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Avaliação da função cardíaca e de preditores do desenvolvimento da síndrome dos ovários policísticos em meninas com pubarca precoce

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Esta Tese de Doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de introdução, dois manuscritos sobre o tema da tese e considerações finais:

- Introdução

- Artigo original 1: Association between left ventricular mass, androgens, adiposity, and insulin resistance in girls with precocious pubarche: a case-control study

- Artigo original 2: Risk factors for polycystic ovary syndrome in a cohort of girls with precocious pubarche

- Considerações finais

RESUMO

Pubarca precoce (PP), definida como o surgimento de pelos pubianos antes dos oito anos de idade em meninas após exclusão de causas secundárias, tem sido associada a maior prevalência de componentes da síndrome metabólica e desenvolvimento da síndrome dos ovários policísticos (PCOS) na adolescência. Em mulheres com PCOS, foi relatado aumento da massa ventricular esquerda (MVE) e disfunção diastólica, associados à resistência insulínica (RI).

Os objetivos dos estudos foram: 1) avaliar a MVE e a função cardíaca sistólica e diastólica através de ecocardiografia em meninas com PP e controles, além de analisar a associação entre os parâmetros cardíacos com androgênios e marcadores de RI; 2) determinar a frequência da PCOS em uma coorte de meninas com PP na pós-menarca e avaliar se existem preditores para o desenvolvimento da PCOS.

Variáveis clínicas, hormonais e metabólicas, ecocardiografia e composição corporal foram obtidas em 35 meninas com PP e 35 controles saudáveis pareadas pela idade (artigo 1). Trinta e quatro meninas com mais de dois anos pós-menarca da coorte de PP da Unidade de Endocrinolgia Ginecológica do Hospital de Clínicas de Porto Alegre (UEG/HCPA) foram classificadas em PCOS e não-PCOS e seus dados pré-puberais foram analisados para detectar preditores do desenvolvimento de PCOS e comparados com os de 17 meninas controles saudáveis da mesma idade (artigo 2).

Após ajuste para gordura corporal total, a MVE foi maior no grupo PP (97,31 ± 33,37 vs. 81,25 ± 19,06 g, p = 0,017), bem como a onda A’ (5,66 ± 1,34 vs. 5,09 ± 0,98 cm / s, p = 0,025), uma medida da função diastólica. O índice de androgêncios livres (FAI) e gordura corporal total foram preditores independentes de maior MVE e, juntamente com HOMA-IR contribuíram com 72% da variabilidade da MVE no grupo PP (artigo 1). No

segundo artigo, 44,1% das meninas com PP foram diagnosticadas com PCOS na pós-menarca. Na pré-puberdade, esse grupo apresentou maior índice de massa corporal (IMC) e HOMA-IR em relação às controles, bem como níveis mais elevados de testosterona, escore de hirsutismo e insulina em jejum quando comparadas às não-PCOS e às controles. O risco para o desenvolvimento de PCOS entre as PPs esteve associado ao IMC z-escore ≥ 2 (odds ratio [OR] = 4; intervalo de confiança 95% [IC] 1.33 – 18.66); escore de Ferriman-Galwey ≥ 4 (OR = 2,7; IC 95% 1,15-5,14); HOMA-IR $\geq 2,42$ (OR = 7; IC 95% 1,39-12,0) e volume ovariano $\geq 1,17$ mL (OR = 8; IC 95% 1,60-39,9) no período pré-puberal.

Em conclusão, meninas com PP apresentaram maior MVE associada aos níveis de androgênios, RI e gordura corporal total. Além disso, foi encontrada alta freqüência de PCOS na coorte de PP da UEG/HCPA. Obesidade, maior escore de hirsutismo, RI e volume ovariano na pré-puberdade foram preditores de desenvolvimento da PCOS na adolescência.

Palavras-chave: pubarca precoce, adrenarca precoce, risco cardiovascular, síndrome dos ovários policísticos, fatores de risco, resistência insulínica, hiperandrogenismo, adiposidade.

ABSTRACT

Precocious pubarche (PP) in girls, defined as idiopathic appearance of pubic hair before the age of eight years, has been associated with higher prevalence of components of metabolic syndrome and post pubertal development of polycystic ovary syndrome (PCOS). Increased left ventricular mass (LVM) and diastolic dysfunction have been reported in women with PCOS associated with insulin resistance (IR).

The aims of the studies were: 1) to assess LVM and cardiac systolic and diastolic function using echocardiography in girls with PP and controls, and to analyze the relationship between cardiac parameters with androgens and IR; 2) to determine the frequency of PCOS in a cohort of postmenarcheal PP girls and to assess whether possible predictors exist for development of PCOS

Clinical, hormonal and metabolic profiles, echocardiography and body composition were examined in 35 PP girls and 35 healthy age-matched controls (article 1). Thirty-four postmenarcheal girls with PP of the Gynecological Endocrinology Unit, Division of Endocrinology of Hospital de Clínicas de Porto Alegre (GEU/HCPA) clinic cohort were classified as PCOS and non-PCOS and prepubertal data were analyzed to detect PCOS development predictors and compared with data of 17 age-matched controls (article 2).

After adjusting for total body fat, LVM was higher in the PP group (97.31 ± 33.37 *vs.* 81.25 ± 19.06 g, p = 0.017) as well as A' wave (5.66 ± 1.34 vs. 5.09 ± 0.98 cm/s, p=0.025), a measurement of diastolic function. Free androgen index (FAI) and total body fat were independent predictors of higher LVM, and together with HOMA-IR contributed with 72% of LVM variability in the PP group (article 1). In the second article, fifteen (44.1%) PP girls were classified as PCOS and had higher body mass index standard deviation score (BMI SDS) and HOMA-IR than controls, as well as higher testosterone, hirsutism score and fasting

insulin than non-PCOS girls and controls in prepubertal period. The risk for PCOS development among PP girls increased with prepubertal BMI SDS ≥ 2 (odds ratio [OR] = 4; 95% confidence interval [CI] 1.33 – 18.66), Ferriman-Galwey hirsutism score ≥ 4 (OR = 2.7; 95% CI 1.15 - 5.14), HOMA-IR ≥ 2.42 (OR = 7; 95% CI 1.39 - 12.0) and ovarian volume ≥ 1.17 mL (OR= 8; 95 CI 1.60 – 39.9).

In conclusion, PP girls had greater LVM associated with higher androgen levels, IR, and total body fat, occurred early in pubertal development. In addition, we found high frequency of PCOS in Southern Brazil PP cohort. Obesity, clinical androgenism, IR and ovarian volume were predictors of development of PCOS later.

Keywords: precocious pubarche, premature adrenarche, cardiovascular risk, polycystic ovary syndrome, risk factors, insulin resistance, hyperandrogenism, adiposity.

SUMÁRIO

INTRODUÇÃO

A pubarca precoce (PP) é definida como o surgimento de pelos pubianos antes dos oito anos de idade nas meninas e nove anos nos meninos, após exclusão de outras causas de desenvolvimento puberal e hiperandrogenismo, tais como: puberdade precoce central (PPC), hiperplasia adrenal congênita forma não clássica (HAC-NC), tumores produtores de androgênios adrenais ou gonadais, exposição a androgênio exógeno, síndrome de Cushing. Normalmente é atribuída à maturação prematura da zona reticular do córtex adrenal, chamada adrenarca prematura (AP), cujas outras manifestações clínicas são: odor axilar, pelos axilares, acne/comedões e seborréia[1-3]. A pubarca é o sinal clínico mais comum da AP, tem prevalência de 0,8 – 17.7% [4, 5] variando conforme a população estudada, sendo mais comum em afrodescendentes e em meninas, tendo uma proporção de até 10:1 em relação aos meninos[1]. Bioquimicamente a AP se manifesta como níveis de androgênios, principalmente dos precursores de androgênios adrenais (PAA) (dehidroepiandrosterona [DHEA] e sulfato de dehidroepiandrosterona [S-DHEA]) acima dos valores pré-puberais, porém compatíveis com o estágio puberal de pelos pubianos[1-3]. A etiologia da AP não é definida, mas é independente da maturação gonadal e provavelmente multifatorial (genética e ambiental)[6]. Crianças nascidas pequenas para idade gestacional (PIG)[7] ou que apresentam excesso de peso na infância possuem maior prevalência de elevação precoce dos níveis de S-DHEA [8, 9]. Os casos que apresentam manifestações androgênicas prematuras com níveis de PAA pré-puberais devem-se ao aumento na atividade de enzimas periféricas ou à maior afinidade entre os androgênios e seus receptores periféricos[2].

As crianças com AP podem apresentar aceleração na idade óssea e no crescimento, além de pequena antecipação na telarca e/ou menarca devido à conversão periférica dos

androgênios em estrogênio e a associação com a obesidade[10-12]. Porém evidências demostram não haver impacto significativo na progressão normal da puberdade e na altura final dessas crianças[13].

Normalmente a AP é considerada uma variante do desenvolvimento puberal e não requer uma forma especial de tratamento, entretanto um número crescente de estudos tem demonstrado que a elevação precoce de androgênios possui associação com alterações metabólicas[1, 2, 14] e pode preceder a síndrome dos ovários policísticos (PCOS)[15-17].

Estudos em diferentes populações demonstraram que meninas com AP, a maioria apresentando como manifestação clínica a PP, possuem maior prevalência de sobrepeso/obesidade[18], adiposidade visceral e resistência insulínica (RI)[1, 2, 19] quando comparadas a meninas da mesma idade. Foi encontrado aumento da frequência de síndrome metabólica em comparação a meninas pareadas pelo índice de massa corporal (IMC): 24 *vs.* 10% (pelos critérios do *National Cholesterol Education Program Adult Treatment Panel III* [NCEP/ATP III] modificado) e 16 *vs*. 5% (pelos critérios da Organização Mundial de Saúde [OMS])[14]. Em uma coorte brasileira com 52 meninas com PP, foi encontrada prevalência aumentada de obesidade (25 *vs*. 4 %) e dislipidemia (63,5% *vs*. 48,8 %) entre as PPs quando comparadas à população de referência, embora não se tenha achado aumento na prevalência de RI[18]. Outros autores descreveram níveis mais elevados de fator de crescimento semelhante à insulina (IGF-1)[20], leptina[8, 21] e marcadores inflamatórios nas meninas com AP[3, 22].

Embora não haja na literatura estudos avaliando função cardíaca através da ecocardiografia em meninas com PP, alguns autores demonstraram alterações ecocardiográficas subclínicas em mulheres jovens com PCOS[23-25], apesar desses achados não serem encontrados em todos os estudos[26-28]. Orio et al. demonstraram aumento da massa ventricular esquerda (MVE) e redução de função diastólica em mulheres com PCOS com

idade média de 24 anos quando comparadas a controles da mesma idade. As alterações foram relacionadas à RI e independentes do peso[23]. Outro estudo reportou alterações subclínicas na função sistólica e diastólica do ventrículo esquerdo em mulheres obesas com e sem PCOS e as associou à RI secundária à obesidade[24]. No estudo *Coronary Artery Risk Development in Young Adults* (CARDIA) que estudou grupos de mulheres com PCOS, oligomenorréia isolada e hirsutismo idiopático (HI), foi encontrado aumento da MVE no grupo PCOS e HI. Porém, após ajuste para peso, pressão arterial, perfil lipídico, glicemia e insulinemia, a MVE permaneceu aumentada apenas no grupo PCOS[25].

A MVE foi preditora de eventos cardiovasculares fatais e não fatais em dois grandes estudos, o Framingham Heart Study[29] e o Multi-Ethnic Study of Atherosclerosis (MESA)[30]. A hipertrofia ventricular esquerda que, dentre outras repercussões clínicas, pode levar à disfunção sistólica e diastólica, está associada principalmente à hipertensão arterial sistêmica e à obesidade[31], além de estudos mostrarem associação com RI[32, 33] e componentes da síndrome metabólica[34].

Com relação aos estudos em meninas com PP que reportaram alterações encontradas na PCOS, Ibanez et al. publicaram uma série de artigos demonstrando que meninas Catalãs com PP, especialmente as com história de baixo peso ao nascer, possuíam níveis mais elevados de androgênios no momento do diagnóstico e ao longo da puberdade[35], bem como de 17-hidroxiprogesterona (17-OHP) e androstenediona (A4) após estímulo com hormônio liberador de gonadotrofina (GnRH) no estágio 4 de mamas de Tanner (M4)[36], sugerindo hiperandrogenismo funcional ovariano (HFO). Quando os autores compararam 35 adolescentes com PP a 12 controles pareadas pela idade, encontraram uma prevalência de 45% de hirsutismo, oligomenorréia e hiperandrogenemia entre as PPs[37]. Em outro estudo, o mesmo grupo documentou aumento da frequência de ciclos anovulatórios nas PPs com mais

de 3 anos após a menarca[38]. Posteriormente, Battaglia et al. reportaram uma frequência de 41% de alterações ovarianas ao ultrassom (aumento de volume ovariano, presença de folículos) em meninas italianas pré-puberes com PP[39]. Um estudo reportou níveis elevados do hormônio anti-mulleriano[40], frequentemente aumentado na PCOS devido à maior foliculogênese, enquanto outro não reproduziu este achado, ambos em meninas no período pré-puberal[41].

A PCOS é o distúrbio endócrino mais comum em mulheres na idade reprodutiva com prevalência entre 6 – 20%, variando conforme a população estudada e os critérios diagnósticos utilizados[42]. Sua apresentação clínica é heterogênea e relacionada ao hiperandrogenismo, à irregularidade menstrual, ovulatória e reprodutiva, bem como à presença frequente de obesidade e outras alterações metabólicas, tornando essas mulheres um grupo de maior risco para doenças cardiovasculares[42-44].

O diagnóstico de PCOS, em mulheres adultas, pode ser realizado de acordo com três principais consensos: National Institute of Health (NIH, 1990)[45], Rotterdam (2003)[46] e Androgen Excess Society (AES, 2006)[47]. Segundo o consenso de Rotterdam, o diagnóstico é feito na presença de dois de três critérios: 1) hiperandrogenismo clínico e/ou laboratorial; 2) oligo-amenorréia/anovulação crônica; 3) aparência policística dos ovários (PCO) na ultrassonografia[46]. Entretanto, o diagnóstico segundo o NIH requer apenas oligomenorréia e hiperandrogenismo[45], enquanto que para o consenso da AES necessita haver a combinação de hiperandrogenismo clínico e/ou bioquímico com anovulação ou aparência policística dos ovários[47]. Todos requerem exclusão de outras causas de hiperandrogenismo e irregularidade menstrual, tais como: HAC-NC, síndrome de Cushing, tumores secretores ou fontes exógenas de androgênios, hiperprolactinemia, distúrbios da tireoide, insuficiência ovariana primária ou outras causas de hipogonadismo[45-47].

Apesar das manifestações clínicas da PCOS geralmente iniciarem na adolescência associadas ao aumento da RI e da atividade do IGF-1[15, 16, 48], o diagnóstico de PCOS nesta faixa etária ainda é controverso, uma vez que muitas das manifestações da síndrome se sobrepõem a mudanças fisiológicas da idade (acne, irregularidade menstrual nos primeiros anos após a menarca, etc.)[49]. A prevalência de PCOS na adolescência varia entre 0,56 – 3% conforme a população e critérios usados[50]. Devido a isso, recomenda-se que o diagnóstico seja feito com no mínimo dois anos após a menarca e que haja a presença concomitante dos três critérios (hiperandrogenismo, irregularidade menstrual e PCO)[2]. Com relação ao critério de PCO, o recomendado é usar o volume ovariano maior ou igual a 10 mL uma vez que múltiplos cistos são achados frequentes na adolescência[43, 51, 52]. Contudo, evidências demonstram que meninas que apresentam hiperandrogenismo e irregulidade menstrual no período pós-puberal, independente do critério ultrassonográfico, frequentemente evoluem com a confirmação do diagnóstico de PCOS na vida adulta[53]. Portanto, utilizar os critérios do NIH[45] para diagnóstico de PCOS na adolescência poderia ser mais sensível e proporcionar um acompanhamento mais próximo dessas meninas até a confirmação na vida adulta.

A etiologia da PCOS ainda é desconhecida. O *link* patogenético comum entre PP e PCOS parece envolver RI, hiperandrogenismo e aumento de adiposidade [1, 2, 54]. Esses fatores podem ter origem genética ou ambiental e estão interligado, um podendo desencadear ou agravar o outro[55]. A RI pode ser genética, envolvendo defeitos nos primeiros passos na sinalização ou no receptor de insulina[56], ou secundária à obesidade. A hiperinsulinemia compensatória aumenta a produção ovariana e adrenal de androgênios e reduz a produção hepática da globulina ligadora dos esteroides sexuais (SHBG), aumentando a fração livre e, portanto, ativa dos androgênios[54]. O hiperandrogenismo pode ser primário, causado por defeitos enzimáticos ou secundário ao hiperinsulinismo e à obesidade[54, 57]. A obesidade por sua vez, pode ser exógena ou agravada pelo hiperinsulinismo e hiperandrogenismo. Ela

também pode produzir maiores níveis de androgênios, provavelmente mediado por hiperinsulinismo e níveis elevados de IGF-1 que aumentam a esteroidogênese nas células adrenais e da teca ou ainda por maior conversão periférica de precursores a androgênios ativos[58].

Além disso, a teoria da programação fetal sugere que mudanças no microambiente intrauterino ocasionem alterações epigenéticas no indivíduo[57, 59-61]. A adaptação fetal, vantajosa na vida pré-natal, pode tornar-se desajustada ao ambiente pós-natal, aumentando o risco de doenças cardiovasculares/metabólicas ao longo da vida[62]. Em fetos com crescimento intrauterino restrito (CIUR), para garantir a sobrevivência e a redução de gasto energético, o fluxo sanguíneo fetal é direcionado para órgãos essenciais (coração, cérebro e glândulas adrenais). Ocorre aumento da produção de glicocorticoides, devido à hiperatividade do eixo hipotálamo-hipófise-adrenal, e redução da secreção de insulina com aumento da resistência periférica à mesma, redistribuindo a glicose disponível para o cérebro e coração em detrimento dos tecidos periféricos, como o músculo esquelético (hipótese do fenótipo poupador)[63]. Após o nascimento, a maioria das crianças nascidas PIG apresenta um padrão rápido de crescimento e de ganho de peso (*catch up growth*) durante os primeiros anos de vida[64]. O ganho de peso exagerado está associado à hiperinsulinemia, obesidade central, disfunção de tecido adiposo[57], maiores níveis de S-DHEA peripuberais[7], início precoce da adrenarca[3, 7, 9, 65], PCOS[66], síndrome metabólica e doenças cardiovasculares na vida adulta[62, 67, 68]. Além disso, o excesso de esteroides (glicocorticoide ou androgênios) na vida intrauterina pode provocar desregulação dos eixos hipotálamo-hipófise-adrenal e/ou gonadal e, na infância e adolescência, levar à maturação prematura adrenal e/ou ao aumento dos níveis de hormônio luteinizante (LH), maior secreção de androgênios pelas células da teca e prejuízo na foliculogênese/ ovulação na vida adulta[61, 69]. Contudo, nem todas as pacientes com PP ou PCOS nascem com peso inadequado ou são filhas de mães hiperandrogênicas. Além disso, há

estudos que não demonstraram associação entre o baixo peso ao nascer e o desenvolvimento de PP[70] ou PCOS ao longo da vida[71] reforçando o conceito da etiologia multifatorial da PCOS[66].

Em conclusão, as evidências demonstram que meninas com PP possuem maior prevalência de alterações metabólicas e desenvolvimento de PCOS na adolescência que a população em geral. O aumento de fatores de risco cardiovascular em mulheres com PCOS já está estabelecido[72]. Neste contexto, os objetivos dos nossos estudos foram analisar as dimensões do ventrículo esquerdo e função cardíaca das meninas com PP quando comparadas a controles, reproduzindo estudos já existentes em PCOS, e estudar a frequência de PCOS nas meninas com PP na nossa população, além de identificar fatores preditores para o desenvolvimento da síndrome.

Entre os anos 1998 e 2015, 83 meninas consultaram no ambulatório de Endocrinologia Ginecológica do Serviço de Endocrinologia do Hospital de Clínicas de Porto Alegre (UEG/HCPA) devido ao surgimento de pelos pubianos antes dos 8 anos de idade. Dessas, 68 tiveram diagnóstico de PP e foram acompanhadas através de consultas médicas e exames complementares periódicos, cujos dados foram registrados em protocolo específico, constituindo-se uma coorte de meninas com pubarca precoce da UEG.

Entre 2010 e 2014, foi realizado um estudo de caso-controle aninhado à coorte, no qual participaram as meninas com PP que possuíam idade entre 5 e 15 anos, não obesas, sem uso de medicamentos ou comorbidades e meninas saudáveis que frequentaram os ambulatórios do HCPA devido à vacinação, pós-operatório tardio de pequenas cirurgias ou atendimento psicológico. Foi realizada avaliação ecocardiográfica pela mesma cardiologista pediátrica não conhecedora do diagnóstico, em 35 meninas com PP e 35 meninas controles pareadas pela idade, com o objetivo de avaliar a MVE, bem como a função sistólica e

diastólica cardíaca, além de possíveis associações dos parâmetros com RI e androgênios (artigo original 1).

No início do ano de 2015, com o objetivo de avaliar a frequência de PCOS na coorte de PP, foram aplicados os critérios diagnósticos de PCOS naquelas meninas com mais de 2 anos de menarca, separando-as nos grupos PCOS e não-PCOS. Foram analisados os dados pré-puberais das meninas que tinham desenvolvido PCOS em comparação com as não-PCOS com objetivo de identificar fatores preditores para o desenvolvimento da síndrome na PP. Além disso, os dados pré-puberais foram comparados com um grupo de meninas saudáveis pareadas pela idade (artigo original 2).

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Capítulo I

Artigo Original 1:

Association between left ventricular mass, androgens, adiposity, and insulin resistance in girls with precocious pubarche: a case-control study

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Association between left ventricular mass, androgens, adiposity, and insulin resistance in girls with precocious pubarche: a case-control study

Short title: Subclinical cardiovascular alterations in precocious pubarche

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ABSTRACT

Objective: Precocious pubarche (PP) has been linked to higher prevalence of metabolic disturbances and polycystic ovary syndrome (PCOS). The aim of the study was to assess echocardiographic parameters in PP girls and to analyze their relationship with androgens and insulin resistance (IR).

Design: Case-control study.

Patients: 35 PP girls and 35 healthy age-matched controls.

Measurements: Clinical, hormonal and metabolic profiles, echocardiography, body composition, and oral glucose tolerance test.

Results: Chronological age (10.04 ± 2.6 years in PP *vs.* 10.13 ± 2.56 years in controls, p=0.227), and pubertal stage at the time of the study were similar between the groups. PP girls had higher free androgen index (FAI) [1.39 (0.48 – 3.64) vs. 1.06 (0.39 – 1.7), p = 0.005] and lower QUICKI (0.33 ± 0.020 *vs.* 0.35 ± 0.03, p = 0.021). However, HOMA-IR was not significantly different between the groups [2.79 (1.84 – 4.05) vs. 2.15 (1.09 – 3.23), p = 0.085]. After adjusting for total body fat, left ventricular mass (LVM) was higher in the PP group (97.31 ± 33.37 *vs*. 81.25 ± 19.06 g, p = 0.017) as well as A' wave (5.66 ± 1.34 vs. 5.09 ± 0.98 cm/s, p=0.025), a measurement of diastolic function. FAI and total body fat were independent predictors of higher LVM, and together with HOMA-IR contributed with 72% of LVM variability in the PP group.

Conclusion: In this study with PP girls, greater LVM, associated with higher androgen levels, IR, and total body fat, occurred early in pubertal development.

INTRODUCTION

Precocious pubarche (PP) in girls refers to the appearance of pubic hair before 8 years of age in the absence of central precocious puberty, non-classical congenital adrenal hyperplasia, androgen-secreting tumors, or exogenous sources of androgens.[1] It is the most pronounced sign of premature adrenarche (PA), a condition in which the levels of adrenal androgen precursors rise above the usually low prepubertal levels.[2,3]

PA is generally regarded as a benign condition; however, in girls, especially in those presenting precocious pubic hair,[2] PA has been linked to higher risk of metabolic disturbances,[2,3] including polycystic ovary syndrome (PCOS).[1,4-6] Girls born small for gestational age manifest a high incidence of PA[7,8] and may, later in adolescence, develop PCOS.[9] Frequent metabolic disturbances found in PCOS, such as overweight or obesity, hyperinsulinism, dyslipidemia, and chronic inflammation are also prevalent among adolescents with PP.[2,3]

Increased left ventricular mass (LVM) [10-12] and subclinical diastolic dysfunction have also been reported in young women with PCOS[10, 11] independently of body weight.[10,12] These cardiac changes have been associated, at least in part, with insulin resistance (IR).[10,11] While other studies did not confirm a PCOS-related increase in LVM,[13,14] some diastolic dysfuntion parameters have been connected with IR and central obesity in both PCOS and non-PCOS women.[15]

In the general population, left ventricular hypertrophy (LVH) is associated with hypertension, obesity, and IR, and has been shown to be an important predictor of cardiovascular morbidity and mortality.[16,17] Higher LVM in young women may be signaling

early remodeling that precedes the onset of LVH and cardiac dysfunction.[12] Because common features of PCOS, such as overweight or obesity and IR, are also prevalent among adolescents with PP,[2,3] we hypothesized that girls with PP might also present increased LVM and subtle changes in diastolic function. However, no studies are available in the literature assessing echocardiographic parameters in this population.

Therefore, the aims of the present study were: 1) to compare LVM using echocardiography in girls with PP and in healthy controls; 2) to assess systolic/diastolic function using echocardiography in girls from both groups; and 3) to analyze whether LVM is associated with androgen levels, adiposity, and IR.

SUBJECTS AND METHODS

Subjects

Forty-six girls with a history of PP, aged 5-15 years and consulting with or referred to the outpatient Gynecological Endocrinology clinic at Hospital de Clínicas de Porto Alegre (HCPA) between January 2010 and June 2014 were eligible for this case-control study. Subjects were excluded if they had central precocious puberty (n=2), non-classical congenital adrenal hyperplasia (NC- CAH) (n=2), androgen producing tumors, or other secondary causes of PP. Additional exclusion criteria applied to both girls with PP and controls were body mass index (BMI) $\geq 97^{th}$ percentile (n=7), heart or endocrinological comorbidities, or treatment with medications that interfere with biochemical analysis within 3 months before the study (i.e. hormonal contraceptive, metformin, antiandrogens, glucocorticoids). After application of exclusion criteria, 35 girls with idiopathic PP were included in this study. The control group

consisted of 35 age-matched healthy girls seen in pediatric/adolescent outpatient clinics and a primary care unit at HCPA for vaccination, late postoperative follow-up (i.e., tonsillectomy among others), and psychological assessments.

Approval for this study was obtained from the Institutional Review Board and the local Ethics Committee. Written informed consent was obtained from every subject or from a legal guardian.

Study protocol

Pubertal development was scored according to Tanner staging.[18] Standing height and weight were measured to calculate body mass index [BMI (kg/m^2) = weight /height^2]. Percentile and standard deviation score (SDS) were determined as recommended by the World Health Organization.[19] Blood pressure was measured after a 10-minute rest, in the sitting position, with feet on the floor and the arm supported at heart level. Waist circumference was obtained at the midpoint between the lower rib margin and the iliac crest with the subjects in a standing position. The result was divided by height. Risk for central obesity was defined as a waist circumference-to-height ratio $\geq$ 0.5 cm/m.[20] Skeletal maturation was staged according to Greulich and Pyle.[21] Hirsutism was evaluated according to the Ferriman and Gallwey method.[22]

Body composition was assessed with the use of electrical bioimpedance (Inbody 230; Biospace; Seoul, Korea). This device directly measures the impedance of each body segment to 20 kHz and 100 kHz. Patients were assessed in the morning after a fast of $\geq$ 4 hours, with an empty bladder, wearing a standard lab coat and not carrying any metal objects. Participants were instructed not to practice vigorous exercise the day before and on the day of the test.

Blood samples were obtained after an overnight fast from an antecubital vein between 8 a.m. and 10 a.m. Glucose and insulin levels were assessed at 0, 30, 60, 90, and 120 minutes after ingestion of 1.75 g/m^2 (maximum 75 g) of oral anhydrous glucose for the oral glucose tolerant test (OGTT). Postmenarchal girls were studied in the follicular phase of their menstrual cycles (days 2–8) or on any day if they were amenorrheic. Upon diagnosis, all girls with PP underwent the ACTH and GnRH tests to rule out 21-hydroxylase deficiency and central puberty respectively.[23]

The Haycock formula for body surface area (BSA) [BSA (m^2) = 0.024265 x weight (kg)] $^{0.5378}$ x height (cm)$^{0.3964}$] was used to adjust echocardiographic parameters.[24] The free androgen index (FAI) was estimated by the formula: [total testosterone (nmol/L) / SHBG (nmol/L)] x 100. To estimate insulin resistance, the homeostasis model assessment index (HOMA-IR) was calculated by multiplying insulin (mUI/L) by glucose (mmol/L) and dividing the product by 22.5. The quantitative insulin sensitivity check index (QUICKI) was calculated as follows: 1/[log fasting insulin (mUI/L) + log fasting glucose (mg/dL)]. The results of the OGTT were used to calculate the area under the curve (AUC) for glucose and insulin. Low-density lipoprotein (LDL) cholesterol was estimated indirectly using the Friedewald formula: total cholesterol – high-density lipoprotein (HDL) cholesterol – (triglycerides/5).

Assays

Total testosterone (TT) levels were measured by chemiluminescence (Centaur XP Siemens), with a sensitivity of 0.35 nmol/L and intra- and interassay coefficients of variation (CVs) of 3.3% and 7.5% respectively. Sex hormone-binding globulin (SHBG) was measured by chemiluminescence (Immulite 2000 Siemens), with a sensitivity of 0.02 nmol/L and intra-

and interassay CVs of 5.3% and 6.6%, respectively. Plasma insulin levels were measured by electrochemiluminescence (Centaur XP Siemens), with a sensitivity of 3.47 pmol/L and intra- and interassay CVs of 2.8% and 2.1% respectively. Total cholesterol, HDL cholesterol, triglycerides, and glucose were determined by colorimetric-enzymatic methods (Advia 1800 Siemens). Dehydroepiandrosterone sulfate and androstenedione (A4) were measured by chemiluminescence (Immulite 2000 Siemens). High-sensitivity C-reactive protein (hs-CRP) was determined by turbidimetry (Advia 1800 Siemens), and 17-hydroxyprogesterone (17-OHP) by radioimmunoassay (Genesys LTi 1001).

Echocardiography

M-mode, two-dimensional and Doppler echocardiographic studies were performed by the same pediatric cardiologist (CF) who was blinded with respect to patients and controls. Inter and intra-operator variability was 1.7% for this operator in a previous study.[25] All echocardiographic evaluations were obtained in the left lateral decubitus using a Vivid 5 Dimension® system (GE Healthcare, Horten, Norway) with a 3 MHz transducer during at least three consecutive cardiac cycles after a 10-minute resting period.

Parameters were measured according to American Society of Echocardiography guidelines.[24] Measurements of the left ventricle (LV) were recorded in M-mode, parasternal long axis view to obtain left ventricle end-diastolic diameter (LVEDD), left ventricle end-systolic diameter (LVESD), interventricular septum (IVS) thickness, and posterior wall (PW) thickness. The Teichholz method was used to assess left ventricle ejection fraction (LVEF). Left ventricle mass was determined according to Devereux's formula and indexed for BSA (LVMi -BSA) and height$^{2.7}$ (LVMi-height$^{2.7}$). The calculation of left atrium (LA) volume was

obtained from apical 4-chamber view at end-systole just before the mitral valve opened, using planimetered areas in orthogonal views.

Pulsed-wave Doppler recordings of the left ventricle inflow were obtained from the apical four-chamber view to measure transmitral peak flow velocities: mitral peak E velocity at rapid ventricular filling (early diastole) and mitral peak A velocity at atrial contraction (late diastole), their rate (E/A), and E wave deceleration time (DT) at the mitral tips. Isovolumetric relaxation time (IVRT) was measured using simultaneous continuous-wave Doppler of LV inflow and outflow.

The peak velocity of early (E') and late (A') diastolic annular motion was measured at the septal annulus of the mitral valve using tissue Doppler echocardiography. The ratio of early mitral inflow to early mitral annular diastolic tissue Doppler velocity (E/E') was calculated to evaluate left ventricle filling pressure.

Statistical analysis

LVM was the primary outcome measure. Variables related to ventricular function, such as E/A and E'/A' ratios were secondary outcome measures. Exploratory outcome measures were those related to the other echocardiographic variables. The sample size was estimated based on the study by Orio et al.[10] Considering a power of 80% and alpha of 5% to detect a difference of 10 g/m^2 in LVMi-BSA between the groups, the sample size was calculated as 32 girls per group.

Categorical variables were presented as percentages (%), and the significance of differences was examined with the Fisher's exact test. Continuous variables were tested for normal distribution using the Shapiro-Wilk test and were described as means ± standard

deviation (SD) or median and interquartile range. The paired, two-tailed Student's t-test was used to compare group means for data with Gaussian distribution. The Wilcoxon two-related-samples test was used to compare group medians for data with non-Gaussian distribution. The generalized estimating equation was used to adjust size or volume echocardiographic parameters for total body fat. Pearson's or Spearman's rank correlation coefficients were calculated between variables. Multivariable linear regression models were developed using LVM as the dependent variable and androgens, total body fat, and IR markers as independent variables. Log10 transformation was used to normalize the distribution of non-Gaussian variables when necessary. Data were considered statistically significant when $p < 0.05$. The Statistical Package for the Social Sciences v. 20 (SPSS, Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Clinical and anthropometric data

The mean age of girls with PP at the time of the study was 10.04 ± 2.60 years vs. 10.13 ± 2.56 years for the control group ($p = 0.227$). Ethnicity was similar in both groups: 26 (74.2 %) vs. 23 (65.7%) Caucasians, 8 (22.9%) vs. 11 (31.4%) Afro-descendants, and 1 (2.9%) vs. 1 (2.9%) Brazilian Indians in the PP and control groups respectively.

Table 1 presents clinical and anthropometric features of girls with PP and age-matched healthy controls. Birth weight, blood pressure, and stage of puberty at the time of inclusion in the study (according to Tanner breast stage), as well as percentage of prepubertal

and postmenarchal girls did not differ between the groups. PP girls started thelarche before controls (9.19 ± 1.03 vs. 10.43 ± 0.69 years, p< 0.001).

Despite a normal median weight and BMI SDS in the two groups, the PP group had higher prevalence of overweight – 16 PP girls (45.7%) vs. 8 control girls (22.8%) (p = 0.039). Total body fat percentage was also higher in PP than controls. While height SDS was similar in both groups, bone age SDS was higher in PP cases than in control girls [1.43 (0.17 – 2.09) vs. 0.37 (-0.09 – 0.62), p =0.006].

Hormonal and metabolic assessments

Table 2 shows hormonal and metabolic variables in the two groups. PP girls presented significantly higher Ferriman-Gallwey scores. Serum TT, DHEA-S, A_4, FAI, and 17-OHP levels were also higher in PP than in control girls.

While differences between the groups were not detected in HOMA-IR and in glucose and insulin AUC, QUICKI was significantly lower in PP girls than in controls. Lipid profile and insulin like growth factor (IGF-1) did not differ between the groups.

Echocardiographic findings

Echocardiographic data are listed in Table 3. LVM, LVMi-BSA, and LVMi- height$^{2.7}$ were higher in the PP group in all analyses. PW and IVS thickness and LA volume were also higher in PP girls, but LA size lost statistical significance after correction for total body fat.

Regarding diastolic function, E'/A' ratio was not statistically different between the groups. A' wave velocity (5.66 ± 1.34 vs. 5.09 ± 0.98 cm/s, p=0.025) was higher in PP girls.

LVM was correlated with FAI in both PP ($r = 0.613$, $p < 0.001$) and control girls (0.583, $p < 0.001$). It was also correlated with HOMA-IR ([$r = 0.497$, $p = 0.005$] and [$r = 0.482$, $p = 0.004$]) and total body fat ([$r = 0.690$, $p < 0.001$] and [$r = 0.430$, $p = 0.014$] respectively for the PP and control groups).

Multivariable linear regressions are presented in Table 4. FAI and total body fat were independent predictors of LVM increase in PP girls. Together with HOMA-IR, FAI and total body fat contributed with 72% of LVM variability in PP girls. Total body fat was also an independent predictor of LVM variability in the control group. The entire model [including total body fat, FAI and HOMA-IR] contributed with 41% of LVM variability.

DISCUSSION

In the present study, an increase in LVM associated with androgens, total body fat, and IR was found in girls with PP as compared with healthy age-matched controls. In addition, A' wave was also higher in PP girls, suggesting subclinical diastolic dysfunction in these girls. To the best of our knowledge, this is the first report of echocardiographic parameters in PP.

LVM was higher in PP girls even after anthropometric adjustments. FAI and total body fat were independent predictors of LVM increase in PP girls accounting for 72% of LVM variability.

LVM is often increased in children with hypertension and elevated BMI. In normotensive individuals (particularly women), [26] elevated BMI, and especially increased abdominal adiposity, seems to account for most of the LVM increase. Indeed, obesity contributes to LVM increase through various mechanisms, including overproduction of

cardio-inhibitory cytokines, myocardial fibrosis, and chronic volume overload through ventricles.[27]

A recent report has shown significantly higher LVM indexed for height in children and adolescents with central obesity (waist circumference/height ≥ 0.55) as compared with those with waist circumference/height < 0.50.[20] Metabolic factors such as IR and inflammation may also play a role in cardiovascular growth or injury in the young.[28] IR has been associated with LVM independently of blood pressure, even in non-obese subjects.[29] Moreover, fetuses of diabetic mothers may present myocardial hypertrophy as a result of intra-uterine hyperinsulinism and impaired cardiac function, especially diastolic, caused by modifications in left ventricular compliance.[30]

LVM predicts cardiovascular risk in adults.[16,17] In the Framingham Heart Study, risk of death was greater in subjects with LVH than in the general population.[16] Subclinical diastolic dysfunction remains poorly understood and may progress to symptomatic heart failure.[31] Mitral inflow and annular tissue velocities are used to evaluate diastolic function.[24]

In the present study, no participant had a diagnosis [32] of LVH, but A' wave was higher in PP girls. The clinical relevance of this finding is not clear and this result should be interpreted with caution. Although diastolic dysfunction more frequently involves reduced early diastolic filling velocity, some authors have also observed an increase in late velocities in patients with impaired LV relaxation and normal filling pressures.[33,34] It is speculated that these subjects have an increased LA preload given the reduced early diastolic LV filling. This increased LA preload would lead to increased atrial muscle contraction (by the Frank Starling mechanism) and to increased A' wave velocity.[34]

Previous studies have shown distinct effects of PCOS on left cardiac structures and diastolic dysfunction. While some reports have shown no significant differences in LVM

and/or diastolic function between women with PCOS and healthy controls, [13-15] Orio et al. showed increased LVM and decreased E/A ratio in 30 young PCOS women (mean age 24 years) when compared with 30 age- and BMI-matched controls; the observed changes were related to HOMA-IR, but not to BMI.[10] Kosmala et al. studied 150 obese women with mean age of 30 years classified into three groups: a group with both PCOS and IR; a group without PCOS with IR; and a third group without either PCOS or IR. The authors found subclinical LV systolic and diastolic dysfunction in both groups with IR, suggesting that LV function abnormalities are linked to IR in obese PCOS women rather than to the sex hormone changes associated with the syndrome.[11] In the Coronary Artery Risk Development in Young Adults (CARDIA) cohort, 984 women were included: 42 with PCOS diagnosis based on NIH criteria, 67 with isolated oligomenorrhea, 178 with isolated hyperandrogenism (IH), and 697 controls. LVM was higher in the PCOS and IH groups as compared to controls when echocardiographic parameters were adjusted for age and race. When parameters were also adjusted for cardiovascular risk factors (BMI, blood pressure, fasting glucose, insulin, and lipid profile), LVM was higher only in the PCOS group, suggesting early adverse cardiac remodeling in PCOS women independently of traditional risk factors. The authors did not explore the possibility of an influence of hyperandrogenism on these findings.[12]

The hypothesis that androgens impact the cardiovascular system is not new; cardiometabolic risk factors are known to be more prevalent in hyperandrogenic PCOS phenotypes.[35] Androgen excess is thought to favor abdominal adiposity from an early age by stimulating the differentiation of preadipocytes into mature adipocytes and consequently facilitating insulin resistance. Also, androgen excess seems to influence the biochemical pathways of lipid and carbohydrate metabolism, as well adipose tissue dysfunction and low grade chronic inflammation.[36] Evidence indicates that the relationship between hyperandrogenism and hypertension may be explained at least in part by changes in

components of the renin-angiotensin system.[37] In the present study, even though blood pressure was not higher in the PP group than in controls, we found a correlation between FAI and systolic/diastolic blood pressure (data not shown).

Our PP girls had higher IR markers and were heavier, with higher prevalence of overweight and fat mass, than age-matched controls at a similar pubertal stage, confirming previous reports.[2,3] Androgens were also higher in the PP group than in controls, as previously reported.[2,3]

Obesity, IR, and hyperandrogenism are associated with chronic inflammation and adipose tissue dysfunction, [36] and consequently with prevalent cardiovascular risk factors. An increase in circulating adipokines, low-gade chronic inflammation markers, serum lipids, and blood pressure has been reported in girls with a history of PA.[2,3] In the present study, however, no significant differences were found in hs-CRP, lipids, or blood pressure between groups, probably because of the early age of these girls.

Finally, PP has been described as a condition with no effect on the normal progression of puberty and final height, although telarche or menarche may occur slightly earlier than in the general population and bone age is often advanced in PP girls.[2,3] These findings may be related to circulating androgen levels, which might be slightly higher for age in these girls, and to the conversion of androgens into estrogen, which impacts bone growth.[38]

In the present study, androgen levels were measured by chemiluminescence assay with quality control standards, and not by liquid chromatography mass spectrometry, which has been proposed as the preferred assay for quantifying serum testosterone levels in women. The main limitation of chemiluminescence is a lack of sensitivity to measure low androgen levels in women; however, the simplicity, speed, and relatively low cost of carefully selected and maintained direct assays still warrant their use for that purpose. In fact, we were able to detect

significant differences in testosterone levels between PP and control girls, which indicates sufficient accuracy of our chemiluminescence assay.

Limitations of this study are its cross-sectional design, which precludes our ability to describe causal relations between echocardiography findings, androgens, and IR, and the relative small sample size, which may have decreased the power of some analyses. However, it should be noted that our sample was similar in size to the samples of other case-control studies focusing on this condition. Also, large samples are often difficult to obtain given the relatively low prevalence of PP in the general population. Despite these limitations, the present findings provide novel and relevant insights into a potential subclinical predictor of diastolic dysfunction in PP.

In conclusion, a geometric LVM increase and possible subclinical alterations in diastolic function were found in PP girls when compared to age-matched controls in the same pubertal stage after anthropometric adjustments. This LVM increase, which may represent early adverse cardiac remodeling, was associated with androgens, IR, and adiposity in the PP group. This important finding sheds light on the presence of potential risk factors for cardiovascular disease starting in childhood. Further studies are needed to determine whether these risk factors will actually develop into cardiac disease in the future, as well as to identify interventions to prevent adverse outcomes in girls with PP.

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Table 1. Clinical and Anthropometric Features of Girls with Precocious Pubarche and Age-Matched Healthy Controls[a]

Feature	PP (n=35)	Controls (n= 35)	*p value*
Chronological age, y	10.04 ± 2.60	10.13 ± 2.56	0.227
Caucasians, n (%)	26 (74.2)	23 (65.7)	0.499
Age at pubarche, y	**5.76 ± 1.16**	**9.88 ± 0.66**	**<0.001**
Age at telarche, y	**9.19 ± 1.03**	**10.43 ± 0.69**	**<0.001**
Age at menarche, y	11.27±1.17	12.04 ± 0.69	0.141
Pre pubertal, n (%)	17 (48.58)	17 (48.58)	1.000
Post menarche, n (%)	11 (28.57)	7 (20)	0.413
Tanner breast stage	2.49 ± 1.68	2.09 ± 1.35	0.279
Tanner hair stage	**3.49 ± 1.12**	**2.03 ± 1.42**	**<0.001**
Ferriman-Galwey score	**4 (2 – 9.5)**	**0 (0 – 1)**	**<0.001**
Birth weight, g	3140 ± 770	2930 ± 600	0.231
BSA, m^2	**1.25 ± 0.27**	**1.19 ± 0.28**	**0.045**
Weight SDS	**0.88 (0.37 – 1.37)**	**0.50 (-0.18 – 0.94)**	**0.011**
Height SDS	0.31 (-0.31 – 0.93)	0.18 (-0.83 – 0.99)	0.432
BMI SDS	**0.95 (0.65 – 1.29)**	**0.44 (-0.32 – 1.03)**	**0.018**
Elevated waist circumference[b], n (%)	12 (34.28)	6 (17.14)	0.096
Total body fat, kg	**12.59 ± 5,53**	**10.08 ± 5.57**	**0.041**
Total body fat percentage, %	**29.58 ± 6.66**	**25.28 ± 8.35**	**0.045**
Fat free mass, kg	28.69 ± 8.75	27.94 ± 8.81	0.167
SBP, mmHg	104.50 ± 14.48	107.64 ± 11.69	0.285
DBP, mmHg	63.23 ± 13.7	63.58 ± 7.45	0.865
CF, b/min	87.08 ± 13.40	83.94 ± 10.96	0.467
Bone age SDS	**1.43 (0.17 – 2.09)**	**0.37 (-0.09 – 0.62)**	**0.006**

[a] Results are expressed as percentage, mean ± standard deviation or median (25%-75% interquartile ranges).
[b] Waist circumference/height ≥ 0.5[(20)]
$p < 0.05$ is statistically significant.
PP, precocious pubarche; y, years; BSA, body surface area; SDS, standard deviation score; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CF, cardiac frequency.

Table 2. Hormonal and metabolic profile in girls with and without precocious pubarche[a]

Variable	PP (n=35)	Controls (n=35)	*p value*
Total testosterone, nmol/L	**1.35 (0.76 – 2.25)**	**0.93 (0.31 – 1.52)**	**0.001**
SHBG, nmol/L	65.89 ± 29.82	70.63 ± 33.42	0.626
Androstenedione, nmol/L	**3.07 (1.95 – 7.15)**	**2.82 (0.69 – 4.18)**	**0.001**
DHEA-S, µmol/L	**2.19 (1.09 – 4.41)**	**1.30 (0.50 – 2.58)**	**0.002**
17-OHP, nmol/L	**2.42 (1.72 – 4.73)**	**1.88 (1.33 – 3.33)**	**0.037**
FAI	**1.39 (0.48 – 3.64)**	**1.06 (0.39 – 1.70)**	**0.005**
IGF-1, nmol/L	49.13 (31.44 – 80.04)	39.43 (23.32 – 58.82)	0.155
Hs-CRP, nmol/L	4.76 (1.81 – 8.76)	2.10 (1.24 – 4.57)	0.115
HOMA-IR	2.79 (1.84 – 4.05)	2.15 (1.09 – 3.23)	0.085
QUICKI	**0.33 ± 0.02**	**0.35 ± 0.03**	**0.021**
AUC glucose	100 (94 – 107)	103 (94 - 115)	0.186
AUC insulin	80 (46 – 125)	57 (38 – 109)	0.831
Total cholesterol, mmol/L	4.17 ± 0.77	3.93 ± 0.66	0.191
HDL cholesterol, mmol/L	1.34 ± 0.39	1.21 ± 0.23	0.136
LDL cholesterol, mmol/L	2.45 ± 0.67	2.27 ± 0.55	0.268
Triglycerides, mmol/L	0.82 ± 0.30	0.95 ± 0.43	0.178

[a] Results are expressed as mean ± standard deviation or median (25%-75% interquartile ranges).
$p < 0.05$ is statistically significant.
PP, precocious pubarche; SHBG, sexual hormone biding globulin; DHEA-S dehydroepiandrosterone sulfate; 17-OHP, 17 hydroxy-progesterone; FAI, free androgen index; IGF-1, insulin like growth factor type 1; Hs-CRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment index for insulin resistance; QUICKI, quantitative insulin sensitivity check index; AUC, area under the curve.

Table 3. Echocardiographic measurements in girls with precocious pubarche and controls[a]

Measurement	PP (n=35)	Controls (n=35)	*p value*
LVM, g	**97.31 ± 33.37**	**81.25 ± 19.06**	**0.017**[b]
LVMi/BSA, g/m^2	**77.00 ± 16.60**	**69.63 ± 11.59**	**0.008**
LVMi/height$^{2.7}$, g/m$^{2.7}$	**38.45 ± 9.58**	**34.15 ± 6.53**	**0.022**
Posterior wall , mm	**6.45 ± 1.09**	**5.82 ± 0.61**	**0.019**[b]
IVS, mm	**6.46 ± 1.19**	**5.86 ± 0.64**	**0.010**[b]
LVEF (%)	67.55± 6.39	66.27 ± 5.80	0.396[b]
LVSF (%)	37.51 ± 5.07	35.91 ± 4.40	0.202[b]
LVEDD, mm	42.82 ± 5.17	41.60 ± 4.08	0.699[b]
LVESD, mm	26.22 ± 4.69	26.42 ± 2.94	0.224[b]
LV systolic volume, mL	44.15 ± 8.04	43.30 ± 7.67	0.292[b]
LA size, mm	28.40 ± 4.57	26.40 ± 3.54	0.294[b]
LA volume, mL	33.77 ± 12.73	33.70 ± 13.43	0.213[b]
Aorta size, mm	21.28 ± 2.75	21.20 ± 2.78	0.565[b]
RV size, mm	15.00 ± 3.72	14.57 ± 4.07	0.832[b]
E wave DT, ms	164.58 ± 43.89	160.21 ± 31.70	0.615
IVRT, ms	80.80 ± 9.90	77.30 ± 8.80	0.120
E/A	1.79 ± 0.30	1.69 ± 0.39	0.218
E´/A´	2.39 ± 0.54	2.54 ± 0.51	0.357
E', cm/s	13.0 ± 2.0	12.5 ± 1.7	0.245
A', cm/s	5.6 ± 1.3	5.0 ± 0.9	**0.025**
E/E´	7.24 ± 1.23	7.26 ± 1.62	0.958

[a] Results are expressed as mean ± standard deviation; [b] *p* adjusted for total body fat; $p < 0.05$ is statistically significant.
PP, precocious pubarche; LVM, left ventricular mass; BSA, body surface area; LVMi/BSA, LVM indexed to BSA; LVMi/height$^{2.7}$, LVM indexed to height$^{2.7}$**;** IVS, interventricular septum; LVEF, left ventricular ejection fraction; LVSF, left ventricular shortening fraction; LVEDD, left ventricular end- diastolic diameter; LVESD, left ventricular end- systolic diameter; LA, left atrium; E wave DT, E wave

deceleration time; IVRT, isovolumetric relaxation time from blood flow Doppler evaluation; E/A, early to late mitral flow velocity; E'/A', early to late diastolic annular motion; E/E' early mitral inflow to early mitral annular diastolic tissue Doppler velocity.

Table 4. Multivariable Linear Regression Analysis of Factors Associated with Left Ventricular Mass

Group	Model	B	95% CI	β	*p value*
PP (n = 35)	**LVM (g)** $R^2 = 0.72$				$< 0.001^a$
	(constant)	31.808	10.424 – 53.192		<0.001
	FAI	5.431	1.787 – 9.075	0.376	0.005
	HOMA-IR	6.630	-0.349 – 13.610	0.237	0.062
	Total body fat, kg	2.824	1.092 – 4.555	0.454	0.003
Controls (n = 35)	**LVM (g)** $R^2 = 0.41$				0.002^a
	(constant)	62.707	50.323 – 75.091		<0.001
	FAI	7.052	-0.273 – 14.378	0.441	0.059
	HOMA-IR	-2.405	-8.599 – 3.700	-0.187	0.421
	Total body fat, kg	1.474	0.166 – 2781	0.432	0.029

PP, precocious pubarche; LVM, left ventricular mass; FAI, free androgen index, HOMA-IR, homeostasis model assessment index for insulin resistance; CI, confidence interval

[a] ANOVA *p value*

$p < 0.05$ is statistically significant.

Capítulo II

Artigo Original 2:

Risk factors for polycystic ovary syndrome in a cohort of girls with precocious pubarche

Artigo submetido à revista Journal of Pediatric and Adolescent Gynecology

Risk factors for polycystic ovary syndrome in a cohort of girls with precocious pubarche

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Authorship

FS and PMS contributed to conception and design, acquisition and interpretation of data and drafting the article. RAV contributed to data acquisition. All authors approved the final version of the manuscript.

ABSTRACT

Study Objective: To determine the frequency of polycystic ovary syndrome (PCOS) in a cohort of girls with precocious pubarche (PP) and to investigate predictors of PCOS development in this population.

Design: Historical cohort study.

Participants: Thirty-four girls from a cohort of idiopathic PP in southern Brazil and 17 healthy prepubertal controls.

Outcome Measures: Frequency of PCOS (according to NIH consensus and Endocrine Society Guideline criteria) among PP girls evaluated two or more years after menarche and prepubertal predictors of the development of PCOS. To determine predictors, the historical prepubertal data from PP girls were compared with data from the 17 prepubertal controls.

Results: Fifteen (44.1%) PP girls were classified as PCOS and had higher body mass index standard deviation score (BMI SDS) and HOMA-IR than controls. They also had higher testosterone, hirsutism score and fasting insulin than non-PCOS girls and controls in prepubertal period. The risk for PCOS development among PP girls increased with prepubertal BMI SDS ≥ 2 (odds ratio [OR] = 4.0, 95 % confidence interval [CI], 1.33-18.66), Ferriman-Gallwey hirsutism score ≥ 4 (OR = 2.7, 95% CI, 1.15 - 5.14), HOMA- ≥ 2.42 (OR = 7.0, 95% CI, 1.39 - 12.0) and ovarian volume ≥ 1.17 mL (OR= 8.0, 95% CI, 1.60 – 39.9).

Conclusions: PP girls presented a high frequency of PCOS. Prepubertal obesity, clinical androgenism, insulin resistance, and ovarian volume were associated with the risk of developing PCOS in the postmenarcheal period.

Keywords: Polycystic ovary syndrome, risk factors, precocious pubarche, insulin resistance, hyperandrogenism

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a multifactorial endocrine disorder that seems to arise from interactions between genetic, intra-uterine, and postnatal environmental factors.[1] PCOS affects 6-15% of women of reproductive age[2] and often emerges during the peripubertal years.[3]

Intrauterine exposure to androgen excess in offspring of mothers with PCOS or other hyperandrogenic conditions[4, 5] has been related to increased risk for PCOS later in life. Pre and postnatal animal models have also confirmed the association between androgen excess and metabolic and reproductive disorders.[6] Indeed, intra-uterine growth restriction, especially when associated with exaggerated weight gain during the first years of life, may lead to more prevalent visceral obesity during childhood, precocious pubarche (PP), and PCOS after puberty.[1, 6] Hyperinsulinism plays an important role in the pathogenic link between these conditions.[7] Early knowledge regarding predisposition to develop PCOS facilitates primary or secondary prevention, reducing the risk of future reproductive and metabolic comorbidities.

A link has also been proposed between PCOS and precocious pubarche (PP), a condition characterized by development of pubic hair before 8 years of age due to premature production of adrenal androgens in the absence of secondary causes, such as congenital adrenal hyperplasia (CAH), central precocious puberty (CPP) and androgen-secreting tumors.[8, 9] PP shares many features of PCOS; it is associated with higher prevalence of overweight/obesity, insulin resistance (IR), chronic inflammation,[10, 11] and, especially in those born with low birth weight (LBW),[12] ovarian hyperandrogenism, hirsutism, and oligomenorrhea in adolescence.[11, 13-15] Despite this

evidence, there is controversy regarding the connection between PP and PCOS, and the association between these entities seems to depend upon the assessed population and the criteria used to diagnose PCOS.[16, 17]

Therefore, the aims of the present study were twofold: 1) to determine the frequency of PCOS in a sample of PP girls from southern Brazil and 2) to assess whether possible predictors exist for PCOS development among pre- and/or peri-pubertal PP girls in comparison to control girls.

MATERIALS AND METHODS

Subjects and Study Protocol

Thirty-four postmenarcheal girls were selected from a cohort of 68 patients with idiopathic PP for inclusion in the present nested case-control study. The overall cohort consisted of patients at different pubertal stages who were followed in the Gynecological Endocrinology outpatient clinic at Hospital de Clínicas de Porto Alegre (HCPA) from May 1998 to April 2015. The median follow-up period was 3.9 years, ranging from 0.2 to 17 years.

All patients in the cohort underwent periodic evaluations including medical interview and physical examination during the follow-up period. Hormonal, metabolic, and bone age assessments were done once a year. Pelvic ultrasound and blood collection were performed at least twice: upon PP diagnosis and two years after the menarche in the follicular phase of the menstrual cycle (days 2-8) or on any day if oligo/amenorrhea was present.

The group of 34 PP girls included in the present study had had their menarche at least two years earlier and did not use any medications that could interfere with biochemical analysis (i.e., hormonal contraceptive, metformin, antiandrogens, glucocorticoids) during the 3 months before the evaluation. They were stratified according to the presence or absence of PCOS.

Prepubertal data of these 34 girls were obtained from the records made during the last follow-up visit before they had reached Tanner 2 breast stage and compared with data from 17 age-matched prepubertal control girls, selected among cases consulting in pediatric/adolescent outpatient clinics at HCPA for vaccination, late postoperative follow-up (i.e., tonsillectomy among others), and psychological assessments. Inclusion criteria for controls were absence of pubertal or menstrual disorders, no signs of hyperandrogenism, and no use of medications that interfere with biochemical analysis.

Figure 1 shows the study flowchart.

Diagnosis of idiopathic PP was based on adrenocorticotropic hormone (ACTH) and gonadotropin-releasing hormone (GnRH) stimulation tests to rule out CAH due to 21-hydroxylase deficiency and CPP respectively.[18] Androgen-secreting tumors, exogenous androgens source or other secondary causes of hyperandrogenism were also excluded.

The diagnosis of PCOS was defined as follows: clinical and/or biochemical hyperandrogenism in the presence of persistent oligomenorrhea, with or without polycystic ovary (PCO) appearance; and exclusion of other androgen-excess-related disorders, such as thyroid disease, hyperprolactinemia, androgen-secreting tumor, or Cushing´s syndrome.[19, 20] Hyperandrogenism was defined as total testosterone above the upper limit of the reference range and/or presence of hirsutism (Ferriman-Galwey[21]

score ≥ 8). Oligomenorrhea was defined as less than 9 menses per year. PCO was defined by the presence of 12 or more follicles 2–9mm in diameter and/or ovarian volume higher than 10 mL (without a cyst or dominant follicle) in either ovary at ultrasound.

Pubertal development was scored according to Tanner staging.[22] Height (*Holtain Ltd.* ® stadiometer, Crymych, UK) and weight (Filizola®, São Paulo, Brazil) were measured and body mass index (BMI) was assessed using the formula: weight (kg)/height $(m)^2$. BMI scores were transformed into SDS and percentiles.[23] Skeletal maturation was staged according to Greulich and Pyle.[24] Blood samples were obtained after an overnight fast from an antecubital vein between 8 a.m. and 10 a.m.in the morning.

Homeostasis model assessment index (HOMA-IR) was calculated by [fasting insulin (mUI/L) x fasting glucose (mg/dL)]/405. Free androgen index was estimated by the formula: [total testosterone (ng/mL) x 2,476/ SHBG (nmol/L)] x 100.

Assays

Total testosterone (TT) levels were measured by radioimmunoassay (RIA) (DPC, Los Angeles, CA) with sensitivity of 0.04 ng/mL and intra- and interassay coefficients of variation (CVs) of 8.5% and 10.3% respectively. Due to changes in laboratory methodology during the cohort follow-up, specifically for the evaluation at 2 or more years post-menarche a chemiluminescent assay (Centaur XP Siemens) was used, with sensitivity of 0.1 ng/mL, upper limit of 0.8 ng/mL and intra- and interassay coefficients of variation (CVs) of 3.3% and 7.5% respectively. Sex hormone-binding globulin (SHBG) was measured by chemiluminescence (Immulite 2000 Siemens), with

a sensitivity of 0.02 nmol/L and intra- and interassay CVs of 5.3% and 6.6% respectively. Plasma insulin levels were measured by electrochemiluminescence (Centaur XP Siemens), with a sensitivity of 0.50 mUI/L and intra- and interassay CVs of 2.8% and 2.1%, respectively. Glucose was determined by colorimetric-enzymatic methods (Advia 1800 Siemens). Dehydroepiandrosterone sulfate (DHEAS) and androstenedione (A4) were measured by chemiluminescense (Immulite 2000 Siemens) and 17-hydroxyprogesterone (17-OHP) by RIA (Genesys LTi 1001).

The study protocol was approved by the local institutional review board and conducted in accordance with the Declaration of Helsinki. The purpose of the study was explained to all participants. Written informed consent was obtained from participants or legal guardians, and an assent form was also signed by participants younger than 18 years of age.

Statistical Analysis

Categorical variables were presented as percentages (%) and the significance of differences was examined with Fisher's exact test. Continuous variables were tested for normal distribution using the Shapiro-Wilk test and were described as means ± standard deviation (SD) or medians and interquartile range. Log10 transformation was used to normalize the distribution of non-Gaussian variables and re-transformed for presentation in graphs and tables. One-way analysis of variance (ANOVA) was carried out for comparing the control, non-PCOS, and PCOS groups. Bonferroni post-hoc test was used as adjustment procedure for multiple comparisons. The *p* for trend was estimated for comparisons among the three groups. Binary logistic regression was carried out to estimate the odds ratio for PCOS development according to prepubertal

risk factors. The cutoff values to indicate PCOS risk are the upper tertile of the sample for each variable, except for BMI SDS, for which a value ≥ 2 SDS is the standard for childhood obesity.

Data were considered statistically significant when $p < 0.05$. The Statistical Package for the Social Sciences v. 20 (SPSS, Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Participants were mostly of Caucasian descent: 76.5% of postmenarcheal PP girls and 64.7% of control girls. In addition, 20.6% of PP girls and 32.4% of control girls were Afro-descendants, and 2.9% in each group were Native Brazilian Indians.

Table 1 shows the prevalence of each PCOS diagnostic criterion or of different combinations in the 34 postmenarcheal PP girls. Fifteen (44.1%) girls were diagnosed with PCOS.

Table 2 shows data for PP participants at the time of the diagnostic evaluation for PCOS. No differences were observed for non-PCOS vs. PCOS participants in terms of age (15.4 ± 3.3 *vs*. 16.4 ± 2.7, $p = 0.644$ respectively) and years of follow-up (6.4 [4.5-8.9] vs. 7.6 [1.2-9.3] years, $p = 0.899$ respectively). All PCOS girls were oligomenorrheic and had higher hirsutism score and higher frequency of ovarian volume > 10 mL.

Table 3 presents prepubertal data of control girls and historical prepubertal data of PP girls stratified according to their PCOS status at the time of the study. The age at

evaluation, ethnicity, and birth weight did not differ between the groups. PCOS girls had higher prepubertal weight and bone age SDS as well as lower SHBG than controls. PP girls who developed PCOS also had higher hirsutism score and fasting insulin as compared to PP girls who did not develop PCOS and to controls in the prepubertal stage. Figure 2 shows higher values of BMI SDS, TT, and HOMA-IR in PCOS and intermediate values in non- PCOS group as compared to controls, with statistically significant p for trend.

Table 4 shows odds ratios of prepubertal variables for PCOS development in PP girls. The risk of PCOS in PP girls increased with prepubertal obesity, hirsutism score ≥ 4, HOMA-IR ≥ 2.42, and ovarian volume ≥ 1.17. Prepubertal total testosterone did not significantly influence the risk of PCOS.

DISCUSSION

In the present study, postmenarcheal PP girls presented a high frequency of PCOS. In addition, prepubertal obesity, IR, hyperandrogenism signs, and ovarian volume were associated with risk of developing PCOS later in the postmenarcheal period. To the best of our knowledge, this is the first report on clinical factors related to PCOS risk in PP girls from southern Brazil.

The frequency of PCOS in our sample was 44.1% based on NIH consensus[19] and Endocrine Society Guideline criteria for PCOS in adolescence.[20] Had we considered the concomitant presence of oligomenorrhea, hyperandrogenism, and PCO, as recommended by others for diagnosis of PCOS in adolescence,[2, 25] the frequency would

be 29%, still higher than expected for the general population. Other studies have also reported prepubertal characteristics as predictors of functional ovarian hyperandrogenism (FOH) or PCOS in girls with PP. Ibanez et al. found that 46% of Spanish PP girls presented FOH in adolescence,[13] and described increased baseline DHEAS, A4, and 17-OHP response to ACTH at the time of diagnosis of idiopathic PP.[26] Paterson et al. reported increased anti-mullerian hormone (AMH) levels, a marker of ovarian follicular development, in a Scottish cohort of prepubertal premature adrenarche girls, as well as a tendency to overweight and mild hyperinsulinemia and no evidence of reduced fetal growth.[27] By contrast, Utriainen et al. did not find abnormalities in AMH concentrations in 52 prepubertal premature adrenarche girls when compared with 48 age-matched healthy girls.[17]

The choice of defining PCOS based on the presence of persistent oligomenorrhea and hyperandrogenism is consistent with the evidence that these clinical features indicate higher risk for PCOS.[28] Recently, Rosenfield et al. showed that FOH detected in adolescence predicted PCOS in adulthood.[29] In that study, the combination of hirsutism and abnormal anovulatory symptoms, which was present in 75% of patients with FOH, was highly predictive of ongoing PCOS.[29]

Indeed, while prepubertal TT was not associated with risk for PCOS in PP girls, participants in the highest tertile of hirsutism score had twice the risk of developing PCOS in adolescence. In fact, it is well known that one limitation of testosterone assays is the lack of sensitivity to measure low androgen levels in women.

Ovarian morphological alterations may overlap physiological changes occurring in puberty, making the diagnosis of PCOS during adolescence a challenge.[30] In contrast,

we observed that slight increases in ovarian volume during the prepubertal period[31] in PP girls were related to later development of PCOS.

We also found that prepubertal obesity and IR in PP girls (BMI ≥ 2 SDS and HOMA-IR ≥ 2.42) were associated with higher risk of PCOS in adolescence. Indeed, obesity, IR, and hyperinsulinism may be genetic and intrinsic to PCOS, involving the early steps of insulin receptor-mediated signaling,[32] or else these features may be related to fetal programing triggered by intrauterine growth restriction.[6] It has also been postulated that obesity predisposes to the developmental phase of adolescent PCOS by causing androgen-mediated premenarcheal LH excess.[33]

In the present study, prepubertal bone age was more advanced in PP girls than in controls. This finding may be related to the circulating androgen levels, which are slightly higher for age in these girls, and to the conversion of androgen to estrogen, acting on bone epiphysis.[34]

Despite the frequent association of LBW and increased adrenal androgens[6, 35] and metabolic disturbances,[36-38] the birth weight of PP girls in our study was similar to that of controls, independent of PCOS status. This may reflect the small sample size and/or the multifactorial etiology of LBW.

Limitations of our study are the small size that may decrease the power of some analyses and preclude adjustments in the regression analysis for confounding variables. In addition, referral bias may have contributed to the high prevalence of overweight/obese and more hyperandrogenic girls at the time of PP diagnosis. The results might be representative of a circumscribed population and reflect the burden of this disease within a specific outpatient clinic. Because of this, the high prevalence of PCOS among adolescent PP girls should be interpreted with caution.

In conclusion, our data are consistent with previous evidence showing that PP is related to higher prevalence of PCOS later in adolescence, especially in girls presenting prepubertal overweight/obesity, IR, and androgen excess. These findings strengthen the view that PP may be an initial stage of PCOS, justifying the importance of carefully monitoring these girls. Further studies are needed to determine whether PCOS characteristics will last until adulthood, as well as to identify interventions to prevent adverse outcomes in girls with PP.

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Figure legends

Figure 1. Study flowchart

Figure 2. Assessment of prepubertal data from healthy controls and girls with precocious pubarche according to later development of polycystic ovary syndrome

Table 1. Polycystic ovary syndrome features in postmenarcheal girls with precocious pubarche

Feature	**PP (n=34)**
Hyperandrogenism	25 (73.5)
Oligomenorrhea	15 (44.1)
PCO	16 (47)
H + O + PCO	10 (29.4)
H + O	5 (14.7)
H + PCO	4 (11.8)
O + PCO	0 (0)
Isolated H	6 (17.7)
Isolated O	0 (0)
Isolated PCO	2 (5.9)
Without any PCOS criteria	7 (20.6)

Results are expressed as number (%).
PP, precocious pubarche; PCO, polycystic ovary appearance at ultrasound; H, hyperandrogenism; O, oligomenorrhea; PCOS, polycystic ovary syndrome.

Table 2. Characteristics of 34 girls with precocious pubarche according to polycystic ovary syndrome status

Characteristic	**Non-PCOS (n=19)**	**PCOS (n=15)**	***p value***
Age at evaluation, y	15.5 ± 2.5	16.4 ± 2.7	0.644
Age at menarche, y	10.95 ± 1.1	11.09 ± 1.4	0.730
Weight, kg	56.8 ± 13.4	67.0 ± 25.3	0.101
Height, cm	157.3 ± 5.6	158.0 ± 3.7	0.683
BMI, kg/m^2	22.8 ± 4.7	27.0 ± 11.1	0.109
WC, cm	74.0 ± 9.0	82.8 ± 15.4	0.098
Oligomenorrhea, n (%)	**0 (0)**	**15 (100)**	**<0.001**
Ferriman-Gallwey score	**6.2 (4.8 – 8.6)**	**13.4 (11.9 – 14.0)**	**0.012**
Ovarian volume > 10 mL, n (%)[a]	**1 (8.3)**	**5 (50)**	**0.043**
Total testosterone, ng/mL	0.4 (0.3 – 0.6)	0.7 (0.5 – 0.9)	0.113
SHBG, nmol/L	49.2 (38.23 – 53.2)	39.3 (24.7 – 48.7)	0.264
FAI	3.5 (2.1 – 4.7)	4.5 (2.9 – 4.8)	0.214
Androstenedione, ng/mL	1.9 (1.1 – 3.0)	2.4 (1.5 – 3.4)	0.323
DHEA-S, µg/dL	146.0 (119.1 – 211.5)	165.5 (98.8 – 269.2)	0.169
17 OHP, ng/mL	0.7 (0.48 – 0.83)	1.5 (1.28 – 2.26)	0.293
HOMA IR	2.2 (1.9 – 3.47)	4.2 (3.9 – 4.6)	0.183
Fasting insulin, mU/L	10.5 (9.5 – 17.95)	19.9 (17.7 – 22.5)	0.222

Results are expressed as mean ± standard deviation, median and interquartile range (25-75%), or number (%).

[a] Data of a subsample of girls with calculated ovarian volume, n = 12 non-PCOS and n = 10 PCOS.

$p < 0.05$ is statistically significant.

PCOS, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; SHBG, sex hormone-binding globulin; FAI, free androgens index; DHEA-S, dehydroepiandrosterone sulfate;17 OHP, 17-hydroxyprogesterone; HOMA IR, homeostasis model assessment index.

Table 3. Prepubertal data of healthy controls and girls with precocious pubarche according polycystic ovary syndrome status

Data	Controls (n=17)	Non-PCOS (n=19)	PCOS (n=15)	*p value*	*p for trend*
Age at evaluation, y	7.8 ± 0.7	7.9 ± 1.0	7.8 ± 0.9	0.923	
Caucasians	11 (64.7)	13 (68.4)	13 (86.7)	0.572¶	
Birth weight, g	2941 ± 596	3112 ± 485	2913 ± 756	0.574	
LBW, n (%)	4 (23.5)	4 (21)	4 (26.7)	0.906¶	
Weight SDS	-0.1 (-0.7 – 0.6)[a]	2.5 (0.7 – 2.9)	2.5 (0.9 – 3.6)[a]	0.015	0.006
Height SDS	0.1 (-0.7 – 0.7)	0.7 (0.3 – 1.4)	0.6 (-0.3 – 2.5)	0.053	
Ferriman-Gallwey score	0 (0 – 5)[a,b]	2 (0 – 4)[a,c]	6.5 (4.3 – 8)[b,c]	<0.001	<0.001
Ovarian volume, mL¥	NA	0.65 (0.34 – 0.86)	1.64 (0.62 – 1.85)	0.016§	
SHBG, nmol/L	89.1 (65 – 100)[a,b]	49 (22.7 – 75.1)[a]	42.7 (0.23 – 66.9)[b]	0.006	
FAI	0.4 (0.2 – 0.9)[a,b]	1.71 (0.9 – 2.8)[a]	3.5 (1.7 – 6.2)[b]	<0.001	<0.001
Androstenedione, ng/mL	0.3 (0.2 – 0.3)[a,b]	0.7 (0.7 – 1.2)[a]	0.7 (0.3 – 1.7)[b]	0.002	
DHEA-S, µg/dL	20 (14 – 34.8)[a]	80.3 (49.8 – 99.8)[a]	45.5 (22.3 – 119)	0.002	
17 OHP, ng/mL	0.5 (0.4 – 0.9)	0.8 (0.4 – 2.0)	1.05 (0.3 – 1.5)	0.208	
Fasting insulin, mU/L	5.2 (4.5 – 7.2)[a]	10.9 (6.1 – 12.5)[b]	18.9 (11.4 – 72.7)[a,b]	0.003	<0.001
Bone age SDS	0.2 (-0.4 – 0.5)[a,b]	2.5 (0.7 – 2.9)[a]	2.5 (0.9 – 3.6)[b]	0.001	0.017

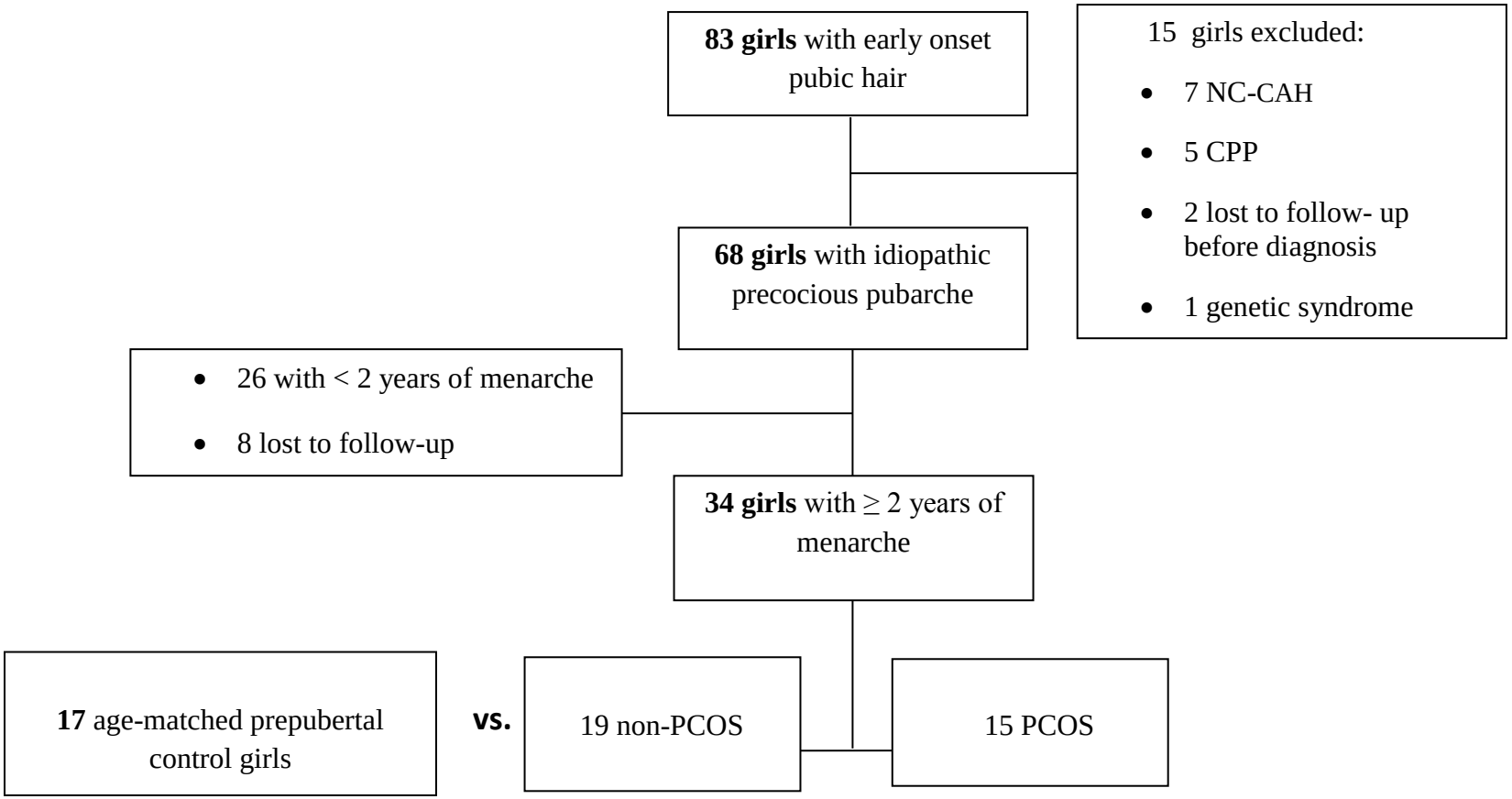
Results are expressed as number (%), mean ± standard deviation, or median and interquartile range (25-75%).
One-way analysis of variance (non-parametric variables were log-converted for statistical analysis and recovered for presentation in table format).
Equal superscript letters denote statistical difference according to Bonferroni multiple-comparison correction test.
$p < 0.05$ is statistically significant.
¶ Fisher's exact test
¥Data of a subsample of girls with calculated ovarian volume, n = 14 non-PCOS and n = 11 PCOS.
§Mann-Whitney *U* test.
PCOS, polycystic ovary syndrome; LBW, low birth weight; SDS, standard deviation score; SHBG, sex hormone-binding globulin; FAI, free androgens index; DHEA-S, dehydroepiandrosterone sulfate; 17-OHP, 17-hydroxyprogesterone; SDS, standard deviation score; NA, not available.

Table 4. Odds ratio for development of polycystic ovary syndrome in precocious pubarche girls according to prepubertal risk factors

Prepubertal risk factor	**OR**	**95%CI**	***p value***
BMI SDS ≥ 2	4.0	(1.33 – 18.66)	0.027
Ferriman-Gallwey score ≥ 4[a]	2.7	(1.15 - 5.14)	0.040
Total testosterone ≥0.38, ng/mL[a]	8.9	(0.73 - 10.3)	0.080
HOMA-IR ≥ 2.42[a]	7.0	(1.39 - 12.0)	0.020
Ovarian volume ≥1.17, mL[a]	8.0	(1.60 – 39.9)	0.011

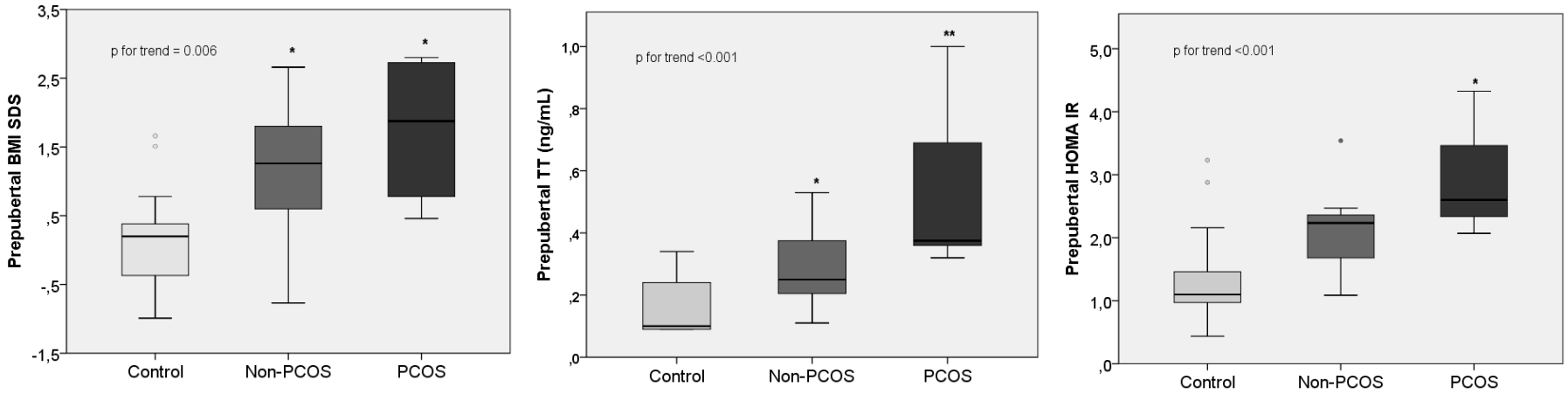
[a]Cutoff values are the superior tertile for the sample.
OR, odds ratio; BMI, body mass index; SDS, standard deviation score; HOMA IR, homeostasis model assessment index.

Figure 1. Study flowchart



NC-CAH, non-classical congenital adrenal hyperplasia; CPP, central precocious puberty; PP precocious pubarche; PCOS, polycystic ovary syndrome.

Figure 2. Assessment of prepubertal data from healthy controls and girls with precocious pubarche according to later development of polycystic ovary syndrome



A **B** **C**

Results are expressed as median and interquartile range (diagram graphs).
One-way analysis of variance (non-parametric variables were log-converted for statistical analysis and recovered for presentation in graphs).
*p < 0.05 for difference in relation to control group; **p < 0.001 for difference in relation to control and non-PCOS groups.
A. Comparison of prepubertal body mass index standard deviation score (BMI SDS) in the three groups; **B**. Comparison of prepubertal total testosterone (TT) in the three groups; **C.** Comparison of prepubertal homeostasis model assessment insulin resistance (HOMA IR) in the three groups.
PCOS, polycystic ovary syndrome.

CONSIDERAÇÕES FINAIS

Demonstrou-se que na amostra de meninas com pubarca precoce (PP) estudada, as mesmas apresentaram maior índice de massa corporal (IMC), percentual de gordura corporal, níveis de androgênios e resistência insulínca (RI); idade óssea com maior avanço e telarca mais antecipada, além do aumento da massa do ventrículo esquerdo (MVE) e uma possível disfunção diastólica subclínica quando comparadas a meninas saudáveis da mesma idade e no mesmo estágio puberal. O aumento da MVE se associou aos níveis de androgênios, adiposidade e RI. No grupo PP, estes três fatores contribuíram com 72% da variação da massa de VE, sendo os androgênios e a gordura corporal fatores independentes e a RI covariável da obesidade.

As meninas com PP acompanhadas na Unidade de Endocrinologia Ginecológica do Hospital de Clínicas de Porto Alegre (UEG/HCPA) possuem frequência aumentada de PCOS na adolescência. No entanto, a frequência de 44,1% de PCOS na coorte, apesar de estar de acordo com dados da literatura, deve ser interpretada com precaução, pois se trata de uma amostra acompanhada em ambulatório especializado, cuja procura por atendimento tente a ser feita por meninas mais sintomáticas e, portanto, com mais predisposição ao desenvolvimento de PCOS.

As meninas com PP obesas; com volume ovariano aumentado para a idade (≥ 1,17 mL), HOMA-IR ≥ 2,42 e escore de hirsutismo ≥ 4 no período pré-puberal apresentam predisposição a ter o diagnóstico de PCOS 4; 8; 7 e 2,7 vezes maior que as meninas com PP com valores abaixo desses, respectivamente.

Com relação à associação entre o peso ao nascer, PP e PCOS, o peso ao nascer entre os grupos PP e controle, bem como entre as PP com ou sem desenvolvimento de PCOS não

diferiu. A divergência deste resultado com o de outros autores pode dever-se à falta de poder do estudo em detectar uma diferença ou à variabilidade genética e ambiental entre as populações estudadas.

Os resultados sugerem que, apesar da PP ser considerada em muitos casos uma variação normal da puberdade, as meninas que apresentam o surgimento precoce de pelos pubianos associado a excesso de peso, maior RI, níveis de androgênios e/ou pontuações maiores no escore de hirsutismo estão mais propensas a ter o diagnóstico de PCOS e alterações cardiometabólicas posteriormente, necessitando, portanto, de acompanhamento com mais atenção.

Novos estudos serão necessários para verificar se as alterações cardíacas subclínicas e as características de PCOS persistirão na vida adulta das meninas com PP, bem como se medidas para controle de peso, melhora da RI e/ou do hiperandrogenismo peripuberal podem ser benéficas e evitar tais desfechos.