differ significantly between the groups. No statistically significant differences were seen in menstrual cycles between adolescents of the two age groups. In both, 43% of respondents indicated that they suffer from amenorrhea. The eight weeks of nutritional intervention was too short to show recovery of the menstrual cycle in adolescents with PCOS (data not shown).

In the MN group, following 8 weeks of nutritional intervention, a significant decrease was seen in the daily dietary energy value (mean 303 kcal/d), associated with the decrease in intakes of fat (36 g/d) and sugar (31 g/d). Comparison of the adolescents' diet before and after the eight-week nutritional intervention with metformin revealed significant differences with regard to the increased intake of plant protein (31 g/d), calcium (285 mg/d), magnesium (137 mg/d), vitamin D (2.7 μg/d), vitamin B12 (1.4 mg/d). Similar results were achieved in the NI group (Table 2).

The changes in the energy and nutrition value of the daily diets resulted in significant changes in body weight and body composition in adolescents from both

Table 2. Energy and nutrients intake at the 0 and 8 week measurement points; M ± SD

	All (n = 35)			MN (n = 16)			NI (n = 19)			p-value*	
Energy and nutrients	**0**	**8**	**p-value**	**0**	**8**	**p-value**	**0**	**8**	**p-value**	**0 vs. 0**	**8 vs. 8**
Energy (kcal)	2785 ± 302	2482 ± 222	< 0.0001	2833 ± 228	2493 ± 205	0.006	2722 ± 242	2435 ± 221	0.03	NS	NS
Fat (g)	107 ± 16	71 ± 16	<0.0001	107 ± 14	68 ± 8	< 0.0001	109 ± 26	71 ± 22	0.03	NS	NS
Protein (g)	97 ± 21	116 ± 18	0.07	104 ± 19	109 ± 23	NS	82 ± 17	117 ± 13	0.008	NS	NS
Plant protein (g)	23.3 ± 7.4	53.8 ± 10.3	< 0.0001	24.2 ± 9.5	52.1 ± 9.2	0.0003	22.4 ± 3.9	55.1 ± 11.9	< 0.0001	NS	NS
Carbohydrate (g)	353 ± 59	335 ± 40	NS	353 ± 56	336 ± 34	NS	354 ± 63	337 ± 49	NS	NS	NS
Sugar (g)	92 ± 10	61 ± 8	<0.0001	95 ± 11	65 ± 9	< 0.0001	87 ± 11	56 ± 7.0	NS	NS	NS
Dietary fiber (g)	23.1 ± 7.9	27.0 ± 4.1	0.02	24.3 ± 7.1	29.0 ± 4.1	NS	20.9 ± 8.1	25.9 ± 3.9	NS	NS	NS
Calcium (mg)	619 ± 248	904 ± 192	< 0.0001	665 ± 218	940 ± 120	0.04	567 ± 300	860 ± 239	0.04	NS	NS
Magnesium (mg)	205 ± 45	342 ± 48	< 0.0001	293 ± 38	342 ± 36	0.03	282 ± 51	338 ± 52	NS	NS	NS
Vitamin D (mg)	2.1 ± 2.1	4.76 ± 1.72	0.005	2.0 ± 1.0	4.53 ± 1.92	0.03	2.2 ± 3.9	4.9 ± 1.7	NS	NS	NS
Foliate (mg)	199 ± 72	352 ± 35	0.0001	302 ± 85	356 ± 34	NS	250 ± 42	332 ± 24	0.005	NS	NS
Vitamin B_{12} (mg)	2.5 ± 1.4	3.9 ± 1.4	0.005	2.2 ± 1.3	3.7 ± 1.7	0.02	2.8 ± 1.6	3.6 ± 1.2	0.004	NS	NS
Vitamin C (mg)	153 ± 111	192 ± 73	NS	128 ± 74	267 ± 36	NS	176 ± 152	222 ± 93	NS	NS	NS

* before nutritional intervention (0) vs. after 8 weeks of nutritional intervention (8)

Table 3. Anthropometric characteristics at the 0 and 8 week measurement points; M ± SD

	All (n = 35)			MN (n = 16)			NI (n = 19)			p-value*	
Parameters	**0**	**8**	**p-value***	**0**	**8**	**p-value***	**0**	**8**	**p-value***	**0 vs. 0**	**8 vs. 8**
Body weight (kg)	93.0 ± 7.8	85.9 ± 4.2	<0.001	93.2 ± 6.3	85.1 ± 2.6	<0.001	92.4 ± 8.9	85.8 ± 9.7	<0.001	NS	NS
FM (%)	46.9 ± 3.8	42.8 ± 4.4	<0.001	47.1 ± 4.1	42.6 ± 3.1	<0.001	46.2 ± 3.7	42.9 ± 3.2	<0.001	NS	NS
Waist circumference (cm)	104 ± 26	93 ± 16	<0.001	101 ± 28	90 ± 18	< 0.001	109 ± 22	97 ± 19	0.01	NS	NS

* before nutritional intervention (0) vs. after 8 weeks of nutritional intervention (8)

groups (MN group: mean 8.1 kg; NI group: mean 6.6 kg) Most significantly, the reduction in participants' body weight was caused by decrease in fat mass (MN group: mean 4.5%; NI group: mean 3.3%; Table 3).

Fasting glucose and fasting insulin concentrations at the 2 hour time point pointed to insulin resistance; HOMA-IR also pointed to insulin resistance in both groups. However, fasting insulin concentrations measured after eight weeks of nutritional intervention were found to be significantly lower in the MN group than at the beginning of the study (MN group: fasting glucose mean 4.1 mg/dl, fasting insulin 2.9 mU/ml). In group NI, the eight weeks of intervention proved insufficient for the changes in fasting insulin level to reach statistical significance (Table 4).

Discussion

Lifestyle intervention should be always the first-line treatment in overweight or obese women with PCOS, and weight loss should be achieved through

Table 4. Carbohydrate metabolism at the 0 and 8 week measurement points; M ± SD

Carbohydrate profile OGTT test	All (n = 35)			MN (n = 16)			NI (n = 19)			p-value*	
	0	8	p-value*	0	8	p-value*	0	8	p-value*	0 vs. 0	8 vs. 8
Fasting glucose (mg/dl)	92.4 ± 5.9	89.5 ± 5.6	0.0003	91.2 ± 7.6	87.1 ± 4.3	0.03	92.9 ± 4.9	91.0 ± 3.0	0.009	NS	NS
Glucose at 2 hours(mg/dl)	115.7 ± 24.4	—	—	106.0 ± 19.9	—	—	121.0 ± 22.4	—	—	NS	—
Fasting insulin (mU/ml)	28.1 ± 14.2	25.0 ± 11.2	0.0007	29.0 ± 11.2	26.1 ± 9.3	0.005	26.2 ± 14.7	24.2 ± 13.9	NS	NS	NS
Insulin at 2 hours(mU/ml)	149.9 ± 92.3	—	—	134.4 ± 99.2	—	—	172.1 ± 90.4	—	—	NS	—
HOMA—IR index	4.5 ± 3.4	4.2 ± 3.1	NS	5.1 ± 3.6	4.6 ± 2.9	NS	4.0 ± 3.8	3.8 ± 3.2	NS	NS	NS

* before nutritional intervention (0) vs. after 8 weeks of nutritional intervention (8)

proper nutrition and physical exercise. Yet there are few studies examining nutritional intervention as the first approach to treatment of PCOS in adolescents; the basic mode treatment in this group of patients remains pharmacological therapy (14, 15).

In this study, we evaluated the effects of a nutritional intervention that provided a negative energy balance and an adequate intake of minerals and vitamins on insulin resistance and hyperandrogenism in obese adolescents with PCOS. The total planned study period is six months, and this study provides the results obtained after eight weeks, which was the first measurement time-point after the beginning of the dietary intervention.

The energy and nutritional intakes measured prior to the nutritional intervention were comparable to those reported by Zhang et al. who found positive energy balances in obese adolescents with PCOS and increased intake of fat and sugar (15). One hypothesis was that the excessive intake of energy by women with PCOS resulted from disturbances in the sensations of hunger and satiety, which were in turn the consequences of a problem in leptin synthesis. Such dietary habits result in the impairment of carbohydrate metabolism and PCOS-associated hormonal status; this has been confirmed in this study where the level of insulin determined in the OGTT test and the HOMA-IR values confirmed the occurrence of insulin resistance in the study group.

It should be underlined that disturbances in carbohydrate metabolism are also intensified by obesity, which occurs most frequently in its android form. This was also a feature of the participants in this study, whose mean waist circumference before nutritional intervention was 110 cm.

Other authors' studies have shown that a reduction in body mass in women is associated with a drop in the concentration of insulin in the serum, as well as in testosterone and leptin levels in adult women with PCOS (15, 16). Also, a small number of studies carried have shown that adequate nutritional therapy can be effective in treating obesity in adolescents with PCOS. The work of Carolo et al. points to the effectiveness of regular dietary consultations in adolescent patients with PCOS who are overweight or obese (17). One advantage of our work in this area is that we additionally determined insulin and glucose levels after eight weeks of intervention, further confirming the value of nutritional intervention in treating insulin resistance among adolescents with PCOS. To underline the crucial importance of treating adolescent PCOS with a low glycemic index weight-reduction diet, rather than of pharmacologically, the study population was divided into two subgroups, one of which received pharmacological treatment with metformin alongside the nutritional intervention. It is worth noting that we deliberate did not divide the participants into three subgroups, with one being be treated with metformin alone, as we considered it unethical to deprive these obese teenagers with PCOS of the attention of a dietitian and regular dietary consultations. However, it should be noted that after eight weeks of nutritional intervention

in the MN group, there was a reduction in the insulin fasting level in addition to a significant decrease in body weight and adipose tissues. In the NI group, these changes were not statistically significant, which indicates that the results of the nutritional intervention in combination with pharmacological treatment prevailed. The results described here are from a preliminary study that lasted only eight weeks. We can thus assume that that if nutritional treatment alone is used to treat insulin resistance, longer intervention times would be needed to achieve a significant improvement. In the study of Salama et al., changes were found in the levels of insulin and insulin resistance only after twelve weeks in a group of adult women with PCOS, while in the study of Marsh et al. twelve months were needed to obtain such results (4, 18). Further, recent reviews have shown that at least a 5% reduction in weight is needed to improve insulin resistance, reduce androgen levels, and help with reproductive system dysfunctions in women with PCOS (19, 20, 21). In the MN group, the loss in body weight was 8.6% and the fasting insulin level after eight weeks of intervention was also statistically lower. On the other hand, a 7.1% decrease in body weight was observed in the NI group, while the insulin level after eight weeks of intervention had not changed significantly. Furthermore in both groups, eight weeks of nutritional intervention proved insufficient to achieve a significant improvement in the HOMA-IR index.

The ineffectiveness of metformin in the treatment of insulin resistance has also been confirmed by Wilson et al. (22). Also, according to Bridger et al., Hoeger et al., Arslania et al. and Erensoy et al. treatment of adolescents with metformin had no effect on body weight, body fat, or levels of androgenic hormones (23, 24, 25, 26).

A major limitation of this study was the very small number of volunteers who adhered to the nutritional intervention. However, all the subjects were considered adequate for this age group, and similar adherence rates have been reported elsewhere. Secondly, androgen and SHBG level were assessed only once, before the dietary intervention began. They were not determined at the eight-week time point, so we do not know what effect the eight week of dietary intervention had on the level of hyperandrogenism, and whether the results in the metformin group exceeded those in group NI.

This report provides further support for the role of nutritional habits in the treatment of polycystic ovary syndrome in young obese adolescents. Continuation of the controlled nutritional intervention is needed to assess the extent to which long-term improvements in nutritional status can result in reduced body weight, improvements in insulin resistance, and improved hormonal status among adolescents with PCOS.

Contribution of authors: Both authors have participated in the conception and design, analysis and interpretation of data. KK has participated in recruitment of the patients. KŁ have participated in data collection. KŁ was responsible for drafting the manuscript. All authors are in agreement with the manuscript and declare that the content has not been published elsewhere.

Disclosure of interest: The authors declare that they have no competing interest.

Funding source: The authors received no funding for the present study.

Conflicts of interests: The authors declare that they have no conflicts of interests.

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