Towards a circulating marker of hepato-visceral fat excess: S100A4 in adolescent girls with polycystic ovary syndrome – Evidence from randomized clinical trials

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Summary

S100A4 is a marker of subcutaneous adipose tissue dysfunction. Polycystic ovary syndrome (PCOS) is often driven by hepato-visceral adiposity. PCOS phenotypes are normalized more by reduction of central fat with spironolactone/pioglitazone/metformin (SPIOMET) than by oral contraceptive (OC) treatment. We studied whether circulating S100A4 concentrations are high in adolescents with PCOS and, if so, whether they normalize more with OC or SPIOMET. Assessments included circulating S100A4, endocrine markers, body composition, abdominal fat partitioning in controls ($n = 12$) and girls with PCOS ($n = 51$; age 15.8 y; body mass index [BMI] 24.5 kg/m²), and 1-year changes in girls with PCOS randomized for OC ($n = 27$) or SPIOMET ($n = 24$) treatment. Mean S100A4 concentrations were 71% higher ($P < 0.001$) in girls with PCOS than in controls and associated with hepato-visceral adiposity ($r = 0.47$; $P = 0.001$); S100A4 concentrations decreased more ($P < 0.01$) with SPIOMET, those decreases associating to hepato-visceral fat loss ($r = 0.50$; $P < 0.0001$). S100A4 may become a circulating marker of hepato-visceral fat excess in adolescents with PCOS.

KEYWORDS

liver fat, PCOS, S100A4, visceral fat

1 | INTRODUCTION

S100A4 is a member of the S100 calcium-binding protein family and was first identified and categorized as a metastasis-associated protein.¹ Subsequently, S100A4 was found to be involved in controlling cell growth, differentiation, survival, and migration in different cell types in physiological and pathological conditions, including inflammation, immune response, neuroprotection, and angiogenesis.^{2–4}

Abbreviations: BMI, body mass index; HMW, high-molecular weight; HOMA-IR, homeostasis model assessment-insulin resistance; LDL-cholesterol, low-density lipoprotein cholesterol; MRI, magnetic resonance imaging; OC, oral contraceptive; PCOS, polycystic ovary syndrome; SPIOMET, spironolactone/pioglitazone/metformin; WAT, white adipose tissue

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Recently, S100A4 was identified as an adipokine whose secretion by subcutaneous white adipose tissue (WAT) is increased in case of WAT dysfunction, for example, when there is subcutaneous adipocyte hypertrophy, WAT inflammation, and/or insulin resistance, independently of body mass index (BMI).⁵

Polycystic ovary syndrome (PCOS) is a frequent cause of hirsutism and menstrual irregularity in adolescent girls and young women, is commonly accompanied by insulin resistance with compensatory hyperinsulinemia and hepato-visceral fat excess, independently of BMI,⁶ and is followed by comorbidities such as subfertility and type 2 diabetes.^{7,8} In nonobese adolescent girls with PCOS, a treatment with low-dose spironolactone, pioglitazone, and metformin (SPIOMET) normalizes the PCOS phenotype more broadly than does treatment with an oral contraceptive (OC).⁹

We studied whether circulating S100A4 concentrations are elevated in adolescents with PCOS and, if so, whether they are normalized more with OC or SPIOMET treatment, and whether they relate to central fat excess.

2 | SUBJECTS AND METHODS

Subsequent to the recent report on S100A4 in subcutaneous WAT,⁵ we have added circulating S100A4 as a secondary end point to the registration of two controlled trials (with essentially identical design) primarily exploring ovulation rates after randomized open-label treatment with OC versus SPIOMET in nonobese adolescent girls with PCOS; the first of these trials (ISRCTN29234515) has been completed,⁹ and the second (ISRCTN11062950) is scheduled to be completed in late 2019. Circulating S100A4 was measured in spare serum samples available from 22 (of 36) girls in the first trial, and from 29 (of 29) girls in the second trial (Figure S1), for a total population of 51 girls (average age 15.8 y; BMI 24.5 kg/m²). Both trials were initiated in the Adolescent Endocrinology Unit of Sant Joan de Déu University Hospital, Barcelona, Spain. The inclusion criteria were hirsutism (score >8 on modified Ferriman and Gallwey scale), oligomenorrhea (menstrual intervals >45 d), gynaecological age >2.0 years, and absence of sexual activity (no need for contraception). Exclusion criteria were 21-hydroxylase deficiency; glucose intolerance or diabetes mellitus; evidence of thyroid, liver, or kidney dysfunction; hyperprolactinemia; and prior use of medications affecting gonadal or adrenal function, or carbohydrate or lipid metabolism.⁹ Mediterranean diet and regular exercise were recommended to all participating girls; OC treatment for 1 year consisted of 20 µg of ethinylestradiol plus 100 mg of levonorgestrel for 21/28 days and placebo for 7/28 days; SPIOMET treatment for 1 year consisted of a low-dose combination⁹ of spironolactone 50 mg/d, pioglitazone 7.5 mg/d, and metformin 850 mg/d.

Twelve age-matched, healthy girls (age 15.8 y) recruited in nearby schools served as controls. All had regular menstrual cycles, and none was hirsute or taking medication.

Birth weight and BMI (and their Z-scores) were retrieved, and endocrine-metabolic variables were assessed as described.⁹⁻¹¹ Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated as [fasting insulin in mU/L] × [fasting glucose in mg/dL]/405. Serum S100A4 was measured using a Human Protein S100A4 ELISA kit (CSB-EL02032HU, Cusabio Biotech, Wuhan, China) with a lower detection limit of 0.2 ng/mL and with intraassay and interassay variation coefficients of <0.8% and <10%, respectively. Body composition was assessed by dual X-ray absorptiometry with a Lunar Prodigy and Lunar software (version 3.4/3.5, Lunar Corp, Madison, Wisconsin); abdominal fat (subcutaneous and visceral) and hepatic fat were assessed by magnetic resonance imaging (MRI) using a multiple-slice MRI 1.5 Tesla scan (Signa LX Echo Speed Plus Excite, General Electric, Milwaukee, Wisconsin).⁹ Central fat was arbitrarily defined here as the sum of visceral fat (in cm²) and hepatic fat (in %).

All statistical analyses were performed using SPSS version 23.0 (IBM, Armonk, New York). Baseline differences in S100A4 concentrations between controls and girls with PCOS were tested with unpaired

t test, adjusting for age and BMI. Longitudinal changes in quantitative variables between groups were compared by repeated-measures general linear model. Differences in longitudinal changes between groups were tested by the interaction term among between- and within-subject effects. Associations between quantitative variables were sought by partial correlation analyses after adjustment for potentially confounding factors. $P < 0.05$ was considered statistically significant. Data are presented as mean ± standard error of the mean (SEM).

The studies were conducted after approval by the Institutional Review Board of Sant Joan de Déu University Hospital, after written informed consent by the parents, and after assent by each of the participants.

3 | RESULTS

The overall results are summarized in Table 1. SPIOMET treatment was accompanied by more broadly normalizing effects than OC, including on circulating insulin, high-molecular weight (HMW) adiponectin, ultra-sensitive C-reactive protein (usCRP), and central (hepato-visceral) fat, as previously published.⁹

Figure 1A and 1B depicts that circulating S100A4 concentrations were 71% (or 2.1 Z-score) higher in girls with PCOS than in control girls and associated with hepato-visceral adiposity ($N = 63$; $r = 0.47$; $P = 0.001$).

Figure 1C and 1D shows also that the elevated S100A4 concentrations in girls with PCOS decreased more with SPIOMET than OC treatment and that those decreases associated most closely (Table S1) with the reduction of hepato-visceral fat ($r = 0.50$; $P < 0.0001$). One-year changes in circulating S100A4 correlated also with those in fasting insulin, HOMA-IR, low-density lipoprotein cholesterol (LDL-cholesterol), and HMW adiponectin (Table S1).

In multiple regression analysis, S100A4 and BMI were major determinants of central fat, together explaining 47% of its variance (beta: 0.419, $P < 0.0001$; beta: 0.505, $P < 0.0001$; respectively), adjusting for age as potential confounding factor.

4 | DISCUSSION

Adolescent girls with PCOS—diagnosed by the latest international criteria allowing for a relatively homogeneous phenotype¹²—were found to have elevated concentrations of circulating S100A4, which are lowered by a central fat reducing intervention with SPIOMET for 1 year. Baseline serum S100A4 levels in the total study population associated positively with central fat; in girls with PCOS, on-treatment changes in S100A4 associated most closely with those of markers of insulin resistance and central fat.

Adolescent PCOS appears to be essentially driven by an exhaustion of the capacity to expand subcutaneous WAT in a metabolically safe way, resulting in a lipotoxic state and in lipid deposition in central/ectopic depots (liver and viscera).¹³ In a study of adipose tissue from women with obesity, high presence of S100A4 has been associated with subcutaneous WAT dysfunction, inflammation and insulin resistance, and adipocyte hypertrophy, independently of BMI.⁵ In contrast, S100A4 could be exerting a protective role in mice experiencing

TABLE 1 Auxological, endocrine-metabolic, body composition, and abdominal fat partitioning assessments in girls with PCOS (n = 51)

	OC ^b (n = 27)			SPIOMET (n = 24)		
	Start ^a	1 y	Δ 0-1 y	Start ^a	1 y	Δ 0-1 y
Auxology						
Age	15.7 ± 0.3	16.7 ± 0.3***		15.8 ± 0.3	16.8 ± 0.3***	
Birth weight Z-score	-0.5 ± 0.2			-0.6 ± 0.2		
BMI, kg/m ²	24 ± 1	25 ± 1**	0.8 ± 0.3	25 ± 1	25 ± 1	-0.04 ± 0.41
BMI Z-score	0.8 ± 0.2	1.1 ± 0.3*	0.2 ± 0.1	1.1 ± 0.2	1.0 ± 0.2	-0.1 ± 0.1
Δ Z-score birth weight-BMI	1.3 ± 0.3			1.7 ± 0.3		
Endocrinology and metabolism						
Testosterone, nmol/L ^c	1.2 ± 0.1	0.7 ± 0.1***	-0.5 ± 0.1	1.3 ± 0.1	0.8 ± 0.1***	-0.4 ± 0.1
SHBG, nmol/L ^c	32 ± 3	58 ± 5***	26 ± 5	31 ± 2	29 ± 2	-2 ± 2*****
FAI ^c	4.4 ± 0.4	2.0 ± 0.6**	-2.4 ± 0.7	5.0 ± 0.6	3.2 ± 0.3***	-1.8 ± 0.5
Fasting insulin, pmol/L ^d	86 ± 10	103 ± 12*	17 ± 8	71 ± 9	48 ± 5**	-23 ± 8*****
HOMA-IR ^d	2.6 ± 0.3	3.1 ± 0.4	0.5 ± 0.3	2.1 ± 0.3	1.3 ± 0.1**	-0.8 ± 0.3*****
HDL-cholesterol, nmol/L ^c	1.3 ± 0.0	1.3 ± 0.1	0.0 ± 0.0	1.3 ± 0.1	1.4 ± 0.0*	0.1 ± 0.0****
LDL-cholesterol, nmol/L ^c	2.3 ± 0.1	2.6 ± 0.1**	0.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	0.0 ± 0.1****
Triglycerides, nmol/L ^c	0.6 ± 0.0	0.7 ± 0.0*	0.1 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	0.0 ± 0.1
HMW adiponectin, mg/L ^d	7 ± 1	9 ± 2	2 ± 1	5 ± 1	12 ± 2***	7 ± 2****
usCRP, mg/L ^d	1.1 ± 0.2	2.9 ± 0.5***	1.8 ± 0.4	1.7 ± 0.3	0.8 ± 0.2**	-0.9 ± 0.3*****
S100A4, ng/mL ^c	103 ± 9	104 ± 8	0.3 ± 7.7	113 ± 9	84 ± 6***	-28 ± 6*****
S100A4 Z-score	1.9 ± 0.4	2.0 ± 0.4	0.0 ± 0.4	2.4 ± 0.4	1.0 ± 0.3***	-1.4 ± 0.3*****
Body composition (DXA) ^e						
Bone mineral density, g/cm ²	1.18 ± 0.02	1.19 ± 0.02	0.01 ± 0.01	1.21 ± 0.02	1.21 ± 0.02	0.01 ± 0.01
Lean mass, kg	35 ± 1	36 ± 1	0.9 ± 0.4	36 ± 1	37 ± 1	0.3 ± 0.4
Fat mass, kg	22 ± 2	24 ± 2**	2 ± 1	24 ± 2	24 ± 2	0 ± 1
Abdominal fat, kg	6.0 ± 0.4	6.1 ± 0.4	0.1 ± 0.2	6.4 ± 0.4	6.1 ± 0.4	-0.3 ± 0.2
Abdominal fat (MRI) ^f						
Subcutaneous fat, cm ²	173 ± 20	196 ± 22*	23 ± 10	193 ± 25	196 ± 22	3 ± 14
Visceral fat, cm ²	40 ± 4	43 ± 4	3 ± 3	44 ± 3	35 ± 2*	-9 ± 4****
Hepatic fat, %	17 ± 1	20 ± 1	3 ± 2	18 ± 1	10 ± 1***	-9 ± 1*****
Central fat (hepato-visceral fat)	58 ± 4	63 ± 5	5 ± 4	61 ± 4	44 ± 2***	-17 ± 4*****

Abbreviations: BMI, body mass index; DXA, dual X-ray absorptiometry; FAI, free androgen index; HDL, high-density lipoprotein; HMW, high-molecular weight; HOMA-IR, homeostasis model assessment insulin resistance; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; OC, oral contraceptive; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; SPIOMET, spironolactone, pioglitazone, and metformin; usCRP, ultra-sensitive C-reactive protein.

Values are mean ± standard error of the mean (SEM).

P values are adjusted for age and BMI.

^aNo significant differences between randomized subgroups at start.

^bEthinylestradiol 20 µg plus levonorgestrel 100 mg (21/28 d).

^cIndicative values in healthy adolescents (n = 12; age 15.8 y; BMI 23 kg/m²): testosterone 0.9 ± 0.1; SHBG 61 ± 4 nmol/L; FAI 1.6 ± 0.2; HDL-cholesterol 1.4 ± 0.1 nmol/L; LDL-cholesterol 2.4 ± 0.2 nmol/L; triglycerides 0.7 ± 0.1 nmol/L; S100A4 63 ± 6 ng/mL.

^dIndicative values in healthy adolescents (n = 18; age 16.9 y; BMI 21 kg/m²): fasting insulin 21 ± 2 pmol/L; HOMA-IR 0.7 ± 0.1; HMW adiponectin 13 ± 2 mg/L; C-reactive protein 0.7 ± 0.2 mg/L.⁹

^eIndicative DXA values in healthy adolescents, matched for age and height (n = 41): lean mass 35.1 ± 1.0 kg; fat mass 17.6 ± 1.4 kg.⁹

^fIndicative MRI values in healthy adolescents (n = 15; age 15.7 y; BMI 22 kg/m²): subcutaneous fat 97 ± 11 cm²; visceral fat 32 ± 2 cm²; hepatic fat 12 ± 1%.⁹

*P ≤ 0.05 for within-subgroup changes (Δ) from start.

**P ≤ 0.01 for within-subgroup changes (Δ) from start.

***P ≤ 0.001 for within-subgroup changes (Δ) from start.

****P ≤ 0.05 for between-subgroup changes (Δ) 0-1 year.

*****P ≤ 0.01 for between-subgroup changes (Δ) 0-1 year.

*****P ≤ 0.001 for between-subgroup changes (Δ) 0-1 year.

FIGURE 1 A, Baseline serum S100A4 concentrations in healthy controls (n = 12) and in girls with polycystic ovary syndrome (PCOS, n = 51). B, Correlation between baseline S100A4 levels and central fat in all subjects (n = 63). C, On-treatment (0-1 y) changes in circulating S100A4 concentrations after oral contraception (OC, n = 27) or spironolactone, pioglitazone, and metformin (SPIOMET, n = 24). D, Correlation between on-treatment (0-1 y) changes in circulating S100A4 and changes in central fat in patients with PCOS (n = 51). Red dots correspond to girls receiving OC (n = 27); blue dots represent girls treated with SPIOMET (n = 24). Data are mean ± standard error of the mean (SEM). Central fat is the sum of visceral fat (by magnetic resonance imaging [MRI], in cm²) and hepatic fat (by MRI, in %)

obesity and inflammation induced by a high-fat diet.¹⁴ Here, serum S100A4 was found to be linked to central fat; circulating S100A4 levels in girls with PCOS and central fat excess were increased as compared with healthy controls, and dropped to near-normal levels after insulin sensitization with low-dose SPIOMET, paralleling the fall in hepato-visceral fat, and the improvement of the markers of insulin resistance and adipose tissue inflammation and dysfunction. S100A4 could become a marker of central (hepato-visceral) fat excess, independently of whether the major origin of circulating S100A4 is subcutaneous or nonsubcutaneous adipose tissue. An inflammatory subset of macrophages in the liver may be an alternative or an additional source of circulating S100A4 in girls with hepatic fat excess¹⁵; a partially hepatic origin of circulating S100A4 in girls with PCOS would contribute to explain the fall of circulating S100A4 levels after SPIOMET-enhanced reduction of liver fatness, as well as the absence of such a fall after OC treatment, which fails to reduce liver fatness.

The present findings establish adolescent PCOS as condition combining central fat excess (even in the absence of obesity) with high circulating concentrations of S100A4, a protein that is long known to enhance the metastatic progression of a variety of cancers. These findings may be relevant in view of the evidence that young women with PCOS are at high risk (up to 18-fold) for developing endometrial carcinoma,¹⁶ which happens to be the most BMI dependent among human cancers.^{17,18} If future studies indicate that chronic hepato-visceral adiposity and prolonged elevation of circulating S100A4 should no longer be viewed as innocent epiphenomena but as core PCOS features, then there may be a rationale to explore whether early SPIOMET-like interventions can reduce the incidence and/or improve the outcome of endometrial carcinoma in young women with PCOS¹⁹

and perhaps of other cancers related to hepatic and/or visceral adiposity.^{17,18} The new concept that S100A4 may serve as a key link between hepato-visceral fat excess and cancer merits further testing.

Study limitations include the relatively low number of participants, which may have prevented the detection of smaller differences between subgroups, and the absence of an untreated subgroup. Study strengths include the strict inclusion and exclusion criteria and the availability of a broad spectrum of advanced outcomes allowing to identify associations between circulating S100A4 and surrogates of adipose tissue dysfunction.

In conclusion, S100A4 may become a circulating marker of hepato-visceral fat excess, at least in adolescent girls with PCOS; the pathophysiological relevance of high concentrations of circulating S100A4 remains to be defined, in PCOS and also in other conditions.

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CONFLICTS OF INTEREST

No conflict of interest was declared.

AUTHORS' CONTRIBUTIONS

R. Malpique researched and analysed data, contributed to data interpretation, and wrote the manuscript. D. Sánchez-Infantes contributed to study design, supervised the S100A4 assessments, and wrote the manuscript. C. García-Beltrán researched and analysed data and contributed to data interpretation. A. López-Bermejo contributed to data analysis and interpretation, contributed to study design, and reviewed/edited the manuscript. D. Taxeras performed the S100A4 assessments. F. de Zegher contributed to study design and data interpretation and wrote/reviewed/edited the manuscript. L. Ibáñez contributed to study design, researched data, and wrote/reviewed/edited the manuscript. Each author listed in the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the contents.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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