SÍNDROME DOS OVÁRIOS POLICÍSTICOS E FATORES DE RISCO CARDIOVASCULAR

Denusa Wiltgen

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Tese apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, como requisito parcial para obtenção do título de Doutor

Orientadora: Profª. Drª. Poli Mara Spritzer

Porto Alegre, setembro de 2009

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"É melhor ser alegre que ser triste, alegria é a melhor coisa que existe, é assim como a luz no coração. Mas pra fazer um samba com beleza é preciso um bocado de tristeza, é preciso um bocado de tristeza senão, não se faz um samba não.... Feito essa gente que anda por aí brincando com a vida.. Cuidado companheiro! A vida é pra valer! E não se engane não, tem uma só. Duas mesmo que é bom, ninguém vai me dizer que tem, sem provar muito bem provado, com certidão passada em cartório do céu e assinado embaixo: Deus. E com firma reconhecida! A vida não é brincadeira, amigo. A vida é a arte do encontro, embora haja tanto desencontro pela vida.. Ponha um pouco amor na sua vida. Como no meu samba..."

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

- Artigo de revisão: "Síndrome dos Ovários Policísticos e risco para doença cardiovascular: uma revisão crítica"

- Artigo original 1: "Lipid accumulation product (LAP) index: a reliable marker of cardiovascular risk in polycystic ovary syndrome" (publicado em Julho de 2009 – Human Reproduction)

- Artigo original 2: "Variation in metabolic and cardiovascular risk in women with different polycystic ovary syndrome phenotypes" (submetido à Fertilty Sterility, 2009)

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RESUMO

A Síndrome dos Ovários Policísticos (PCOS) é a endocrinopatia mais prevalente em mulheres em idade reprodutiva cuja as principais características clínicas são hiperandrogenismo e anovulação crônica. Apesar de jovens, as pacientes com PCOS apresentam freqüência elevada de alterações metabólicas como resistência insulínica, dislipidemia e maior risco para desenvolver diabete tipo 2. Evidências indicam uma maior prevalência de fatores de risco cardiovascular em pacientes com a Síndrome dos Ovários Policísticos, sugerindo uma maior chance de desenvolvimento prematuro de aterosclerose e, possivelmente, doença cardiovascular estabelecida. Contudo a associação entre PCOS e eventos cardiovasculares primários, como infarto agudo do miocárdio e acidente vascular cerebral, ainda precisa ser confirmada. Os estudos publicados até o momento apresentam limitações metodológicas e resultados controversos. Novos estudos prospectivos são portanto necessários, com maior tempo de seguimento e delineados a partir de uma definição clara dos critérios diagnósticos de PCOS. Isto possibilitará uma melhor caracterização dos riscos associados à síndrome.

A presença de resistência insulínica, independente do peso corporal parece ser o ponto central das alterações metabólicas encontradas nas pacientes com PCOS. A identificação da presença de resistência insulínica é importante pois o tratamento pode ser melhor individualizado. Neste sentido, achados clínicos sugestivos de resistência insulínica, como acantose *nigricans*, hipertensão, bem como alterações no perfil lipídico devem ser valorizados. . Neste trabalho buscou-se avaliar a acurácia do índice LAP – *lipid accumulation product* -[cintura(cm) -58] x [triglicerídeos (mg/dl) x 0,01536], na identificação de pacientes PCOS em maior risco metabólico e cardiovascular, por estar associado à presença resistência insulínica. Observou-se uma correlação forte e positiva entre o índice HOMA e índice LAP. O valor de LAP 34,5 determinou sensibilidade de 84% e especificidade de 79% para identificar pacientes em maior risco metabólico. Esses resultados sugerem que o índice LAP pode ser uma ferramenta útil na identificação de pacientes PCOS com resistência insulínica e maior risco cardiovascular.

Tendo em vista os critérios atuais para o diagnóstico da Síndrome dos Ovários Policísticos, com a recente valorização da aparência policística do ovário (PCO), novos

fenótipos surgiram, em especial, aqueles associados com ovulação. No presente estudo, foram comparadas pacientes com PCOS típico, constituído por anovulação, hirsutismo e excesso de androgênios, e outros dois grupos de pacientes ovulatórias, um com hirsutismo e PCO e outro hirsutismo isolado. Foi incluído também um grupo controle de mulheres ovulatórias e não hirsutas. Verificou-se que o grupo de pacientes com PCOS típico apresentou alterações metabólicas e maior prevalência de fatores de risco CV do que os outros grupos, mesmo quando os dados foram ajustados pelo IMC, enquanto que as pacientes ovulatórias com hirsutismo e PCO não diferiram daquelas com hirsutismo isolado. Estes resultados sugerem que na ausência de anovulação e androgênios aumentados o risco metabólico e cardiovascular pode não diferir de mulheres normais.

PARTE I

SÍNDROME DOS OVÁRIOS POLICÍSTICOS E RISCO PARA DOENÇA CARDIOVASCULAR: REVISÃO CRÍTICA

Síndrome dos Ovários Policísticos e risco para doença cardiovascular: uma revisão crítica

Denusa Wiltgen , Poli Mara Spritzer

Unidade de Endocrinologia Ginecológica, Serviço de Endocrinologia, Hospital de Clínicas de Porto Alegre, Brasil; Laboratório de Endocrinologia Molecular, Departamento de Fisiologia, Universidade Federal do Rio Grande do Sul (UFRGS), Brasil; Instituto Nacional de Hormônios e Saúde da Mulher – CNPq, Brasil

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Resumo

A Síndrome dos Ovários Policísticos (PCOS) é a endocrinopatia mais prevalente em mulheres em idade reprodutiva cuja as principais características clínicas são hiperandrogenismo e anovulação crônica. Além dos distúrbios reprodutivos, as pacientes com PCOS apresentam alterações metabólicas associadas à presença de resistência insulínica e maior prevalência de fatores de risco cardiovascular. Por outro lado, ainda não é claro se esta exposição precoce a um perfil metabólico desfavorável aumenta a freqüência de desfechos cardiovasculares neste grupo de mulheres, especialmente no período pós- menopáusico. O objetivo deste trabalho é revisar os dados disponíveis na literatura a respeito de um potencial risco cardiovascular aumentado em pacientes com PCOS. A análise dos estudos publicados até o momento apresenta resultados controversos. Limitações metodológicas como número restrito de pacientes avaliados e diferentes critérios de inclusão para pacientes com PCOS não permitem conclusões definitivas sobre o tema. Serão necessários novos estudos prospectivos, com maior tempo de seguimento e delineados a partir de uma definição clara dos critérios diagnósticos de PCOS para que se possa avançar nesta questão.

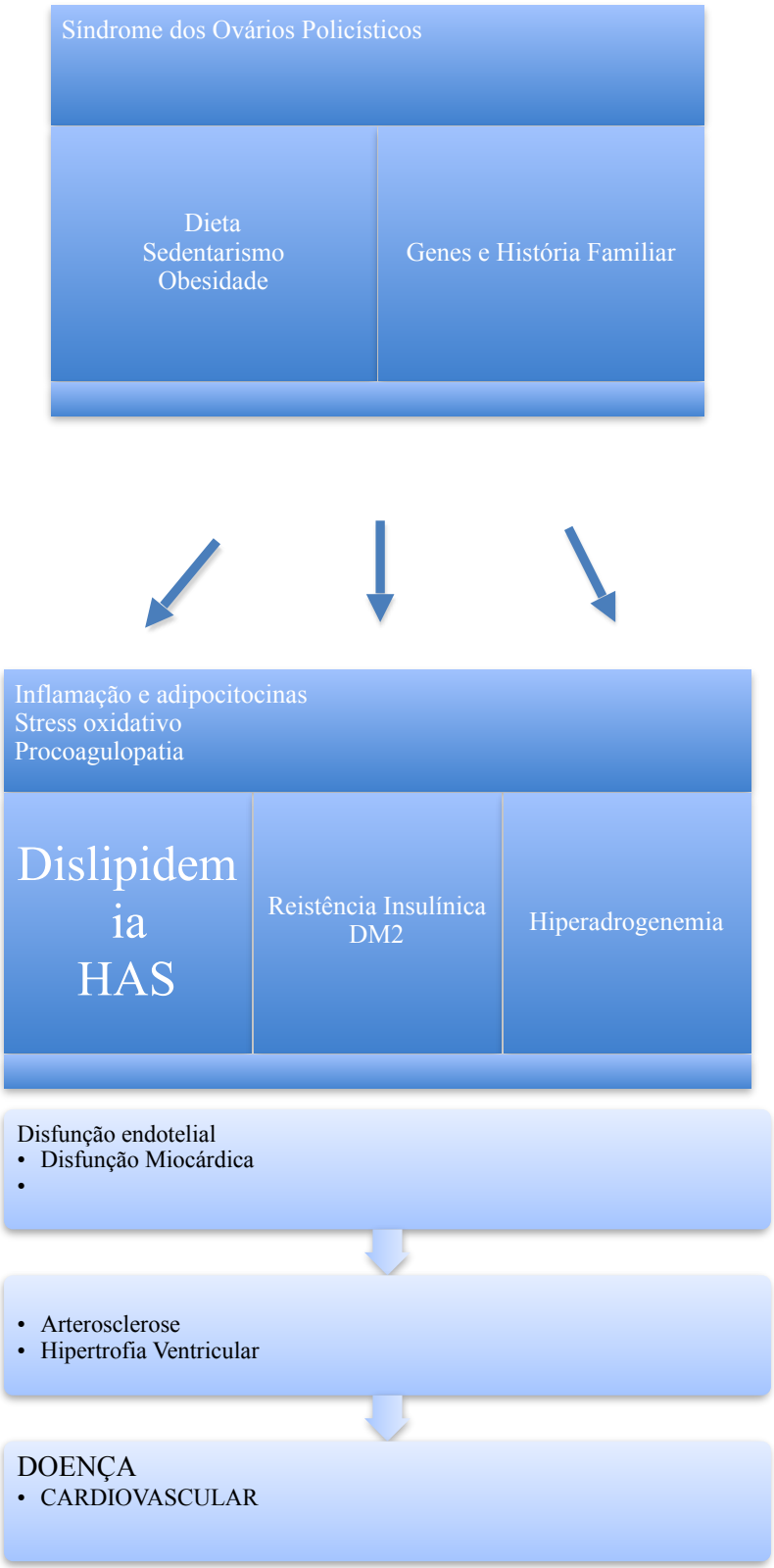
Síndrome dos Ovários Policísticos e risco para doença cardiovascular: uma revisão crítica

Introdução

A Síndrome dos Ovários Policísticos (PCOS) é uma doença de apresentação clínica heterogênea, cujas principais características são anovulação crônica e manifestações de hiperandrogenismo. Apesar de freqüente em populações não-selecionadas (5-10% em mulheres na fase reprodutiva) e de ser reconhecida há mais de 70 anos [1-4], sua etiopatogenia ainda não foi totalmente esclarecida. Evidências indicam que as anormalidades encontradas nesta síndrome são multifatoriais e que a susceptibilidade individual para o desenvolvimento da doença seja determinada pela ação conjunta de fatores genéticos e ambientais. A elevada prevalência de fatores de risco metabólicos e cardiovasculares e a presença de comorbidades metabólicas relacionadas com a obesidade, observadas em pacientes com PCOS, são um exemplo desta interação entre genética e ambiente. Assim, a presença de resistência insulínica e o aumento da adiposidade central, mesmo em pacientes de peso normal, e em outros membros da família, indica o potencial genético da síndrome para distúrbios metabólicos [5-8] . Além disso, quando comparadas a mulheres obesas de mesma faixa etária, as pacientes com PCOS apresentam maior freqüência de hipertensão arterial sistêmica (HAS), síndrome metabólica (SM), tolerância diminuída à glicose (TDG) e diabete tipo 2 (DM2), demonstrando que a obesidade acentua uma característica que parece ser intrínseca à própria síndrome [9-12] . Por outro lado, ainda não é claro se este tempo prolongado de exposição a um perfil metabólico desfavorável aumenta a freqüência de desfechos cardiovasculares neste grupo de mulheres, especialmente no período pós- menopáusico.

Desta forma, o objetivo deste trabalho é revisar e analisar os dados disponíveis na literatura a respeito de um potencial risco cardiovascular aumentado em pacientes com PCOS.

Figura 1: Esquema hipotético para o surgimento da doença cardiovascular na Síndrome dos Ovários Policísticos



Adaptado de Cussons & col, 2006 [13]

Fatores de risco cardiovascular (FRCV) em pacientes com PCOS

Existem várias evidências que indicam uma maior prevalência de fatores de risco cardiovascular em pacientes com a Síndrome dos Ovários Policísticos. Há, portanto, uma noção de que a precocidade dessas alterações, muitas vezes desde a adolescência, aumentariam a chance de desenvolvimento prematuro de aterosclerose e, possivelmente, doença cardiovascular estabelecida [14-17] . A resistência à ação da insulina e a hiperinsulinemia compensatória parecem ser o ponto central da maior frequência das alterações metabólicas e dos FRCV observada nestas pacientes. Entre estes, pode-se citar os seguintes:

1. <u>*Obesidade:*</u> a prevalência de obesidade varia de acordo com a origem étnica da população e de sua localização geográfica. No entanto, estima-se que aproximadamente 50% das mulheres com PCOS são obesas [18]. Além de maior índice de massa corporal, as pacientes com PCOS apresentam maior acúmulo de gordura central/visceral, caracterizada clinicamente por maior prevalência de circunferência da cintura aumentada (≥88cm) [19]. Quando presente, a obesidade agrava as manifestações secundárias à resistência insulínica.

2. <u>*Dislipidemia:*</u> é a anormalidade metabólica mais frequente nas mulheres com PCOS, podendo alcançar prevalência em torno de 70%, segundo os critérios do *"National Cholesterol Education Program"* [19]. . As alterações mais comuns são aumento dos níveis dos triglicerídeos e do LDL-colesterol, e diminuição dos níveis do HDL-colesterol [20,21,22], independente do peso corporal. Tanto a resistência insulínica quanto a hiperandrogenemia contribuem para um perfil lipídico mais "aterogênico" nas PCOS: a testosterona diminui a atividade da enzima lipoproteína lípase nos adipócitos abdominais e a resistência insulínica prejudica a atividade lipolítica da própria insulina . [23]

3. <u>*Tolerância diminuída à glicose e diabete tipo 2:*</u> devido à presença de resistência insulínica, as pacientes com PCOS apresentam maior risco de TDG e DM2 quando comparadas a controles normais. Estima-se que 30 a 40 % das pacientes com PCOS apresentam TDG e em torno de 10% desenvolvem DM2 antes da quarta década de vida [10, 24].

4. *Hipertensão arterial sistêmica, disfunção endotelial/vascular e atividade pró-inflamatória:* HAS é incomum em pacientes PCOS jovens mas sua prevalência aumenta consideravelmente durante a vida adulta, podendo alcançar índices como 40% na perimenopausa. [25,26] Estudos clínicos também demonstram alterações na complacência vascular e na função endotelial das mulheres com PCOS como, por exemplo, o aumento na velocidade de onda de pulso da artéria braquial [25] e na espessura da íntima média carotídea [27], ambos sinais de aterosclerose precoce, quando comparadas à mulheres controles normais. Estudos mais recentes têm verificado também alterações em fatores pró-inflamatórios, marcadores de função endotelial e de fibrinólise, considerados, em conjunto, como marcadores de risco cardiovascular. As alterações mais frequentemente observadas na PCOS estão relacionadas com proteína C-reativa [28,29], fator de Von-Willebrand [30], homocisteína [31], endotelina-1 e óxido nítrico [32,33]. Outros estudos identificaram que estas pacientes, mesmo que jovens e de peso normal, apresentam um aumento significativo do tamanho cardíaco e diminuição da fração de ejeção do ventrículo esquerdo quando comparadas a mulheres normais de mesma faixa etária e peso [20]. Novamente, a presença de resistência insulínica parece estar diretamente relacionada com estas alterações da estrutura e funcionamento cardíacos.

Risco de Doença Cardiovascular em mulheres com Síndrome dos Ovários Policísticos

Para a busca da literatura na base de dados *Pubmed* foram utilizados os termos *"PCOS and death", "PCOS and cardiovascular disease", "PCOS and myocardial infarction"* e "*PCOS and cerebrovascular disease"*. Em sua maioria, os estudos citados avaliaram desfecho cardiovascular e presença de PCOS, e estão sumarizados na tabela 2.

O primeiro estudo a tratar deste assunto data de 1992 foi uma coorte retrospectiva de 1462 mulheres suecas. A partir desta população, foi aplicado um modelo de risco cardiovascular a uma amostra de 33 pacientes perimenopáusicas, com diagnóstico prévio de PCOS por achados histopatológico obtidos de ressecção em cunha ovariana, e a um grupo de referência de 132 mulheres[34] . O modelo contemplou vários fatores de risco para infarto agudo do miocárdio (IAM) e os autores encontraram um risco 7,1 vezes maior de IAM no grupo PCOS em relação às mulheres sem PCOS de mesma faixa etária. No entanto, é

importante enfatizar que este risco foi relacionado com obesidade e que o delineamento foi retrospectivo e de estimativa de risco, não tendo sido avaliada a presença de doença cardiovascular propriamente dita.

Posteriormente, Pierpoint & col realizaram um estudo retrospectivo avaliando 786 pacientes com diagnóstico de PCOS estabelecido por evidência histológica ou macroscópica de ovários policísticos entre os anos de 1930 à 1979. Nos casos em que houve descrição das características clínicas das pacientes, estes dados foram adicionados às características morfológicas ovarianas para que os casos fossem classificados em "PCOS definitivo"(evidência clínica ou histológica de disfunção ovariana), ou "PCOS provável" (evidência histológica sem informações clínicas, alteração macroscópica ovariana com evidência clínica de disfunção ovariana ou diagnóstico clínico definido por especialista) [35] . Após um período médio de 30 anos de seguimento, não houve aumento de mortalidade cardiovascular por doença isquêmica nas pacientes com PCOS quando comparadas a população geral das mulheres do Reino Unido [RR 1,4 (0,75-2,40)]. A crítica a este trabalho foi um possível viés de seleção, já que parte das pacientes incluídas teve um diagnóstico questionável de PCOS utilizando, por exemplo, a aparência macroscópica do ovário descrita em procedimentos cirúrgicos. Além disso, apenas um número restrito de pacientes tinha registro de dosagens hormonais, história de irregularidade menstrual ou hirsutismo. Em estudo subseqüente o mesmo grupo analisou uma amostra menor de pacientes nas quais houve registro de características clínicas e laboratoriais sugestivas de PCOS [36]. Os resultados demonstraram que apesar da maior prevalência de fatores de risco cardiovascular (p ex: dislipidemia e DM2), a morbimortalidade por doença arterial coronariana foi semelhante em PCOS e controles. Porém, as pacientes com PCOS apresentaram maior incidência de doença cerebrovascular não-fatal (RR 2,8; IC 1,1-7,1). Esta coorte apresentou um número elevado de perdas no seguimento (maior 40%). Os autores citam que as duas populações (avaliadas e não-avaliadas), eram muito semelhantes em suas características clínicas basais. Contudo, a possibilidade de que estas perdas tenham interferido na prevalência dos desfechos coronarianos nas pacientes PCOS não pode ser desprezada.

Em outro estudo realizado em mulheres caucasianas, avaliadas no período perimenopaúsico, 28 pacientes com história de PCOS (ressecção em cunha do ovário entre

as décadas de 60 a 80, associada à história de oligomenorréia/amenorréia, hiperandrogenismo, infertilidade) apresentaram maior prevalência de DM2 (32% *vs* 8%) e doença arterial coronariana (21% *vs* 5%) quando comparadas a uma população de referência de 782 mulheres [37] . Um aspecto interessante foi a observação de que, no momento da avaliação, as variáveis antropométricas, os níveis de glicose, colesterol total e frações e pressão arterial eram semelhantes entre os dois grupos, sugerindo que o perfil de risco metabólico das pacientes PCOS durante sua juventude influenciam na incidência posterior de doença cardiovascular. Entretanto, uma crítica a este estudo é que a maior prevalência de doença coronária não foi ajustada à presença de diabete 2, cuja presença reconhecidamente aumenta risco para doença cardiovascular.

Já Elting & col publicaram em 2001 um estudo retrospectivo comparando 346 pacientes com PCOS (presença de ciclos oligomenorreicos/amenorreicos e níveis elevados de hormônio luteinizante) com dados da população geral alemã [38]. As pacientes eram contatadas por telefone e respondiam a um questionário geral de saúde. Os dados foram estratificados por faixa etária, mas não foram pareados por índice de massa corporal. Novamente as pacientes com PCOS apresentavam maior prevalência de DM2 e HAS, especialmente nas mulheres entre 35 e 55 anos, mas a prevalência de queixas cardiovasculares não diferiu da população geral. É relevante comentar que o grupo de pacientes com PCOS apresentou um percentual de obesidade menor do que o esperado (mediana de IMC 24.4) e talvez este achado tenha influenciado nos resultados quanto a desfechos cardiovasculares.

Recentemente, 3 estudos foram publicados sobre o seguimento de coortes de pacientes com diagnóstico de PCOS e incidência de desfechos cardiovasculares. Lunde & Tanbo, [39] em estudo retrospectivo com mulheres norueguesas, 15 a 25 anos após ressecção em cunha do ovário por sintomas como hiperandrogenismo, irregularidade menstrual e infertilidade, observaram que não houve maior prevalência de doença cardiovascular nas pacientes com PCOS quando comparadas aos dados da população geral. Já Shaw & col encontraram maior prevalência de eventos cardiovasculares em mulheres com PCOS [40] . Estudando uma população de 390 mulheres pós-menopáusicas seguidas por 6 anos após a realização de cateterismo coronariano por suspeita de IAM, identificaram 104 com características sugestivas de PCOS. Em comparação com as 286 mulheres sem alterações de

ciclo menstrual ou manifestações de hiperandrogenismo, as pacientes PCOS eram mais frequentemente diabéticas, obesas, com maior prevalência de SM e de doença arterial coronariana (DAC). Além disso, a curva de sobrevida ou curva livre de eventos (IAM) foi significativamente menor nas pacientes com PCOS. Os autores também montaram um modelo de prognóstico para risco cardiovascular, e PCOS permaneceu um preditor importante de DCV ($p<0,01$), mesmo incluindo variáveis como circunferência da cintura, HAS e alterações angiográficas sugestivas de DAC. Este foi o primeiro estudo prospectivo com critérios diagnósticos bem definidos para a Síndrome dos Ovários Policísticos com poder suficiente para medir risco de eventos cardiovasculares. Como também observado por Krentz & col [41], o risco de DCV e doença coronariana parece ser maior nas pacientes com níveis mais elevados de androgênios e de fatores pró-inflamatórios séricos.

Os androgênios circulantes parecem diminuir espontaneamente após os 35 anos mesmo nas pacientes com PCOS [42] o que tende a minimizar o pior perfil de risco cardiovascular com o passar dos anos. Contudo, estudos em mulheres pós-menopáusicas não selecionadas pelo diagnóstico prévio de PCOS já evidenciaram uma associação entre maiores níveis de androgênios, mesmo que em valores considerados dentro da faixa da normalidade, e elevada freqüência de fatores de risco cardiovascular [42-45]. Esta associação poderia explicar, pelo menos em parte, o insucesso dos estudos prévios em pacientes com PCOS que sofreram ressecção em cunha do ovário, em determinar maior risco cardiovascular, já que este procedimento modifica significativamente a secreção de androgênios.

Tabela 1: Estudos que avaliaram risco para desfechos cardiovasculares em mulheres com a Síndrome dos Ovários Policísticos

AUTORES	DELINEAMENTO	N TOTAL / N DE PCOS	CRITÉRIO DE PCOS	RESULTADO	RISCO ASSOCIADO À PCOS

Dahlgren & col., 1992	Estudo Populacional	1462 /33	Morfologia ovariana [A]	**Positivo** para risco de IAM	RR 7,1
Pierpoint & col., 1998	Estudo Populacional	Dados populacionais / 786	Morfologia ovariana ou diagnóstico clínico [B]	**Negativo** para mortalidade por DCV	RR 1,4 (0,75-2,40)
Wild & col., 2000	Estudo populacional	1738 /678	Morfologia ovariana ou diagnóstico clínico	**Positivo** para doença cerebrovascular	RR 2,8 (1,1-7,1)
Cibula & col., 2000	Transversal	780/28	Morfologia ovariana e diagnóstico clínico	**Positivo** para DAC	não reportado
Elting & col., 2001	Estudo populacional	Dados populacionais /346	Diagnóstico clínico	**Negativo** para DCV	RR 1,5 (0,7-2,9)
Krentz & col, 2007	Transversal	713 /66	Diagnóstico clínico	**Positivo** para DCV e DAC	OR 1,36 (1,05-1,79)
Tanbo & col., 2007	Estudo populacional	Dados populacionais/ 149	Morfologia ovariana e diagnóstico clínico	**Negativo** para DCV	RR 2,8 (0,10-71)

SHAW & COL., 2008	PROSPECTIVO, MULTICÊNTRICO	390 / 104	DIAGNÓSTICO CLÍNICO	**POSITIVO** PARA MORTALIDADE POR DCV E IAM NÃO-FATAL	RR 1,61 (1,22-2,12)

[A] MORFOLOGIA OVARIANA: achados histopatológicos típicos de PCOS a partir de ressecção em cunha ovariana; [B] DIAGNÓSTICO CLÍNICO: história de irregularidade menstrual e/ou hiperandrogenismo e/ou infertilidade

Conclusões e Perspectivas Futuras

A prevalência e a magnitude das alterações metabólicas e dos fatores de risco cardiovascular nas pacientes com a Síndrome dos Ovários Policísticos são bem estabelecidas na literatura em diferentes populações. Contudo a associação entre PCOS e eventos cardiovasculares primários, como infarto agudo do miocárdio e acidente vascular cerebral, ainda precisa ser confirmada. Os estudos publicados até o momento apresentam limitações metodológicas e resultados controversos. A dificuldade de definição da própria Síndrome explica em parte os achados conflitantes e o impacto variável do risco a ela associado. É possível que fenótipos menos graves de PCOS, com diferentes manifestações clínicas quanto à anovulação e hiperandrogenismo, tenham menor incidência de eventos cardiovasculares [46,47]. Assim, novos estudos prospectivos são necessários, com maior tempo de seguimento e delineados a partir de uma definição clara dos critérios diagnósticos de PCOS. Isto possibilitará, adicionalmente, que dados de diferentes estudos possam ser analisados em conjunto, em metanálises e/ou revisões sistemáticas e, consequentemente, se possa melhor definir os riscos associados à síndrome. Em termos de manejo clínico, a identificação das alterações metabólicas nas pacientes com PCOS devem ser cuidadosamente pesquisadas. Da mesma forma, o tratamento deve incluir medidas simples como aumento de atividade física e cuidados dietéticos desde as primeiras manifestações clínicas da PCOS, usualmente na adolescência, e associadas ao tratamento farmacológico. Espera-se que um melhor conhecimento das relações entre comorbidades metabólicas, fatores de risco cardiovascular e

eventos cardiovasculares em pacientes com PCOS possa definir novas estratégias terapêuticas.

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Parte II

Artigo Original 1: "Lipid accumulation product index: a reliable marker of cardiovascular risk in polycystic ovary syndrome"

Lipid accumulation product (LAP) index: a reliable marker of cardiovascular risk in polycystic ovary syndrome

Short title: LAP as marker of cardiovascular risk in PCOS

Wiltgen D[1,2,3], Benedetto IG[1,2], Mastella LS[1,2], Spritzer PM[1,2,3]

[1]Gynecological Endocrinology Unit, Division of Endocrinology, Hospital de Clínicas de Porto Alegre, Brazil; [2]Laboratory of Molecular Endocrinology, Department of Physiology, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil; [3] National Institute of Hormones and Women's Health- CNPq, Brazil.

Correspondence:
Poli Mara Spritzer, MD, PhD
Gynecological Endocrinology Unit, Division of Endocrinology, Hospital de Clínicas de Porto Alegre and Department of Physiology, Universidade Federal do Rio Grande do Sul
Rua Ramiro Barcelos, 2350
CEP 90035-003 – Porto Alegre, RS – Brazil
Phone/Fax: +55-51-3308-3671
E-mail: spritzer@ufrgs.br

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Abstract

BACKGROUND: Metabolic disturbances are common features of polycystic ovary syndrome (PCOS), which possibly enhance the risk of cardiovascular disease. The aim of this study was to assess the accuracy of lipid accumulation product (LAP) [waist (cm) - 58] x triglyceride concentration (mmol/l)] index as a marker of cardiovascular risk in PCOS patients.

METHODS: Case-control study including 51 PCOS patients with age between 14 and 35 years and 41 body mass index (BMI)-matched controls in the same age range.

RESULTS: LAP index was positively correlated with homeostasis model assessment (HOMA) index in all subjects (r 0.70; $p < 0.001$). Waist circumference ($p=0.002$), HOMA index ($p<0.001$), and LAP index ($p=0.035$) were higher in PCOS patients. On receiver operating characteristic curve analysis, a LAP index of 34.5 (sensitivity: 84%; specificity 79%) showed a better performance than non-HDLc, waist circumference or BMI to identify insulin resistance in all subjects. In PCOS patients, the positive and negative predictive values for LAP ≥ 34.5 were 91% and 74%, respectively, compared with 73% and 61%, respectively, for waist circumference ≥80cm, and 43% and 20%, respectively, for waist circumference ≥88cm.

CONCLUSIONS: LAP index, an easily obtainable measure, may be regarded as a useful screening tool to identify IR. LAP ≥ 34.5 is an additional risk factor for cardiovascular disease in PCOS patients.

Introduction

Polycystic ovary syndrome (PCOS), a disorder characterized by ovulatory dysfunction and hyperandrogenism, is the most prevalent endocrinopathy in women of reproductive age. PCOS is also considered a metabolic disorder, since insulin resistance (IR), an independent risk factor for cardiovascular disease, is a common feature in these patients (Ehrmann, 1997; Legro *et al.*, 1999; Dunaif *et al.*, 1997; Wild *et al.*, 2000). The early recognition of an "insulin-resistant phenotype" is important to prevent cardiovascular involvement in a subset of young and susceptible PCOS patients without other signs of IR.

Euglycemic hyperinsulinemic clamping is currently the gold standard for measuring IR. However, it is not suitable for clinical practice since it is complex, time-consuming and not feasible in large populations (DeFronzo *et al.*, 1979). On the other hand, alternative methods for identifying IR that rely on the measurement of insulin itself may be misleading, due to substantial interassay and inter-laboratory variations (Laakso, 1993). Taking into consideration the practical and technical limitations of these methods, we hypothesized that the presence of IR, and therefore cardiovascular risk, could also be determined on the basis of variables associated with insulin action, rather than on direct insulin measurements. The lipid accumulation product (LAP) index (Kahn, 2005), which combines waist circumference and triglyceride concentration, could be useful in this situation. Therefore, the aim of this study was to verify the accuracy of LAP index as a marker of cardiovascular risk in PCOS patients.

Materials and methods

Patients and controls

This is a case-control study carried out with women consulting at the Gynecological Endocrinology Unit at Hospital de Clínicas de Porto Alegre, Brazil. Fifty-one hirsute women with age between 14 and 35 years, oligo/amenorrheic cycles (<9 cycles/year), increased levels of serum testosterone and/or free androgen index (FAI), and absence of other disorders causing hirsutism (Spritzer *et al*., 1990) were enrolled in the study (The Rotterdam ESHRE/ ASRM-Sponsored PCOS Consensus Workshop Group, 2003; Azziz *et al.*, 2006). Transabdominal or transvaginal ovarian ultrasound was performed in all patients. Enlarged, cystic ovaries were detected in most PCOS patients.

Forty-four body mass index (BMI)-matched, non-hirsute women in the same age range, with regular ovulatory cycles (luteal phase progesterone levels higher than 12 nmol/L), were included in the study as a control group. None of the women from either group had received any drugs known to interfere with hormonal levels for at least 3 months before the study. Almost 95% of the sample was Caucasian. The remaining 5% were of mixed (African and European) descent. Women with BMI higher than 40 kg/m^2 or type 2 diabetes were excluded. The study protocol was approved by the local Ethics Committee (IRB-equivalent), and written informed consent was obtained from all subjects.

Study protocol

Anthropometric measurements were performed in duplicate by two investigators (WD and BIG), and included body weight, height, BMI (current measured weight in kg divided by height in m^2) and waist circumference (waist measured at the midpoint between the lower rib margin and the iliac crest in a plane that is perpendicular to the long axis of the body, with the subject standing balanced on both feet, approximately 20 centimeters apart, with both arms hanging freely) (WHO, 1995; Donato *et al.*, 2006; Toscani *et al.*, 2007). Obesity was defined as BMI $\geq$ 30. Hirsutism was defined as a modified Ferriman–Gallwey score of 8 or more (Ferriman and Gallwey, 1961). Blood pressure was measured after a 10-minute rest in the supine position. The hormonal and metabolic evaluation was made between days 2 and 10 of the menstrual cycle or on any day if the patient was amenorrheic. After an overnight 12-hour fast, blood samples were drawn from an antecubital vein for determination of plasma cholesterol, HDL-cholesterol and triglycerides at baseline and glucose and insulin before and 2 hours after the ingestion of a75-g oral glucose load. Impaired glucose tolerance (IGT) was determined by glucose levels between 140 and 200 mg/mL, as defined by the World Health Organization (WHO, 1999).

Blood samples were also drawn for measurement of sex hormone binding globulin (SHBG) and total testosterone (TT). All samples were obtained between 8 and 10 a.m. FAI was estimated by dividing TT (nmol/L) by SHBG (nmol/L) x 100. Homeostasis model assessment index (HOMA index) was calculated by multiplying insulin (μIU/mL) by glucose (mmol/L) and dividing this product by 22.5 (Wallace *et al.*, 2004). The cutoff point to define IR was arbitrarily defined as a HOMA index $\geq$ 3.8 (Toscani *et al.*, 2007). Metabolic syndrome was defined in accordance with NCEP/ATPIII criteria (NCEP/ATPIII, 2001). LAP

index for women was calculated using the formula [waist (cm) - 58] x triglyceride concentration (mmol/L), as previously reported (Kahn, 2005).

Assays

Total cholesterol, HDL-cholesterol, triglycerides, and glucose were determined by colorimetric-enzymatic methods using the Bayer 1650 Advia System (Mannheim, Germany). Non-HDL cholesterol levels were calculated by subtracting HDL-cholesterol from total cholesterol values. LDL cholesterol was estimated indirectly using the formula LDL = total cholesterol - HDL - triglycerides / 5. Serum LH was measured by a specific immunometric assay (Diagnostic Products Corporation-DPC, Los Angeles, CA, USA) with sensitivity of 0.05 mIU/mL, and intra- and interassay coefficients of variation (CV) of 3.6% and 6.7%, respectively. Total serum testosterone (T) levels were measured with the RIA method (ICN, Costa Mesa, CA, USA) with an intra- and interassay CV of 10% and 11.6%, respectively. SHBG was measured by chemiluminescent enzyme immunoassay (DPC, Los Angeles, CA, USA) with a sensitivity of 0.2 nmol/L, and intra and interassay CV of 6.1% and 8.0%, respectively. Serum insulin levels were measured with an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, D-68298 Mannhein, Germany) with sensitivity of 0.20 μIU/mL and intra- and interassay CV of 1.8% and 2.5%, respectively.

Statistical analysis

Results are presented as means ± standard deviation (SD), or median and interquartile range. Log10 transformation was used to normalize the distribution of non-Gaussian variables in order to allow comparisons between the groups using the Student's t test. χ^2 was calculated for comparisons of dichotomous variables. Receiver operating characteristic (ROC) curves were generated for LAP index and non-HDL cholesterol, waist circumference and BMI using a HOMA index $\geq$ 3.8 as the reference value to define IR, as previously reported.[12] Sensitivity and specificity for LAP and non-HDL cholesterol were calculated based on the point of inflection in these ROC curves. Sensitivity and specificity for BMI and waist circumference were calculated using validated cutoffs (25 for BMI, 80 and 88 for waist circumference). The correlation between variables was tested using the two-tailed Spearman rank correlation test considering the non-Gaussian distribution of variables. All analyses were

performed using the Statistical Package for the Social Sciences (SPSS version 14.0, Chicago, IL, USA). Data were considered to be significant at $p < 0.05$.

Results

Hirsutism score was 14.5 (ranging from 12 to 23) in the women with PCOS. Table 1 summarizes the clinical, hormonal and metabolic profile of both groups. Controls were older than PCOS patients. As expected, BMI was similar in both groups (normal 35.3% vs. 25%; overweight 17.7% vs. 22.7%; obese 47% vs. 52.3%; respectively in PCOS and controls, $p = 0.429$), but PCOS patients had greater waist circumference. The metabolic syndrome was about four times more frequent in PCOS. The groups had similar glucose levels, but other IR parameters, such as HOMA and LAP index, were strikingly higher in PCOS patients (Figure 1), even when adjusted for age (Table 1). The frequency of hypertension was 19.6% in PCOS vs. 9.1% in controls. Five (9.8%) PCOS patients and one (2.3%) control presented impaired glucose tolerance.

A positive and significant correlation was found between HOMA index and LAP (r 0.70; $p < 0.001$), waist circumference (r 0.71; $p < 0.001$) and BMI (r 0.82; $p < 0.001$) in both the PCOS and control groups (Figure 2).

ROC curve analysis revealed that the best cutoff values for LAP and non-HDL cholesterol to define the presence of IR were 34.5 (sensitivity: 84%; specificity 79%) and 3.02 mmol/L (sensitivity: 81%; specificity 63%), respectively (Figure 3). ROC curves were also generated for BMI and waist circumference, using the standard cutoffs of 25 for BMI (sensitivity: 83%; specificity: 38%), and of 80cm (sensitivity: 84%; specificity: 40%) and 88 cm (sensitivity: 71%; specificity: 61%) for waist circumference (Figure 3). Comparing all these ROC curves, we observed that a LAP index of 34.5 was the best marker of insulin resistance. This result was confirmed by analyzing the PCOS and control groups separately (Figure 3).

Table 2 shows the predictive values for markers of central adiposity/IR according to HOMA index in PCOS patients. The positive and negative predictive values (PPV and NPV) for LAP $\geq$ 34.5 were 91% and 74%, respectively – again showing that LAP was more accurate than the other studied markers to determine the presence of insulin resistance (PPV of 73% and NPV of 61% for waist circumference of 80 cm; PPV of 43% and NPV of 20% for

waist circumference of 88cm; PPV of 76% and NPV of 68% for non-HDLc of 3.02 mmol/L; and PPV of 72% and NPV of 76% for BMI 25).

Discussion

The present study shows that, despite being younger than controls, our PCOS patients had a worse metabolic profile and were more insulin resistant. The metabolic syndrome was also more prevalent in PCOS patients, even though they were in the same BMI range as controls.

While in the last years it has become clear that IR plays a central role in both the reproductive and metabolic disturbances observed in women with PCOS (Ehrmann, 1997; Legro *et al.*, 1999; Dunaif *et al.*, 1997; Wild *et al.*, 2000), identification of IR in these patients is challenging. The gold standard, euglycemic hyperinsulinemic clamping, is clearly inadequate for clinical practice since it is expensive, time-consuming, and requires complex technical skills such as bilateral cannulation and arterializations of blood flow to the vein. In turn, simpler, alternative methods that use fasting insulin levels as a diagnostic tool can lead to misdiagnosis of IR. In addition to the large intra and interassay variability that complicates direct insulin measurements, there is no reference range for normal insulin levels (Olefsky *et al.*, 1973; Chevenne *et al.*, 1999). Therefore, indexes that have a good correlation with the clamp but which depend on fasting insulin levels are difficult to employ as a clinical test for predicting the insulin resistance of individual patients.

Previous studies have evaluated enlarged waist circumference and elevated triglycerides (EWET) in different populations as a surrogate marker of cardiovascular. Amongst the criteria that define the metabolic syndrome, the association of these two variables was more sensitive than the metabolic syndrome itself to demonstrate higher cardiovascular risk. (Tankó *et al.*, 2005; Lemiex *et al.*, 2000). The presence of EWET was related to higher HOMA index at all ages, including in young women (Kahn and Valdez, 2003), and this dichotomous risk marker presented a moderate association with metabolic syndrome criteria in premenopausal women (Alhassan et al, 2008).

The LAP index, an ordinal scale combining waist circumference and triglycerides, was first tested in 2005 in a study using data from National Health and Nutrition Examination Survey sample database (NHANES III). The authors compared the LAP index to BMI in

terms of their ability to identify cardiovascular risk in adults. The subpopulation with ordinal LAP quartile higher than BMI quartile (adjusted for sex, race-ethnicity and age) had more adverse levels in 9 out of the 11 cardiovascular risk factors assessed in the study, suggesting that LAP might be a better predictor of the incidence of cardiovascular disease (Kahn, 2005).

In the present study, we propose that the LAP index may be an accurate method for estimating the presence of IR in PCOS patients and, in consequence, to precociously screen a subset of young women who are susceptible to the development of diabetes and other insulin resistance-related comorbidities, including cardiovascular disease. The fact that PCOS patients have higher LAP index values compared with controls with same BMI is further evidence of the potential metabolic implications of this disorder. In addition to a strong association with HOMA, the ROC curve showed that a LAP index $\geq$ 34.5 had adequate sensitivity and specificity for detecting a state of insulin resistance. Similar to other multifactorial diseases with heterogeneous clinical manifestations, creating prediction diagrams based on tests with good predictive values should be useful to make clinical decisions in PCOS. In the present study, a LAP index $\geq$ 34.5 showed a better performance to accurately discriminate IR in PCOS women when compared with the cutoff points defined for BMI (25) and waist circumference (80 cm and 88 cm) (NCEP/ATPII, 2001; Donato *et al.*, 2006). Therefore, our results suggest that LAP index $\geq$ 34.5 could be considered as a risk factor for metabolic disturbances and cardiovascular disease in PCOS patients and perhaps guide clinicians in the decision-making for treatment with insulin-sensitizer drugs.

As stated in the literature, the metabolic syndrome is closely related to IR; it is also more prevalent in PCOS patients than in women from the general population considering all age groups (Apridonidze *et al.*, 2005; Glueck *et al.*, 2003; Cussons *et al.*, 2008). However, the syndrome is largely influenced by the presence of obesity, which means that young, non-obese PCOS women may present IR even without the metabolic syndrome. We have previously observed a 58.5% prevalence of IR, evaluated by HOMA index, vs. 27.9% for the metabolic syndrome in a sample of PCOS patients that was similar to that of the present study (Spritzer and Wiltgen, 2007).

One limitation of the present study is the fact that euglycemic hyperinsulinemic clamping was not performed. However, previous studies in susceptible populations have shown that the HOMA index, used in our study as reference standard, is closely correlated

with euglycemic hyperinsulinemic clamp results (Bonora *et al.*, 2000). In addition, the HOMA index has been shown to predict cardiovascular disease in Caucasian individuals from the general population (Bonora *et al.*, 2007).

In conclusion, our results show that the LAP index, an easily obtainable measure, may be regarded as a useful tool to screen for the presence of IR and a reliable marker of risk for cardiovascular disease in PCOS. The early recognition of PCOS women who are prone to develop IR-related metabolic disturbances in the absence of other signs will allow the introduction of therapeutic interventions to ameliorate IR and probably reduce the risk of cardiovascular disease in the future.

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Table I. Clinical, hormonal and metabolic features of patients with PCOS and non-hirsute ovulatory controls

	PCOS (n = 51)	Controls (n=44)	P	P[b]
Age (years)	20.61± 5.15	28.91 ± 5.63	< 0.001	
Body mass index (kg/m^2)	29.50 ± 7.52	29.46 ± 5.40	0.976	
Waist circumference (cm)	90.65 ± 16.15	85.50 ± 11.61	0.002	0.002
Metabolic syndrome (NCEP/ ATPIII)	9 (17.6%)	2 (4.5%)	0.047	
LH[a] (IU/L)	6.45 (3.13-11.8)	5.47 (3.65-7.98)	0.93	
Total testosterone (nmol/L) [a]	0.03 (0.02 –0.04)	0.02 (0.01 – 0.027)	<0.001	<0.001
SHBG (nmol/L) [a]	22.45 (13.27 – 41.36)	36.59 (26.57 – 50.97)	0.002	0.001
Free androgen index[a]	16 (9-28)	4 (3-8)	< 0.001	<0.001
Total cholesterol (mmol/L)	4.86 ± 1.11	4.21 ± 0.8	0.002	<0.001
LDL-c (mmol/L)	3.08 ± 0.97	2.46 ± 0.69	0.005	0.001
HDL-c (mmol/L)	1.35 ± 0.027	1.32 ± 0.30	0.693	
Non-HDL-c (mmol/L)	3.51±1.13	2.88± 0.78	0.003	<0.001
Triglycerides (mmol/L)[a]	1.10 (0.77-1.48)	0.73 (0.54-1.21)	0.008	0.014
Glucose (mmol/L)	4.84 ± 0.46	4.92 ± 0.42	0.359	
HOMA index[a]	5.27 (2.82-8.09)	2.14 (1.42-3.09)	< 0.001	<0.001
LAP index[a]	37.87 (13.51-68.11)	20.89 (11.37-38.06)	0.035	0.001

Values are expressed as mean± SD (Student's t-test) or[a] median and 25-75 interquartile range (Mann-Whitney test)

P[b] adjusted for age (linear regression)

HOMA = homeostasis model assessment; LAP = lipid accumulation product; PCOS = polycystic ovary syndrome; BMI= body mass index; non-HDL-c: non-HDL cholesterol

Table II. Predictive values for markers of central adiposity/ insulin resistance according to HOMA index in PCOS patients

	HOMA index			
Cutoff point	**≥ 3.8**	**< 3.8**	**Predictive value**	**p**
LAP				
≥ 34.54	82.1%	12.5%	Positive: 91%	
< 34.54	17.9%	87.5%	Negative: 74%	< 0.001
Waist circumference (cm)				
≥ 88	31%	68.7%	Positive: 43%	
< 88	69%	31.3%	Negative: 20%	0.015
≥ 80	82.8%	50%	Positive: 73%	
< 80	17.2%	50%	Negative: 61%	0.02
Non-HDL-c				
≥ 3.02	79.3%	35%	Positive: 76%	
< 3.02	20.7%	65%	Negative: 68%	0.003
BMI				
≥ 25	80%	42.9%	Positive: 72%	
< 25	20%	57.1%	Negative: 66%	0.009

HOMA = homeostasis model assessment; LAP = lipid accumulation product; PCOS = polycystic ovary syndrome; BMI= body mass index; non-HDL-c: non-HDL cholesterol

Figure I. LAP values in women with PCOS and normal controls.

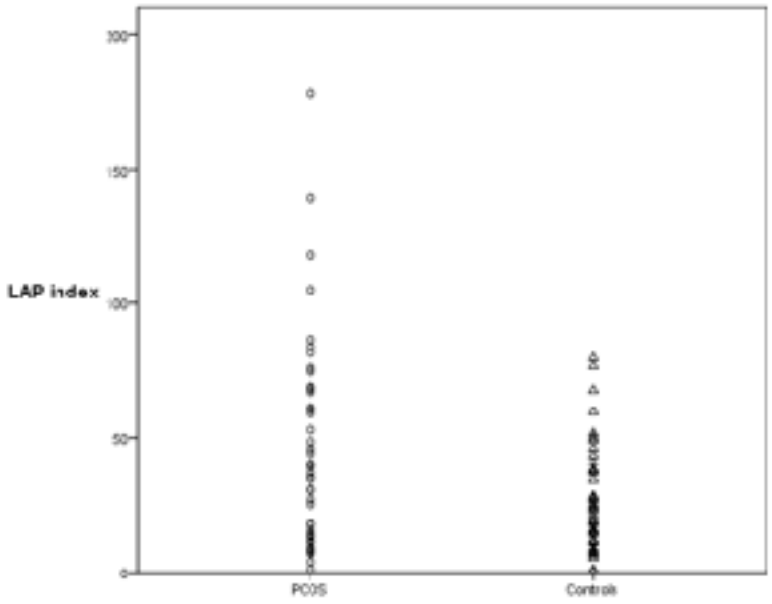
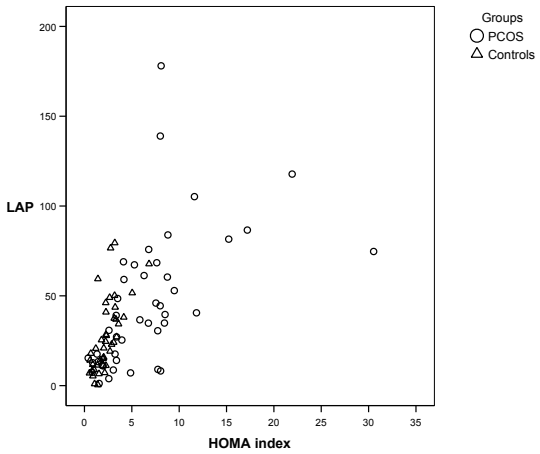
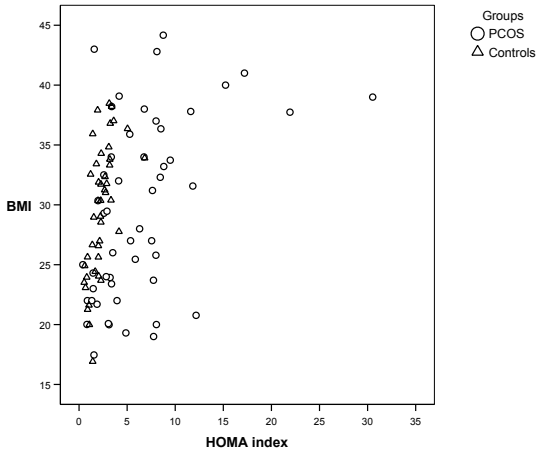


Figure II. Correlation between HOMA index and A) LAP index , B) BMI and c) waist circumference in PCOS and normal controls.

A) r = 0.7 p < 0.001



B) r = 0.82 p < 0.001



C) r = 0.71 p < 0.001

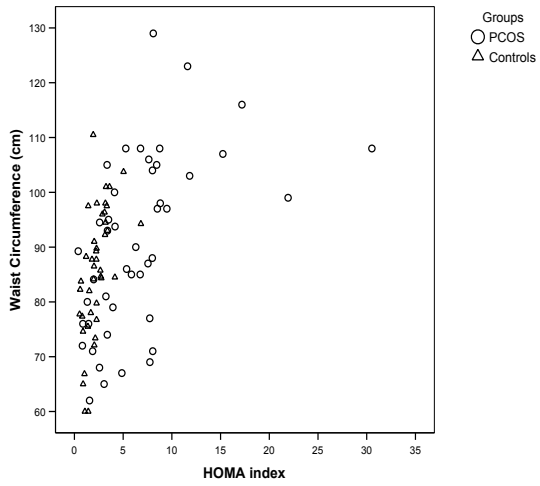
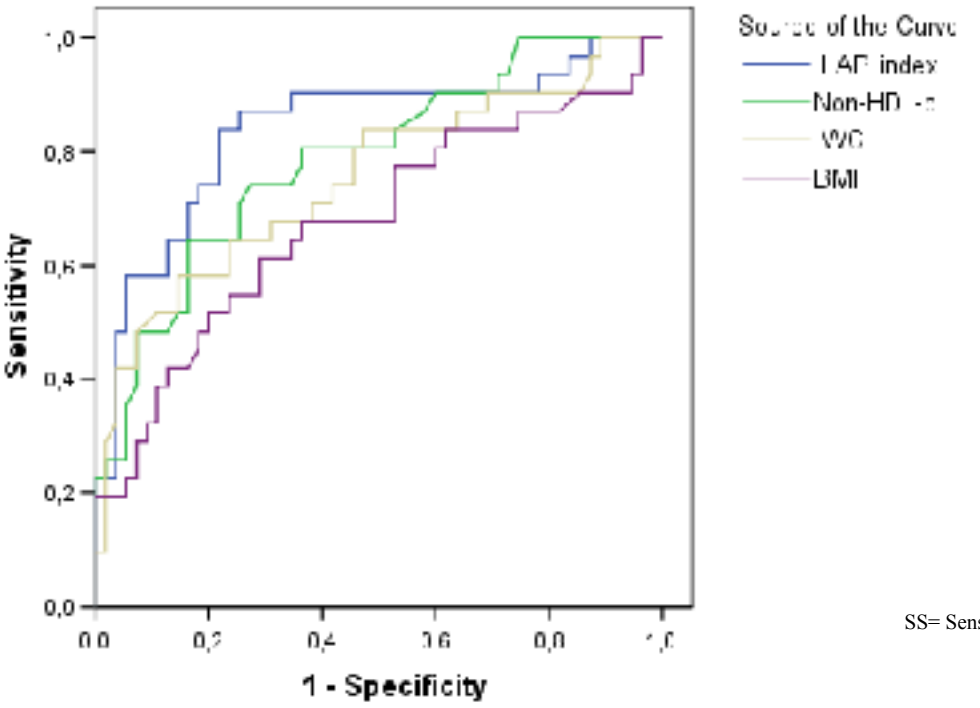


Figure III. ROC for LAP index, Non –HDL-c, waist circumference and body mass index (BMI) with HOMA index of 3.8 as marker of insulin resistance.

	All		PCOS		Controls	
	SS (%)	SP (%)	SS (%)	SP (%)	SS (%)	SP (%)
LAP 34.5	84	79	82	87	100	79
Non-HDL-c 3.02	81	63	78	68	100	61
WC 80 cm	84	40	82	56	100	46
WC 88 cm	71	61	88	58	67	54
BMI 25	83	38	82	52	100	25



SS= Sensitivity; SP= Specificity

Parte III

ARTIGO ORIGINAL 2: "VARIATION IN METABOLIC AND CARDIOVASCULAR RISK IN WOMEN WITH DIFFERENT POLYCYSTIC OVARY SYNDROME PHENOTYPES"

Variation in metabolic and cardiovascular risk in women with different polycystic ovary syndrome phenotypes

Denusa Wiltgen, MD

Poli Mara Spritzer, MD, PhD

Gynecological Endocrinology Unit, Division of Endocrinology, Hospital de Clínicas de Porto Alegre, Brazil; Laboratory of Molecular Endocrinology, Department of Physiology, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil. National Institute of Hormones and Women's Health - CNPq, Brazil.

Correspondence:

Dr. Poli Mara Spritzer

Division of Endocrinology, Hospital de Clínicas de Porto Alegre

Rua Ramiro Barcelos, 2350

CEP 90035-003 – Porto Alegre, RS – Brazil

Phone/Fax: +55-51-3308-3671

E-mail: spritzer@ufrgs.br

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Capsule

In the presence of ovulation and normal androgen levels, polycystic ovaries and hirsutism may not be associated with the metabolic and cardiovascular risk factors that are common in PCOS.

Abstract

Objective

To compare anovulatory women with hyperandrogenism, with or without polycystic ovaries (PCO) (classic phenotype); ovulatory women with normal androgens, hirsutism (H), and PCO (H+PCO phenotype); and ovulatory women with isolated hirsutism (IH) presenting normal ovaries and androgens.

Design

Case-control study.

Setting

University hospital.

Patients

195 classic phenotype patients, 45 H+PCO patients, 68 IH women, and 25 non-hirsute controls with regular ovulatory cycles.

Interventions

Anthropometric and hormone measurements.

Main outcome measures

Hormone levels, glucose and lipid profile, free androgen index (FAI), homeostasis model assessment index (HOMA-IR), and lipid accumulation product index (LAP).

Results

Classic PCOS patients were younger and had higher body mass index (BMI) vs. other groups ($p<0.001$). Glucose levels were similar. Waist circumference ($p=0.002$), triglycerides ($p<0.001$), LAP and HOMA ($p<0.007$) were significantly higher in classic PCOS even after BMI adjustment. The metabolic syndrome was three times more frequent in classic PCOS than in H+PCO or IH (31.3% vs. 11.9% vs. 9%, $p<0.001$).

Conclusions

H+PCO patients with normal androgens were similar to IH patients regarding metabolic profile and cardiovascular risk factors. In the presence of ovulation and normal androgen levels, PCO and hirsutism may not be associated with the metabolic and cardiovascular risk factors that are common in PCOS.

Key words: Polycystic ovary syndrome, hyperandrogenism, hirsutism, metabolic syndrome.

Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder in women of reproductive age, affecting 6-8% of women worldwide.[1-5] One of the hallmarks of PCOS is a great variability of signs and symptoms as well as a wide spectrum of severity, usually based on three major characteristics: androgen excess, anovulation, and, more recently, ovarian morphology (ovaries exhibiting a polycystic appearance). Also important is the recognition of PCOS as a metabolic disease, since these patients are about 50% more insulin resistant than controls, with higher prevalence of the metabolic syndrome and increased risk for type 2 diabetes.[6-9]

Recent publications have underscored the heterogeneous nature of PCOS and the relevance of describing different phenotypes in order to identify specific risks and individualize treatments.[10-12] Distinct PCOS phenotypes have been recently established based on a combination of National Institutes of Health (NIH)[13] and Rotterdam consensus[14] criteria. These phenotypes include the classic syndrome, that is, hyperandrogenism (hyperandrogenemia and/or hirsutism) and ovulatory dysfunction, with or without polycystic ovaries (PCO) on ultrasound; and what has been called an "ovulatory PCOS" phenotype, characterized by hirsutism and/or hyperandrogenemia, polycystic ovaries, and absence of anovulation.

Evidence suggests that the clinical impact and severity of metabolic manifestations is intermediate in ovulatory patients with hyperandrogenism, even in the presence of polycystic ovaries.[10, 11] However, no studies so far have assessed the hormone and metabolic profile of hirsute ovulatory women with PCO presenting normal androgen levels. Therefore, the aim of the present study was to compare clinical, hormonal and metabolic variables in women with classic PCOS, in ovulatory women presenting hirsutism, normal androgen levels and PCO and in a group with isolated hirsutism.

Subjects and methods

Three hundred and eight outpatients aged between 14-35 years and consulting for hirsutism at the Gynecological Endocrinology Unit at Hospital de Clínicas de Porto Alegre, Brazil were included in this cross-sectional study. Of this sample, 240 patients fulfilled the NIH and Rotterdam criteria for PCOS.[13, 14] They were stratified into two groups according to

the following phenotypes: 195 patients presenting biochemical and/or clinical hyperandrogenism and ovulatory dysfunction (OD), with or without polycystic ovaries (classic PCOS group); and 45 hirsute women with normal androgen levels, regular and ovulatory cycles (luteal phase progesterone > 3.8 ng/ml) and polycystic ovaries (H+PCO group). Ovulatory dysfunction was defined by the presence of oligo/amenorrheic cycles (<9 cycles/year), and/or ovarian volume greater than 10mm^3 in at least one ovary. Hirsutism was defined as a modified Ferriman Gallwey score ≥ 8.[15] A group of 68 women presenting isolated hirsutism (normal androgens levels, regular and ovulatory cycles and normal ovarian volume) was also included (group IH).[16-18]

Controls

Twenty-five non-hirsute women in the same age range, with regular and proven ovulatory cycles were included in the study as a control group. None of the subjects or controls had received any drugs known to interfere with hormonal levels for at least 3 months before the study. Women with other hyperandrogenic disorders were excluded, as well as patients with BMI higher than 40 kg/m^2. The study protocol was approved by the local Ethics Committee (IRB-equivalent), and written informed consent was obtained from all subjects.

Study protocol

Anthropometric measurements included body weight, height, BMI (current measured weight in kg divided by height in m^2) and waist circumference (waist measured at the midpoint between the lower rib margin and the iliac crest, in a plane that is perpendicular to the long axis of the body).[17, 19, 20] Obesity was defined as BMI ≥ 30. Blood pressure was measured after a 10-minute rest with the woman in the supine position. The hormonal, metabolic and ultrasound evaluation were made between days 2 and 10 of the menstrual cycle or on any day if the patient was amenorrheic. All samples were obtained between 8 and 10 a.m. after an overnight fast. Blood samples were drawn from an antecubital vein for determination of plasma cholesterol, HDL-cholesterol and triglycerides at baseline and glucose and insulin before and 120 minutes after a 75g oral glucose tolerance test (OGTT). Impaired glucose tolerance (IGT) was determined by glucose levels between 140 and 200 mg/ml 120 minutes after the oral glucose load, as defined by the World Health Organization (WHO).[21]

Blood samples were also drawn for measurement of sex hormone binding globulin

(SHBG) and total testosterone (TT). The free androgen index (FAI) was estimated by dividing TT (nmol/L) by SHBG (nmol/L) x 100. Homeostasis model assessment index (HOMA-IR index) was calculated by multiplying insulin (μIU/ml) by glucose (mmol/l) and dividing this product by 22.5.[22] The cutoff point to define IR was arbitrarily defined as a HOMA index ≥ 3.8.[17, 23] Lipid accumulation product index (LAP index) for women was calculated using the formula [waist (cm) - 58] x triglyceride concentration (mmol/L)], as previously reported.[24, 25] Metabolic syndrome was defined in accordance with NCEP ATP III criteria.[26]

Assays

Total cholesterol, HDL-cholesterol, triglycerides, and glucose were determined by colorimetric-enzymatic methods using the 400 Roche Centricichem System. LDL cholesterol was determined indirectly using the formula LDL = total cholesterol - HDL + triglycerides / 5. Serum LH was measured by a specific immunometric assay (Diagnostic Products Corporation-DPC, Los Angeles, CA, USA) with sensitivity of 0.05 mIU/mL, and intra- and interassay coefficients of variation (CV) of 3.6% and 6.7%, respectively. Total serum testosterone (T) levels were measured with the RIA method (ICN, Costa Mesa, CA) with an intra- and interassay CV of 10% and 11.6%, respectively. SHBG was measured by chemiluminescent enzyme immunoassay (DPC, Los Angeles, CA) with a sensitivity of 0.2 nmol/L, and intra- and interassay CV of 6.1% and 8.0%, respectively. Serum insulin levels were measured with an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, D-68298 Mannhein) with sensitivity of 0.20 μIU/ml and intra- and interassay CV of 1.8% and 2.5%, respectively.

Statistical analysis

Results are presented as means ± standard deviation (SD), or median and interquartile range. Log_{10} transformation was used to normalize distribution of non-Gaussian variables in order to allow comparisons between the groups by one-way analysis of variance (ANOVA/ ANCOVA), followed by Bonferroni test. χ^2 was calculated for comparisons of dichotomous variables. The correlation between variables was performed by two-tailed Spearman rank correlation test considering the non-Gaussian distribution of variables. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 16, Chicago, IL, USA). Data were considered to be significant at $p < 0.05$.

Results

Table 1 summarizes the clinical, hormonal and metabolic profile of all groups. Hirsutism score was similar in the PCOS, H+PCO, and IH groups. As expected by definition, androgen levels were higher in the PCOS phenotype. Patients in this group were younger and had higher BMI than the other groups. They also had higher systolic and diastolic blood pressure and a worse lipid profile in comparison to the other groups. However, after adjustment for BMI, only androgen levels, waist circumference, glucose levels 120 minutes after OGTT, and triglycerides remained significantly higher in the PCOS group. In addition, markers of insulin resistance, such as HOMA-IR and LAP index, were strikingly higher in the classic PCOS phenotype even after adjustment for BMI (Figure 1).

Table 2 shows the prevalence of the metabolic syndrome and its individual components in the four groups. The metabolic syndrome was three times more frequent in PCOS than in H+PCO and IH patients. Waist circumference > 88 cm and HDL-c < 50 mg/dl were the most prevalent components of the metabolic syndrome in all groups. Also noteworthy was the high prevalence of hypertension in the PCOS group (40.2%). In contrast, the metabolic syndrome was absent in control subjects, even though their BMI was similar to that of H+PCO and IH women (Figure 2). Type 2 diabetes affected 6.2% of PCOS patients, 2.2% of H+PCO patients, 1.4% of IH patients, and 0% of controls. Impaired glucose tolerance was present in 11.3% of PCOS, 2.2% of H+PCOS and 7.6% of IH patients. LAP index $\geq$ 34.5 and HOMA-IR > 3.8 were also significantly more prevalent in PCOS (Figure 2). When considering all subjects, both LAP index and HOMA-IR showed a moderate and significant correlation with TT ($r = 0.322$ and $r = 0.31$, respectively; $p < 0.001$).

Discussion

In the present study, women with the H+PCO phenotype (known as ovulatory PCOS) were very similar to patients with isolated hirsutism in terms of clinical, hormonal and metabolic characteristics. Moreover, both these groups had a normal lipoprotein and glucose profile, as well as waist circumference, LAP and HOMA in the same range as control women. This observation differs from the results of previous studies reporting that the risk for metabolic comorbidities in women with the H+PCO phenotype is intermediate[11] or equal[12,

[27] to that faced by women with the classic, hyperandrogenic and anovulatory PCOS phenotype when compared to isolated hirsutism or normal women. However, while most studies evaluating the so-called ovulatory PCOS phenotype include women presenting hirsutism and/or hyperandrogenemia,[11, 12, 27] this study appears to be the first specifically evaluating hirsute ovulatory PCO patients with normal androgen levels.

After the introduction, in 2003, of ovarian morphology as a new criterion to be considered in the diagnosis of PCOS,[14] much controversy has emerged, mostly concerning the threat of overdiagnosing PCOS and the establishment of metabolic risks associated with the new phenotypes, especially those including ovulatory patients. Our data show that PCOS diagnosis based on the presence of hyperandrogenism and ovulatory dysfunction, with or without PCO, is associated with a worse metabolic profile and more insulin resistance than that observed in ovulatory women with the H+PCO phenotype or with isolated hirsutism, and in ovulatory control women without hirsutism. Our patients with classic PCOS also had higher prevalence of metabolic syndrome and isolated cardiovascular risk factors, such as increased waist circumference, hypertension and a more adverse lipid profile.[11, 27-29] Moreover, even after adjustment for BMI (since the prevalence of obesity was higher in the classic PCOS group), the adverse metabolic profile found in PCOS patients remained significantly worse.

In this sense, hyperandrogenism seems to be a key element in the pathophysiology of PCOS. Despite the known technical limitations of measuring androgen concentrations and the ethnic differences in hair body distribution and severity of hirsutism, it is becoming clear that the diagnosis of PCOS should imply clinical or biochemical hyperandrogenism.[10] In turn, whether or not hirsute ovulatory patients with normal androgen levels are at risk of developing metabolic comorbidities and cardiovascular risk remains to be confirmed.

Obesity, which affects both androgen secretion and insulin sensitivity, is a major element in the PCOS phenotype.[30, 31] The prevalence of obesity in PCOS has been shown to be higher than in control subjects in the same age range. Obesity further exacerbates metabolic and reproductive dysfunctions often seen in these anovulatory and hyperandrogenic women.[12, 32-35] In addition, data from the present study, showing a moderate correlation between androgen levels and insulin resistance surrogate markers, like HOMA and LAP, support the idea that PCOS and the metabolic abnormalities observed in

these patients might share a common pathogenetic pathway.[36-39]

Our patients with the classic PCOS phenotype were younger than those with the H+PCO phenotype. As a result of a higher number of early abnormalities, especially menstrual irregularities, women with PCOS are prone to seek medical assistance more promptly than those presenting a less severe clinical presentation. Previous studies have also reported that the variation in the clinical manifestations observed in older women with other phenotypes of PCOS in relation to those with classic PCOS are probably due to a difference in disease etiology rather than to the amelioration of the disturbances along the years.[27]

In conclusion, our data indicate that hirsute ovulatory patients with PCO – a recently defined PCOS phenotype – presenting normal androgen levels have a normal metabolic profile and low prevalence of cardiovascular risk factors, being similar in that regard to women with isolated hirsutism. This finding suggests that PCO and isolated hirsutism, in the absence of anovulation and hyperandrogenemia, may not be associated with the metabolic and cardiovascular risk factors that are commonly observed in PCOS patients.

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Table 1. Clinical, hormonal and metabolic features of patients with PCOS, ovulatory H+PCO phenotype, isolated hirsutism
and normal non-hirsute, ovulatory controls[a]

	PCOS (n = 195)	H+PCO phenotype (n = 45)	Isolated hirsutism (n = 68)	Controls (n = 25)	p	p (BMI adjusted)
Age (yr)	22.31 ± 6.7[a]	25.89 ± 7.56[b]	24.73 ± 8.35[a,b]	29.68 ± 4.29[b]	< 0.001	
BMI (kg/m^2)	31.06 ± 7.98[a]	26.96 ± 6.38[b]	26.91 ± 7.48[b]	26.97 ± 3.64[b]	< 0.001	
Waist circumference (cm)	93.79 ± 18.81[a]	83.42 ± 13.37[b]	84.07 ± 16.92[b]	79.83 ± 8.37[b]	< 0.001	0.019
SBP (mmHg)	123.09 ± 16.92[a]	114.73 ± 21.02[b]	116.33 ± 15.30[b]	115.21 ± 9.51[b]	0.002	0.24
DBP (mmHg)	78.9 ± 12.29[a]	73.48 ± 12.84[b]	74.22 ± 13.10[b]	73.6 ± 8.27[a,b]	0.005	0.41
Fasting glucose (mg/dL)	90.52 ± 21.42	84.58 ± 10.66	86.79 ± 11.86	88.67 ± 8.19	0.09	
Glucose 120 (mg/dL)	119.56 ± 48.21 [a]	100.82 ± 26.98 [b]	97.73 ± 26.72 [b]	90.25 ±16.9 [b]	0.001	0.017
Total cholesterol (mg/dL)	182.4 ± 43.79 [a]	162.8 ± 27.47 [b]	177.52 ± 32.62 [a,b]	165.28 ± 36. 82 [a,b]	0.009	0.12
HDL-c (mg/dL)	48.43 ± 11.18 [a]	53.6 ±11.28 [b]	52.38 ± 11.85 [a,b]	54.96 ± 13.71 [a,b]	0.002	0.15

LDL-c (mg/dL)	110.15 ± 37.28 [a]	93.68 ± 24.39 [b]	108.5 ± 29.36 [a,b]	95.5 ± 31.49 [a,b]	0.01 1	0.09
Triglycerides (mg/ dL)	99.5 (67.25-142) [a]	68 (52-96.5) [b]	70.5 (51.5 -93.2) [b]	60 (42 - 93) [b]	< 0.00 1	< 0.001
Ferriman-Gallwey score	13 (9-18)[a]	13 (11-15.5)[a]	13 (10-17.5)[a]	2 (0-5.5)[b]	< 0.00 1	
FAI	15.64 (8.5-22.87)[a]	6.06 (3.64-8.81)[b]	6.93 (3.82-9.84)[b]	4.03 (3.47-4.66) [b]	< 0.00 1	< 0.001
Total testosterone (nmol/L)	3.467 (2.53-4.36)[a]	2.08 (1.38-2.87)[b]	2.18 (1.59-2.87)[b]	2.08 (1.59-2.39) [b]	< 0.00 1	< 0.001
Ovarian volume (cm^3)	10.12 (7.41-14)[a]	11.32 (9.6-13.45) [a]	6.67 (5.41-7.84)[b]	8.18 (6.43-10.54)[b]	< 0.00 1	< 0.001

[a]PCOS: hyperandrogenism + ovarian dysfunction (anovulation with or without PCO); H: hirsutism; PCO: polycystic ovaries.

Values are expressed as mean ± SD or median and 25-75 interquartile range. Different superscript letters indicate statistical difference by ANOVA and Bonferroni tests.

Table 2. Prevalence (%) of metabolic syndrome and its individual components in PCOS, hirsute ovulatory patients with PCO, isolated hirsutism and control groups[a]

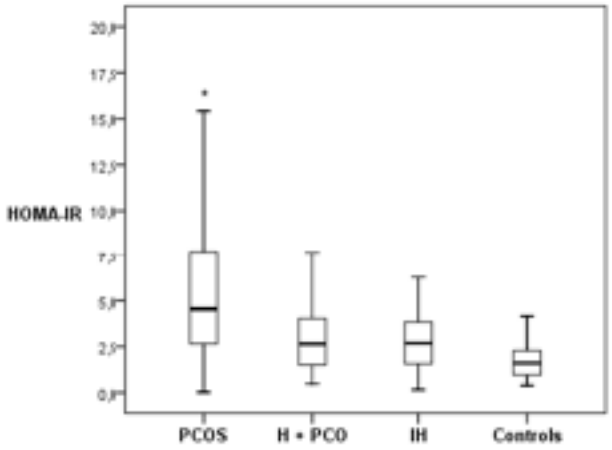
	PCOS	H+PCO	IH	Controls	p
Metabolic syndrome (3 of 5)	31.3	11.9	9.0	0	< 0.001
Waist circumference > 88cm	59.9	33.3	34.3	16	< 0.001
Hypertension (≥ 130/ ≥ 85 mmHg)	40.2	28.9	20.3	13.0	0.003
HDL-c < 50 mg/dl	58.8	35.3	40.3	32	< 0.001
Triglycerides ≥ 150 mg/dl	22.9	6.8	7.4	8.0	0.003
Fasting glucose ≥ 110 mg/dl	6.8	2.2	4.4	0	0.354

[a]PCOS: hyperandrogenism + ovarian dysfunction (anovulation with or without PCO); H: hirsutism; PCO: polycystic ovaries.

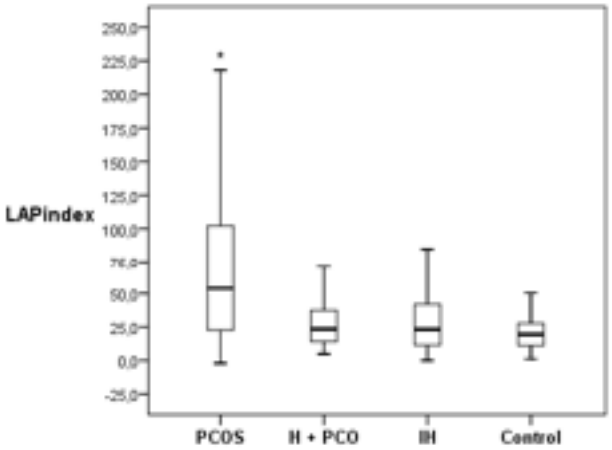
Figure legends

Figure 1 – A) HOMA IR and B) LAP index in PCOS, hirsute ovulatory patients with PCO, isolated hirsutism and control groups.

A)



B)



* p< 0.007 vs H +PCO, IH and control groups

Figure 2. Prevalence of obesity, insulin resistance and diabetes in PCOS, hirsute ovulatory patients with PCO, isolated hirsutism and control groups