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Markers of gastrointestinal permeability and dysbiosis in premenopausal women with PCOS: a case–control study

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ABSTRACT

Objectives Altered intestinal permeability and gut barrier dysfunction have been suggested to play a role in the pathogenetic mechanism of polycystic ovary syndrome (PCOS), the most common endocrine and metabolic condition in reproductive-aged women. However, data on intestinal permeability and dysbiosis of the gut microbiota in PCOS is still limited, with conflicting results. To this end, the concentrations of gastrointestinal permeability and gut dysbiosis markers were analysed in women with PCOS.

Design Case–control study.

Setting General community.

Participants 104 women with PCOS and 203 body mass index (BMI) matched control women at age 46.

Primary and secondary outcome measures Serum levels of zonulin, fatty acid-binding protein 2 (FABP2), urinary levels of indican, and hormonal and metabolic parameters.

Results Serum levels of zonulin (128.0±17.0 vs 130.9±14.0 ng/mL, p=0.13) and FABP2 (1.5±0.9 vs 1.5±0.7 ng/mL, p=0.63) and urinary levels of indican (9.5±5.5 vs 8.4±4.2 mg/dL, p=0.07) were comparable in women with PCOS and controls in the whole study population. Likewise, when the study population was divided into different BMI groups as normal weight, overweight and obese, the levels of the above markers were comparable between the study groups. After BMI adjustment, zonulin levels correlated with the levels of high-sensitivity C reactive protein and homoeostasis model assessment of insulin resistance (p<0.05) both in women with PCOS and controls.

Conclusions Intestinal permeability markers zonulin and FABP2, and the dysbiosis marker indican do not seem to be altered in women with PCOS at age 46 compared with BMI-matched controls. Serum zonulin levels correlated with BMI, insulin resistance and inflammatory marker levels, but did not segregate women with PCOS and controls. This suggests that metabolic factors, but not PCOS per se, is the driving force of dysbiosis in premenopausal women with PCOS.

Strengths and limitations of this study

► This is the first study to report on fatty acid-binding protein 2 and indican in women with polycystic ovary syndrome (PCOS), and the largest study by far to report on serum levels of zonulin in women with PCOS.
► This is the first study in which the study subjects have been matched for body mass index and age as regards the measurement of gastrointestinal permeability markers in women with PCOS.
► Urinary indican used for the assessment of dysbiosis is a surrogate marker and may not reflect the actual microbiome status.
► The age of the study subjects at the time of sampling was 46 years, and the metabolic profile did not differ significantly between women with PCOS and controls.
► The diagnosis of PCOS was based on self-reported symptoms of oligo-amenorrhoea and hirsutism. Ovarian ultrasonography data were not available.

mediated via junctional complexes, including tight-junction proteins. In recent years, disturbances of the intestinal barrier and increased intestinal permeability in various diseases have become evident. Indeed, increased intestinal permeability, often referred to as 'leaky gut', has been thought to be a common underlying mechanism in the pathogenesis of several inflammatory and autoimmune disorders.[1 2]

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder, affecting 6%–15% of reproductive-aged women.[3] It is a complex disorder with heterogeneity of characteristics, including chronic anovulation, hyperandrogenism, obesity and insulin resistance, all having significant long-term metabolic and cardiovascular health implications. Even though metabolic, neuroendocrine and genetic factors have been suggested to play important roles in the development of PCOS,[4] the underlying aetiology remains unclear. It has been hypothesised that gut

INTRODUCTION

The intestinal epithelium acts as a selectively permeable barrier, allowing absorption of nutrients and water while maintaining effective defence against micro-organisms and toxins. Intestinal mucosal permeability is

barrier dysfunction may play a role in the pathogenesis of PCOS. Disturbances in intestinal bacterial flora cause increased intestinal mucosal permeability, with a consequent increase in lipopolysaccharide passage into the systemic circulation, which is thought to trigger a chronic inflammatory response and subsequent insulin resistance, and increased ovarian androgen production.[5] Furthermore, chronic low-grade inflammation is often present in women with PCOS.[6] In addition, ovarian theca cells, responsible for ovarian androgen synthesis, have been shown to have an altered steroid-hormone response in the presence of an inflammatory environment, linking low-grade inflammation to hyperandrogenism.[7]

The most commonly accepted method of assessing intestinal permeability is measurement of the urinary excretion ratio of lactulose/rhamnose or lactulose/mannitol.[8] Even though considered as an accurate, repeatable and non-invasive method, it does, however, have some limitations. It requires ingestion of probes and a period of fasting urine collection. Furthermore, analysis of urinary ratios is complex and time-consuming. Alternatively, assessment of intestinal permeability is possible by measuring the levels of biomarkers of intestinal barrier integrity.[2]

Zonulin, a human protein analogue of *Vibrio cholerae*-derived zonula occludens toxin, is the only physiological mediator known to reversibly regulate intestinal permeability by modulating intercellular tight junctions.[9] Zonulin induces tight-junction disassembly, consequently increasing permeability of the intestinal epithelia and activating immune reactions.[10] Zonulin is considered to be a non-invasive biomarker of intestinal permeability, and serum levels of zonulin have been shown to strongly correlate with the lactulose/mannitol ratio.[10 11] Studies have shown that zonulin levels are increased in obesity, type 2 diabetes, gestational diabetes mellitus and positively correlated with insulin resistance and levels of inflammatory markers such as interleukin-6.[12–14]

Fatty acid-binding protein 2 (FABP2), also known as intestinal (I)-FABP, is an intracellular protein involved in the uptake and metabolism of fatty acids, and it is predominantly expressed in small intestinal enterocytes. FABP2 is released into the circulation following small intestinal mucosal injury, and elevated levels indicate intestinal epithelial cell damage.[15] Since intestinal epithelial cell injury is considered a key pathway in altered intestinal permeability,[16] FABP2 can be used as an indirect measure of increased intestinal permeability. Elevated FABP2 levels have been reported in coeliac disease and necrotising enterocolitis.[17 18] However, data are limited as regards metabolic diseases. Increased FABP2 levels have been reported in severely obese subjects with chronic hyperglycaemia.[19]

Changes in the composition of gut microflora have been associated with chronic diseases such as obesity, type 2 diabetes mellitus and metabolic syndrome.[20 21] Furthermore, recent studies have suggested that hyperandrogenaemia in PCOS may be related to changes in the gut microbiota or gut dysbiosis.[22] The growth of gut microbiota is associated with the release of bacterial metabolites, which enter the systemic circulation via intestinal absorption and are excreted in the urine.[23] One such metabolite is indican, also known as indoxyl sulfate. Dietary tryptophan is metabolised into indole by the gut microbiota, which after systemic absorption, is sulfated in the liver to indoxyl sulfate, and is excreted in the urine. Determining the urinary concentrations of indican can be used as a simple measure to assess dysbiosis.

Given that previous studies reporting gastrointestinal permeability markers in women with PCOS have performed the comparisons using controls not matched for age or body mass index (BMI), the aim of the present study was to determine the levels of these markers in women with PCOS and in BMI-matched and age-matched controls in a large community-based population. We further aimed to evaluate the possible associations of these gastrointestinal permeability markers with hormonal and metabolic parameters. In addition, concentrations of urinary indican, as a surrogate marker of altered gut microbiota, were also analysed.

MATERIALS AND METHODS
Study population
The study population consisted of women of the same age (46 years): 104 women with PCOS and 203 BMI-matched non-PCOS controls from the prospective Northern Finland Birth Cohort 1966 (NFBC1966). Data collection in connection with NFBC1966 included postal questionnaires and clinical health examinations at 31 and 46 years. The questionnaire at age 31 included questions on oligo/amenorrhoea and hirsutism (OA+H): 'Is your menstrual cycle often (more than twice a year) longer than 35 days?' and 'Do you have bothersome, excessive body-hair growth?' The questionnaire at age 46 included the question 'Have you ever been diagnosed as having polycystic ovaries and/or PCOS?' Accordingly, PCOS was diagnosed in women reporting OA+H at age 31 and/or having a diagnosis of PCOS by age 46. Women without OA+H at age 31 and without diagnosis of PCOS by age 46 were classified as controls. The NFBC1966 flow chart is shown in figure 1. The PCOS population has been validated and published previously.[24–26] Only the women who had a full set of serum, urine and faecal samples available were included in the present study. The BMI-matched control women were included at a ratio of 1:2 (PCOS: controls). The subjects included in the present study had not been on any antibiotics within the previous 3 months at the time of sample collection.

The study participants provided written informed consent for the study and took part on a voluntary basis.

Patient and public involvement statement
The patients, the public or any third parties were not involved in the design, conduct, reporting or dissemination of our research.
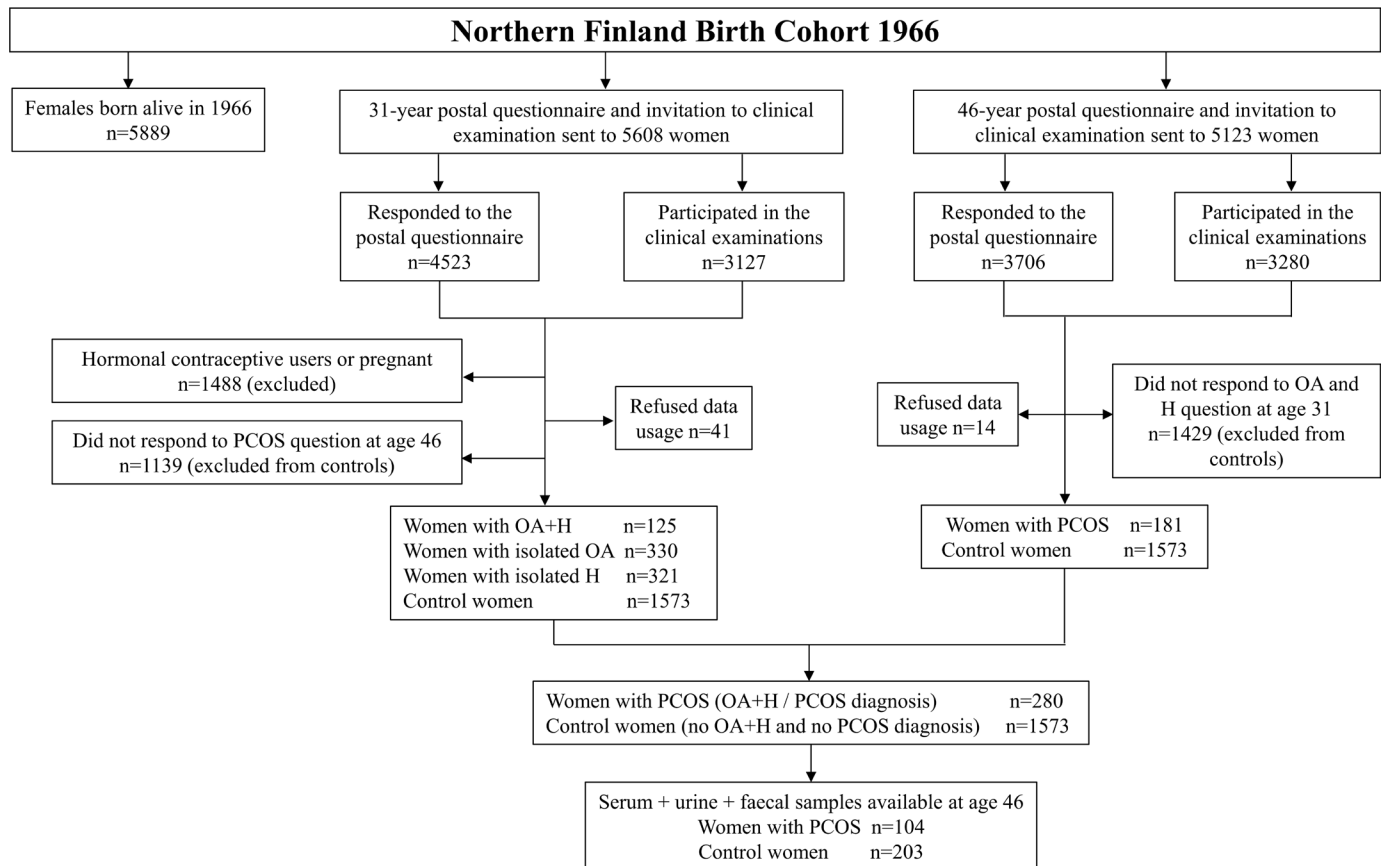
Northern Finland Birth Cohort 1966

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Northern Finland Birth Cohort 1966
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Females born alive in 1966
n=5889

31-year postal questionnaire and invitation to clinical examination sent to 5608 women

46-year postal questionnaire and invitation to clinical examination sent to 5123 women

Responded to the postal questionnaire
n=4523

Participated in the clinical examinations
n=3127

Responded to the postal questionnaire
n=3706

Participated in the clinical examinations
n=3280

Hormonal contraceptive users or pregnant
n=1488 (excluded)

Did not respond to PCOS question at age 46
n=1139 (excluded from controls)

Refused data usage n=41

Refused data usage n=14

Did not respond to OA and H question at age 31
n=1429 (excluded from controls)

Women with OA+H   n=125
Women with isolated OA n=330
Women with isolated H   n=321
Control women   n=1573

Women with PCOS   n=181
Control women   n=1573

Women with PCOS (OA+H / PCOS diagnosis)   n=280
Control women (no OA+H and no PCOS diagnosis)   n=1573

Serum + urine + faecal samples available at age 46
Women with PCOS n=104
Control women   n=203

Figure 1 Flow chart of the study. OA+H, oligo/amenorrhoea and hirsutism; PCOS, polycystic ovary syndrome.

Methods

The study has been conducted and reported applying the criteria of the Strengthening the Reporting of Observational Studies in Epidemiology statement.[27]

Anthropometric measurements

All clinical assessments were performed by an experienced research nurse. Weight and height were measured at the time of clinical examination at age 46. BMI was calculated as the ratio of weight (kg) and height squared (m^2). Waist and hip circumferences were measured (at the level midway between the lowest rib margin and the iliac crest, and at the widest part of the gluteal region respectively) and the waist-to-hip ratio (WHR) was calculated.

Lifestyle and social characteristics

Categorisation of lifestyle variables and social descriptives was based on the responses to the postal questionnaire. Smoking was categorised as non-smoker, former/occasional smoker or active smoker. Alcohol consumption was categorised into three groups: abstainer, low-risk drinker (≤20 g/day) or high-risk drinker (>20 g/day).[28 29] Leisure time physical activity (PA) was self-reported with questions on the frequency and duration of light and brisk PAs, as described previously.[30] PA was calculated as metabolic equivalent of task (MET) scores in hours per week from the reported frequency and duration of leisure-time activities (light PA=3 METs, and brisk PA=5 METs).[31] Marital status was categorised

as either single or in a relationship and education level was classified into three categories as basic, secondary or tertiary (online supplemental table 1).

Oral glucose tolerance test

A standard 2-hour oral glucose tolerance test (OGTT) was performed after an overnight fast (12 hours). Plasma glucose and serum insulin were measured at baseline and at 30, 60 and 120 min after a 75 g glucose intake. Glucose tolerance was classified according to American Diabetes Association guidelines as normal glucose tolerance (NGT) (fasting glucose ≤5.5 mmol/L and 2 hours glucose <7.8 mmol/L), impaired fasting glucose (IFG) (fasting glucose 5.6–6.9 mmol/L and normal 2-hour glucose) and impaired glucose tolerance (IGT) (normal fasting glucose and 2 hours glucose 7.8–11.0 mmol/L).

Laboratory methods

Serum concentrations of FABP2 and zonulin were analysed by ELISA (R&D Systems, Minneapolis, USA, and Immundiagnostik AG, Bensheim, Germany) according to the manufacturers' instructions, with intra-assay and interassay coefficients of variation of 4.1% and 11.1% for FABP2, and 6% and 13.6% for zonulin. Urinary concentrations of indican were measured by colorimetric assay according to the instructions of the manufacturer (Sigma-Aldrich, Missouri, USA), with a detection range between 0.2 and 20 mg/dL.

Table 1 Anthropometric, hormonal and metabolic parameters in the whole study population

Parameter	Control		PCOS		P value
	n	Mean (SD)	n	Mean (SD)	
BMI (kg/m^2)	203	27.9 (5.5)	104	27.9 (5.5)	0.994
Waist (cm)	202	90.0 (13.0)	103	89.8 (13.2)	0.929
WHR	202	0.87 (0.06)	103	0.87 (0.06)	0.923
Systolic BP (mm Hg)	203	124 (18)	104	125 (18)	0.027
Diastolic BP (mm Hg)	203	85 (12)	104	87 (12)	0.040
Testosterone (nmol/L)	202	0.9 (0.3)	104	0.9 (0.3)	0.057
SHBG (nmol/L)	201	58.9 (31.0)	104	55.5 (27.0)	0.289
FAI	201	1.8 (1.1)	104	2.1 (1.1)	**0.011**
Total cholesterol (mmol/L)	203	5.1 (1.0)	104	5.2 (0.7)	0.367
HDL (mmol/L)	203	1.6 (0.4)	104	1.6 (0.3)	0.068
LDL (mmol/L)	203	3.2 (0.9)	104	3.4 (0.8)	0.095
Triglycerides (mmol/L)	203	1.1 (0.5)	104	1.2 (0.6)	**0.039**
hs-CRP (mg/L)	191	1.7 (2.2)	101	1.8 (3.2)	0.805
Fasting glucose (mmol/L)	198	5.3 (0.5)	103	5.6 (1.4)	0.104
Fasting insulin (mIU/L)	198	9.1 (5.3)	102	11.0 (10.4)	0.150
HOMA-IR	197	2.2 (1.5)	102	3.3 (6.3)	0.091
Matsuda index	196	5.3 (3.2)	96	5.8 (3.6)	0.242

Data shown as mean (SD). Statistically significant P values are in bold.
P values according to independent samples t-tests.
BMI, body mass index; BP, blood pressure; FAI, Free Androgen Index; HDL, high-density lipoprotein; HOMA-IR, homoeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C reactive protein; LDL, low-density lipoprotein; SHBG, sex hormone-binding globulin; WHR, waist hip ratio.

Serum testosterone concentrations were analysed by using Agilent triple quadrupole 6410 liquid chromatography–mass spectrometry equipment with an electrospray ionisation source operating in positive-ion mode (Agilent Technologies, Wilmington, Delaware, USA). The normal upper limit for testosterone was 2.3 nmol/L at age 31 and 1.70 nmol/L at age 46, based on 97.5 percentiles calculated in the women of the NFBC1966. Serum sex hormone-binding globulin (SHBG) concentrations were analysed by chemiluminometric immunoassay (Immulite 2000, Siemens Healthcare Diagnostics, Llanberis, UK). The Free Androgen Index was calculated by using the equation T/SHBG (both in nmol/L)×100.

Serum levels of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides were determined by using an enzymatic assay method (Advia1800, Siemens Healthcare Diagnostics). High-sensitivity C reactive protein (hs-CRP) was analysed by nephelometric assay (BN ProSpec, Siemens Healthcare Diagnostics). Plasma glucose was analysed by an enzymatic dehydrogenase method (Advia 1800, Siemens Healthcare Diagnostics) and serum insulin by a chemiluminometric immunoassay (Advia Centaur XP, Siemens Healthcare Diagnostics). Insulin resistance was defined by the homoeostasis model assessment of insulin resistance (HOMA-IR) and insulin sensitivity by the composite Insulin Sensitivity Index or the Matsuda

index, as described earlier.[32 33] All samples were analysed for hormonal and metabolic parameters at NordLab, Oulu (formerly known as Oulu University Hospital laboratory). Of note, the number of subjects varied between analyses owing to lack of measurements in some cases or non-response to some questions.

Statistical analysis
Statistical analyses were performed using SPSS V.25.0 software (IBM). Variables with a skewed distribution were logarithmically transformed before statistical analyses. Independent samples t-tests were used to assess differences between the PCOS and control groups. To evaluate the hormonal and metabolic changes in connection with BMI, the PCOS and control groups were subgrouped as normal weight (BMI<25 kg/m^2), overweight (25–30 kg/m^2) and obese (>30 kg/m^2). One-way analysis of variance (ANOVA) and post hoc Tukey test was used to assess the possible BMI-related changes in the levels of gut permeability markers in the three different BMI subgroups. PA, calculated as MET score, was included in covariate analysis to control for its confounding effects on gastrointestinal permeability markers. Correlations between different parameters were assessed by using Pearson's correlation and adjustment for BMI was carried out by way of partial correlation analyses. The lowest (<25th percentile) and highest (>75th percentile) quartiles for

Table 2 Anthropometric, hormonal and metabolic parameters in the study population stratified according to different BMI categories

Parameter	BMI <25			BMI 25–30			BMI >30		
	Control	PCOS	P value	Control	PCOS	P value	Control	PCOS	P value
BMI (kg/m^2)	22.9 (1.7)	22.9 (1.7)	1.000	27.1 (1.5)	27.1 (1.5)	0.915	35.2 (3.8)	35.2 (4.0)	0.946
Waist (cm)	78.2 (6.1)	79.3 (5.7)	0.362	89.4 (6.1)	87.5 (5.5)	0.112	105.6 (8.9)	107.0 (10.6)	0.545
WHR	0.84 (0.05)	0.84 (0.05)	0.717	0.88 (0.05)	0.87 (0.05)	0.301	0.90 (0.05)	0.91 (0.06)	0.346
Systolic BP (mm Hg)	116 (15)	120 (18)	0.180	124 (15)	126 (17)	0.530	134 (19)	131 (16)	0.397
Diastolic BP (mm Hg)	79 (11)	82 (13)	0.171	85 (10)	87 (12)	0.283	93 (10)	92 (9)	0.477
Testosterone (nmol/L)	0.9 (0.3)	1.0 (0.3)	0.145	0.8 (0.3)	0.9 (0.3)	0.765	0.9 (0.4)	1.0 (0.3)	0.118
SHBG (nmol/L)	69.4 (33.4)	66.5 (23.0)	0.853	57.9 (31.5)	53.3 (26.2)	0.577	46.5 (20.9)	44.4 (28.4)	0.244
FAI	1.4 (0.7)	1.6 (0.6)	0.216	1.9 (1.4)	1.9 (1.0)	0.321	2.1 (1.1)	2.8 (1.3)	**0.031**
Total cholesterol (mmol/L)	5.0 (0.9)	5.3 (0.8)	0.077	5.3 (1.0)	5.0 (0.6)	0.094	5.0 (1.0)	5.3 (0.8)	0.097
HDL (mmol/L)	1.8 (0.4)	1.6 (0.2)	**0.001**	1.6 (0.3)	1.6 (0.4)	0.313	1.4 (0.3)	1.5 (0.4)	0.273
LDL (mmol/L)	2.9 (0.7)	3.4 (0.8)	**0.002**	3.4 (0.9)	3.1 (0.8)	0.166	3.4 (0.9)	3.6 (0.8)	0.205
Triglycerides (mmol/L)	0.8 (0.3)	1.0 (0.4)	**0.014**	1.1 (0.6)	1.1 (0.6)	0.504	1.4 (0.5)	1.5 (0.5)	0.272
hs-CRP (mg/L)	1.0 (2.2)	1.2 (3.2)	0.621	1.2 (1.5)	1.3 (1.1)	0.561	2.9 (2.6)	3.3 (4.5)	0.639
Fasting glucose (mmol/L)	5.1 (0.4)	5.5 (2.1)	0.275	5.3 (0.4)	5.4 (0.5)	0.504	5.6 (0.7)	5.9 (1.1)	0.187
Fasting insulin (mIU/L)	6.3 (2.7)	8.4 (11.5)	0.270	8.2 (3.5)	9.1 (4.3)	0.379	13.9 (6.5)	17.1 (12.3)	0.307
HOMA-IR	1.5 (0.7)	3.1 (9.4)	0.202	2.0 (0.9)	2.2 (1.2)	0.362	3.5 (1.9)	4.9 (5.0)	0.231
Matsuda index	7.1 (3.6)	6.9 (2.6)	0.920	5.1 (2.4)	6.3 (4.5)	0.427	3.0 (1.4)	3.8 (2.6)	0.130

Data shown as mean (SD). Statistically significant P values are in bold.
P value according to independent samples t-test.
BMI, body mass index; BP, blood pressure; FAI, Free Androgen Index; HDL, high-density lipoprotein; HOMA-IR, homoeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C reactive protein; LDL, low-density lipoprotein; SHBG, sex hormone-binding globulin; WHR, waist hip ratio.

each gut permeability marker were estimated, and clinical and hormonal profiles of the study subjects were compared in each quartile. A value of p<0.05 was considered statistically significant.

RESULTS
Characteristics of the study population
The anthropometric, hormonal and metabolic parameters of the study population are shown in table 1. BMI matching was successful, as there was no difference in BMI between the respective study populations. Women with PCOS had higher levels of triglycerides and higher FAI values compared with the control women. The BMI-stratified anthropometric, hormonal and metabolic parameters are shown in table 2. Normal-weight women with PCOS had decreased levels of HDL, and increased levels of LDL and triglycerides compared with normal-weight controls. Obese women with PCOS had higher FAI values compared with obese controls.

Concentrations of serum FABP2 and zonulin, and urinary indican
Serum levels of FABP2 and zonulin and urinary levels of indican were comparable among women with PCOS and controls in the whole study population as well as in the different BMI groups (figure 2). BMI-stratified analysis using one-way ANOVA showed that serum concentrations of zonulin increased with BMI in both women with PCOS (p<0.001) and controls (p=0.001), while no changes were observed in serum levels of FABP2 or urinary levels of indican.

Correlation analyses
Serum levels of zonulin correlated positively with BMI, waist circumference and WHR in both control women and in women with PCOS, but remained statistically significant after adjusting for BMI only in control women (table 3). Moreover, in control women the levels of zonulin correlated with the levels of total cholesterol, LDL, triglycerides, FABP2 and indican, and the Matsuda
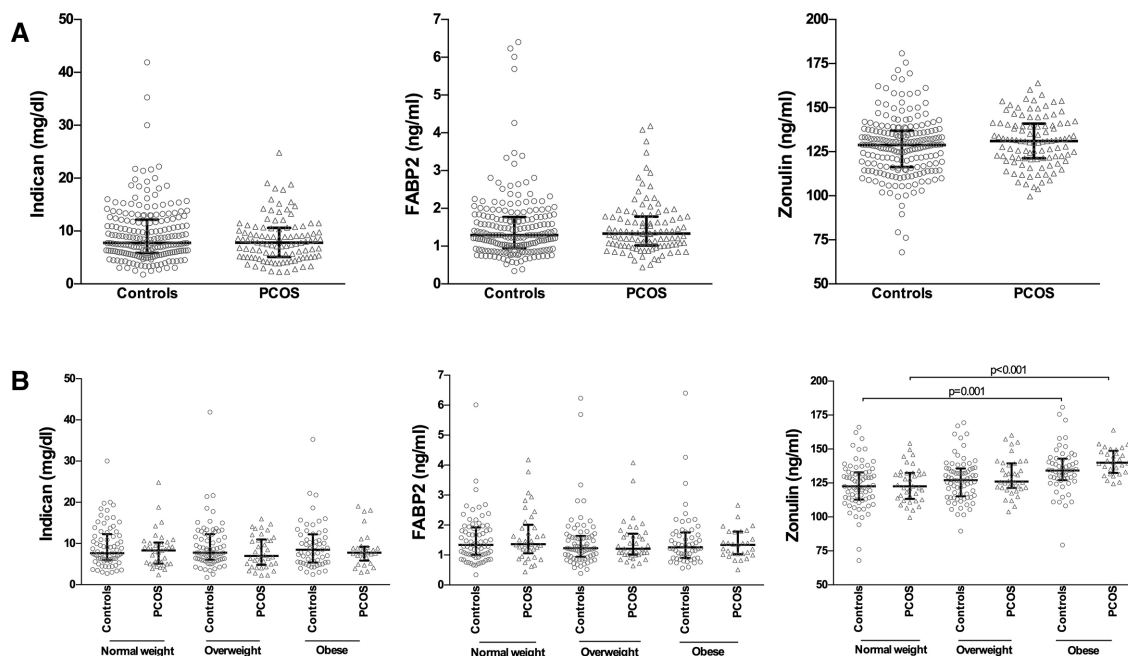
Figure 2 Concentrations of indican, FABP2 and zonulin in (A) whole study population (B) women stratified according to BMI as normal weight, overweight and obese (BMI <25, 25–30 and >30 kg/m², respectively). The horizontal line in each plot corresponds to the median value. The vertical line represents the 25th–75th percentile range. P values according to one-way ANOVA in controls and women with PCOS. ANOVA, analysis of variance; BMI, body mass index; FABP2, fatty acid-binding protein 2; PCOS, polycystic ovary syndrome.

index after adjustment for BMI. Furthermore, in both control women and women with PCOS, serum levels of zonulin correlated positively with levels of hs-CRP, fasting glucose, fasting insulin and HOMA-IR after adjustment for BMI.

Concentrations of FABP2 were negatively correlated with those of testosterone (r=−0.220, p=0.002), and with the FAI (r=−0.228, p=0.001) in the control women after adjusting for BMI. No significant correlations between the levels of FABP2, indican and other parameters were observed in women with PCOS (data not shown).

Concentrations of urinary indican, serum FABP2 and zonulin based on androgen status, glucose tolerance and metabolic syndrome

When women with PCOS were classified on the basis of their androgenic status as normoandrogenic or hyperandrogenic, the levels of indican (8.4±3.4 (mean±SD) vs 8.4±5.0 mg/dL), FABP2 (1.5±0.7 vs 1.5±0.7 ng/mL) and zonulin (129.5±12.4 vs 132.7±5.7 ng/mL) were comparable in the two groups. No significant differences were found in the levels of indican, FABP2 or zonulin when the women were classified on the basis of their glucose tolerance as NGT, IGT or IFG (data not shown). Furthermore, when women with PCOS were classified on the basis of their metabolic syndrome status[34] no significant differences were found in the levels of indican, FABP2 or zonulin (data not shown).

Quartile analysis

When quartiles of hormonal and metabolic parameters in women with PCOS were compared, testosterone levels

were increased in the lower indican quartile compared with the highest quartile (1.0±0.3 vs 0.8±0.4 nmol/L; p=0.030), whereas no differences were observed as regards FABP2. Significant differences were observed in anthropometric, hormonal and metabolic parameters as regards zonulin quartiles (online supplemental table 2).

DISCUSSION

To the best of our knowledge, this is the first study to report on FABP2 and indican in women with PCOS, and the largest study to report on serum levels of zonulin in women with PCOS. Moreover, this is also the first study in which the study subjects have been matched for BMI and age. The present study showed that serum levels of zonulin and FABP2, and urinary levels of indican were comparable in women with PCOS and non-PCOS control women at age 46. Furthermore, serum concentrations of zonulin increased with BMI in both women with PCOS and in controls, while no changes were observed in serum levels of FABP2 or urinary levels of indican.

Data on intestinal permeability in women with PCOS and its role in the pathogenesis of the condition is limited. Previous, although few, studies have analysed gut permeability in women with PCOS by determining serum levels of zonulin and reported conflicting results. In line with the present results, in one study, no differences in the levels of serum zonulin were reported in women with PCOS without metabolic syndrome, compared with controls.[35] In contrast, Zhang et al reported elevated levels of serum zonulin in women with PCOS compared with healthy

Table 3 Correlations between zonulin and various parameters in the study population

Parameter	Controls R	Controls P value	PCOS R	PCOS P value
BMI	0.268	<0.001	0.512	<0.001
Waist	0.314	**<0.001***	0.507	<0.001
WHR	0.286	**<0.001***	0.320	0.001
Testosterone	0.031	0.658	0.159	0.106
SHBG	−0.154	0.029	−0.191	0.053
FAI	0.126	0.074	0.277	0.004
Total cholesterol	0.148	**0.035***	0.171	0.083
HDL	−0.145	0.039	−0.104	0.291
LDL	0.229	**0.001***	0.213	0.030
Triglycerides	0.360	**<0.001***	0.385	<0.001
hs-CRP	0.269	**<0.001***	0.409	**<0.001***
Fasting glucose	0.257	**<0.001***	0.292	**0.003***
Fasting insulin	0.377	**<0.001***	0.459	**<0.001***
HOMA-IR	0.382	**<0.001***	0.463	**<0.001***
Matsuda index	−0.326	**<0.001***	−0.320	0.002
FABP2	0.155	**0.027***	0.054	0.588
Indican	0.159	**0.024***	−0.029	0.769

*P<0.05 after adjustment for BMI.
BMI, body mass index; FABP2, fatty acid-binding protein 2; FAI, Free Androgen Index; HDL, high-density lipoprotein; HOMA-IR, homoeostatic model assessment of insulin resistance; hs-CRP, high sensitivity C reactive protein; LDL, low-density lipoprotein; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; WHR, waist-hip ratio.

controls.[36] Of note, there were significant differences in the metabolic profiles of women with PCOS compared with the controls in their study, whereas in the present study metabolic profiles were comparable in the study groups. This may reflect the greater age of subjects in the present study and the possibility that the groups may become metabolically closer to each other with advancing age.

It has been reported that obesity is associated with increased intestinal permeability[37 38] and studies have shown elevated levels of serum zonulin in obese subjects when compared with non-obese.[12 39] In line with these results, our study showed increased levels of serum zonulin with increasing BMI in both women with PCOS and controls. The highest levels were observed in obese women when compared with normal-weight women, suggesting that serum levels of zonulin may be determined by the degree of obesity, not PCOS per se.

Insulin resistance is a common feature in women with PCOS.[40] Few studies have examined the association of serum zonulin levels with insulin resistance, but the results have been conflicting; both no correlation[35] and a positive correlation[12 36] have been reported. In the present study, serum zonulin levels were correlated with HOMA-IR values in both women with PCOS and in controls after adjustment for BMI. Furthermore, the levels of hs-CRP correlated with those of zonulin in both study groups, suggesting that zonulin may be a marker of insulin resistance and inflammation.

There have been no previous studies in which the levels of FABP2, another marker of intestinal permeability, have been assessed in women with PCOS. Serum levels of FABP2 were similar in women with PCOS and controls, and there was no correlation between concentrations of FABP2 and hs-CRP. It has been suggested that FABP2 is not a direct marker of altered intestinal permeability per se, but a marker of enterocyte injury, and enterocyte damage contributes to altered intestinal barrier function.[16] Our findings suggest that there was no altered intestinal permeability in our PCOS study population, as reflected in the levels of gastrointestinal permeability markers. This may indicate not only that disturbances in intestinal permeability are not associated with the pathogenesis of PCOS, but also the fact that the women in our study did not present with extreme metabolic and/or hormonal dysfunction.

Levels of urinary indican, a marker of dysbiosis, were comparable in women with PCOS and controls, suggesting that dysbiosis of the gut microbiota might not be linked to PCOS in our study population. This is supported by the findings of a microbiome study conducted in the same cohort, which revealed no differences in the gut microbiome profile between women with PCOS and control women at late reproductive age.[41] This is in contrast to the results of studies indicating an association between gut microbiota and PCOS.[22 42] However, it has to be noted that urinary indican is only a surrogate marker in the assessment of dysbiosis and may not reflect the actual status of the microbiome.

There are strengths as well as limitations in the present study. The study population was homogeneous, with all participants being Caucasian and from the unique NFBC 1966. Our study included BMI-matched and age-matched women with PCOS and controls, thus minimising the effects of BMI and age on case–control comparisons. The fact that the diagnosis of PCOS was based on questionnaire data and no ovarian ultrasonography was performed can be considered as a limitation. Women with relatively mild PCOS symptoms could have been included in the study population, possibly decreasing differences between the study groups. However, it has been shown previously that self-reported OA+H can identify women with the typical endocrine and metabolic profiles of PCOS.[24–26] The data may also have a selection bias as only those women with PCOS who had faecal samples were selected from the NFBC1966 for the present study. The controls, however, were chosen to match the BMI of women with PCOS, which minimised the confounding effects of BMI. Due to the matching, the control women also had relatively high BMIs, and consequently more metabolic disturbances, which may narrow differences between the groups. The age of the study subjects at the time of sampling was 46

years and thus some women may have been premenopausal. However, given that menopausal age is delayed in PCOS, the effect of menopause, at least among women with PCOS, was most likely minimal.[43 44]

In conclusion, serum levels of gastrointestinal permeability markers, namely zonulin and FABP2, and levels of urinary indican, a marker of dysbiosis, did not differ between BMI-matched women with PCOS and controls at late reproductive age. Zonulin presented as a metabolic and inflammatory marker, although its concentrations did not associate with PCOS.

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REFERENCES

1. Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 2009;124:3–2.
2. Bischoff SC, Barbara G, Buurman W, *et al*. Intestinal permeability--a new target for disease prevention and therapy. *BMC Gastroenterol* 2014;14:189.
3. March WA, Moore VM, Willson KJ, *et al*. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* 2010;25:544–51.
4. Dumesic DA, Oberfield SE, Stener-Victorin E, *et al*. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocr Rev* 2015;36:487–525.
5. Tremellen K, Pearce K. Dysbiosis of Gut Microbiota (DOGMA)--a novel theory for the development of Polycystic Ovarian Syndrome. *Med Hypotheses* 2012;79:104–12.
6. González F. Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. *Steroids* 2012;77:300–5.
7. Fox CW, Zhang L, Sohni A, *et al*. Inflammatory stimuli trigger increased androgen production and shifts in gene expression in Theca-Interstitial cells. *Endocrinology* 2019;160:2946–58.
8. de Kort S, Keszthelyi D, Masclee AAM. Leaky gut and diabetes mellitus: what is the link? *Obes Rev* 2011;12:449–58.
9. Fasano A, Not T, Wang W, *et al*. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. *Lancet* 2000;355:1518–9.
10. Wang W, Uzzau S, Goldblum SE, *et al*. Human zonulin, a potential modulator of intestinal tight junctions. *J Cell Sci* 2000;113 Pt 24:4435–40.
11. Sapone A, de Magistris L, Pietzak M, *et al*. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes* 2006;55:1443–9.
12. Moreno-Navarrete JM, Sabater M, Ortega F, *et al*. Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. *PLoS One* 2012;7:e37160.
13. Demir E, Ozkan H, Seckin KD, *et al*. Plasma zonulin levels as a non-invasive biomarker of intestinal permeability in women with gestational diabetes mellitus. *Biomolecules* 2019;9:24.
14. Jayashree B, Bibin YS, Prabhu D, *et al*. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Mol Cell Biochem* 2014;388:203–10.
15. Pelsers MMAL, Namiot Z, Kisielewski W, *et al*. Intestinal-Type and liver-type fatty acid-binding protein in the intestine. tissue distribution and clinical utility. *Clin Biochem* 2003;36:529–35.
16. March DS, Marchbank T, Playford RJ, *et al*. Intestinal fatty acid-binding protein and gut permeability responses to exercise. *Eur J Appl Physiol* 2017;117:931–41.
17. Thuijls G, Derikx JPM, van Wijck K, *et al*. Non-invasive markers for early diagnosis and determination of the severity of necrotizing enterocolitis. *Ann Surg* 2010;251:1174–80.
18. Adriaanse MPM, Tack GJ, Passos VL, *et al*. Serum I-FABP as marker for enterocyte damage in coeliac disease and its relation to villous atrophy and circulating autoantibodies. *Aliment Pharmacol Ther* 2013;37:482–90.
19. Verdam FJ, Greve JWM, Roosta S, *et al*. Small intestinal alterations in severely obese hyperglycemic subjects. *J Clin Endocrinol Metab* 2011;96:379.
20. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest* 2011;121:2126–32.
21. Tilg H, Moschen AR. Microbiota and diabetes: an evolving relationship. *Gut* 2014;63:1513–21.
22. Thackray VG, Sex TVG. Sex, microbes, and polycystic ovary syndrome. *Trends Endocrinol Metab* 2019;30:54–65.
23. Lord RS, Bralley JA. Clinical applications of urinary organic acids. Part 2. dysbiosis markers. *Altern Med Rev* 2008;13:292–306.
24. Taponen S, Martikainen H, Järvelin M-R, *et al*. Hormonal profile of women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab* 2003;88:141–7.
25. Taponen S, Ahonkallio S, Martikainen H, *et al*. Prevalence of polycystic ovaries in women with self-reported symptoms of oligomenorrhoea and/or hirsutism: Northern Finland birth cohort 1966 study. *Hum Reprod* 2004;19:1083–8.
26. Taponen S, Martikainen H, Järvelin M-R, *et al*. Metabolic cardiovascular disease risk factors in women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab* 2004;89:2114–8.
27. von Elm E, Altman DG, Egger M, *et al*. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Int J Surg* 2014;12:1495–9.
28. Laitinen J, Pietiläinen K, Wadsworth M, *et al*. Predictors of abdominal obesity among 31-y-old men and women born in northern Finland in 1966. *Eur J Clin Nutr* 2004;58:180–90.
29. Fawehinmi TO, Ilomäki J, Voutilainen S, *et al*. Alcohol consumption and dietary patterns: the FinDrink study. *PLoS One* 2012;7:e38607.
30. Kiviniemi AM, Perkiömäki N, Auvinen J, *et al*. Lifelong physical activity and cardiovascular autonomic function in midlife. *Med Sci Sports Exerc* 2016;48:1506–13.
31. Suija K, Timonen M, Suviola M, *et al*. The association between physical fitness and depressive symptoms among young adults: results of the Northern Finland 1966 birth cohort study. *BMC Public Health* 2013;13:535.
32. Matthews DR, Hosker JP, Rudenski AS, *et al*. Homeostasis model assessment: insulin resistance and beta-cell function from fasting

plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–9.

33 Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462–70.

34 Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;81:19–25.

35 Cetin Z, Kosem A, Can B, *et al*. Serum zonulin level is not elevated in patients with polycystic ovary syndrome without metabolic syndrome. *Arch Gynecol Obstet* 2019;300:1785–90.

36 Zhang D, Zhang L, Yue F, *et al*. Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation. *Eur J Endocrinol* 2015;172:29–36.

37 Lam YY, Ha CWY, Campbell CR, *et al*. Increased gut permeability and microbiota change associate with mesenteric fat inflammation and metabolic dysfunction in diet-induced obese mice. *PLoS One* 2012;7:e34233.

38 Gummesson A, Carlsson LMS, Storlien LH, *et al*. Intestinal permeability is associated with visceral adiposity in healthy women. *Obesity* 2011;19:2280–2.

39 Zak-Gołąb A, Kocełak P, Aptekorz M, *et al*. Gut microbiota, microinflammation, metabolic profile, and zonulin concentration in obese and normal weight subjects. *Int J Endocrinol* 2013;2013:674106.

40 Ovalle F, Azziz R. Insulin resistance, polycystic ovary syndrome, and type 2 diabetes mellitus. *Fertil Steril* 2002;77:1095–105.

41 Lüll K, Arffman RK, Sola-Leyva A, *et al*. The gut microbiome in polycystic ovary syndrome and its association with metabolic traits. *J Clin Endocrinol Metab* 2021;106:858–71.

42 Liu R, Zhang C, Shi Y, *et al*. Dysbiosis of gut microbiota associated with clinical parameters in polycystic ovary syndrome. *Front Microbiol* 2017;8:324.

43 Forslund M, Landin-Wilhelmsen K, Schmidt J, *et al*. Higher menopausal age but no differences in parity in women with polycystic ovary syndrome compared with controls. *Acta Obstet Gynecol Scand* 2019;98:320–6.

44 Li J, Eriksson M, Czene K, *et al*. Common diseases as determinants of menopausal age. *Hum Reprod* 2016;31:2856–64.