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DIZERTAČNÍ PRÁCE

Hyperandrogenní stavy u žen: problematika adrenální hyperandrogenémie

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Obsah : **strana**

Úvod

Neklasická forma adrenální hyperplázie (NCAH)

Hyperandrogenní stavy jsou nejčastější endokrinopatií žen ve fertilním věku, postihující přibližně 7% této populace (1, 2, 3). Převážná většina je vyvolána poruchou steroidogeneze na úrovni vaječníků (hyperandrogenní syndrom – HAS, syndrom polycystických vaječníků - PCOS). U části hyperandrogenních žen je příčinou zvýšených hladin androgenních hormonů úplný nebo částečný blok enzymů uplatňujících se v adrenální steroidogenezi. Naprostá většina případů (přes 95%) je způsobena deficitem 21-hydroxylázy. V důsledku snížené aktivity enzymu se zpomaluje konverze 17-hydroxyprogesteronu na 11-deoxykortizol (větev glukokortikoidní) a progesteronu na 11-deoxykortikosteron (mineralokortikoidní metabolická větev).

Existují dvě základní formy onemocnění - klasická forma (classic congenital adrenal hyperplasia - CAH) a neklasická (pozdní) forma adrenální hyperplázie (nonclassic adrenal hyperplasia - NCAH). Klinické projevy onemocnění jsou pestré, od asymptomatického průběhu až po ženský pseudohermafroditismus s deplecí natria, manifestující se již v prvních týdnech života (CAH).

Klasická forma kortikální adrenální hyperplázie (CAH) je charakterizována závažným deficitem 21-hydroxylázy (kompletní blok), virilizací již v prenatálním období a různým stupněm adrenální insuficience. Vyskytuje se ve dvou subtypech : s deplecí sodíku, označovaného SW-CAH (salt-wasting), charakterizovaným depleční krizí v již prvních týdnech života, a jako prostá virilizující forma, SV-CAH (simple virilizing). Incidence CAH se pohybuje mezi 1 na 12100 – 23000 (4).

Léčba vyžaduje substituci glukokortikoidy i mineralokortikoidy a supresi nadměrné produkce ACTH. Ve zvlášť těžkých případech, kdy není možno dosáhnout dobré kompenzace, je metodou volby bilaterální adrenalektomie.

V budoucnosti lze očekávat využití antagonistů corticotropin-releasing hormonu (CRH) a genové terapie.

Incidence a prevalence

Incidence NCAH se liší u jednotlivých etnických skupin. Nejvyšší výskyt byl zjištěn mezi Aškenázskými Židy (1:27), v nežidovské bílé populaci je odhadován 1:1000-2000 (5). Nosičství alel pro mírnou nebo pro neklasickou formu CAH kolísá podle etnicity vyšetřeného souboru mezi 1:5 až 1:50 (New Annu Rev med 1998 (6). V největším publikovaném souboru 873 hyperandrogenních žen byla zjištěna prevalence NCAH v 1.6% (7). Za vznik onemocnění může být odpovědný i deficit jiných steroidogenních enzymů (zejména 11β- hydroxylázy), přes 95% případů NCAH je ale způsobeno sníženou aktivitou 21-hydroxylázy (5, 8). Kontroverzní je otázka, jaká jsou hormonální kriteria pro NCAH způsobenou defektem 3β- hydroxylázy (20).

Genetika

Příčinou jak klasických, tak neklasických forem kongenitální adrenální hyperplázie, je geneticky podmíněná nedostatečnost enzymů steroidogeneze: 1) 21- hydroxylázy (*CYP21, pseudogen CYP21P*), 2) 11 β -hydroxylázy (*CYP11B1*), 3) dehydrogenázy 3 β -hydroxysteroidů a delta 5-4 izomerázy (*HSD3B1, HSD3B2*), 4) steroidogenního akutního regulačního proteinu (*StAR*) a 5) 17α-hydroxylázy (*CYP17*), přitom u neklasických forem jde vždy o mírnější enzymatickou poruchu. Jedná se o onemocnění s autozomálně recesívním typem dědičnosti.

Dominantní příčinou jak klasických forem CAH, tak pozdní formy CAH, je deficit enzymu 21-hydroxylázy, který je nacházen až u 90% případů.

Již koncem 70. let byla popsána asociace různých forem deficitu 21-hydroxylázy s charakteristickými haplotypy HLA (human leukocyte antigen), čehož se využívalo pro nepřímou molekulárně genetickou diagnostiku CAH. Později byly identifikovány dva geny kódující 21-hydroxylázu u člověka – funkční gen *CYP21* a nefunkční, tzv. pseudogen *CYP21P*. Oba leží na

krátkém raménku 6. chromozómu, v těsné blízkosti oblasti kódující alely HLA. Právě tato blízká chromozomální lokalizace genu a pseudogenu predisponuje ke vzniku mutací, může docházet k delecím, ke genovým konverzím mezi funkčním genem a pseudogenem. Nejčastěji jsou u pacientů nalézány delece různého rozsahu v genu *CYP21* (delece malých úseků až po kompletní deleci jedné alely), duplikace genu (jsou častou příčinou NCAH), či bodové mutace. Fenotyp u většiny pacientů dobře koreluje s typem mutace. Mutace *CYP21* vedoucí k úplné deaktivaci 21-hydroxylázy jsou příčinou virilizace spojené se solnou poruchou, druhá skupina mutací *CYP21*, kdy produkt má reziduální enzymatickou aktivitu, je spojena s prostou virilizací a třetí skupina mutací, které snižují enzymatickou aktivitu o 50-70 %, je nalézána převážně u neklasické formy CAH (21, 22).

U pacientek s NCAH je obvykle vyšetřováno 10 frekventních mutací a delecí (identifikace 90-95% defektních alel).V etnické skupině Aškenázských Židů byla potvrzena vysoká frekvence „NCAH alel", v subpopulaci pacientek nežidovského původu byl zaznamenán vyšší výskyt „klasických" mutací (9). Specifické mutace pro pozdní – neklasickou formu CAH byly zatím identifikovány pouze při deficitu 21-hydroxylázy (*CYP21*), nikoliv u ostatních výše zmíněných enzymopatií, které mohou být příčinou minoritních forem NCAH.

Laboratorní diagnostika

V důsledku deficitu aktivity enzymu 21-hydroxylázy (u NCAH snížení aktivity na 20 – 50%) se u pacientek s adrenální hyperplázií zvyšují hladiny jeho substrátu, 17OH-progesteronu.

Za základní laboratorní test ke stanovení diagnózy NCAH je považován ACTH stimulační test. Provádí se v ranních hodinách folikulární fáze menstruačního cyklu, po bazálním odběru je aplikováno 0.25 mg ACTH a za 60 min. stanovena hladina stimulovaného 17OH-progesteronu. Hladina nad 30.3 nmol/l je diagnostická pro NCAH. S cílem omezit nutnost provádění ACTH testu (z důvodů ekonomických, časových, nutnosti venepunkce) stanovil Azziz hraniční hodnoty bazální hladiny 17OH-progesteronu, které vylučují či potvrzují diagnózu NCAH při zachování odpovídající senzitivity a

pozitivní či negativní prediktivní hodnoty. Odběr by měl být proveden ve folikulární fázi cyklu, v ranních hodinách (7.30-9.30 hod.) vzhledem k cirkadiánnímu rytmu adrenální sekrece. Diagnózu NCAH prakticky vylučuje hladina bazálního 17OH-progesteronu nižší než 6nmol/l (2ng/ml) – negativní prediktivní hodnota se přibližuje 100%. Naopak při bazální hladině 17OH-progesteronu nad 10nmol/l je diagnóza NCAH pravděpodobná (pozitivní prediktivní hodnota (PPV) +/- 40%, senzitivita +/- 90%). Těchto vylučovacích hodnot lze využít screeningově v populaci hyperandrogenních žen. ACTH test tak může být proveden jen u žen s hladinami bazálního 17OH-progesteronu mezi 6 a 10 nmol/l; zastoupení těchto hyperandrogenních pacientek se odhaduje mezi 3 - 6 % (10, 23).

Z dalších laboratorních nálezů většina studií zjistila vyšší hladiny celkového i volného testosteronu, přestože testosteron není přímým ukazatelem aktivity 21-hydroxylázy, dále dehydroepiandrosteronsulfátu (DHEAS) a androstendionu (11, 7, 12).

V již zmiňované Azzizově práci sledující 873 hyperandrogenních žen měly pacientky s NCAH signifikantně vyšší hladiny celkového i volného testosteronu ve srovnání se ženami s ovariální hyperandrogenémií (hyperandrogenním syndromem, HAS). Dalším paradoxním nálezem byly u žen s NCAH významně vyšší hladiny SHBG (7).

Vyšší hladiny androgenů u NCAH lze vysvětlit pravděpodobně současnou nadprodukcí androgenů v nadledvinách a ve vaječnících. Teorii o kombinované příčině zvýšených hladin androgenů podporuje i jejich pokles a zlepšení hirzutismu u pacientek s NCAH po podání agonistů gonadoliberinu (13).

NCAH a inzulínová senzitivita

Řada prací se zabývá inzulínovou senzitivitou u hyperandrogenních žen s hyperandrogenním syndromem (HAS). Důvodem jsou pozdní metabolická rizika HAS (metabolický syndrom). V literatuře však není dostupná studie sledující parametry inzulínové senzitivity u NCAH pomocí diagnostického vyšetření, které je považováno za zlatý standard - euglykemického hyperinzulínemického clampu. I jinak byla zatím pozornost inzulínové senzitivitě u žen s NCAH věnována jen okrajově. Speiser pomocí

tolbutamidem modifikovaného intravenózního glukózového tolerančního testu zjistil v malém souboru žen s NCAH (n = 6) sníženou inzulínovou senzitivitu (14).

Ve výše citované Azzizově studii byla popsaná tendence k horším parametrům inzulínové senzitivity ve skupině HAS ve srovnání se skupinou NCAH (u HAS vyšší hladina inzulínu nalačno, vyšší HOMA index). Přesto i další faktory (obezita není častá u žen s NCAH,) podporují předpoklad příznivějších parametrů inzulínové senzitivity u NCAH než u HAS.

Klinická symptomatologie

Klinické projevy NCAH připomínají symptomy ovariální hyperandrogenémie, tedy nepravidelný menstruační cyklus charakteru oligo/amenorey, kožní androgenní obtíže – hirzutismus, akné, ev. alopecie. Ve studiích zabývajících se klinickou symptomatologií a léčbou NCAH je za dominující příznak obvykle považován hirzutismus. K manifestaci symptomů dochází nejčastěji v pozdní pubertě či časné dospělosti, medián věku, ve kterém je onemocnění diagnostikováno, se pohybuje mezi 22.3 – 24.5 roky (11, 13).

O možné časnější manifestaci (pod 10 let věku) a progresivním charakteru onemocnění vypovídá Moranova multicentrická studie sledující 218 pacientek s NCAH z 11 center. Ve skupině dívek mladších deseti let zjistil u 92% předčasnou pubarché. Ve starších věkových skupinách byly dominujícími příznaky hirzutismus a oligomenorea. Na progresivní charakter ukazuje dle Morana stoupající prevalence hirzutismu přímo úměrně věku – 10-19 let 70%, 20-29 let 82%, 30-39 let 90%, 40-49 let 94%. Zajímavý je fakt, že vzrůstá prevalence, nikoli stupeň zvýšeného ochlupení (dle Ferimann-Gallwey skore) mezi uvedenými věkovými skupinami (11).

Další androgenní příznak, akné, nepatří mezi častý projev NCAH. Ve zmiňované Moranově studii se vyskytoval s prevalencí mezi 20 - 40%, konstantně ve všech věkových skupinách (11). Obdobnou prevalenci akné mezi ženami s NCAH potvrdil Azziz (22.2%). Rovněž v jeho práci byl hlavním příznakem hirzutismus (prevalence 72.2%). Oba soubory se naopak liší ve

výskytu poruch menstruačního cyklu charakteru oligo/amenorey (89% vs. 50-58%). Mezi typický klinický příznak nepatřila obezita, její prevalence se pohybuje mezi 20 a 50%, mediány BMI mezi 23.7 a 29 kg/m^2.

Vztah mezi genotypem a fenotypem u pacientek s NCAH porovnává Speiserova multicentrická studie, ve které sledoval 34 žen. Z výsledků vyplývá, že tzv. smíšení heterozygoti, tedy nositelky „klasické" mutace na jedné alele a „neklasické" mutace na druhé, mají vyšší hodnoty 17OH-progesteronu spolu se zvýšeným výskytem hirzutismu než homozygoti pro mírné mutace (9). Naopak starší práce Knochenhauera nezjistila vyšší riziko manifestace hirzutismu u heterozygotních nositelů mutace genu *CYP21* (15).

Léčba

Ačkoliv se vzhledem k mechanizmu onemocnění nabízí možnost terapeuticky zasáhnout kauzálněji než v případě hyperandrogenního syndromu, tedy kortikoidy suprimovat osu hypotalamus - hypofýza - nadledviny a tím redukovat excesívní sekreci androgenů, nejsou klinické účinky této léčby spolehlivé. Strategie léčby je obdobná individuálním postupům u HAS. Rovněž v případě NCAH zvažujeme, které projevy chceme terapeuticky ovlivnit : kožní androgenní příznaky (akné, hirzutismus, alopecie), nepravidelný menstruační cyklus, ev. anovulaci - sterilitu. Dosud existuje málo intervenčních studií na větších souborech a tedy jen málo spolehlivých dat o léčbě NCAH.

Konvenční léčba NCAH, substituce kortikoidy, je odvozena od léčby klasické formy adrenální hyperplázie. V případě CAH substituce koriguje deficit kortizolu, inhibuje sekreci ACTH a snižuje stimulaci adrenální steroidogeneze. Hlavním cílem kortikoidní léčby u neklasické formy je rovněž suprimovat adrenální produkci androgenů. V případě NCAH však není třeba korigovat hladiny kortizolu; jeho hladiny jsou u částečného deficitu 21-hydroxylázy ve fyziologických mezích. Nejčastěji používaným kortikoidem používaným v léčbě NCAH je hydrokortizon v dávce 10 – 20 mg denně. Efekt

léčby na hladiny androgenů je zřejmý, byl prokázán významný pokles hladin testosteronu, androstendionu a samozřejmě i 17OH-progesteronu (12).

Naopak účinek léčby kortikoidy na klinické projevy není výrazný. Ve studii srovnávající efekt léčby hydrokortizonem (2x10 mg, n=16) a antiandrogenem cyproteronacetátem (CPA, 50mg denně, n=14) na laboratorní parametry a dominující klinický projev, hirzutismus, bylo ve skupině léčené glukokortikoidy zaznamenáno pouze velmi pozvolné a mírné snížení ochlupení (FG skore 22, 21.3, 19.5 a 16.5 v 0. 3., 6. a 12. měsíci léčby). Ve skupině léčené CPA byla signifikantní redukce ochlupení zjištěna již ve 3. měsíci (FG skore 23, 17, 12.9a 10 v 0., 3., 6. a 12. měsíci léčby). Ve skupině léčené CPA došlo pouze k mírnému poklesu hladin androgenů, které potvrdilo jeho periferní účinek – kompetitivní inhibicí vazby androgenů v cílových tkáních (12).

Další zkoušenou léčebnou modalitou v terapii NCAH byla ovariální suprese agonisty gonadoliberinu. Po 6měsíčním podávání byl zaznamenán pokles hladin 17OH-progesteronu, celkového i volného testosteronu a androstendionu. DHEAS zůstal nezměněn. Signifikantně bylo zlepšeno zvýšené ochlupení – FG skore z 16.4 na 8.4 (13). Přestože se jedná o malý soubor (n=6), výsledky potvrzují významný podíl ovariální hyperandrogenémie.

S volbou léčebné modality souvisí otázka monitorování účinku intervence. Logické je hodnocení podle vývoje hladin androgenů. Kortikoidní substitucí lze současně se snížením hladin testosteronu a androstendionu očekávat pokles hlavního markeru adrenální hyperandrogenémie, 17OH- progesteronu. Přesto jsou při této léčbě často zjišťovány přetrvávající zvýšené hladiny, při normalizaci hladin ostatních androgenů. Je to nejspíše způsobeno vyšší citlivostí androgenů ke kortikoidní supresi ve srovnání s C-21 steroidy.
Při monitorování efektu léčby by měly být sledovány hladiny testosteronu, androstendionu, DHEAS, jejichž adekvátní suprese může být dosaženo i při přetrvávajících zvýšených hladinách 17OH-progesteronu. Sledování hladin 17OH- progesteronu je považováno za nejméně významné; naopak snaha o normalizaci jeho hodnot může vést až k „overtreatmentu" a rozvoji cushingoidních znaků. Nejsilnější androgenní účinek má testosteron,

androgenní potenciál 17OH-progesteronu je slabší. Není jasné, zda zvýšené hladiny 17OH-progesteronu zvyšují riziko pozdních komplikací (metabolický syndrom).

Dalším ukazatelem efektu léčby je zlepšení klinických příznaků.

NCAH a gravidita

Ačkoliv se u pacientek s NCAH vyskytují často anovulační cykly, část z nich otěhotní spontánně (16).

U sterilních žen je po vyloučení dalších možných příčin obvykle nejdříve doporučována ve snaze o nastolení ovulačních cyklů kortikoidní substituce na 4 měsíce. Není-li úspěšná, postupuje se obdobně jako v léčbě anovulační sterility u HAS, tedy indukcí ovulace antiestrogeny, event. gonadotropiny.

Další otázkou související s těhotenstvím je prekoncepční příprava a management gravidity žen s NCAH. Riziko porodu novorozence s klasickou formou adrenální hyperplázie (CAH) u žen s NCAH závisí kromě genotypu matky i na tom, zda je otec nosičem mutace genu CYP21. Riziko je vyšší pouze v případě otcova nosičství a matčiny mutace genu způsobující těžký deficit 21-hydroxylázy. V případě mutací genů kódujících mírný či střední deficit tohoto enzymu je riziko kongenitálního postižení dítěte extrémně nízké (pokud není zároveň přítomna další neidentifikovaná mutace, či mutace pro těžký deficit vzniklý „de-novo"). Neznáme- li genotyp CYP21 u rodičů, je riziko porodu dítěte postiženého CAH u pacientek s NCAH odhadováno na 1.7-2.3/1000 (4). Takto nízká míra rizika neopravňuje k invazívní prenatální diagnostice ani ke kortikoidní substituci v průběhu gravidity (23).

Závěr

Deficit aktivity 21-hydroxylázy patří mezi nejčastější autozomálně recesívní genetické choroby. Přesto je incidence klasické i neklasické formy adrenální hyperplázie v celé populaci i v populaci hyperandrogenních žen velmi nízká.

Ke stanovení diagnózy NCAH v naprosté většině případů stačí vyšetření bazální hladiny 17OH-progesteronu, provedení ACTH-stimulační testu je vhodné u přibližně 6% hyperandrogenních žen s hodnotami bazálního 17OH-progesteronu mezi 6 a 10 nmol/l.

Klinická symptomatologie může být velmi chudá, často připomíná obraz hyperandrogenního syndromu; typický příznak pro NCAH neexistuje.

Léčba substitucí kortikoidy je odvozena od klasické formy adrenální hyperplázie. V korekci kožních androgenních obtíží lze využít antiandrogeny. Vzhledem k normálním hladinám kortizolu je racionální i využití kombinované hormonální antikoncepce. Cílem i ukazatelem adekvátní intervence by mělo být v první řadě zlepšení klinických obtíží.

Léčebný postup při anovulační sterilitě se v podstatě neliší od HAS. V současnosti nejsou k dispozici spolehlivá data o riziku metabolických pozdních komplikací.

Popis projektu

Hyperandrogenní stavy jsou nejčastější endokrinopatií žen ve fertilním věku. Převážná většina je vyvolána poruchou steroidogeneze na úrovni vaječníků (hyperandrogenní syndrom – HAS, syndrom polycystických vaječníků - PCOS). Na druhém místě v diferenciální diagnostice hyperandrogenních stavů je tradičně uváděna neklasická forma adrenální hyperplázie (NCAH), tedy porucha adrenální steroidogeneze. Zatímco o ovariální hyperandrogenémii existuje v literatuře mnoho údajů a zabývá se jí drtivá většina studií, o NCAH je k dispozici málo dostupných dat.

Uváděný rozptyl prevalence NCAH u hyperandrogenních žen (mezi 1 a 10 %) souvisí s odlišným výskytem v různých geografických regionech a etnických skupinách obyvatel. Dosud největší soubory hyperandrogenních žen, které sledovaly prevalenci NCAH a její klinické a endokrinní parametry, pocházejí z USA a Sicílie. V našich geografických podmínkách a v naší populaci hyperandrogenních žen se neklasickou formou adrenální hyperplázie dosud žádná práce nezabývala.

Nedostatek spolehlivých dat existuje o léčbě NCAH. Konvenční léčba substitucí kortikoidy (hydrokortizonem) je odvozena od léčby klasické formy adrenální hyperplázie (CAH), kde koriguje deficit kortizolu a inhibuje sekreci ACTH. Suprese ACTH tak vede i ke snížení produkce adrenálních androgenů. V souvislosti s managementem NCAH je třeba brát v úvahu následující faktory: 1) vzhledem k pouze částečnému snížení aktivity enzymu 21-hydroxylázy není NCAH spojena s deficitem kortizolu a nadprodukcí ACTH (na rozdíl od CAH), 2) nebyl prokázán uspokojivý efekt léčby kortikoidy na klinickou manifestaci NCAH (nepravidelný menstruační cyklus, kožní androgenní obtíže), 3) v léčbě NCAH byl zjištěn efekt i léčby analogy GnRH (gonadotropin-releasing hormonu) - na androgenní nadprodukci se tak u pacientek s NCAH podílejí pravděpodobně i vaječníky. Přestože je znám účinek podávání kombinované hormonální antikoncepce nejen na redukci ovariální, ale i adrenální produkce steroidů, nebyla k dispozici dosud žádná studie, která by sledovala její efekt v managementu NCAH, nebo ji srovnávala

s klasickou léčbou kortikoidy. Účinek kombinované hormonální antikoncepce na kožní androgenní symptomy je přitom prokázán mnoha studiemi.

Cílem retrospektivně/prospektivní části bylo z našeho velkého souboru hyperandrogenních žen stanovit prevalenci neklasické formy adrenální hyperplázie, zjistit její laboratorní, klinické a endokrinní parametry a porovnat je s naší populací žen s ovariální hyperandrogenémií (PCOS, HAS) a dosud největším publikovaným americkým souborem.

Cílem intervenční části bylo zjistit účinek léčby kombinovanou hormonální antikoncepcí na klinické a laboratorní NCAH a porovnat jej s léčbou kortikoidy.

Cíle projektu. Zjistit, zda je odlišná manifestace klinických symptomů u nejčastějších hyperandrogenních stavů (ovariální a adrenální etiologie), zda se liší naše populace hyperandrogenních žen od dosud nejrozsáhlějšího amerického souboru v antropometrických parametrech a manifestaci klinických symptomů, zda je nutné rutinně provádět ke stanovení diagnózy NCAH ACTH test, zda je možné k léčbě NCAH využít stejně jako v případě ovariální hyperandrogenémie přípravky kombinované hormonální antikoncepce s minimální reziduální androgenní aktivitou.

Očekávaným přínosem projektu je kromě zjištění prevalence, laboratorních a klinických charakteristik NCAH v našich geografických poměrech, ověření, zda ovariální a adrenální suprese androgenní produkce kombinovanou hormonální antikoncepcí může být terapeutickou alternativou ke kortikoidní substituci (jejíž efekt na klinickou manifestaci je slabý) v managementu NCAH.

Výsledky projektu

I. Prevalence neklasické formy adrenální hyperplázie (NCAH) u hyperandrogenních žen

Naprostá většina hyperandrogenních stavů u žen je ovariální etiologie, označované jako syndrom polycystických vaječníků (PCOS) nebo hyperandrogenni syndrom (HAS). Jeho prevalence je odhadována na přibližně 7% (1, 2, 3).

V diferenciální diagnostice je vždy (kromě vzácných androgen-secernujících tumorů diagnostikovaných na základě rychlé progrese klinických známek hyperandrogenizmu) doporučováno vyloučit adrenální původ hyperandrogenémie, tedy neklasickou (pozdní) formu adrenální hyperplázie (NCAH).

NCAH je ve více něž 95% případů způsobena částečným deficitem aktivity enzymu 21-hydroxylázy (4, 5), snížené na 20 – 50%.

Prevalence NCAH se liší u jednotlivých etnických skupin. Nejvyšší výskyt byl zjištěn mezi Aškenázskými Židy (1:27), v nežidovské bílé populaci je odhadován 1:1000-2000 (5, 26).

Předpokládá se, že více než 1% populace je nositelem alely pro neklasickou formu adrenální hyperplázie (24).

Rozptyl prevalence mezi hyperandrogenními ženami se pohybuje mezi 1 až 10%. V dosud největším publikovaném souboru 873 hyperandrogenních žen byla zjištěna prevalence NCAH v 1.6% (25). Data z naší populace dosud chybějí; cílem studie bylo zjistit skutečnou prevalenci NCAH mezi hyperandrogenními ženami naší populace.

Soubor a metodika

Od roku 1999 do 2006 byla u 298 pacientek endokrinologické ambulance naší kliniky zjištěna zvýšená hladina alespoň jednoho z androgenů (testosteronu, dehydroepiandrosteronu, dehydroepiandrosteronsulfátu, androstendionu) v kombinaci s některým z klinických projevů hyperandrogenizmu (nepravidelný menstruační cyklus, zvýšené ochlupení, akné).

Analýzy hormonálních hladin a sex hormone binding proteinu (SHBG) byly prováděny v Centrální laboratoři Všeobecné fakultní nemocnice. Plazmatické koncentrace luteinizačního hormonu (LH), folikulostimulačního hormonu (FSH) a testosteronu jsou stanovovány chemoluminiscenční reakcí (ACS:180 auto analyzér, Bayer Diagnostics GmbH, Německo). Koncentrace dehydroepiandrosteronu (DHEA), dehydroepiandrosteronsulfátu (DHEAS), androstendionu, 17OH-progesteronu RIA metodou (Immunotech, Francie). SHBG je měřen použitím IRMA kitů (Orion, Finsko).

Kromě androgenů, LH, FSH a SHBG byly stanoveny hladiny prolaktinu (PRL), thyreoideu stimulačního hormonu (TSH) a provedeno vyšetření ultrazvukem (vaginální sondou).

V hodnocení klinických projevů byla oligo až amenorea definována jako menstruační cyklus delší než 35 dní. Hirzutizmus byl definován na základě skóre dle Ferrimana – Gallweyové vyššího než 8.

Zároveň byly hodnoceny antropometrické parametry pacientek – body mass index (BMI), obvod pasu a boků.

Diagnóza NCAH byla stanovena na základě bazální a stimulované hladiny 17OH-progesteronu 60 min. po stimulaci v krátkém ACTH stimulačním testu (Controsyn/Synacthen 0,25 mg i.v.) vyšší než 30 nmol/l. ACTH test byl prováděn u pacientek s bazální hodnotou 17OH-progesteronu vyšší než 6 nmol/l. Odběry krve byly prováděny ve folikulární fázi cyklu, mezi 8 a 10 hod. ranní.

Výsledky

Výsledky studie a jejich srovnání s naším souborem pacientek s PCOS a největším dosud publikovaným souborem jsou shrnuty v tabulkách I a II.

Deficit enzymu 21-hydroxylázy a diagnóza NCAH byla stanovena z celého souboru 298 hyperandrogenních žen u osmi pacientek.

Průměrný body mass index (BMI) u žen s NCAH byl 22.67 (20.64 –27.27), hirzutismus a akné byly zjištěny pouze u dvou pacientek, oligomenoreu mělo šest žen.

Sedm žen mělo zvýšenou bazální i stimulovanou hladinu 17OH-progesteronu, jedna pouze stimulovanou. U všech pacientek s NCAH byl zjištěn zvýšený testosteron, u šesti dehydroepiandrosteron (DHEA), ve 4 případech snížená hladina SHBG. Překvapivě žádná z pacientek s NCAH neměla zvýšenou hladinu dehydroepiandrosteronsulfátu (DHEAS). U poloviny pacientek byl poměr LH/FSH vyšší než 1.

Pouze u jedné pacientky byly nalezeny polycystické vaječníky při ultrazvukovém vyšetření.

Diskuze

Přestože je NCAH druhým nejčastějším hyperandrogenním stavem žen ve fertilním věku, je k dispozici zcela minimum studií (a většinou na omezeném počtu případů) zaměřených na prevalenci, diagnostiku a symptomatologii NCAH. Široký rozptyl prevalence NCAH u hyperandrogenních žen (mezi 1 a 10 %) souvisí s odlišným výskytem v různých geografických regionech a etnických skupinách obyvatel (4, 5, 26, 27). V naší populaci hyperandrogenních žen se prevalencí NCAH dosud žádná práce nezabývala.

V naší studii předkládáme zkušenosti z vyšetření velkého dobře definovaného souboru téměř tří set pacientek se zjištěnou nadprodukcí některého z androgenů a klinickým příznakem hyperandrogenizmu a srovnání s našimi pacientkami s PCOS a s velkým americkým souborem.

V populaci našich hyperandrogenních žen jsme zjistili prevalenci NCAH 2.68%. Tedy stejně jako Azziz v dosud nejrozsáhlějším, téměř tisícovém souboru hyperandrogenních žen spíše při dolní hranici uváděného rozmezí (25).

NCAH byla diagnostikována na základě bazální či stimulované hladiny 17OH-progesteronu; kromě jedné měly pacientky v našem souboru zvýšené jeho bazální i stimulované hladiny. Screeningově u drtivé většiny hyperandrogenních žen v diagnostice NCAH díky adekvátní senzitivitě a

pozitivní i negativní prediktivní hodnotě vylučujících či potvrzujících hladin (<6nmol/l, resp. >10nmol/l) postačuje stanovení bazální hladiny 17OH-progesteronu (27).

K manifestaci symptomů dochází nejčastěji v pozdní pubertě či časné dospělosti, medián věku stanovení diagnózy NCAH se pohybuje mezi 22.3 – 24.5 (28, 29, 30). Tomu odpovídají i naše zkušenosti; průměrný věk při stanovení diagnózy byl 23.5 (17-28 let).

Náš soubor se od amerického lišil v manifestaci klinických symptomů a výskytu endokrinních odchylek (tab. I). V obou souborech byla vedoucím symptomem oligomenorea; častěji se vykytovala u amerických NCAH pacientek (88.9% vs. 62.5%). Nejvýznamnější rozdíly byly zjištěny v antropometrických parametrech – rozdílné BMI (29.0 vs. 22.67), zatímco v americkém souboru byla obézních polovina pacientek, žádná pacientka s NCAH z našeho souboru obézní nebyla. V americkém souboru byl častým příznakem hirzutismus (72.2%), v našem souboru bylo zvýšené ochlupení minoritním problémem (pouze třetina pacientek). Diskrepance v klinických symptomech je velmi pravděpodobně způsobena rozdílnou prevalencí obezity mezi oběma soubory. Obezita je tak nezávislým faktorem, který významně ovlivňuje fenotyp syndromu a zhoršuje metabolické a endokrinní parametry.

Z laboratorních charakteristik jsme potvrdili zvýšené hladiny testosteronu u NCAH (všechny pacientky), častěji než u PCOS jsme v našem souboru zaznamenali zvýšení jak testosteronu tak androstendionu. Naopak jsme nezaznamenali zvýšené hladiny DHEAS, tedy androgenu dominantně produkovaného nadledvinami. Narozdíl od Azzizovy studie, kde byly zjištěny signifikantně vyšší DHEAS oproti dalším skupinám hyperandrogenních žen (PCOS, hyperandrogennímu inzulínorezistentnímu acanthosis nigricans - HAIRAN, idiopatickému hirzutismu). Rovněž snížené hladiny SHBG u 50% pacientek v našem souboru nekorespondují s výsledky Azzizovy práce, v níž zjistil signifikantně vyšší hladiny tohoto vazebného proteinu ve skupině NCAH pacientek oproti pacientkám s PCOS (25).

Poměr LH/FSH větší než 1 u 50% pacientek našeho souboru potvrzuje nižší specifitu tohoto laboratorního kritéria pro hyperandrogenní syndrom.

V našem souboru nebylo zjištěno častější zastoupení ultrazvukového morfologického nálezu polycystických vaječníků, ačkoliv je tento nález často popisován i u žen s NCAH (4).

V našem ani Azzizově souboru nebyly zjištěny významné rozdíly v klinické symptomatologii mezi pacientkami s PCOS a NCAH. Obě diagnózy jsou tedy na základě příznaků nerozlišitelné.

Naše výsledky mohou mít význam pro klinickou praxi v diferenciální diagnostice hyperandrogenních stavů. Pokud bychom stanovovali samotnou bazální hladinu 17-hydroxyprogesteronu, nezachytili bychom pouze jeden případ NCAH z celého souboru téměř tří set hyperandrogenních žen. Měření bazální hladiny 17-hydroxyprogesteronu tak patří mezi základní diagnostické nástroje u pacientek s androgenní nadprodukcí, ve shodě s Azzizem jsme potvrdili, že ACTH- test je možné omezit pouze na ženy s bazální hladinou 17-hydroxyprogesteronu mezi 6 a 10nmol/l (27).

Závěr

V naší studii byla prevalence NCAH mezi hyperandrogenními ženami 2.68%, tedy stejně jako v dosud největším publikovaném souboru na dolní hranici obvykle uváděného rozmezí. Ačkoliv je tradičně v diferenciální diagnostice hyperandrogenních stavů uváděná po hyperandrogenním syndromu (PCOS) na druhém místě, znamená spíše minoritní problém. Detekce NCAH je komplikovaná tím, že její klinická manifestace je od dalších hyperandrogenních stavů (hlavně PCOS) neodlišitelná. Vedoucím klinickým symptomem v našem souboru byl nepravidelný menstruační cyklus charakteru oligomenorey, naopak kožní androgenní obtíže nebyly významněji zastoupeny. Téměř u všech pacientek s NCAH byly zvýšené bazální hladiny 17-hydroxyprogesteronu, který tak může být využit jako základní test k detekci pacientek s deficiencí 21-hydroxylázy. U žádné z pacientek s NCAH nebyla zjištěna zvýšená hladina DHEAS, androgenu dominantně produkovaného nadledvinami.

Tabulka I. Charakteristiky hyperandrogenních pacientek s NCAH a PCOS – srovnání se souborem publikovaným Azzizem.

	NCAH Fanta, n=8	**PCOS Fanta, n=290**	NCAH Azziz, n=18	PCOS Azziz, n=716
prům. věk (l.)	23.5	24.6	28.28	27.67
prům. BMI (kg/m^2)	22.67	25.29	29.0	33.39
prům. WHR	0.74	0.8	0.83	0.83
oligo/amenorea	62.5 %	71 %	88.9 %	100 %
hirsutismus	37.5 %	49 %	72.2 %	72.2 %
akné	25.0 %	32.0 %	22.2 %	14.5 %
obezita	0	11 %	50 %	60 %

BMI = body-mass index, WHR = poměr pas/boky (waist to hip ratio)

Tabulka II. Laboratorní parametry a morfologický nález polycystických ovárií u žen s NCAH a PCOS.

		NCAH, n=8		**PCOS, n=290**	
↑ 17- OHP^0	(nmol/l)	87.5 %	12.8	1 %	2.58
↑ 17- OHP^{60} (nmol/l)		100 %	111.2	n.a.	
↑ testosteron	(nmol/l)	100 %	4.0	63 %	3.26
↑ DHEA	(μg/l)	75 %	27.5	66 %	29.01
↑ DHEAS	(μmol/l)	0	11.44	3 %	8.9
↑ androstenedion	(nmol/l)	75 %	13.35	14 %	8.58
↓ SHBG	(nmol/l)	50 %	32.1	23 %	41.8
LH/FSH > 1		50 %		59 %	
polycystická ovária		12.5 %		47 %	

n.a. = not applicable, 17- OHP^0 = bazální 17-hydroxyprogesteron, 17- OHP^{60} = stimulovaný 17-hydroxyprogesteron, DHEA = dehydroepiandrosteron, DHEAS = dehydroepiandrosteron sulfát, SHBG = sex hormone-binding globulin, LH = luteinizačníhormon, FSH = folikulo-stimulační hormon

II. Srovnání účinku léčby kortikoidní substitucí a kombinovanou hormonální antikoncepcí v managementu neklasické formy adrenální hyperplázie (NCAH)

Konvenční léčba NCAH kortikoidní substitucí (hydrokortizon – HCT) je odvozena od klasické formy adrenální hyperplázie (CAH), kde koriguje deficit kortizolu a inhibuje produkci ACTH. Tím je dosaženo i poklesu androgenní produkce v nadledvinách (12, 31). Kortikoidní terapie má ale pouze omezený efekt na klinickou manifestaci NCAH (12). Na kožní androgenní symptomy (hirzutismus) u pacientek s NCAH byl prokázán účinek antiandrogenu cyproteronacetátu (12). Rovněž byl zjištěn účinek ovariální suprese analogy GnRH (gonadotropin-releasing hormonu), který potvrzuje i ovariální účast na abnormální androgenní produkci (13).

Přestože účinek kombinované hormonální antikoncepce na inhibici ovariálních i adrenálních steroidů je znám poměrně dlouho, nebyla dosud publikována žádná studie, která by sledovala její efekt, nebo ho porovnávala s účinkem léčby kortikoidy v managementu NCAH (17, 18, 19). Cílem studie bylo zjistit efekt léčby nízkodávkovanou hormonální antikoncepcí s minimální reziduální androgenní aktivitou (obsahující gestagen 3. generace) na hladiny androgenů a klinické symptomy a porovnat jej s účinkem léčby kortikoidy.

Soubor a metodika

Subjekty byly vybírány ze souboru 298 pacientek, u nichž byla zjištěna elevace alespoň jednoho z androgenů (testosteronu, dehydroepiandrosteronu, dehydroepiandrosteronsulfátu, androstendionu) v kombinaci s některým z klinických projevů hyperandrogenizmu (nepravidelný menstruační cyklus, zvýšené ochlupení, akné).

Diagnóza NCAH byla stanovena na základě bazální a stimulované hladiny 17-hydroxyprogesteronu 60 min. po stimulaci v krátkém ACTH stimulačním testu (Controsyn/Synacthen 0,25 mg i.v.) u osmi pacientek. Odběry krve byly prováděny ve folikulární fázi cyklu, mezi 8 a 10 hod. Kritériem pro zařazení do souboru pacientek s deficiencí 21-hydroxylázy (NCAH) z celé populace téměř tří set hyperandrogenních žen byly bazální

hladiny 17-hydroxyprogesteronu vyšší než 10nmol/l, nebo stimulované hladiny nad 30.3nmol/l.

Průměrný věk pacientek v souboru byl 23.5 roku, průměrný body mass index (BMI) 22.67. Charakteristika souboru je shrnuta v tabulce I. Vzhledem k malému souboru byl k získání reprezentativních výsledků zvolen cross-over design studie. Osm pacientek bylo rozděleno do dvou stejných podskupin podle pořadí aplikace léčebné modality. Hodnocení laboratorních a klinických parametrů bylo prováděno vždy po každé terapeutické intervenci HCT nebo COC (tab. II). Léčba (proměnná *Treatment*) tak byla testována na každé pacientce (proměnná *subject*) dvakrát. HCT (20mg denně) byl podáván podskupině 1 (*Group 1*) v první periodě léčby po dobu 4 měsíců, poté následovala 3 měsíční wash-out perioda, a nakonec druhá čtyřměsíční perioda léčby přípravkem COC (30 μg ethinylestradiolu a 150 μg desogestrelu). Paralelně byla podávána léčba v inverzním schématu podskupině 2 (*Group 2*). Tímto formálním přístupem byl eliminován vliv pořadí léčby.

Před léčbou a po každé z period byly prováděny krevní odběry mezi 8 a 10 hod. ranní a stanovovány tyto parametry: LH, FSH, 17-hydroxyprogesteron, testosteron, androstendion, dehydroepiandrosteron (DHEA), dehydroepiandrosteronsulfát (DHEAS), sex hormone binding globulin (SHBG), prolactin a TSH. Rovněž byly hodnoceny po každé periodě antropometrické parametry a klinické symptomy.

Analýzy hormonálních hladin a sex hormone binding proteinu (SHBG) byly prováděny v Centrální laboratoři Všeobecné fakultní nemocnice. Plazmatické koncentrace luteinizačního hormonu (LH), folikulostimulačního hormonu (FSH) a testosteronu jsou stanovovány chemoluminiscenční reakcí (ACS:180 auto analyzér, Bayer Diagnostics GmbH, Německo). Koncentrace dehydroepiandrosteronu (DHEA), dehydroepiandrosteronsulfátu (DHEAS), androstendionu, 17OH-progesteronu RIA metodou (Immunotech, Francie). SHBG je měřen použitím IRMA kitů (Orion, Finsko). Kromě androgenů, LH, FSH a SHBG byly stanoveny hladiny prolaktinu (PRL), thyreoideu stimulačního hormonu (TSH) a provedeno vyšetření ultrazvukem (vaginální sondou).

V hodnocení klinických projevů byla oligo a amenorea definována jako menstruační cyklus delší než 35 dní, resp. 3 měsíce. Hirzutizmus byl definován na základě skóre dle Ferrimana – Gallweyové vyššího než 8.

Zároveň byly hodnoceny antropometrické parametry pacientek – body mass index (BMI), obvod pasu a boků.

Cílem studie bylo porovnat efekt léčby obou sledovaných léčebných modalit – HCT 2x10 mg denně vs. COC s obsahem 30 μg ethinylestradiolu a 150 μg desogestrelu – na laboratorní a klinické parametry NCAH.

Statistické zpracování

Hodnoty parametrů z posledních osmi sloupců tabulky II byly jako nezávislé proměnné y_{ijk} zpracovány v ANOVA modelu (1)

$$y_{ijk} = \mu + Group_i + Subject_{ij} + Period_k + Treatment_h + e_{ijk} \quad (1)$$

μ označuje celkový účinek (Intercept), e_{ijk} je symbol pro chyby měření. Normální distribuce pro (yk) bylo dosaženo použitím Shapiro-Wilkova testu a z-score testu. Z důvodu nesymetrie dat a nekonstantního rozptylu byla před vlastní analýzou provedena mocninná nebo logaritmická transformace k přiblížení Gaussovskému rozdělení. Data tak byla dostatečně homogenní pro všechny každou proměnnou, absolutní hodnota studentizovaného rezidua nikdy nedosáhla hodnoty 4.

Výsledky

Z velké kohorty téměř tří set hyperandrogenních žen dokončilo studijní protokol všech osm pacientek s NCAH. Obě hodnocené terapeutické modality (HCT vs. COC) byly podávány každé pacientce po dobu čtyř měsíců s tříměsíční wash-out periodou.

Akné a hirzutismus měly před intervencí pouze dvě pacientky, šest žen ze souboru mělo oligomenoreu. Všechny pacientky s NCAH měly zvýšenou hladinu testosteronu, šest androstendionu a DHEA, čtyři sníženou hladinu SHBG. Žádná neměla zvýšenou hladinu DHEAS.

Jak je patrné z obr. 1 léčba COC vedla k významnému zvýšení hladin SHBG (graf 1G) a úpravě nepravidelného menstruačního cyklu (graf 1D). Významné interindividuální rozdíly byly zjištěny u hladin 17-hydroxyprogesteronu (graf 1B), androstendionu (graf 1F) a FG-skóre (graf 1H). Vliv skupiny (*Group*) a periody intervence (*Period*) nebyly signifikantní pro žádnou ze závislých proměnných, což potvrzuje dostatečnou délku wash-out periody, resp. abscenci vlivu změn doby užívání na efekt léčby.

Shrnutím kompletní statistické analýzy jsme zjistili významný pokles celkových hladin androgenů v obou terapeutických skupinách; výsledky se u obou léčebných modalit signifikantně nelišily. Naopak významně vyšší hladiny SHBG a tedy snížení hladin volných androgenů bylo zaznamenáno ve skupině léčené COC ve srovnání s HCT. Logicky byl zjištěn příznivý vliv COC na nejfrekventnější symptom NCAH, oligomenoreu, naopak účinek HCT byl neuspokojivý.

Diskuze

NCAH je druhou nejčastější příčinou hyperandrogenizmu u žen, po syndromu polycystických ovárií. Studie zaměřené na její léčbu jsou vzácné a na velmi malých souborech pacientek (12, 13).

Konvenční léčba glukokortikoidní substitucí je odvozena od léčby klasické formy adrenální hyperplázie, která je způsobena významným enzymatickým defektem. Substituce tak upravuje deficit kortizolu, inhibuje sekreci ACTH a vede tak k supresi adrenální produkce androgenů. V managementu NCAH by měly být brány v úvahu následující specifické faktory: 1) díky pouze parciální deficienci aktivity enzymu 21-hydroxylázy jsou u pacientek s NCAH normální hladiny kortizolu i ACTH, 2) standardní dávka HCT nemusí přesně korigovat adrenální steroidní produkci ("over/under- treatment"), 3) substituce HCT vede k efektivní redukci androgenní produkce, ale vliv na klinickou symptomatologii je velmi omezený (12).

Dosud byly publikovány pouze dvě studie srovnávající tradiční léčbu kortikoidy s jinou léčebnou modalitou. Spritzer a kol. srovnával účinek hydrokortizonu (HCT) a cyproteronacetátu (CPA) na klinický a hormonální profil pacientek s NCAH (n = 30). Zaznamenal významné zlepšení hirzutismu,

ale pouze nevýznamný pokles hladin androgenů ve skupině pacientek léčených CPA a naopak, snížení produkce androgenů, ale minimální efekt na hirzutismus ve skupině léčené HCT (12).

Carmina a kol. sledovala na malém souboru pacientek s NCAH (n = 6) účinek ovariální suprese analogy GnRH. Po šestiměsíční léčbě bylo dosaženo snížení hladin androgenů i zlepšení hirzutismu (13). Tyto výsledky potvrzují podíl ovariální steroidogeneze na nadprodukci androgenů i u pacientek s NCAH. Na základě výše uvedených údajů byl založen náš předpoklad, že i ovariální suprese kombinovanou hormonální antikoncepcí s minimální reziduální androgenní aktivitou (COC) může být účinná v léčbě NCAH, navíc je vhodná pro dlouhodobé podávání, je dobře tolerována a je levnější než analoga GnRH.

Podle našich znalostí je naše studie první prací, která srovnávala účinek léčby COC s konvenční kortikoidní substitucí v managementu NCAH. Hlavním limitem naší práce je malý počet subjektů, který je dán (při užití striktních diagnostických kritérií) nízkou incidencí NCAH. Z velké kohorty téměř tří set hyperandrogenních žen jsme v průběhu osmiletého follow-up identifikovali pouze osm pacientek s NCAH. Obdobným nedostatkem jsou zatíženy i ostatní studie zabývající se managementem NCAH. V naší práci jsme tento nedostatek překonali užitím cross-over designu, který umožnil získání reprezentativních výsledků.

Zjistili jsme srovnatelný pokles celkových androgenů v obou skupinách, ale signifikantně výraznější pokles hladin volných androgenů v každé periodě s aplikací COC (vzestup SHBG) - tento účinek nebyl silněji přítomen v periodách HCT. Zřejmé bylo i zlepšení dominantního klinického symptomu, oligomenorey, v periodách s COC. Hirzutismus byl pouze minoritním problémem v našem souboru, který tak neumožnil hodnotit účinek obou modalit. Žádná z pacientek souboru nevykazovala známky adrenální insuficience v průběhu podávání COC nebo wash-out periody.

Závěr

Výsledky naší studie ukazují, že podávání COC může být využito v dlouhodobé léčbě NCAH. U pacientek s nedostatečným účinkem na kožní

androgenní symptomy (hirzutismus, akné) je indikovaná kombinace COC s antiandrogenem CPA, stejně jako v případě PCOS. Substituce kortikoidy by tak mohla být vyhrazena pro pacientky s NCAH, u nichž nedošlo k adekvátní supresi androgenních hladin během léčby COC, nebo pro pacientky se známkami adrenální insuficience.

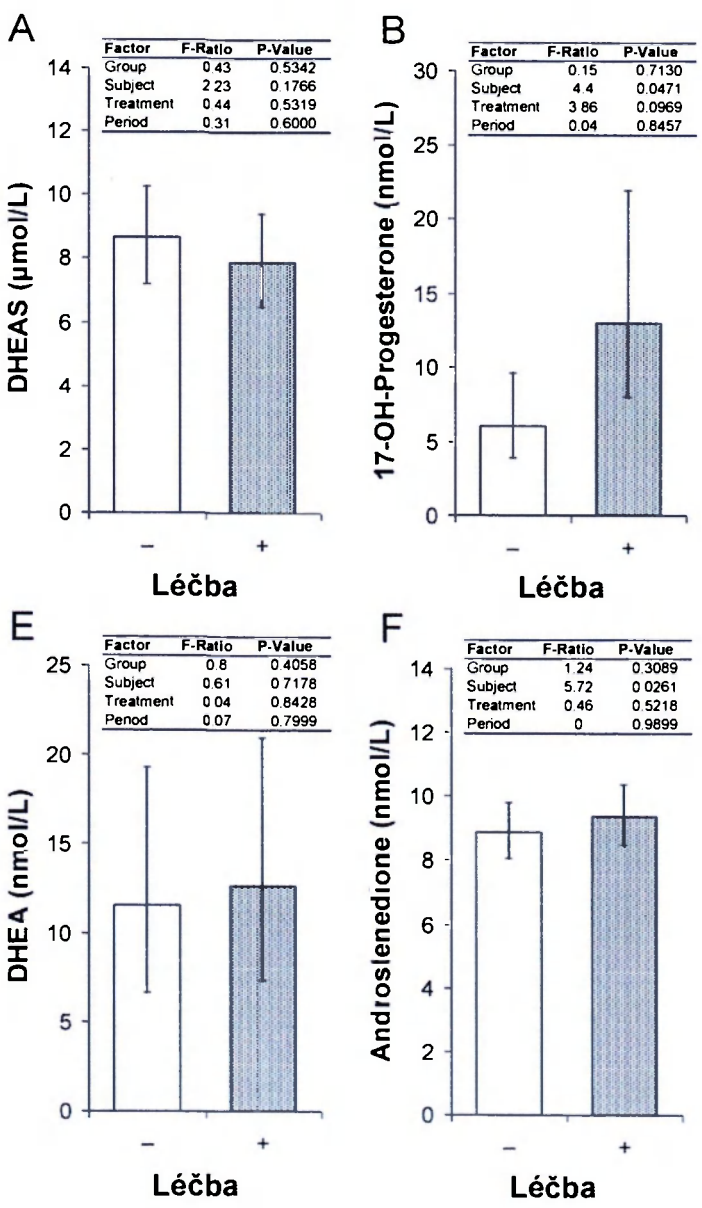
Tabulka I. Charakteristika souboru pacientek s NCAH.

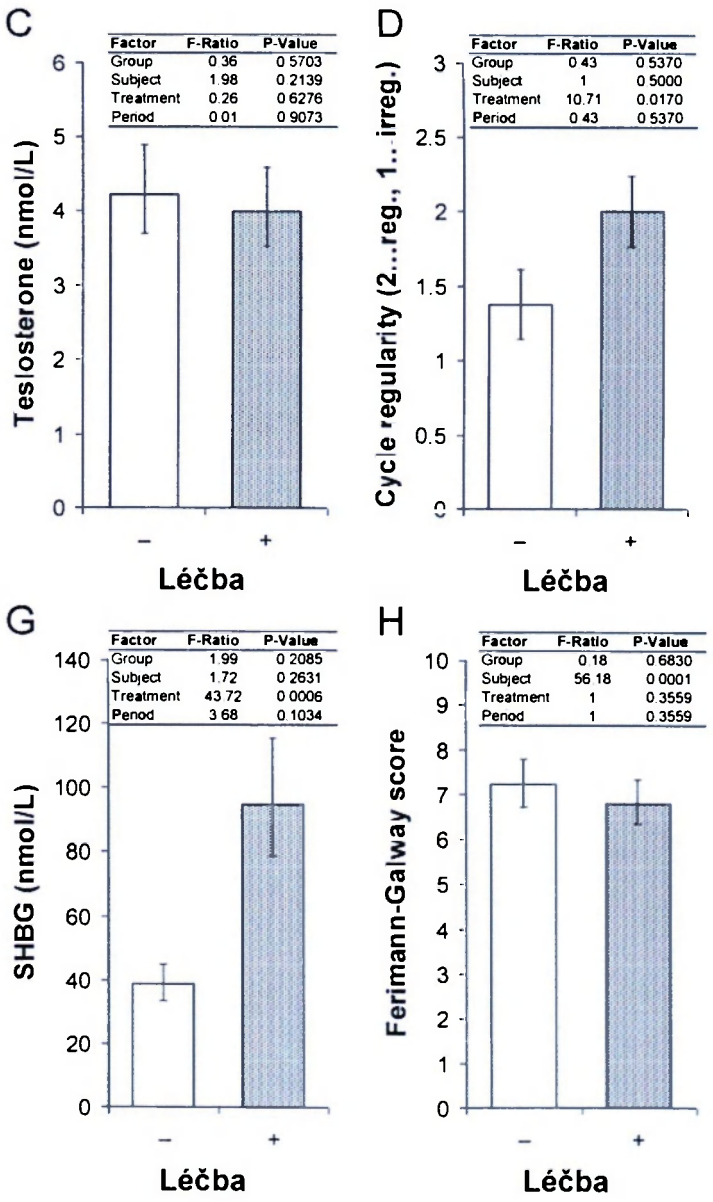
Laboratorní a klinické parametry	Jednotky	Průměrné hodnoty	Zastoupení subjektů s patol. hodnotami
17-OH-progesteron (0)	nmol/l	12.8 ± 3.92	87,50%
17-OH-progesteron (60)	nmol/l	111.2 ± 104	100%
Testosteron	nmol/l	4.0 ± 0.12	100%
DHEA	µg/l	27.5 ± 7.7	75%
DHEAS	µmol/l	11.44 ± 2.26	0%
Androstenedion	nmol/l	13.35 ± 0.55	75%
SHBG	nmol/l	32.1 ± 5.95	50%
Age	years	23.5 ± 3.04	
BMI	kg/m^2	22.67 ± 1.91	
WHR		0.74 ± 0.03	
LH/FSH > 1		50,0%	
Oligo/amenorrhea		62,5%	
Hirzutismus		37,5%	
Akné		25,0%	
Polycystická ovária		12,5%	
Obezita		0,0%	
NCAH, n = 8			

BMI = body-mass index,WH WHR= poměr pas/boky (waist to hip ratio)

Tabulka II. Hodnoty vložených diagnostických parametrů (input experimental data)

Subjekt	Skupina/Group	Perioda	Léčba/Treatment (1=HCT, 2=COC)	17-OH-Progesteron	Testosteron	DHEAS	DHEA	Androstendion	SHBG	Cyklus (1=nep.av d.; 2=prav.d.)	FG-skóre
1	1	1	1	6,48	5,33	3,7	2,5	13,43	43,1	1	6
2	1	1	1	13,9	3,47	14,5	9,1	11,6	41	2	17,5
3	1	1	1	2,86	4,93	10,83	28,1	7,34	36,7	1	8
4	1	1	1	9,05	4,31	8,72	9,8	8,45	35,3	1	3
5	2	1	2	1,38	4,3	5,23	11,8	5,63	176	2	16
6	2	1	2	160,1	6,6	6,1	40,1	13,3	128,3	2	5
7	2	1	2	16,3	2,9	8,8	9,2	7,6	105,3	2	5
8	2	1	2	8,5	3,1	8,1	7,5	9	119,1	2	10
1	1	2	2	58,6	7,61	8,71	16,5	15,44	97,8	2	6
2	1	2	2	18,4	3,4	10,4	27	11,3	54	2	17,5
3	1	2	2	2,71	4,74	9,89	4,15	8,43	46,6	2	5
4	1	2	2	35,6	3,2	6,5	8,3	8,26	116,6	2	3
5	2	2	1	2,08	4,4	5,23	11,8	5,63	50,1	2	16
6	2	2	1	12,5	3,83	9,44	20,8	8,3	45,8	1	5
7	2	2	1	3,71	3,81	13,1	19,1	10,15	23,1	1	5
8	2	2	1	10,68	4,2	6,89	8,4	8,63	44,2	2	10


A
Factor F-Ratio P-Value
Group 0.43 0.5342
Subject 2.23 0.1766
Treatment 0.44 0.5319
Period 0.31 0.6000
DHEAS (µmol/L)
Léčba
B
Factor F-Ratio P-Value
Group 0.15 0.7130
Subject 4.4 0.0471
Treatment 3.86 0.0969
Period 0.04 0.8457
17-OH-Progesterone (nmol/L)
Léčba
E
Factor F-Ratio P-Value
Group 0.8 0.4058
Subject 0.61 0.7178
Treatment 0.04 0.8428
Period 0.07 0.7999
DHEA (nmol/L)
Léčba
F
Factor F-Ratio P-Value
Group 1.24 0.3089
Subject 5.72 0.0261
Treatment 0.46 0.5218
Period 0 0.9899
Androstenedione (nmol/L)
Léčba


C
Factor F-Ratio P-Value
Group 0.36 0.5703
Subject 1.98 0.2139
Treatment 0.26 0.6276
Period 0.01 0.9073
Teslosterone (nmol/L)
0
1
2
3
4
5
6
– +
Léčba
D
Factor F-Ratio P-Value
Group 0.43 0.5370
Subject 1 0.5000
Treatment 10.71 0.0170
Period 0.43 0.5370
Cycle regularity (2...reg., 1...irreg.)
0
0.5
1
1.5
2
2.5
3
– +
Léčba
G
Factor F-Ratio P-Value
Group 1.99 0.2085
Subject 1.72 0.2631
Treatment 43.72 0.0006
Period 3.68 0.1034
SHBG (nmol/L)
0
20
40
60
80
100
120
140
– +
Léčba
H
Factor F-Ratio P-Value
Group 0.18 0.6830
Subject 56.18 0.0001
Treatment 1 0.3559
Period 1 0.3559
Ferimann-Galway score
0
1
2
3
4
5
6
7
8
9
10
– +
Léčba

Legenda ke grafům

Prázdné a tečkované sloupce reprezentují retransformované průměrné hodnoty na jejich 95% hladině významnosti pro neléčené a léčené subjekty, vložené tabulky výsledky ANOVA testu.

Závěry

V naší populaci je prevalence NCAH mezi hyperandrogenními ženami 2.68%, tedy stejně jako v dosud největším publikovaném souboru na dolní hranici obvykle uváděného rozmezí. Ačkoliv je tradičně v diferenciální diagnostice hyperandrogenních stavů uváděná po hyperandrogenním syndromu (PCOS) na druhém místě, znamená spíše minoritní problém. Klinická manifestace NCAH je od dalších hyperandrogenních stavů (hlavně PCOS) neodlišitelná. Vedoucím klinickým symptomem v našem souboru byl nepravidelný menstruační cyklus charakteru oligomenorey, naopak kožní androgenní obtíže nebyly významněji zastoupeny. Ve srovnání s dosud největším publikovaným souborem hyperandrogenních žen z USA se soubory lišily výrazně v antropometrických parametrech a výskytu hirzutismu. Tento fakt potvrzuje, že obezita může být nezávislým faktorem ovlivňujícím manifestaci klinických symptomů hyperandrogenních stavů.

Téměř u všech pacientek s NCAH byly zvýšené bazální hladiny 17-hydroxyprogesteronu, který tak může být využit jako základní test k detekci pacientek s deficiencí 21-hydroxylázy. Provádění ACTH testu tedy není nutné provádět rutinně u všech žen s podezřením na hyperandrogenémii. U všech pacientek s NCAH byly zvýšené hladiny testosteronu, u většiny v kombinaci s dalšími androgeny (androstendion, DHEA). U žádné z pacientek s NCAH nebyla zjištěna zvýšená hladina DHEAS, androgenu dominantně produkovaného nadledvinami.

Výsledky intervenční části projektu prokázali, že podávání COC může být využito v dlouhodobé léčbě NCAH. U pacientek s nedostatečným účinkem na kožní androgenní symptomy (hirzutismus, akné) je indikovaná kombinace COC s antiandrogenem CPA, stejně jako v případě PCOS. Substituce kortikoidy by tak mohla být vyhrazena pro pacientky s NCAH, u nichž nedošlo k adekvátní supresi androgenních hladin během léčby COC, nebo pro pacientky se známkami adrenální insuficience.

Conclusions

We found out a low prevalence of NCAH in a large group of well defined women with androgen excess (2.68%) in our study. Although adrenal etiology is often declared as a common cause of hyperandrogenism, diagnosis of NCAH is rarely established. The detection of NCAH patients is complicated by the fact that their clinical presentation is indistinguishable from that of other hyperandrogenic women, such as those with PCOS. In comparison with our PCOS patients both study groups did not significantly differ in anthropometric parameters and dominant clinical symptom, while only skin androgenic problems were more frequent in PCOS. Manifestation of clinical symptoms and occurence of endocrine abnormalities differs between our group and the largest American one. Oligomenorrhea is the leading symptom in both groups. The most significant differences exist in anthropometric parameters. BMI was significantly different, none of NCAH patients was obese in our group, compared to 50 % in American study. Hirsutism was frequent symptom in NCAH American group but was found only in one third of patients in our study. Discrepancies in clinical symptoms can be most probably caused by different prevalence of obesity. It was well documented that obesity itself is a factor that influences phenotype of the syndrome and worsens endocrine and metabolic parameters

Basal 17-hydroxyprogesterone level was elevated in almost all women with NCAH and can be used as an effective screening test for detection of patients with 21-hydroxylase deficiency. ACTH stimulation test can be performed only exceptionally, when basal levels are moderately elevated (6 – 10 nmol/l). None of the NCAH patients had an elevated DHEAS, the androgen dominantly produced by the adrenal glands.

Our results indicate that COC administration can be used for long-term treatment of NCAH. In patients with inadequate effect on skin androgenic disorders (hirsutism) COC treatment should be combined with CPA, as in PCOS patients. Corticosteroid substitution can be limited to patients with inadequate response to COC on plasma androgen levels or with signs of adrenal insufficiency.

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Přílohy

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ADRENAL HYPERPLASIA

Prevalence of nonclassic adrenal hyperplasia (NCAH) in hyperandrogenic women

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Abstract

Objective. The clinical symptoms of nonclassic adrenal hyperplasia (NCAH) are identical with polycystic ovary syndrome (PCOS). The aim of our study was to determine the prevalence of nonclassic adrenal hyperplasia (21-hydroxylase-deficiency) in hyperandrogenic women, its biochemical, endocrine and clinical characteristics and to compare them with parameters of patients with ovarian hyperandrogenism.

Methods. Since 1999, 298 patients with elevation of at least one androgen and manifestation of one of the clinical androgenic symptoms (oligo/amenorrhea, hirsutism or acne) have been identified in our database. A diagnosis of NCAH was considered when the basal or stimulated 17-hydroxyprogesterone was elevated.

Results. Only eight patients were identified as having 21- hydroxylase deficient NCAH in the whole group of 298 hyperandrogenic women. Hirsutism and acne were found only in three, two patients, five of them had oligo/amenorrhea. Seven patients had both elevated basal and stimulated 17-hydroxyprogesterone, while in one case only elevation of stimulated level was found. All of the NCAH patients had elevated concentrations of testosterone, six DHEA, lower SHBG was found in four patients. Surprisingly, none of the NCAH patients had increased DHEAS.

Conclusion. In our study, the prevalence of NCAH in hyperandrogenic women was 2.68%. Their leading symptom was oligomenorrhea, skin androgenic disorders were a minor clinical problem. None of the NCAH patients had an elevated DHEAS, the androgen dominantly produced by the adrenal glands.

Keywords: *21-hydroxylase deficiency, 17-hydroxyprogesterone, PCOS, hyperandrogenemia, oligomenorrhea*

Introduction

Androgen excess is one of the most common endocrine disorders of reproductive-aged women. Hyperandrogenic disorders are dominantly caused by overproduction of ovarian androgens in polycystic ovary syndrome (PCOS). Prevalence of PCOS in women of reproductive age is estimated to be about 7% [1,2,3].

Less frequent cause of androgen excess disorders which is recommended to exclude (except of extremely rare androgen-secreting neoplasms detected by rapid progress of clinical presentation) is adrenal etiology of hyperandrogenemia. In peripubertal or later period it concerns nonclassic (late-onset) adrenal hyperplasia (NCAH). NCAH is in more than 95% cases caused by partial deficiency of enzyme 21-hydroxylase activity [4,5], which is decreased to 20–50%.

The incidence of NCAH due to 21-hydroxylase deficiency varies especially according to ethnicity or race, being the highest among Ashkenazi Jews (1:27) In non-Jewish Caucasians it is estimated at 1:1000–2000 [5,6]. It is suggested that about 1% of population are carriers of allele for NCAH [7]. Data concerning prevalence of NCAH among hyperandrogenic women are limited and they fluctuate in published articles between 1 to 10% [6,8,9,10,11]. Except ethnicity, differences can be caused by smaller study groups and selection bias. Prevalence of NCAH was often established in groups of patients with some clinical signs of hyperandrogenism (hirsutism,

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alopecia, etc.), but without evidence of androgen excess in laboratory parameters [9,10,11]. There are only two large studies published so far by Azziz et al., on American group of hyperandrogenic women, and by Carmina et al., on population of hyperandrogenic women from Southern Europe [12,13].

As far as we are aware this is the first study evaluating the prevalence of NCAH on well defined hyperandrogenic women in Central Europe and comparing their clinical and endocrine aspects to American population of hyperandrogenic women.

Subjects and methods

All patients with hyperandrogenemia investigated at the university department from 1999 to 2006 were included. A total of 298 patients were enrolled in the study, based on the following diagnostic criteria : 1) increased concentration of testosterone, androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS) or 17-hydroxyprogesterone and 2) clinical manifestation of hyperandrogenism (oligomenorrhea or amenorrhea, hirsutism, acne or both).

All analytical determinations were performed at the National Reference Laboratory. Serum LH, FSH, testosterone concentrations were measured by chemiluminescent assay using an ACS: 180 autoanalyzer (Bayer Corp. Diagnostics GmbH, Leverkusen, Germany). The concentrations of DHEA, DHEAS, androstenedione and 17-hydroxyprogesterone were determined by RIA methods (Immunotech, Marseille, France). SHBG was measured using immunoradiometric assays kits (Orion, Turku, Finland). Concentrations of prolactin (PRL-kit) and thyroid-stimulating hormone (TSH-kit) were also measured. All subjects underwent transvaginal ultrasound of ovarian morphology.

Oligomenorrhea was defined as menstrual cycles $\geq$ 35 days, amenorrhea $\geq$ 3 months. Body and facial terminal hair growth was assessed using Ferriman-Gallwey score; hirsutism was defined as a score equal or higher than 8. Anthropomethric parameters (body mass index, waist and hip circumference) of subjects were measured.

Diagnosis of NCAH was established by measuring basal and stimulated concentrations of 17-hydroxyprogesterone (Controsyn/Synacthen 0.25 mg i.v.). ACTH stimulation was performed in patients with basal concentration of 17-hydroxyprogesterone higher than 6 nmol/l. Diagnostic criteria for NCAH were either basal 17-hydroxyprogesterone equal or above 10 nmol/l, or ACTH-stimulated 17-hydroxyprogesterone level (60 min.) equal or above 30 nmol/l. Blood samples were taken in follicular phase of cycle from 8 a.m. till 10 a.m.

Results

Results of our study and its comparison with the largest published group are summed up in Tables I, II.

Diagnosis of NCAH was established in our group of 298 hyperandrogenic women in eight subjects (2.68%).

The mean body mass index (BMI) in NCAH subjects was 22.67 $\pm$ 1.91, hirsutism and acne were found only in two patients, six patients had oligomenorrhea.

Both basal and ACTH- stimulated level of 17-hydroxyprogesterone was elevated in seven subjects. One patient had elevated only ACTH-stimulated level. Testosterone was elevated in all patients and DHEA in six patients with NCAH. In four cases level of SHBG was decreased. Surprisingly, none of the NCAH patients had elevated DHEAS. In half of the subjects LH/FSH ratio was greater than 1. Polycystic ovaries on transvaginal ultrasound (TVS) were found only in one subject.

Our PCOS group was comparable with that with NCAH in anthropometric parameteres (BMI 25.29 vs. 22.67, WHR 0.8 vs. 0.74). A leading clinical symptom was oligomenorrhea in both groups (71% vs. 62.5%). Skin androgenic disorders were slightly more frequent in PCOS group (hirsutism 49% vs. 37.5%, acne 32% vs. 25%). In PCOS group fewer patients had increased level of testosterone and

Table I. Characteristics (mean + SD) of hyperandrogenic patients with NCAH and PCOS – comparison with the group published by Azziz.

	NCAH Fanta, $n=8$	PCOS Fanta, $n=290$	NCAH Azziz, $n=18$	PCOS Azziz, $n=716$
Age (y)	23.5 $\pm$ 3.04	24.6 $\pm$ 4.68	28.28 $\pm$ 10.65	27.67 $\pm$ 7.26
BMI (kg/m^2)	22.67 + 1.91	25.29 $\pm$ 5.32	29.0 $\pm$ 6.32	33.39 $\pm$ 9.26
WHR	0.74 $\pm$ 0.03	0.8 + 0.36	0.83 $\pm$ 0.069	0.83 $\pm$ 0.39
Oligo/amenorrhea	62.5%	71%	88.9%	100%
Hirsutism	37.5%	49%	72.2%	72.2%
Acne	25.0%	32.0%	22.2%	14.5%
Obesity	0	11%	50%	60%

BMI = body-mass index, WHR = waist to hip ratio.

Table II. Laboratory parameters (mean + SD) and finding of polycystic ovaries in women with NCAH and PCOS.

	NCAH, $n=8$		PCOS, $n=290$	
↑ 17- OHP^0 (nmol/l)	87.5%	12.8 ± 3.92	1%	2.58 ± 1.69
↑ 17- OHP^{60} (nmol/l)	100%	111.2 ± 104.0	n.a.	
↑ testosterone (nmol/l)	100%	4.0 ± 0.12	63%	3.26 ± 1.35
↑ DHEA (μg/l)	75%	27.5 ± 7.7	66%	29.01 ± 15.32
↑ DHEAS (μmol/l)	0	11.44 ± 2.26	3%	8.9 ± 4.65
↑ androstenedione (nmol/l)	75%	13.35 ± 0.55	14%	8.58 ± 3.68
↓ SHBG (nmol/l)	50%	32.1 ± 5.95	23%	41.8 + 22.53
LH/FSH > 1	50%		59%	
polycystic ovaries	12.5%		47%	

n.a. = not applicable, 17- OHP^0 = basal 17-hydroxyprogesterone, 17- OHP^{60} = stimulated 17-hydroxyprogesterone, DHEA = dehydroepiandrosterone, DHEAS = dehydroepiandrosterone sulphate, SHBG = sex hormone-binding globulin, LH = luteinizing hormone, FSH = follicle-stimulating hormone.

androstenedione (63% vs. 100%, 14% vs. 75%), SHBG was decreased more frequently in NCAH group (23% vs. 50%). Higher levels of DHEAS were found in 3% of PCOS patients while in none of NCAH. Half of PCOS women and only one patient in NCAH subgroup had polycystic ovaries on ultrasound.

Discussion

NCAH is the second most frequent cause of hyperandrogenism in fertile women following the PCOS. Surprisingly, studies focusing on prevalence, symptomatology and diagnostics of NCAH are rare, and mostly on limited number of cases [9,10,11]. A wide range of NCAH prevalence in current literature (from 1% till 10%) can be caused by different geographic regions and ethnic groups, but inclusion criteria and limited size of study groups can be significant factors, too. There are only two large studies published so far by Azziz et al., on American group of hyperandrogenic women, and by Carmina et al., on population of hyperandrogenic women from Southern Europe [12,13]. We present in our study analysis of NCAH prevalence, and clinical manifestation in well defined group of almost three hundred hyperandrogenic women from different European region and compare it to the American population, in the above study, and to our PCOS patients.

Clinical symptoms of hyperandrogenemia generally develop in late pubertal or early adult age. The median age at presentation varies in literature between 22.3 and 24.5 years [14,15,16]. This corresponds with our experience; median age of referred patients was 23.5 years (17–28 years) in both NCAH and PCOS groups.

Manifestation of clinical symptoms and occurence of endocrine abnormalities differs between our group and the American one published by Azziz (Table I). Oligomenorrhea is the leading symptom in both groups, appearing more often in American NCAH patients (88.9% vs. 62.5%). The most significant differences exist in anthropometric parameters. BMI was significantly different (29.0 vs. 22.67), none of NCAH patients was obese in our group, compared to 50% in American study. Hirsutism was frequent symptom in NCAH American group (72.2%) but was found only in one third of patients in our study. Discrepancies in clinical symptoms can be most probably caused by different prevalence of obesity. It was well documented that obesity itself is a factor that influences phenotype of the syndrome and worsens endocrine and metabolic parameters [17,18].

In laboratory analysis we confirmed elevated testosterone in all patients with NCAH, increased both testosterone and androstenedione were found more often in NCAH in comparison with PCOS. Surprisingly, none of the NCAH patients had an elevated DHEAS, the androgen dominantly produced by the adrenal glands. In Azziz's group significantly higher levels of DHEAS were found in comparison with other groups of hyperandrogenic patients (PCOS, HAIRAN, idiopathic hirsutism). Decreased levels of SHBG in 50% of NCAH vs. 23% of PCOS patients in our group also did not correspond with Azziz's study, where significantly higher levels of SHBG in NCAH group than in PCOS group were found [12].

Although finding of polycystic ovaries on ultrasound is often declared also in NCAH patients (4), this ovarian morphology was present in one case only.

Neither in our nor in American group were present significant variations in clinical symptoms between NCAH and PCOS patients. It implies that both diagnoses are indistinguishable based on clinical signs only.

Our results might have a direct implication on clinical practise, specifically on differential diagnosis of hyperandrogenic states. If only basal level of 17-hydroxyprogesterone had been evaluated, only one case of NCAH would have been missed in the whole group of almost three hundred hyperandrogenic patients. This legitimates the measurement of basal 17-hydroxyprogesterone level in the evaluation of patients with androgen excess. In accordance with Azziz we may confirm that ACTH stimulation test can be limited only to patients with basal 17-hydroxyprogesterone levels between 6 and 10 nmol/l [19].

The main aim of our study was to evaluate the prevalence of NCAH among women with androgen

excess. This was low in our study, reaching 2.68% only. This is in accordance with the study by Azziz who also found prevalence close to the lower level of declared prevalence range [12].

Conclusion

We found out a low prevalence of NCAH in a large group of well defined women with androgen excess (2.68%) in our study. Although adrenal etiology is often declared as a common cause of hyperandrogenism, diagnosis of NCAH is rarely established. The detection of NCAH patients is complicated by the fact that their clinical presentation is indistinguishable from that of other hyperandrogenic women, such as those with PCOS. In comparison with our PCOS patients both study groups did not significantly differ in anthropometric parameters and dominant clinical symptom, while only skin androgenic problems were more frequent in PCOS, basal 17-hydroxyprogesterone level was elevated in almost all women with NCAH and can be used as an effective screening test for detection of patients with 21-hydroxylase deficiency. ACTH stimulation test can be performed only exceptionally, when basal levels are moderately elevated (6–10 nmol/l). Surprisingly, none of the NCAH patients had an elevated DHEAS, the androgen dominantly produced by the adrenal glands.

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ADRENAL HYPERANDROGENEMIA

Comparison of corticoid substitution *versus* combined oral contraception administration in the treatment of non-classic adrenal hyperplasia: A prospective study

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Abstract

Objective. The clinical symptoms of non-classic adrenal hyperplasia (NCAH) are the same as those in patients with polycystic ovary syndrome (PCOS). The aim of our study was to compare conventional corticoid treatment of NCAH with the effect of combined oral contraception (COC) administration (used in treatment of PCOS) on clinical and laboratory parameters of NCAH.
Design. A prospective clinical study, cross-over design.
Material and methods. Since 1999 from 298 hyperandrogenic women, eight patients having 21-hydroxylase deficient NCAH have been identified. They were divided equally into two groups according to the order of application treatment modality (hydrocortison *vs.* COC). Effect of treatment of both modalities on clinical symptoms (hirsutism – FG score, acne, menstrual cycle) and laboratory parameters (testosterone, androstenedione, dehydroepiandrosterone, dehydroepiandrosterone sulphate, sex hormone binding globulin (SHBG)) were evaluated.
Results. We observed the decrease of plasma androgens in both groups, which did not differ significantly. Significant increase of SHBG (i.e. decrease of free androgens) was, however, documented in each period with COC administration. Not surprisingly, improvement of the most frequent clinical symptom of NCAH in our study group, oligomenorrhea, was also more apparent in COC. Hirsutism was only a minor problem in our group that did not allow to evaluate treatment effect of both the modalities
Conclusion. Our results indicate that ovarian suppression by COC administration can effectively suppress androgen production and improve the most frequent clinical symptom (irregular cycle) in patients with NCAH, so can be successfully used for the treatment at least under basal conditions. Whether corticosteroid substitution can be limited to patients with inadequate response to COC on plasma androgen levels or with signs of adrenal insufficiency requires further data.

Keywords: *NCAH, hyperandrogenemia, combined oral contraception, corticosteroid, SHBG*

Introduction

Androgen excess is one of the most common endocrine disorders in reproductive-aged women. Hyperandrogenic disorders are dominantly caused by overproduction of ovarian androgens in polycystic ovary syndrome (PCOS). Less frequent cause of androgen excess is adrenal aetiology of hyperandrogenemia, non-classic (late-onset) adrenal hyperplasia (NCAH). Although there are a lot of studies concerning PCOS, data focused on treatment of NCAH are very limited. Main reason is very low incidence [1,2]. The conventional treatment by glucocorticoids substitution (hydrocortisone – HCT) is derived from classical form of adrenal hyperplasia, where it corrects the cortisol deficiency and inhibits ACTH secretion, thereby decreasing adrenal androgen secretion [3,4]. The effects of corticosteroid therapy on clinical manifestation of NCAH, especially on skin androgenic disorders, are disappointing [4]. Antiandrogen therapy with cyproterone acetate (CPA) has been used successfully for

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these patients with hirsutism [4]. As there may be a significant ovarian contribution to the abnormal androgen production of patients with NCAH, ovarian suppression with GnRH agonists was also demonstrated to be an effective treatment modality [5].

Despite the known effect of combined oral contraceptives (COC) on reduction of ovarian and adrenal androgenic steroids [6–8], there is no study following this treatment option and comparing it with corticoid therapy in the patients with NCAH. The aim of our study was to determine the efficacy of low-dose COC with minimal residual androgenic potential (containing 3rd generation progestin) on the levels of androgens and clinical symptoms in comparison with the treatment using corticosteroids (HCT).

Subjects and methods

Patients were selected from a large group of 298 hyperandrogenic women with elevated androgens and clinical symptom of hyperandrogenism (oligo/amenorrhea, skin androgenic disorders). Diagnosis of NCAH was established by measuring basal and stimulated concentrations of 17-hydroxyprogesterone (Controsyn/Synacthen 0.25 mg i.v.) in eight of them. Blood samples were taken in follicular phase of cycle from 8 a.m. to 10 a.m. Basal levels above 10 nmol/l and/or stimulated levels of 17-hydroxyprogesterone above 30.3 nmol/l identified as having 21-hydroxylase deficient NCAH in the whole group of 298 hyperandrogenic women were enrolled in the study.

Median age of referred patients was 23.5 years, the median body mass index (BMI) was 22.67. Characteristics of study group are summed up in Table I.

Because of small study group cross-over designed experiment was chosen to gain representative results.

Table I. Characteristics of hyperandrogenic patients with NCAH.

Indice	Unit	Median, incidence	Comparison with normal values (%)
17-OH-progesterone (0)	nmol/l	12.8 ± 3.92	87.50
17-OH-progesterone (60)	nmol/l	111.2 + 104	100
Testosterone	nmol/l	4.0 ± 0.12	100
DHEA	μg/l	27.5 ± 7.7	75
DHEAS	μmol/l	11.44 ± 2.26	0
Androstenedione	nmol/l	13.35 ± 0.55	75
SHBG	nmol/l	32.1 ± 5.95	50
Age	years	23.5 ± 3.04	
BMI	kg/m^2	22.67 + 1.91	
WHR		0.74 ± 0.03	
LH/FSH > 1		50.0%	
Oligo/amenorrhea		62.5%	
Hirsutism		37.5%	
Acne		25.0%	
Polycystic ovaries		12.5%	
Obesity		0.0%	
NCAH, $n = 8$			

BMI, body-mass index; WHR, waist to hip ratio.

Eight patients were divided equally into two groups according to the order of application treatment modality. Laboratory and clinical parameters were evaluated after the first and second application of HCT or COC (alternatively) (Table II). The therapy (variable *Treatment*) was tested on each patient (variable *Subject*) twice. HCT (HCT 20 mg daily) was preliminary administered to group 1 in the 1st period for 4 months, then 3-month wash-out period followed, after that during 4 months 2nd period low-androgenic COC (30 μg ethinylestradiol and desogestrel 150 μg) were administered. The parallel treatment in inverse therapeutic sequence has been applied to group 2. By this formal approach the influence of 'order of therapy' was eliminated. (without fulfilment of this sequence of steps the real effect of therapies stays hidden).

Before treatment and after each of the period, blood samples were obtained between 8 and 10 a.m. for the following parameters: LH, FSH, 17-hydroxyprogesterone, testosterone, androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), sex hormone binding globulin (SHBG) prolactin and TSH. Clinical manifestation and anthropometric parameters were also evaluated after each period.

All analytical determinations were performed at the National Reference Laboratory. Serum LH, FSH, testosterone concentrations were measured by chemiluminescent assay using an ACS: 180 autoanalyser (Bayer Corp. Diagnostics GmbH, Leverkusen, Germany). The concentrations of DHEA, DHEAS, androstenedione and 17-hydroxyprogesterone were determined by RIA methods (Immunotech, Marseille, France). SHBG was measured using immunoradiometric assays kits (Orion, Turku, Finland). Concentrations of prolactin (PRL-kit) and thyroid-stimulating hormone (TSH-kit) were also measured. All subjects underwent transvaginal ultrasound of ovarian morphology.

Oligomenorrhea was defined as menstrual cycles ≥35 days, amenorrhea ≥3 months. Body and facial terminal hair growth was assessed using Ferriman-Gallwey score; hirsutism was defined as a score ≥8. Anthropomethric parameters (BMI, waist and hip circumference) of subjects were measured.

The aim of our study was to compare effects of both treatment modalities – HCT 2 × 10 mg daily *vs.* COC containing 30 μg ethinylestradiol and desogestrel 150 μg – on laboratory and clinical parameters.

Statistical evaluation

The values of the parameters from last eight columns of Table II were used as independent variables y_{ijk} in ANOVA model (1)

$$y_{ijk} = \mu + \text{Group}_i + \text{Subject}_{ij} + \text{Period}_k + \text{Treatment}_h + e_{ijk} \quad (1)$$

Table II. The values of input diagnostic parameters (input experimental data).

Subject	Group	Period	Treatment (1 = HCT, 2 = COC)	17-OH-Progesterone	Testosterone	DHEAS	DHEA	Androstendion	SHBG	Cycle (1 = irregular; 2 = regular)	FG-score
1	1	1	1	6.48	5.33	3.7	2.5	13.43	43.1	1	6
2	1	1	1	13.9	3.47	14.5	9.1	11.6	41	2	17.5
3	1	1	1	2.86	4.93	10.83	28.1	7.34	36.7	1	8
4	1	1	1	9.05	4.31	8.72	9.8	8.45	35.3	1	3
5	2	1	2	1.38	4.3	5.23	11.8	5.63	176	2	16
6	2	1	2	160.1	6.6	6.1	40.1	13.3	128.3	2	5
7	2	1	2	16.3	2.9	8.8	9.2	7.6	105.3	2	5
8	2	1	2	8.5	3.1	8.1	7.5	9	119.1	2	10
1	1	2	2	58.6	7.61	8.71	16.5	15.44	97.8	2	6
2	1	2	2	18.4	3.4	10.4	27	11.3	54	2	17.5
3	1	2	2	2.71	4.74	9.89	4.15	8.43	46.6	2	5
4	1	2	2	35.6	3.2	6.5	8.3	8.26	116.6	2	3
5	2	2	1	2.08	4.4	5.23	11.8	5.63	50.1	2	16
6	2	2	1	12.5	3.83	9.44	20.8	8.3	45.8	1	5
7	2	2	1	3.71	3.81	13.1	19.1	10.15	23.1	1	5
8	2	2	1	10.68	4.2	6.89	8.4	8.63	44.2	2	10

where μ denotes the overall effect (intercept), Group_i, Subject_{ij}, Period_k and Treatment_h are the main effects for the model created by nominal crossing values from first four columns of Table II and e_{ijk} is a symbol for measurement errors. The ANOVA model requires the measurement normally distributed error vector e_{ijk} with zero mean vector and diagonal variance matrix of the same constants. The assumption of normal distribution for (yk) was evaluated using Shapiro-Wilk test and skewness z-score test. Using these tests, we have found unacceptable departures from normality in most variables. Accordingly, the original data were treated by power transformations to attain Gaussian distribution and homoscedasticity before ANOVA testing. The data after power transformation were sufficiently homogeneous for every variable; the absolute values of studentised residuals never exceed the value 4.

Results

All eight subjects with diagnosis of NCAH from large cohort of almost 300 hyperandrogenic patients enrolled in our study completed the cross-over designed study protocol. Both analysed treatment modalities (HCT *vs.* COC) were administered to each patient for 4 month lasting treatment period, with 3 month wash-out period in between.

Before treatment hirsutism and acne were found only in two patients, and six patients had oligomenorrhea. Testosterone was elevated in all patients and DHEA in six patients with NCAH. In four cases, level of SHBG was decreased. Surprisingly, none of the NCAH patients had elevated DHEAS.

As shown in Figure 1, the treatment with COC significantly increased circulating SHBG (Figure 1G) and reduced an incidence of cycle irregularities (Figure 1D). Significant inter-individual differences were found in 17-OH-Progesterone (Figure 1B), androstenedione (Figure 1F) and FG-score (Figure 1H). The Group and the Period effects were insignificant for all dependent variables, which indicate sufficient duration of wash-out period and absence of time changes independent of the treatment, respectively.

In summarising complete statistical analysis, we found a significant decrease of total androgen levels in both treatment groups, the results did not differ significantly between both treatment modalities. Significantly higher levels of SHBG, i.e. decrease of free androgens, were found in group treated with COC in comparison with HCT. Not surprisingly, the effect of COC on the most frequent symptom of hyperandrogenism in our study group, oligomenorrhea, was significantly more pronounced than in HCT periods.

Discussion

NCAH is the second most frequent cause of hyperandrogenism in fertile women following the PCOS. Surprisingly, studies focusing on treatment of NCAH are rare, and mostly on limited number of cases [4,5].

The conventional treatment by glucocorticoid substitution is derived from classical form of congenital adrenal hyperplasia resulting from a major enzyme defect. Therapy corrects the cortisol deficiency, inhibits ACTH secretion, thereby decreasing adrenal androgen production. However, there are some specific factors which should be considered in NCAH management: (1) because ofpartial defect in 21-hydroxylase there are normal levels of cortisol and ACTH, (2) standard HCT dose cannot accurately correct adrenal hormonal secretion, which can lead to over- or under-treatment, (3) HCT therapy is

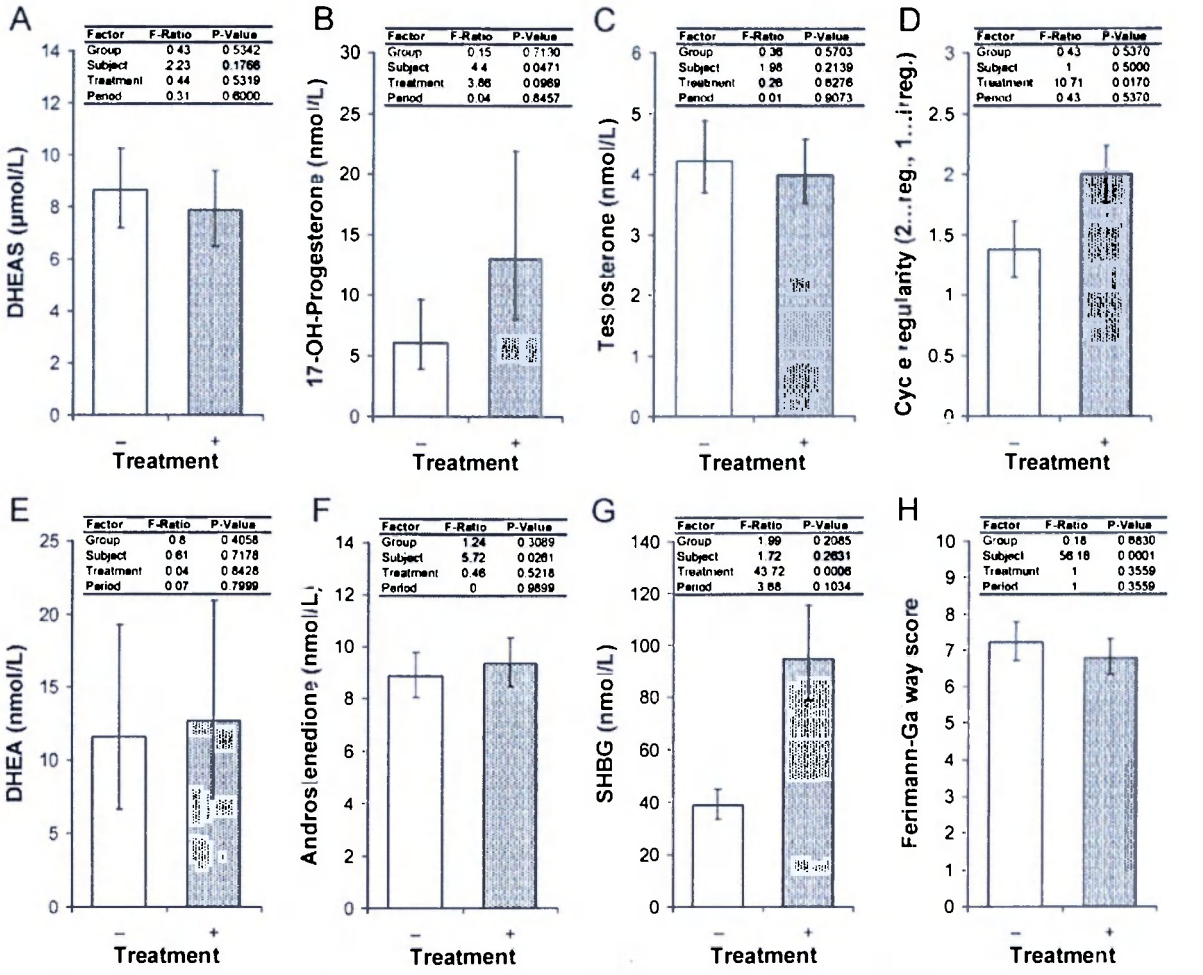

Figure 1. The empty and dotted bars with error bars represent the re-transformed mean values with their 95% confidence intervals for untreated and treated subjects, respectively. The embedded tables represent the results of ANOVA testing.

effective in reduction of androgen levels, but effect on clinical endpoints, especially oligo/amenorrhea and hirsutism is poor [4].

There are only two studies comparing traditional therapy with other treatment options. Spritzer et al. compared effect of HCT and CPA on clinical and hormonal profiles of the two groups of NCAH patients ($n = 30$). Significant improvement of hirsutism, but only slight decrease of plasma androgens in the group treated with CPA and conversely, reduction of plasma androgens to normal levels in contrast with only slight decrease in hirsutism in HCT-treated group were observed [4].

Carmina et al. determined the effectiveness of ovarian suppression with GnRH-agonist on a small study group ($n = 6$). Androgen levels were suppressed and hirsutism was improved significantly during 6 months treatment [5]. These results document contribution of ovarian steroidogenesis to the androgen overproduction in NCAH patients. On the basis of the above arguments, we postulated that ovarian suppression with low-androgenic COC may also be an effective treatment, more suitable for long-term therapy, cheaper and well-tolerated by patients (in comparison with both HCT and GnRH agonists).

Our study is, to our knowledge, the first report comparing COC with conventional treatment of NCAH. Main limit of our study is a small number of subjects, which is caused by very low incidence of NCAH, if strict diagnostic criteria are used. Despite a large cohort of hyperandrogenic patients, only eight cases were identified during 8 years of follow-up. Similar published studies concerning treatment of NCAH suffer from the same limitation [4,5]. This was however overcome by using cross-over design.

We observed the decrease of plasma androgens in both groups, which did not significantly differ. But we documented significant increase of SHBG, i.e. decrease of free androgens, in each period with COC administration, whereas no such effect was present with corticoids. Not surprisingly, improvement of the

most frequent clinical symptom of NCAH in our study group, oligomenorrhea, was also more apparent in COC. Hirsutism was only a minor problem in our group, that did not allow to evaluate treatment effect of both modalities. Treatment with COC was better tolerated than with HCT. None of the patients showed signs of adrenal insufficiency during COC administration.

Conclusion

Our results indicate that COC administration can be used for long-term treatment of NCAH. In patients with inadequate effect on skin androgenic disorders (hirsutism) COC treatment should be combined with CPA, as in PCOS patients. Corticosteroid substitution can be limited to patients with inadequate response to COC on plasma androgen levels or with signs of adrenal insufficiency. Administration of HCT to the patients exposed to stress will require further data.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Pozdní (neklasická) forma kortikální adrenální hyperplazie

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Late (Non-classic) Adrenal Hyperplasia

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Structured Abstract

Objective: To summarize available data concerning adrenal hyperandrogenemia caused by 21-hydroxylase deficiency, non-classic adrenal hyperplasia (NCAH).

Design: Review article.

Setting: Department of Gynecology and Obstetrics, General Faculty Hospital and 1st Medical Faculty, Prague.

Methods: Compilation of published data from scientific literature.

Conclusion: Although 21-hydroxylase deficiency is one of the most frequent autosomal recessive genetic disorders, prevalence of NCAH in the whole population and among hyperandrogenic women is very low. The measurement of 17OH-progesterone should be incorporated into the standard evaluation of all hyperandrogenic patients to establish or exclude the diagnosis of NCAH. There is no typical clinical sign of NCAH, and clinical symptoms are to similar to patients with PCOS. Corticoid substitution as a treatment modality of NCAH is derived from therapy of classic congenital adrenal hyperplasia (CAH). Antiandrogen therapy is effective in skin disorders (hirsutism). Due to normal cortisol value there is to use of combined oral contraceptives in the treatment of choice. An improvement of clinical symptoms is a key parameter for the evaluation of treatment effectiveness. There are no data about risk of late metabolic complications in NCAH patients.

Key words: classic adrenal hyperplasia (CAH), non-classic adrenal hyperplasia (NCAH), polycystic ovary syndrome, 17OH-progesterone, corticoids

Strukturovaný souhrn

Cíl práce: Shrnutí současných znalostí o problematice adrenální hyperandrogenémie na podkladě deficitu aktivity enzymu 21-hydroxylázy, neklasické formě kortikální adrenální hyperplazie (NCAH) u žen.

Typ studie: Přehledový článek.

Název a sídlo pracoviště: Gynekologicko-porodnická klinika 1. LF UK a VFN v Praze.

Metodika: Zpracování údajů z literatury.

Závěr: Deficit aktivity 21-hydroxylázy patří mezi nejčastější autozomálně recesivní genetické choroby. Přesto je incidence klasické i neklasické formy adrenální hyperplazie v celé populaci i v populaci hyperandrogenních žen velmi nízká. Ke stanovení diagnózy NCAH v naprosté většině případů stačí vyšetření bazální hladiny 17OH-progesteronu. Klinická symptomatologie může být velmi chudá, často připomíná obraz hyperandrogenního syndromu; typický příznak pro NCAH neexistuje. Léčba pomocí substituce kortikoidy je odvozena od klasické formy adrenální hyperplazie. V korekci kožních androgenních obtíží lze využít antiandrogeny. Vzhledem k normálním hladinám kortizolu je racionální i využití kombinované hormonální antikoncepce. Cílem i ukazatelem adekvátní intervence by mělo být v první řadě zlepšení klinických obtíží. V současnosti nejsou k dispozici spolehlivá data o riziku metabolických pozdních komplikací.

Klíčová slova: klasická forma kortikální adrenální hyperplazie (CAH), neklasická forma adrenální hyperplazie (NCAH), hyperandrogenní syndrom, 17OH-progesteron, kortikoidy

ÚVOD

Hyperandrogenní stavy jsou nejčastější endokrinopatií žen ve fertilním věku, postihující přibližně 7 % této populace [1, 2, 3]. Převážná většina je vyvolána poruchou steroidogeneze na úrovni vaječníků (hyperandrogenní syndrom – HAS, syndrom polycystických vaječníků – PCOS). U části hyperandrogenních žen je příčinou zvýšených hladin androgenních hormonů úplný nebo částečný blok enzymů uplatňujících se v adrenální steroidogenezi. Naprostá většina případů (přes 95 %) je způsobena deficitem 21-hydroxylázy. V důsledku snížené aktivity enzymu se zpomaluje konverze 17-hydroxyprogesteronu na 11-deoxykortizol (větev glukokortikoidní) a progesteronu na 11-deoxykortikosteron (mineralokortikoidní metabolická větev).

Existují dvě základní formy onemocnění - klasická forma (classic congenital adrenal hyperplasia - CAH) a neklasická (pozdní) forma kortikální adrenální hyperplazie (non-classic adrenal hyperplasia - NCAH). Klinické projevy onemocnění jsou pestré, od asymptomatického průběhu až po ženský pseudohermafroditismus s deplecí natria, manifestující se již v prvních týdnech života (CAH).

KLASICKÁ FORMA KORTIKÁLNÍ ADRENÁLNÍ HYPERPLAZIE (CAH)

CAH je charakterizována závažným deficitem 21-hydroxylázy (kompletní blok), virilizací již v prenatálním období a různým stupněm adrenální insuficience. Vyskytuje se ve dvou subtypech: s deplecí sodíku, označovaného SW-CAH (salt-wasting), charakterizovaného depleční krizí již v prvních týdnech života, a jako prostá virilizující forma, SV-CAH (simple virilizing). Incidence CAH se pohybuje mezi 1 na 12 100–23 000 [4].

Léčba vyžaduje substituci glukokortikoidy i mineralokortikoidy a supresi nadměrné produkce ACTH. Ve zvlášť těžkých případech, kdy není možno dosáhnout dobré kompenzace, je metodou volby bilaterální adrenalektomie.

V budoucnosti lze očekávat využití antagonistů corticotropin-releasing hormonu (CRH) a genové terapie.

NEKLASICKÁ FORMA KORTIKÁLNÍ ADRENÁLNÍ HYPERPLAZIE (NCAH)

Incidence a prevalence

Incidence NCAH se liší u jednotlivých etnických skupin. Nejvyšší výskyt byl zjištěn mezi aškenázskými židy (1:27), v nežidovské bílé populaci je odhadován 1:1000-2000 [5]. Nosičství alel pro mírnou nebo pro neklasickou formu CAH kolísá podle etnicity vyšetřeného souboru mezi 1:5 až 1:50 [6]. V největším publikovaném souboru 873 hyperandrogenních žen byla zjištěna prevalence NCAH v 1,6 % [7]. Za vznik onemocnění může být odpovědný i deficit jiných steroidogenních enzymů (zejména 11ß- hydroxylázy), přes 95 % případů NCAH je ale způsobeno sníženou aktivitou 21-hydroxylázy [5, 8]. Kontroverzní je otázka, jaká jsou hormonální kritéria pro NCAH způsobenou defektem 3ß-hydroxylázy [20].

GENETIKA

Příčinou jak klasických, tak neklasických forem kongenitální adrenální hyperplazie je geneticky podmíněná nedostatečnost enzymů steroidogeneze:

1. 21- hydroxylázy (*CYP21, pseudogen CYP21P*),
2. 11 ß -hydroxylázy (*CYP11B1*),
3. dehydrogenázy 3 ß - hydroxysteroidů a delta 5-4 izomerázy (*HSD3B1, HSD3B2*),
4. steroidogenního akutního regulačního proteinu (*StAR*) a
5. 17-hydroxylázy (*CYP17*), přitom u neklasických forem jde vždy o mírnější enzymatickou poruchu. Jedná se o onemocnění s autozomálně recesivním typem dědičnosti.

Dominantní příčinou jak klasických forem CAH, tak pozdní formy CAH je deficit enzymu 21-hydroxylázy, který je nacházen až u 90 % případů.

Již koncem 70. let byla popsána asociace různých forem deficitu 21-hydroxylázy s charakteristickými haplotypy HLA (human leukocyte antigen), čehož se využívalo pro nepřímou molekulárně genetickou diagnostiku CAH. Později byly identifikovány dva geny kódující 21-hydroxylázu u člověka – funkční gen *CYP21* a nefunkční, tzv. pseudogen *CYP21P*. Oba leží na krátkém raménku 6. chromozomu, v těsné blízkosti oblasti kódující alely HLA. Právě tato blízká chromozomální lokalizace genu a pseudogenu predisponuje ke vzniku mutací, může docházet k delecím, ke genovým konverzím mezi funkčním genem a pseudogenem. Nejčastěji jsou u pacientů nalézány delece různého rozsahu v genu *CYP21* (delece malých úseků až po kompletní deleci jedné alely), duplikace genu (jsou častou příčinou NCAH), či bodové mutace. Fenotyp u většiny pacientů dobře koreluje s typem mutace. Mutace *CYP21* vedoucí k úplné deaktivaci 21-hydroxylázy jsou příčinou virilizace spojené se solnou poruchou, druhá skupina mutací *CYP21*, kdy produkt má reziduální enzymatickou aktivitu, je spojena s prostou virilizací a třetí skupina mutací, které snižují enzymatickou aktivitu o 50-70 %, je nalézána převážně u neklasické formy CAH (21, 22).

U pacientek s NCAH je obvykle vyšetřováno 10 frekventních mutací a delecí (identifikace 90-95 % defektních alel). V etnické skupině aškenázských židů byla potvrzena vysoká frekvence „NCAH alel", v subpopulaci pacientek nežidovského původu byl zaznamenán vyšší výskyt „klasických" mutací [9].

Specifické mutace pro pozdní – neklasickou formu CAH byly zatím identifikovány pouze při deficitu 21-hydroxylázy (*CYP21*), nikoliv u ostatních výše zmíněných enzymopatií, které mohou být příčinou minoritních forem NCAH.

LABORATORNÍ DIAGNOSTIKA

V důsledku deficitu aktivity enzymu 21-hydroxylázy (u NCAH snížení aktivity na 20–50 %) se u pacientek s adrenální hyperplazií zvyšují hladiny jeho substrátu, 17OH-progesteronu.

Za základní laboratorní test ke stanovení diagnózy NCAH je považován ACTH stimulační test. Provádí se v ranních hodinách folikulární fáze menstruačního cyklu, po bazálním odběru je aplikováno 0,25 mg ACTH a za 60 minut je stanovena hladina stimulovaného 17OH-progesteronu. Hladina nad 30,3 nmol/l je diagnostická pro NCAH. S cílem omezit nutnost provádění ACTH testu (z důvodů ekonomických, časových, nutnosti venepunkce) stanovil Azziz hraniční hodnoty bazální hladiny 17OH-progesteronu, které vylučují či potvrzují diagnózu NCAH při zachování odpovídající senzitivity a pozitivní či negativní prediktivní hodnoty. Odběr by měl být proveden ve folikulární fázi cyklu, v ranních hodinách (7.30-9.30 hod.) vzhledem k cirkadiánnímu rytmu adrenální sekrece. Diagnózu NCAH prakticky vylučuje hladina bazálního 17OH-progesteronu nižší než 6 nmol/l (2 ng/ml) – negativní prediktivní hodnota se přibližuje 100 %. Naopak při bazální hladině 17OH-progesteronu nad 10 nmol/l je diagnóza NCAH pravděpodobná (pozitivní prediktivní hodnota (PPV) je +/- 40 %, senzitivita je +/- 90 %). Těchto vylučovacích hodnot lze využít screeningově v populaci hyperandrogenních žen. ACTH test tak může být proveden jen u žen s hladinami bazálního 17OH-progesteronu mezi 6 a 10 nmol/l; zastoupení těchto hyperandrogenních pacientek se odhaduje mezi 3-6 % [10].

Z dalších laboratorních nálezů většina studií zjistila vyšší hladiny celkového i volného testosteronu, přestože testosteron není přímým ukazatelem aktivity 21-hydroxylázy, dále dehydroepiandrosteronsulfátu (DHEAS) a androstendionu [7, 11, 12].

V již zmiňované Azzizově práci sledující 873 hyperandrogenních žen měly pacientky s NCAH signifikantně vyšší hladiny celkového i volného testosteronu ve srovnání se ženami s ovariální hyperandrogenémií (hyperandrogenním syndromem, HAS). Dalším paradoxním nálezem byly u žen s NCAH významně vyšší hladiny SHBG (7).

Vyšší hladiny androgenů u NCAH lze vysvětlit pravděpodobně současnou nadprodukcí androgenů v nadledvinách a ve vaječnících. Teorii o kombinované příčině zvýšených hladin androgenů podporuje i jejich pokles a zlepšení hirzutismu u pacientek s NCAH po podání agonistů gonadoliberinu [13].

NCAH a inzulinová senzitivita

Řada prací se zabývá inzulinovou senzitivitou u hyperandrogenních žen s hyperandrogenním syndromem (HAS). Důvodem jsou pozdní metabolická rizika HAS (metabolický syndrom). V literatuře však není dostupná studie sledující parametry inzulinové senzitivity u NCAH pomocí diagnostického vyšetření, které je považováno za zlatý standard - euglykemického hyperinzulinemického clampu. I jinak byla zatím pozornost inzulinové senzitivitě u žen s NCAH věnována jen okrajově. Speiser pomocí tolbutamidem modifikovaného intravenózního glukózového tolerančního testu zjistil v malém souboru žen s NCAH (n = 6) sníženou inzulinovou senzitivitu [14].

Ve výše citované Azzizově studii byla popsána tendence k horším parametrům inzulinové senzitivity ve skupině HAS ve srovnání se skupinou NCAH (u HAS vyšší hladina inzulinu nalačno, vyšší HOMA index). Přesto i další faktory (obezita není častá u žen s NCAH) podporují předpoklad příznivějších parametrů inzulinové senzitivity u NCAH než u HAS.

KLINICKÁ SYMPTOMATOLOGIE

Klinické projevy NCAH připomínají symptomy ovariální hyperandrogenémie, tedy nepravidelný menstruační cyklus charakteru oligo/amenorey, kožní androgenní obtíže – hirzutismus, akné, ev. alopecie. Ve studiích zabývajících se klinickou symptomatologií a léčbou NCAH je za dominující příznak obvykle považován hirzutismus. K manifestaci symptomů dochází nejčastěji v pozdní pubertě či časné dospělosti, medián věku, ve kterém je onemocnění diagnostikováno, se pohybuje mezi 22,3–24,5 roky [11, 13].

O možné časnější manifestaci (pod 10 let věku) a progresivním charakteru onemocnění vypovídá Moranova multicentrická studie sledující 218 pacientek s NCAH z 11 center. Ve skupině dívek mladších deseti let zjistil u 92 % předčasnou pubarché. Ve starších věkových skupinách byly dominujícími příznaky hirzutismus a oligomenorea. Na progresivní charakter ukazuje podle Morana stoupající prevalence hirzutismu přímo úměrně věku – 10-19 let 70 %, 20-29 let 82 %, 30-39 let 90 %, 40-49 let 94 %. Zajímavý je fakt, že vzrůstá prevalence, nikoli stupeň zvýšeného ochlupení (podle Ferimann-Gallwey skóre) mezi uvedenými věkovými skupinami [11].

Další androgenní příznak, akné, nepatří mezi častý projev NCAH. Ve zmiňované Moranově studii se vyskytoval s prevalencí mezi 20–40 %, konstantně ve všech věkových skupinách [11]. Obdobnou prevalenci akné mezi ženami s NCAH potvrdil Azziz (22,2 %). Rovněž v jeho práci byl hlavním příznakem hirzutismus (prevalence 72,2 %). Oba soubory se naopak liší ve výskytu poruch menstruačního cyklu charakteru oligo/amenorey (89 % vs. 50-58 %). Mezi typický klinický příznak nepatřila obezita, její prevalence se pohybuje mezi 20 a 50 %, mediány BMI mezi 23,7 a 29 kg/m^2.

Vztah mezi genotypem a fenotypem u pacientek s NCAH porovnává Speiserova multicentrická studie, ve které sledoval 34 žen. Z výsledků vyplývá, že tzv. smíšení heterozygoti, tedy nositelky „klasické" mutace na jedné alele a „neklasické" mutace na druhé, mají vyšší hodnoty 17OH-progesteronu spolu se zvýšeným výskytem hirzutismu než homozygoti promírné mutace [9]. Naopak starší práce Knochenhauera nezjistila vyšší riziko manifestace hirzutismu u heterozygotních nositelů mutace genu *CYP21* (15).

LÉČBA

Ačkoliv se vzhledem k mechanismu onemocnění nabízí možnost terapeuticky zasáhnout kauzálněji než v případě hyperandrogenního syndromu, tedy kortikoidy suprimovat osu hypotalamus - hypofýza - nadledviny, a tím redukovat excesivní sekreci androgenů, nejsou klinické účinky této léčby spolehlivé. Strategie léčby je obdobná individuálním postupům u HAS. Rovněž v případě NCAH zvažujeme, které projevy chceme terapeuticky ovlivnit: kožní androgenní příznaky (akné, hirzutismus, alopecie), nepravidelný menstruační cyklus, ev. anovulaci - sterilitu. Dosud existuje málo intervenčních studií na větších souborech, a tedy jen málo spolehlivých dat o léčbě NCAH.

Konvenční léčba NCAH, substituce kortikoidy, je odvozena od léčby klasické formy adrenální hyperplazie. V případě CAH substituce koriguje deficit kortizolu, inhibuje sekreci ACTH a snižuje stimulaci adrenální steroidogeneze. Hlavním cílem kortikoidní léčby u neklasické formy je rovněž suprimovat adrenální produkci androgenů. V případě NCAH však není třeba korigovat hladiny kortizolu; jeho hladiny jsou u částečného deficitu 21-hydroxylázy ve fyziologických mezích. Nejčastěji používaným kortikoidem používaným v léčbě NCAH je hydrokortizon v dávce 10–20 mg denně. Efekt léčby na hladiny androgenů je zřejmý, byl prokázán významný pokles hladin testosteronu, androstendionu a samozřejmě i 17OH-progesteronu [12].

Naopak účinek léčby kortikoidy na klinické projevy není výrazný. Ve studii srovnávající efekt léčby hydrokortizonem (2x10 mg, n=16) a antiandrogenem cyproteronacetátem (CPA, 50 mg denně, n=14) na laboratorní parametry a dominující klinický projev, hirzutismus, bylo ve skupině léčené glukokortikoidy zaznamenáno pouze velmi pozvolné a mírné snížení ochlupení (FG skóre 22, 21,3, 19,5 a 16,5 v 0., 3., 6. a 12. měsíci léčby). Ve skupině léčené CPA byla signifikantní redukce ochlupení zjištěna již ve 3. měsíci (FG skóre 23, 17, 12,9 a 10 v 0., 3., 6. a 12. měsíci léčby). Ve skupině léčené CPA došlo pouze k mírnému poklesu hladin androgenů, které potvrdilo jeho periferní účinek – kompetitivní inhibicí vazby androgenů v cílových tkáních [12].

Další zkoušenou léčebnou modalitou v terapii NCAH byla ovariální suprese agonisty gonadoliberinu. Po 6měsíčním podávání byl zaznamenán pokles hladin 17OH-progesteronu, celkového i volného testosteronu a androstendionu. DHEAS zůstal nezměněn. Signifikantně bylo zlepšeno zvýšené ochlupení – FG skóre z 16,4 na 8,4 [13]. Přestože se jedná o malý soubor (n=6), výsledky potvrzují významný podíl ovariální hyperandrogenémie.

Léčba COC způsobuje rovněž pokles hladin ovariálních i adrenálních androgenů [17, 18, 19]. Účinek na 17OH-progesteron je velmi individuální, často během intervence přetrvávají jeho zvýšené hladiny (dosud nepublikovaná data).

S volbou léčebné modality souvisí otázka monitorování účinku intervence. Logické je hodnocení podle vývoje hladin androgenů. Kortikoidní substitucí lze současně se snížením hladin testosteronu a androstendionu očekávat pokles hlavního markeru adrenální hyperandrogenémie, 17OH-progesteronu. Přesto jsou při této léčbě často zjišťovány přetrvávající zvýšené hladiny, při normalizaci hladin ostatních androgenů. Je to nejspíše způsobeno vyšší citlivostí androgenů ke kortikoidní supresi ve srovnání s C-21 steroidy.

Při monitorování efektu léčby by měly být sledovány hladiny testosteronu, androstendionu, DHEAS, jejichž adekvátní suprese může být dosaženo i při přetrvávajících zvýšených hladinách 17OH-progesteronu. Sledování hladin 17OH-progesteronu je považováno za nejméně významné; naopak snaha o normalizaci jeho hodnot může vést až k „overtreatmentu" a rozvoji cushingoidních znaků. Nejsilnější androgenní účinek má testosteron, androgenní potenciál 17OH-progesteronu je slabší. Není jasné, zda zvýšené hladiny 17OH-progesteronu zvyšují riziko pozdních komplikací (metabolický syndrom).

Dalším ukazatelem efektu léčby je zlepšení klinických příznaků.

NCAH a gravidita

Ačkoliv se u pacientek s NCAH vyskytují často anovulační cykly, část z nich otěhotní spontánně [16].

U sterilních žen je po vyloučení dalších možných příčin obvykle nejdříve doporučována ve snaze o nastolení ovulačních cyklů kortikoidní substituce na 4 měsíce. Není-li úspěšná, postupuje se obdobně jako v léčbě anovulační sterility u HAS, tedy indukcí ovulace antiestrogeny, event. gonadotropiny.

Další otázkou související s těhotenstvím je prekoncepční příprava a management gravidity žen s NCAH. Riziko porodu novorozence s klasickou formou adrenální hyperplazie (CAH) u žen s NCAH závisí kromě genotypu matky i na tom, zda je otec nosičem mutace genu *CYP21*. Riziko je vyšší pouze v případě otcova nosičství a matčiny mutace genu způsobující těžký deficit 21-hydroxylázy. V případě mutací genů kódujících mírný či střední deficit tohoto enzymu je riziko kongenitálního postižení dítěte extrémně nízké (pokud není zároveň přítomna další neidentifikovaná mutace, či mutace pro těžký deficit vzniklý „de-novo"). Neznáme-li genotyp *CYP21* u rodičů, je riziko porodu dítěte postiženého

CAH u pacientek s NCAH odhadováno na 1,7-2,3/1000 [4]. Takto nízká míra rizika neopravňuje k invazivní prenatální diagnostice ani ke kortikoidní substituci v průběhu gravidity.

ZÁVĚR

Deficit aktivity 21-hydroxylázy patří mezi nejčastější autozomálně recesivní genetické choroby. Přesto je incidence klasické i neklasické formy adrenální hyperplazie v celé populaci i v populaci hyperandrogenních žen velmi nízká.

Ke stanovení diagnózy NCAH v naprosté většině případů stačí vyšetření bazální hladiny 17OH-progesteronu, provedení ACTH-stimulačního testu je vhodné u přibližně 6 % hyperandrogenních žen s hodnotami bazálního 17OH-progesteronu mezi 6 a 10 nmol/l.

Klinická symptomatologie může být velmi chudá, často připomíná obraz hyperandrogenního syndromu; typický příznak pro NCAH neexistuje.

Léčba substitucí kortikoidy je odvozena od klasické formy adrenální hyperplazie. V korekci kožních androgenních obtíží lze využít antiandrogeny. Vzhledem k normálním hladinám kortizolu je racionální i využití kombinované hormonální antikoncepce. Cílem i ukazatelem adekvátní intervence by mělo být v první řadě zlepšení klinických obtíží.

Léčebný postup při anovulační sterilitě se v podstatě neliší od HAS. V současnosti nejsou k dispozici spolehlivá data o riziku metabolických pozdních komplikací.

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ERRATA

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The effect of combination therapy with metformin and combined oral contraceptives (COC) versus COC alone on insulin sensitivity, hyperandrogenaemia, SHBG and lipids in PCOS patients

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BACKGROUND: Neither oral contraceptives (COC) nor metformin are an optimal modality for the long-term treatment of polycystic ovary syndrome (PCOS). The aim of this study was to evaluate whether a combination of both is beneficial over COC monotherapy. METHODS: Altogether, 30 women were included in the study and 28 finished the protocol. The patients were randomly assigned to two groups treated with either COC (COC group) or COC and metformin (1500 mg/day) (METOC group) for 6 months. Anthropometric parameters, androgens, lipids, fasting insulin, glucose and sex hormone binding globulin (SHBG) concentrations were measured before and at the end of the sixth cycle of treatment. The insulin sensitivity index was evaluated using the euglycaemic clamp. RESULTS: There were no significant changes in anthropometric parameters, fasting glucose or insulin sensitivity in either group. Total testosterone, free androgen index, androstenedione and dehydroepiandrosterone decreased and SHBG increased significantly in both groups. When comparing the effect of both treatments, only a more pronounced deerease in free androgen index was found in the METOC group. CONCLUSIONS: Adding metformin slightly modified the treatment effect of COC, causing a more significant decrease in the free androgen index but having no additional positive impact on lipids, insulin sensitivity, SHBG or testosterone. The available data do not offer enough evidence to advocate the standard use of combined treatment in PCOS. Whether the combination might be beneficial for specific subgroups of patients is of further interest.

Key words: androgens/insulin sensitivity/metformin/oral contraceptives/polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome with a wide variety of endocrine and metabolic abnormalities and clinical symptoms. The optimal modality for long-term treatment should favourably influence androgen synthesis, sex hormone binding globulin (SHBG) production, the lipid profile, insulin sensitivity, and clinical symptoms including acne, hirsutism and irregular menstrual cycle. The above requirements are difficult to meet with a single form of treatment.

The two widely used options for long-term treatment, combined oral contraceptives (COC) and metformin, have different effects. There are data showing a direct comparison of metformin and COC in women with PCOS. Two studies separately evaluated the treatment effect in obese and non-obese patients, with comparable results (Morin-Papunen *et al.*, 2000; 2003). Metformin caused no changes in insulin sensitivity in non-obese patients, a slight improvement in obese patients and a significant decrease in fasting insulin in both groups. During COC treatment, no changes in insulin sensitivity or fasting insulin were found. The latter treatment caused a more significant decrease in total androgens and highly significant increase in SHBG in both subgroups of patients.

To date, only two studies have focused on treatment with COC and metformin in combination. Both included non-obese subjects only. Elter *et al.* (2002) found a greater decrease in androstenedione and more pronounced increase in SHBG in the group receiving combination treatment. In a recent study, COC was administered to adolescent PCOS women receiving continuous metformin and flutamide treatment (Ibáñez and Zegher, 2003). Addition of COC was followed by an increase in SHBG only. None of these studies directly evaluated insulin sensitivity.

The aim of our study was to evaluate the effect of treatment with COC alone or in combination with metformin on insulin sensitivity, total androgens, SHBG and lipids. A priority of our study was the direct measurement of insulin sensitivity, which should be a key argument for adding metformin.

Materials and methods

Subjects

The subjects were recruited from the Unit of Reproductive Endocrinology. Patients fulfilling the diagnostic criteria for PCOS were consecutively enrolled in the study. PCOS was defined as follows: (i) oligomenorrhea from menarche (menstrual cycle >35 days); (ii) an increased concentration of at least one androgen above the upper reference limit [testosterone 0.5–2.63 nmol/l, androstenedione 1.57–5.4 nmol/l, dehydroepiandrosterone (DHEA) 0.8–10.5 nmol/L]; and (iii) clinical manifestation of hyperandrogenism (acne, hirsutism or both). Women presenting with a secondary endocrine disorder, such as hyperprolactinaemia, thyroid dysfunction or a non-classical form of congenital adrenal hyperplasia, those wishing to conceive within the next 6 months, or women with contraindications to oral contraceptive use were excluded from the study. The study was approved by the local ethics committees of the First Faculty of Medicine and General Teaching Hospital, and written informed consent was obtained from all subjects.

Protocol of the study

All patients were randomly assigned to two groups using a generator of random values with a uniform distribution within the interval 0 to 1 (statistical software NCSS 2002). The values obtained were transformed into rank values. The subjects with ranks 1–15 were assigned to the COC group and received a monophasic COC (EE 35 μg/NGM 250 μg) in a cyclic regimen (21 days of active pills followed by 7 days of pill-free interval) for 6 months. The remaining 15 subjects received an identical COC in combination with metformin (1500 mg/day) for 6 months (METOC group). All laboratory tests were performed prior to treatment and after the sixth cycle of treatment.

The weight and height of all women were taken to calculate the body mass index (BMI). The waist and hip circumferences were measured in the standing position at the levels of the umbilicus and spina iliaca anterior superior and the waist-to-hip ratio (WHR) was calculated. Blood samples were taken in the early follicular phase, i.e. between days 3 and 6 of the menstrual cycle.

Assays

All analytic determinations were performed at the National Reference Laboratory. Serum LH, FSH and testosterone concentrations were measured by chemiluminiscent assay using an ACS:180 Autoanalyzer (Bayer Diagnostics, GmbH, Germany). The concentrations of DHEA, dehydroepiandrosterone sulphate (DHEA-S) and androstenedione were determined by radioimmunoassay methods (Immunotech, Fullerton, CA, USA). SHBG was measured using IRMA kits (Orion, Finland). The free androgen index (FAI) was calculated according to the following formula: FAI = 100 × testosterone (nmol/l)/SHBG (nmol/l). Plasma glucose concentration was determined by the glucose oxidase method (Olympus Diagnostica, GmbH, Germany). Plasma insulin concentrations were measured by radioimmunoassay kits (CIS Bio International, France; normal range 4–20 mIU/l; inter-assay %CV <5; intra-assay %CV <8.5). Serum cholesterol and triglycerides were analysed using CHOD-PAP and GPO-PAP-based kits, respectively (Oxochrome; Lachema a.s., Czech Republic). High-density lipoprotein (HDL) cholesterol was determined by an immunoinhibition method (HDL-C Direct; Wako Chemicals GmbH, Neuss, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula (LDL cholesterol = total cholesterol-HDL cholesterol-triglycerides/2.19 mmol/l) (Friedewald *et al.*, 1972).

Euglycaemic hyperinsulinaemic clamp

The hyperinsulinaemic euglycaemic clamp was performed as described previously (De Fronzo *et al.*, 1979). Briefly, to obtain blood for biochemical analyses during the clamp, one cannule was inserted into the wrist vein. For continuous blood glucose determination, a double-lumen catheter was inserted into the cubital vein of the ipsilateral arm. A third cannule was inserted into the contralateral forearm vein for insulin and glucose administration by Biostator (GCIIS, Elkhart, IN, USA). After a 30-min washout period, a hyperinsulinaemic euglycaemic state was attained during the next 45 min and the clamp was then performed using a constant insulin infusion rate (1 mU/kg/min) over 120 min. The glucose solution (40% w/v) was sampled by Biostator (mode 7:1) to maintain blood glucose levels at baseline value. During the clamp, blood glucose levels were repeatedly determined by glucose analyser (ESAT 6660-2; PWG, Medingen, Germany). Two blood samples for insulin determination were collected in the last 20 min of the clamp.

The following characteristics of insulin action were calculated: glucose disposal rate (M), defined as the amount of glucose supplied by the Biostator to maintain blood glucose levels during the last 20 min of the clamps (in mmol/kg/min); the insulin sensitivity index (ISI), defined as the ratio of glucose disposal rate to insulin concentration at the end of the clamps (in mmol/kg/min/mU/l × 100); and the metabolic clearance rate of glucose (MCRg), expressed as the ratio of glucose disposal rate to blood glucose concentration (ml/kg/min).

Statistical analysis

With respect to deviations from the Gaussian distribution of data and the occurrence of severe non-homogeneities in some variables, the treatment effect was evaluated using the robust paired Wilcoxon's test. For the same reason, the differences between the groups were tested with the Mann–Whitney robust test. Spearman's robust correlations were used for evaluating the differences before and after treatment. Besides the Mann–Whitney test, a general linear model was applied with adjustment of FAI, DHEA and BMI to eliminate the differences in androgens and BMI between the groups found at the beginning of the experiment in relation to ISI. With respect to the skewed data distribution in FAI and DHEA, the variables were transformed by a power transformation to attain a Gaussian distribution. The minimum value of mean square error of the linear fit in the normal probability plot (the plot of experimental fractiles versus theoretical fractiles of Gaussian distribution) was used as a criterion for optimal transformation parameters.

Results

A total of 30 women were enrolled in the study and 28 completed the protocol. Two subjects were excluded from the study, both from the METOC group, for unacceptable adverse events (gastrointestinal problem in one case and for non-compliance with the study protocol in the other).

The characteristics of both groups before treatment as well as the changes in the parameters during treatment are

Table I. Summary statistics of anthropometric parameters, lipids, hormones and insulin sensitivity in the groups treated with combined oral contraceptives (COC) and COC with metformin (METOC)

Variable	Indices at the beginning of the experiment							Difference after treatment compared with before treatment								
	COC			METOC				COC				METOC				
	Mean	SD	95% CI of mean	Mean	SD	95% CI of mean	Differences between the groups	Mean	SD	95% CI of mean	Differences from zero	Mean	SD	95% CI of mean	Differences from zero	Differences between the groups
Age	23.2	4.6	20.7–25.7	23.8	5.4	20.3–27.2	NS	–	–	–	–	–	–	–	–	–
Waist	74.1	10.5	68.3–79.9	80.0	12.6	72.0–88.0	NS	–	–	–	–	–	–	–	–	–
WHR	0.752	0.079	0.708–0.796	0.794	0.086	0.740–0.849	NS	–	–	–	–	–	–	–	–	–
Weight	63.3	11.9	56.7–69.9	68.6	13.3	60.1–77.0	NS	1.8	3.5	−0.2–3.7	NS	−0.9	4.7	−3.9–2.1	NS	NS
BMI	22.1	3.1	20.4–23.8	24.7	4.9	21.6–27.8	NS	0.6	1.2	−0.1–1.3	NS	−0.3	1.7	−1.4–0.7	NS	NS
Io	9.4	6.7	4.9–13.9	11.2	4.9	7.7–14.7	NS	5.40	2.67	3.3–7.5	$P < 0.01$	3.90	6.31	−1.0–8.8	NS	NS
Go	4.60	0.45	4.35–4.85	4.68	0.50	4.36–5.00	NS	−0.22	0.67	−0.6–0.2	NS	−0.26	0.59	−0.63–0.12	NS	NS
ISI	59.2	29.7	42.0–76.3	44.4	27.6	25.8–62.9	NS	−6.6	37.3	−28.2–14.9	NS	−3.5	30.8	−24.2–17.2	NS	NS
MCRg	8.54	2.50	7.16–9.93	6.83	1.86	5.65–8.00	NS	0.66	3.96	−1.5–2.9	NS	0.43	3.41	−1.73–2.60	NS	NS
CHOL	4.63	0.70	4.24–5.02	4.81	0.80	4.30–5.32	NS	0.62	0.72	0.2–1.0	$P < 0.007$	0.71	0.67	0.29–1.14	$P < 0.006$	NS
TGD	0.941	0.420	0.708–1.173	1.168	0.480	0.863–1.473	NS	0.381	0.547	0.1–0.7	$P < 0.02$	0.29	0.65	−0.12–0.700	NS	NS
HDL	1.55	0.21	1.44–1.67	1.47	0.40	1.22–1.73	NS	0.13	0.40	−0.1–0.3	$P < 0.05$	0.29	0.24	0.14–0.44	$P < 0.008$	NS
LDL	2.67	0.58	2.34–2.99	2.81	0.58	2.44–3.18	NS	0.31	0.54	0.0–0.6	$P < 0.05$	0.29	0.67	−0.14–0.72	NS	NS
LH	6.82	3.96	4.63–9.02	6.88	5.56	3.35–10.41	NS	−4.77	4.37	−7.2–2.4	$P < 0.003$	−4.07	5.71	−7.69–0.44	$P < 0.003$	NS
FSH	4.83	1.57	3.96–5.70	4.65	1.92	3.43–5.88	NS	−1.47	3.97	−3.7–0.7	NS	−0.91	2.41	−2.44–0.62	NS	NS
PRL	11.8	5.5	8.7–14.8	11.7	5.6	8.2–15.3	NS	1.3	5.9	−2.0–4.5	NS	1.1	6.8	−3.2–5.4	NS	NS
T	3.94	1.49	3.11–4.76	4.84	1.16	4.10–5.57	NS	−0.45	1.01	−1.0–0.1	$P < 0.05$	−0.92	2.10	−2.25–0.42	$P < 0.05$	NS
FAI	11.8	7.9	7.5–16.2	19.2	6.9	14.6–23.8	$P < 0.02$	−9.0	7.2	−13.0–5.0	$P < 0.001$	−15.5	8.2	−21.1–10.0	$P < 0.004$	$P < 0.04$
A	11.1	5.8	7.8–14.3	12.6	3.5	10.4–14.9	NS	−4.0	6.4	−7.5–0.5	$P < 0.04$	−4.8	2.6	−6.4–3.1	$P < 0.003$	NS
DHEA	25.6	13.3	18.3–33.0	40.4	16.6	29.8–50.9	$P < 0.02$	−7.8	14.6	−13.9–2.3	$P < 0.05$	−14.3	12.1	−22.1–6.6	$P < 0.009$	NS
DHEA-S	10.5	2.2	9.3–11.8	12.2	3.8	9.8–14.6	NS	−4.1	3.0	−5.8–2.4	$P < 0.002$	−2.5	4.0	−5.0–0.1	NS	NS
Prog17	3.74	3.32	1.73–5.74	3.47	1.17	2.73–4.22	NS	−1.69	3.16	−3.6–0.2	$P < 0.02$	−0.62	1.79	−1.82–0.58	NS	NS
SHBG	32	13	31–58	27	9	21–33	NS	108	63	73.2–143.3	$P < 0.001$	116	71	68–164	$P < 0.004$	NS

CI = confidence interval; NS, not significant; –, not available; Io = fasting insulin; Go = fasting glucose; CHOL = total cholesterol; TGD = triglycerides; PRL = prolactin; T = testosterone; A = androstenedione; Prog17 = 17OH-progesterone.

demonstrated in Table I. There were no differences in anthropometric parameters, ISI, lipid profile, SHBG concentration and hormones, except for a higher DHEA concentration and higher free androgen index in the METOC group, of borderline significance. Owing to significant differences in androgens and an insignificant difference in BMI between both groups, a general linear model was applied with adjustment of FAI, DHEA and BMI to eliminate the effect of these differences on the change of ISI during treatment. Even after this adjustment, no differences were found in the change of ISI between the COC and METOC groups.

As demonstrated in Table I, there was a slight weight gain and increase of BMI in the COC group and the opposite tendency in the METOC group, although this was not significant in either group. Insulin sensitivity did not change significantly in either group, but fasting insulin increased in the COC group. Both treatment protocols caused an increase in total cholesterol, triglycerides, HDL and LDL cholesterol; changes in triglycerides and LDL cholesterol did not reach significance in the METOC group. During treatment, there was a significant decrease in testosterone, androstenedione, DHEA, DHEA-S and 17OH-progesterone in the COC group. More pronounced changes in androstenedione and DHEA and a lack of significance in changes of DHEA-S and 17OH-progesterone were found in the METOC group. Highly significant changes in SHBG were found in both groups. Comparing the effect of treatment in both groups, only a decrease in free androgen index was significantly different and more pronounced in the METOC group.

Discussion

There is an ongoing discussion in the literature concerning the role of metformin in the treatment of PCOS (Homburg, 2002; Legro, 2002). Promising results were not fully confirmed in prospective randomized studies (Harborne *et al.*, 2003). The most consistent effect of metformin is an improvement in the ovulation rate (Lord *et al.*, 2003). However, changes in insulin, glucose tolerance, BMI and androgens vary. Stimulation of SHBG production, which is one of the key mechanisms in acne and hirsutism improvement, is not seen or is insignificant in the vast majority of studies. Restoration of a regular menstrual cycle usually occurs in $< 50\%$ of patients. Based on the above data it is difficult to consider metformin alone as a first-line option for the treatment of PCOS.

A combination of metformin with COC would seem to be a justifiable solution for many reasons. Treatment with COC enables significant inhibition of androgen production and a significant increase in SHBG synthesis (Cibula *et al.*, 2002; Elter *et al.*, 2002). As a consequence, it is successfully used in the treatment of acne and hirsutism (Redmond *et al.*, 1997). Restoration of a regular menstrual cycle is reliable. On the other hand, a beneficial influence on glucose metabolism or insulin action is unlikely, although some recent papers showed neutral or even positive effects of COC with low androgenic progestins on insulin sensitivity (Cibula *et al.*, 2002; Cagnacci *et al.*, 2003). The significant effect of COC on SHBG, androgen production, skin androgenic symptoms and irregular menstrual cycle might be successfully combined with the effects of metformin on anthropometric parameters, glucose tolerance and insulin sensitivity. An additional argument for combination therapy is the need for effective contraception in women while on metformin.

Two studies have been published to date that used combined treatment with COC and metformin in patients with PCOS. In the first study, from 2002, the authors found a significant decrease in BMI and WHR only in the group on combined treatment, and a more pronounced effect on androstenedione, SHBG and glucose-to-insulin ratio in the same group (Elter *et al.*, 2002). When comparing both groups, only changes in androstenedione and SHBG remained significantly higher in the group on combination treatment. The authors concluded that adding metformin to the COC treatment might improve insulin sensitivity and further suppress hyperandrogenaemia in non-obese women with PCOS. However, insulin sensitivity was not measured directly.

The second paper, from 2003, focused on adolescent girls with PCOS (Ibáñez and Zegher, 2003). The design of the study was different. COC was randomly added to a continuous treatment with metformin and flutamide. The additive effect of COC was investigated as such. The only significant change following addition of COC was an increase in SHBG and consequently a decrease in the free androgen index.

In our study, we compared COC monotherapy with combination therapy of COC with metformin for 6 months. This was the first study using the euglycaemic clamp for insulin sensitivity evaluation during combination treatment.

In agreement with the paper by Elter *et al.* (2002), we showed a slight decrease in fasting glucose in both groups, although the above changes did not reach significance in our study. A slight rise in fasting insulin was significant only in the COC group. While Elter and colleagues evaluated insulin sensitivity indirectly using a calculation of glucose-to-insulin ratio, which improved significantly in the group on combined treatment, we measured insulin sensitivity by the clamp technique and found no significant changes. It should be mentioned, however, that the effect of metformin on glucose metabolism is mostly exerted through the inhibition of glucose production, and this mechanism might be masked by the increased levels of insulin during the clamp. There were no differences between the two treatments in the effect on glucose or insulin in our study, as in the study by Elter *et al.* (2002). In conclusion, we were not able to show an expected improvement in insulin sensitivity while on combined treatment, and the trends in fasting insulin and glucose concentrations were comparable in both groups.

Besides a potential improvement in insulin sensitivity, another argument for metformin is its beneficial effect on anthropometric parameters. While BMI and weight increased in the COC group, these were decreased during combined treatment. This is in accordance with Elter *et al.* (2002), although the changes were not significant in our study in either group. However, it is difficult to conclude whether those changes are caused by direct metabolic effect of

metformin or by frequent gastrointestinal problems at the beginning of metformin treatment. The positive trend in weight and body fat distribution should be confirmed in a long-term follow-up.

Modification of the COC effect on androgens by metformin is difficult to interpret. Randomized prospective studies have documented a direct effect of metformin on ovarian steroidogenesis (Pasquali *et al.*, 2000; Ng *et al.*, 2001; Vrbíková *et al.*, 2001; Kocak *et al.*, 2002). A more pronounced decrease in androstenedione in the group on combined treatment was described previously by Elter and colleagues. In our study, comparable effects on testosterone and androstenedione were found in both groups, and a change in DHEA of higher significance in the women on combined treatment might rather be explained by higher basal concentrations at the beginning of the study.

Stimulation of SHBG production is one of the key mechanisms in the treatment of skin androgenic symptoms by COC. Elter and colleagues found a greater increase in SHBG in the group with combined treatment. This was not confirmed in our study. We showed a comparable significant increase in SHBG in both groups. Our results are in agreement with many papers that show insignificant changes or even a decrease in SHBG with metformin treatment (Nestler *et al.*, 1998; Pasquali *et al.*, 2000; Ng *et al.*, 2001; Fleming *et al.*, 2002).

In summary, our study confirmed a significant positive effect of COC on androgens and SHBG. Combination with metformin caused an additional decrease in FAI. The beneficial trends in anthropometric parameters in the METOC group are in accordance with other studies, but weight reduction and positive changes of body fat distribution should be confirmed with long-term follow-up. Besides a few positive trends, combined treatment with metformin did not cause added beneficial effects on lipids, insulin sensitivity, SHBG or testosterone. It should be emphasized, however, that for evaluation of insulin sensitivity the number of subjects needed to reach enough power is very high (>300 patients), and was not fulfilled in our study. We conclude that the available data do not offer enough evidence to advocate the standard use of COC in combination with metformin in the long-term treatment of PCOS due to unsatisfactory improvement of endocrine and metabolic abnormalities that characterize the syndrome. However, it might be argued that the value of metformin could be different in specific subgroups of PCOS patients, especially in obese ones. This can not be addressed by our study and remains an area of future interest.

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Insulin sensitivity in non-obese women with polycystic ovary syndrome during treatment with oral contraceptives containing low-androgenic progestin

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BACKGROUND: Combined oral contraceptives (COC) effectively suppress hyperandrogenism in women with polycystic ovary syndrome (PCOS), though deterioration of insulin sensitivity during treatment is assumed. The study aim was to investigate insulin action and androgen production during treatment with COC containing low-androgenic progestin. METHODS: A total of 13 PCOS women and nine controls was enrolled into the study. Only non-obese women with a body mass index (BMI) <30 kg/m^2 were included. Hyperinsulinaemic euglycaemic clamp techniques were performed before and after 6 months of treatment with a monophasic COC containing norgestimate. RESULTS: Anthropometric parameters [BMI, waist:hip ratio (WHR)] remained unaltered during the study in both groups. No deterioration in glucose disposal rate (M), insulin sensitivity index (ISI) or metabolic clearance rate of glucose (MCRG) was observed during treatment in PCOS subjects. Fasting glucose decreased significantly ($P < 0.01$), but fasting insulin remained unchanged. Significant decreases in concentrations of testosterone ($P < 0.001$), androstenedione ($P < 0.01$) and dihydroepiandrosterone (DHEA) ($P < 0.001$), a decrease in the free androgen index, and an increase in concentrations of sex hormone-binding globulin were found in PCOS subjects. CONCLUSIONS: The norgestimate-containing COC significantly decreased androgen production and concentrations of free androgens, without reducing insulin sensitivity in non-obese PCOS subjects.

Keywords: androgens/insulin sensitivity/norgestimate/oral contraceptives/PCOS

Introduction

The polycystic ovary syndrome (PCOS) is one of the most common endocrinopathics in women of reproductive age (Knochenhauer *et al.*, 1998; Asuncion *et al.*, 2000). It is the leading cause of menstrual cycle disturbances, skin androgenic complaints and anovulatory infertility. One of the principal endocrine abnormalities of the syndrome is overproduction of androgens in the ovaries, though many other endocrine and metabolic disturbances characterize the syndrome. PCOS women have been found to have lipid profile deterioration, increased concentrations of plasminogen activator inhibitor (PAI-1), and decreased concentrations of sex hormone-binding globulin (SHBG) (Dunaif *et al.*, 1987; Holte *et al.*, 1994; Sampson *et al.*, 1996; Atiomo *et al.*, 1998; Talbott *et al.*, 1998). A frequent finding in non-obese and obese PCOS women is hyperinsulinaemia secondary both to increased peripheral insulin resistance and abnormal insulin secretion (Chang *et al.*, 1983; Dunaif *et al.*, 1989, 1992; Dunaif and Finegood, 1996). The major consequence of these abnormalities is a high incidence of impaired glucose tolerance and type 2 diabetes in these patients (Ehrmann *et al.*, 1999; Legro *et al.*, 1999). An increased risk of ischaemic heart disease and hypertension in the perimenopausal age has also been demonstrated (Dahlgren *et al.*, 1992; Cibula *et al.*, 2000).

Estrogen-progestogen products (combined oral contraceptives; COC) are an effective modality in the long-term treatment of hyperandrogenism in PCOS patients. They significantly suppress ovarian androgen synthesis and increase the binding capacity for circulating androgens by increasing SHBG concentrations (Falsetti and Pasinetti, 1995; Coenen *et al.*, 1996). COC containing progestins with a low androgenic potency have a neutral or positive effect on the lipid profile, and sufficiently improve skin androgenic symptoms (Gevers Leuven *et al.*, 1990; Kuhl *et al.*, 1990). Apart from these beneficial effects a deterioration of insulin action is assumed. The impairment of insulin sensitivity in healthy women using COC has been demonstrated by several authors (Skouby *et al.*, 1987; Kasdorf and Kalkhoff, 1988; Godsland *et al.*, 1992).

To our knowledge, only one report has been published to date determining the changes in insulin action during COC

administration in PCOS patients, with insulin sensitivity being significantly decreased during three cycles of treatment in nine patients (Korytkowski *et al.*, 1995). Norethindrone with a relatively high androgenic potency was used in this study as the progestogenic component. However, a less pronounced effect of agents with low-androgenic progestins on carbohydrate metabolism was demonstrated (Godsland *et al.*, 1992). Hence, the present study was conducted in order to determine the effect of COC containing progestin with low androgenic potency on insulin sensitivity in PCOS women. The euglycaemic glucose clamp technique was used to study insulin action.

Materials and methods

Study group

A total of 14 patients meeting the diagnostic criteria of PCOS were enrolled into the study. PCOS was defined as follows: (i) oligomenorrhoea from menarche (menstrual cycle longer than 35 days); (ii) an increased concentration of at least one androgen above the upper reference limit [testosterone 0.5–2.63 nmol/l; androstenedione 1.57–5.4 nmol/l; dihydroepiandrosterone (DHEA) 0.8–10.5 nmo/l; dihydroepiandrosterone sulphate (DHEAS) 2.4–14.5 μmol/l]; and (iii) clinical manifestation of hyperandrogenism (acne, hirsutism, or both). Only non-obese women with a body mass index (BMI) <30 kg/m^2, aged >18 years, and who had not used hormonal therapy during the previous 6 months, were included. Women presenting with a secondary endocrine disorder, such as hyperprolactinaemia, thyroid dysfunction or a non-classical form of congenital adrenal hyperplasia, those wishing to conceive within the next 6 months, or women with contraindications to oral contraceptives use were excluded from the study. Normal glucose tolerance was established according to both the criteria of the World Health Organization and the revised criteria of the American Diabetes Association using the 2-h, 75-g oral glucose tolerance test (OGTT) (World Health Organization, 1985; The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Only one patient did not complete the study protocol of COC treatment because of the occurrence of side effects (nausea, breast tenderness) requiring discontinuation. All patients were informed about the study protocol and provided their informed consent. The study was approved by the Local Ethics Committee of both institutions.

Control group

A control group was selected from women willing to use COC, who agreed with the study protocol. All subjects met the following inclusion criteria: (i) concentrations of androgens and SHBG within the reference limits; (ii) a regular menstrual cycle from menarche to the present; and (iii) absence of skin androgenic symptoms (hirsutism, persistent acne). Only non-obese women with a BMI <30 kg/m^2, age >18 years and who had not used hormonal therapy during the previous 6 months, were included. Patients with an established endocrine disorder or with contraindications to hormonal contraception use were excluded. Normal glucose tolerance was established before the study. All controls provided their informed consent.

Study protocol

All patients and controls received monophasic COC pills containing ethinyl estradiol (35 μg/day) and low-androgenic progestin (norgestimate 250 μg/day) for seven cycles. The COC agent was given in a cyclic regimen of 21 active pills followed by 7 days without hormonal use. All clinical investigations and laboratory tests were performed prior to treatment and after the sixth cycle of treatment.

Blood samples taken prior to treatment were withdrawn during the early follicular phase, i.e. between days 3 and 6 of the menstrual cycle. Provided that menstrual bleeding failed to occur until day 45 of the cycle, bleeding was induced by progesterone administration. The second blood sample was taken after six cycles of COC administration.

Euglycaemic hyperinsulinaemic clamp technique

The hyperinsulinaemic euglycaemic clamp was performed as described previously (Flier, 1992). A flexible cannula was inserted into the forearm vein to obtain blood samples for the determination of basal insulin, and plasma glucose and potassium concentrations. The cannula was then connected to an infusion module of a Biostator (GCSII; Elkhart, IN, USA) to administer an insulin solution (160 units of Actrapid HM®; Novo-Nordisk, in 500 ml 0.9% sodium chloride saline solution), 40% glucose solution, and wash-out sodium chloride saline solution (0.9% w/v). At the same time, 7.5% potassium chloride solution diluted with physiological saline solution 1:4 was delivered by perfusor (Infusor Secura FT; B. Braun, Germany) to another channel of the cannula at a rate of 0.1 ± 0.05 ml/min to maintain basal potassium concentrations. The rate of this infusion was adjusted according to the results of repeatedly determined serum potassium concentrations. A double-lumen catheter was inserted into the contralateral arm for continuous blood glucose determination. A third cannula placed into a wrist vein was used to collect blood samples for biochemical measurements. After a 30 min washout period, the hyperinsulinaemic euglycaemic clamp was performed using the Biostator (mode 7:1) over 120 min using a constant insulin infusion rate (1 mU/kg per min) (Fogt *et al.*, 1978). The glucose solution (40% w/v) was sampled by the Biostator to maintain blood glucose concentration at baseline value. During the clamp, blood glucose concentrations were repeatedly determined using a glucose analyser (ESAT 6660-2; Melsungen, Germany). Two blood samples were collected for insulin determination during the last 20 min of clamping.

The following characteristics of insulin action were calculated: (i) glucose disposal rate (M, μmol/kg per min), defined as the amount of glucose supplied by the Biostator to maintain blood glucose concentrations during the clamps; (ii) the insulin sensitivity index (ISI, μmol/kg per min per mU/l×100), defined as the ratio of glucose disposal rate to insulin concentration at the end of the clamps; and (iii) metabolic clearance rate of glucose (MCRG, ml/kg per min), expressed as the ratio of glucose disposal rate to blood glucose concentration.

Analyses

All analyses were performed at the National Reference Laboratory. Serum LH, FSH and testosterone concentrations were measured with a chemiluminescence assay (ACS:180 auto-analyser; Bayer Diagnostics GmbH, Germany). The concentrations of DHEA, DHEAS and androstenedione were determined using radioimmunoassay methods (Immunotech, France). SHBG was measured using an IRMA kit (Orion, Finland). The intra- and interassay coefficients of variation (CV) were respectively: <3.7 and <6.7% for LH, <2.6 and <4.2% for FSH, <4.0 and <8.0% for testosterone, <7.9 and <11.9% for DHEA, <7.4 and <10.6% for DHEAS, <8.9 and <10.3% for androstenedione, and <5.5 and <6.9% for SHBG. The free androgen index (FAI) was calculated according to the following formula: FAI = 100×testosterone (nmol/l)/SHBG (nmol/) (Carlstrom *et al.*, 1987). Plasma glucose concentration was determined by the glucose oxidase method (Olympus Diagnostica GmbH, Germany). Plasma insulin concentrations were measured using radioimmunoassay kits (CIS Bio International, France). Normal range was 4–20 μIU/ml; interassay

Table I. Summary statistics of age, body mass index (BMI), waist:hip ratio (WHR), glucose, insulin, HbA1, LH, FSH and steroids before treatment in controls and in PCOS patients

Parameter	Controls						PCOS patients						*P*
	Count	Average	SEM	Median	Lower quartile	Upper quartile	Count	Average	SEM	Median	Lower quartile	Upper quartile	
Age (years)	9	23.4	0.8	22.5	21.5	25.5	13	25.4	1.6	23.0	21.0	29.0	NS
BMI (kg/m^2)	9	20.8	0.6	21.0	19.4	22.2	13	22.7	0.9	22.7	20.4	23.0	NS
WHR	9	0.707	0.011	0.713	0.693	0.724	13	0.682	0.013	0.683	0.656	0.707	NS
Glucose (mmol/l)	9	4.35	0.15	4.25	4.20	4.65	13	4.48	0.12	4.30	4.20	4.70	NS
Insulin (mIU/l)	9	17.1	2.6	15.0	13.1	21.3	13	15.7	1.4	16.4	14.5	17.2	NS
HbA1 (%)	8	4.75	0.05	4.72	4.65	4.91	10	5.43	0.12	5.48	5.35	5.73	<0.01
LH (IU/l)	9	4.81	0.66	4.60	3.10	6.60	13	10.16	1.25	8.40	7.10	10.70	<0.001
FSH (IU/l)	9	5.53	0.51	5.55	4.60	6.65	13	5.57	0.57	4.80	4.40	6.70	NS
Testosterone (nmol/l)	9	1.32	0.33	1.15	0.84	1.59	12	3.18	0.22	3.10	2.55	3.60	<0.001
Androstenedione (nmol/l)	9	9.69	1.43	8.97	7.47	9.66	13	13.73	1.79	12.40	9.55	15.60	NS
DHEA (nmol/l)	9	12.1	1.0	12.7	11.8	13.8	13	12.3	2.2	9.6	7.6	13.8	NS
DHEAS (μmol/l)	9	7.78	0.98	8.94	4.94	9.53	13	7.98	0.78	8.80	5.50	10.10	NS
SHBG (nmol/l)	9	63.2	5.2	59.0	54.3	67.2	13	52.1	5.5	50.4	39.2	66.0	NS

DHEA = dihydroepiandrosterone; DHEAS = dihydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin.

CV was <5%, and intra-assay CV <8.5%. Glycated haemoglobin $A1_c$ (HbA1) concentrations were measured using an IM kit (Abbott analyser; Assay System Abbot IMX, IL, USA). Normal range was 4.4–6.4%; intra-assay CV was <4.5%, and interassay CV <8.5%.

Statistical analysis of data

Each variable was tested for normality using the Kolmogorov–Smirnov test, the Martinez–Iglewicz test and the D'Agostino test. If at least one of the tests indicated non-Gaussian distribution, or if the variances were not homogeneous, the data were treated by square-root or by logarithmic transformation. The raw or the treated data were submitted to repeated measures two-way analysis of variance (ANOVA), using the control or patient status as the between-subject effect and the treatment effect (before versus after treatment) as the within-subject effect. This test answered the question whether or not there were differences between patients and controls, differences before and after treatment, and if there was any interaction among within-subject and between-subject effects.

When the between-subject effects were significant, the differences were identified using unpaired *t*-tests both before and after treatment. Significant within-subject effects were further explored by paired *t*-test. Patients and controls were considered as a whole if the between-subject effect was not significant. If the between-subject effect was significant, the patients and controls were evaluated separately.

Results

The basic characteristics of the patients and controls before treatment are shown in Table I. The mean age of both groups was not significantly different; neither did patients and controls differ in their mean BMI or waist:hip ratio (WHR). Anthropometric parameters remained stable during the study in both groups and did not differ at the end of the study. The PCOS group entered the study with significantly higher serum concentrations of LH and testosterone ($P < 0.001$).

Concentrations of gonadotrophins, androgens and SHBG before and after treatment are shown in Figure 1 (retransformed means with 95% confidence intervals). A significant decrease was observed in the concentrations of LH ($P < 0.001$), androstenedione ($P < 0.01$), DHEA ($P < 0.01$) and DHEAS ($P < 0.001$), a significant decrease in FAI ($P < 0.01$), and a significant increase in SHBG concentrations ($P < 0.001$) during treatment in both groups. The concentration of testosterone decreased significantly in the patients ($P < 0.001$), but no changes were observed in controls. LH concentrations decreased more significantly in patients than in controls ($P < 0.01$).

No significant differences were observed in either fasting plasma glucose, fasting insulin, M, ISI or MCRG between the groups (Figure 2). A significant decrease in fasting glucose was determined during treatment in both groups ($P < 0.01$). The mean HbA1 concentration was higher in patients before treatment ($P < 0.05$); however, no difference was seen between the two groups after treatment. No deterioration of either M, ISI or MCRG was observed during treatment in PCOS subjects. Moreover, none of the latter parameters was changed significantly during treatment in controls.

Discussion

The most important consequence of endocrine and metabolic disturbances associated with PCOS is an increased risk for

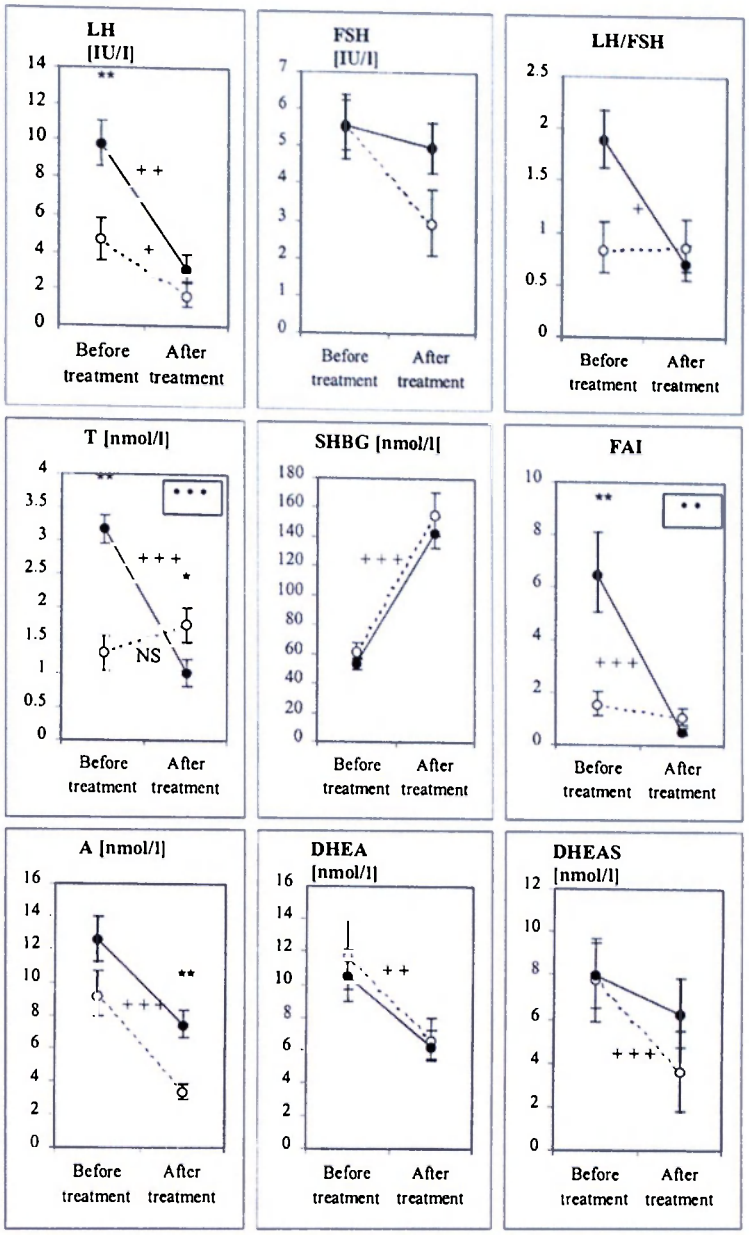

Figure 1. Changes in LH, FSH, LH/FSH, sex hormone-binding globulin (SHBG), steroids and the free androgen index (FAI) during treatment. Empty and full circles with error bars represent retransformed mean value ± SEM in controls and patients respectively. Crosses denote the significance of the differences in subjects between the stages of the study (before/after treatment) (+, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$). Asterisks denote the significance of the differences between controls and patients (*, $P < 0.05$; **, $P < 0.01$). Closed circles in the frame denote the significance of interactions (oo, $P < 0.01$; ooo, $P < 0.001$). A = androstenedione; DHEA = dihydroepiandrosterone; DHES = dihydroepiandrosterone sulphate; T = testosterone.

impaired glucose tolerance and type 2 diabetes mellitus (Dahlgren *et al.*, 1992; Ehrmann *et al.*, 1999; Legro *et al.*, 1999; Cibula *et al.*, 2000). Insulin resistance independent of obesity, abnormal insulin secretion and dyslipidaemia are risk factors participating in the pathogenesis of the above conditions (Dunaif *et al.*, 1987, 1989, 1992; Holte *et al.*, 1994; Talbott *et al.*, 1998). New findings regarding the metabolic consequences have changed the concepts for the long-term treatment of PCOS. The optimal therapeutic modality should correct the increased androgen production, yet at the same time exert a beneficial, or at least neutral, effect on insulin action.

One of the most frequent treatment modalities—combined oral contraceptives—fulfilled almost all the criteria for optimal treatment. However, the impairment of insulin sensitivity, as described in healthy users, might be a significant weakness. The first study examining insulin sensitivity during COC use was published in 1987 (Skouby *et al.*, 1987). These authors performed the euglycaemic clamp technique to demonstrate a decrease in insulin sensitivity in six healthy subjects after a 6-month administration of a levonorgestrel-containing COC. Similar results were reported after 3 months of COC use; however, insulin sensitivity returned toward control values

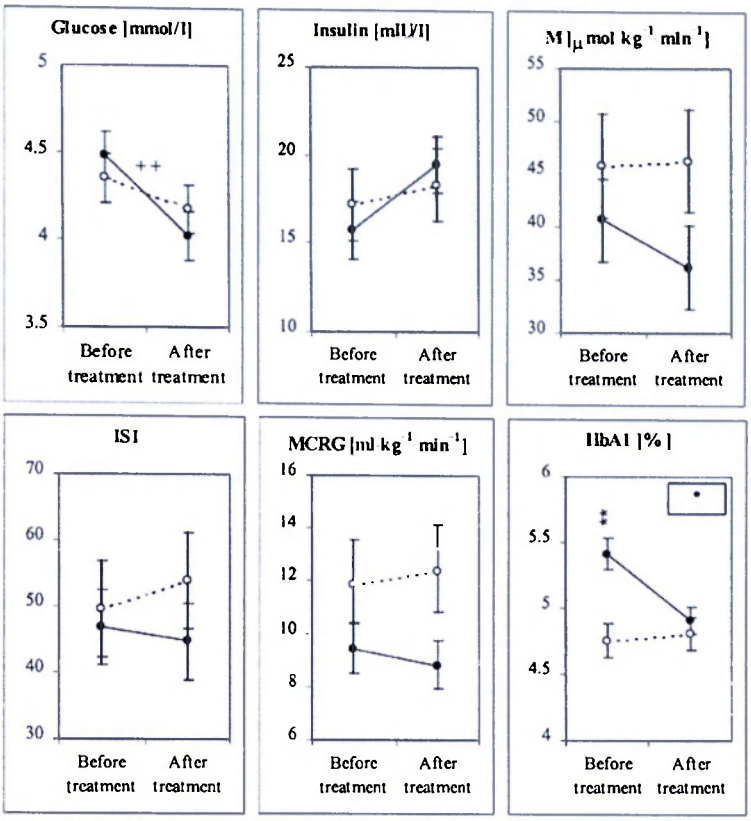

Figure 2. Changes in glucose, insulin, glucose disposal rate (M), insulin sensitivity index (ISI), metabolic clearance rate of glucose (MCRG) and glycosylated haemoglobin A1 (HbA1) concentration during treatment. Empty and full circles with error bars represent retransformed mean value ± SEM in controls and patients respectively. Crosses denote the significance of the differences in subjects before and after treatment (++, $P < 0.01$). Asterisks denote the significance of the differences between controls and patients (**, $P < 0.01$). Closed circles in the frame denote the significance of interactions (●, $P < 0.05$).

after 6 months (Kasdorf and Kalkhoff, 1988). A decrease in insulin sensitivity was also demonstrated after application of a minimal model approach in 296 contraceptive users (Godsland *et al.*, 1992).

To the best of our knowledge, only one report has been published to date addressing the changes in insulin sensitivity during COC administration in PCOS patients (Korytkowski *et al.*, 1995), with insulin sensitivity being studied in nine women with PCOS, and in 10 controls. The hyperglycaemic clamp technique was performed before and after 3 months of therapy with triphasic oral contraceptives containing norethindrone, a progestogen with a relatively high androgenic potency. Despite the short time of intervention, a decline in insulin sensitivity was shown in both groups.

The present study is the first designed to investigate changes in insulin sensitivity in PCOS women receiving a COC containing a progestin with a low androgenic potency. The minimal androgenicity of progestins is reflected in a more pronounced increase in the concentrations of SHBG and a decrease in free testosterone concentrations in COC users (Hammond *et al.*, 1984; van der Vange *et al.*, 1990). Moreover, a number of reports have documented only slight changes in glucose tolerance, fasting insulin, fasting glucose and areas under glucose or insulin curves in users of COC with low-androgenic progestins (Petersen *et al.*, 1988; Corson, 1990; Godsland *et al.*, 1992; Crook *et al.*, 1993). Studies with norgestimate, the progestin used in the present study, showed virtually no effect on carbohydrate metabolism. In two US multicentre trials, no significant changes in fasting blood glucose, or in the results of a 3-h glucose tolerance test, were demonstrated (Corson, 1990), whilst a 12-month German study documented no adverse effect of treatment on the insulin, glucose, or $HbA1_c$ concentrations (Becker, 1990). The effect of two products containing progestins with a low androgenic potency (norgestimate and gestodene) on insulin action was evaluated in a recent study (Petersen *et al.*, 2000). Insulin sensitivity was determined by a frequently sampled intravenous glucose tolerance test. The users of either product showed increased concentrations of fasting plasma insulin and a decrease in insulin sensitivity. However, the relationship between the ISI and insulin response remained unchanged.

An important inclusion criteria in the present study was that of BMI. Only non-obese patients were eligible for inclusion, as it was well recognized that obesity is an independent risk factor worsening the parameters of insulin resistance (Acién *et al.*, 1999; Ciampelli *et al.*, 1999). Every minor shift in BMI during the study may dramatically influence insulin sensitivity in obese patients. On the other hand, impaired insulin action has been repeatedly documented in both non-obese and obese PCOS patients (Dunaif *et al.*, 1989; Dunaif and Finegood, 1996).

Higher insulin concentrations in PCOS patients compared with controls were not observed prior to treatment. The value of the ISI was lower, though not significantly so, in the PCOS group. Importantly, no adverse effect of COC administration was observed on either insulin sensitivity, glucose disposal rate or the metabolic rate of glucose. It might be speculated therefore that COC could have had an adverse effect on ISI, but on the other hand there was a compensatory improvement in ISI due to a decrease in androgen concentrations. However, the control group—where no such marked effect on androgen concentrations was seen—also did not show any deterioration in insulin sensitivity. The only relevant effects of treatment on carbohydrate metabolism were significantly decreased concentrations of fasting glucose. Unlike the study of Petersen (Petersen *et al.*, 2000), who used a frequently sampled intravenous glucose tolerance test, no deterioration of insulin sensitivity could be demonstrated in the group of healthy users.

The present study confirmed a marked suppressive effect of COC treatment on androgen concentrations that has been observed in other relevant investigations (Falsetti and Pasinetti, 1995; Coenen *et al.*, 1996). In addition to reduced androgen production, the treatment significantly raised the serum androgen binding capacity. The significant increase in SHBG concentrations reflects the minimal androgenicity of the progestogenic component used in COC.

It can be concluded that, in the present study there was no deterioration of insulin sensitivity in non-obese PCOS women during administration of COC containing low-androgenic progestin. In addition, the beneficial effect of COC administration reducing the concentrations of total and free androgens was confirmed. It is clear that whilst the present study was of insufficient duration to draw any authoritative conclusions, the results obtained provide significant evidence supporting the use of COC containing low-androgenic progestins in the long-term treatment of PCOS patients.

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Does obesity diminish the positive effect of oral contraceptive treatment on hyperandrogenism in women with polycystic ovarian syndrome?

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Polycystic ovarian syndrome (PCOS) is an obvious indication for long-term treatment. Combined oral contraceptives (COC) remain the first choice for the treatment of hyperandrogenism in most patients. However, differences in endocrine and metabolic parameters between obese and lean patients have been postulated. This is the first study evaluating the effect of COC treatment in obese versus non-obese PCOS patients. In total, 28 lean [body mass index (BMI) <25 kg/m^2)] and 15 obese (BMI >30 kg/m^2) women patients were enrolled in the study. The concentrations of androgens, sex hormone-binding globulin (SHBG) and lipids were measured before and after 6 months of treatment with COC containing low-androgenic progestins. Clinical androgenic symptoms were monitored. There was a lower concentration of SHBG in obese patients, but there were no differences in androgen concentrations between both groups before the study. Highly significant changes in concentrations of testosterone ($P < 0.001$), androstenedione ($P < 0.0001$), SHBG ($P < 0.001$) and LH ($P = 0.01$) were demonstrated in lean patients, with only less significant changes in SHBG ($P < 0.01$) and testosterone ($P < 0.05$) in obese patients during the study. Clinical androgenic symptoms improved significantly ($P = 0.05$) only in the group of lean women. No reduction in low-density lipoprotein-cholesterol/high-density lipoprotein-cholesterol ratio was observed in either group. In conclusion, the positive effect of COC treatment on androgen production, serum androgen binding capacity, and clinical androgenic symptoms was negatively influenced by an increased BMI.

Key words: androgens/lipids/obesity/oral contraceptives/polycystic ovarian syndrome

Introduction

Polycystic ovarian syndrome (PCOS) is an evident indication for long-term treatment as a proven risk factor for non-insulin-dependent diabetes mellitus (NIDDM) and coronary artery disease (Dahlgren *et al.*, 1992; Ehrmann *et al.*, 1999; Legro *et al.*, 1999; Cibula *et al.*, 2000). Moreover, improvement of skin androgenic symptoms also requires long-term medication. Combined oral contraceptives (COC) remain the first choice for the treatment of hyperandrogenism, as a key endocrine disturbance, for most women with PCOS. COC have been shown to inhibit significantly the ovarian androgens production and to increase serum androgen binding capacity (Falsetti and Galbignani, 1990; Dahlgren *et al.*, 1998; Gjonnaess, 1999). An effect on adrenal steroidogenesis has also been proposed (Wild *et al.*, 1991). In consequence, treatment with COC significantly reduces serum concentrations of both total and free androgens.

PCOS is a heterogeneous syndrome in that it presents with a broad spectrum of clinical manifestations and endocrine or metabolic disturbances. One of the most important factors determining the phenotype of the disease is the presence or absence of obesity. Approximately 50% of women suffering from PCOS are obese (Yen, 1980). Obesity itself negatively influences the synthesis of sex hormone-binding globulin (SHBG), androgen production, and insulin sensitivity (Kurtz *et al.*, 1987; Pasquali and Casimirri, 1993). Higher concentrations of androgens and more pronounced insulin resistance were demonstrated in obese PCOS women compared with lean patients (Dunaif *et al.*, 1989; Acién *et al.*, 1999; Ciampelli *et al.*, 1999). The increased concentrations of androgens and insulin stimulate the synthesis of insulin-like growth factor-I (IGF-I) and suppress SHBG and insulin-like growth factor binding protein-1 (IGFBP-1) production in the liver (Buyalos *et al.*, 1995; Morales *et al.*, 1996). As a result, higher amounts of free, non-bound forms of insulin and IGF-I might influence ovarian steroidogenesis in obese PCOS patients.

Other than the documented differences in endocrine and metabolic parameters in obese and lean patients, little is known

about the effect of the treatment of PCOS women with different body mass index (BMI). This is the first study evaluating the effect of COC treatment in obese versus non-obese women. The aim of this study was to compare the effect of COC, containing low-androgenic progestins on clinical androgenic symptoms, on androgen production, and on lipid profiles in two groups of PCOS patients selected on the basis of their BMI.

Materials and methods

Patients

Overall, 46 women with PCOS were enrolled in the study. PCOS was defined as follows: (i) oligomenorrhoea (menstrual cycle longer than 35 days) from menarche; (ii) increased concentrations of at least one androgen; and (iii) clinical manifestations of hyperandrogenism (acne, hirsutism, or both). Only lean patients with a BMI <25 kg/m^2 and obese patients with a BMI >30 kg/m^2 were selected. All women were aged over 18 years, had not used hormonal therapy or systemic treatment of acne for the preceding 6 months, and were not using any long-term medication. Women presenting with a secondary endocrine disorder, such as non-classic congenital adrenal hyperplasia, hyperprolactinaemia or thyroid dysfunction, those wishing to conceive within the next 6 months, those with contraindications to COC use, or with a blood pressure >140/90 mmHg were excluded from the study. Altogether, three women withdrew from the study (intolerance of COC treatment in two cases, personal reasons in one case). A total of 28 lean and 15 obese women completed the study protocol. All participants were studied before and after 6 months of therapy with monophasic low-dose oral contraceptives containing 30–35 μg ethinyl oestradiol in combination with a low-androgenic progestin (norgestimate, desogestrel or gestodene). All women participating in the study gave their informed written consent. The study was approved by the local Ethics Committee.

Clinical examination

The weight and height of all women were taken to calculate the BMI. Waist and hip circumference were measured in the standing position at the levels of the umbilicus and spina iliaca anterior superior to calculate the waist-to-hip ratio (WHR). Blood pressure readings were taken twice in the sitting position after a 10 min rest. Increased body hair was graded using a previously described method (Ferriman and Gallwey, 1961); hirsutism was defined as a Ferriman–Gallwey score >8. Acne severity was graded in three locations (face, back, and chest) using a modification of a published technique (Burke and Cunliffe, 1984). The value of total grade was analysed in this study. Blood was collected from all women during the early follicular phase, i.e. between days 3 and 6 of the menstrual cycle. Blood was centrifuged and frozen at –20°C. Provided that spontaneous menstrual bleeding failed to occur until day 45 of the cycle, bleeding was induced by progesterone administration. A shortened adrenocorticotrophic hormone (ACTH) test was performed to exclude non-classic congenital adrenal hyperplasia (NCCAH).

Laboratory investigations

Concentrations of LH, FSH, testosterone, androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), SHBG, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides were evaluated before and after the treatment. The concentrations of LH, FSH and testosterone were determined by chemiluminescent assay using an ACS:180 autoanalyser (Bayer Diagnostics GmbH, Munich, Germany). The concentrations of DHEA, DHEAS and androstenedione were established using radioimmunoassay methods (Immunotech, IOT, Marseille, France). SHBG was determined using immunoradiometric assay (IRMA) kits (Orion, Finland). The value of the free androgen index (FAI) was calculated according to the following formula: FAI = 100×testosterone (nmol/l)/SHBG (nmol/l). Serum cholesterol and triglycerides were analysed using CHOD-PAP and GPO-PAP-based kits respectively (Oxochorome, Lachema a.s., Brno, Czech Republic). HDL-cholesterol was determined using an immunoinhibition method (HDL-C Direct, Wako Chemicals GmbH, Neuss, Germany). LDL-cholesterol was calculated according to using the Friedewald formula (LDL- cholesterol = total cholesterol – HDL-cholesterol – triglycerides/2.19 mmol/l) (Friedewald *et al.*, 1972).

The reference values for normal concentrations were as follows: testosterone 0.5–2.63 nmol/l, androstenedione 1.57–5.4 nmol/l, DHEA 0.8–10.5 nmol/l, DHEAS 2.4–14.5 μmol/l and SHBG 43.2–96.0 nmol/l.

Statistical analysis

Due to skewed distribution of the data, the sign test and Wilcoxon's robust paired test were used for the evaluation of intra-individual differences. Student's *t*-test after transformation of original variables to minimum skewness and the Mann–Whitney robust test in the non-transformed variables were used for the evaluation of differences between subjects with BMI <25 kg/m^2 and those with BMI >30 kg/m^2. For the evaluation of the pre/post-treatment differences in the two groups with different BMI, the sign test and Wilcoxon's paired test were used.

Results

The two groups did not differ in mean age (23.5 ± 0.925 versus 25.4 ± 1.362 years). Mean BMI and WHR remained stable during the study in obese women (BMI 32.3 ± 0.262; WHR 0.82 ± 0.017) and lean women (BMI 20.3 ± 1.39; WHR 0.65 ± 0.013). Obese women exhibited significantly lower concentrations of SHBG ($P < 0.05$), and a higher FAI ($P < 0.05$) (Table I). However, there were no significant differences in lipid profile in the concentrations of androgens, or in the severity of clinical symptoms between lean and obese PCOS women before the treatment.

The significance of changes in monitored parameters during the study in both groups is demonstrated in Table II. In the group of lean women, highly significant changes in circulating concentrations of testosterone ($P < 0.001$), androstenedione ($P < 0.0001$), LH ($P < 0.001$) and SHBG ($P < 0.01$) were observed. Obese patients showed a less pronounced yet significant increase in SHBG concentrations ($P < 0.01$), suppression of testosterone production of borderline significance ($P < 0.05$), and non-significant changes in androstenedione and LH concentrations. The acne score ($P < 0.05$) and the severity of increased body hair ($P = 0.05$) were significantly improved in lean women only. The concentration of total cholesterol increased significantly ($P < 0.05$) during the treatment in both groups; an increase in LDL-cholesterol ($P < 0.05$) was demonstrated only in lean patients.

Discussion

The BMI exceeds the limit for obesity in about 50% of PCOS patients. An increased BMI impairs many endocrine

Table I. Summarized statistics of the two groups with different body mass index (BMI) before the treatment

	Acne score	F-G score	LH (IU/l)	FSH (IU/l)	Testosterone (nmol/l)	SHBG (nmol/l)	Log IFA	Androstene-dione (nmol/l)	DHEAS (μmol/l)	DHEA (nmol/l)	Total-C (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)
BMI <25 kg/m^2														
Number	13	14	28	28	27	27	27	28	27	27	27	28	28	28
Mean	2.38	9.1	7.79	5.29	2.72	73.0	4.47	13.1	7.72	9.51	4.35	2.34	1.62	0.97
SEM	0.213	1.193	1.0	0.444	0.24	7.37	0.56	0.79	0.67	0.79	0.16	0.13	0.09	0.12
Median	3.0	9.0	6.7	5.3	2.7	66.7	4.09	12.4	8.0	8.8	4.21	2.37	1.62	0.77
Skewness	−1.25	0.888	1.0	0.378	0.795	1.285	1.86	0.848	0.322	1.209	0.232	−0.314	0.830	2.276
BMI > 30 kg/m^2														
Number	7	14	15	15	15	15	15	15	15	15	15	15	15	15
Mean	1.67	9.8	5.23	5.83	2.91	51.0	10.32	11.4	9.36	10.75	4.69	2.68	1.57	1.15
SEM	0.333	1.017	0.73	0.485	0.31	10.51	2.42	1.10	1.23	1.37	0.18	0.11	0.11	0.17
Median	1.5	9.5	4.6	5.5	2.8	36.7	8.16	11.9	9.03	10.2	4.62	2.61	1.53	1.04
Skewness	0.857	0.92	1.03	1.249	0.326	1.053	1.57	0.462	0.552	1.492	0.558	1.181	0.380	0.621
Significance of the differences between subjects with BMI <25 kg/m^2 and those with BMI >30 kg/m^2														
Student's *t*-test	NS	NS	NS	NS	NS	$P < 0.01$	$P < 0.05$	NS	NS	NS	NS	NS	NS	NS
Mann–Whitney test	NS	NS	NS	NS	NS	$P < 0.05$	$P < 0.05$	NS	NS	NS	NS	NS	NS	NS

DHEA = dehydroepiandrosterone; DHEA-S = DHEA sulphate; FAI = free androgen index; F-G = Ferriman–Gallwey; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NS = not significant; SHBG = sex hormone binding globulin; TG = triglycerides.

Table II. Statistical significance of the pre- and post-treatment differences (change in values) in the two groups with different body mass index (BMI)

	Acne score	F-G score	LH (IU/l)	FSH (IU/l)	Testosterone (nmol/l)	SHBG (nmol/l)	Log IFA	Androstene-dione (nmol/l)	DHEAS (μmol/l)	DHEA (nmol/l)	Total-C (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)
BMI <25 kg/m^2														
Number	13	14	28	28	27	27	27	28	27	27	25	24	25	25
Mean	0.92	2.17	4.14	0.15	1.28	−77.3	3.34	4.0	1.45	0.38	−0.36	−0.47	−0.03	−0.06
SEM	0.358	0.60	1.18	0.655	0.25	13.0	0.51	0.78	0.66	1.04	0.31	0.13	0.13	0.09
Median	1.0	2.0	3.1	−0.6	1.10	−79.5	2.98	3.2	1.06	0.00	−0.54	−0.31	−0.11	−0.17
Skewness	−0.845	1.473	0.46	0.643	0.328	0.782	1.46	1.035	1.041	0.60	−2.51	0–1.334	−1.613	−1.592
Statistical significance of the pre-/post-treatment differences (paired tests, P <...)														
Sign-test	0.05	0.05	0.001	NS	0.001	0.0001	0.001	0.0001	NS	NS	NS	0.05	NS	NS
Wilcoxon's test	0.05	0.01	0.01	NS	0.001	0.001	0.01	0.0001	NS	NS	0.05	0.01	NS	NS
BMI >30 kg/m^2														
Number	8	14	15	15	15	15	15	15	15	15	11	11	11	11
Mean	0.88	2.58	1.62	1.12	1.26	−54.51	8.09	2.37	1.03	0.83	−0.55	−0.16	−0.15	−0.38
SEM	0.295	0.857	0.788	0.741	0.44	13.82	2.41	1.33	1.04	1.48	0.28	0.29	0.13	0.23
Median	1.0	1.5	1.8	0.9	1.80	−59.60	6.98	1.3	0.48	1.30	−0.37	−0.12	−0.16	−0.11
Skewness	0.277	1.080	0.326	0.005	0.064	0.891	1.280	0.182	−0.259	−0.610	−2.24	−0.484	−0.452	−0.991
Statistical significance of the pre/post treatment differences (paired tests, P <...)														
Sign-test	NS	0.05	NS	NS	NS	0.01	0.01	NS	NS	NS	0.05	NS	NS	NS
Wilcoxon's test	NS	NS	NS	NS	0.05	0.01	0.01	NS	NS	NS	0.05	NS	NS	NS

DHEA = dehydroepiandrosterone; DHEA-S = DHEA sulphate; FAI = free androgen index; F-G = Ferriman–Gallwey; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NS = not significant; SHBG = sex hormone binding globulin; TG = triglycerides.

and metabolic parameters. First, obesity significantly influences circulating concentrations of SHBG. BMI is inversely correlated with SHBG in healthy women (Glass *et al.*, 1981; Purifoy *et al.*, 1981; Pasquali *et al.*, 1987), as well as in women with PCOS (Dunaif *et al.*, 1987; Graf *et al.*, 1990).

Conflicting results have been reported in terms of a correlation between BMI and androgen production. Significantly increased production rates of androstenedione and DHEA were demonstrated in obese non-PCOS women versus normal-weight women (Kirschner *et al.*, 1983; Kurtz *et al.*, 1987). Higher concentrations of androgens in obese PCOS patients were documented by several authors (Dunaif *et al.*, 1988; Conway *et al.*, 1989), but not confirmed by others (Golland *et al.*, 1990; Graf *et al.*, 1990). Recently, a negative effect of obesity on plasma concentrations of testosterone and SHBG in PCOS patients was reported (Ciampelli *et al.*, 1999). These findings were supported by Acién *et al.* who found a correlation between BMI and testosterone concentrations (Acién *et al.*, 1999).

Moreover, the presence of obesity has a major impact on the lipid profile and insulin metabolism. PCOS and obesity exert a synergistic deleterious effect on glucose tolerance and insulin sensitivity (Ciampelli *et al.*, 1999). Many studies have demonstrated a more atherogenic lipid profile in obese

PCOS patients (Falsetti and Pasinetti, 1995). Furthermore, a positive relationship between BMI and plasminogen activator inhibitor-1 (PAI-1) activity and a negative relationship between BMI and IGFBP-1 were found in PCOS women (Morales *et al.*, 1996; Atiomo *et al.*, 2000).

As a result, obesity is one of the main factors determining the phenotype of the disease. There are many pathogenic mechanisms which could adversely influence the treatment of obese PCOS women. However, little is known about the influence of BMI on the effect of different treatment modalities. One exception documented a significantly worsened response by obese PCOS women undergoing ovarian electrocautery (Gjonnaess, 1994).

This study appears to be the first evaluating a different response by PCOS patients to COC on the basis of their obesity. Oral contraceptives are a therapeutic modality which addresses many of the endocrine disturbances associated with PCOS. The treatment corrects androgen overproduction by several mechanisms, including gonadotrophin suppression, stimulation of the androgen binding capacity, and suppression of ovarian and adrenal androgen synthesis. Reducing the concentrations of free and total androgens during COC treatment of PCOS patients has been demonstrated in many studies (Falsetti and Pasinetti, 1990; Dahlgren *et al.*, 1998; Gjonnaess, 1999).

Agents containing norgestimate, desogestrel, and gestodene were used in the current study. All of the above gestagens are classified into the same group of what is referred to as low-androgenic progestins or 'new progestins' (Speroff and DeCherney, 1993; Collins, 1994). The minimal androgenicity of these progestins is reflected in significant increases in SHBG concentrations in users of combined COC (Bergink *et al.*, 1981; Hammond *et al.*, 1984; Palatsi *et al.*, 1984; van der Vange, *et al.*, 1990). A consequence of decreased concentrations of free androgens and minimal affinity of new progestins alone to the androgenic receptor is a beneficial effect of COC on skin androgenic symptoms (Mango *et al.*, 1996; Lucky *et al.*, 1997; Redmond *et al.*, 1997). The low androgenic potency of progestins also influences the effect of COC on the lipid profile. A number of reports showed that COC containing new progestins elevated cholesterol slightly, exert a neutral or positive effect on LDL-cholesterol, and substantially increase concentrations of HDL-cholesterol (Petersen *et al.*, 1988; Gevers Leuven *et al.*, 1990; Kuhl *et al.*, 1990; Falsetti and Pasinetti, 1995). Besides reduction of the LDL-cholesterol/HDL-cholesterol ratio, a significant elevation of triglycerides has been reported.

The objective of the current study was to evaluate the effect of COC treatment on hyperandrogenism, on clinical androgenic symptoms, and on lipid profile. A limitation of the study was that it did not determine changes in insulin action, which might be an important factor in the pathogenesis of the disease, at least in part of PCOS subjects. In accordance with the literature, significantly lower concentrations of SHBG and lower FAI values were found in the group of obese women. On the other hand, it was not possible to confirm any differences in lipid parameters or in the concentrations of androgens between the two groups. Although no differences were found in androgen production between lean and obese women before treatment, highly significant changes in androgen concentrations were demonstrated in lean women, but only a minor positive effect of the treatment in obese patients. The most marked difference in treatment outcome between the two groups was found in androstenedione, with lean women showing a highly significant decline in serum concentrations whereas circulating concentrations were not significantly altered in obese women. SHBG production likewise was increased in a more pronounced fashion in lean women. The different effect of treatment on androgen production is consistent with a different clinical outcome. The acne score and the grade of increased body hair growth improved significantly only in non-obese subjects.

Surprisingly, no beneficial effect of COC treatment on lipid profile was observed. The slightly increased concentrations of total cholesterol in both groups, like the raised LDL-cholesterol concentrations in lean women, are consistent with published data (Petersen *et al.*, 1988; Kuhl *et al.*, 1990). However, an increase in HDL-cholesterol, reportedly associated with new progestin-containing agents by healthy users and PCOS women alike (Peterson *et al.*, 1988; Gevers Leuven *et al.*, 1990), was not demonstrated in either of the patient groups.

The finding in the current study of an adverse effect of obesity on treatment outcome underlines the need for an individualized approach to long-term treatment of PCOS women. The optimal treatment modality in obese women seems to be weight reduction, where the effect on androgen production and insulin sensitivity has been well demonstrated (Pasquali *et al.*, 1989; Andersen *et al.*, 1995; Holte *et al.*, 1995). However, other possibilities should be considered for the future, such as a combination of COC with insulin-receptor sensitizers. Future studies are warranted to evaluate the efficacy of these combinations in obese women.

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Prediction of Insulin Sensitivity in Nonobese Women with Polycystic Ovary Syndrome

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Insulin resistance is a frequent (although not constant) abnormality in both obese and nonobese women with polycystic ovary syndrome (PCOS). It plays a key role in the predisposition to type 2 diabetes, which is the most important health consequence of the syndrome. Identification of patients with insulin resistance is significant both for follow-up and for therapeutic reasons. The aim of the study was to evaluate the relationships between insulin sensitivity, measured by euglycemic clamp, and both endocrine and metabolic indices and to identify the best model for predicting insulin sensitivity. A total of 41 nonobese women fulfilling the diagnostic criteria for PCOS were enrolled in the study. None of the androgens correlated with the insulin sensitivity index. All clamp parameters correlated with SHBG, triglycerides, and body mass index, although no correlation was found with waist to hip ratio or waist circumference. The close relationship between insulin sensitivity and SHBG was documented by factor analysis and by its presence in all prediction models as the most significant (or even the single) predictor of the insulin sensitivity index. In conclusion: 1) a decreased level of SHBG can be used as a single reliable parameter in the prediction of insulin sensitivity in nonobese women with PCOS; and 2) waist to hip ratio, waist circumference, and androgen concentrations have no predictive value. (*J Clin Endocrinol Metab* 87: 5821–5825, 2002)

DECREASED INSULIN SENSITIVITY has been documented in both obese and nonobese women with polycystic ovary syndrome (PCOS), but the prevalence of insulin resistance is not known (1, 2). Although there is no prospective study, it is assumed that it is the subgroup of women with PCOS and concurrent insulin resistance that is at increased risk of diabetes and possibly cardiovascular disease (3, 4). Identification of patients with impaired insulin sensitivity is significant, both for follow-up and for long-term therapy.

In our study, we investigated a large group of nonobese women fulfilling the generally accepted diagnostic criteria of PCOS, using the euglycemic clamp. The aim of the study was to evaluate the relationships between insulin sensitivity and endocrine and metabolic indices, and possibly to identify a parameter or its combination, that would be applicable in clinical practice to predict insulin sensitivity.

Subjects and Methods

Subjects

A total of 41 patients were enrolled in the study, based on the following diagnostic criteria of PCOS: 1) oligomenorrhea from menarche; 2) an increased concentration of testosterone (0.5–2.63 nM/liter), androstenedione (1.57–5.4 nM/liter), or dehydroepiandrosterone (DHEA) (0.8–10.5 nM/liter); and 3) clinical manifestation of hyperandrogenism (acne, hirsutism, or both). Only nonobese women with a body mass index (BMI) under 30 kg/m^2, above 18 yr of age, who had not used hormonal therapy during the previous 6 months, were included. All patients were informed about the study protocol and signed an informed consent. The Local Ethics Committee approved the study protocol.

Euglycemic hyperinsulinemic clamp

The hyperinsulinemic euglycemic clamp was performed as described previously (5). Briefly, one cannule, to obtain blood tor biochemical analyses during the clamp, was inserted into the wrist vein. A double-lumen catheter for continuous blood glucose determination was inserted into the cubital vein of the ipsilateral arm. A third cannule for insulin and glucose administration by Biostator (GCIIS, Elkhart, IN) was inserted into the contralateral forearm vein. After a 30-min washout period, hyperinsulinemic euglycemic state was reached during the next 45 min, and the clamp was then performed using a constant insulin infusion rate (1 mU/kg·min) over 120 min. The glucose solution (40% wt/vol) was sampled by Biostator (mode 7:1) to maintain the blood glucose level at baseline value. During the clamp, blood glucose levels were repeatedly determined by glucose analyzer (ESAT 6660–2, PGV, Freital, Germany). Two blood samples for insulin determination were collected in the last 20 min of the clamp.

The following characteristics of insulin action were calculated: glucose disposal rate (M), defined as the amount of glucose supplied by the Biostator to maintain blood glucose levels during the last 20 min of the clamps (M, mM/kg·min); the insulin sensitivity index (ISI), defined as the ratio of M to insulin concentration at the end of the clamps (ISI, mM/kg·min per mU/liter·100); metabolic clearance rate of glucose (MCRg), expressed as the ratio of M to blood glucose concentration (MCRg, ml/kg·min).

Assays

All analytical determinations were performed at the National Reference Laboratory. Serum LH, FSH, and testosterone concentrations were measured by chemiluminescent assay using an ACS:180 autoanalyzer (Bayer Corp. Diagnostics GmbH, Leverkusen, Germany). The concentrations of DHEA, dehydroepiandrosterone-sulfate (DHEAS) and androstenedione were determined by RIA methods (Immunotech, Marseille, France). SHBG was measured using immunoradiometric

Abbreviations: BMI, Body mass index; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-sulfate; Go, fasting glucose; Go/Io, Go-to-Io ratio; HDL, high-density lipoprotein; HOMA, homeostasis model assessment index; ISI, insulin sensitivity index; Io, fasting insulin; LDL, low-density lipoprotein; M, glucose disposal rate; MCRg, metabolic clearance rate of glucose; PCOS, polycystic ovary syndrome; TGD, triglycerides; WHR, waist to hip ratio.

assay kits (Orion, Turku, Finland). The free androgen index (FAI) was calculated according to the following formula: FAI = 100 × testosterone (nM/liter) ÷ SHBG (nM/liter). Plasma glucose concentration was determined by a glucose oxidase method (Olympus Corp. Diagnostica GmbH, Hamburg, Germany). Plasma insulin concentrations were measured by RIA kits (CIS-Bio International, Gif-sur-Yvette, France); normal range, 4–20 mIU/ml; interassay CV < 5%; intraassay CV < 8.5%. The homeostasis model assessment index (HOMA) was calculated using the following formula: fasting serum insulin (mIU/ml) × fasting plasma glucose (mM/liter) − 22.5 (6). Serum cholesterol and triglycerides (TGD) were analyzed using CHOD-PAP and GPO-PAP-based kits, respectively (Oxochrome, Lachema a.s., Czech Republic); HDL-cholesterol was determined by an immunoinhibition method (HDL-C Direct, Wako Pure Chemical Industries Ltd. GmbH, Neuss, Germany). Low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald formula [LDL-cholesterol = (total cholesterol − HDL-cholesterol − TGD) ÷ 2.19 mM/liter].

Statistical analyses

For evaluation of the relationships between indices of insulin resistance, several methods were applied, such as correlation analysis followed by factor analysis and multiple regression analysis. To approximate the Gaussian data distribution and to straighten the relationships between variables, the data were transformed by power transformation to minimum skewness in individual dimensions. To avoid the influence of univariate outliers, all data with absolute studentized values greater than 2 were excluded. The multivariate outliers were searched using F-distributed Mahalanobis distance (statistical software NCSS 2000). The data treated as described above underwent correlation analysis followed by factor analysis (the principal factor method) followed by VARIMAX factor rotation (statistical software Stagraphics Plus 3.1). Besides factor analysis, multiple regression models were used to find the best predictors of insulin sensitivity represented by the indices of the euglycemic clamp. Respecting the limited number of experiments, the correlation matrix of transformed variables was used for choosing the preliminary set of predictors. These were further evaluated using backward stepwise multiple regression, with F-statistics less than 4 as the exclusion criterion.

Results

The characteristics of the entire group are demonstrated in Table 1. Table 2 summarizes the results of the correlation analysis. There was a strong correlation of statistical significance among ISI and fasting insulin (Io), the ratio of fasting glucose (Go) to Io (Go/Io), and HOMA. None of the androgens (testosterone, androstenedione, DHEA, or DHEAS) correlated with ISI; only a correlation of borderline significance was found among M, MCRg, and androstenedione. All pa-

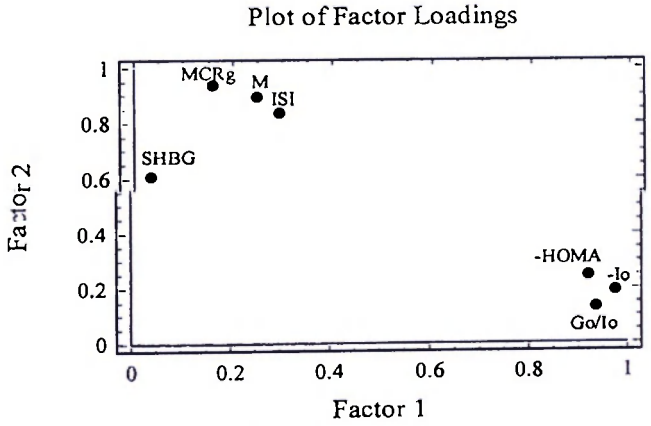

FIG. 1. Plot of factor loadings.

TABLE 1. Characteristics of investigated group

	Age	BMI	WHR	WC	Chol	HDL	LDL	TGD	LH	FSH	SHBG	DHEAS	DHEA	A	T	Go	Io	Go/Io	ISI	M	MCRg	HOMA
Count	41	41	41	41	39	40	40	39	41	41	41	41	40	41	40	41	41	41	41	41	41	41
Mean	24.2	24.1	0.737	74.4	4.57	1.59	2.54	0.98	7.01	5.04	45.1	9.62	17.3	12.6	3.49	4.49	16.3	0.341	47.1	39.1	9.18	3.32
SD	4.5	4.3	0.072	11.8	0.67	0.45	0.62	0.42	4.60	1.95	25.3	3.59	12.0	5.4	1.28	0.42	8.4	0.178	26.3	13.7	3.72	1.95
Median	23.0	22.9	0.739	74.0	4.59	1.46	2.57	0.92	5.95	4.60	37.0	9.43	15.7	11.9	3.11	4.50	15.9	0.294	45.0	36.4	8.90	3.00
Lower quartile	21.0	21.5	0.684	65.0	4.11	1.30	2.26	0.66	3.50	3.70	24.0	7.64	7.8	9.0	2.70	4.20	12.0	0.239	25.5	28.8	6.20	2.38
Upper quartile	27.0	25.9	0.800	80.0	5.00	1.73	2.89	1.23	8.40	6.10	67.0	12.00	22.7	15.6	4.33	4.70	17.0	0.383	55.5	48.4	11.50	3.46

WC, Waist circumference; Chol, total cholesterol.

TABLE 2. Pearson's correlations after power transformations

Go	0.227	−0.128	−0.211	−0.305	−0.336	0.426	0.170	0.158	0.218	−0.271	−0.055	0.037	−0.040	0.020
0.171	Io	−0.943	−0.542	−0.470	−0.447	0.972	0.230	0.107	0.175	−0.336	−0.259	0.221	0.058	0.168
0.438	0.000	Go/Io	0.538	0.541	0.401	−0.852	−0.272	−0.172	−0.192	0.267	0.135	−0.116	0.197	0.024
0.191	0.000	0.000	M	0.863	0.922	−0.547	−0.451	−0.158	−0.242	0.598	0.107	−0.334	−0.208	−0.216
0.062	0.003	0.000	0.000	ISI	0.852	−0.503	−0.388	−0.098	−0.197	0.550	0.156	−0.062	−0.010	0.013
0.036	0.005	0.011	0.000	0.000	MCRg	−0.488	−0.452	−0.120	−0.284	0.613	0.042	−0.332	−0.147	−0.243
0.008	0.000	0.000	0.000	0.002	0.002	HOMA	0.219	0.116	0.183	−0.372	−0.279	0.214	0.061	0.147
0.308	0.172	0.099	0.004	0.018	0.004	0.192	BMI	0.439	0.724	−0.168	0.029	0.193	0.211	0.269
0.331	0.515	0.290	0.323	0.552	0.460	0.481	0.005	WHR	0.814	−0.167	−0.047	0.082	0.275	0.181
0.177	0.286	0.247	0.127	0.230	0.076	0.264	0.000	0.000	WC	−0.250	−0.009	0.113	0.213	0.279
0.091	0.037	0.096	0.000	0.000	0.000	0.020	0.306	0.297	0.115	SHBG	0.072	0.147	−0.073	−0.194
0.742	0.121	0.418	0.519	0.356	0.802	0.094	0.866	0.778	0.957	0.662	T	−0.321	0.013	0.330
0.823	0.183	0.481	0.035	0.710	0.039	0.197	0.238	0.613	0.488	0.367	0.049	A	0.295	0.195
0.811	0.730	0.237	0.205	0.954	0.380	0.716	0.210	0.090	0.192	0.659	0.939	0.072	DHEA	0.518
0.907	0.312	0.886	0.187	0.938	0.141	0.378	0.107	0.270	0.085	0.236	0.043	0.241	0.001	DHEAS

The values *above* and *below the diagonal* represent the correlation coefficients and their statistical significance, respectively. *Shaded values* denote significant correlations. T, Testosterone; A, androstenedione.

TABLE 3. Factor analysis of 36 subjects and 7 variables (power transformation)

Factor number	Eigenvalue	Proportion of explained		Variable	Communality	Factor loadings	
		%	Cumulative %			Factor 1	Factor 2
1	4.04	68.0	68.0	−Io	0.992	0.977	0.191
2	1.69	28.4	96.4	Go/Io	0.898	0.938	0.131
3	0.19	3.2	99.6	−HOMA	0.910	0.922	0.243
4	0.02	0.4	100	M	0.864	0.250	0.895
5	0.00	0.0	100	ISI	0.785	0.295	0.836
6	0.00	0.0	100	MCRg	0.904	0.159	0.938
7	0.00	0.0	100	SHBG	0.371	0.035	0.608

rameters calculated on the basis of the euglycemic clamp correlated significantly with SHBG. No significant correlation of M or ISI with Go was found. From the anthropometric parameters, we found a correlation of clamp parameters with BMI, whereas the waist to hip ratio (WHR) and waist circumference did not correlate.

The mutual relationships between the parameters of insulin action (Io, Go/Io, HOMA, M, ISI, and MCRg) and SHBG were evaluated by factor analysis (Table 3 and Fig. 1). Factor loadings representing the correlation coefficients between the factor and the individual variables were used as a measure of the informative value in individual markers. Two factors fulfilled Kaiser's criterion (which is commonly used for determination of factor number, *i.e.* only the factors with eigenvalue more than 1 should be considered). The first factor demonstrates the similarity among Io, Go/Io, and HOMA; whereas the second expresses the close relationship between clamp parameters and SHBG. The close alliance between insulin resistance and SHBG is documented by its location in the cluster of clamp indices close to the 2nd factor axis.

Using stepwise regression analysis, prediction models for the individual clamp parameters were developed. The strong relationship between SHBG and clamp indices is documented by its presence in all of the prediction models, as the most significant or even the single predictor for the ISI as described by the expression $ISI^{0.7} = -2.73\ (3.39) + 5.61\ (1.16)\cdot SHBG^{0.49}$ ($R = 0.623$, $P < 0.0001$, $n = 37$), where the numbers *in parentheses* represent SDs of the parameters, R is a correlation coefficient of the regression, P is a statistical significance of the model, and n is the number of subjects investigated. The optimal prediction model for M comprises SHBG in combination with androstenedione (A), as demonstrated by the equation $M^{0.79} = 23.9\ (5.7) + 6.56\ (0.96)\cdot SHBG^{0.29} - 14.1\ (3.1)\cdot A^{0.24}$ ($R = 0.794$, $P < 0.0001$, $n = 37$); and the model for MCRg consists of SHBG in combination with androstenedione and TGD, as documented by the model $MCRg^{0.74} = 2.69\ (2.24) + 1.48\ (0.36)\cdot SHBG^{0.29} - 2.83\ (1.01)\cdot A^{0.24} - 2.89\ (1.21)\cdot TGD^{-0.37}$ ($R = 0.769$, $P < 0.0001$, $n = 37$).

Discussion

The present study demonstrates SHBG as a single reliable parameter in the prediction model of insulin sensitivity in nonobese women with PCOS.

Women with PCOS are at significantly increased risk for type 2 diabetes and impaired glucose tolerance (7, 8). Because of its high prevalence, which ranges between 4–7% in unselected population groups, PCOS is one of the greatest risk factors at a young age (9, 10). Insulin resistance plays a key role in the predisposition of women with PCOS to diabetes mellitus (4, 11). Identification of the subgroup of women with PCOS and concurrent insulin resistance is very significant, for their follow-up but also for possible therapeutic measures using insulin sensitizers.

The authors are aware that the study does not include a control group. However, it was not the aim of the study to determine the normal values for the ISI. The objective was to evaluate the relationships between the studied parameters and the insulin sensitivity measured by the euglycemic clamp. For this purpose, it is of no importance whether ISI

values lie within the normal range or not. Furthermore, we included only nonobese women with a BMI lower than 30. The obesity itself is a factor that influences the phenotype of the syndrome and worsens endocrine and metabolic parameters, including insulin action (12–14). It is apparent that in obese women, obesity is one of the most significant predictive parameters of insulin sensitivity (15). We assumed that the model of nonobese women would enable us to identify other relationships than those with anthropometric parameters.

In 2000, Gennarelli *et al.* (15) suggested three prediction models for insulin resistance in PCOS. The diagnosis of PCOS was made on the basis of ovarian morphology rather than endocrine criteria. The study included both nonobese and obese women. In all suggested models, waist circumference was represented as an independent predictor in combination with Io, serum TGD, or subscapularis skin fold. In nonobese women, we confirmed a close relationship between insulin sensitivity and BMI; on the other hand, we did not find any relationship between insulin sensitivity and visceral fat. Our results are in accord with other studies that did not find an association between WHR and insulin sensitivity in healthy nonobese women (16).

Although the euglycemic insulin clamp and a minimal model method applied to a frequently sampled iv glucose tolerance test are considered to be the gold standards in evaluating insulin sensitivity, several other indices based on oral glucose tolerance test values or on Go and insulin levels are discussed. In our study, besides the clamp parameters, we also evaluated Go, Io, Go/Io, and the homeostasis model assessment. Although a very strong correlation was found among all of the above-mentioned parameters with the clamp results, factor analysis demonstrated weaker relationships. This result is confirmed by the prediction models, in which none of the parameters calculated from the fasting values are present.

The most significant result of our study is the finding of a very close relationship between SHBG and insulin sensitivity. Insulin is an important regulator of hepatic SHBG production. In physiological concentrations, insulin decreases SHBG production by cultured hepatoma cells (17). OK as edited? In spite of stimulating hepatic production, the acute effect of insulin on SHBG is possibly attributable to changing of binding affinity (18). Low SHBG concentration is considered a risk factor for type 2 diabetes in women (19, 20). A direct correlation between insulin sensitivity and SHBG has been demonstrated in men with type 2 diabetes (21). In patients with PCOS, one of the characteristic features is low SHBG concentration (11). A direct relationship between insulin and SHBG in obese PCOS patients was documented by Nestler (22). Suppression of insulin by diazoxide in combination with a GnRH agonist was followed by an increase in SHBG, although no change was found after the administration of a GnRH agonist alone. In our study, SHBG significantly correlated with all clamp parameters. The results of the factor analysis show a clustering of clamp parameters with SHBG. It is apparent that SHBG is a much better marker of insulin sensitivity, in comparison with Go/Io or the HOMA index. SHBG is present in all prediction models for ISI, M, or MCRg as the most significant or even the single predictor for ISI. Based on our results, we may conclude that SHBG is a single reliable predictor of insulin sensitivity in lean PCOS women. Considering its simple evaluation, it could be used in clinical practice as an indicator for further tests of insulin sensitivity (preferably the euglycemic clamp or iv glucose tolerance test) and potentially for treatment with insulin sensitizers.

In summary, in nonobese women with PCOS, we did not find a correlation between insulin sensitivity and WHR, waist circumference, or androgen concentrations. Parameters of insulin action that were calculated based on fasting values correlated poorly with insulin sensitivity measured by the euglycemic clamp. On the other hand, a very strong relationship was found between insulin sensitivity and SHBG. Although our study unables us to indicate the cutoff value for SHBG, we may conclude that a decreased level of SHBG can be used as a single predictor to define the subgroup of patients with an increased risk of insulin resistance, among nonobese women with PCOS.

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Insulin Sensitivity in Women with Polycystic Ovary Syndrome

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The aim of our study was to compare insulin sensitivity in lean and obese European polycystic ovary syndrome (PCOS) women with lean healthy women. We performed the euglycemic hyperinsulinemic clamp in 83 women with PCOS [53 lean with body mass index (BMI) of 21.5 ± 1.8 kg/m^2 and 30 obese with BMI of 29.6 ± 3.7 kg/m^2] and in 15 healthy women with BMI of 21.6 ± 1.8 kg/m^2 to determine glucose disposal (M) and the insulin sensitivity index (ISI). Statistical evaluation was done using Kruskal-Wallis ANOVA followed by Kruskal-Wallis multiple-comparison z-value test. The basal blood glucose was significantly higher in lean and obese PCOS women than in controls ($P < 0.02$). Fasting insulin was significantly higher in both lean and obese PCOS women than in controls ($P < 0.000001$). Obese PCOS women were more insulin resistant than controls ($P < 0.02$ for M and $P < 0.0008$ for ISI); lean PCOS women did not differ from controls in M or ISI. Posthepatic insulin delivery was significantly higher in both lean and obese PCOS women compared with controls ($P < 0.000008$). We conclude that lean PCOS women are not more insulin resistant than healthy controls. Insulin hypersecretion, on the other hand, is present even in lean PCOS women. (*J Clin Endocrinol Metab* 89: 2942–2945, 2004)

IT IS GENERALLY accepted that women with polycystic ovary syndrome (PCOS) have a high prevalence of insulin resistance, with a consequent increased risk of metabolic diseases later in life. It is well established that obese PCOS women are insulin-resistant in excess of their adiposity (1, 2). In lean PCOS women, however, the situation is not so clear. To date, there have been two studies using the euglycemic hyperinsulinemic clamp that were not able to demonstrate a difference in insulin sensitivity between lean PCOS women and healthy controls in Europe (3, 4), whereas a study in the United States described significantly impaired insulin action in lean PCOS in comparison with healthy women matched for body fat and fat-free mass (2). Similar results were obtained by another group in Turkey (5). All these studies were conducted on small groups of lean women with PCOS (from 7–11 patients). These discrepancies are not easy to explain; ethnic origin of the patients could probably account for part of the differences.

In the present study, we thus examined large groups of both lean and obese women fulfilling the generally accepted diagnostic criteria of PCOS, using an euglycemic clamp, which is considered the gold standard in evaluating insulin sensitivity. The aim of our study was to compare insulin sensitivity in lean and obese European PCOS women with lean controls.

Abbreviations: A, Androstenedione; BMI, body mass index; CV, coefficient of variance; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; ISI, insulin sensitivity index; M, glucose disposal rate; MCRI, posthepatic clearance rate of plasma insulin; PCOS, polycystic ovary syndrome; PHD, posthepatic insulin delivery; T, testosterone.

* G.Š. died tragically in 2001.

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Subjects and Methods

The study group consisted of 83 oligo/amenorrheic women with PCOS matching the National Institutes of Health criteria (6). All had clinical manifestation of hyperandrogenemia presenting as hirsutism and/or acne and with the elevation of the free testosterone (T) index more than six and/or androstenedione (A) above the upper limit of the normal range. Women were in good health condition, without any other serious disorder. Women with epilepsy or migraine were excluded. In all patients, 17-OH-progesterone was determined in the early follicular phase of their cycle, and if levels were between 5 and 10 nmol/liter, an ACTH test was undergone to exclude late-onset congenital adrenal hyperplasia. Hyperprolactinemia (prolactin levels), hypercortisolism (plasma cortisol, and, if necessary, urinary cortisol excretion per 24 h or short dexamethasone suppression test with 1 mg of dexamethasone at 2200–2300 h), and thyroid dysfunction (TSH, free T_4, antithyroglobulin, and anti-thyroid-peroxidase antibodies) were excluded. In one patient, cytologically benign nodular goiter was present; she was on thyroid-suppressive medication with TSH, 0.04 mIU/liter; free T_4, 19 pmol/liter (normal range, 12–22 pmol/liter), and T_3, 1.83 nmol/liter (normal range, 1.30–3.10 nmol/liter). None of the patients had taken oral contraceptives or any other steroid or glucose-metabolism-affecting medication during the preceding 3 months.

The control group consisted of 15 healthy women with regular menstrual cycles (28–33 d) who also had not used oral contraceptives for at least the preceding 3 months and had no clinical signs of hyperandrogenemia. Their age and BMI were 27.6 ± 6.4 yr and 22.0 ± 2.7 kg/m^2, respectively. The controls were recruited from the healthcare personnel of the hospital and their acquaintances.

The local ethical committees of the Institute of Endocrinology and the Faculty Hospital of Charles University in Prague (Prague, Czech Republic) approved the protocol of the study.

The patients and controls were evaluated at the clinical departments of both institutions as outpatients. After signing a written informed consent, they underwent blood sampling for hormonal and biochemical examinations between d 3 and 6 of the menstrual cycle or, in the case of secondary amenorrhea, at any time. After basal blood samples were taken, a 2-h euglycemic hyperinsulinemic (1 mIU/kg·min) clamp was performed as described previously (7). Insulin sensitivity was determined from the values obtained during the steady-state period, between the 100th and 120th minute. Target blood glucose level was 5.0 mmol/liter, with the coefficient of variance (CV) less than 5%.

The following parameters were calculated based on clamp results: glucose disposal rate (M) was defined as the amount of glucose supplied by the infusion to maintain the desired blood glucose level (M, μmol/kg·min), and the insulin sensitivity index (ISI) was defined as the ratio of M and the average insulin concentration during the observed period (ISI, μmol/kg·mm per mIU/liter × 100). The posthepatic clearance rate of plasma insulin (MCRI, liter/kg·min) was calculated as the ratio of the insulin infusion rate by the steady-state plasma insulin. Fasting posthepatic insulin delivery (PHD, mIU/kg·min) was obtained as the product of MCRI and fasting plasma insulin (8).

Blood glucose was determined in whole blood by electrochemical method (Super GL; Dr. Muller Gerate Bau, Freital, Germany). Insulin was estimated by immunoradiometric assay kit (Immunotech, Marseille, France) or by RIA kits (CIS Bio International, Marseille, France) (interassay CV < 5%; intraassay CV < 8.5%; correlation coefficient between RIA and immunoradiometric assay was 0.92). T, A, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S), LH, and SHBG were determined as stated previously (9).

Statistical analysis

Kruskal-Wallis robust ANOVA was used for evaluation of the differences between controls, lean PCOS patients, and obese PCOS patients. The individual differences between the subgroups were evaluated by Kruskal-Wallis robust multiple-comparison z-value test. NCSS 2001 (Number Cruncher Statistical Systems, Kaysville, UT) was used for the calculations.

Results

The basal demographical, biochemical, and hormonal parameters in controls and lean and obese PCOS women are given in Table 1. As expected, significantly higher levels of T, A, and DHEA-S were found in both lean and obese PCOS compared with controls. SHBG levels were significantly lower in both lean and obese PCOS than in controls.

Basal blood glucose was significantly higher in lean and obese PCOS than in controls ($P < 0.02$). Basal insulin was significantly higher in obese and lean PCOS women than in controls ($P < 0.000001$). M was significantly lower in obese than in lean PCOS and controls ($P < 0.02$), and the same was observed for ISI ($P < 0.0008$). On the other hand, lean PCOS did not differ in M or ISI from controls. MCRI was significantly lower in obese than in lean PCOS ($P < 0.03$) and showed no difference between lean PCOS and controls. PHD was higher in both lean and obese PCOS compared with controls ($P < 0.000008$; Fig. 1).

Discussion

PCOS is a heterogeneous syndrome. In the phenotypic spectrum, we can find obese, invariably insulin-resistant women, and at the other extreme, lean PCOS patients with marked hyperandrogenemia but without insulin resistance. Nevertheless, until now, there have been few studies (3, 10) conducted on larger groups of PCOS women to evaluate whether insulin resistance is invariably connected with PCOS and, consequently, whether the majority of lean PCOS women is thus insulin resistant or whether insulin resistance is only an epiphenomenon related to body weight. Few studies found decreased insulin sensitivity only in association with abdominal obesity (3) or in lean PCOS women if they were also hyperinsulinemic (10).

Insulin resistance is a well-known risk factor for diabetes mellitus type 2. It is closely associated with syndrome X and is probably one of its central features (11). PCOS women show a pattern of cardiovascular risk factors (12) putting them at a greater risk of cardiovascular events. Thus, the early identification of insulin-resistant PCOS women could be advantageous from the perspective of possible early preventive measures.

In the present study, we documented that lean PCOS women are not insulin resistant in comparison with healthy controls. Lower insulin sensitivity was found only in obese PCOS women when compared with both controls and lean PCOS. This result is in accordance with others (3, 4) examining European PCOS women, who found no difference in insulin sensitivity between the PCOS women and healthy women with BMI of 21 kg/m^2 but a significant reduction at BMI of about 28 and 35 kg/m^2, respectively. After adjustment for truncoabdominal fat, both groups have similar insulin sensitivities over the whole range of BMI. On the contrary, there is good evidence in American or Asian PCOS women that they are insulin resistant independent of BMI (5) and body composition (2). These discrepancies cannot be easily explained and deserve future investigations. Probably, ethnic background, dietary composition, and a more sedentary lifestyle in the United States could play a role.

In addition to normal insulin sensitivity in lean PCOS, we found insulin hypersecretion in both lean and obese PCOS women compared with healthy women. Although basal insulin secretion was not measured directly in our study, our data accord well with the values obtained by others in general population using PHD as an estimate of insulin secretion (8) or with the results of C-peptide deconvolution analysis (13). The data related to insulin secretion in PCOS published

TABLE 1. Differences between group of controls (C) and lean (L) and obese (O) PCOS women in anthropometric characteristics and biochemical markers

	Controls			Lean			Obese			Kruskal-Wallis test	Significant differences between groups ($P < 0.05$)[a]
	Count	Mean	SD	Count	Mean	SD	Count	Mean	SD		
Age (yr)	15	28.1	6.6	53	24.2	4.6	30	26.2	4.8	NS	
BMI (kg/m^2)	15	21.5	2.0	53	21.5	1.8	30	29.6	3.7	$P < 0.000001$	C-O, L-O
LH (IU/liter)	10	5.39	2.12	53	7.55	5.22	29	5.85	2.89	NS	
SHBG (nmol/liter)	15	68.5	21.3	53	48.6	24.3	30	36.0	22.7	$P < 0.0002$	C-L, C-O, L-O
T (nmol/liter)	15	1.78	0.64	53	3.44	1.27	29	3.31	1.51	$P < 0.00003$	C-L, C-O
A (nmol/liter)	15	6.1	4.1	52	10.7	5.0	30	9.9	5.7	$P < 0.002$	C-L, C-O
DHEA (nmol/liter)	15	23.7	14.3	52	21.4	12.6	27	26.5	14.6	NS	
DHEA-S (μmol/liter)	15	4.68	2.20	52	8.72	3.58	30	10.09	4.18	$P < 0.00004$	C-L, C-O
17-OH-Progesterone (nmol/liter)	15	2.57	1.71	53	2.52	1.05	30	2.62	1.31	NS	

[a] Kruskal-Wallis multiple comparisons were used for evaluation of individual between-group differences.

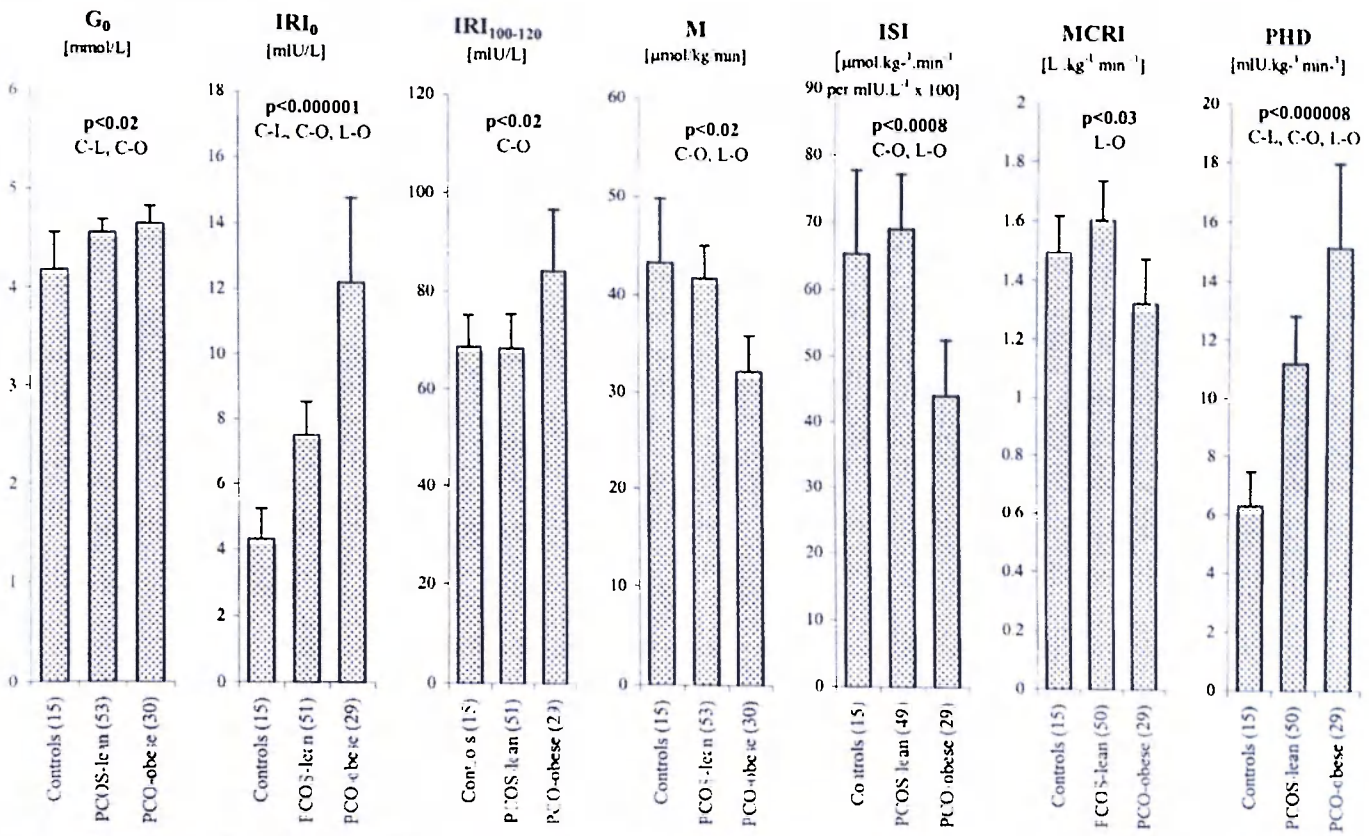

FIG. 1. Relationships between clamp indices and patient groups (C, controls; L, lean PCOS patients; O, obese PCOS patients). *Dotted bars with error bars* represent means with their 95% confidence intervals, whereas $P<\ldots$ denotes statistical significance of the overall trend as found by Kruskal-Wallis z-value test, multiple comparisons on the level of statistical significance of $P < 0.05$, were symbolized as follows: C-L, difference between controls and lean PCOS patients; C-O, difference between controls and obese PCOS patients; L-O, difference between lean and obese PCOS patients. The *numbers in parentheses* at the end of the labels in the x-axis represent the numbers of subjects in the groups. G_0, Basal blood glucose; IRI_0, basal insulinemia.

so far are inconsistent. There is evidence supporting defective glucose-stimulated insulin secretion in PCOS using a minimal model and disposition index (1, 14). On the other hand, an increase in basal insulin secretion rate and a decrease in meal-stimulated pulses was found (15), as well as insulin hypersecretion independent of obesity (3). We have recently described increased glucose-stimulated β-cell function even in lean individuals with PCOS (16). Using the oral glucose tolerance test and euglycemic clamp, insulin resistance and hypersecretion were found to be two distinct features of PCOS (10). Higher basal insulin levels described in lean PCOS women could thus be explained by basal insulin hypersecretion rather than by insulin resistance. The exact cause of basal insulin hypersecretion is not known. It could be speculated that hypersecretion could be determined genetically because heritability of fasting plasma insulin was found even after adjustment for BMI and insulin resistance (17). On the other hand, early phase of insulin secretion during iv glucose tolerance test was shown to be correlated with androgenicity (3) in PCOS, and thus, environmental factors also could play a role.

Decreased hepatic clearance of insulin may also influence basal insulinemia. We found lower hepatic clearance of insulin only in obese PCOS patients. This finding is in accordance with others (10), who found impaired insulin clearance defined by the ratio of basal C-peptide to insulin in obese but not in lean PCOS women. We are aware of the fact that the MCRI could be underestimated, especially in obese individuals when not corrected for the endogenous insulin secretion. Thus, to what extent basal insulin levels in obese PCOS patients are influenced by insulin resistance, defective hepatic insulin clearance, and increased insulin secretion remains to be established by other studies using direct measurement.

In conclusion, we were not able to confirm insulin resistance in lean PCOS women in comparison with lean healthy controls. On the other hand, lean PCOS women had higher basal insulin levels, probably caused by the increased basal insulin secretion. Obese PCOS women were more insulin resistant than both lean PCOS and lean controls.

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