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Vlastnosti velkých tepen ve vztahu ke krevnímu tlaku a ke genetickému pozadí hypertenze

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Doktorandská práce
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Poděkování

Ráda bych poděkovala všem, bez kterých by tato dizertační práce buď nevznikla, nebo alespoň ne v takové podobě, v jaké ji můžete číst.

Zvláště bych chtěla poděkovat za odborné vedení a cenné rady k tématu práce svému školiteli **doc. MUDr. Janu Filipovskému, CSc.**

Dík patří také kolegyním, kolegům a zdravotním sestrám za jejich spolupráci na výzkumných projektech.

V neposlední řadě chci poděkovat celé své rodině, zejména rodičům a manželovi, za jejich trpělivost, porozumění a podporu v mé práci i v životě.

Milena Dolejšová

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OBSAH

Seznam použitých zkratek

ABPM	Ambulatory Blood Pressure Monitoring, 24-h monitorace TK
ACE	angiotensin-konvertující enzym
ADD	adducin
AGT	angiotensinogen
AI	Augmentation Index, index navýšení krevního tlaku
AS	aldosteron syntáza
AT1R	receptor angiotensinu II
BMI	Body Mass Index
C	compliance, poddajnost
CAI	Central Augmentation Index, centrální augmentační index
CNS	centrální nervový systém
D	průměr
Di	distenzibilita, roztažnost
DTK	diastolický krevní tlak
EHT	esenciální arteriální hypertenze
eNOS	endoteliální syntáza oxidu dusnatého
EPOGH	**European Project on Genes in Hypertension**
fT4	volný thyroxin
Hcy	homocystein
HDL	High Density Lipoproteid (HDL cholesterol)
ICHS	ischemická choroba srdeční
I/D	inzerčně deleční polymorfismus
IMT	Intima-Media Thickness, tloušťka komplexu intima-media na krkavici
LDF	laser-dopplerovská flowmetrie
LKS	levá komora srdeční
MONICA	**Monitoring of Trends and Determinants in Cardiovascular Diseases**
mV	milivolt
NO	oxid dusnatý
P	tlak
PCR	Polymerase Chain Reaction, polymerázová řetězová reakce
PAI	Peripheral Augmentation Index, periferní augmentační index
PWA	Pulse Wave Analysis, analýza pulzní vlny
PWV	Pulse Wave Velocity, rychlost šíření pulzní vlny
RAS	renin-angiotensinový systém
STK	systolický krevní tlak
TK	krevní tlak
V	objem daného cévního oddílu

Úvod

Předmětem mé postgraduální práce byla problematika studia a klinického využití vyšetřování vlastností cév neinvazivními metodami. Tyto metody byly zavedeny na naší klinice v roce 1999 mým školitelem doc. MUDr. Janem Filipovským, CSc. jako na prvním pracovišti v České republice. Pracuji především na dvou velkých výzkumných úkolech:

EPOGH: Evropský projekt o dědičnosti hypertenze (European Project on Genes in Hypertension) – projekt zahájený v roce 1998, koordinovaný dr. Janem A. Staessenem z Katolické univerzity v Leuvenu, Belgie. Tento projekt stále pokračuje a má za cíl vytvořit databázi genetického materiálu rodin hypertoniků a normotoniků. Naše pracoviště vytvořilo podprojekt zaměřený na tepenné vlastnosti (další podrobnosti k tomuto projektu jsou rozvedeny v oddílu Vlastní výsledky).

MONICA: Monitoring Trends and Determinants of Cardiovascular Diseases. Naše studie navázala na projekt Světové zdravotnické organizace prováděný v mnoha zemích světa; ten však byl v 90. letech zastaven a v České republice pokračuje dále podle stejného protokolu na základě národních grantů. Koordinátorem této studie, která má za cíl monitorovat kardiovaskulární nemoci a jejich rizikové faktory v náhodně vybraných vzorcích obecné populace, je doc. MUDr. Renata Cífková z Institutu klinické a experimentální medicíny v Praze. V našem centru jsme opět uskutečnili podprojekt zaměřený na tepenné vlastnosti.

Na základě našich výsledků jsme se zabývali především následujícími tématy:

- zavedení studia vlastností tepen ve výzkumných programech pomocí neinvazivních a poměrně jednoduchých vyšetřovacích metod, jmenovitě studia rigidity tepenné stěny a studia odrazu tlakové vlny
- studium tepenných vlastností v náhodně vybraném vzorku obecné populace a zvláště určení jejich vztahu k běžným rizikovým faktorům kardiovaskulárních nemocí

- vyšetření a analýza vlastností tepen v rodinách hypertoniků a normotoniků s otázkou, nakolik se dědičnost obecně podílí na těchto vlastnostech a na krevním tlaku (TK)
- zkoumání vztahu kandidátních genů hypertenze a jejich polymorfismů k tepenným vlastnostem a ke krevnímu tlaku
- studium dalších speciálních otázek v některých menších souborech, např. srovnání tepenných vlastností u zdravých a nemocných seniorů nebo závislost tepenných vlastností na hladinách homocysteinu.

Všechny tyto práce se vztahují k problematice arteriální hypertenze, která je základním klinickým i výzkumným zaměřením naší skupiny.

OBECNÁ ČÁST

I. Obecná patogeneze arteriální hypertenze

Arteriální hypertenze je multifaktoriální onemocnění, které patří mezi nejčastější kardiovaskulární choroby; její prevalence se v průmyslově vyspělých zemích odhaduje na 20 – 50 % dospělé populace se zřetelným nárůstem ve vyšších věkových skupinách a představuje závažný zdravotní problém - je rizikovým faktorem pro kardiovaskulární onemocnění, cévní mozkové příhody a renální selhání.

Podle nejnovějších doporučení Světové zdravotnické organizace se za hypertenzi v dospělosti (bez ohledu na věk) považuje trvalé zvýšení krevního tlaku nad hodnoty 140/90 mmHg včetně. Diagnózu lze stanovit teprve při opakovaném naměření TK 140/90 mmHg - alespoň dvakrát ze tří měření při minimálně dvou návštěvách u lékaře. Při hypertenzi obvykle dochází k současnému zvýšení jak systolického (STK), tak diastolického TK (DTK) – systolicko-diastolická hypertenze. Ve stáří se však často setkáváme se zvýšením pouze systolického TK a hovoříme pak o izolované systolické hypertenzi (STK nad 140 mmHg, DTK do 90 mmHg), která však rovněž zhoršuje životní prognózu. Současná klasifikace do jednotlivých kategorií TK a další podrobnosti jsou publikovány v Doporučeních diagnostických a léčebných postupů u arteriální hypertenze - verze 2004 *(Cífková et al., 2004)*.

Podle etiologie a patogeneze se arteriální hypertenze rozděluje na:

- Esenciální (primární) hypertenzi
- Sekundární (symptomatickou) hypertenzi, kdy je zvýšený TK pouze symptomem jiného primárního onemocnění s identifikovatelnou příčinou; vyskytuje se asi ve 2 – 5 % všech případů arteriální hypertenze.

U esenciální arteriální hypertenze (EHT) neznáme vlastní vyvolávající příčinu, ale známe řadu patogenetických mechanismů. Jedná se o multifaktoriální

onemocnění, kde se kombinují genetické faktory, vlivy zevního prostředí a poruchy vnitřních regulačních mechanismů.

V současné době se výzkum stále více zaměřuje na studium dědičnosti arteriální hypertenze a tím na zkoumání jednotlivých kandidátních genů (viz oddíl V. Genetické aspekty arteriální hypertenze).

Z faktorů zevního prostředí se na patogenezi primární hypertenze uplatňují především nadměrný přívod kuchyňské soli, nedostatečný přívod draslíku, vápníku, nadměrný přísun potravy s nedostatečnou fyzickou aktivitou a následným vývojem obezity, nadměrná konzumace alkoholu, kouření a opakující se stresové situace.

Z endogenních vlivů se vedle centrálního sympatoadrenálního nervového systému podílí na patogenezi EHT řada humorálních působků, a to s účinkem vazokontrikčním a zároveň natrium-retenčním a růst stimulujícím: katecholaminy, renin-angiotenzinový systém, vazopresin, endotelin, tromboxan A2, prostaglandin H2, či vazodilatačním, natriuretickým a růst inhibičním účinkem: dopamin, kalikrein-kininový systém, atriální natriuretický peptid, oxid dusnatý (EDRF/NO), prostaglandin E2 a I2. Hypertenze může vzniknout v důsledku absolutního nebo relativního nadbytku vazopresorických nebo nedostatku vazorelaxačních působků *(Klener et al., 1998)*.

Systémové a humorální působky ovlivňují hemodynamiku, průtok krve tkáněmi a periferní cévní rezistenci. V oblasti cévního endotelu ovlivňují agregabilitu a adhezivitu trombocytů, migraci monocytů subendotelově a abluminálně pak ovlivňují kontraktibilitu hladkého svalstva cév, jeho případnou hypertrofii, hyperplazii a remodelaci. Jejich působení, mající za důsledek dysfunkci endotelu, může být spojovacím článkem mezi hypertenzí, urychleným rozvojem ateroskleróza a ischemickou chorobou srdeční (ICHS) u hypertoniků.

Z dalších endogenních změn se na patogenezi hypertenze mohou podílet odchylky v elektrolytových mechanismech přes buněčnou membránu, jako jsou sodíková pumpa (Na-K-ATPáza), Na-K-kotransport, Na-Li-protitransport a další.

Důsledkem vrozené nebo získané odchylky některého z těchto transportních mechanismů je intraceleulární zvýšení Na a druhotně i Ca. To má za následek zvýšenou citlivost hladkého svalstva cév na presorické podněty a tím i větší pohotovost k vazokonstrikci a zvýšení TK.

Z hemodynamických faktorů se na vzniku a dalším rozvoji hypertenze mohou podílet změny srdečních funkcí a změny funkce tepenného systému. Srdeční funkce jsou již dlouho studovány, a to jak invazivními metodami, tak neinvazivně především pomocí echokardiografie. Prioritní práce prof. *Widimského et al. (1957)* ukázala, že u počínající hypertenze je přítomna hyperdynamická cirkulace v důsledku zvýšeného minutového srdečního objemu. Tato koncepce je dodnes platná.

Problematika struktury a funkce cév je složitá. Rozdílné oddíly cév se díky svému specifickému složení cévní stěny a specifické funkci chovají odlišně za fyziologických i patologických okolností. Prozatím neexistuje žádné univerzální vyšetření, které by podalo informaci o funkci tepenného systému jako celku. Teprve rozvoj některých neinvazivních metodik s dostatečnou reprodukovatelností otevřel v posledních zhruba 15 letech nové možnosti studia v této oblasti.

Hemodynamicky nacházíme na začátku hypertenze zvýšený minutový výdej při normální periferní cévní rezistenci. Ta je však vzhledem ke zvýšení minutového výdeje nepřiměřeně vysoká. Později dochází k normalizaci minutového srdečního výdeje a vysoký TK je udržován vysokou periferní rezistencí. Tento vzestup periferní cévní rezistence je dán zpočátku především vazokostrikcí arteriol. Později se však mění obsah sodíku a vody ve stěně arteriol a artérií, a tím se mění poměr lumina k cévní stěně. Další změny jsou již strukturální - dochází k hypertrofii a remodelaci cévní stěny. K trvalému zvýšení cévní rezistence dochází v důsledku těchto změn na úrovni rezistenčního řečiště. V našich pracech jsme se zaměřili především na studium vlastností velkých tepen.

II. Funkce tepenného systému

a) Funkce tepenného systému za fyziologických podmínek

Z funkčního hlediska můžeme cévy rozdělit do 6 kategorií:

- Pružník - velké tepny elastického typu. Jejich výrazné elastické vlastnosti mají význam v přeměně nárazového přítoku krve v systole na její kontinuální proudění - to zajišťuje hlavně aorta.
- Velké tepny muskulárního typu (např. a. femoralis, poplitea, brachialis, radialis) – zajišťují přívod krve do jednotlivých oblastí organismu. To je označováno jako rozvodná funkce („conduit function").
- Rezistenční cévy - regulují přítok krve k orgánům a tkáním. Patří k nim:
  - malé tepny a tepénky (arterioly), tzv. prekapilární rezistenční cévy, které mají malý průsvit a silnou stěnu s vysokým podílem hladkého svalstva. Díky této svalovině mohou značně měnit svůj průsvit a tím prakticky rozhodovat o distribuci minutového srdečního výdeje mezi různé orgány. Na jejich konci se nacházejí prekapilární sfinktery. Jejich konstrikce nebo dilatace rozhoduje o počtu otevřených kapilár a tím o velikosti kapilární plochy, na níž dochází k výměně tekutiny mezi kapilárami a intersticiem
  - venuly, tzv. postkapilární rezistenční cévy, které však vytvářejí jen velmi malou část rezistence. Jejich význam je především v tom, že poměrem mezi tonem prekapilárních a postkapilárních rezistenčních cév je určován kapilární hydrostatický tlak, který umožňuje výměnu tekutiny na úrovni kapilár, tedy filtraci a resorpci.
- Kapiláry - představují styčnou plochu mezi krví a tkání. Na jejich úrovni probíhá přesun látek z krve do intersticiální tekutiny a naopak. Nemají schopnost kontrakce a jejich průsvit se mění pasivně jako výsledek pre- a postkapilárních rezistenčních cév a prekapilárních sfinkterů.
- Arteriovenózní zkraty - jsou jen v některých tkáních, kde zabezpečují rychlý převod krve z tepenného řečiště do žilního s obejitím kapilár. Jsou-li tedy tyto

cévy otevřeny, průtok kapilárami se v dané tkáni snižuje nebo úplně zastavuje, průtok tkání se současně zrychluje.

- Kapacitní cévy - především žíly, které díky své výrazné roztažnosti mohou pojmout značný objem krve. Slouží proto jako rezervoár, který je v uzavřeném systému cév nutný pro stále se měnící distribuci krve v jednotlivých orgánech, zabezpečují žilní návrat a ovlivňují tak funkci srdce.

Jednotlivé oddíly tepenného řečiště se liší morfologicky především ve složení cévní médie. V centrálním oběhu převažuje vazivová složka nad hladkou svalovinou. Stěny těchto tepen elastického typu (aorta, a. carotis) obsahují především velké množství elastických vláken, která jsou pružná a reagují roztažením již na nižší tlak, a menší množství pevnějších kolagenních vláken. Směrem do periferie, např. v a. brachialis, a. femoralis a jejich větvích, přibývá v cévní stěně hladkých svalových buněk a také kolagenních vláken. Nejčetnější zastoupení současně s největší celkovou plochou představují arterioly a kapiláry. V tomto rezistenčním řečišti převažuje hladká svalovina, takže již při malé změně tonu svaloviny dochází při malém průměru arteriol k velkému vzestupu periferní cévní rezistence.

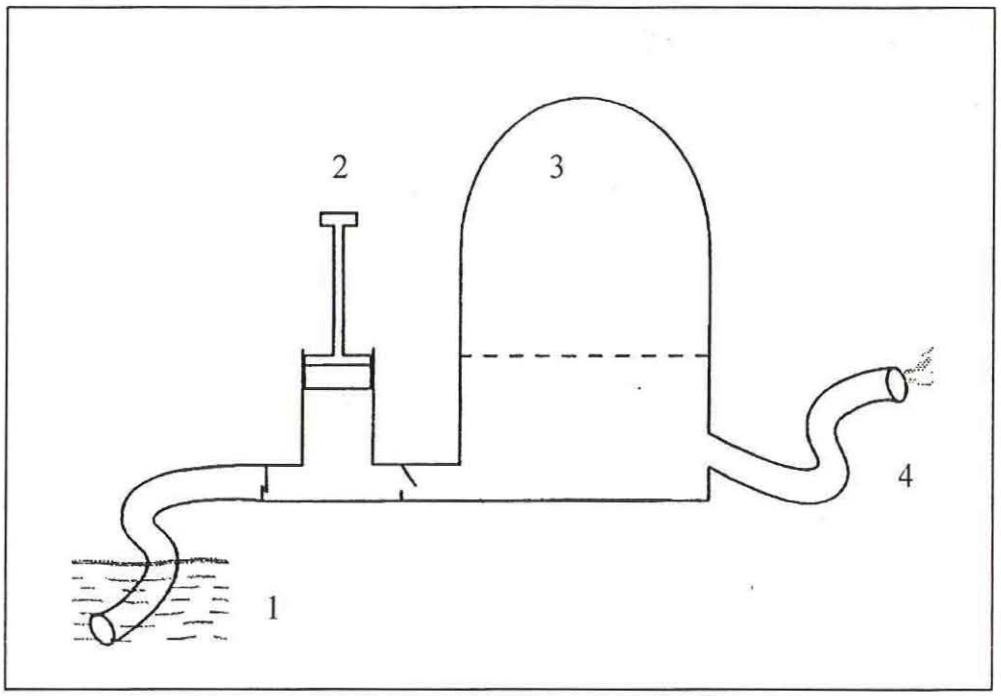
Za normálních okolností se střední arteriální tlak udržuje na stejné úrovni ve velkých tepnách a prudce klesá na úrovni rezistenčních cév, kde narůstá cévní odpor.

Funkcí arteriální části systémového oběhu je dopravit krev pod tlakem do tkání a přeměnit nárazový tok krve z levé komory na kontinuální. Krev proudí tepnami značně rychle: v klidu se krev od okamžiku svého okysličení dostane do tkání asi za 10 s, při maximální fyzické zátěži za 2 - 3 s, tato hodnota vyjadřuje tzv. oběhovou rychlost.

Do aorty je krev z levé komory vypuzována během ejekční fáze systoly. Lineární rychlost krevního proudu v aortě po otevření semilunárních chlopní prudce vzrůstá a dosahuje hodnoty až 100 cm/s. V důsledku této vysoké rychlosti

má proudění turbulentní charakter. Průměrná lineární rychlost krevního proudu v aortě je oproti maximálním hodnotám výrazně nižší (zejména proto, že krev v úseku bezprostředně za semilunárními chlopněmi proudí jen po dobu ejekční fáze) a pohybuje se v klidu okolo 20 cm/s. Při fyzické zátěži pak roste přímo úměrně minutovému výdeji. S rostoucí vzdáleností od levé komory se maximální dosažená rychlost krevního proudu zmenšuje (jinými slovy se snižuje amplituda proudového pulzu) a současně se prodlužuje doba, po kterou krev cévami proudí. Ve vzestupné aortě proudí krev pouze v období ejekční fáze a na začátku diastoly se dokonce na chvíli směr jejího proudu obrací a uzavírá semilunární chlopně. V tepnách vzdálenějších od srdce krev proudí po celou dobu srdeční akce, nedosahuje však takové rychlosti jako ve vzestupné aortě. Směrem ke kapilárám se přeměňuje charakter krevního proudu z nárazového na kontinuální. Je třeba odlišit proudovou vlnu, jejíž rychlost je zde popsána, od tlakové vlny, která vyvolává vydouvání cévní stěny a může být snímána jako pulzní vlna. Rychlost této pulzní vlny je daleko vyšší a dosahuje zhruba od 5 do 20 m/s v závislosti na tepenném úseku, kde provádíme měření, a v závislosti na kvalitě cévní stěny (podrobnosti viz dále v oddílu III. Přehled metod vyšetření tepenného systému).

Přeměna nárazového proudu v kontinuální je umožněna funkcí pružníku velkých tepen a je způsobena elasticitou jejich stěny: krev vypuzená z levé komory roztáhne stěnu aorty a tak se část kinetické energie krve přemění na potenciální energii stěny aorty. Poté, co odteče hlavní proud krve, elastické síly roztažené stěny aorty způsobí návrat stěny do původních rozměrů a krev obsažená v rozšíření je přitom vypuzována od srdce - směrem nejmenšího odporu. Elastická energie se přeměňuje zpět na kinetickou energii krve. Činnost srdce a nárazníková funkce velkých tepen je v podstatě analogií principu středověké hasičské stříkačky *(obr. 1)*. Tekutina je nasávána z rezervoáru {1} pumpou {2} za použití ventilů a odtud se dostává do nádrže {3}. Tekutina proudí dále hadicí {4} víceméně kontinuálně, velikost proudu závisí na hydrostatickém tlaku v nádržce. Tepny tedy plní svou nárazníkovou funkci obdobně jako nádrž.



Obr. 1
Princip hasičské stříkačky – analogie fungování srdce a nárazníkové funkce tepen. Bližší specifikace viz text.

Za normálních okolností se během systoly dostává do periferních tkání asi 40 % tepového objemu, zbylá krev zůstává ve velkých tepnách a je vypuzována během diastoly. Zatímco při selhání funkce vedení krve trpí především tkáně distálně od postižené cévy, při selhání nárazníkové funkce centrálních tepen dochází k nadměrnému zvýšení systolického TK v centrálním řečišti a tím ke zvýšení dotížení (afterloadu) levé komory srdeční. Důsledky se pak projeví proximálně od postiženého cévního řečiště.

Cévní stěna je komplexní tkáň, která je schopna měnit se na základě působení mechanických nebo jiných stimulů; trvalou přestavbu cévní stěny nazýváme remodelací. Mechanické podněty pro remodelaci tepen jsou dvojího druhu: tenzní stres a střižní stres (tensile stress, shear stress).

Tenzní stres je síla způsobená tlakovou vlnou a působí kolmo na cévní stěnu. Je přímo úměrná krevnímu tlaku a poloměru cévy, nepřímo úměrná tloušťce cévní stěny, jak lze odvodit z Laplaceova zákona. Vysoký tenzní stres je příčinou hypertrofie cévní stěny.

Střižní stres je dán frikční silou způsobenou proudovou vlnou a působí podél cévní stěny. Je přímo úměrný rychlosti proudící krve a její viskozitě, nepřímo úměrný poloměru cévy.

Na tenzní stres reaguje především cévní médie, naproti tomu střižní stres je vnímán především endotelem. Střižní stres určuje stav cév v mnoha ohledech: ovlivňuje metabolismus endoteliálních buněk, morfologickou stavbu stěny a rozměry cévy. Je stimulem pro produkci vazodilatačních substancí cévní stěnou, tj. především oxidu dusnatého (NO). K patologickým změnám dochází především při abnormálně nízkém střižním stresu, jak lze demonstrovat při změně tvaru proudové vlny při větvení cév: čelo proudové vlny naléhá na vnitřní stěnu cév, a proto je vnější stěna vystavena nízkému střižnímu stresu. Důsledkem je nedostatečná produkce vazodilatačních substancí a vyšší tonus hladké svaloviny médie. Zpomalený proud může mít také za následek snazší usazování

aterogenních substancí do cévní stěny. Proto se tato místa stávají predilekcí ateroskleróz y.

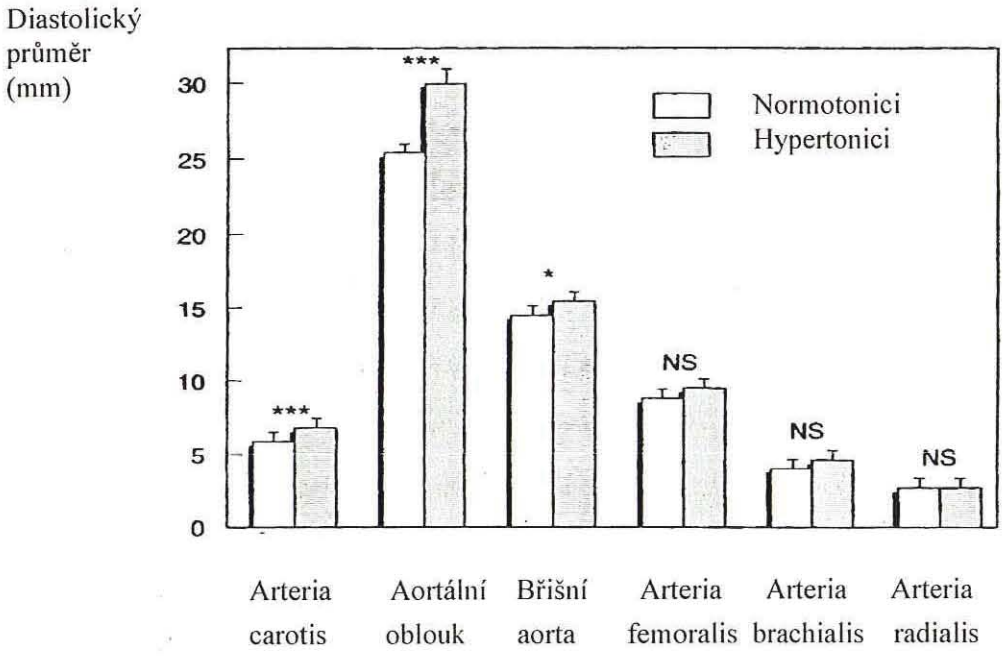
Zatímco vlastnosti velkých tepen souvisí především s pulzním tlakem, stav rezistenčního řečiště je určující pro střední krevní tlak.

b) Změny tepenného systému u hypertenze

Změny velkých tepen typické pro hypertenzi jsou dány především opotřebením při výrazněji působících mechanických stresech. Jedná se tedy o urychlené stárnutí tepen a jsou obdobou změn, k nimž dochází s věkem *(Filipovský, 2004)*. Věkové změny byly dříve nazývány arterioskleróza na rozdíl od aterosklerózy, jejíž příčiny jsou komplexní a kde hypertenze je pouze jedním z patogenetických faktorů. V současné době nejsou tyto pojmy přesně odlišovány. **Ateroskleróza** je proces lokalizovaný na určitý úsek cévy, zatímco **arterioskleróza** je difúzní změnou, k níž dochází především v centrálních tepnách elastického typu. Ateroskleróza vzniká a rozvíjí se hlavně v intimě, změny při stárnutí a hypertenzi jsou primárně procesem cévní médie. Ateroskleróza vede ve většině případů ke zúžení cévy (výjimku tvoří např. břišní aorta, kde sklerotický proces může mít za následek její aneurysmatické rozšíření). Důsledky aterosklerózy se projeví především distálně ischémií, neboť jde především o poruchu vedení cévou. Naproti tomu změny podmíněné stárnutím a hypertenzí vedou spíše k dilataci cév a snížení jejich poddajnosti – je porušena hlavně nárazníková funkce. To má důsledky především proximálně, kdy dochází ke zvýšení zátěže levého srdce. Zesílení médie při arterioskleróze je spojeno nejen s rozšířením cévy, ale také s jejím prodloužením (proto se aorta stává vinutou). Tyto změny jsou kontinuální v průběhu života a začínají již kolem 20. roku věku. Ve věku nad 80 let je plocha aorty 3 – 4 krát větší než ve 20 letech. Ateroskleróza je v některých zemích velmi častá a její následky představují nejčastější příčinu smrti, jinde je zcela vzácná. Naproti tomu věkové změny tepen

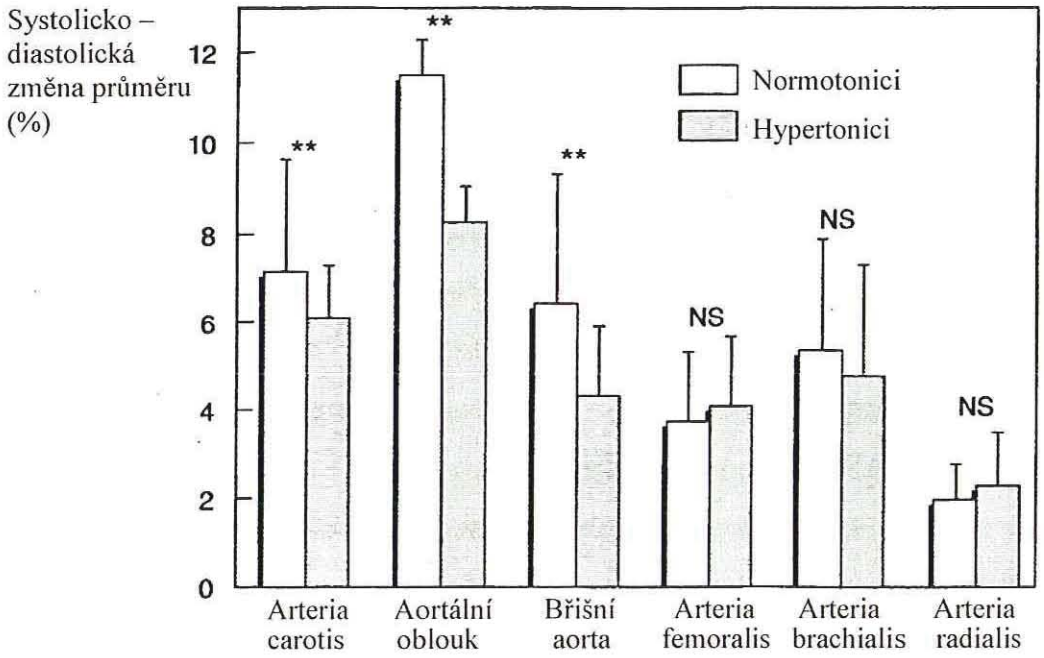
jsou přítomné ve všech populacích, i když jejich stupeň je závislý na výskytu hypertenze.

Morfologické změny cévní stěny při dlouhotrvající hypertenzi jsou tedy lokalizovány především v médii. Zatímco u mladých normotenzních jedinců jsou elastinová vlákna uspořádána rovnoběžně, dochází u hypertoniků k jejich dezorganizaci, ztenčování a fragmentaci. Stoupá obsah kolagenního materiálu a často dochází k depozici vápníku, a to jak do kolagenu, tak do elastinových vláken. Funkční důsledky těchto změn ukazuje studie *Boutouyrie et al. (1992)*, v níž autoři studovali 50 neléčených esenciálních hypertoniků a 32 kontrolních osob vyšetřených ultrazvukovým přístrojem s vysokou rozlišovací schopností. Zjistili, že aorta (zvláště na úrovni oblouku) a karotida měly významně větší vnitřní průměr měřený v diastole u hypertoniků ve srovnání s kontrolními jedinci, naproti tomu průměr distálních velkých tepen (femorální, brachiální a radiální) byl stejný. U distálních tepen byla míra pulzace stejná jako u kontrol, ale u centrálních tepen docházelo k významnému snížení pulzace. Z těchto výsledků vyplývá, že účinek hypertenze na proximální a distální velké tepny je zcela odlišný. V centrálních arteriích jsou alterovány geometrické vlastnosti (zvětšený průměr) i mechanické vlastnosti (změna průměru během srdečního cyklu - pulzatilita). U distálních tepen není prokazatelná změna těchto vlastností při hypertenzi (alespoň pokud jsou soubory hypertoniků a normotenzních kontrol ponechány se svým přirozeným TK jako v případě citované práce), ale jejich funkci nelze rovněž pokládat za normální: protože jsou u hypertoniků vystaveny vyššímu TK (tenznímu stresu), který se sem přenáší z centrálních tepen, bylo by možno očekávat, že jejich diastolický průměr bude zvýšen. Jelikož tomu tak není *(obr. 2, 3)*, znamená to, že tyto tepny reagují aktivně na hypertenzi vazokonstrikcí, která může být důsledkem dysfunkce endotelu tohoto cévního úseku.



Obr. 2
Diastolický průměr jednotlivých tepen u hypertoniků a normotoniků.

Skupiny mají stejné složení podle věku a pohlaví. *p<0,05, ***p<0,001.
Podle Boutouyrieho et al., 1992.



Obr. 3
Relativní změny průměru tepen v systole oproti diastole u hypertoniků a normotenzních kontrol.

Skupiny mají stejné složení podle věku a pohlaví. **p<0,01.
Podle Boutouyrieho et al., 1992.

III. Přehled metod vyšetření tepenného systému

V současné době existuje velké množství neinvazivních metod používaných k vyšetření tepen. Protože tepenný systém je velmi komplexní a jeho jednotlivé části mají rozdílnou strukutru i funkci, neexistuje žádná univerzální metoda, která by postihovala vlastnosti tepenného systému jako celku. Mezi nejčastěji používané parametry vyšetření tepen patří:

1. Pulzní tlak (rozdíl systolického a diastolického TK - oscilační tlaková komponenta), je nejjednodušší parametr, který nás informuje především o pružníkové funkci centrálních tepen. Je určen na jedné straně srdeční kontraktilitou a tepovým objemem, tj. schopností srdce generovat primární tlakovou vlnu, a na druhé straně vlastnostmi tepen. Je-li rigidita centrálních tepen zvýšena, selhává nárazníková funkce tohoto řečiště a krevní proud vycházející z levé komory srdeční vyvolá vyšší vzestup TK během systoly a větší pokles TK během diastoly.

Protože se zvláště ve vyšším věku uplatňuje fenomén odrazu tlakových vln, který je částečně závislý na stavu rezistenčního řečiště, je výsledný pulzní tlak závislý také na jeho funkci a struktuře. Pulzní tlak je významným prediktorem kardiovaskulárního rizika, nezávislým na ostatních tlakových hodnotách, jak ukázala řada studií *(např. Darné et al., 1989, Benetos et al., 2000)*. Jeho vliv je nejsilnější u jedinců po 55. roce života, kdy v obecné populaci dochází k poklesu průměrného diastolického TK a ten se stává špatným prediktorem rizika. Existují práce ukazující na prognostický význam pulzního tlaku i v mladším věku (*Benetos et al., 2000*). Výše pulzního tlaku má vztah k riziku srdečních i mozkových příhod, přičemž predikce koronárních příhod se zdá být silnější. Význam pulzního tlaku dokazují také intervenční studie u nemocných s izolovanou systolickou hypertenzí, kde je systolickodiastolické rozpětí vždy zvýšené *(SHEP Cooperative Research Group, 1991, Staessen et al., 1997)*. Za hranici normálu pulzního tlaku je většinou považována hodnota 50 mmHg odpovídající hraniční hodnotě hypertenze 140/90 mmHg.

2. Rychlost šíření pulzní vlny - PWV (Pulse Wave Velocity) odráží rigiditu určitého úseku tepenného řečiště. Stanovujeme ji podle časového posunu pulzní vlny mezi dvěma místy při známé vzdálenosti těchto míst (např. aortální PWV, tzn. karotido-femorální, nebo PWV na dolní končetině mezi a. femoralis a a. tibialis posterior/a. dorsalis pedis). Čím rychleji se tlaková vlna šíří, tím rigidnější je cévní stěna. Rychlost je však také závislá na aktuálním středním TK. Její výhodou je, že lze studovat větší arteriální segment jako celek (např. aortu, řečiště dolní nebo horní končetiny). Tato metoda je poměrně jednoduchá a má dobrou reprodukovatelnost (rozdíly mezi dvěma vyšetřujícími jsou kolem 5 %). Díky tomu jsou k dispozici velmi cenná data z populačních studií. Jiný způsob, který se využívá zvláště pro měření PWV na aortě, je měření ultrazvukem. PWV měřená na periferních velkých tepnách je vyšší než PWV na aortě. Aortální PWV však stoupá s věkem, zatímco periferní PWV se mění jen málo, proto se ve vyšším věku rychlosti prakticky vyrovnávají. *Avolio (1995)* provedl měření PWV v Austrálii a v Číně (zde u městské a venkovské populace). PWV rostla s věkem obdobně v australské a čínské městské populaci, a to přesto, že v Číně je daleko menší výskyt ateroskleróza a nižší průměrná hladina cholesterolu. Naproti tomu byla PWV výrazně nižší u venkovské čínské populace. Hlavním rozdílem, jemuž je připisován tento nález, je rozdílná strava s nižším obsahem soli ve venkovských oblastech. Tyto výsledky jsou kompatibilní s jinými studiemi ukazujícími, že sůl je důležitým rizikovým faktorem hypertrofie cévní médie, obdobně jako je nezávislým faktorem hypertrofie levé srdeční komory. Navíc z této studie vyplývá, že stupeň manifestních aterosklerotických změn již příliš neovlivňuje rychlost pulzní vlny, která je dána především změnami cévní médie, a proto se tato metoda hodí k detekci tepenných změn způsobených hypertenzí a stárnutím.

PWV byla rovněž použita ve studiích u hypertenzních pacientů a je dnes dobře zdokumentováno, že se u nich vyskytují abnormality, a to jak u stabilní hypertenze, tak v jejích raných studiích. *Girerd et al. (1989)* studovali PWV

u mladých hypertoniků s hraniční hypertenzí a u stejně starých kontrolních normotenzních jedinců, a to aortální PWV (karotido-femorální) a PWV na horní končetině (vycházející z časového posuvu mezi brachiální a radiální pulzací). Zjistili, že hypertonici měli oproti kontrolám významně vyšší obě PWV: 7,6 vs. 6,8 m/s pro aortální PWV a 12,0 vs. 10,7 m/s pro PWV horní končetiny, $p<0,001$ v obou případech. Regresní přímky PWV v závislosti na aktuálním TK se v obou skupinách významně lišily, což nasvědčuje tomu, že zvýšení PWV při hraniční hypertenzi není dáno pouze zvýšeným krevním tlakem, ale odráží strukturální nebo funkční změny cévního řečiště. *Asmar et al. (1995)* analyzovali aortální PWV v rozsáhlé populaci nemocných s trvalou hypertenzí a normotoniků. Ukázali, že PWV byla vyšší u hypertoniků: 11,8 vs. 8,5 m/s. Regresní přímky PWV podle věku byly rovněž významně odlišné, přímka pro hypertoniky byla strmější. Z těchto výsledků vyplývá, že rigidita cévní stěny je zvýšená již u počínající hypertenze. I když k těmto změnám může docházet druhotně při dlouhodobém zvýšení TK, tento nález zvyšuje pravděpodobnost toho, že se může jednat o primární poruchu cévní stěny. Jak již bylo uvedeno, PWV je závislá na aktuálním TK – jeho zvýšení vede k roztažení cévní stěny a tím zvýšení její tuhosti. Ve výše uvedených studiích je PWV vyšší u hypertoniků i při stejném TK jako u kontrol. Z toho vyplývá, že hypertenze je sama o sobě spojena se zvýšením arteriální rigidity.

3. Analýza pulzní vlny - PWA (Pulse Wave Analysis) nám umožňuje určit přesný tvar pulzní vlny. Definitivní tvar je výsledkem komplexních změn vlastností velkých a drobných tepen i srdečních funkcí. Výsledný tvar pulzní vlny je dán sumací primární tlakové vlny s vlnami vyššího řádu. Primární tlaková vlna vzniká při kontrakci levé komory, její velikost a tvar závisí především na funkční zdatnosti levé komory a na vlastnostech stěn centrálních tepen. Na periferii dochází k odrazu tlakové vlny, která pak interferuje s vlnou primární. V centrálním oběhu poté dochází opět k odrazu, vzniká terciární vlna atd. Protože

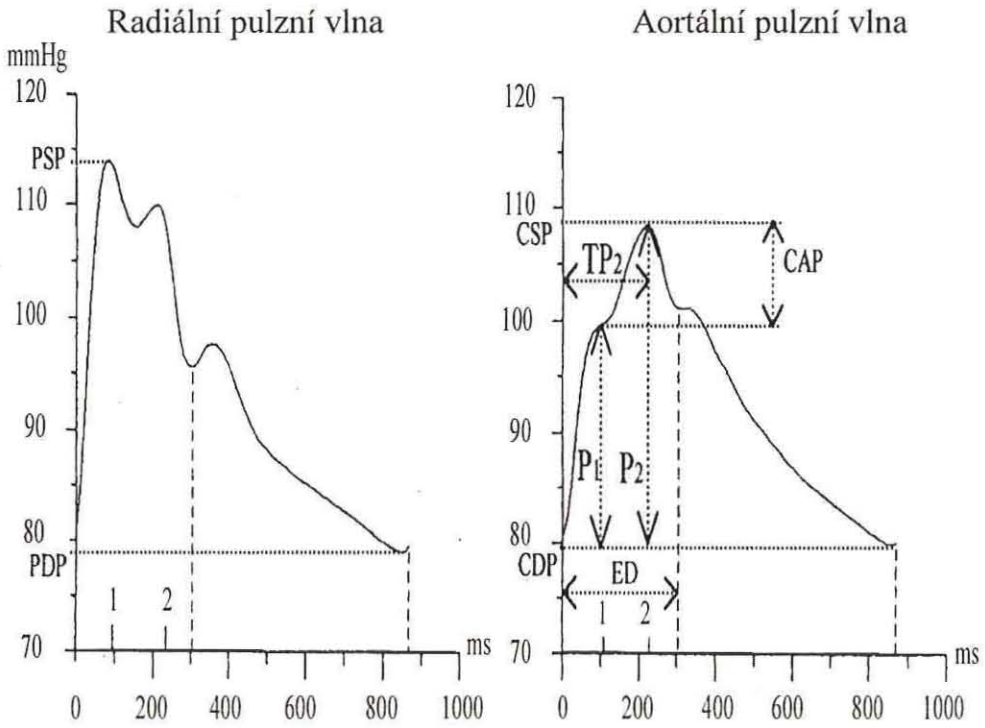
tlaková vlna se šíří velmi rychle, podílí se na tvaru definitivní tlakové vlny až zhruba vlna 20. řádu. Vlastnosti odražených vln, tj. velikost a načasování, závisí především na rychlosti šíření tlakové vlny, tedy na tepenné rigiditě, a na místě odrazu. Soudí se, že místem odrazu je hlavně začátek rezistenčních cév, kde dochází k největšímu větvení řečiště. Poměr odražené a primární tlakové vlny se nazývá v anglické literatuře Augmentation Index (AI, index navýšení TK). Tvar tlakové vlny se zcela liší u mladých a starých jedinců: zatímco u mladých je odražená vlna spíše nízká a objevuje se v pozdní systole, takže nasedá až na sestupnou část tlakové křivky, u starých jedinců se vrací dříve a zvyšuje tlakovou amplitudu. Vezmeme-li výšku primární vlny jako 100 %, pak odražená vlna může zvyšovat tlakovou amplitudu až o dalších 80 % *(obr. 4, 5)*.

Důvody, proč je ve stáří odražená vlna vyšší, jsou komplexní a existuje několik mechanismů, které se patrně na tomto jevu podílejí:

- rychlost šíření tlakové vlny zvláště po aortě je větší, a proto je návrat časnější,
- protože rychlost šíření po aortě se s věkem přibližuje rychlosti tlakové vlny na velkých tepnách horních i dolních končetin, je odraz vln v celém těle synchronní,
- v důsledku morfologických změn malých cév se hlavní místa odrazu patrně přesouvají více proximálně.

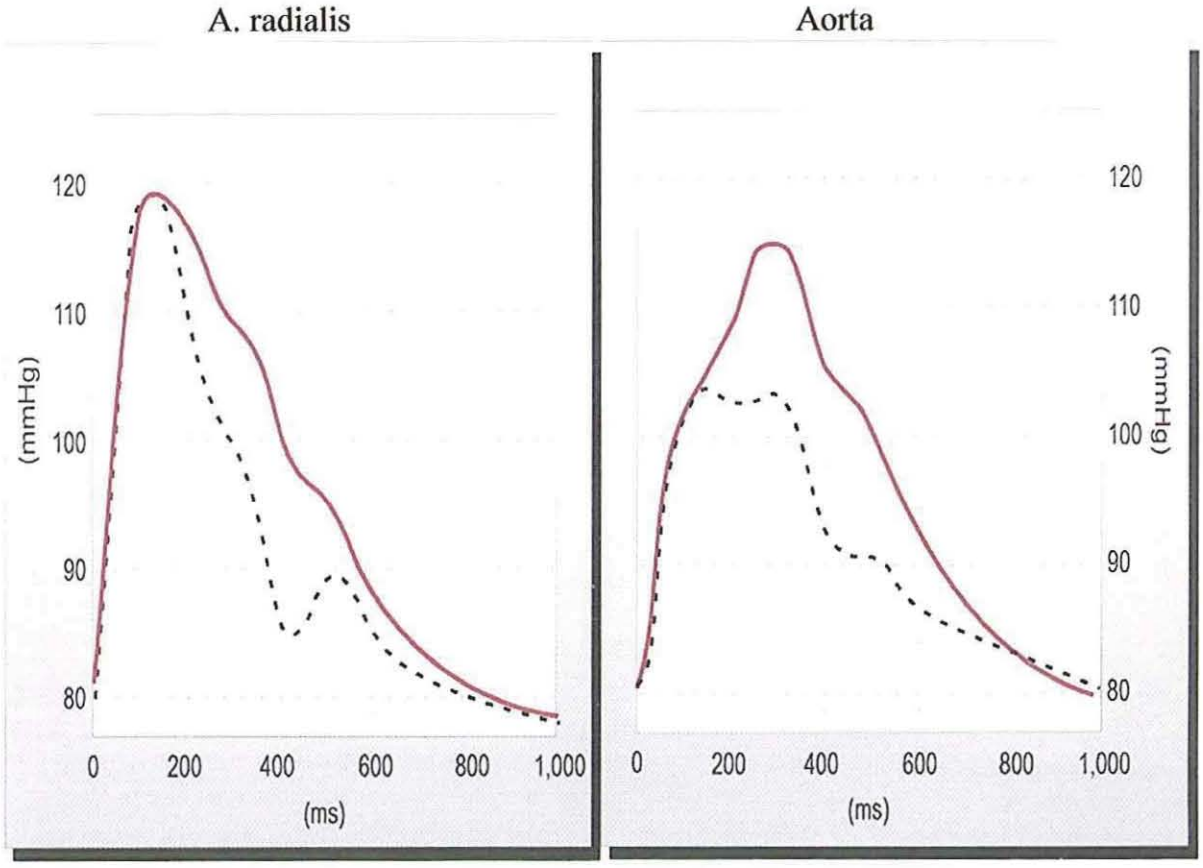
Z hlediska hypertenze má odraz tlakových vln několik významů:

- Vysoký stupeň odrazu zvyšuje dotížení levé srdeční komory – svědčí pro to data *Marchaise et al. (1993)* prokazující silnou korelaci indexu navýšení TK s masou levé komory. Velká odražená vlna tedy zvyšuje pravděpodobnost hypertrofie levé srdeční komory.



Obr. 4
Záznam pulzní vlny na a. radialis (vlevo), tvar pulzní vlny v aortě získaný matematickou transformací (vpravo).

Vysvětlivky: PSP – periferní systolický tlak (mmHg), PDP – periferní diastolický tlak (mmHg), 1 – vrchol primární vlny, 2 – vrchol sekundární vlny. CSP – centrální systolický tlak (mmHg), CDP – centrální diastolický tlak (mmHg), P1 – amplituda primární vlny (mmHg), P2 – amplituda sekundární vlny (mmHg), CAP – centrální augmentační tlak (central augmentation pressure, CAP=P2-P1, mmHg), ED – trvání ejekční fáze (ejection duration, ms), TP2 – čas do vrcholu sekundární vlny (time to peak, ms)



Obr. 5
Rozdíl v registrované pulzní vlně u mladého (tečkovaně) a staršího člověka (plná linka).

V levé části záznam pulzní vlny na a. radialis, vpravo získaná pulzní vlna na aortě. Přesto, že periferní TK měřený na a. brachialis je u obou jedinců shodný, je patrný rozdíl ve tvaru pulzní vlny na a. radialis a zejména v aortě, kde je vrchol pulzní vlny dán sekundární vlnou. Blíže viz text.

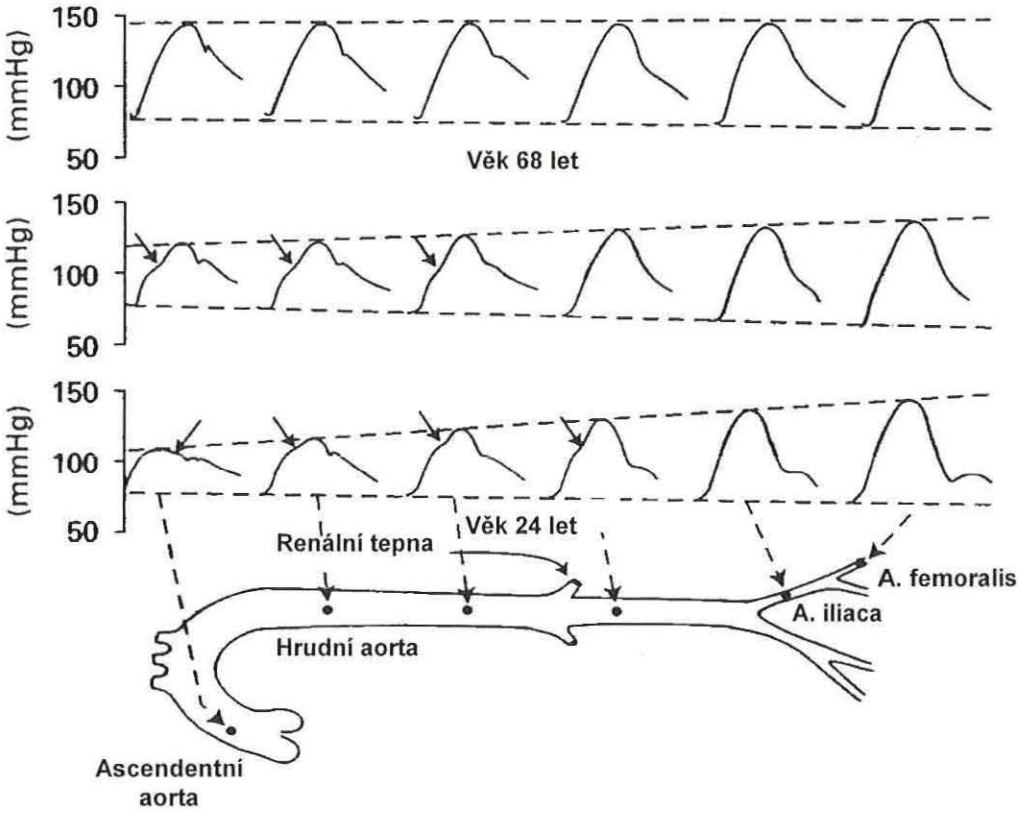
- Pro hypertenzi ve stáří je typický vzestup především systolického TK, jehož nárůst je u řady nemocných způsoben právě odraženou vlnou. To otevírá prostor pro terapeutickou strategii, protože izolovanou systolickou hypertenzi by bylo možno léčit látkami, které by dlouhodobě snižovaly odraz tlakových vln. Takové preparáty (na bázi nitrátů) jsou ve vývoji.
- Odražené vlny se podílejí na amplifikaci TK, tj. rozdílu mezi TK v centrálním řečišti a v periferních velkých tepnách, např. v brachiální tepně, kde běžně měříme TK *(obr. 6)*. Brachiální TK je u starších jedinců stejný jako centrální TK, mj. díky vysokým a časným odraženým vlnám, zatímco u mladých jedinců je běžně měřený brachiální TK vyšší než centrální. Tlakové zatížení centrálního oběhu ve stáří je tedy při běžném měření brachiálního TK podhodnoceno a mělo by být bráno v úvahu.

Technika registrace PWA a PWV

Analýzu pulzní vlny metodou aplanační tonometrie (mikromanometr, Millar Instruments, Inc., Houston, Texas, USA) a stanovení rychlosti šíření pulzní vlny na naší klinice provádíme pomocí australského přístroje SphygmoCor Px (AtCor Medical Pty., West Ryde, Australia). Tyto metody používáme v našich výzkumných pracích.

Zcela nedávno byl publikován konsenzus odborníků v oblasti vlastností velkých tepen *(Laurent et al., 2006)* a jsou zde shrnuta základní doporučení ke správné metodice měření. Z tohoto konsenzu vycházíme i my a při vyšetřování se snažíme zachovat doporučené standardní podmínky *(tab. 1)*.

Před vlastním měřením je vždy nutno zjistit aktuální hodnotu TK na paži (na našem pracovišti používáme digitální tonometr značky OMRON M4-I) a zadat ji do programu, automaticky se v programu vypočítá střední arteriální tlak. Dále do programu zadáváme nacionále vyšetřovaného, výšku a váhu, můžeme zadat i další parametry, např. současnou medikaci.



Obr. 6
Amplifikace TK.

Tlakové vlny registrované ve velkých tepnách u různě starých jedinců.
U nejmladšího jedince se zvyšuje amplituda tlakové vlny přibližně o 60 % během postupu po tepenném řečišti do periferie. U nejstaršího jedince není prakticky žádná amplifikace TK.
Podle Nicholse a spol., 1998.

Periferní stanovení centrálního aortálního tlaku

Analýza a vyšetření pulzní vlny spočívá v registraci tlakové vlny nejčastěji nad a. radialis, ze které matematickou transformací získáme tvar pulzní vlny v centrálním řečišti (v aortě) spolu s hodnotou centrálního TK. Technika vyžaduje určitou zručnost – je nutno najít nejvhodnější místo k registraci nad tepnou a získat tak záznam několika po sobě jdoucích pulzních vln s dostatečnou amplitudou (nad 80 mV) a zároveň s co nejnižší variabilitou jejich tvaru (dle našich požadavků do 5 % pro a. radialis, do 10 % pro a. carotis).

Tab. 1

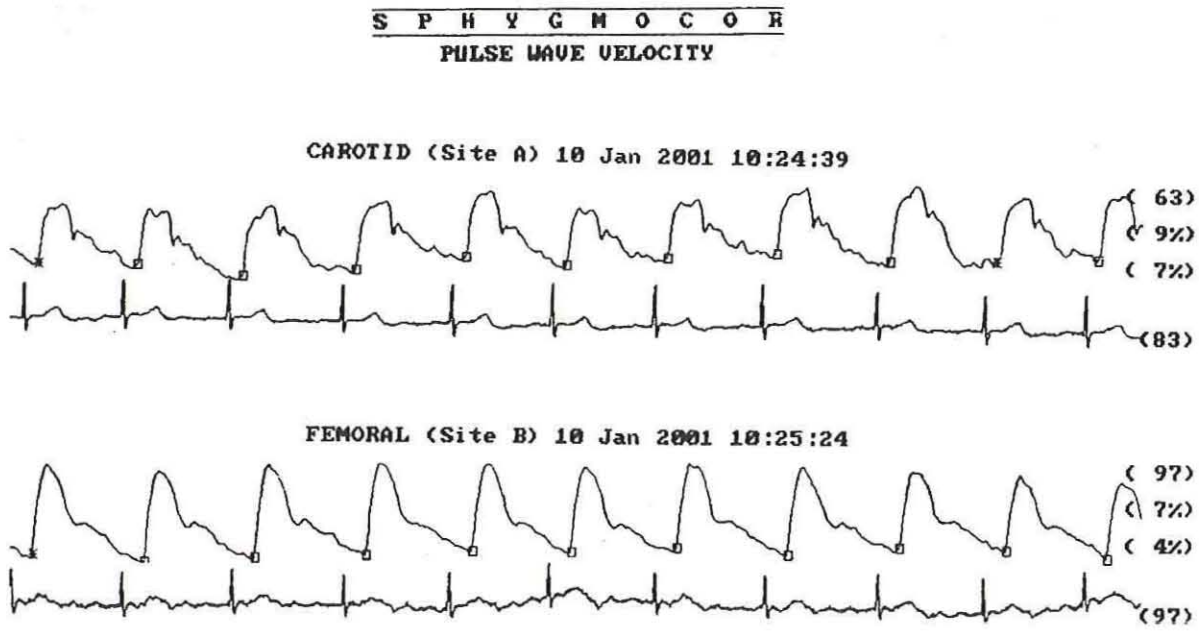
Doporučení ke sjednocení podmínek vyšetřování *(Laurent et al., 2006)*:

Nepříznivé faktory:	**Doporučení:**
Pokojová teplota	optimálně 22 +/- 1 °C
Klid	vyšetřovaný leží nejméně 10 minut na zádech
Denní doba	stejná denní doba při opakovaném měření
Kouření, jídlo	minimálně 3 hodiny před měřením by vyšetřovaný neměl pít zejm. nápoje s obsahem kofeinu
Alkohol	vyšetřovaný by neměl pít alkohol alespoň 10 hodin před měřením
Mluvení, spánek	vyšetřovaný by neměl během měření mluvit ani spát
Poloha	přednostně vleže na zádech, pozice (vleže, vsedě) by měla být uvedena v protokolu
Fenomén bílého pláště	ovlivňuje TK a tlakově závislou cévní tuhost
Srdeční arytmie	uvědomit si možné komplikace při měření

Rychlost šíření pulzní vlny (PWV)

Při PWV registrujeme pulzní vlnu (za současného EKG záznamu – *obr. 7*), a to na dvou místech, mezi kterými chceme rychlost šíření změřit. Nejčastěji se jedná o zjišťování rychlosti šíření vlny po aortě - karotido-femorální rychlost, a na dolní končetině – femoro-distální rychlost. Opět je nutno předem zadat hodnotu aktuálního TK a tentokrát i délku vyšetřovaného cévního úseku (krejčovským metrem měříme vzdálenost např. od místa registrace nad a. carotis k jugulu a od jugula k místu registrace nad a. femoralis). Nejčastěji registrujeme nad a. carotis, a. femoralis a a. tibialis posterior, event. nad a. dorsalis pedis.

4. Poddajnost (compliance C) a roztažnost (distenzibilita Di). Tyto parametry jsou určovány na základě speciálního ultrazvukového vyšetření (zejm. tzv. echo-tracking). Poddajnost je definována jako změna objemu daného cévního oddílu (V) při změně tlaku (P), tj. C=deltaV/deltaP; za předpokladu, že daná céva má konstantní délku, lze objem nahradit průměrem (D), tedy C=deltaD/deltaP. Vztah tlaku a objemu není lineární, to je dáno vlastnostmi cévní médie. Ta sestává z hladké svaloviny a pojivové tkáně obsahující elastin a kolagen. Při nízkém tlaku se uplatňují především elastinová vlákna, zatímco při vysokém kolagenová, která kladou tlakové vlně větší odpor, a proto je cévní stěna v této situaci méně poddajná. Z toho vyplývá, že poddajnost může být definována pouze v souvislosti s konkrétní hodnotou tlaku. Poddajnost představuje sklon křivky v grafu závislosti objemu (průměru) na tlaku v určitém bodu grafu. Lze odvodit, že poddajnost je úměrná druhé mocnině PWV. Podobně jako PWV je i tepenná poddajnost závislá na aktuálním TK. **Distenzibilita** je relativní změna objemu (nebo průměru – obdobně jako poddajnost) vztažená na změnu tlaku: Di=deltaV/V x deltaP.



Obr. 7
Záznam při měření aortální PWV pomocí přístroje Sphygmocor.

V horní části registrace průběhu pulzní vlny na a. carotis, a poté na a. femoralis, vždy současně se záznamem EKG. V dolní části je výsledek – rychlost pulzní vlny po aortě. Blíže viz text.

5. Měření tloušťky komplexu intima–media na krkavici - IMT (Intima-Media Thickness) se provádí pomocí duplexní sonografie. Ta dnes poskytuje spolehlivé informace nejen o závažnosti morfologických změn na cévách, ale i o možném hemodynamickém dopadu těchto změn. Kromě změření tloušťky komplexu intima-médie nám dává možnost potvrzení či vyloučení přítomnosti plátu a posouzení kalcifikace a charakteru plátu. V identifikaci časných aterosklerotických změn se uplatňuje především ultrasonografie karotid. Důvodem je snadná vyšetřitelnost vzhledem k jejich povrchovému uložení. Zásadní je dále fakt, že tyto tepny zásobují centrální nervový systém, jehož cévní zásobení hraje významnou roli pro prognózu jedince. Opakovaně bylo prokázáno, že aterosklerotické změny na karotidách korelují se změnami na věnčitých tepnách i na tepnách dolních končetin. Ateroskleróza je děj, který se odehrává v původně nejtenčí vrstvě cévní stěny – intimě.

Za první morfologický projev aterosklerózy je považováno rozšíření intimo-mediální vrstvy. Měření tloušťky komplexu intima - media se nejčastěji provádí v úseku 1 cm pod začátkem bifurkace společné karotidy na straně více vzdálené od ultrazvukové sondy. IMT stoupá s věkem a s přítomností rizikových faktorů aterosklerózy. U hypertoniků koreluje s mírou hypertrofie levé komory. Určitým omezením této metody je reprodukovatelnost, protože velmi záleží na zkušenostech vyšetřujícího. Jde např. o to, v jakém místě je měření prováděno, dále aby do měření nebyl zavzat aterosklerotický plát atd. Proto se jedná o metodu, která není příliš vhodná pro větší multicentrické studie (v takovém případě je nutné centrální odečítání nálezů ze všech center).

IMT u zdravých jedinců se pohybuje pod 0,75 mm, avšak neexistuje jednoznačně definovaná norma. Hodnota nad 0,9 mm je podle nejnovějších společných doporučení Evropské hypertenzní a kardiologické společnosti *(ESH-ESC Guidelines Committee, 2003)* považována za specifickou orgánovou komplikaci hypertenze.

6. Vyšetření mikrocirkulace a rezistenčního řečiště. Zlatým standardem je invazivní vyšetření – **biopsie** – která se provádí většinou z gluteální oblasti. To umožňuje vyšetření tepének a kapilár tukové tkáně. V kontextu s hypertenzí jsou klasické práce skupiny prof. *Heagertyho (1995)*. V jedné ze svých prací autoři studovali rezistenční řečiště u lidské hypertenze pomocí biopsií gluteální tkáně, a to u 56 nemocných s neléčenou esenciální hypertenzí a u 56 normotenzních kontrolních jedinců. Zjistili, že hypertonici měli oproti normotonikům silnější médii a menší lumen arteriol, tj. poměr media/lumen byl zvýšen *(Korsgaard et al., 1993)*. U hypertoniků koreloval s tímto poměrem především střední a diastolický TK ($p<0,01$), slaběji systolický TK ($p<0,02$). Tlaková amplituda a věk nebyly v žádné asociaci s poměrem media/lumen. U hypertoniků byl tedy hlavní determinantou stavu rezistenčního řečiště TK, zatímco věk nebyl významný. U normotoniků tomu bylo naopak: žádný z tlakových parametrů nekoreloval s poměrem media/lumen, ale věk byl jeho významnou determinantou.

Při hypertenzi se na změnách rezistenčního řečiště uplatňují především tři mechanismy:

- hypertrofie buněk hladké svaloviny, která způsobuje zesílení cévní médie a zmenšení lumina cév
- zvýšená citlivost hypertrofované stěny na vazoaktivní substance
- řídnutí (rarefakce) arteriol a kapilár.

Další metodou studia mikrocirkulace je **kapilaroskopie**, kdy kapiláry jsou přímo pozorovány, např. v nehtovém lůžku; je to stará metoda, která dnes nemá významné využití. Nejnovější neinvazívní metodou je **laser-dopplerovská flowmetrie** (LDF), kdy je posuzováno prokrvení kůže v bazálních podmínkách i např. po aplikaci tepla k posouzení schopnosti vazodilatace. Tato metoda se zatím používá spíše v klinické angiologii, ale je možné, že najde své uplatnění i při studiu struktury a funkce oběhu v kontextu s krevním tlakem (naše centrum připravuje projekt zaměřený na tuto problematiku).

IV. Význam arteriální rigidity měřené pomocí PWV

Jak již bylo uvedeno, rigiditu cévní stěny lze měřit na různých tepenných segmentech. Byly provedeny studie s měřením PWV mezi karotidou a radiální tepnou nebo mezi radiální tepnou a a. tibialis posterior. Je třeba říci, že taková měření mají jen omezený význam, protože v sobě zahrnují tuhost zcela odlišných tepenných úseků, především úseků tepen elastického a muskulárního typu. Z hlediska poznání etiologie kardiovaskulárních nemocí má zdaleka největší význam měření na aortě: jednak tuhost aorty přímo ovlivňuje srdeční funkce prostřednictvím hemodynamických změn, jednak nám dává určité informace o rozsahu morfologického poškození aorty a v jejích hlavních větvích se odehrávají kardiovaskulární příhody. Kromě aorty považujeme za přínosné studovat tuhost tepen dolních končetin (mezi a. femoralis a a. dorsalis pedis/tibialis posterior): získáváme tak informaci o víceméně homogenním úseku muskulární tepny, kde je navíc častý výskyt aterosklerózy. Toto vyšetření jsme zavedli v rámci našeho výzkumu (blíže viz oddíl Vlastní výsledky) a podle našich znalostí nejsou o nich žádná předchozí literární data.

Bylo ukázáno v několika studiích, že aortální rigidita měřená pomocí PWV koreluje s různými dalšími parametry cévního postižení, jako jsou index systolického TK kotník/paže, IMT měřená na karotidě nebo index aortální kalcifikace *(Blacher et al., 2001, Oren et al., 2003, Zureik et al., 2002)*. Aortální tuhost má také vztah ke klasickým rizikovým faktorům aterosklerózy, jak bylo např. ukázáno u starších jedinců v Rotterdamské studii *(van Popele et al, 2001)*. V práci *Lehmanna et al. (1998)* bylo zjištěno, že pacienti s největším počtem faktorů kardiovaskulárního rizika a příhod mají nejvyšší tuhost, měřenou pomocí PWV. K poznání těchto vztahů také významně přispěly naše vlastní výsledky (viz dále). Důležité interakce byly stanoveny také mezi PWV a tzv. malými faktory kardiovaskulárního rizika – pulzní tlak, srdeční frekvence, obvod pasu, poměr pas/boky, hypertrofie levé komory srdeční, mikroalbuminurie, homocystein a sedavé zaměstnání.

Blacher et al. (1999) vyšetřoval skupinu pacientů s esenciální hypertenzí s aterosklerotickými změnami nebo bez nich a analyzoval aortální PWV jako prediktor kardiovaskulárního rizika. Výsledky potvrdily konstantní nárůst aortální PWV se všemi fatálními a nefatálními příhodami – infarkt myokardu, koronární onemocnění, cévní mozkové příhody. Pro všechny typy kardiovaskulárního rizika je aortální PWV silnějším prediktorem než plazmatický kreatinin, hypertrofie levé komory srdeční a poměr celkový/HDL cholesterol. Navíc je aortální PWV nejlepším prediktorem kardiovaskulární mortality v jakémkoli věku. Pro předpovídání desetileté kardiovaskulární mortality byla při tomto diagnostickém testu stanovena optimální hraniční (cut-off) hodnota PWV 13 m/s.

Další významná práce studující význam PWV u hypertenze je práce *Laurenta et al. (2001)*. V letech 1980 až 1996 sledovali 1980 pacientů s esenciální hypertenzí (průměrný věk 50 +/- 13 let). Vstupně jim byla změřena aortální PWV. Pro odhad relativního rizika celkové a kardiovaskulární mortality použili model logistické regrese. Během průměrného devítiletého sledování zemřelo 107 pacientů (z toho bylo 46 úmrtí kardiovaskulární etiologie). PWV byla významně spojena s celkovou i kardiovaskulární mortalitou, byla nezávislá na předchozím kardiovaskulárním onemocnění, věku a diabetu. Naproti tomu pulzní tlak nebyl s mortalitou signifikantně spojen. Tato studie poskytuje první přímý důkaz, že aortální tuhost je nezávislým prediktorem celkové a kardiovaskulární mortality u hypertoniků.

Aortální rigidita má také vztah k rozvoji srdeční hypertrofie u hypertenze. Ta je kromě hodnoty krevního tlaku závislá také na vlastnostech centrálních tepen, různých neurohumorálních faktorech a na přívodu soli. *Bouthier et al. (1985)* analyzoval u normotoniků a hypertoniků vztah mezi karotido-femorální PWV a echokardiografickými parametry – poměr masa/objem levé komory srdeční a end-systolické napětí levé komory srdeční. Jeho výsledky ukázaly vysoce významné korelace mezi PWV a všemi sledovanými echokardiografickými

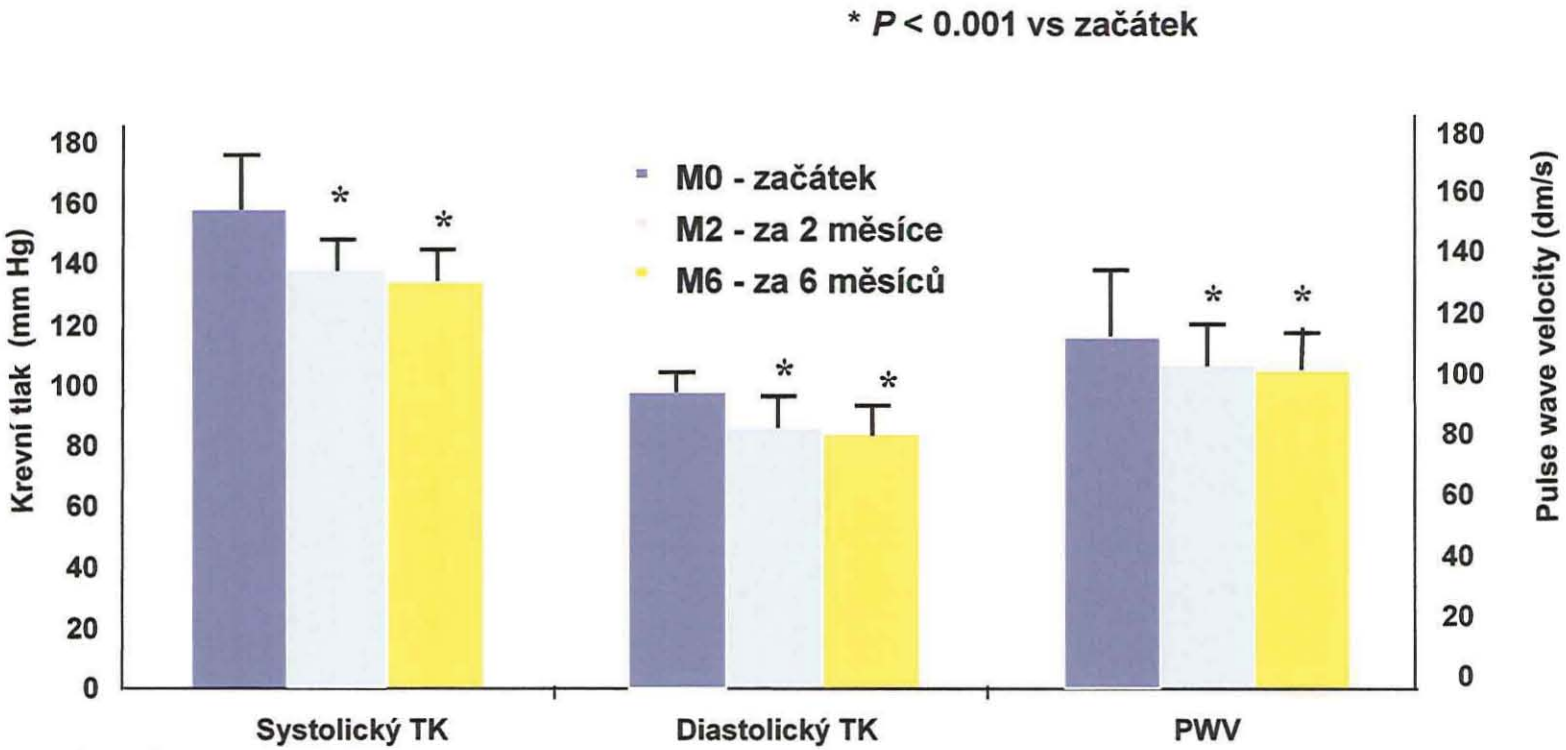
parametry dokonce i po adjustaci na věk. Na základě těchto výsledků lze konstatovat, že aortální tuhost stanovená pomocí karotido-femorální PWV hraje důležitou roli v plnění levé komory srdeční, její hypertrofii a hemodynamice.

Několik studií analyzovalo vztah mezi PWV a diabetickými komplikacemi. *Takegoshi e al. (1991)* zjistil vyšší PWV u diabetiků s mikroalbuminurií než bez ní, a poukázal na významné korelace mezi PWV a albuminem v moči. Ze svých nálezů vyvozuje, že PWV může být spolehlivým indexem diabetické mikroangiopatie vyjádřeným mikroalbuminurií. *Taniwaki et al. (1998)* vyšetřovali diabetiky 2. typu a označili arteriální rigiditu nezávislým faktorem souvisejícím s poměrem glomerulární filtrace.

Lehmann et al. (1998) ve své longitudinální studii sledoval diabetiky 2. typu. Pacienti, kteří zemřeli měli signifikantně tužší aortu než diabetici dosud žijící, a to i vzhledem k věku, pohlaví, kouření a terapii. Měření aortální PWV tak dovolilo časnou identifikaci pacientů diabetiků ohrožených vysokým rizikem vaskulárních komplikací a náhlé smrti.

Pomocí aortální PWV byl stanoven i vliv aortální rigidity na přežití u nemocných v konečném stadiu renálního selhání *(Guerin et al., 2001)*. Hodnota PWV se ukázala jako nejsilnější prediktor mortality, následovaná věkem a předchozím kardiovaskulárním onemocněním.

Existuje jen málo prospektivních studií, kde je testován vliv léčby na aortální rigiditu. Nejvýznamnější z nich je studie Complior *(Asmar et al., 2001)*. Pacienti s mírnou esenciální hypertenzí byli po dobu 6 měsíců léčeni perindoprilem v kombinaci s indapamidem. Arteriální rigidita byla měřena pomocí aortální PWV 2 a 6 měsíců po léčbě. Výsledky ukázaly signifikantní pokles TK a PWV navozeného léčbou *(obr. 8)*. Přestože aktuální TK ovlivňuje PWV a interpretace výsledků není jednoduchá, ukázalo se, že snížení PWV bylo statisticky nezávislé na snížení TK. Antihypertenzní terapie ACE inhibitory tedy dokázala oddálit



Obr. 8

Vliv ACE inhibitoru na aortální PWV u hypertoniků léčených po dobu 6 měsíců. Podle studie Complior.

poškození tepen mechanismy, které jsou částečně nezávislé na redukci TK. Existují také krátkodobé úspěšné studie s blokátory receptorů pro angiotenzin II.

Lze shrnout, že určení aortální PWV má následující význam pro praxi:

- PWV koreluje s dalšími parametry cévního poškození, jako jsou index systolického TK kotník/paže, aortální kalcifikace, IMT a další

- PWV má významný vztah k velkým i malým faktorům kardiovaskulárního rizika v různých populacích

- PWV je v asociaci s hypertrofií levé srdeční komory; důvodem je fakt, že aortální rigidita zvyšuje afterload levého srdce

- PWV je také vyšší u diabetiků se známkami mikrovaskulárního poškození zachyceného např. mikroalbuminurií a předpovídá u nich riziko úmrtí

- Hraniční hodnota PWV, nad kterou je třeba ji považovat za patologickou, byla u hypertoniků stanovena na 13 m/s

- V případě hypertoniků byla PWV stanovena jako silný prediktor pro jakýkoli typ kardiovaskulárního rizika a jako nejlepší prediktor kardiovaskulární mortality v jakémkoli věku

- PWV je silným a nezávislým prediktorem celkové a kardiovaskulární mortality u hemodialyzovaných pacientů v konečném stadiu renálního selhání

- Ovlivnění aortální rigidity léčbou, která je dnes k dispozici, je nedostatečné; zdá se, že nejúčinnější jsou ACE inhibitory

V. Genetické aspekty arteriální hypertenze

Genetická informace se podle současných poznatků podílí na variabilitě krevního tlaku 30 – 60 %. Esenciální arteriální hypertenze je onemocnění polygenní: na vzestupu TK se podílí celá řada genů, které interagují navzájem mezi sebou, ale i s vlivy životního prostředí, a komplexně ovlivňují humorální a strukturální mechanismy zasahující do regulace TK. Vyvážený krevní tlak odráží koordinované vzájemné působení srdečního výdeje, periferní vaskulární rezistence, kontroly renálního objemu a zapojení CNS do odpovědí na krátkodobé a dlouhodobé vnější stimuly.

Nejzřejmější příklady tohoto genetického podílu se objevují u vzácných forem monogenní hypertenze, kde vzestup TK je důsledkem změny jediného genu (glukokortikoidy léčitelný-suprimovatelný hyperaldosteronismus, Liddleův syndrom, zdánlivý nadbytek mineralokortikoidů, hypertenzní formy adrenogenitálního syndromu, pseudohyperaldosteronismus typ II s hyperkalémií atd.).

Při průkazu genetických vlivů u esenciální hypertenze jsou určitým problémem rozdíly v jednotlivých rodových, geografických a etnických skupinách, protože různé geny mohou přispívat k rozvoji hypertenze různou měrou. U experimentální hypertenze, kde fenotyp studovaného zvířecího modelu je homogenní, jsou poznatky mnohem jednoznačnější než u lidské hypertenze.

Jedním z hlavních směrů současného výzkumu jsou polymorfismy genů, které se mohou podílet na aktivitě regulačních mechanismů TK - tzv. **kandidátní geny**. Mezi nejsledovanější patří geny kódující jednotlivé komponenty renin-angiotenzinového systému (RAS), především geny pro angiotensinogen (AGT), receptory angiotensinu II (AT1R), angiotensin-konvertující enzym (ACE) a aldosteron syntázu, dále pak geny pro endoteliální syntázu oxidu dusnatého (eNOS), geny pro adducin alfa, beta a gama, pro adrenergní nervový systém (především G-protein beta1-receptorů), inzulinové receptory, natriuretické peptidy a další.

V krátkosti uvádím některé poznatky o nejdůležitějších polymorfismech souvisejících s hypertenzí.

Gen pro ACE – inzerčně-deleční (I/D) polymorfismus

Z dosavadních výsledků je gen pro ACE významný zejména ve vztahu k ischemické chorobě srdeční, srdečnímu selhání, infarktu myokardu a hypertrofii levé komory srdeční *(Ludwig et al., 1995, Cambien et al., 1992, Samani et al., 1996)*. DD polymorfismus genu pro ACE je spojen s vyšší sérovou hladinou ACE a vyšší četnost D alely byla nalezena u pacientů s hypertrofickou kardiomyopatií a s hypertrofií levé komory srdeční *(Iwai N et al., 1994, Tesson et al., 1997, Saeed et al., 2005)*, u diabetiků 1. typu předpovídá přítomnost D alely rozvoj diabetické nefropatie *(Hadjadj et al., 2001, Vleming et al., 1999)*. Tento polymorfismus by také mohl být potenciálním genetickým markerem u hypertoniků s rizikem renálních komplikací.

Ve vztahu polymorfismu genu pro ACE k esenciální hypertenzi nejsou výsledky zcela jednotné. V roce 1997 provedl Staessen metanalýzu dat od téměř 50 000 probandů a nezávislou asociaci tohoto polymorfismu k hypertenzi nezjistil *(Staessen et al., 1997)*, podobně ve většině dalších prací nebyla přímá asociace prokázána *(např. Schmidt et al., 1993, Harrap et al., 1993, Morise et al., 1994)*. *Jáchymová et al. (1998)* studovali rozdíly mezi syny hypertoniků a normotoniků – podle jejich výsledků genotyp DD (a AA polymorfismu genu pro angiotensinogen) u potomků z hypertenzních rodin může představovat určitou rizikovou situaci pro vznik hypertenze, protože tito jedinci měli vyšší plazmatické koncentrace noradrenalinu a vyšší hodnoty imunoreaktivního inzulinu po zátěži glukózou. Ve shodě s většinou dostupných publikací však neprokázali statisticky významný vztah výšky TK k jednotlivým genotypům genu pro ACE, ani rozdíl ve frekvenci či celkovém počtu jednotlivých alel.

Rozdíl ve vztahu mezi masou levé komory srdeční (LKS) a ACE I/D polymorfismem v závislosti na příjmu soli byl hodnocen ve studii EPOGH u tří populací (382 rodičů a 436 potomků). Výsledky nebyly jednoznačné:

homozygotní stav II v polské a ruské populaci byl jasně spojen s vyšším indexem masy LKS, naopak v italské populaci byl index masy LKS lehce vyšší u DD homozygotů. Index masy LKS rostl s vyšší exkrecí sodíku do moče u potomků s ACE II polymorfismem ve všech třech populacích, avšak za přítomnosti D alely byl nárůst indexu LKS pozorován jen u slovanských jedinců. *(Kuznetsova et al., 2004)*

Spiering a spol. (2005) se nedávno vyjádřili v tom smyslu, že ke stanovení vazby I/D polymorfismu s hypertenzí je základem časté měření TK - ve své studii používali ABPM a zjistili pozitivní asociaci výšky TK s tímto polymorfismem (více vyjádřeno u mužů než u žen).

Gen pro angiotensinogen

Bylo objeveno několik genetických variant genu pro AGT, z nichž jako nejdůležitější se jeví polymorfismus M235T. TT genotyp bývá spojován se zvýšenými hladinami sérového angiotensinogenu u bělochů, předpokládá se spojitost s hypertenzí a koronárním postižením.

Ve studii *Winkelmanna et al. (1999)* se hladina cirkulujícího AGT ukázala jako nejdůležitější prediktor diastolického TK; hodnoty plazmatického AGT se postupně zvyšovaly v závislosti na počtu přítomných T alel. Homozygoti s T235 měli zvýšené riziko koronárního postižení a infarktu myokardu. Signifikantní vztahy varianty M235T pro AGT tak poskytují důkaz o možné roli zvýšené hladiny AGT v patogenezi koronárního onemocnění.

Nedávno však němečtí autoři *(Mondry et al., 2005)* prezentovali zcela opačné výsledky - TT genotyp vykazoval významně nižší riziko vývoje hypertenze u žen studované populace. Na základě těchto kontroverzních výsledků je nutné brát v úvahu rozdíl v genomu jednotlivých populací.

Gen pro receptory angiotenzinu II (AT1R)

Vzhledem k tomu, že receptory typu 1 pro angiotensin II hrají klíčovou roli ve zprostředkování vasokonstrikce a růstového efektu angiotensinu II, věnuje se polymorfismu jejich kódujícího genu velká pozornost. Data z mnoha studií jsou

však často protichůdná. *Castellano et al. (1996)* ve své Vobarno studii shrnuje, že A1166C polymorfismus je pravděpodobně s hypertenzí spojen, ale neprokázali jeho spojitost s kardiálním onemocněním či cévními změnami. U japonské populace podle výsledků rozsáhlé studie Ohasama souvislost tohoto polymorfismu s hypertenzí chybí *(Sugimoto et al., 2004)*.

Mezi nejnovější studie efektu kandidátních genů na prevalenci a incidenci hypertenze patří prospektivní studie skupiny prof. Staessena, do které byla zařazena i naše data. Sledovali tzv. epistázi – supresi či potlačení genu jinými non-alelickými geny. V mnoha případech totiž nelze popsat efekt jednoho či více genů pouhým součtem vlivu jednotlivých genů na výsledném fenotypu. Oproti ostatním pracím se v této studii zaměřili na sledování jednotlivého působení, ale i interakcí tří polymorfismů (pro ACE, alfa-adducin a aldosteron syntázu), které ovlivňují krevní tlak přes sodíkovou homeostázu. Zjistili, že v případě kombinace homozygocity ACE/DD, současně s aldosteron syntázou AS/CC a přítomností Trp alely pro alfa-adducin (ADD/Trp) je incidence hypertenze o 252 % vyšší než u ostatních ACE genotypů. Incidence hypertenze je až o 30 % vyšší u homozygotů pro D alelu genu pro ACE *(Cwynar et al., 2005)*.

Geny pro adducin alfa, beta a gama

Adducin je proteinem buněčného skeletu ovlivňující aktivitu sodíko-draslíkové pumpy a tím vylučování sodíku ledvinami u experimentální i u lidské hypertenze. Podle většiny provedených studií jsou hypertonici s polymorfismem Gly/Trp pro alfa-adducin výrazněji citliví na restrikci soli a na léčbu diuretiky *(Wojciechowska et al., 2004)*. Bližší údaje jsou uvedeny v sekci vlastních výsledků. Tento polymorfismus je nadějný z hlediska volby antihypertenzní léčby podle genotypu.

G-protein beta-3-subunit je protein intracelulárního přenosu signálu. Jeho polymorfismus C825T na exonu 10 byl popsán nedávno a bylo zjištěno, že nositelé T alely mají významně vyšší riziko vzniku hypertenze *(Schunkert et al., 1998, Siffert et al., 1998)*. Přítomnost T alely je spojena se zvýšenou perfúzí

ledvin a je četnější u osob s rodinnou anamnézou hypertenze *(Benjafield et al., 1998)*. V italské studii zjistili, že T alela zvyšuje riziko nejen hypertenze, ale i obezity, inzulinové rezistence a hypertrofie levé komory srdeční, a dále, že nositelé T alely vykazovali lepší odezvu na terapii thiazidovými diuretiky a clonidinem *(Sartori et al., 2004)*.

VI. Genetické faktory a vlastnosti velkých tepen

Výše krevního tlaku je dána působením mnoha systémů a orgánů (mozek, srdce, tepny, ledviny, intermediární metabolismus, sympatická nervová aktivita atd.). Proto je hypertenze heterogenní onemocnění a v genetickém výzkumu je zapotřebí studovat vliv na parametry, které jsou specifičtější než krevní tlak, tj. na tzv. intermediární fenotypy. Jedním z těchto důležitých fenotypů jsou vlastnosti velkých tepen. Dědičnost může ovlivnit vulnerabilitu cévní stěny k rizikovým faktorům, jako je hypertenze, stárnutí, cholesterol a kouření. Zvýšená tepenná rigidita může být faktorem, který vyvolává hypertenzi, nebo faktorem, který hypertenzi udržuje, fixuje, a dále přispívá k její progresi. Identifikace genetických markerů pro vysokou tepennou tuhost pak může být na předním místě v identifikaci vysoce rizikových pacientů.

Polymorfismus NO syntázy

Díky mohutnému efektu oxidu dusnatého (NO) na krevní tlak a cévní stěnu je polymorfismus genu pro endoteliální NO syntázu (eNOS) kandidátním genem pro hypertenzi a arteriální rigiditu. Provedené studie jsou však víceméně negativní. *Lacolley et al. (1998)* zhodnotil vztah arteriální rigidity (PWV) se dvěma nedávno popsanými polymorfismy genu pro eNOS – G10T na intronu 23 a G298T na exonu 7 (Glu298Asp). Zjistili, že u hypertoniků je prevalence alely 298G vyšší oproti normotonikům, zatímco rozdíly u polymorfismu G10T nebyly mezi jednotlivými skupinami přítomny. Nezjistili žádnou asociaci mezi genotypy eNOS a TK nebo arteriální rigiditou.

Genetické faktory a RAS

Ovlivnění aortální rigidity v souvislosti s I/D polymorfismem genu pro ACE popsal *Benetos et al. (1996)*. Jejich výsledky ukázaly, že tento polymorfismus je spojen se vzestupem rigidity tím více, čím je větší počet přítomných I alel. Naopak ve Vobarno studii zjistil *Castellano et al. (1996)* vyšší tloušťku karotické intimy a medie za přítomnosti D alely. Tyto kontroverzní výsledky poukazují na předpokládaný rozdílný mechanismus rozvoje cévní hypertrofie a arteriální rigidity. Bylo skutečně zjištěno, že zesilování stěny velkých cév není bezpodmínečně spojeno se vzestupem cévní rigidity. Vzhledem k nízké incidenci II genotypu nelze jasně uzavřít předchozí výsledky a je zapotřebí provést rozsáhlejší studie. Stanovení role polymorfismu genu pro AT1R ukázala, že u normotoniků tento polymorfismus nemá významný vztah k aortální rigiditě. Výsledky týkající se polymorfismu AT1R lze shrnout tak, že

- arteriální hypertenze a s ní spojené mechanické nebo strukturální změny mohou zesílit vliv tohoto polymorfismu na aortální rigiditu.
- asociace mezi AT1R genotypem a aortální rigiditou byla sledována u mladých a starých hypertoniků, ale tento efekt byl více vyjádřen u starých pacientů. Starší s C alelou měli mnohem horší cévní rigiditu než pacienti bez C alely, a předpokládá se, že tento polymorfismus může modifikovat efekty věku na cévní rigiditu.
- byly nalezeny interakce mezi AT1R genotypem a poměrem celkový cholesterol/HDL-cholesterol, působící na aortální rigiditu: hypertonici s C alelou mají pozitivní korelace mezi tímto poměrem a PWV. Naopak pacienti bez C alely (AA homozygoti) nemají asociaci mezi zvýšeným poměrem celkový/HDL-cholesterol a vyšší rigiditou.

Tyto výsledky ukazují, že polymorfismus genu pro AT1R může být důležitý rizikový faktor pro arteriální rigiditu a může modulovat efekty hypertenze, stárnutí a lipidů na velké cévy *(Asmar, 1999)*.

Polymorfismus genu pro aldosteron syntázu

Gen pro aldosteron syntázu (CYP11B2) a jeho vliv na hladiny plazmatického reninu a aldosteronu, na krevní tlak a arteriální rigiditu byl studován u pacientů s esenciální hypertenzí *(Pojoga et al., 1998)*. Autoři zjistili, že přítomnost C alely (-344C) je spojena se zvýšenými hladinami plazmatického aldosteronu a vyšší PWV. Tyto asociace nebyly pozorovány pro T4896C polymorfismus.

Stella et al. (2004) ve své nejnovější studii zjišťovali vztah mezi polymorfismem CYP11B2 -344C/T a hodnotou masy levé komory srdeční u pacientů esenciální hypertenzí a jejich výsledky naznačují, že s počtem přítomných T alel roste u mírné až střední hypertenze velikost a tloušťka levé komory srdeční.

Wojciechowska et al. (2004) nedávno prezentovala výsledky ze studie EPOGH. Stejně jako autoři ve výše zmíněné práci potvrdili, že oproti homozygotům s -344T alelou zvyšuje přítomnost C alely (CYP11B2 -344C) arteriální rigiditu. Tento jev se však projevil u osob s vyšší než průměrnou exkrecí sodíku do moče, tzn. že přívod soli ve stravě s velkou pravděpodobností ovlivňuje tento genetický efekt.

Lze shrnout, že výsledky genetických studií, v nichž je studována role polymorfismů kandidátních genů pro hypertenzi, jsou často nejednoznačné. Důvodem je fakt, že zde patrně hraje roli nikoli jeden izolovaný polymorfismus, ale vzájemná interakce polymorfismů různých genů. Kromě toho se na výsledném efektu podílejí další faktory životního stylu, jako jsou přívod soli, obezita, kouření apod. Výsledky mohou být také rozdílné u jedinců rozdílné rasy nebo pohlaví. Genetické vyšetření by v budoucnu mohlo pomoci identifikovat nemocného s vysokým rizikem zhoršování hypertenze nebo vzniku jejích orgánových komplikací. Důležitým potenciálním přínosem genetického výzkumu hypertenze v budoucnosti je lepší znalost odpovědi na antihypertenzní léčbu daného pacienta s určitým typem polymorfismu (farmakogenetický přístup).

Zatím nemáme prakticky žádné ukazatele, podle kterých by bylo možno zvolit nejefektivnější lék u konkrétního nemocného. Stanovením genetického polymorfismu pacienta bychom mohli identifikovat jak jeho riziko, tak by byla snadnější volba vhodné terapie.

VLASTNÍ VÝSLEDKY

Zkoumání vlastností velkých tepen ve vztahu ke krevnímu tlaku a ke genetickému pozadí hypertenze jsme uplatnili především ve dvou velkých výzkumných mezinárodních projektech:

• EPOGH

European **P**roject **O**n **G**enes in **H**ypertension

- evropský projekt o dědičnosti hypertenze probíhal v České republice v roce 2000 - 2001 (pokračování proběhlo na podzim roku 2006).

• MONICA

Multinational **MONI**toring of Trends and Determinants in **CA**rdiovascular Diseases

- projekt Světové zdravotnické organizace prováděný ve 21 zemích Evropy, Severní Ameriky, Asie a Austrálie v letech 1984 – 1993.

Česká část projektu probíhala v šesti okresech České republiky: Benešov, Cheb, Chrudim, Jindřichův Hradec, Pardubice, Praha-východ. Ačkoliv celosvětově studie nepokračuje, v ČR probíhá jako **Post-MONICA** podle stejného protokolu na základě národních grantů. K původním šesti okresům byly do studie zapojeny další tři okresy: Kroměříž, Litoměřice a Plzeň-město.

EPOGH a vlastnosti velkých tepen v rodinách hypertoniků a normotoniků.

EPOGH = **E**uropean **P**roject **O**n **G**enes in **H**ypertension.

Cíle studie:

- určit, do jaké míry souvisí genetická determinace krevního tlaku a hypertenze s následnými kardiovaskulárními komplikacemi,
- identifikovat polymorfismy, které významně souvisejí s hypertenzí nebo s kardiovaskulárním rizikem,
- vytvořit databázi, která popíše genetické pozadí a kardiovaskulární fenotypy u pěti východoevropských a dvou západoevropských populací,
- zřídit stálý, okamžitě dostupný fond genetického materiálu určený pro potřeby nově se rozšiřujícího genetického výzkumu.

Metodika:

Do studie bylo zahrnuto cca 600 rodin (cca 2400 osob) ze 7 evropských zemí (Belgie, Bulharsko, Česká republika, Itálie, Polsko, Rumunsko, Rusko). Byly vybírány jednak náhodným výběrem z obecné populace a jednak ze specializovaných klinických pracovišť zaměřených na hypertenzi. Zařazeny byly rodiny s minimálně dvěma potomky a v hypertenzních rodinách byl alespoň jeden rodič hypertonik. Věkové omezení všech subjektů pro zařazení do projektu bylo 18 – 59 let. Krevní tlak byl měřen obvyklým způsobem při dvou domácích návštěvách (tj. dvakrát 5 měření) a při klinickém vyšetření, které mimo jiné zahrnovalo spektrální analýzu variability srdeční frekvence a v některých centrech vyšetření vlastností velkých tepen pomocí analýzy pulzní vlny. Součástí bylo i 24-hodinové monitorování krevního tlaku, 24-hodinový sběr moče a vyplnění validizovaného dotazníku zaměřeného na osobní i rodinnou anamnézu a důležité faktory životního stylu. V současné době jsou zpracovávána data z druhé fáze projektu – kontrolní vyšetření po 5 - 6 letech.

Komentáře k přiloženým článkům

1. Kontrola kvality měření kreního tlaku ve studii EPOGH
2. Souvislost mezi odrazem pulzní vlny s alelou CYP11B2 -344C a vylučováním sodíku
3. Interakce mezi alfa- a gama-adducinem ovlivňuje periferní a centrální pulzní tlak
4. Tepenné vlastnosti potomků normotenzních a hypertenzních rodičů

ad 1. Kontrola kvality měření krevního tlaku ve studii EPOGH

Pro stanovení komplexních fenotypů skládajících se z TK v kombinaci s ostatními znaky byly v projektu EPOGH použity standardizované epidemiologické metody. V článku je prezentována kvalita kontroly jednoho z fenotypů TK.

V 7 evropských státech (8 výzkumných pracovišť – v ČR Plzeň a Praha) byl vybrán náhodný vzorek "celých" rodin. Vyškolení pracovníci měřili TK dle stávajících doporučení jednotlivým členům rodiny pětkrát po sobě, vsedě, při dvou domácích návštěvách (v průběhu 1 až 3 týdnů).

Výsledky: Do 31. 8. 2001 byla k dipozici data od 2476 změřených subjektů. Méně než 5 plánovaných měření TK při domácí návštěvě se vyskytlo v jednom z osmi center, ale jen v 0,4 % domácích návštěv. Ve všech centrech se relativní frekvence identických po sobě jdoucích odečtů STK a DTK pohybovala od 0 do 6 %. Výskyt lichých čísel v odečtech TK se pohyboval od 0 do 0,1 %. Z 49 488 odečtů STK a DTK končilo nulou 24 % (předpoklad byl 20 %). Ve většině centrech byl patrný postupný pokles hodnot TK během 1. a 2. návštěvy (průměrný pokles STK 2,36 mmHg a DTK 1,74 mmHg).

Z á v ě r : Zabezpečení kvality a kontroly by mělo být plánováno ve fázi návrhu projektu zahrnujícího měření TK a uskutečňováno již od samého začátku až do konce studie. Postupy pro zabezpečení kvality měření TK v rámci projektu

EPOGH měly za následek přesně stanovený fenotyp TK, který byl shodný ve všech centrech.

ad 2. Souvislost mezi odrazem pulzní vlny s alelou CYP11B2 -344C a vylučováním sodíku

Angiotensin II a aldosteron vznikající pomocí angiotensin konvertujícího enzymu (ACE) a aldosteron syntázy (CYP11B2), nejen regulují sodíkovou a vodní homeostázu, ale pravděpodobně též ovlivňují vaskulární remodelaci jako odpověď na vysoký krevní tlak. V naší práci jsme se zaměřili na zjištění, zda polymorfismy ACE I/D a CYP11B2 C-344T ovlivňují odraz pulzní vlny – parametr tepenné rigidity.

Pomocí aplanační tonometrie jsme měřili periferní a centrální augmentační index systolického tlaku u 622 jedinců (160 rodin, a navíc 64 osob bez příbuzenského vztahu), kteří byli náhodně vybráni ze 3 evropských zemí. Průměrný odpad sodíku do moče se v jednotlivých zemích pohyboval od 196 do 245 mmol/den.

Výsledky: periferní i centrální AI byly výrazně vyšší u jedinců s CYP11B2 -344C alelou než u TT homozygotů. Tento efekt CYP11B2 polymorfismu se objevil pouze u jedinců s vyšším než středním odpadem sodíku do moče (210 mmol/l). Polymorfismus ACE I/D augmentační index neovlivnil.

Z á v ě r : Polymorfismus CYP11B2 C-344T ovlivňuje arteriální tuhost, příjem natria tento efekt moduluje.

ad 3. Interakce mezi alfa- a gama-adducinem ovlivňuje periferní a centrální pulzní tlak

V uvedené práci jsme zkoumali, zda polymorfismy genů kódující alfa- (Gly460Trp), beta- (C1797T) a gama-adducin (A386G) ovlivňují vylučování sodíku, draslíku, a dále pulzní tlak, který odráží rigiditu cévního řečiště.

Analýza byla provedena u 642 jedinců ze 3 evropských populací (Belgie, Polsko, Česká republika). Periferní pulzní tlak byl stanoven z běžného měření TK tonometrem, centrální pulzní tlak byl odvozen z měření pulzní vlny přístrojem SphygmoCor. Polymorfismy byly stanoveny standardním způsobem pomocí PCR metody.

Výsledky: Průměrný periferní pulzní tlak byl 46,1 mmHg, průměrný centrální pulzní tlak byl 32,6 mmHg. Mezi jedinci, kteří byli nositeli alely Trp pro alfa-adducin, měli ti, kteří zároveň byli homozygoty GG pro gama-adducin, periferní pulzní tlak o 5,8 mmHg vyšší než ti, kteří byli homozygoty AA pro gama-adducin. Zvýšení pulzního tlaku bylo dáno především zvýšením systolického tlaku, odvozený centrální pulzní TK vykazoval obdobné výsledky. Tito jedinci (nositelé alely Trp pro alfa-adducin a homozygoti GG pro gama-adducin) měli také nižší poměr Na/K v moči, což svědčí pro zvýšenou aktivitu sodíko-draslíkové pumpy.

Z á v ě r : Výsledky svědčí pro významnou interakci polymorfismů alfa- a gama-adducinu ovlivňující solné hospodářství v ledvinách a následně tepennou rigiditu. U nositelů Trp alely genu pro alfa-adducin roste periferní i centrální pulzní tlak v závislosti na přítomnost G alely genu pro gama-adducin.

ad 4. Tepenné vlastnosti potomků normotenzních a hypertenzních rodičů

Cílem práce bylo porovnat vlastnosti velkých tepen a hodnoty TK normotenzních potomků hypertenzních a normotenzních rodičů.

Do analýzy bylo zařazeno 174 potomků hypertoniků (věk 17 - 40 let, alespoň jeden rodič hypertonik) a 59 potomků normotenzních rodičů (16 - 34 let). Vyšetřili jsme centrální a periferní augmentační indexy (CAI a PAI), centrální pulzní tlak a PWV. Analyzovali jsme TK měřený konvenčně i pomocí 24-hodinového monitorování TK.

Z á v ě r : Potomci hypertoniků měli vyšší hodnoty konvenčního TK, periferní pulzní tlak i 24-hodinový průměr TK. Měli též vyšší centrální pulzní tlak, oba

augmentační indexy a PWV. Po komplexní adjustaci, včetně adjustace na střední arteriální tlak i na věk, rozdíly v PAI, CAI a PWV ztratily statistickou významnost. Důvodem může být malý počet zařazených jedinců.

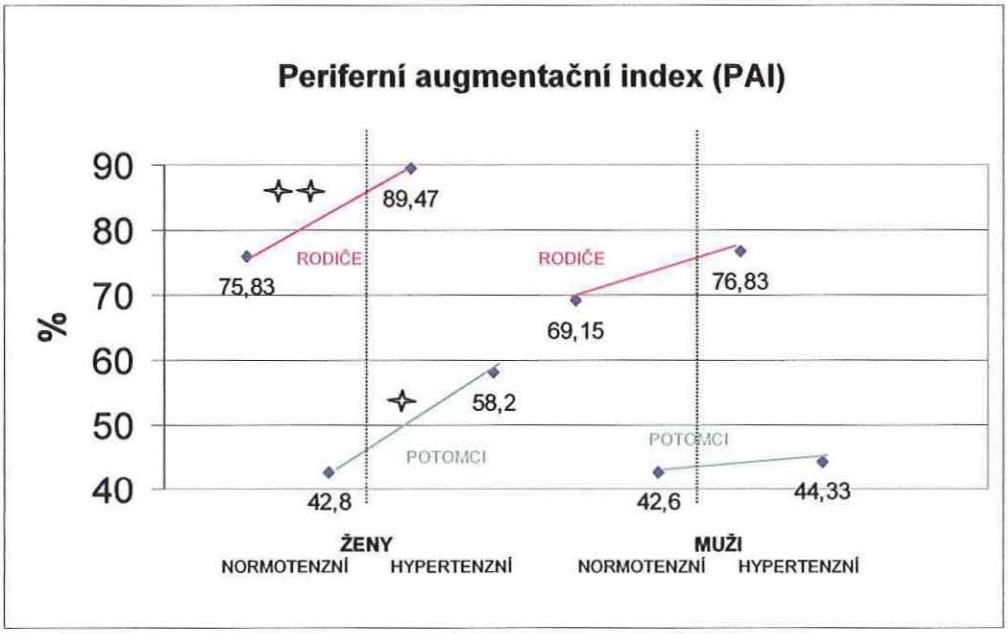
Naše výsledky ze studie EPOGH ve stručnosti

Přestože u hypertenzních rodičů byla arteriální hypertenze v průměru dobře kontrolovaná (průměrný TK 137/86 mmHg), měli systolický TK signifikantně vyšší než kontrolní rodiče (137 vs. 117 mmHg). Významný rozdíl v hodnotách STK jsme zjistili i mezi potomky vyšetřovaných skupin (120 vs. 113 mmHg).

Diastolický TK byl opět u hypertenzních rodičů významně vyšší oproti kontrolním rodičům (86 vs. 76 mmHg), u potomků se statistický rozdíl neprojevil (76 vs. 70 mmHg).

Rychlost šíření aortální pulzní vlny byla statisticky vyšší u hypertoniků než u kontrolních subjektů (7,5 vs. 6,44 m/s, p=0,0076), byla jednoznačně vyšší u rodičů než u jejich dětí, ale mezi potomky obou skupin nebyl rozdíl statisticky významný při komplexní adjustaci.

Hodnocení indexu odrazu na periferii (relativní parametr odrazu tlakové vlny v periferních tepnách, v %) jsme prováděli po rozdělení skupin podle pohlaví. Statisticky významný rozdíl byl mezi hypertenzními a kontrolními matkami – u hypertoniček je odražená vlna výrazně vyšší (89,47 vs. 75,83 %, p=0,0175), značný rozdíl byl též mezi dcerami obou skupin (58,2 vs. 42,8 %, p=0,0145). U mužů jsme statisticky významný rozdíl neprokázali *(obr. 9, 10)*.

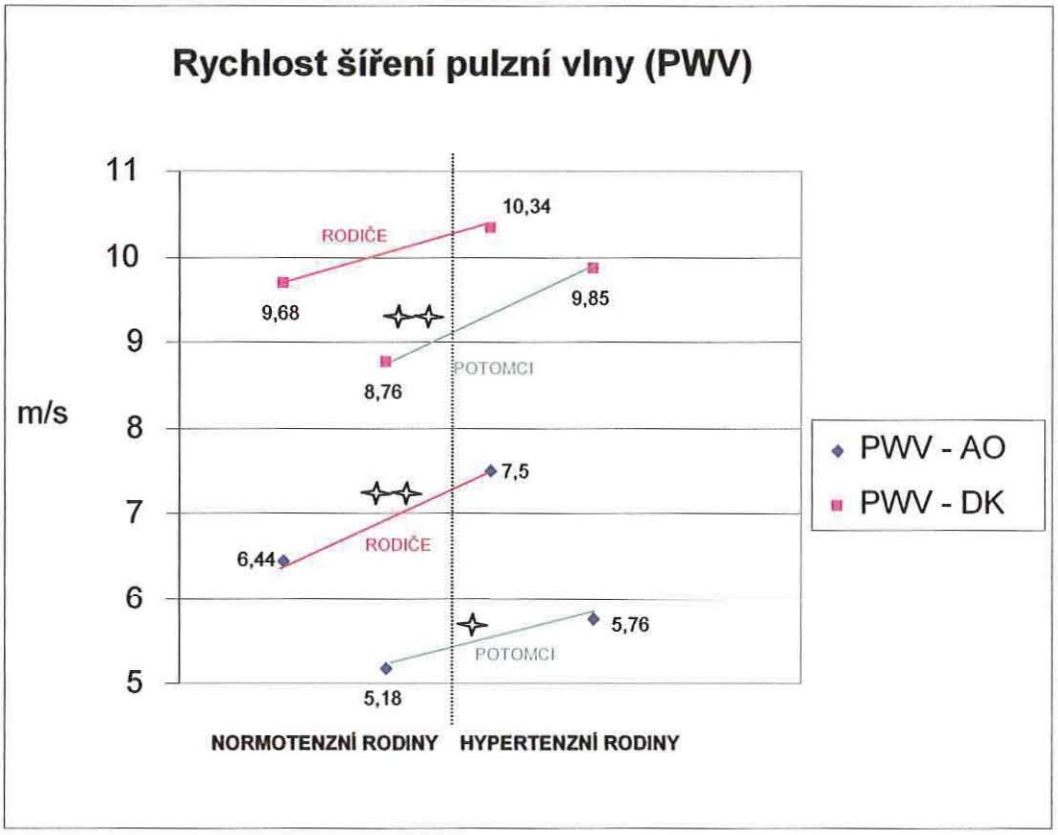


✧✧ Signifkantní rozdíl po adjustaci

✧ Signifikantní rozdíl bez adjustace

Adjustace zahrnuje pohlaví, věk, BMI, tepovou frekvenci, kouření.

Obr. 9
Výsledky ze studie EPOGH. Rozdíly mezi hodnotami periferního augmentačního indexu (PAI) u jednotlivých skupin rozdělených podle pohlaví. Blíže viz text.



✧✧ Signifikantní rozdíl po adjustaci

✧ Signifikantní rozdíl bez adjustace

Adjustace zahrnuje pohlaví, věk, BMI, tepovou frekvenci, kouření.

Obr. 10

Výsledky ze studie EPOGH. Rozdíly mezi hodnotami šíření pulzní vlny - aortální PWV a PWV na dolní končetině u jednotlivých skupin podle pohlaví. Blíže viz text.

Vysvětlivky: PWV (Pulse Wave Velocity) – rychlost šíření pulzní vlny,
PWV-AO – aortální rychlost šíření pulzní vlny, PWV-DK – rychlost šíření pulzní vlny po dolní končetině.

Mezinárodní projekt MONICA a vlastnosti cév v obecné populaci

MONICA = Multinational **MONI**toring of Trends and Determinants in **CA**rdiovascular Diseases.

Cíle studie:

Mezinárodní projekt koordinovaný Světovou zdravotnickou organizací byl zaměřen na zjištění výskytu kardiovaskulárního rizika a na objasnění hlavní příčiny snížení úmrtnosti na ICHS v mnoha zemích světa.

U obyvatelstva naší republiky bylo vyšetření systematicky prováděno od roku 1985 v tří- a čtyřletých intervalech v šesti okresech. Od roku 1997 se přidaly další tři okresy a vyšetřování bylo součástí nového výzkumného projektu podporovaného z grantu Ministerstva zdravotnictví České republiky (Post-MONICA) - cílem bylo pokračovat v monitoraci úrovně rizika nemocí oběhové soustavy u české populace.

Metodika:

Vyšetření rizikových faktorů bylo provedeno u osob ve věku 25 - 64 let, byli zváni ti, kteří se účastnili již předešlých vyšetření v rámci projektu MONICA a vždy byly vybrány i nové vzorky populace podle věku a pohlaví. Vyšetření v plzeňském centru (vyplňování dotazníků, měření TK, výšky a váhy, výpočet BMI a odběr žilní krve) byla zajišťována personálem LF a FN Plzeň spolu s kolegy z Institutu klinické a experimentální medicíny v Praze.

Při vyšetřování plzeňské populace v roce 2000 jsme k základnímu protokolu studie přidali vyšetření vlastností velkých cév analýzou pulzní vlny a stanovení PWV *(Tab. 2)*.

Věk	Muži		Ženy	
	Celkem	Vyš. cév	Celkem	Vyš. cév
25-34	58	29	49	26
35-44	67	29	72	30
45-54	78	49	79	47
55-65	81	36	89	45
Celkem	284	143	289	147

Tab. 2

Post-MONICA. Vyšetřená populace v plzeňském centru, rok 2000.

Komentáře k přiloženým pracím ze studie Post-MONICA

5. Rigidita velkých tepen a odraz pulzní vlny: výsledky z populační studie

6. Odpovídá klasifikace podle brachiálního systolického krevního tlaku kategoriím podle odhadnutého systolického tlaku v aortě?

7. Mírná hyperhomocysteinémie je v obecné populaci spojena se zvýšenou aortální rigiditou

8. Vztah mezi hladinami volného tyroxinu, aortální rigiditou a A1166C mutací genu pro AT1-receptor angiotensinu II v obecné populaci (pouze abstrakt)

ad 5. Rigidita velkých tepen a odraz pulzní vlny: výsledky z populační studie

Ve vyšetřovaném vzorku populace jsme ve shodě s výsledky autorů publikovaných prací zjistili stoupající hodnoty aortální PWV u obou pohlaví s věkem. PWV na dolní končetině však rostla pouze u žen, i když průměrná hodnota byla vyšší u mužů. Také hodnota PAI se zvyšovala u obou pohlaví v závislosti na věku, celkově byla vyšší u žen ve všech věkových skupinách. U mužů kuřáků se odražená vlna vracela dříve a jejich hodnota PAI výrazně převyšovala hodnoty nekuřáků. Z uvedených výsledků byla patrna závislost PAI zejména na věku, kouření, systolickém TK, celkovém cholesterolu a ženském pohlaví. Porovnáním korelací jsme zjistili, že PAI korelovala s CAI mnohem více (0,94) než s aortální PWV (0,22). PAI se tak jeví jako nejtěsnější parametr vlastností cév ve vztahu ke kardiovaskulárním rizikovým faktorům a vzhledem k tomu, že je měřen přímo nad a. radialis bez nutnosti dalšího matematického přepočtu, je vhodný k dalšímu studiu odrazu pulzní vlny a může poskytnout hodnotné údaje o tomto fenomenu.

ad 6. Odpovídá klasifikace podle brachiálního systolického krevního tlaku kategoriím podle odhadnutého systolického tlaku v aortě?

Při zpracovávání a hodnocení našich dat jsme si položili otázku, zda odpovídá klasifikace podle brachiálního systolického krevního tlaku kategoriím

podle odhadnutého systolického tlaku v aortě. Pokusili jsme se osoby rozdělit do tercilů podle hodnot jejich periferního TK a podle vypočítaných centrálních tlaků. Jako hraniční hodnoty jednotlivých kategorií pro brachiální TK jsme zvolili hodnotu optimálního TK a hodnotu pro definici arteriální hypertenze. Následně z tohoto rozdělení vyplynuly hranice pro kategorie centrálního TK, které byly odlišné pro ženy a muže *(Tab. 3, 4)*.

Z á v ě r : Je patrné, že určité procento jedinců, kteří podle hodnot periferního TK patří do skupiny normotoniků, má již hodnoty centrálního TK v nejvyšší kategorii. Naopak některé osoby v nejvyšší skupině periferního TK by podle centrálního TK mohly patřit do kategorie nižší.

ad 7. Mírná hyperhomocysteinémie je v obecné populaci spojena se zvýšenou aortální rigiditou

Homocystein (Hcy) je považován za silného a nezávislého ukazatele kardiovaskulárního onemocnění. Hledali jsme asociaci mezi Hcy a mechanickými vlastnostmi velkých tepen ve vzorku obecné populace (251 osob, průměrný věk 48 let). Měřili jsme aortální PWV a PWV na dolní končetině. Aortální PWV pozitivně korelovala s Hcy ($r=0,28$, $p<0.0001$), významně stoupající trend aortální PWV byl zaznamenán v kvartilech hodnot Hcy ($p=0,0003$, ANOVA). Osoby s mírnou hyperhomocysteinémií (Hcy>=15 mikromol/l) měli více než 2,5 krát vyšší riziko aortální PWV nad 8,42 m/s. Tyto vztahy se nepotvrdily pro PWV na dolní končetině ani pro periferní augmentační index.

Z á v ě r : V našem vzorku obecné populace jsme našli silný a nezávislý vztah mezi hladinou homocysteinu a rigiditou centrálního řečiště měřenou pomocí aortální PWV.

centrální / periferní	<=107	108-125	>125	celkem	
<120	**14 (9.8%)**	5 (3.5%)	0	19 (13.3%)	***15 (10.5%)***
120-139	5 (3.5%)	**45 (31.5%)**	10 (7.0%)	60 (42.0%)	
>=140	0	5 (3.5%)	**59 (41.3%)**	64 (44.7%)	***10 (7%)***
celkem	**19 (13.3%)**	**55 (38.5%)**	**69 (48.2%)**	**143 (100%)**	

Tab. 3
Rozdělení populace podle STK (mmHg) – muži.

Hranice centrálního STK 107 a 125 mmHg odpovídají stejným percentilám brachiálního STK 120 a 140 mmHg.

centrální / periferní	<=108	109-130	>130	celkem	
<120	**36 (24.3%)**	11 (7.4%)	0	47 (31.8%)	***14 (9.4%)***
120-139	8 (5.4%)	**50 (33.8%)**	3 (2.0%)	61 (41.2%)	
>=140	0	2 (1.4%)	**38 (25.7%)**	40 (27.0%)	***10 (6.8%)***
celkem	**44 (29.7%)**	**63 (42.6%)**	**41 (27.7%)**	**148 (100%)**	

Tab. 4
Rozdělení populace podle STK (mmHg) – ženy.

Hranice centrálního STK 108 a 130 mmHg odpovídají stejným percentilám brachiálního STK 120 a 140 mmHg.

ad 8. Vztah mezi hladinami volného tyroxinu, aortální rigiditou a A1166C mutací genu pro AT1-receptor angiotensinu II v obecné populaci (pouze abstrakt)

Hormony štítné žlázy mají přímý proliferační účinek na kardiovaskulární systém, ovlivňují i expresi sympatiku a renin-angiotensinového systému. Cílem naší analýzy bylo zjistit, zda i mírné změny hladin volného tyroxinu (fT4) budou spojeny se změnami aortální rigidity (měřené pomocí PWV) a zda tento vztah může být modifikován polymorfismem genu pro AT1-receptor angiotensinu II.

Jako podvzorek plzeňského souboru studie post-MONICA bylo vybráno 249 osob (121 mužů, 128 žen, průměrný věk 48 let). Rychlost pulzní vlny byla měřena pomocí zařízení SphygmoCor, mutace A1166C pomocí PCR. Sledované parametry byly porovnány podle kvintilů fT4: optimální (II.-IV. kvintil), nízký-normální (I. kvintil) a vysoký-normální (V. kvintil).

U osob s homo- či heterozygotní mutací genu (A1166C) byl mezi skupinami optimálního, nízkého-normálního a vysokého-normálního kvintilu zjištěn statisticky významný rozdíl v hodnotách aortální PWV: 7,26+/-0,20 vs. 7,46+/-0,42 ($p=0,88$), vs. 8,63+/-0,72 m/s ($p<0,004$). V ostatních parametrech se skupiny statisticky významně nelišily.

Z á v ě r : V našem souboru relativně zdravých probandů jsme nalezli, že i mírně zvýšené hladiny fT4 jsou spojeny se zvýšenou aortální rigiditou, tento vztah však dosáhl statistické významnosti pouze u nositelů homo- či heterozygotní A1166C mutace genu pro AT1-receptor angiotensinu II.

Celkové shrnutí

V lidské společnosti je jedním z hlavních sociálních faktorů zdraví a péče o něj. Za posledních 100 let bylo ve zdravotnictví dosaženo výrazného pokroku. Nejen díky tomu se průměrná délka lidského života ve vyspělých zemích takřka zdvojnásobila.

Hlavním rysem zdravotnictví budoucnosti - dle názorů a zkušeností předních odborníků uvedených v „Medivizi 2000" - bude především preventivní a osvětová činnost. V oblasti léčebné prevence a léčby se budou stále více uplatňovat přístupy, kde zásadní roli sehraje znalost úplné lidské genetické informace.

Vezmeme-li v úvahu uvedené tvrzení, pak by k prevenci a léčbě měla směřovat a přispět též naše aktivní účast na mezinárodních či národních projektech. Konkrétní závěry ze studií, na kterých jsme se účastnili, jsou následující:

ze studie EPOGH

- Hypertonici středního věku měli vyšší rigiditu aorty a v případě žen (matek i dcer) signifikantně vyšší odraženou tlakovou vlnu navzdory dobré kontrole TK
- Děti hypertoniků měly signifikantně vyšší systolický TK než děti kontrol, nelišily se rigiditou aorty

ze studie Post-MONICA

- Periferní augmentační index (PAI), který je přímo měřeným parametrem, se díky velmi těsné korelaci s centrálním augmentačním indexem (CAI) jeví jako nejvhodnější parametr ke studiu reflekčních vlastností tepen
- Kuřáctví zvyšuje odraz tlakových vln
- Pokud považujeme hodnotu centrálního systolického krevního tlaku za jeden z nejdůležitějších hemodynamických parametrů, pak podle našich výsledků má konvenční klasifikace osob do jednotlivých skupin podle brachiálního TK omezenou platnost

- Hyperhomocysteinémie je u obecné populace spojena se zvýšenou aortální rigiditou, toto riziko se projevuje již u mírně zvýšených hodnot homocysteinu
- Mírně zvýšené hladiny fT4 (v obecné populaci ještě v mezích normálu) zvyšují aortální rigiditu; statistická významnost se projevila při kombinaci s mutací genu pro AT1-receptor angiotensinu II

Doufám, že naše práce na výše uvedených projektech bude přispívat nejen dalšímu teoretickému rozvoji, ale že dojde i k většímu využití neinvazivních metodik při vyšetřování kardiovaskulárního systému v klinické praxi. Chce se mi věřit, že získané výsledky budou významným příspěvkem jak pro lékaře edukující své pacienty o zásadách správného životního stylu, tak i pro vývoj nové léčby v rámci farmakogenetického přístupu.

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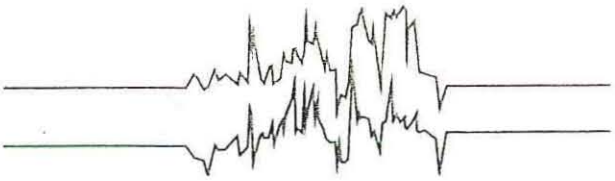
9. Citační ohlas dle databáze web of science – pro obě studie.

Příloha 1

ISSN 1359-5237

Blood Pressure Monitoring



http://www.bpmonitoring.com

Quality control of the blood pressure phenotype in the European Project on Genes in Hypertension

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Objectives In the European Project on Genes in Hypertension (EPOGH) standardized epidemiological methods were used to determine complex phenotypes consisting of blood pressure (BP) in combination with other traits. In this report, we present the quality control of one of the BP phenotypes.

Methods In seven European countries eight different research groups recruited random samples of nuclear families. Trained observers measured the BP five times consecutively with the participants in the seated position at each of two separate home visits, 1 to 3 weeks apart, according to the guidelines of the British Hypertension Society. Quality assurance and quality control of this BP phenotype were implemented according to detailed instructions defined in the protocol of the EPOGH study.

Results On 31 August 2001, BP measurements of 2476 subjects were available for analysis. Fewer BP readings than the five planned per visit occurred in one of the eight centres, but only in 0.4% of the home visits. Across centres the relative frequency of identical consecutive readings for systolic or diastolic blood pressure varied from 0 to 6%. The occurrence of odd readings ranged from 0 to 0.1%. Of the 49 488 systolic and diastolic BP readings, 24.0% ended on a zero (expected 20%). In most EPOGH centres there was a progressive decline in the BP from the first to the second home visit. Overall, these decreases averaged 2.36 mmHg [95% confidence interval (CI): 1.98–2.74, $P < 0.001$] for systolic BP and 1.74 mmHg (95% CI: 1.46–2.02, $P < 0.001$) for diastolic BP.

Conclusions Quality assurance and control should be planned at the design stage of a project involving BP measurement and implemented from its very beginnings until the end. The procedures of quality assurance set up in the EPOGH study for the BP measurements resulted in a well-defined BP phenotype, which was consistent across centres. *Blood Press Monit* **7**: 215–224 © 2002 Lippincott Williams & Wilkins.

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Introduction

Accurate measurement of the blood pressure phenotype is of paramount importance in studies on the genetic determination of hypertension [1]. In most studies in humans, the blood pressure phenotype is the mean of three to five readings obtained in a single occasion. A number of automated electronic devices have become available for blood pressure measurement under static or ambulatory conditions [2–4]. However, until now auscultatory blood pressure measurement using the Riva-Rocci/Korotkoff technique remains the standard in clinical and epidemiological research.

The European Project on Genes in Hypertension (EPOGH) involves eight centres in seven European countries. Its main objective is to identify genetic polymorphisms that are significantly associated with blood pressure as a continuous or dichotomous trait. In addition to ambulatory blood pressure monitoring and measurement of the clinic blood pressure, the blood pressure phenotype

in the EPOGH study also consists of five consecutive blood pressure readings obtained with the subjects seated at each of two separate home visits. Intra- and inter-observer variability in blood pressure measurements may already be large in single-centre studies and may even further increase in large-scale epidemiological projects, involving multiple centres. In this article we present the initial progress of the EPOGH study together with the results of the quality control programme of the blood pressure phenotype measured at the participants' homes.

Methods

Fieldwork

Random samples of nuclear families were recruited in Hechtel-Eksel [Belgium (B)], Sofia [Bulgaria (BU)], Pilsen and Prague [Czech Republic (CZ)], Mirano [Padova, Italy (I)], Cracow [Poland (PL)], Bucharest [Romania (RO)], and Novosibirsk [Russian Federation (RF)]. To increase the number of hypertensive patients, four groups (those of Padova, Cracow, Bucharest, and Novosibirsk) also recruited approximately 30% of the required number of nuclear families via specialized clinics for hypertensive patients.

Nuclear families had to include at least one parent and two siblings. The minimum age for participation was 10 years. Family members had to live within a distance of no more than approximately 10 km to make repeated home visits feasible.

Trained observers measured blood pressure with a standard mercury sphygmomanometer five times consecutively during each of two home visits. The guidelines of the British Society of Hypertension [5] were applied. Standard cuffs had a 12×24 cm inflatable bladder, but, if upper arm circumference exceeded 31 cm, larger cuffs with 15×35 cm bladder were used. After at least 10 min rest, five consecutive blood pressure readings were obtained in the sitting position with an interval of 30 to 60 s between readings. The cuff was deflated at approximately 2 mmHg per second, and systolic and phase V diastolic blood pressure were recorded to the nearest 2 mmHg. Each subject's conventional blood pressure was the mean of the 10 readings obtained at home.

Quality assurance procedures

Quality assurance refers to the procedures set up at all centres to ensure high quality blood pressure measurements throughout the project. At the start of the EPOGH study (November 1998), we organized a 1-week workshop at the Coordinating Office in Leuven, Belgium. Subsequently, during the course of the study, investigators of four centres requested further training and visited the Coordinating Office, respectively, in June 1999, June 2000, October 2000, and February 2001. On each occasion, the investigators took a refresher course on the procedures of blood pressure measurement.

At each field centre, quality assurance sessions were organized at three to six monthly intervals to reinforce the theoretical concepts and to rehearse the practical procedures of sphygmomanometric blood pressure measurement. At each session the observers had to pass a test requiring them to read blood pressures from videotape featuring a falling mercury column with simultaneous Korotkoff sounds (Measuring Blood Pressure; British Medical Association, London, 1990). For each session a specific standard was computed which reflected the acoustic conditions under which the test had taken place, and which was computed by averaging the film readings of experienced senior clinical researchers. All readings from each observer had to be within 5 mmHg of the standard. If an observer failed to pass the test, she/he did not participate in blood pressure measurement until re-tested successfully at a later session. The intra-observer reproducibility was studied by comparing 10 pairs of identical video simulations of blood pressure measurements and was calculated as twice the standard deviation of the differences between duplicate readings. Moreover, the reproducibility coefficient was expressed as a percentage of the mean of the identical readings. Digit preference was not evaluated at the training sessions, but computed from the blood pressure measurements in study participants.

All observers involved in the study completed a questionnaire providing information on their gender, age, and qualification (paramedic, nurse, or medical doctor). In addition, in the same questionnaire, the observers provided information on the technical characteristics of the sphygmomanometers used for blood pressure measurement at subjects' homes (aneroid versus mercury; cuff size).

Quality control

The present analysis includes the blood pressure readings obtained at the participants' homes and made available to the Coordinating Office before 31 August 2001. From published guidelines [5,6] and six previous studies [7–12] we selected six criteria applicable to the design and the multicentre character of the EPOGH study:

1. In each participant, the blood pressure measurements were considered as complete if all five systolic and diastolic blood pressure readings at each of the two separate home visits were available in the database.
2. Five consecutive blood pressure readings obtained in a subject were considered as identical, if there was no single difference between any of the systolic or diastolic blood pressure values among the five readings. The frequency of consecutive five identical readings was determined.

4. The proportion of odd blood pressure readings with as terminal digit 1, 3, 5, 7, or 9 was determined for systolic and diastolic measurements separately.
4. Digit preference, i.e., the distribution of the last digits of all single systolic and diastolic blood pressure readings was monitored at three-monthly intervals throughout the EPOGH project.
5. To assess the consistency between centres, we evaluated the blood pressure changes from the first to the second home visit and across the ten readings obtained at the two home visits.
5. To investigate the pattern of variation between observers, the mean for each individual observer's blood pressure readings was computed and compared with the overall within-centre mean.

Statistical analysis

We used the SAS software package, version 6.12. (SAS Institute, Cary, North Carolina, USA) for database management and statistical analysis. Comparison of means and proportions were performed with the standard normal z-test and the χ^2-statistic, respectively. To assess intra-observer variability, Bland and Altman's technique [13] was applied. We used analysis of covariance to compare blood pressure measurements between observers with adjustment for sex, age, body mass index, antihypertensive treatment, smoking, alcohol intake, and the use of oral contraceptives. To compare trends in the consecutive blood pressure measurements between centres we used repeated measures analysis of variance and we determined the significance of the interaction terms between centre and the order of the blood pressure readings.

Results

Characteristics of the study population

The 2476 participants included 1173 men (47.4%) and 605 hypertensive patients (24.4%) of whom 328 were on antihypertensive drug treatment (Table 1). The subjects ranged in age from 10 to 84 years. Among the men, 32.5% ($n = 374$) were current smokers, and 57.8% ($n = 661$) reported intake of alcohol. In women, these proportions were 21.4% ($n = 273$) and 36.1% ($n = 460$), respectively. Among women, 20.0% ($n = 261$) used oral contraceptives.

Characteristics of the observers

The number of observers employed per centre ranged from one to six (Table 2). The observers' age ranged from 25 to 62 years. Most observers (84%) were female and/or medical doctors (77%). Two centres employed only nurses for measuring blood pressure during the study (Table 2). The number of blood pressure readings per observer ranged from 60 to 4560. All centres used standard mercury sphygmomanometers and adjusted cuff size according to arm circumference.

Video test

Table 3 summarizes the results of all training sessions by centre and gives for each centre the distribution of the differences between the observers' film readings and the standard (20 differences per observer during one training session) and the intra-observer reproducibility. Overall, 88% of the observers' systolic pressure readings were within $\pm$ 5 mmHg of the standard. For diastolic pressure this proportion was 87.4%. The repeatability coefficient across seven centres and 29 observers was 5.4% for systolic pressure and 6.4% for diastolic pressure.

Quality control according to six predefined criteria

Fewer blood pressure readings than the five projected per visit occurred in one of the eight centres, but only in 0.4%. of the home visits (Table 4). The frequency of identical consecutive readings for systolic or diastolic blood pressure varied across the centres from 0 to 6%. The occurrence of odd readings which represented a deviation from the study protocol, ranged from 0 to 0.1%. Of 49 488 systolic and diastolic blood pressure readings, 24.0% ended on a zero (Fig. 1). The difference with the expected frequency of 20% was statistically significant ($\chi^2 = 390.5$; $P < 0.001$).

Table 1 Characteristics of the participants

Characteristics	Hechtel-Eksel (B)	Sofia (BU)	Pilsen (CZ)	Prague (CZ)	Mirano (I)	Cracow (PL)	Bucharest (RO)	Novosibirsk (RF)	Total
Number[a]	1024	40	190	42	346	325	193	316	2476
Female (%)	513 (50.1)	21 (52.5)	101 (53.2)	17 (40.5)	187 (54.0)	176 (54.2)	111 (57.5)	177 (56.0)	1303 (52.6)
Age (years)	37.4 ± 16.8	37.1 ± 14.5	37.4 ± 13.5	39.2 ± 13.8	41.1 ± 14.0	35.5 ± 13.9	39.4 ± 16.0	38.9 ± 15.0	38.1 ± 15.5
Body mass index (kg/m^2)	24.6 ± 4.5	23.7 ± 4.1	26.2 ± 4.9	24.3 ± 4.2	25.3 ± 4.3	25.5 ± 4.9	24.8 ± 5.5	25.3 ± 5.0	25.0 ± 4.8
Systolic pressure (mmHg)[b]	121.4 ± 14.4	123.7 ± 17.6	123.5 ± 15.5	116.9 ± 10.9	126.5 ± 16.1	129.3 ± 18.1	124.6 ± 21.5	124.6 ± 17.6	123.9 ± 16.5
Diastolic pressure (mmHg)[b]	74.8 ± 10.7	79.6 ± 10.9	77.9 ± 10.1	74.7 ± 8.0	80.6 ± 9.8	80.8 ± 11.5	79.3 ± 13.5	79.4 ± 11.3	77.7 ± 11.2
Heart rate (beats/min)	66.8 ± 8.2	76.0 ± 10.6	70.7 ± 8.6	73.8 ± 8.9	73.2 ± 8.8	73.8 ± 9.0	75.6 ± 8.2	74.1 ± 7.4	70.7 ± 9.1
Hypertensive (%)	189 (18.5)	11 (27.5)	46 (24.2)	4 (9.8)	106 (30.6)	112 (34.5)	52 (26.9)	85 (26.9)	605 (24.4)
Taking antihypertensive drugs (%)	106 (56.1)	7 (63.6)	35 (76.1)	2 (50.0)	50 (47.2)	63 (56.3)	20 (38.5)	45 (52.9)	328 (54.1)

Values are arithmetic means (SD), or number of subjects (%). [a]Number of subjects with data available at the Coordinating Office. [b]Average of 10 readings at the first and second home visit.

Table 2 Characteristics of observers

Centre	Age group (years)		Gender		Qualification	
	25–35	> 35	Male	Female	Nurse	Doctor
Hechtel-Eksel (B) $n = 4$	2 (50%)	2 (50%)	–	4 (100%)	4 (100%)	–
Sofia (BU) $n = 2$	2 (100%)	–	1 (50%)	1 (50%)	–	2 (100%)
Pilsen (CZ) $n = 3$	2 (67%)	1 (33%)	–	3 (100%)	1 (33%)	2 (67%)
Prague (CZ) $n = 1$	–	1	–	1	1	–
Mirano (I) $n = 6$	4 (67%)	2 (33%)	2 (33%)	4 (67%)	–	6 (100%)
Cracow (PL) $n = 6$	4 (67%)	2 (33%)	1 (17%)	5 (83%)	1 (17%)	5 (83%)
Bucharest (RO) $n = 6$	2 (33%)	4 (67%)	1 (17%)	5 (83%)	–	6 (100%)
Novosibirsk (RF) $n = 3$	2 (67%)	1 (33%)	–	3 (100%)	–	3 (100%)
Total $n = 31$	18 (58%)	13 (42%)	5 (16%)	26 (84%)	7 (23%)	24 (77%)

Table 3 Results of training sessions for observers

Centre	Hechtel-Eksel (B)		Sofia (BU)†		Pilsen (CZ)		Prague (CZ)		Mirano (I)		Cracow (PL)		Bucharest (RO)		Novosibirsk (RF)	
Number of sessions	5		1		1		1		7		5		2		5	
	SBP (%)	DBP (%)	SBP (%)	DBP (%)	SBP (%)	DBP (%)	SBP (%)	DBP (%)	SBP (%)	DBP (%)	SBP (%)	DBP (%)	SBP (%)	DBP (%)	SBP (%)	DBP (%)
Deviation of observers' readings *vs.* reference																
> 10 mmHg	1.5	0.5	0	0	0	0	0	0	3.3	3.3	1.3	3.0	1.9	4.4	0	0
8–10 mmHg	1.8	2.8	5.0	5.0	0	0	0	0	0	1.3	2.5	0	3.8	3.7	0	0
5–7 mmHg	5.5	3.5	5.0	0	0	0	0	0	1.5	0.8	4.3	1.8	5.6	7.5	0	0
2–4 mmHg	31.5	30.8	5.0	20.0	16.7	30.0	10.0	25.0	4.8	11.9	12.2	15.0	25.0	21.3	25.0	16.0
−1 to 1 mmHg	39.3	38.3	80.0	65.0	53.3	45.0	35.0	45.0	62.7	45.8	51.2	50.7	47.5	35.0	65.0	68.0
−2 to −4 mmHg	14.8	21.3	5.0	10.0	30.0	25.0	50.0	30.0	20.8	27.1	23.7	23.2	11.8	13.1	10.0	14.0
−5 to −7 mmHg	0.8	0.8	0	0	0	0	5.0	0	5.0	8.1	1.8	4.0	1.9	4.4	0	2.0
−8 to −10 mmHg	2.3	0.8	0	0	0	0	0	0	1.3	1.0	1.5	1.2	1.3	3.1	0	0
< −10 mmHg	2.8	1.5	0	0	0	0	0	0	0.6	0.6	1.5	1.2	1.2	7.5	0	0
Reproducibility‡	5.2 (3.3)	6.8 (6.3)	4.0 (2.4)	9.5 (8.7)	2.7 (1.6)	3.6 (3.4)	2.8 (1.9)	2.8 (2.6)	8.8 (5.3)	6.6 (6.0)	13.7 (8.1)	5.7 (5.3)	9.4 (5.3)	10.9 (10.4)	2.2 (1.4)	3.0 (2.9)

Observers read 20 blood pressures per session from a video movie showing a falling mercury column with Korotkoff sounds. †One observer's result not yet available at the Coordinating Office at the time of writing of this article. ‡2SD of the changes between identical blood pressures from a video movie. See Methods for further explanations. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 4 Qualitative indicators for control of blood pressure measurement

	Hechtel-Eksel (B)	Sofia (BU)	Pilsen (CZ)	Prague (CZ)	Mirano (I)	Cracow (PL)	Bucharest (RO)	Novosibirsk (RF)
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Number of home visits	2103	80	380	84	692	650	398	632
Incomplete BP measurements†	8 (0.4%)	0	0	0	0	0	0	0
Number of five consecutive BP readings‡	4206	160	760	168	1384	1300	796	1264
Identical readings	66 (1.6%)	1 (0.6%)	0	10 (6.0%)	41 (3.0%)	1 (0.1%)	1 (0.1%)	0
Total number of BP readings‡	21018	800	3800	840	6920	6500	3980	6320
Odd readings	7 (0.03%)	1 (0.1%)	0	1 (0.1%)	6 (0.08%)	6 (0.09%)	0	0

†Less than five systolic or five diastolic measurements per home visit. ‡Systolic and diastolic readings were counted as separate measurements.

Among individual observers the proportion of blood pressure readings with a terminal zero ranged from 15.5 to 47.6% (Table 5). Five observers in three centres were found to record blood pressure values with a terminal zero in excess of 30% (47.6, 32.9, 31.8, 31.5 and 31.4%).

In most EPOGH centres there was a significant and progressive decline in the conventional blood pressure from the first to the second home visit (Fig. 2). Across all centres, blood pressure decreased by 2.36 mmHg (95% confidence interval (CI): 1.98–2.74, $P < 0.001$) systolic and by 1.74 mmHg (95% CI: 1.46–2.02, $P < 0.001$) diastolic. However, as illustrated in Figure 3, there were significant trend differences between the EPOGH centres in the blood pressure changes across the ten readings of systolic ($F = 5.32$, $P < 0.001$) and diastolic blood pressure ($F = 3.71$, $P < 0.001$). Moreover, the decline of blood pressure on repeat measurement depended on the level of pressure (Figs 2 and 3). For instance, in comparison with other EPOGH centres, we observed more prominent

ecreases in systolic (5.25; 95% CI: 3.91–6.59) and diastolic 3.24; 95% CI: 2.32–4.16) blood pressures from the first to ıe second home visit in Cracow. In this centre blood pressure at initial home visit was higher then in all other centres.

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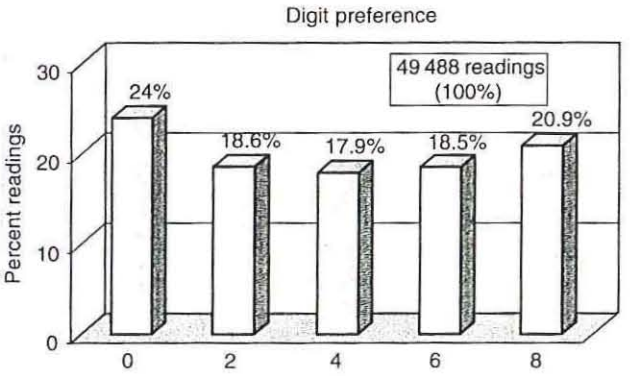


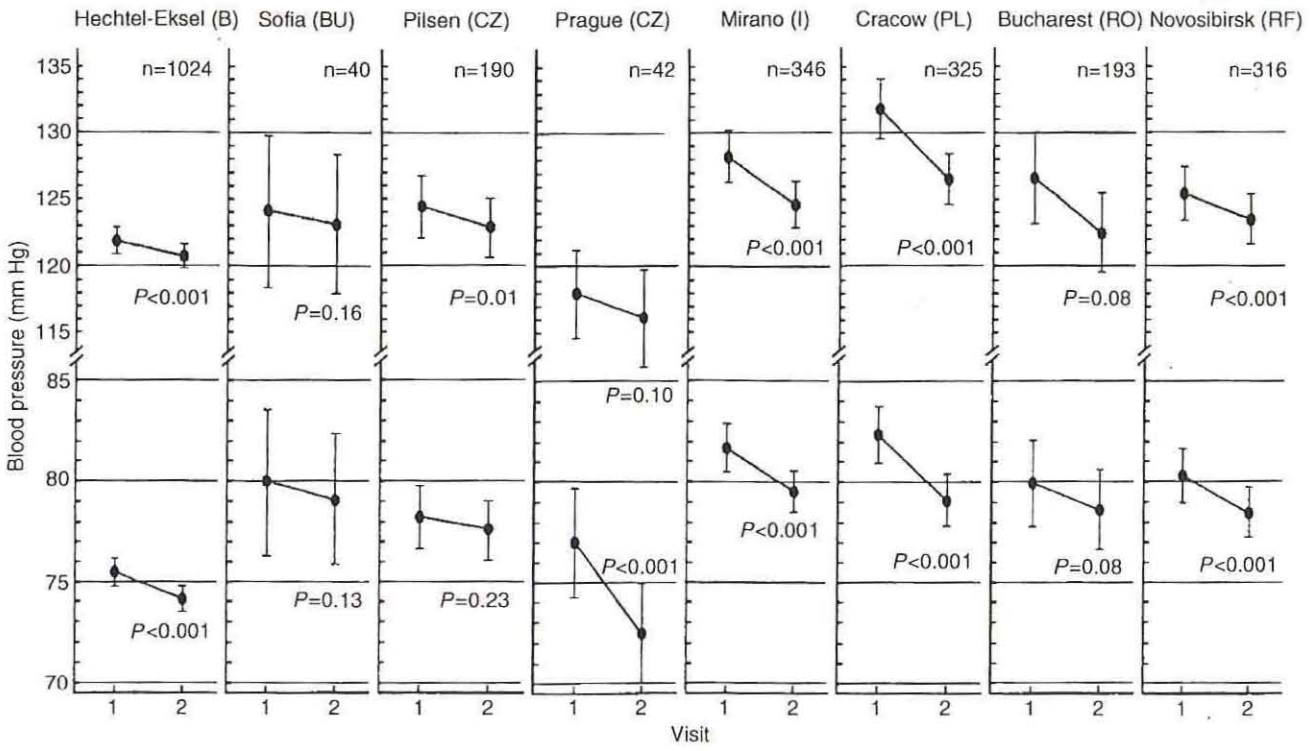
)igit preference in the blood pressure readings across six EPOGH centres. The proportion of systolic or diastolic blood ›ressure readings with an even terminal digit is presented.

Figure 4 shows the deviations between the mean of each individual observer's blood pressure readings and the overall within-centre mean for the eight EPOGH centres. These deviations were adjusted for sex, age, body mass index, antihypertensive treatment, smoking, alcohol intake, and the use of oral contraceptives. For systolic blood pressure, the deviations ranged from −1.48 to +2.07 mmHg in Hechtel-Eksel (Belgium), from −0.50 to +0.50 mmHg in Sofia (Bulgaria), from −1.17 to +0.99 mmHg in Pilsen (Czech Republic), from −4.86 to +7.33 mmHg in Mirano (Italy), from −1.46 to +3.19 mmHg in Cracow (Poland), from −1.80 to +2.79 mmHg in Bucharest (Romania), and from −2.86 to +2.01 mmHg in Novosibirsk (Russia). For diastolic blood pressure, the deviations ranged from −3.00 to +2.90 mmHg in Hechtel-Eksel (Belgium), from −0.33 to +0.33 mmHg in Sofia (Bulgaria), from −1.45 to +1.03 mmHg in Pilsen (Czech Republic), from −2.04 to +4.33 mmHg in Mirano (Italy), from −1.11 to +0.62 mmHg in Cracow (Poland), from −4.80 to

Table 5 Digit preference

Observer	0		2		4		6		8		Total number of BP readings
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Hechtel-Eksel (B)											
1	743	47.6	162	10.4	261	16.7	197	12.6	197	12.6	1560
2	2687	31.8	1436	17.0	1107	13.1	1623	19.2	1595	18.9	8448
3	1416	15.5	1667	18.3	1963	21.5	2021	22.2	2047	22.4	9120
4	415	22.0	342	18.1	344	18.2	353	18.7	431	23.0	1890
Pilsen (CZ)											
1	420	20.8	414	20.5	428	21.2	368	18.2	390	19.3	2020
2	200	20.4	184	18.8	186	19.0	185	18.9	225	23.0	980
3	189	23.6	143	17.9	149	18.6	148	18.5	171	21.4	800
Prague (CZ)											
1	246	31.4	166	19.8	119	14.2	105	12.5	185	22.0	840
Sofia (BU)											
1	68	17.0	97	24.3	94	23.5	72	18.0	69	17.3	400
2	87	21.8	60	15.0	59	14.8	86	21.5	107	26.8	400
Mirano (I)											
1	435	25.3	331	19.2	316	18.4	239	13.9	398	23.1	1720
2	60	27.3	42	19.1	40	18.2	29	13.2	48	21.8	220
3	666	18.8	768	21.7	719	20.3	732	20.7	652	18.4	3540
4	30	25.0	24	20.0	22	18.3	23	19.2	21	17.5	120
5	100	17.9	134	23.9	109	19.5	93	16.6	123	22.0	560
6	134	19.1	116	16.6	157	22.4	146	20.9	147	21.0	700
Cracow (Poland)											
1	288	24.8	213	18.4	202	17.4	188	16.2	268	23.1	1160
2	288	18.9	272	17.9	293	19.3	304	20.0	362	23.8	1520
3	200	29.4	101	14.9	146	21.5	89	13.1	143	21.0	680
4	170	31.5	85	15.7	86	15.9	92	17.0	107	19.8	540
5	306	17.2	426	23.9	339	19.0	277	15.6	432	24.3	1780
6	270	32.9	129	15.7	130	15.9	98	12.0	190	23.2	820
Bucharest (RO)											
1	40	28.6	34	24.3	25	17.9	20	14.3	21	15.0	140
2	201	22.3	187	20.8	142	15.8	148	16.4	222	24.7	900
3	378	27.4	256	18.6	205	14.9	254	18.4	287	20.8	1380
4	338	21.7	307	19.7	266	17.1	309	19.8	340	21.8	1560
Novosibirsk (RF)											
1	808	26.2	566	18.4	524	17.0	525	17.0	657	21.3	3080
2	36	22.5	34	21.3	36	22.5	31	19.4	23	14.4	160
3	827	26.9	602	19.5	514	16.7	508	16.5	629	20.4	3080

Fig. 2



Mean systolic and diastolic blood pressure values at the first and second home visits. *P* values for the differences between the two visits are given.

+2.64 mmHg in Bucharest (Romania), and from −0.35 to +0.22 mmHg in Novosibirsk (Russia). The significance of these systolic and diastolic deviations increased with the number of subjects examined by each observer, with the amount that an observer's readings deviated from the overall within-centre population mean, and with the total number of subjects per centre.

Discussion

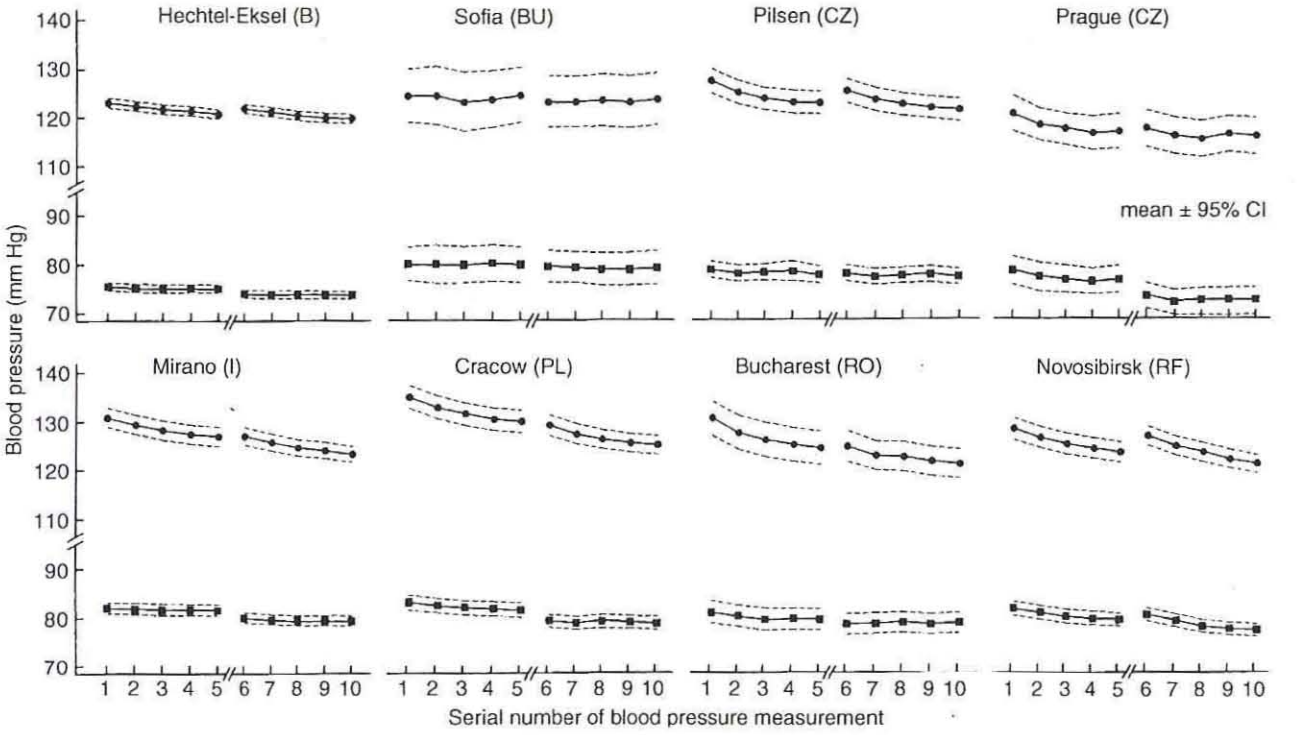
The main objective of the EPOGH study was to investigate a well-standardized blood pressure phenotype in relation to genetic polymorphism. This report focuses on the quality assurance and quality control procedures, which were an essential and intricate part of the EPOGH protocol.

Accurate determination of the blood pressure levels in large-scale surveys or multicentre studies requires central coordination and the implementation of a standardized protocol. The measurement of blood pressure according to Riva-Rocci/Korotkoff technique [14] is dependent on the accurate transmission and interpretation of a signal (Korotkoff sound or pulse wave) from a subject via a device (the sphygmomanometer) to an observer [15,16]. The successful outcome of this complex interaction requires that the observer is competent in performing the technique of blood pressure measurement, that the subject is examined in basal and standardized conditions, that the equipment used for the measurement is well maintained and calibrated, and that the blood pressure readings are archived accurately. This procedure is fraught with sources of potential error, which may arise in the observer, the subject, the sphygmomanometer or in the overall application of the technique [4,15,17,18]. The quality assurance and the quality control in our study focused on these potential sources of inaccuracy.

In our study, subjects were visited at home on two separate occasions and trained observers, either doctors or nurses, measured blood pressure in the relaxed home environment. This procedure of blood pressure measurement tends to increase the participation rate and has been validated in several epidemiological studies in Belgium. Blood pressure, measured this way, shows the expected associations with gender, age, body mass index, social class, and physical activity [19,20].

Our quality assurance programme was based on published guidelines [5,6]. Its objective was to ensure high quality

g. 3



ystolic and diastolic blood pressure values for five consecutive readings at two separate home visits. Values are mean with 95% onfidence intervals.

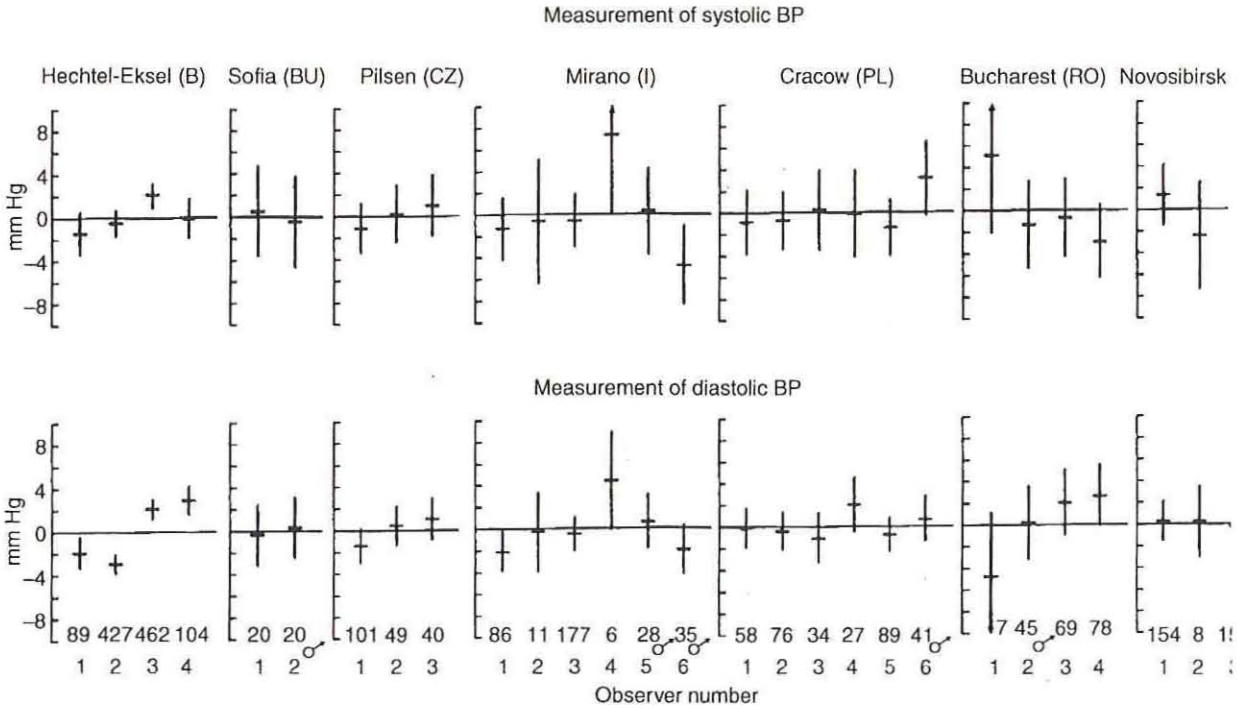
lood pressure measurements throughout the whole roject, in which eight different researches groups and 27 bservers participated. Various training programmes have een developed to minimize observer error [5,6,21], most f which use a film or a video showing a falling mercury olumn with Korotkoff sounds as the main component of aining and quality assessment. The film allows quantify-g inter- and intra-observer variability. In our study quality ssurance was set up via repeated training sessions. The bservers always received the results of their tests, so that ney remained aware of their performance and were ncouraged to improve their measurement technique.

n our quality control programme we used six criteria, ecause they reflect different problems that may occur uring blood pressure measurement. Some of these items re conceived as qualitative rather than quantitative ndicators of the accuracy of blood pressure measurement. The proportion of incomplete measurements is a strong ndicator of validity problems in terms of population epresentativeness [7]. The occurrence of odd digits is kely to reflect forgetfulness of the protocol or a desire for xtra accuracy by the observer [10]. It might also indicate a ss of accuracy. The frequency of identical blood pressure eadings in series of repeated blood pressure readings ight influence the overall shape of the blood pressure distribution and the prevalence of diagnostic categories based on blood pressure thresholds [10,22]. It is well known from clinical and epidemiological studies [9,15,22] that repeated blood pressure measurements in the same subject are in most instances non-identical. The degree, to which the frequency of identical readings can be considered normal or at least acceptable, is not clearly defined. All eight EPOGH centres appeared to have complied well with these predefined quality criteria (Table 4).

Owing to habitation and regression to the mean, blood pressure usually falls when repeated measurements are obtained during a single visit or at consecutive visits over the course of a study. In all centres we noticed a progressive and significant decline in blood pressure when the readings were repeated by the observer at the subjects' homes. This observation underscores the need for multiple assessments of blood pressure over a time in order to avoid over-diagnosis of hypertension among individuals with high initial blood pressure values [2,23].

The maximal between-centre differences in blood pressure were 12.4 mmHg systolic and 6.1 mmHg diastolic (Prague centre versus Cracow centre). The maximal difference in pulse rate was between the Hechtel-Eksel and Sofia centres (9.2 beats per minute). These between-centre

Fig. 4



Mean systolic and diastolic differences between each observer's blood pressure (BP) readings and the overall within-centre popu means. Values for each observer are point estimates with 95% confidence interval adjusted for sex, age, body mass antihypertensive treatment, smoking, alcohol intake, and the use of oral contraceptives. Along with the observer identification nu gender of the observer (♂ if male) and the number of subjects examined by each observer are presented.

differences may be expected on the basis of random variability, small sample size (for instance, in Sofia and Prague), and inclusion of varying proportions of hypertensive subjects.

Another common manifestation of measurement error in epidemiological studies is digit preference for a terminal zero [9,10,24–26]. Because this means that the precision of the measurements is 10 mmHg instead of 2 mmHg, this may result in considerable bias. Digit preference affects the shape of the blood pressure distribution [24] and reduces the power of statistical tests thereby making it more difficult to assess associations between potential risk factors and blood pressure [10,26]. In our report preference for a terminal zero was statistically significant, but of minor importance from a clinical point of view.

The present analysis not only focused on the centres as units of observation, but also included an evaluation of the performance of individual observers. Only a few studies [6,8,10,21,27] have reported data on inter- or intra-observer variability. In our study, we assessed inter-observer variability using the blood pressure readings of expert clinical observers as the standard. Overall, 88% of the observers' systolic pressure readings were within ± 5 mmHg of the standard. For diastolic pressure, proportion was 87.4%. To assess intra-observer varial we used Bland and Altman's technique. The repeata coefficient across seven centres and 29 observers was for systolic pressure and 6.4% for diastolic pressure. H repeatability coefficients indicate worse reproducil Furthermore, we also evaluated differences bet observers, which could not be explained by confour by gender, age, body mass index, antihypertensive t ment, use of oral contraceptives, smoking, and drir habits. Differences between observers, over and be confounding, might be due to systematic error, prej for or against certain blood pressure values, the subje and emotional interaction between subject and obse the white-coat effect, and/or random variability [4,8,1

As opposed, for instance, to biochemical measurem external quality control cannot be easily mounted for b pressure readings. However, quality assurance and co should be planned at the design stage of a project invol BP measurement and implemented from its very be nings until the end. In our opinion, the procedure quality assurance and control set up for the blood pres measurement in the frame of the EPOGH study resu

n a well-defined blood pressure phenotype, which was consistent across centres.

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Appendix

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Příloha 2

Association of peripheral and central arterial wave reflections with the *CYP11B2 −344C* allele and sodium excretion

Wiktoria Wojciechowska[a], Jan A. Staessen[b], Katarzyna Stolarz[a], Tim Nawrot[b], Jan Filipovský[c], Milena Tichá[c], Giuseppe Bianchi[d], Eva Brand[e], Marcin Cwynar[f], Tomasz Grodzicki[f], Tatiana Kuznetsova[b], Harry A. Struijker-Boudier[g], Vlasta Svobodová[c], Lutgarde Thijs[b], Luc M. Van Bortel[h] and Kalina Kawecka-Jaszcz[a] on behalf of the European Project on Genes in Hypertension (EPOGH) Investigators

Objective **Angiotensin II and aldosterone, generated by the angiotensin-converting enzyme (ACE) and aldosterone synthase (CYP11B2), respectively, not only regulate sodium and water homeostasis, but also influence vascular remodeling in response to high blood pressure. In the European Project on Genes in Hypertension (EPOGH), we therefore investigated whether the *ACE I/D* and *CYP11B2 C-344T* polymorphisms influence early arterial wave reflections, a measure of vascular stiffness.**

Methods **We measured the peripheral and central augmentation index of systolic blood pressure by applanation tonometry at the level of the radial artery in 622 subjects (160 families and 64 unrelated individuals) randomly recruited from three European populations, whose average urinary sodium excretion ranged from 196 to 245 mmol/day. In multivariate analyses, with sodium excretion analyzed as a continuous variable, we explored the phenotype–genotype associations by means of generalized estimating equations and the quantitative transmission disequilibrium test.**

Results **The peripheral and central augmentation indexes were significantly higher in *CYP11B2 −344C* allele carriers than in *−344T* homozygotes. In offspring, early wave reflections increased with the transmission of the *−344C* allele. This effect of the *CYP11B2* polymorphism occurred in subjects with a higher than median urinary sodium excretion (210 mmol/day). The *ACE I/D* polymorphism did not influence augmentation of systolic blood pressure.**

Conclusions **The *CYP11B2 C-344T* polymorphism affects arterial stiffness. However, sodium intake seems to modulate this genetic effect.** *J Hypertens* **22:2311–2319 © 2004 Lippincott Williams & Wilkins.**

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Keywords: aldosterone synthase, angiotensin-converting enzyme, augmentation index, genetics, sodium

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Sponsorship: Nuclear families were recruited in the framework of the European Project on Genes in Hypertension, which was supported by the European Union (contract numbers IC15-CT98-0329-EPOGH and QLGI-CT-2000-01 137-EURNETGEN). The study was also supported by research grant G.0424.03 from the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (Brussels, Belgium), by a special research grant (Onderzoekstoelage OT/99 per 28) from the Katholieke Universiteit Leuven (Leuven, Belgium), by research grants (OK 375 and OK 376) from the Czech Ministry of Education, and by the International Scientific and Technological Cooperation between Poland and Flanders (contract number BIL 00/18). E. B. participated in the research programme Innovationen des Therapiekonzeptes für das metabolische Syndrom – mit Fokus auf Hypertonie' (PTJ-BIO/0313040C), funded by the Bundesministerium für Bildung und Forschung (Berlin, Germany).

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Introduction

The arterial pulse wave consists of a forward and reflected component. The amplitude and velocity of the reflected wave increase with cardiac contractility, a more proximal localization of the sites of reflection along the arterial tree and arterial stiffness. The augmentation index, derived from the pulse contour, reflects the degree to which reflected waves enhance

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systolic pressure [1]. Twin studies recently demonstrated that independent of blood pressure, heart rate, height, and age, the heritability of the aortic augmentation index was 37% [2].

Angiotensin II and aldosterone play key roles in the regulation of sodium and water homeostasis. In sodium deplete conditions, these hormones are upregulated, whereas the opposite occurs in the presence of a high salt intake. Circulating angiotensin II is produced by cleavage of angiotensin I by the angiotensin-converting enzyme (ACE) at the endothelial–luminal interface throughout the vasculature, in particular in the pulmonary circulation. Carriers of the *ACE* deletion polymorphism have constitutively increased plasma and tissue levels of ACE [3]. Aldosterone is generated in the adrenal gland by aldosterone synthase (CYP11B2). However, both angiotensin II and aldosterone are also locally produced in the arterial wall, influence vascular structure and function, and mediate vascular remodeling in response to pathological stimuli, such as a high blood pressure [4,5].

In the European Project on Genes in Hypertension (EPOGH), we investigated whether the *ACE I/D* and *CYP11B2 C-344T* polymorphisms impact on the peripheral and central augmentation indexes, as assessed by applanation tonometry at the level of the radial artery. Our analyses accounted for salt intake, estimated from the urinary excretion of sodium and for other host and environmental determinants of cardiovascular function.

Methods

Study population

The European Project on Genes in Hypertension (EPOGH) was conducted according to the principles outlined in the Helsinki declaration for investigations in human subjects. The Ethics Committee of each institution approved the protocol. Participants gave informed written consent.

Three EPOGH centers opted to take part in vascular phenotyping. They randomly recruited nuclear families of Caucasian extraction, including offspring with a minimum age of 10 years in Belgium and 18 years in the two other countries. Overall, the response rate was 82%. Of 870 participants recruited in Cracow (Poland, $n = 302$), Hechtel-Eksel (Belgium, $n = 380$) and Pilsen (Czech Republic, $n = 188$), we discarded 21 from analysis because the recorded pulse wave was of insufficient quality and 60 because of missing genotypes. In addition, we detected six cases of inconsistency in Mendelian segregation. The Belgian sample included seven extended families spanning more than two generations. Because there is no generally agreed algorithm to construct the variance–covariance matrix for correlated data within extended pedigrees using generalized estimating equations (see below), we selected from each complex family the most informative nuclear unit with the largest number of phenotypes and genotypes. This procedure removed 161 Belgian subjects from our analyses. Thus, the overall number of participants analyzed statistically totaled 622.

Phenotypes and genotypes

After subjects had rested for 15 min, we recorded, during an 8-s period, the radial arterial waveform at the dominant arm by applanation tonometry. We used a high-fidelity SPC-301 micromanometer (Millar Instruments, Inc., Houston, Texas, USA) interfaced with a laptop computer running SphygmoCor software, version 6.31 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). We discarded recordings when the systolic or diastolic variability of consecutive waveforms exceeded 5% or when the amplitude of the pulse wave signal was less than 80 mV. We calibrated the pulse wave by measuring blood pressure at the contralateral arm immediately before the recordings. From the radial signal, the SphygmoCor software calculates the aortic pulse wave by means of a validated and population-based generalized transfer function [6]. The radial augmentation index was defined as the ratio of the second to the first peak of the pressure wave expressed as a percentage. The aortic augmentation index was the difference between the second and first systolic peak given as a percentage of the aortic pulse pressure. For statistical analysis, we used the average of the peripheral and central waveforms over the 8-s measurement period.

The blood pressure phenotype was the average of five consecutive readings obtained at one home visit. Peripheral and central pulse pressures were defined as the difference between systolic and diastolic blood pressure derived from the brachial blood pressure measured at the subjects homes and from the aortic pulse wave, respectively. From the home readings, we calculated mean arterial pressure as the diastolic pressure plus one-third of peripheral pulse pressure. We administered a standardized questionnaire to obtain information on each subject's medical history, smoking and drinking habits and use of medications. The participants collected a 24-h urine sample in a wide-neck plastic container for the measurement of sodium, potassium, creatinine and aldosterone. For statistical analysis of the urinary phenotypes, we excluded 18 subjects because according to previously published criteria [7] the urine samples were judged to be under- or over-collected. Genomic DNA from white blood cells was amplified and genotyped for the *ACE I/D* and *CYP11B2 C-344T* polymorphisms, as previously described [8,9]. For the *ACE* gene, all samples initially genotyped as *DD* underwent a second polymerization chain reaction with insertion-specific primers.

Statistical methods

Database management and most statistical analyses were performed with SAS software version 8.1 (SAS Institute Inc., Cary, North Carolina, USA). Population means and proportions were compared by Tukey's multiple means test and the χ^2 statistic with Bonferroni's adjustment for multiple comparisons, respectively. If Shapiro–Wilk's test showed significant departure from normality, we analyzed logarithmically transformed variables. We searched for possible covariates of the augmentation indexes using stepwise multiple regression with the P value for independent variables to enter and stay in the model set at 0.10. We used the methods described by Kleinbaum *et al.* [10] to test the null hypothesis of no differences between the parameters of regression equations.

We performed population-based as well as family-based analyses. In the population-based approach, we tested association of continuous traits with the genotypes of interest by use of generalized estimating equations (GEE). This approach allows adjustment for covariates as well as for the non-independence of observations within families [11]. In GEE, we also tested for heterogeneity between populations, using appropriate interaction terms with the genotypes.

In the family-based analyses, we performed transmission disequilibrium tests for quantitative traits (QTDT) using two different methods. First, we evaluated the within- and between-family components of phenotypic variance, using the orthogonal model as implemented by Abecasis *et al.* in the QTDT software, version 2.3 (http://www.sph.umich.edu/csq/abecasis/QTDT) [12]. In addition, using the model proposed by Allison, we regressed the quantitative phenotypes of informative offspring on their genotypes, while controlling for parental genotypes [13]. To allow for residual correlation among offspring, we implemented Allison's model using GEE.

Results

Characteristics of participants

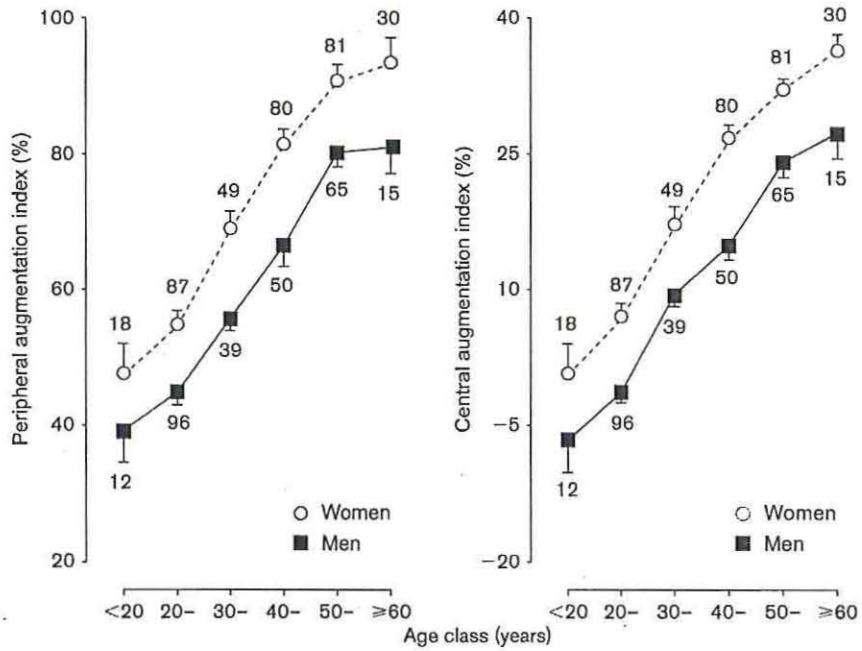
Table 1 gives the characteristics of the participants by country. Overall, the study population included 558 subjects from 160 nuclear families and 64 unrelated individuals. Mean (± SD) age of the 294 founders and 328 offspring was 51.7 ± 8.5 and 29.9 ± 10.6 years, respectively. The number of sibs per family amounted to one in 34 families, two in 102 families, and ranged from three to eight in 24 families. The Belgian participants were older than the subjects from the two other countries. Compared with Polish participants, fewer Belgians were on antihypertensive drug treatment. Czechs more frequently reported regular alcohol intake (≥ 5 g/day) than Belgians and Polish. Urinary sodium excretion was on average 50 and 36 mmol/day higher in Poland than in Belgium and Czechia, respectively. The urinary aldosterone excretion was lower in Poland than in the other two countries. Figure 1 shows the sex and age dependence of the peripheral and central augmentation indexes, which in the whole study population averaged 68.0 ± 23.1 and 16.4 ± 17.1%, respectively. As shown in Figure 2, systolic pressure in

Table 1 Characteristics of the study participants by country

	Belgium	Czechia	Poland
No.	181	147	294
Anthropometrical characteristics			
Age (years)	46.8 ± 14.6	37.9 ± 13.5*	37.2 ± 13.9*
Female gender (%)	57.5	57.1	53.4
Height (cm)	167.4 ± 9.4	171.1 ± 9.1*	169.9 ± 8.7*
Weight (kg)	72.2 ± 14.5	76.7 ± 16.1*	73.3 ± 14.4
Hemodynamic measurements			
Systolic pressure (mmHg)†	125.0 ± 15.7***	123.8 ± 16.6***	128.8 ± 17.5
Diastolic pressure (mmHg)†	78.6 ± 11.4	77.8 ± 10.9	80.3 ± 12.2
Peripheral augmentation index (%)	72.7 ± 21.9	63.7 ± 23.7*	67.3 ± 23.0*
Central augmentation index (%)	21.2 ± 15.5	14.0 ± 17.9*	14.6 ± 17.1*
Pulse rate (beats/min)	62.8 ± 9.4***	67.0 ± 9.7*	73.3 ± 11.4**
Questionnaire data			
Antihypertensive treatment (%)	12.7	21.1	23.8*
Current smokers (%)	28.2	25.8	27.6
Regular alcohol intake (%)	27.1	45.6*	19.1[C]
Urinary excretion			
Volume (l/day)‡	1.64 ± 0.77***	1.93 ± 0.67*	1.45 ± 0.53**
Sodium (mmol/day)	196 ± 65***	210 ± 83	245 ± 87**
Potassium (mmol/day)	70 ± 27	62 ± 29*	65 ± 25
Aldosterone (nmol/day)	20.0 (17.4–22.9)***	17.4 (15.8–19.1)	10.7 (9.8–11.7)**
Creatinine (mmol/day)	10.7 ± 3.5***	13.3 ± 4.1*	12.1 ± 4.1**

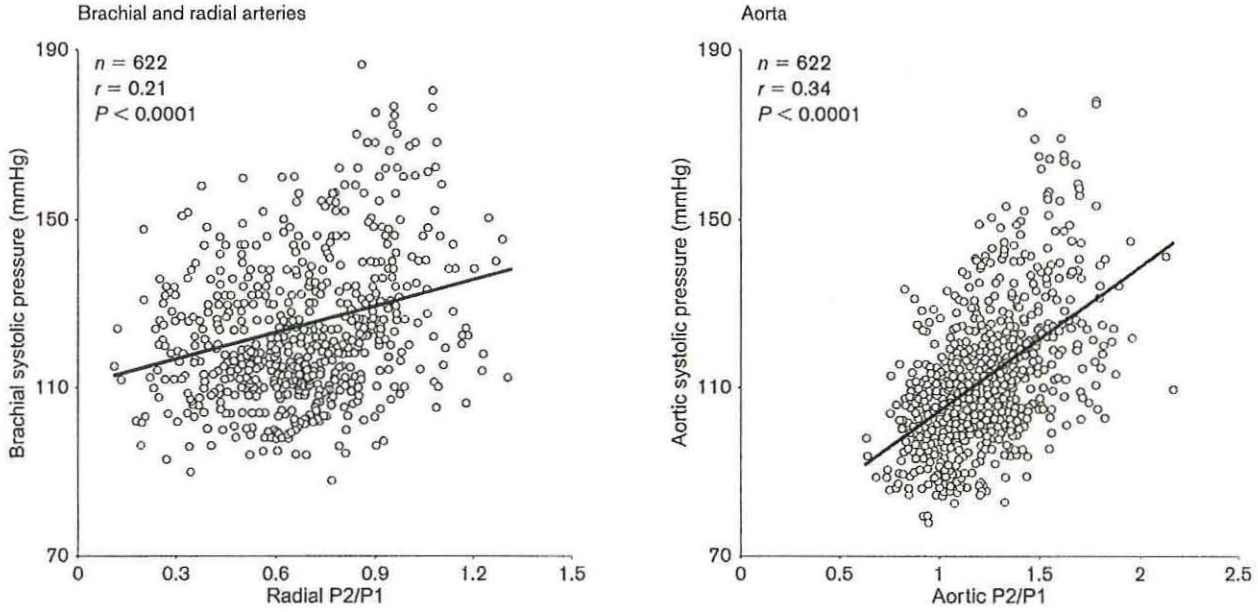
Values are arithmetic means ± SD, geometric means (95% confidence interval) or the percentage of subjects. P values for between-country differences were adjusted for multiple comparisons by Tukey's test (means) or Bonferroni's method (proportions): *$P \leq 0.05$ versus Belgium; **$P \leq 0.05$ versus Czechia; ***$P \leq 0.05$ versus Poland. †Average of five readings obtained at one home visit. ‡The number of subjects with 24 h collection was 181 in Belgium, 133 in Czechia and 290 in Poland.

Fig. 1



Peripheral and central augmentation indexes by sex and age class. Values are non-adjusted means ± SE. The number of subjects contributing to each mean is given.

Fig. 2



Local systolic pressure in the brachial and radial arteries and in the aorta in relation to systolic augmentation calculated as a the ratio of the second (P2) to the first (P1) systolic peak. The aortic measurements were extrapolated from the radial waveform by means of the SphygmoCor software.

the brachial artery and aorta significantly ($P < 0.0001$) increased with the ratio of the second to the first systolic peak. Furthermore, the slope of systolic blood pressure on this ratio was significantly ($P = 0.0002$) steeper in the aorta than in the radial artery.

Stepwise multiple regression demonstrated that the peripheral augmentation index significantly and independently increased with female gender (regression coefficient $\beta \pm SE$; $9.16 \pm 1.58\%$; $P = 0.0001$), age ($0.81 \pm 0.04\%$ per year; $P = 0.0001$), mean arterial pressure ($0.38 \pm 0.05\%$ per mmHg; $P = 0.0001$), current smoking ($5.20 \pm 1.20\%$; $P = 0.0001$), whereas it decreased with body height ($-0.52 \pm 0.09\%$ per cm; $P = 0.0001$) and pulse rate ($-0.63 \pm 0.05\%$ per beat; $P = 0.0001$). Similarly, the central augmentation index increased with female gender ($6.80 \pm 1.17\%$; $P = 0.0001$), age ($0.61 \pm 0.03\%$ per year; $P = 0.0014$), mean arterial pressure ($0.26 \pm 0.04\%$ per mmHg; $P = 0.0001$), current smoking ($2.94 \pm 0.92\%$; $P = 0.0001$), whereas it decreased with body height ($-0.38 \pm 0.07\%$ per cm; $P = 0.0001$), pulse rate ($-0.50 \pm 0.04\%$ per beat; $P = 0.0001$) and current antihypertensive treatment ($-2.10 \pm 1.17\%$; $P = 0.07$). We adjusted all further analyses for the aforementioned covariates as well as for observer (one in Belgium, one in Czechia, and two in Poland). Further analyses also accounted for sodium intake estimated from the 24-h urinary excretion.

The within-country frequencies of genotypes (Table 2) complied with Hardy–Weinberg equilibrium ($0.36 \leq P \leq 0.94$). The genotype and allele frequencies were similar across countries for the *ACE* gene. The *CYP11B2 –344C* allele was more prevalent in Poland than Belgium.

Both before and after adjustment for urinary sodium and potassium, the 24-h urinary aldosterone excretion was not associated with the *CYP11B2 C-344T* polymorphism ($P \geq 0.16$). In all countries, aldosterone excretion adjusted for sex and age was closely correlated with urinary sodium and potassium. With additional adjustment for country, the overall partial correlation coefficients were −0.11 for sodium, 0.30 for potassium and −0.38 for the urinary sodium-to-potassium ratio ($P < 0.01$, for all).

Population-based association study

Because across centers there was no heterogeneity in the phenotype–genotype relations ($0.07 \leq P \leq 0.82$), we combined all countries. Furthermore, for none of the phenotype–genotype relations, we found significant interactions with gender ($0.54 \leq P \leq 0.94$) or generation (parents *versus* offspring; $0.39 \leq P \leq 0.80$).

GEE did not reveal any association between the augmentation indexes and the *ACE I/D* polymorphism. In the whole study population as well as in offspring, the peripheral and central augmentation indexes were significantly higher in the *CYP11B2 –344C* allele carriers than in the *–344TT* homozygotes with similar trends in founders (Table 3). Further analyses (Table 4) demonstrated that in founders central, but not peripheral, pulse pressure was also significantly higher in *CYP11B2–344C* allele carriers than in *–344TT* homozygotes. There was no interaction between the *ACE* and *CYP11B2* genotypes ($P > 0.22$). However, in untreated subjects ($n = 483$), we observed a significant interaction between the *CYP11B2* genotype and sodium excretion, analyzed as a continuous variable, in relation to the peripheral ($P = 0.029$) and central ($P = 0.013$) augmentation indexes. Figure 3 illustrates these interactions according to the country- and sex-specific median of sodium excretion (approximately 210 mmol/day).

Family-based association study

With the exception of the *ACE* gene in Czechia ($P < 0.01$), Abecasis' orthogonal model did not reveal population stratification in any country ($0.19 \leq P \leq 0.34$). In 216 informative offspring, it confirmed significant association between the peripheral augmentation index and the transmission of the *CYP11B2 –344C* allele (effect size, + 3.0%; $P = 0.028$) with a similar trend for the central augmentation index (effect size, + 2.2%; $P = 0.092$). Using Allison's approach, the

Table 2 Genotype and allele frequencies by country ordered according to the prevalence of the major allele

Gene	Country	Allele		Genotypes		
ACE		D	I	DD	DI	II
	Czechia	158 (53.7)	136 (46.3)	40 (27.2)	78 (53.1)	29 (19.7)
	Poland	302 (51.4)	286 (48.6)	72 (24.5)	158 (53.7)	64 (21.8)
	Belgium	184 (50.8)	178 (49.2)	49 (27.1)	86 (47.5)	46 (25.4)
CYP11B2		T	C	TT	TC	CC
	Belgium	216 (59.7)	146 (40.3)	63 (34.8)	90 (49.7)	28 (15.5)
	Czechia	172 (58.5)	122 (41.5)	50 (34.0)	72 (49.0)	25 (17.0)
	Poland	297 (50.5)	291 (49.5)	70 (23.8)	157 (53.4)	67 (22.8)

Values indicate number of alleles or genotypes (%). Braces join countries with similar allele frequencies. ACE, angiotensin-converting enzyme.

Table 3 Peripheral and central augmentation indexes by genotypes in nuclear families

Gene	Augmentation index		n	Adjusted mean ± SE†			
ACE			DD/DI/II	DD	DI	II	P*
	Peripheral	All	161/322/139	68.4 ± 1.0	68.1 ± 0.8	67.9 ± 1.3	0.79
		Founders	79/156/59	80.4 ± 1.5	81.5 ± 1.1	81.1 ± 1.9	0.58
		Offspring	82/166/80	57.3 ± 1.3	56.2 ± 1.1	55.6 ± 1.5	0.39
	Central	All	161/322/139	17.1 ± 0.8	16.1 ± 0.6	16.5 ± 1.0	0.35
		Founders	79/156/59	25.9 ± 1.0	25.7 ± 0.7	25.6 ± 1.3	0.85
		Offspring	82/166/80	9.3 ± 1.2	7.6 ± 0.9	7.7 ± 1.2	0.24
CYP11B2			TT/TC/CC	TT	TC	CC	P**
	Peripheral	All	183/319/120	66.1 ± 1.1	68.8 ± 0.9	69.5 ± 1.1	0.027
		Founders	84/155/55	79.3 ± 1.5	81.3 ± 1.2	83.3 ± 1.4	0.16
		Offspring	99/164/65	53.1 ± 1.4	57.9 ± 1.0	57.4 ± 1.6	0.004
	Central	All	183/319/120	15.1 ± 0.8	16.8 ± 0.6	17.6 ± 0.9	0.050
		Founders	84/155/55	24.4 ± 1.0	25.8 ± 0.7	27.6 ± 1.1	0.12
		Offspring	99/164/65	6.2 ± 1.2	9.1 ± 0.8	8.4 ± 1.5	0.049

†Adjustments included: observer, sex, age, body height, pulse rate, mean arterial pressure, current smoking and antihypertensive treatment. *P value for comparison between *ACE DD* versus *DI + II*. **P value for comparison between *CYP11B2 TT* versus *TC + CC*. ACE, angiotensin-converting enzyme.

Table 4 Peripheral and central pulse pressure in relation to the *CYP11B2* genotype

Pulse pressure		n (CC/TC/TT)	Adjusted mean ± SE† TT	TC	CC	P*
Peripheral	All	161/322/139	47.9	47.5	46.8	0.53
	Founders	79/156/59	49.6	49.4	48.8	0.84
	Offspring	82/166/80	46.1	44.9	45.7	0.45
Central	All	161/322/139	31.9	33.4	32.9	0.06
	Founders	79/156/59	34.8	38.4	36.4	0.01
	Offspring	82/166/80	29.2	28.8	29.5	0.75

†Pulse pressure was adjusted for sex, age, body height, pulse rate, current smoking and antihypertensive treatment. Additional adjustments included observer and mean arterial pressure for the central pulse pressure and country for the peripheral pulse pressure. *P value for comparison between *CYP11B2 TT* versus *TC + CC*.

number of informative offspring decreased to 135, but the test statistic remained significant for the association between the peripheral augmentation index and the *CYP11B2* polymorphism with a similar tendency for the central augmentation index (Table 5). None of the QTDT models provided any evidence for association between the pulse wave phenotypes and transmission of the *ACE D* allele.

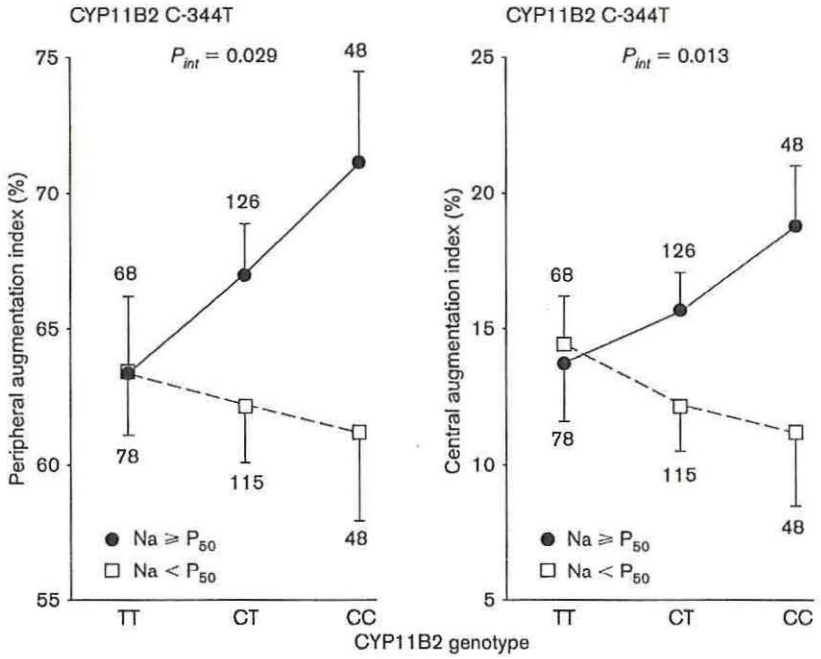
Discussion

The main finding of our study was that in both population-based and family-based analyses, early arterial wave reflections increased with the presence or transmission of the *CYP11B2 –344C* allele. This genetic effect was more pronounced in subjects whose sodium excretion exceeded 210 mmol/day. The frequency of the *–344T* allele was higher in Belgians (60%) than Polish (51%). However, this difference in allele frequency did not impact on our results. Indeed, we neither observed heterogeneity in the phenotype–genotype relation across countries nor stratification within any population. Previous studies reported *CYP11B2 –344T* allele frequencies of 45% [14] in Caucasians and 64% [15] in Japanese.

Aldosterone synthase is a cytochrome P450 enzyme and catalyzes the terminal steps in aldosterone biosyntheses. Chromosome 8 harbors the human gene. The *C-344T* polymorphism is located in an enhancer element, which is also present in the genes of other adrenal hydroxylases [16] and interacts with the regulatory protein steroidogenic factor (SF-1) [17]. Several *in vitro* studies addressed the possible functional role of *CYP11B2* [17–20]. Preliminary gel-shift experiments suggested that on a molar basis the *C* compared with the *T* containing element bound SF-1 about four times more effectively [18]. Subsequent transfection studies of human adrenocortical cells showed that the *C-344T* locus is not essential for basal or regulated expression of human *CYP11B2*, apparently because the human gene contains an additional SF-1 binding site closer to the start of transcription [17,19]. However, these experiments do not exclude a functional role of the *C-344T* polymorphism, because *CYP11B2* is expressed in cardiac [21] and vascular [5,21] tissue and because its expression might be differentially regulated in steroidogenic and non-steroidogenic tissues [20].

Within and across populations, we did not find any

Fig. 3



Peripheral and central augmentation indexes by *CYP11B2* genotype and median sodium excretion (210 mmol/day) in 483 untreated subjects. Values are adjusted means ± SE. The significance of the genotype-by-sodium interaction (P_{int}) was derived from a generalized estimating equations (GEE) model, which included sodium excretion as a continuous variable and which accounted for clustering within families and significant covariates (Table 3).

Table 5 QTDT analyses of peripheral and central augmentation indexes

Gene	Number of offspring informative/all	Peripheral augmentation index β (%)	χ^2	*P*	Central augmentation index β (%)	χ^2	*P*
ACE							
Orthogonal model	212/328	+ 1.7	1.51	0.22	+ 1.8	2.21	0.14
Allison's model	137/328	+ 2.4	0.77	0.37	+ 3.0	1.70	0.19
CYP11B2							
Orthogonal model	216/328	+ 3.0	4.84	0.028	+ 2.2	2.83	0.092
Allison's model	135/328	+ 7.2	4.61	0.031	+ 4.3	2.94	0.086

The orthogonal model accounted for between- and within-family components of phenotypic variability. The parameter estimate (β) for the within-family variability component indicates the direction and size of the association when the *ACE D* allele or the *CYP11B2 –344C* allele were transmitted. Adjustments were similar as in Table 3. ACE, angiotensin-converting enzyme.

relation between the urinary aldosterone excretion and the *CYP11B2 C-344T* polymorphism. Other studies on the putative association between plasma or urinary aldosterone and genetic variation in the *CYP11B2* gene produced inconsistent results. Indeed, they showed higher plasma levels of aldosterone associated with the *–344C* [22] or *–344T* allele [23], higher urinary aldosterone excretion in the presence of the *–344T* allele [24,25] and divergent results for blood pressure analyzed as a binary or continuous phenotype [14,15,22,24–26]. In the light of our present findings, the contradiction in the literature is not surprising, because few studies accounted for sodium intake, which is an important determinant of aldosterone production. We noticed a major interaction between the *CYP11B2 C-344T* polymorphism and sodium excretion in relation to a vascular phenotype.

Arterial stiffness and the velocity and amplitude of reflected arterial waves are the main determinants of the peripheral and central augmentation indexes. In keeping with current physiological concepts [27], we found that the augmentation of systolic pressure was significantly higher in the aorta compared with the

brachial artery. Wave reflections occur at sites of changes of arterial impedance along the arterial tree, such as branching points or atherosclerotic plaques. However, in the present study, we adjusted the augmentation indexes for body height and pulse rate, so that to a large extent these phenotypes probably reflected vascular stiffness rather than changes in the localization of the reflection points. In keeping with our present observations, some researchers reported a positive association of the *CYP11B2 –344C* allele with arterial stiffness [22] or with the prevalence [15] or incidence [26] of hypertension. However, the physiological and molecular pathways via which this polymorphism might impact on vascular stiffness remain to be elucidated.

Blacher and coworkers noticed in hypertensive patients ($r = -0.497$; $P < 0.01$), but not normotensive subjects, a close and inverse correlation between systemic arterial compliance and the plasma aldosterone concentration [28]. The absence of a direct relation between vascular stiffness and plasma aldosterone in our study ($P > 0.25$) and other reports [26,28] might be explained by the local generation of aldosterone in the endothelium or in vascular smooth muscle cells [5]. Indeed, in rats, the concentration of aldosterone in cardiac tissue was 17-fold higher than in plasma [21].

We observed a significant interaction between the *CYP11B2 C-344T* polymorphism and sodium excretion, analyzed as a continuous variable, in relation to the peripheral and central augmentation of systolic blood pressure. Previous studies demonstrated that in humans a high salt intake is associated with increased arterial stiffness and vascular and cardiac hypertrophy [29]. Furthermore, in normotensive Wistar–Kyoto rats, a high salt diet (0.9% NaCl) stimulated the expression of mRNA for CYP11B2 in the myocardium [30]. Sodium loading activated local aldosterone synthesis with elevated tissue levels of the steroid and produced cardiac hypertrophy in the absence of a noticeable increase in blood pressure [30]. Similarly, stroke-prone spontaneously hypertensive rats fed 0.9% NaCl in drinking water, compared with the control group given tap water, had increased expression of mRNA for CYP11B2 in the arterial wall, but lower levels of circulating aldosterone [31]. Thus, an excessive salt intake might contribute to increased arterial stiffness by inappropriately sustaining the expression of the *CYP11B2* gene in the arterial wall, despite a decrease of the angiotensin II and aldosterone levels in the blood.

Because plasma and tissue levels of ACE increase with the number of copies of the *ACE D* allele [3] and because angiotensin II is a potent vasoconstrictor and stimulates vascular growth, the hypothesis that the *ACE* deletion polymorphism might be associated with increased vascular stiffness is plausible. However, in keeping with the contradictory results of previous studies [32,33], we did not observe such a relation. Our null findings with respect to the *ACE I/D* polymorphism might be due to the plasticity of the renin–angiotensin system, as exemplified by the multiple pathways via which angiotensin II can be generated, or to the activation by angiotensin II of counter-regulatory hormonal or paracrine mechanisms [4].

The present study has to be interpreted within the context of its limitations and strengths. One 24-h urine collection is insufficient to characterize an individual's habitual sodium intake, but it does accurately reflect the average salt consumption of groups of subjects [34]. We investigated the interaction between the *CYP11B2* polymorphism and sodium excretion in relation to augmentation of systolic pressure only in untreated subjects, because many antihypertensive drugs, in particular diuretics, influence renal sodium handling. There was consistency between the population-based and family-based statistical approaches and between the results involving the central and peripheral augmentation indexes. The latter was obtained directly from the radial pulse wave and did not involve extrapolation based on the transfer function implemented in the SphygmoCor software.

In conclusion, our study suggests that a common variant of the *CYP11B2* gene, located in the promoter area, may be involved in the pathophysiology of arterial stiffness. However, sodium intake seems to modulate this genetic effect.

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Appendix

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Příloha 3

Epistatic interaction between α- and γ-adducin influences peripheral and central pulse pressures in white Europeans

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Background **Adducin is a membrane skeleton protein consisting of α- and β- or α- and γ-subunits. Mutations in α- and β-adducin are associated with hypertension. In the European Project on Genes in Hypertension, we investigated whether polymorphisms in the genes encoding α-adducin (Gly460Trp), β-adducin (C1797T) and γ-adducin (A386G), alone or in combination, affected pulse pressure (PP), an index of vascular stiffness.**

Methods **We measured peripheral and central PP by conventional sphygmomanometry and applanation tonometry, respectively. We randomly recruited 642 subjects (162 nuclear families and 70 unrelated individuals) from three European populations. In multivariate analyses, we used generalized estimating equations and the quantitative transmission disequilibrium test.**

Results **Peripheral and central PP averaged 46.1 and 32.6 mmHg, respectively. Among carriers of the α-adducin Trp allele, peripheral and central PP were 5.8 and 4.7 mmHg higher in γ-adducin GG homozygotes than in their AA counterparts, due to an increase in systolic pressure. γ-Adducin GG homozygosity was associated with lower urinary Na^+/K^+ ratio among α-adducin Trp allele carriers and with higher urinary aldosterone excretion among α-adducin GlyGly homozygotes. Sensitivity analyses in founders and offspring separately, and tests based on the transmission of the γ-adducin G allele across families, confirmed the interaction between the α- and γ-adducin genes.**

Conclusions **In α-adducin Trp allele carriers, peripheral and central PP increased with the γ-adducin G allele. This epistatic interaction is physiologically consistent with the heterodimeric structure of the protein and its influence on transmembranous sodium transport.** ***J Hypertens*** **23:961–969 © 2005 Lippincott Williams & Wilkins.**

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Introduction

Adducin is an ubiquitously expressed membrane-skeleton protein, which consists of either α- and β- or α- and γ-subunits, which to a large extent are similar in amino acid sequence and domain organization [1]. Point mutations of the α- and β-adducin subunits account for up to 50% of the blood pressure difference between Milan normotensive (MNS) and hypertensive (MHS) rats [2]. Enhanced Na^+,K^+-ATPase activity [3,4] and increased renal tubular sodium reabsorption [5] explain the rise in blood pressure in MHS rats.

In previous studies, we demonstrated that human carriers of the mutated α-adducin 460Trp allele are characterized

by an increased risk of hypertension [6] and slight impairment of renal function [7]. Additional observations in Belgian [8] and other European populations [9] gave rise to the hypothesis that the β-adducin C1797T polymorphism might also be associated with hypertension, especially in post-menopausal women. Furthermore, the Gly460Trp polymorphism in the α-adducin gene seems to confer a genetic background favouring thickening of the intima–media of the femoral artery [10] and influencing the distensibility of the large arteries [11].

Pulse pressure is an age-related index reflecting vascular stiffness, the amplitude and velocity of reflected waves and cardiac stroke volume [12]. In view of the evidence outlined above [6–11], we investigated, in randomly recruited participants of the European Project on Genes in Hypertension (EPOGH), whether genetic variation in the three adducin subunits, alone or in combination, impacted on peripheral and central pulse pressures. Our analysis accounted for salt intake, estimated from the urinary excretion of sodium, and for other host and environmental determinants of cardiovascular function. In the context of this paper, epistasis refers to non-additive gene–gene interactions.

Methods

Study population

The European Project on Genes in Hypertension (EPOGH) was conducted according to the principles outlined in the Helsinki Declaration for investigation of human subjects [13]. The Ethics Committee of each institution approved the protocol. Participants gave informed written consent.

Three EPOGH centres opted to take part in vascular phenotyping. They randomly recruited nuclear families of Caucasian extraction, including offspring with a minimum age of 10 years in Belgium and 18 years in the two other countries. Overall, the response rate was 82%. Of 870 participants recruited in Cracow (Poland, $n = 302$), Hechtel-Eksel (Belgium, $n = 380$) and Pilsen (Czech Republic, $n = 188$), we discarded 17 from analysis because the recorded pulse wave was of insufficient quality, and 26 because of missing genotypes. In addition, we detected nine cases of inconsistency in Mendelian segregation. The Belgian sample included seven extended families spanning more than two generations. Because there is no generally agreed algorithm to construct the variance–covariance matrix for correlated data within extended pedigrees using generalized estimating equations (see below), we selected from each complex family the most informative nuclear unit with the largest number of phenotypes and genotypes. This procedure removed 176 Belgian subjects from our analyses. Thus, the overall number of participants analysed statistically totalled 642.

Phenotypes and genotypes

After subjects had rested for 15 min, four observers (one in Belgium, one in the Czech Republic and two in Poland), recorded the radial arterial waveform at the dominant arm by applanation tonometry for 8 s. We used a high-fidelity SPC-301 micromanometer (Millar Instruments, Inc., Houston, Texas, USA) interfaced with a laptop computer running the SphygmoCor software, version 6.31 (AtCor Medical Pty Ltd, West Ryde, New South Wales, Australia). We discarded recordings when the systolic or diastolic variability of consecutive waveforms exceeded 5%, or when the amplitude of the pulse wave signal was less than 80 mV. We calibrated the pulse wave by measuring blood pressure in the contralateral arm immediately before the recordings. From the radial signal, the SphygmoCor software calculates the aortic pulse wave by means of a population-based and validated transfer function [14]. For statistical analysis, we used the average of the central waveforms over the 8-s measurement period. The blood pressure phenotype was the average of five consecutive readings obtained at one home visit. Peripheral and central pulse pressures were defined as the differences between systolic and diastolic blood pressure, derived from the brachial blood pressure measured at the subjects' homes and from the aortic pulse wave, respectively. From the home readings, we calculated mean arterial pressure as diastolic pressure plus one-third of pulse pressure. We defined hypertension as a blood pressure of at least 140 mmHg systolic or 90 mmHg diastolic, or as the use of antihypertensive drugs. The observers involved in phenotyping were unaware of the subjects' genotype.

We administered a standardized questionnaire to obtain information on each subject's medical history, smoking and drinking habits and use of medications. The participants collected a 24-h urine sample in a wide-neck plastic container, for the measurement of sodium, potassium, creatinine and aldosterone. The participants did not receive any prior advice with regard to their salt intake. One 24-h urine collection might be insufficient to characterize an individual's habitual sodium intake, but it does accurately reflect the average salt consumption of groups of subjects [15]. For statistical analysis of the urinary phenotypes, we excluded 23 subjects because, according to published criteria [16], their urine samples were under- or overcollected or because they were on antihypertensive treatment ($n = 123$).

Blood was sampled in ACD buffer and stored at −80°C. DNA was extracted according to standard methods. Genotyping was carried out on an ABI Prism 7700 Sequence Detection System (Applied Biosystems Inc., Foster City, California, USA) using a 5′ nuclease detection assay. Primers and probes for the α-adducin Gly460Trp (rs4961 dbSNP) and the β-adducin

C1797T (rs4984 dbSNP) polymorphisms have been described previously [6,9].

For γ-adducin (rs3731566 dbSNP), the forward and reverse primers were 5′-TGGAGGTGGGAATTGAAG-AGA-3′ and 5′-CCCGAATCTGAATTGAAAAACAA-3′, respectively. The A and G probes were 5′FAM-TGTCA-AATAGTAAGCTTTT-MGB-3′ and 5′VIC-TGTCAA-GTAGTAAGCTTT-MGB-3′. Each 25 μl of polymerase chain reaction (PCR) fluid contained: 50 ng genomic DNA, 1200 nmol/l primers, 400 nmol/l FAM-probe and 120 nmol/l VIC-probe. The amplification conditions were: 50°C for 2 min; 95°C for 10 min; followed by 42 cycles at 92°C for 15 s and at 60°C for 1 min.

Statistical methods

Database management and most statistical analyses were performed with SAS software version 8.1 (SAS Institute Inc., Cary, North Carolina, USA). Population means and proportions were compared by Tukey's multiple means test and the χ^2 statistic with Bonferroni's adjustment for multiple comparisons, respectively. If Shapiro–Wilk's statistic showed significant departure from normality, we analysed logarithmically transformed variables. We searched for possible covariates of the pulse pressures, using stepwise multiple regression with the P value for independent variables to enter and to stay in the model set at 0.10.

In a population-based approach, we tested the association of continuous traits with the genotypes of interest by use of generalized estimating equations (GEE). This approach allows adjustment for covariates as well as for the non-independence of observations within families [17]. In GEE, we also tested for heterogeneity across populations, using appropriate interaction terms with the genotypes. Furthermore, in family-based analyses, we performed a transmission disequilibrium test for quantitative traits (QTDT). We evaluated the within- and between-family components of phenotypic variance, using the orthogonal model as implemented by Abecasis *et al.* [18] in the QTDT software, version 2.3 (http://www/sph.umich.edu/cgs/abecasis/QTDT/download).

Results

Characteristics of the participants

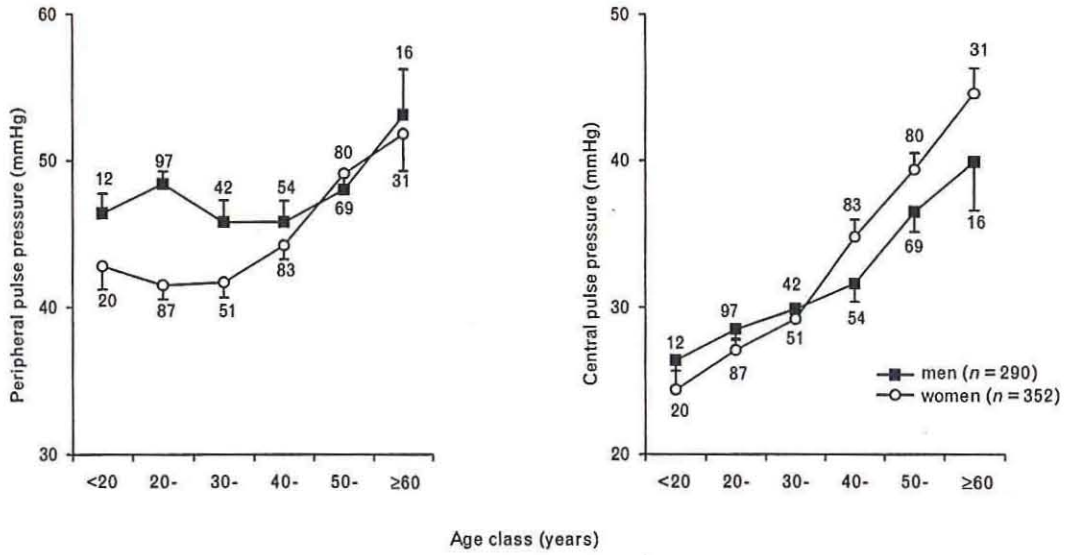
Table 1 gives the characteristics of participants by country. Overall, the study population included 572 subjects from 162 nuclear families and 70 unrelated individuals. Mean (±SD) age of the 303 founders and 339 offspring was 51.8 ± 8.6 years and 30.0 ± 10.7 years, respectively. The number of sibs per nuclear family amounted to 1 in 30 families, 2 in 110 families, and ranged from 3 to 8 in 22 families. The Belgian participants were older than the Slavic subjects. Compared to Polish subjects, fewer Belgians were on antihypertensive drug treatment. Czechs more frequently reported regular alcohol intake (≥5 g/day) than Belgians and Polish subjects. Urinary sodium excretion was on average 48 mmol/day and 32 mmol/day higher in Poland than in Belgium and the Czech Republic, respectively. The urinary aldosterone excretion was lowest in Poland. Figure 1 shows the sex- and

Table 1 Characteristics of the study participants by country

	Belgium	Czech Republic	Poland
Number	194	162	286
Anthropometric characteristics			
Age (years)	46.7 ± 14.6	38.0 ± 13.6[x]	37.3 ± 13.9[x]
Female gender (%)	56.7	54.3	53.9
Body mass index (kg/m^2)	25.8 ± 4.1	26.2 ± 4.7	25.4 ± 4.9
Haemodynamic measurements			
Peripheral pulse pressure (mmHg)[a]	46.7 ± 10.7	45.0 ± 10.3	46.4 ± 9.4
Central pulse pressure (mmHg)	34.4 ± 9.3	29.3 ± 9.1[x]	33.4 ± 10.7[y]
Systolic pressure (mmHg)[a]	123.4 ± 15.2	122.5 ± 15.8	125.7 ± 15.3
Diastolic pressure (mmHg)[a]	76.7 ± 10.9	77.5 ± 10.4	79.3 ± 10.4[x]
Pulse rate (beats/min)	62.7 ± 9.4	67.1 ± 9.8[x]	73.4 ± 11.4[x,y]
Questionnaire data			
Hypertensives (%)[b]	29.4	29.0	39.9[x,y]
Antihypertensive treatment (%)	13.4	20.4	22.7[x]
Current smokers (%)	29.4	25.3	27.3
Alcohol intake ≥ 5 g/day (%)	28.4	43.2[x]	18.9[y]
Urinary measurements[c]			
Volume (l/day)	1.59 ± 0.74	1.92 ± 0.74[x]	1.47 ± 0.55[y]
Sodium (mmol/day)	197 ± 66	213 ± 89	245 ± 89[x,y]
Potassium (mmol/day)	70 ± 28	62 ± 26[x]	64 ± 23[x]
Na^+/K^+ ratio (units)	3.0 ± 1.0	3.6 ± 1.4[x]	4.1 ± 1.4[x,y]
Aldosterone (nmol/day)	21.0 (18.6 – 23.4)	18.0 (16.0 – 20.0)	10.4 (8.4 – 12.4)[x,y]
Creatinine (mmol/day)	10.8 ± 3.6	14.0 ± 4.2[x]	12.0 ± 4.2[x,y]

Values are arithmetic means ± SD, geometric means (95% confidence interval) or the percentage of subjects. P values for the between-country differences were adjusted for multiple comparisons by Tukey's test (means) or Bonferroni's method (proportions). [x]$P \leq 0.05$ versus Belgium; [y]$P \leq 0.05$ versus the Czech Republic. [a]Average of five measurements obtained at one home visit; [b]We defined hypertension as a blood pressure of at least 140 mmHg systolic or 90 mmHg diastolic or as the use of antihypertensive drugs; [c]The number of subjects with a 24-h urine collection was 166 in Belgium, 112 in the Czech Republic and 218 in Poland.

Fig. 1



Peripheral and central pulse pressures by sex and age class. Values are unadjusted means ± SD. The number of subjects contributing to each mean is given.

age-dependency of the peripheral and central pulse pressures which, in the whole study population, averaged 46.1 ± 10.1 and 32.6 ± 10.1 mmHg, respectively.

Stepwise multiple regression demonstrated that peripheral pulse pressure significantly and independently increased with male gender (regression coefficient [β] ± SE; 2.22 ± 0.84 mmHg; $P = 0.008$), mean arterial pressure (0.17 ± 0.04 mmHg/mmHg; $P = 0.0001$) and that it was higher in patients on antihypertensive treatment (3.25 ± 1.10 mmHg; $P = 0.003$). Peripheral pulse pressure also tended to increase with current smoking (1.54 ± 0.86 mmHg; $P = 0.08$) and to decrease with pulse rate (−0.07 ± 0.04 mmHg/beat; $P = 0.07$). Central pulse pressure increased with age (0.25 ± 0.03 mmHg/year; $P = 0.0001$), mean arterial pressure (0.23 ± 0.03 mmHg/mmHg; $P = 0.0001$) and current smoking (1.78 ± 0.71 mmHg; $P = 0.01$), whereas it decreased with male gender (−2.20 ± 0.69 mmHg; $P = 0.002$), pulse rate (−0.22 ± 0.03 mmHg/beat; $P = 0.0001$) and regular alcohol intake (−2.00 ± 0.79 mmHg; $P = 0.01$). We adjusted the population-based and family-based analyses of pulse pressures for all aforementioned covariates as well as for country (peripheral pulse pressure) or observer (central pulse pressure).

In all countries, aldosterone excretion adjusted for sex and age was closely correlated with urinary sodium and potassium. With additional adjustment for country, the overall partial correlation coefficients were −0.11 for sodium, 0.28 for potassium and −0.37 for the Na^+/K^+ ratio ($P < 0.01$, for all). In the population-based and

Table 2 Genotype and allele frequencies by country ordered according to the prevalence of the major allele

Gene	Country	Allele		Genotype		
α-Adducin		Gly	Trp	GlyGly	GlyTrp	TrpTrp
	Belgium	283 (72.9)	105 (27.1)	106 (54.6)	71 (36.6)	17 (8.8)
	Czech Republic	258 (79.6)	66 (20.4)	102 (63.0)	54 (33.3)	6 (3.7)
	Poland	481 (84.1)	91 (15.9)	199 (69.6)	83 (29.0)	4 (1.4)
β-Adducin		C	T	CC	CT	TT
	Czech Republic	271 (83.6)	53 (16.4)	112 (69.1)	47 (29.0)	3 (1.9)
	Belgium	347 (89.4)	41 (10.6)	156 (80.4)	35 (18.0)	3 (1.6)
	Poland	514 (89.9)	58 (10.1)	232 (81.1)	50 (17.5)	4 (1.4)
γ-Adducin		A	G	AA	AG	GG
	Czech Republic	174 (53.7)	150 (46.3)	50 (30.9)	74 (45.7)	38 (23.5)
	Belgium	216 (55.7)	172 (44.3)	69 (35.6)	78 (40.2)	47 (24.2)
	Poland	322 (56.3)	250 (43.7)	90 (31.5)	142 (49.7)	54 (18.9)

Values indicate number of alleles or genotypes (%). Braces join countries with similar allele frequencies.

Table 3 Peripheral and central pulse pressures by genotypes

Polymorphism	Pulse pressures	Number	Adjusted means ± SE			*P*
α-adducin Gly460Trp		GlyGly/GlyTrp + TrpTrp	GlyGly	GlyTrp + TrpTrp		
	Peripheral	407/235	46.4 ± 0.5	45.7 ± 0.7		0.40*
	Central	407/235	32.3 ± 0.4	32.6 ± 0.6		0.69*
β-adducin C1797T		CC/CT + TT	CC	CT + TT		
	Peripheral	500/142	45.9 ± 0.5	47.1 ± 0.9		0.24†
	Central	500/142	32.2 ± 0.4	33.2 ± 0.7		0.24†
γ-adducin A386G		AA/AG/GG	AA	AG	GG	
	Peripheral	209/294/139	45.8 ± 0.7	46.0 ± 0.6	47.1 ± 0.9	0.46‡
	Central	209/294/139	32.3 ± 0.5	32.3 ± 0.4	33.0 ± 0.6	0.34‡

Pulse pressures were adjusted for sex, age, pulse rate, mean blood pressure, current smoking, alcohol intake, antihypertensive treatment and country (peripheral pulse pressure) or observer (central pulse pressure). *P* values were derived by GEE. *, For comparison between GlyGly versus GlyTrp + TrpTrp; †, for comparison between CC versus CT + TT; ‡, for comparison across the γ-adducin genotypes.

family-based analyses involving urinary phenotypes in untreated subjects ($n = 496$), we adjusted for country, sex, age, body mass index, alcohol intake and, in women, also for the use of oral contraceptives. The adjustment of urinary aldosterone additionally accounted for sodium and potassium excretion.

The within-country frequencies of the genotypes (Table 2) complied with Hardy–Weinberg equilibrium ($0.07 \leq P \leq 0.91$). The genotype and allele frequencies were similar across countries for the γ-adducin gene. In Belgium and the Czech Republic, respectively, the α-adducin Trp allele and the β-adducin T allele were more prevalent than in the other countries.

Population-based association study

Because across centres there was no heterogeneity in the phenotype–genotype relations ($0.06 \leq P \leq 0.99$), we combined all countries. Furthermore, for none of the phenotype–genotype associations, did we find significant interactions with gender ($0.28 \leq P \leq 0.96$), age ($0.36 \leq P \leq 0.99$) or generation (parents versus offspring; $0.52 \leq P \leq 0.88$).

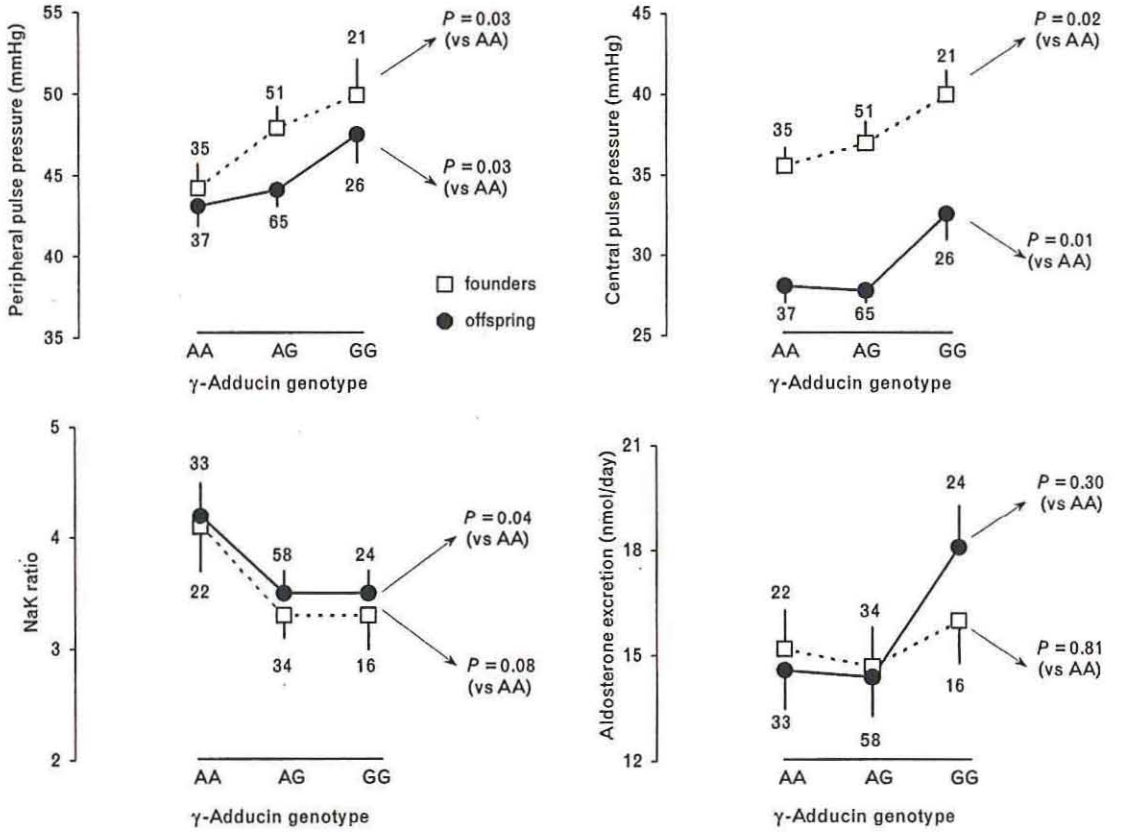
In single-gene analyses involving all subjects (Table 3), founders ($0.29 \leq P \leq 0.86$) or offspring ($0.29 \leq P \leq 0.92$), the GEE approach did not reveal any significant association between peripheral or central pulse pressure and the three adducin polymorphisms. This was also the case for the urinary excretion of sodium, potassium and aldosterone ($0.06 \leq P \leq 0.98$). However, multiple-gene analyses demonstrated significant interactions between the α- and γ-adducin genotypes in relation to the peripheral and central pulse pressures, the urinary Na^+/K^+ ratio and aldosterone excretion (Table 4). Among α-adducin GlyGly homozygotes, the aldosterone excretion decreased with the number of γ-adducin G alleles (Table 4). This trend explained the overall borderline significant interaction between α- and γ-adducin in relation to urinary aldosterone. Among carriers of the α-adducin Trp allele, the peripheral and central pulse pressures were 5.8 mmHg [95% confidence interval (CI) 2.3–9.3; $P = 0.001$] and 4.7 mmHg (CI 2.0–7.4; $P = 0.0002$) higher in γ-adducin GG homozygotes than in their AA counterparts. These genetic effects on pulse pressure were due to increases in systolic pressure, averaging 5.0 mmHg (CI 2.9–7.1; $P = 0.02$) peripherally and 4.9 mmHg (CI 3.3–6.5; $P = 0.002$) centrally. Furthermore, among α-adducin Trp carriers, the urinary Na^+/K^+ ratio was 0.8 units (CI 0.4–1.2; $P = 0.004$) lower in γ-adducin GG homozygotes than in A-allele carriers. In α-adducin GlyGly homozygotes, the γ-adducin polymorphism influenced neither peripheral nor central pulse pressures nor the urinary Na^+/K^+ ratio (Table 4).

Table 4 Pulse pressures and urinary phenotypes by α- and γ-adducin genotypes

		Pulse pressures				Urinary phenotypes				
		Peripheral		Central			Na^+/K^+ ratio		Aldosterone excretion	
α/γ-Adducin genotypes	Number	Mean ± SE	P_{int}	Mean ± SE	P_{int}	Number	Mean ± SE	P_{int}	Mean (95% CI)	P_{int}
GlyGly/AA	137	46.9 ± 0.9		32.8 ± 0.7		103	3.6 ± 0.1		18.1 (15.5–20.7)	
GlyGly/AG	178	46.2 ± 0.7		32.4 ± 0.6		129	3.7 ± 0.1		14.1 (12.3–16.1)	
GlyGly/GG	92	45.9 ± 1.1		31.5 ± 0.7		77	3.6 ± 0.1		12.5 (10.4–14.9)	
GlyTrp + TrpTrp/AA	72	43.6 ± 1.1		31.4 ± 0.9		55	4.2 ± 0.2		14.0 (11.7–16.8)	
GlyTrp + TrpTrp/AG	116	45.7 ± 0.8		32.1 ± 0.7		92	3.4 ± 0.1		14.4 (12.6–16.5)	
GlyTrp + TrpTrp/GG	47	49.4 ± 1.4	0.02	36.1 ± 1.1	0.004	40	3.4 ± 0.1	0.004	15.2 (12.1–19.0)	0.05

Pulse pressures were adjusted as in Table 3. Urinary phenotypes in 496 untreated subjects were adjusted for country, sex, age, body mass index, alcohol intake and, in women, also for the use of oral contraceptives. The adjustment of urinary aldosterone additionally accounted for sodium and potassium excretion. P_{int} is the probability of the interaction between α- and γ-adducin. Braces join trios of means which are similar.

Fig. 2



Peripheral and central pulse pressures and urinary Na^+/K^+ (NaK) ratio and aldosterone excretion by γ-adducin genotype and generation in carriers of the α-adducin Trp allele. For adjustments of pulse pressures and urinary phenotypes, see Tables 3 and 4, respectively. Vertical lines denote SEs. The number of subjects contributing to each mean is given.

Sensitivity analyses in founders and offspring separately, confirmed the associations of peripheral and central pulse pressures (Fig. 2) and the urinary Na^+/K^+ ratio (Fig. 2) with the γ-adducin polymorphism in the presence of the mutated α-adducin Trp allele. There was no significant interaction between α- and β-adducin in relation to any of the aforementioned phenotypes ($0.10 \leq P \leq 0.99$). Furthermore, in all subjects combined, the results for pulse pressure remained consistent if we additionally adjusted for hypertension status, irrespective of whether we kept mean arterial pressure in the model.

Family-based association study

Abecasis' orthogonal model did not reveal population stratification in any country ($0.07 \leq P \leq 0.95$). In all informative offspring, none of the aforementioned phenotypes was significantly associated with the transmission of the α-adducin Trp allele ($P \geq 0.22$), the β-adducin T allele ($P \geq 0.14$) or the γ-adducin G allele ($P \geq 0.07$). However, in offspring carrying the mutated α-adducin Trp allele, transmission of the γ-adducin G allele was associated with significant increases in peripheral pulse pressure (Table 5) and systolic pressure measured at the

Table 5 Association between phenotypes and transmission of the γ-adducin G allele to offspring carrying the α-adducin Trp allele

Phenotypes	Number of offspring (informative/all)	Statistical parameters			
		β	Units	χ^2	P
Pulse pressures					
Peripheral	83/128	+4.6	mmHg	8.75	0.006
Central	83/128	+2.2	mmHg	2.70	0.10
Urinary phenotypes					
Na^+/K^+ ratio	75/115	−0.6	units	5.36	0.02
Aldosterone excretion	75/115	+9.6	% change	0.33	0.56

The orthogonal model accounted for between- and within-family components of phenotypic variability. The parameter estimate (β) for the within-family variability component indicates the direction and size of the association when the γ-adducin G allele was transmitted. For adjustments of the pulse pressures and the urinary phenotypes, see Tables 3 and 4, respectively.

level of the brachial artery (effect size + 5.0 mmHg; $\chi^2 = 9.23$; $P = 0.002$) with a similar, but non-significant trend in central pulse pressure (Table 5). Furthermore, among carriers of the α-adducin Trp allele, transmission of the γ-adducin G allele was associated with a decrease in the urinary Na^+/K^+ ratio (Table 5), while among GlyGly homozygotes G allele transmission was associated with a slight decrease in the aldosterone excretion (effect size −17.4%; $\chi^2 = 3.28$; $P = 0.07$).

Discussion

The key finding of our study was an epistatic interaction between the α- and γ-adducin genes, which in three European populations of Caucasian extraction impacted on pulse pressure and the relative concentrations of sodium and potassium in urine. Indeed, the γ-adducin G allele was associated with higher peripheral and central pulse pressures and lower urinary Na^+/K^+ ratio in α-adducin Trp allele carriers, and with lower aldosterone excretion in α-adducin GlyGly homozygotes. These findings emerged from a population-based association study. Sensitivity analyses in founders and offspring separately and a family-based QTDT approach subsequently confirmed these relations.

Adducin is a heterodimeric cytoskeleton protein and consists of subunits that share a similar structure, but are translated from three different genes [1]. ADD1 (α), ADD2 (β) and ADD3 (γ) map to 4p16.3, 2p13–p14, and 10q24.2–q24.3, respectively [1,19]. The α- and γ-subunits are constituents of cell membranes at the actin–spectrin junctions across all tissues, whereas expression of the β-subunit occurs mainly in the brain and erythropoietic organs [1]. Thus, our present findings subscribe to what is known from a molecular point of view.

Previous studies in populations and patients demonstrated that the α-adducin Gly460Trp polymorphism, alone or in combination with variation in the genes encoding β-adducin [8], angiotensin-converting enzyme or aldosterone synthase, influences the prevalence and incidence of hypertension [6], the serum creatinine concentration [7], proteinuria [7], intima–media thickness of the femoral artery [10], distensibility of the large arteries [11] and the risk of cardiovascular events [20]. On the other hand, other researchers [21–24], mostly in single-gene studies, failed to demonstrate an association between blood pressure analysed as a continuous or dichotomous trait and the α-adducin polymorphism.

The mechanisms underlying our present observations, which link peripheral and central pulse pressures with changes in the urinary Na^+/K^+ ratio and aldosterone excretion, remain to be elucidated. However, based on current knowledge, several pathophysiological pathways might be implicated. First, investigations in rats [2], *in vitro* transfection studies [3], dietary [25] and pharmacological [26] interventions in hypertensive patients, and epidemiological studies [6–8] revealed a coherent sequence of events leading from a point mutation in the α-adducin subunit to a cellular dysfunction characterized by higher activity of the sodium pump [3], hence increased tubular sodium reabsorption in the kidney [27], and ultimately hypertension [6]. In keeping with this body of evidence, we found that the increase in pulse pressure was due to an elevation in systolic pressure without decrease in diastolic pressure. From a haemodynamic point of view, these findings suggested that, in addition to systolic blood pressure, also mean blood pressure was elevated, either due to an increase in stroke volume, peripheral resistance or both factors. Analysis of our data confirmed the elevation of mean blood pressure (data not shown) with the γ-adducin G allele in α-adducin Trp carriers.

Furthermore, it is conceivable that the constitutive activation of the sodium pump in α-adducin Trp allele carriers not only occurs in renal tubular cells, but that it might also be present in vascular smooth muscle cells or adrenal glomerulosa cells. The carboxy-terminal region of the adducin subunits contains the binding site for calmodulin and is required for the interaction with spectrin and actin [1]. In vascular myocytes, enhanced Na^+,K^+-ATPase activity might reduce the sarcolemmal Na^+/Ca^{2+} exchange and, through calcium-dependent pathways, modulate excitation–contraction coupling and the expression of growth-related genes [28]. A similar mechanism might also apply to adrenal glomerulosa cells. Indeed, potassium stimulates the secretion of aldosterone via depolarization of the cell membrane, which activates voltage-dependent calcium channels [29]. Via activation of the Na^+,K^+-ATPase activity, mutations in the adducin genes might influence the cytosolic calcium concentration in adrenal cells and interfere with the regulation of the aldosterone biosynthesis [30].

Dependent on the α-adducin genotype, we noticed dissociation between the urinary Na^+/K^+ ratio and the aldosterone excretion. Indeed, in α-adducin GlyGly homozygotes, the urinary Na^+/K^+ ratio was similar across the γ-adducin genotypes, whereas aldosterone excretion was 31% lower in GG than AA homozygotes. Conversely, with the mutated α-adducin Trp allele in the background, the urinary Na^+/K^+ ratio was 19% lower in γ-adducin G allele carriers than in AA homozygotes, with no significant differences in the aldosterone excretion. The observation, that in young as well as older rats γ-adducin is expressed in the adrenal gland at higher concentrations than in most other tissues, is likely to be relevant to our present findings [31]. In addition to the adrenal aldosterone biosynthesis, adducin might also affect other regulators and hormones underlying the urinary Na^+/K^+ ratio. For instance, in renal tubular cells, the serine–threonine kinase WNK4 acts as a molecular switch of various ion transporters, which can vary the

balance between NaCl reabsorption and K^+ secretion [32]. Although in the distal nephron the electrogenic epithelial sodium channel provides the driving force for K^+ secretion [33], changes in Na^+,K^+-ATPase activity due to mutations in adducin might also impact on transepithelial ion transport. Furthermore, we previously demonstrated that, in the population at large, the plasma concentration of endogenous ouabain, a steroid hormone released from the adrenal gland and possibly from the hypothalamus [34], increases with the number of mutated α-adducin Trp alleles [35]. At very low concentrations within the nanomolar range, endogenous ouabain may enlarge the membrane pool of active sodium pumps [36] and activate mediators of cell growth [28,34]. To what extent the aforementioned pathways might increase pulse pressure, either via renally mediated effects on the circulating plasma volume and cardiac output or via structural or functional alterations in the vasculature, must be further clarified.

The present study has to be interpreted within the context of its limitations and strengths. We measured only one single nucleotide polymorphism per gene and we may therefore have underestimated the full functional impact of the adducin genes on pulse pressures. We did not measure the intermediate phenotypes, which might link pulse pressure with changes in sodium and potassium homeostasis, such as the plasma concentration of endogenous ouabain. Because of this, our pathophysiological interpretations should be considered as hypothesis-generating and should withstand the test of confirmatory evidence and experimental investigation. Peripheral and central pulse pressures are quantitative traits, which arise through complex interaction between multiple genes and environmental factors and are prone to measurement error. In the present study, only four experienced observers performed all measurements of central pulse pressure, with high intra-observer intra-session reproducibility. We derived the peripheral pulse pressure from five conventional blood pressure readings at the subjects' homes, for which a quality assurance and quality control programme was implemented across all EPOGH centres, with satisfactory results [37]. More importantly, the peripheral and central pulse pressures were measured on different occasions with different techniques, but nevertheless both phenotypes were consistently influenced by the polymorphisms in the α- and γ-adducin genes. There was also a high degree of internal consistency between the results of the population-based and family-based analyses, which started from divergent assumptions with implementation of different statistical algorithms, and between the findings within each country (data not shown) and in the whole study sample. The QTDT approach is not sensitive to population stratification or admixture. Our findings can also be readily generalized, because we recruited population samples randomly in three different European countries.

In conclusion, in carriers of the mutated α-adducin Trp allele, peripheral and central pulse pressures increase with the γ-adducin G allele. This epistatic interaction is physiologically consistent with the heterodimeric structure of the cytoskeleton protein adducin. The underlying molecular mechanisms remain to be elucidated, but might involve dysregulation of transmembranous active sodium transport [3,4] at the renal, adrenal or systemic level.

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Appendix

EPOGH centres

Belgium (Hechtel-Eksel)

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Bulgaria (Sofia)

C. Nachev, A. Postadjian, E. Prokopova, E. Shipkovenska and K. Vitljanova.

Czech Republic (Pilzen)

J. Filipovský, J. Kucerová, V. Svobodová and M. Tichá.

Czech Republic (Prague)

O. Beran, L. Golán, T. Grus, Z. Marecková and J. Peleška.

Italy (Padova)

E. Casiglia, A. Pizzioli and V. Tikhonoff.

Poland (Cracow)

A. Adamkiewicz-Piejko, M. Cwynar, J. Gąsowski, T. Grodzicki, K. Kawecka-Jaszcz, W. Lubaszewski, A. Olszanecka, K. Stolarz, B. Wizner, W. Wojciechowska and J. Życzkowska.

Romania (Bucharest)

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Příloha 4

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Arterial Characteristics in Normotensive Offspring of Parents With or Without a History of Hypertension

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Background: In this study we compared the arterial characteristics and blood pressure (BP) of normotensive offspring of two normotensive parents (OFF/NT) and normotensive offspring who had at least one hypertensive parent (OFF/HT).

Methods: A total of 174 OFF/HT (17 to 40 years of age) and 59 OFF/NT (16 to 34 years) were recruited in Cracow, Poland (n = 138) and Pilsen, Czech Republic (n = 95). Peripheral pulse pressure (PPp) was determined from conventional and 24-h ambulatory BP. A SphygmoCor device was used to measure the central (CAIx) and peripheral (PAIx) systolic augmentation indexes, central pulse pressure (PPc), and the aortic pulse wave velocity (PWV). In multivariate analyses family clusters and significant covariates were accounted for.

Results: The OFF/HT had higher ($.14 < P < .0007$) conventional BP and PPp on conventional BP measurement (121/75 v 114/71 mm Hg and 46 v 42 mm Hg) as well as on 24-h ambulatory monitoring (118/70 v 114/67 mm Hg and 48 v 47 mm Hg). OFF/HT, compared with OFF/NT, also had higher ($.05 < P < .0008$) PPc (28 v 26 mm Hg), PAIx (54.7% v 44.9%), CAIx (108.8% v 99.8%), and PWV (7.4 v 6.6 m/sec). However, complex adjustment including mean arterial pressure and age removed the differences between the offspring in the PAIx, CAIx, and PWV.

Conclusions: Large-artery properties are altered in OFF/HT compared with OFF/NT. The findings from this cross-sectional study suggest that the alterations in arterial function in subjects with a family history of hypertension are determined mainly by an increased BP and age-related hemodynamic changes. Am J Hypertens 2006;19: 264–269 © 2006 American Journal of Hypertension, Ltd.

Key Words: Hypertension, augmentation index, pulse wave velocity, offspring.

Offspring of hypertensive parents have higher blood pressure (BP) and increased stiffness of the carotid artery compared with offspring of normotensive parents.[1] Pulse wave analysis, as implemented by O'Rourke and Gallagher using a SphygmoCor device,[2] provides a simple and reproducible method to assess various indexes of arterial stiffness, including the peripheral and central augmentation indexes and aortic pulse wave velocity. To our knowledge only one study based on the SphygmoCor technique[3] reported an increased augmentation index, but not higher brachial pulse wave velocity, in offspring of hypertensive compared with normotensive parents. The goal of the present study was to compare the above-mentioned indexes of arterial stiffness as well as

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The nuclear families in this study were recruited in the framework of the European Project on Genes in Hypertension, which was supported by the European Union (contract number IC15-CT98-0329-EPOGH and QLGI-CT-2000-01137-EURNETGEN) and which is endorsed by the European Society of Hypertension and European Council for Cardiovascular Research. The Czech Ministry of Health provided additional funding (NG 7445-3, to JF). This study was also supported by a grant from the Czech Society of Hypertension and by the International Scientific Collaboration between Poland and Flanders (contract BIL 00/18).

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conventional and ambulatory BP among offspring with a different family history of hypertension.

Methods

Study Population

The European Project on Genes in Hypertension (EPOGH) was ethically approved and conducted according to the principles outlined in the Helsinki declaration for investigations in human subjects.[4] Participants gave informed written consent.

Two EPOGH centers (Cracow, Poland, and Pilsen, the Czech Republic) opted to take part in vascular phenotyping using the SphygmoCor device. They randomly recruited nuclear families of white ethnicity, including offspring with a minimum age of 16 years and parents with a maximum age of 68 years. Overall, the response rate was 82%. Of the 482 participants recruited in Cracow (n = 299) or Pilsen (n = 183), we eliminated 80 subjects from analysis because the recorded pulse wave was of insufficient quality (n = 15), because offspring were hypertensive (n = 12), or because of missing information concerning the hypertensive or normotensive status of parents (n = 53). Among the offspring of hypertensive parents, 25 subjects had both parents who were hypertensive. In those families considered as normotensive, both parents were required to have normal BP according to the 2003 criteria of the European Society of Hypertension.[5] Thus the overall number of participants who were statistically analyzed totaled 402, of whom 233 were offspring. All measurements of BP and arterial parameters were completed in all participants.

Measurements of BP

The conventional BP phenotype in parents and offspring was the average of five consecutive readings obtained at one home visit. Hypertension was defined as a conventional systolic BP of ≥140 mm Hg, diastolic BP of >90 mm Hg, or use of antihypertensive medication.[5] We programmed oscillometric and properly validated 90202 or 90207 SpaceLabs monitors (Redmond, WA) to obtain BP readings at intervals of 20 min from 8 AM until 10 PM and at 45 min from 10 PM to 8 AM. We monitored the ambulatory BP within 1 week of the home visit. From unedited recordings, we calculated the average over 24-h BP with weights according to the time intervals between successive readings. From the conventional and 24-h ambulatory BP, we derived peripheral pulse pressure as the difference between systolic and diastolic BP.

Measurements of Arterial Properties

All arterial measurements were performed in the clinic. After the subjects had rested for 15 min we recorded, during an 8-sec period, the radial arterial waveform at the dominant arm by applanation tonometry. We used a high-fidelity SPC-301 micromanometer (Millar Instruments, Inc., Houston, TX) interfaced with a laptop computer running SphygmoCor software, version 6.31 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). We discarded recordings when the systolic or diastolic variability of consecutive waveforms was >10% or when the amplitude of the pulse wave signal was <80 mV. The pulse wave was calibrated by measuring BP at the contralateral arm immediately before the recordings. From this BP reading, we calculated mean arterial pressure as diastolic pressure plus one third of pulse pressure.

From the radial signal the SphygmoCor software derives the aortic pulse wave by means of a validated generalized transfer function.[6,7] The radial augmentation index was defined as the ratio of the second to the first peak of the pressure wave expressed as a percentage. The aortic augmentation index was the difference between the second and first systolic peak given as a percentage of the aortic pulse pressure. From the aortic pulse wave we derived central pulse pressure. For statistical analysis, the average of the peripheral and central waveforms over the 8-sec measurement period was used.

Aortic pulse wave velocity was measured by sequential recordings of the arterial pressure wave at the carotid and femoral arteries and by measurement of the distance from the carotid sampling site to the suprasternal notch and from the suprasternal notch to the femoral sampling site. Aortic pulse wave velocity was calculated as the ratio of the distance (in meters) to the transit time (in seconds).

Other Measurements

A standardized questionnaire was administered to obtain information on each subject's medical history, smoking and drinking habits and use of medications. After the subject had fasted overnight, a venous blood sample was collected for measurement of blood glucose and serum lipids by means of standardized automated methods.

Statistical Methods

For database management and statistical analysis SAS software version 9.1 (SAS Institute Inc., Cary, NC) was used. Means and proportions were compared by the Student t test and the χ^2 statistic, respectively. Possible covariates of the phenotypes were searched for with the use of stepwise multiple regressions. Those covariates with P values < .15 were used for further analysis. The PROC MIXED procedure of the SAS package[8] was used to account for the nonindependence of observation within families while adjusting for covariates including study center, sex, age, body height, pulse rate and mean arterial pressure (taken immediately before arterial measurements), current smoking, regular alcohol intake (>5 g/day), and serum total cholesterol, as appropriate.

Table 1. General characteristics of offspring in study

Characteristic	Normotensive parents	Hypertensive parents*	*P*
Number of offspring	59	174	
Anthropometrics			
Age (y)	23.0 ± 3.9	25.5 ± 5.4	.001
Female gender (%)	32 (54.2)	87 (50)	.58
Body height (cm)	172.0 ± 7.7	172.0 ± 9.3	.93
Body weight (kg)	66.1 ± 11.0	70.5 ± 14.8	.04
Body mass index (kg/m^2)	22.2 ± 2.9	23.7 ± 4.0	.01
Lifestyle			
Current smoker, *n* (%)	15 (25.4)	41 (23.6)	.77
Alcohol intake >5g/day, *n* (%)	32 (54.2)	79 (45.4)	.24
Biochemistry			
Blood glucose (mmol/L)	4.6 ± 0.8	4.7 ± 0.8	.84
Serum total cholesterol (mmol/L)	4.6 ± 1.0	4.7 ± 1.0	.61
Serum HDL cholesterol (mmol/L)	1.6 ± 0.3	1.6 ± 0.4	.41

Values are arithmetic mean ± SD or percentage of subjects. *P* values refer to the comparison of offspring of normotensive and hypertensive parents. HDL = high-density lipoprotein.

* Hypertension was based on blood pressure measured at home (average of five readings) of ≥140 mm Hg systolic or ≥90 mm Hg diastolic or use of antihypertensive drugs.

Results

Characteristics of Participants

The overall study population included 233 offspring from 123 nuclear families. The number of siblings per family was one sibling in 22 families, two in 94 families, three in six families, and five in one family. Table 1 lists the general characteristics of the offspring according to presence or absence of parental hypertension. Offspring of hypertensive parents compared with offspring of normotensive parents were on average 2.5 years older and 4.4 kg heavier, with body mass index 1.5 kg/m^2 higher. Otherwise there were no differences in lifestyle and biochemical measurements between the two groups of offspring.

Hypertensive parents (n = 110; 60% women) compared with normotensive parents (n = 59; 52.5% women) were slightly older (51.9 *v* 48.7 years; $P < .0001$) and had higher mean values of body mass index (29.6 *v* 26.5 kg/m^2; $P < .0001$), systolic and diastolic BP (144 *v* 121 mm Hg and 90 *v* 78 mm Hg; $P < .0001$), peripheral and central augmentation indexes (87.3 *v* 76.2% and 144.2 *v* 132.7%; $P = .0009$), and pulse wave velocity (9.6 *v* 8.0 m/sec; $P < .0001$). Otherwise the characteristics of the hypertensive and normotensive parents were similar.

Hemodynamic Measurements

In unadjusted analyses most hemodynamic measurements, including BP and peripheral pulse pressure at the subjects' homes, the 24-h ambulatory BP, the central pulse pressure, the central and peripheral augmentation indexes and aortic pulse wave velocity were significantly higher in offspring of hypertensive compared with normotensive parents (Table 2). In contrast the 24-h peripheral pulse pressure and pulse rate (71.6 *v* 72.4; $P = .62$) measured during the vascular examination were similar in the two groups (Table 2).

Based on the results of stepwise regression, we adjusted all hemodynamic measurements for center, sex, age, pulse rate, current smoking, alcohol intake, and serum total cholesterol. In addition we adjusted BP for body mass index and all vascular measurements for mean arterial pressure. The systolic augmentation indexes were also adjusted for body height.

In fully adjusted analyses (Table 3), systolic BP and peripheral pulse pressures on conventional measurement at home and the 24-h systolic and diastolic BP remained significantly higher in offspring of hypertensive parents compared with offspring of normotensive parents. In contrast, in fully adjusted analyses, the differences in the arterial characteristics between the two groups of offspring disappeared. As shown in Fig. 1, this was mainly caused by the introduction in the regression model of age, mean arterial pressure, or both covariates. These results were consistent, when we ran separate analyses in offspring with one (n = 149) or two (n = 25) hypertensive parents (data not shown).

Discussion

The key finding of the present study was that in unadjusted analyses systolic and diastolic BP on conventional as well as 24-h ambulatory measurement, peripheral pulse pressure on conventional BP measurement, the central and peripheral systolic augmentation indexes and aortic pulse wave velocity were significantly higher in normotensive offspring with at least one hypertensive parent compared with normotensive offspring of two normotensive parents. Fully adjusted analyses confirmed these findings for systolic BP irrespective of the type of BP measurement, for

Table 2. Unadjusted hemodynamic measurements in offspring

Characteristic	Normotensive parents	Hypertensive parents	*P*
Number of offspring	59	174	
Measurements at home*			
Systolic BP (mm Hg)	114 ± 2	121 ± 1	.0007
Diastolic BP (mm Hg)	71 ± 1	75 ± 1	.02
Peripheral pulse pressure (mm Hg)	42 ± 1	46 ± 8	.02
24-h Ambulatory measurements			
Systolic BP (mm Hg)	114 ± 1	118 ± 1	.01
Diastolic BP (mm Hg)	67 ± 1	70 ± 1	.05
Peripheral pulse pressure (mm Hg)	47 ± 1	48 ± 1	.14
Arterial measurements			
Central pulse pressure (mm Hg)	26 ± 1	28 ± 1	.05
Peripheral augmentation index (%)	44.9 ± 2.5	54.7 ± 1.4	.0008
Central augmentation index (%)	99.8 ± 2.6	108.8 ± 1.5	.003
Aortic pulse wave velocity (m/s)	6.6 ± 0.3	7.4 ± 0.2	.03

Values are arithmetic mean ± SE adjusted for family clusters. *P* values refer to the comparison of offspring of normotensive and hypertensive parents. For further explanation, see Table 1. BP = blood pressure.
* Average of five measurements obtained at one home visit.

peripheral pulse pressure on conventional measurement, and for the 24-h diastolic BP. However, adjustment for center, sex, age, mean arterial pressure, pulse rate, current smoking, alcohol intake, and serum total cholesterol removed the differences between the two groups of offspring in the systolic augmentation indexes and aortic pulse wave velocity.

To our knowledge only three studies dealt with large artery properties in young subjects at risk of hypertension. Meaney et al[1] studied 100 nonobese offspring aged 10 to 20 years who were descendants of hypertensive or normotensive parents. By means of an ultrasound technique these investigators studied the characteristics of the ascending aorta and the common carotid artery. Carotid but not aortic stiffness and maximum velocity flow in the aorta were significantly higher in the offspring of the hypertensive parents; the comparisons were, however, not adjusted for BP which was already higher in this group.

Yasmin et al[3] recruited offspring of families with essential hypertension (mean age 39 years) and normotensive controls (mean age 43 years). They measured BP at the brachial artery and applied the same SphygmoCor technique as we used. They observed that offspring of hypertensive compared with normotensive parents had higher systolic/diastolic BP (123/75 *v* 118/71 mm Hg). They also reported that offspring of hypertensive parents

Table 3. Adjusted hemodynamic measurements in offspring

Characteristic	Normotensive parents	Hypertensive parents	*P*
Number of offspring	59	174	
Measurements at home*			
Systolic BP (mm Hg)	115 ± 2	120 ± 1	.009
Diastolic BP (mm Hg)	73 ± 1	74 ± 1	.24
Peripheral pulse pressure (mm Hg)	43 ± 1	46 ± 1	.02
24-h Ambulatory measurements			
Systolic BP (mm Hg)	115 ± 1	118 ± 1	.01
Diastolic BP (mm Hg)	67 ± 1	70 ± 1	.02
Peripheral pulse pressure (mm Hg)	47 ± 1	48 ± 0	.24
Arterial measurements			
Central pulse pressure (mm Hg)	27 ± 1	28 ± 1	.87
Peripheral augmentation index (%)	50.9 ± 1.9	52.5 ± 1.1	.50
Central augmentation index (%)	105.8 ± 1.9	106.6 ± 1.1	.72
Aortic pulse wave velocity (m/sec)	6.9 ± 0.1	7.1 ± 0.1	.23

Values are arithmetic mean ± SE, adjusted for study center, sex, age, pulse rate, current smoking, alcohol intake, and serum total cholesterol. Additional adjustments included: 1) for blood pressure; body mass index; 2) for the augmentation indexes: body height and mean arterial pressure; 3) for aortic pulse wave velocity and central pulse pressure: mean arterial pressure. *P* values for the comparison of offspring of normotensive and hypertensive parents also account for family clusters. BP = blood pressure.
* Average of five measurements obtained at one home visit.

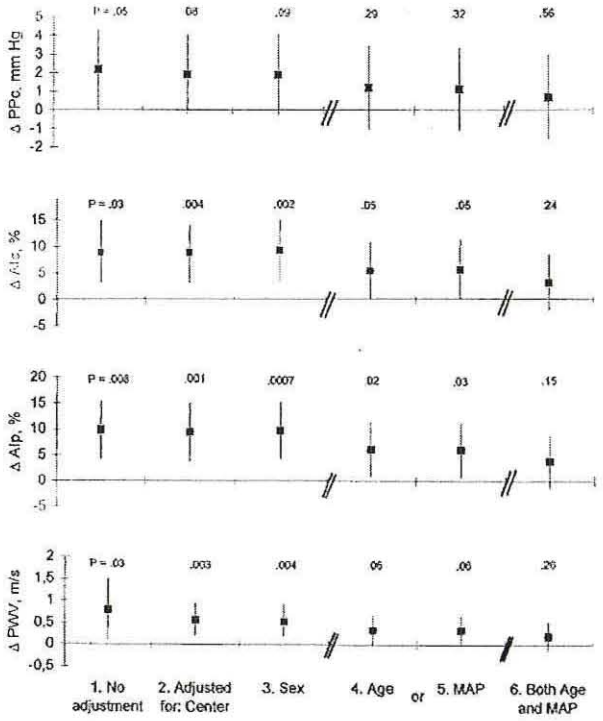

FIG. 1. Effect of cumulative adjustments on the differences in arterial characteristics between offspring of normotensive and hypertensive parents. Mean differences with 95% confidence intervals and corresponding *P* values are given (eg, ΔPPc = 2.2 mm Hg indicates that central pulse pressure (PPc) in offspring of hypertensive parents was higher on average by 2.2 mm Hg than in offspring of normotensive parents). AIc = central augmentation index; AIp = peripheral augmentation index; PPc = central pulse pressure; PWV = aortic pulse wave velocity, respectively. Adjustments: unadjusted (1); adjusted for center (2); center and sex (3); center, sex, and age (4); center, sex, and mean arterial BP (5); center, sex, and both age and mean arterial BP (6).

had higher peripheral pulse pressure (49 *v* 47 mm Hg; $P <$ .01), higher central pulse pressure (35 *v* 35; $P <$ .01), and higher central systolic augmentation index (19.1% *v* 17.8%; $P <$ .01), but similar brachial pulse wave velocity (8.40 *v* 8.24 m/sec); aortic pulse wave velocity was not studied. However when we tried to replicate the calculations reported in Table 1 in the paper by Yasmin et al, we found nonsignificant *P* values for peripheral pulse pressure ($t = 1.82$; $P = .08$), central pulse pressure ($t = 0.23$; $P = .78$), and the systolic augmentation index ($t = 0.85$; $P = .30$). The sample studied by Yasmin et al differed from ours: the mean age was higher in the Yasmin et al study and the offspring of hypertensive parents had higher HDL cholesterol, creatinine, and blood glucose levels and smoking prevalence, whereas in our study these parameters were similar between the two groups.

Rajzer et al[9] studied the effect of selected clinical and biochemical measurements on the pulse wave velocity in 70 young normotensive individuals. The subjects were subdivided into two groups: those with and without a family history of arterial hypertension. They observed that pulse wave velocity did not differ between these two groups (9.7 *v*9.3 m/sec; $P = .52$).

Other authors used the techniques which focus on the functional abnormalities separately in small and large arteries.[10,11] The results showed that especially small artery elasticity (the C2 component of the modified Windkessel model of circulation) may correlate closely with BP[10] and predict future cardiovascular events.[11]

The recently published study by Dernellis and Panaretou provided evidence that increased aortic stiffness precedes hypertension.[12] The investigators examined 2512 subjects (aged 35 to 94 years) who were not hypertensive at baseline and followed them for 4 years. Aortic stiffness measured at baseline by means of echocardiographic technique predicted progression to hypertension in both sexes and in younger as well as older subjects. The results were consistent for all the three aortic stiffness indexes used and for different BP parameters (increase in systolic, in diastolic and in pulse pressure and hypertension incidence) and remained significant after adjustment for baseline BP, age, and all other classic cardiovascular risk factors. Thus aortic wall properties are likely to play a role in the pathogenesis of hypertension already in its early stages. On the other hand, the relationship of BP and arterial properties is reciprocal; it has been shown in the Bogalusa Heart Study[13] that childhood BP predicted arterial stiffness assessed on the mean 26.5 years later by brachial–ankle pulse wave velocity.

Our study was cross-sectional, and therefore the question of cause and consequence cannot be answered on the basis of our data. However it adds to the longitudinal studies[12,13] because it suggests that an increased BP and increased arterial stiffness run in parallel in hypertensive families. This might be caused by genetic factors, shared environmental influences or their interaction. In a study of 225 monozygotic and 594 dizygotic female twin pairs, aged 18 to 73 years Snieder et al[14] noticed that the heritability of the central augmentation index was 37% and was largely independent of age, BP, heart rate and height. In 950 Native Americans from 32 extended families, North et al[15] reported that the heritability of carotid stiffness and the central augmentation index were 23% and 18%, respectively. These two studies underscore the importance of genetic factors in pathogenesis of arterial stiffness.

Our results have to be interpreted within the context of its limitations. Our sample size was relatively small, although still larger than in the three previously published studies with similar design.[1,3,9] This may be one of the reasons why the differences in arterial parameters were no more significant after the complex adjustment. On the other hand we collected six measurements reflecting arterial stiffness and found great consistency among these measurements in the comparison between the two groups of offspring.

In conclusion compared with normotensive offspring of normotensive parents, normotensive offspring of hyperten-

sive parents have increased BP and impaired arterial properties, namely aortic stiffness and pulse wave reflection as measured noninvasively by assessing aortic pulse wave velocity and radial augmentation index. The present cross-sectional findings, in keeping with the large prospective study by Dernellis and Panaretou[12] suggest that the alteration in arterial function is present already in nonhypertensive subjects at risk of hypertension and it may contribute to the progression to hypertension in later life.

Acknowledgments

This study would not have been possible without the voluntary collaboration of the participants.

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RIGINAL ARTICLE

arge artery stiffness and pulse wave reflection: Results of a opulation-based study

AN FILIPOVSKÝ[1], MILENA TICHÁ[1], RENATA CíFKOVÁ[2], VĚRA LÁNSKÁ[3], VLASTA ŤASTNÁ[1] & PATRIK ROUČKA[1]

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stract

jective. To assess the determinants of large artery stiffness and pulse wave reflection in a population sample. *Methods.* A random population sample aged 25–65 years was selected in nine districts of the Czech Republic for a survey of rdiovascular risk factors (Czech post-MONICA). Of 891 individuals screened in the Pilsen centre in the year 2000, erial properties were studied in 291 (143 males and 148 females) using the Sphygmocor device. Pulse wave velocity WV) in the aorta and in the lower limbs was measured to assess large artery stiffness. Wave reflection was assessed from lial pulse wave analysis; the main estimated parameter was peripheral augmentation index (PAI) defined as P2/P1=ratio pulse pressures measured at the peaks of secondary and primary waves. *Results.* Aortic PWV increased with age <0.001) and was similar in both sexes. Lower extremity PWV increased with age in women, but not in men, and its mean ue was higher in men ($p<0.01$). PAI was higher in females in all age groups ($p<0.001$) and increased steeply with age in th sexes ($p<0.001$). PAI was increased in current smokers ($p<0.01$ in both sexes) and in male smokers, the reflected ve returned earlier than in male non-smokers ($p<0.05$). Correlation coefficient of PAI with aortic PWV was 0.22 <0.01), and with central augmentation index (CAI), derived from PAI by mathematical transformation, was 0.94 <0.001). Multiple regression analyses, where age, sex, systolic blood pressure (SBP), total cholesterol level, smoking, cose level and body mass index were included as independent variables, were performed. PAI was better predicted than tic or lower extremity PWV in these models (41%, 14% and 10% of variance explained, respectively). Age, female sex, oking, SBP and total cholesterol predicted PAI level whereas age, SBP and glucose level were the main determinants of tic PWV. *Conclusions.* Of the studied arterial parameters, PAI showed the closest association with cardiovascular risk tors. The correlation between PAI and aortic PWV was loose, and both parameters had partially different determinants. I, which is obtained by direct measurement above radial artery, was practically identical with the mathematically derived I in the studied population sample, and therefore, it is a suitable parameter for studying the phenomenon of wave ection.

y Words: *Aortic stiffness, blood pressure, cardiovascular risk assessment, smoking, wave reflection*

roduction

e structure and function of central large arteries nge with increasing age. The aorta and its main nches, where elastin compounds in the vessel wall dominate over collagen, become stiff as the ount of collagen fibres increases. The underlying nges responsible for the process of stiffening are diffuse and occur mainly in the media of the arterial wall. They lead to enlargement of the central arteries and result in impairment of their buffering function (1). Thus, arterial stiffening, caused by the aging process and accelerated by high blood pressure (BP), is different from atherosclerosis in many aspects. Atherosclerosis develops above all at predisposed

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sites, it is localized in the intima and leads usually to the narrowing of the vessel lumen; therefore, the conduit function is predominantly impaired. Besides the stiffening of elastic-type large arteries, the pulse waveform changes with age. The primary pressure wave, generated by contraction of the left ventricle, propagates towards the periphery where, probably at the level of smallest arteries, it is reflected and runs back. The reflected wave interferes with the primary one, and the definite pulse waveform is therefore composed of primary and secondary wave (the higher-order waves seem to play a minor role). In young individuals, the reflected wave falls typically into early diastole and diminishes the diastolic BP (DBP) drop. As age increases, the wave is higher and tends to return earlier for complex reasons. It produces a late systolic peak, which may be as high as 30–40 mmHg. Arterial stiffness can be assessed non-invasively by measuring the velocity of pulse wave propagation between two sites (pulse wave velocity, PWV), and wave reflection is estimated from pulse waveform analysis using most often augmentation index (AI).

Both large artery stiffness and wave reflection contribute to the increase of systolic BP (SBP) and widening of pulse pressure with age (2). This type of BP is associated with a high risk of cardiovascular events, and SBP has been shown to be associated with risk of cardiovascular events more closely than DBP (3). Therefore, it is important to study the pathogenesis and the risk factors for the SBP increase. Whereas atherosclerosis and its risk factors have been explored in detail, little is known about factors determining the mechanical properties of the arteries. As their changes usually precede the development of atherosclerosis, a detailed knowledge of mechanisms could allow early prevention of vascular changes. This paper presents an analysis of several vascular indices showing arterial stiffness and wave reflection, measured in healthy subjects selected randomly from a general population. Whereas there are some data about aortic stiffness in epidemiological studies (4,5), data about stiffness of peripheral arteries and about wave reflection in normal subjects are rare (6,7). PWV and AI are considered by some authors to give similar information about large artery properties. This presumption need not be correct, as wave reflection is a complex phenomenon, depending not only on large artery stiffness. Therefore, we focused in the present analysis on the relationships between the parameters of stiffness and wave reflection. We further studied the relationships of these parameters with age, sex and classical cardiovascular risk factors

Population and methods

The present study was performed as part of the Czech post-MONICA study as an extension of the WHO MONICA (Multinational MONItoring of trends and determinants in CArdiovascular disease) study. In 2000/2001, a survey for cardiovascular risk factors was conducted in nine districts of the Czech Republic, involving a 1% population random sample aged 25–65 years in each district. Selection (stratified by age, sex and community size) was made from the General Health Insurance Registry keeping, by law, a list of all those insured. The examination included patient's medical history, physical examination, three measurements of BP to the nearest 2 mmHg and taking a fasting blood sample.

In the Pilsen centre, the overall response rate was 67.5%. Besides the screening for cardiovascular risk factors, vascular properties were examined in about one half of the randomly chosen subjects (Table I) using the Sphygmocor device (AtCor Medical Ltd., Australia). The first part of this examination was the registration of radial pulse wave. Radial pulse wave analysis (PWA) was used primarily to evaluate wave reflection (Fig. 1). The parameter showing wave reflection is AI, defined as the ratio of pulse pressure at the peaks of secondary to primary wave (AI=P2/P1). The radial waveform can be mathematically transformed to the form in central circulation (8) and thus, CAI and several other central haemodynamic parameters are estimated. The mathematical transformation using the general transfer function was validated towards invasive measurements by some authors (9); however, others cast doubts on its precision (10). We focused therefore on peripheral parameters, which are directly measured. Immediately before the radial pulse wave registration, an additional BP reading was obtained using the Omron 705CP oscillometric device, validated by an independent centre (11) and recommended by the European Society of Hypertension (12). The probability of observer bias is minimized with this type of device. The second part of the examination was PWV measurement assessing arterial stiffness (7,13). We used the same device and measured it with the patient in supine position in the aorta, i.e. between carotid and femoral arteries, and in the lower extremity, i.e. between femoral and dorsalis pedis/tibialis posterior arteries. Consecutive registrations of the pulse waves are ECG gated and thus, the time shift between the appearance of wave at the first and the second sites can be calculated. The distance between the two sites was measured on the body surface; to determine aortic PWV, we measured the distance from the jugular fossa to the pulsation of the

Table I. Population characteristics.

	Males	Females	*p*
Subjects (n)			
Age 25–34	29	26	
Age 35–44	29	30	
Age 45–54	49	47	
Age 55–65	36	45	
Total number	143	148	0.768
Indices of vascular properties[a]			
Aortic PWV (m/s)	7.8 ± 2.8	7.4 ± 2.3	0.171
Lower extremity PWV (m/s)	13.0 ± 5.2	11.2 ± 3.6	<0.001
PAI (%)	66.2 ± 20.2	77.4 ± 20.1	<0.001
TP1 (ms)	101.3 ± 14.8	102.5 ± 17.9	0.546
TP2(ms)	227.0 ± 23.0	222.2 ± 30.2	0.136
CAP (mmHg)	6.8 ± 6.8	9.6 ± 6.9	<0.001
CAI (%)	123.8 ± 21.4	137.4 ± 22.8	<0.001
Cardiovascular risk factors			
Systolic blood pressure (mmHg)	128.3 ± 15.5	123.0 ± 17.2	0.01
Diastolic blood pressure (mmHg)	82.8 ± 9.8	78.0 ± 9.0	<0.001
Hypertension (%)[b]	39.9	28.6	<0.05
Total cholesterol (mmol/l)	5.9 ± 1.2	5.8 ± 1.1	0.339
HDL-cholesterol (mmol/l)	1.3 ± 0.4	1.6 ± 0.3	<0.001
LDL-cholesterol (mmol/l)	3.7 ± 0.9	3.5 ± 1.0	0.19
Triglycerides (mmol/l)	2.0 ± 1.2	1.5 ± 0.7	<0.001
Hypercholesterolaemia (%)[c]	80.3	71.2	0.105
Glucose (mmol/l)	5.7 ± 1.3	5.5 ± 1.5	0.231
Diabetes (%)[d]	5.3	5.2	0.975
Current smoking (%)	40.6	28.6	0.032
Height (cm)	177.3 ± 7.0	163.6 ± 6.8	<0.001
Weight (kg)	85.8 ± 13.3	70.0 ± 11.5	<0.001
BMI (kg/m^2)	27.3 ± 4.0	26.2 ± 4.4	0.027

Peripheral parameters are those measured on radial artery, central ones are estimated by mathematical transformation in the aorta. [a]Mean ± SD is given. [b]Mean of three measurements ≥140/90 mmHg and/or on antihypertensive treatment. [c]total cholesterol level ≥5 mmol/l and/or on antihyperlipidemic treatment. [d]glucose level ≥7 mmol/l and/or on antidiabetic treatment. PWV, pulse wave velocity; PAI, peripheral augmentation index; CAP, CAI, central augmentation pressure and augmentation index, respectively; TP1, TP2, time to peaks of primary and secondary wave, respectively, measured on radial artery; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index.

emoral artery in the groin, subtracting from this listance the distance from the jugular fossa to carotid ulsation, since the pulse runs here in the direction pposite to that in the aorta. The average of measure-ients over a period of 8 s (9–10 cardiac cycles) was alculated after the exclusion of extreme values. Both WA and PWV were shown to have good reproduci-ility (14,15). Wilkinson et al. found that within-bserver variability was 0.49 ± 5.37% for AI and .07 ± 1.17 m/s for aortic PWV (14). In our previous tudy performed in healthy subjects, AI was found ess variable than BP values when several measure-ients of these parameters were compared (15).

Statistical analysis was performed using Pearson's orrelation coefficients for normally distributed ariables (normality was tested by χ^2 goodness-of-t test), partial correlation coefficients for elimina-on of the age effects and Spearman's correlation oefficients for highly skewed variables. Analysis of variance was applied to calculate age trends and sex differences. The indices of vascular properties were further correlated with the 10-year risk of coronary heart disease calculated according to the equation derived from the Framingham Study; the method-ology published by Anderson et al. was used (16). All calculations were done by statistical SYSTAT 10 software.

Results

Basic characteristics of the sample are given in Table I. Mean aortic PWV was much lower than lower extremity PWV (aortic PWV: 7.8 and 7.4 m/s in males and females, respectively, lower extremity PWV: 13.0 and 11.2 m/s in males and females, respectively). Mean peripheral augmentation index (PAI), showing wave reflection on the radial artery, was 66% in males and 77% in females, i.e. the

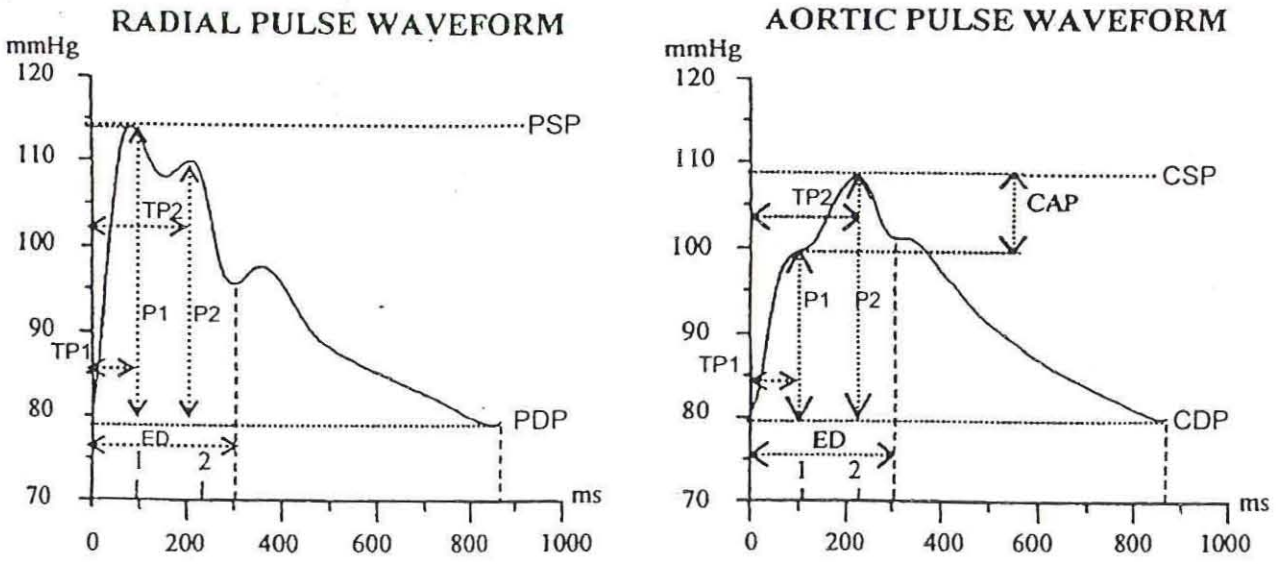


Figure 1. Pulse waveform registered on radial artery (left) and derived by means of mathematical transformation in the aorta (right). Radial pulse wave is calibrated on blood pressure measured conventionally on brachial artery (peripheral systolic and diastolic blood pressure, PSP, PDP). Aortic pulse wave is calibrated on mean pressure, obtained by integration of radial pulse wave, assuming that mean pressure is identical at the two sites. P1, P2, first and second pulse pressure peak, respectively; TP1, TP2, time to the peak of primary and reflected wave, respectively; ED, ejection duration; CSP, CDP, central systolic and diastolic pressure; CAP, central augmentation pressure – increase of pressure over P1 due to wave reflection. Augmentation index is defined as P2/P1 (peripheral, PAI, or central, CAI, measured on the radial or aortic pulse wave, respectively).

secondary wave did not exceed the primary wave in a typical case, whereas, in the aorta, the mean values were 123.8% in males and 137.4% in females (=central augmentation index, CAI). Compared to females, males had significantly higher lower extremity PWV, lower PAI, CAI and central augmentation pressure, higher SBP and DBP, body mass index, triglycerides level and lower high-density lipoprotein cholesterol level. Males were also more often current smokers (40.6% vs 28.6% in females, $p<0.032$).

In Fig. 2, the changes in vascular indices with age are shown separately for males and females. Aortic PWV (panel A) increased significantly with age ($p<0.001$). Lower extremity PWV was found to increase with age in females ($p<0.01$), but not in males (panel B); except for the highest age group (55–65 years), it was lower in females than in males. PAI (panel C) increased with age ($p<0.001$) and was consistently higher in women in all age groups ($p<0.001$). The timing of waves on the radial artery changed significantly with age (panel D): the primary peak was reached later with increasing age ($p<0.001$), whereas the reflected wave arrived earlier ($p<0.05$).

Table II shows associations of vascular indices with age and cardiovascular risk factors. Age correlated most closely with PAI, and further with aortic PWV and time to the primary pressure wave peak. BP values showed the closest age-adjusted correlations with aortic PWV in both sexes. Body height correlated inversely with PAI. Current smoking was associated with higher PAI in both sexes ($p<0.01$ for both sexes) whereas PWV, measured either in the aorta or in lower extremity, was not related to smoking status, and the reflected wave occurred earlier in male, but not female, smokers ($p<0.05$).

Table III shows mutual correlations of vascular indices. PAI and the derived CAI were closely correlated ($r=0.94$, $p<0.001$): this indicates that these two parameters were nearly identical in our setting. On the contrary, the correlations of PWV with parameters of wave reflection were fairly loose.

Multiple linear regressions were further calculated (Table IV). In order to increase statistical power, both sexes were analysed together. Of the vascular parameters tested as dependent variables, PAI was best predicted by the included independent variables (41% of variance explained). PAI was significantly associated with age, sex (higher in females), smoking status, SBP and total cholesterol level and aortic PWV was significantly associated with age, SBP and glucose level.

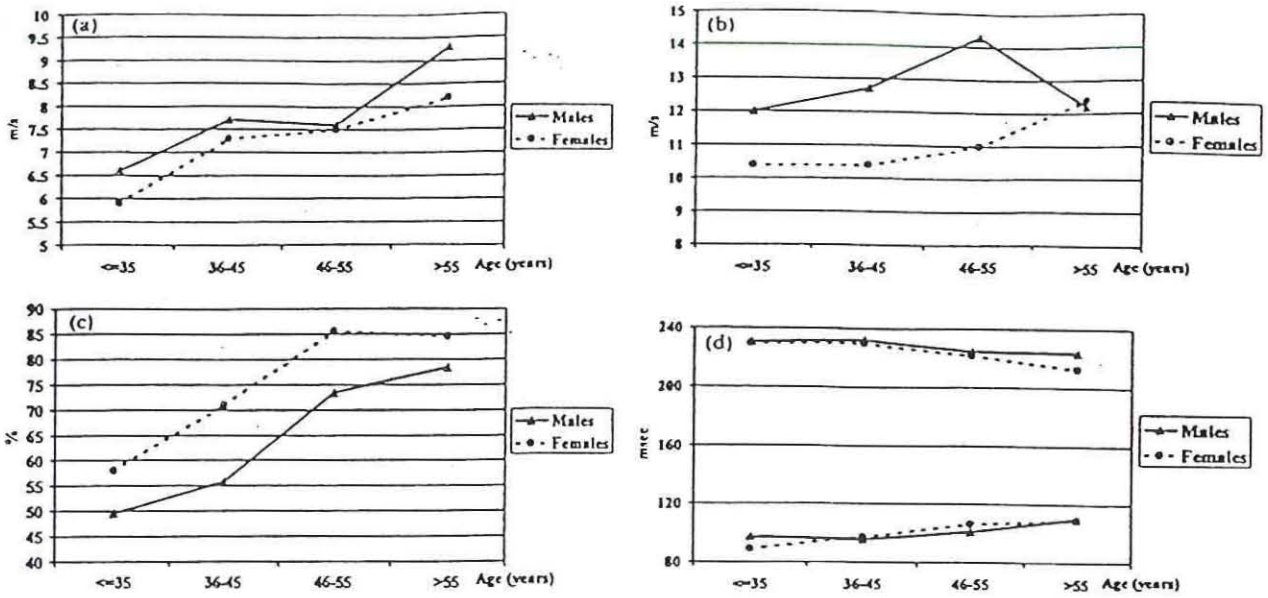


Figure 2. Analysis of vascular indices according to age and sex. Panel A: aortic pulse wave velocity. Age trend: $p<0.001$, sex difference: NS. Panel B: Pulse wave velocity on arteries of lower extremity. Age trend: NS (correlation coefficient of PWV with age NS for males, 0.24 for females, $p<0.01$). Sex difference: $p<0.01$. Panel C: Augmentation index assessed on radial pulse waveform. Age trend: $p<0.001$, sex difference: $p<0.001$. Panel D: Times to reach primary peak (lower curves) and secondary peak (upper curves) on radial pulse wave. Age trends: $p<0.001$ for first peak and $p<0.05$ for second peak, sex difference: NS for both peaks.

Table II. The age-adjusted associations of vascular indices to cardiovascular risk factors and anthropometric measurements.

	Aortic PWV(m/s)		Lower extremity PWV(m/s)		PAI(%)		TP1(ms)		TP2(ms)	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
1. Correlation analysis of continuous variables										
Age[a]	0.30***	0.38***	0.06	0.24**	0.57***	0.52***	0.34***	0.40***	−0.12	−0.22**
Systolic blood pressure[b]	0.29***	0.27***	0.12	0.14	0.16	0.16	0.16	0.13	0.08	−0.17*
Diastolic blood pressure	0.28***	0.24**	0.25**	0.09	0.28**	0.11	0.05	0.01	−0.10	−0.17*
Total cholesterol	−0.12	0.11	0.10	−0.07	0.18	0.17	−0.02	−0.03	−0.21*	−0.03
HDL-cholesterol	−0.04	−0.06	0.16	−0.20	0.19	−0.06	−0.09	−0.10	−0.10	0.14
LDL-cholesterol	−0.14	0.12	0.07	−0.01	0.08	0.23*	−0.03	−0.01	−0.10	−0.07
Triglycerides	−0.02	0.08	0.02	0.02	0.21*	−0.05	0.05	0.04	−0.15	−0.04
Glucose	0.17	0.10	−0.15	−0.09	0.14	−0.11	−0.02	0.02	−0.32**	−0.01
Height	0.05	−0.12	−0.13	−0.07	−0.29***	−0.22*	0.08	−0.15	0.10	0.08
Weight	0.08	0.06	0.02	−0.12	−0.18*	−0.03	0.11	0.01	0.08	−0.11
Body mass index	0.07	0.13	0.09	−0.09	−0.04	0.07	0.08	0.08	0.04	−0.16
2. Analysis of smoking (yes/no)										
Smokers[c]	7.8	7.8	12.5	11.7	71.1**	84.0**	101.5	103.4	220.8*	219.3
Non-smokers	7.9	7.3	13.4	11.0	62.9	74.4	101.2	102.1	231.1	223.7

* $p<0.05$. ** $p<0.01$. *** $p<0.001$ [a]Pearson corelation coefficients are given. [b]Age-adjusted partial corelation coefficients are given. [c]Age-adjusted means are given. PWV, pulse wave velocity; PAI, peripheral augmentation index; TP1, TP2, time to peaks of primary and secondary wave, respectively, measured on radial artery; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

The vascular indices were further related to the 10-year risk of coronary heart disease as predicted by the Framingham equation (Fig. 3). Mean risk was 11.0% (range 0.35–36.1%) in males and 5.2% (range 0.02–26.5%) in females. Both aortic PWV (Panel A) and PAI (Panel C) were significantly associated with the risk; the closest relationship was that of PAI in males ($r=0.51$, $p<0.001$). The correlation of lower extremity PWV with the risk was not significant in males, and low in females.

Table III. The interrelationships of pulse wave velocities and parameters of pulse wave reflection.

	Aortic PWV	Lower extremity PWV	PAI
Aortic PWV			
Lower extremity PWV	0.18**		
PAI	0.22**	0.04	
CAI	0.13*	0.02	0.94***

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ Spearman correlation coefficients are given. PWV, pulse wave velocity; PAI, CAI, peripheral and central augmentation index.

Table IV. Multiple regression analysis of vascular parameters.

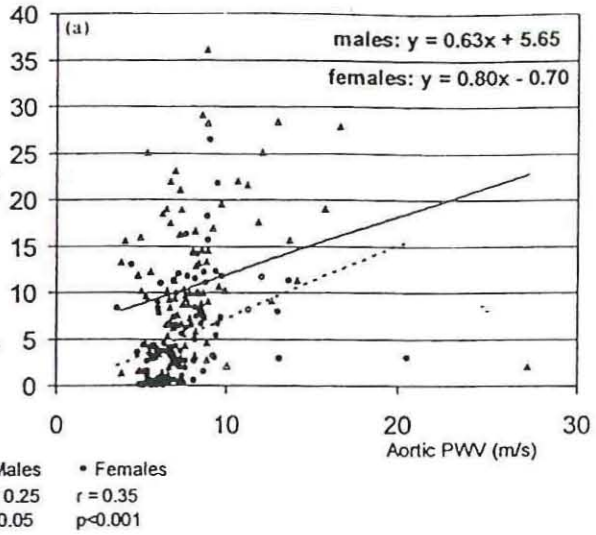
	Aortic PWV	Lower extremity PWV	PAI	TP1	TP2
R^2 (%)	14	10	41	16	5
Age	0.20**	0.09	0.44**	0.31**	−0.06
Sex	−0.06	−0.19**	0.33**	0.09	−0.12
Systolic blood pressure	0.16*	0.15	0.14*	0.11	−0.05
Total cholesterol	−0.06	0.05	0.11*	−0.05	−0.09
Glucose	0.14*	−0.15*	−0.03	−0.01	−0.12
Smoking	0.06	0.06	0.18**	0.04	−0.06
Body mass index	−0.01	−0.01	−0.03	0.10	0.01

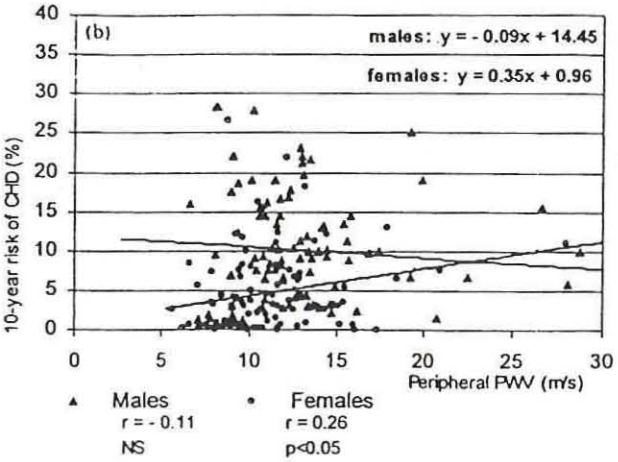
R^2, percentage of variance explained. Standardized regression coefficients are given; * $p<0.05$. ** $p<0.01$. Sex: 0 – male, 1 – female. Smoking: 0 – nonsmoker, 1 – exsmoker (no smoking for a period of more than one year), 2 – current smoker. Other variables are continuous. PWV: pulse wave velocity, PAI: peripheral augmentation index; TP1, TP2, time to peaks of primary and secondary wave, respectively, measured on radial artery.

Discussion

In our study, we analysed the relationships of parameters characterizing arterial properties with age, sex and classical cardiovascular risk factors in a sample of general population. PAI was best predicted in multiple regression analysis where cardiovascular risk factors were included as independent variables (41% of total variance explained; Table IV), mainly through its association with age, smoking and to lesser extent through BP and total cholesterol level. Other vascular parameters, including aortic PWV, were much less predicted by the independent variables. We further correlated the arterial parameters with the Framingham score separately in both sexes and the findings were compatible with the previous ones: the score was most closely associated with PAI in males (Fig. 3). Smoking was significantly associated with higher reflected wave in both sexes and with its earlier return in males (Table II), whereas no association with PWV, measured either above aorta or on lower extremity, was found. The fact that the timing of reflected wave between female smokers and non-smokers was not different (power of the test less than 20%), whereas it differed between male smokers and non-smokers (power of the test 66%), could be probably ascribed to the fact that females smokers were less numerous in our sample and generally have lower cigarette consumption than males.

Our results show, in accordance with the recently published findings of Millasseau et al. (17), a very close relationship between the parameters of wave reflection measured in peripheral circulation on the radial artery and derived in central circulation (correlation coefficient 0.94 between PAI and CAI). This indicates that peripheral, directly measured parameters can be used for studying the phenomenon of wave reflection. Wave reflection is influenced by several factors: (i) contractility of the heart determines the magnitude and duration of the primary wave; (ii) large artery stiffness determines the velocity of primary as well as secondary wave and, therefore, influences the timing of reflected wave; (iii) the status of resistance vessels determines the degree of wave reflection because wave reflection occurs there (with peripheral vasodilation, e.g. by nitrates, there is less reflection); and (iv) the length of the arterial bed from the heart to the reflection sites influences timing – this is why smaller individuals have higher reflected wave as it returns earlier (see also our results in Table II). Wave reflection is therefore a complex phenomenon determined by heart function and the status of the arterial tree as a whole rather than by local properties





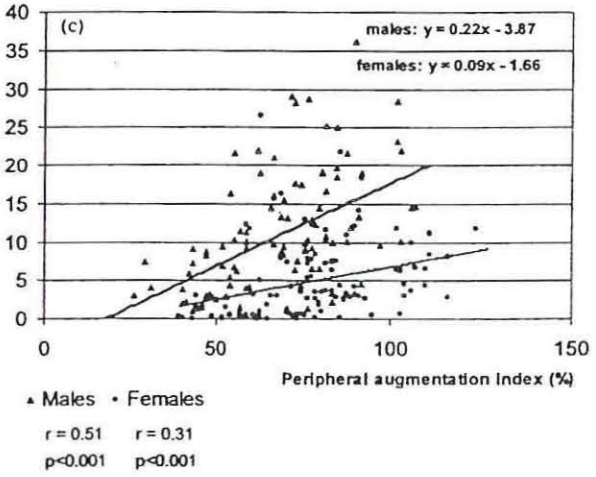


Figure 3. The relationships of vascular indices to 10-year risk of coronary heart disease estimated according to the Framingham equation. Mean risk is 11.0% (range 0.35–36.1%) in males and 5.2% (range 0.02–26.5%) in females. Panel A: aortic pulse wave velocity. Panel B: Pulse wave velocity on lower extremity. Panel C: Augmentation index calculated from the radial pulse wave.

the vascular bed where reflection is measured. In dition, we found rather low associations between gmentation indices and pulse wave velocities able III). These findings confirm the fact that ge artery stiffness is only one factor contributing wave reflection.

PAI was significantly higher in females in all age ups (see panel C of Fig. 2). The same applied for I and absolute height of reflected wave – gmentation pressure (data not shown in detail, also Table I). Similar findings were reported by er authors and they were ascribed to the fact that men have smaller body height. If this were indeed reason then there would be a difference in timing ween males and females, which was not the case our material (see panel D of Fig. 2). This issue studied in detail by Gatzka et al. (18) in 104 s of elderly males and females of identical height. nales also had higher AI in this setting, and they had a smaller aortic diameter and longer duration of systole. Both these phenomena are supposed to account for the gender differences in wave reflection.

Arterial stiffness as assessed by PWV is different in the aorta and lower limb arteries. Mean values were much higher on the lower limb in our material (Table I): this reflects higher stiffness due to the higher content of collagen fibres. Stiffness of these two arterial beds was not closely correlated (r=0.18; Table III). Thus, stiffness of elastic-type and muscular-type arteries is probably regulated by different mechanisms and may have different risk factors. The relationships with age and sex also differ (panels A and B of Fig. 2): whereas aortic PWV increased with age and there was no difference between sexes, lower extremity PWV was higher in men and did not increase with age, though it did increase in women and reached the same value as in men in the oldest age group (55–65 years). The reason for the

difference between males and females is not known and cannot be explained from our data; we can only hypothesize that the change in hormonal status after the menopause could increase the stiffness of distal large arteries in women.

In conclusion, the parameters under study are non-invasive and well reproducible. PAI gives practically the same information about wave reflection as CAI. The phenomenon of wave reflection should always be studied separately in males and females, because it is systematically higher in the latter, because of anatomic conditions and probably related to different mechanic properties of arterial system. The associations of pulse wave velocities and indices of wave reflection are loose; this indicates that the two sorts of parameters show different aspects of arterial properties. Aortic stiffness was shown to predict subsequent morbidity and mortality (19–21) and the same was found for wave reflection in patients with end-stage renal disease (22); these relationships existed in most studies independently of classical risk factors. Therefore, aortic PWV and AI could be used for better stratification of cardiovascular risk, and interventions that would be successful in influencing positively these parameters could represent a progress in cardiovascular disease prevention and treatment.

Acknowledgement

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Mechanical properties of large arteries: results of a population-based study

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Purpose of the Study: To assess the determinants of arterial stiffness and pulse wave reflection in a general population.

Methods: We performed a population-based study according to the protocol of the WHO-study "MONICA". Of 891 subjects, aged 25–67 years, randomly chosen from a population register and screened in our centre, 290 were examined with the Sphygmocor device. Aortic pulse wave velocity (PWV) and PWV on lower limbs were measured. Wave reflection was assessed from radial pulse wave analysis.

Results: Aortic PWV increased with age and was similar in both sexes. PWV measured above lower extremity,i. e.above muscular type arteries, was higher in males and did not increase with age. While lower in younger females, it increased in the highest age group (55–67 years) where it reached the same value as in males. AI was higher in females in all the age groups and increased steeply with age in both sexes. Besides age, the relationship of factors of atherosclerosis to both PWV and AI were weak or non-significant.

Conclusion: The steep increase of both aortic PWV and AI with age shows that these parameters reflect biological aging of arterial system. The weak associations with cardiovascular risk factors reflect the fact that the mechanisms of stiffening and of increased wave reflection are different from atherosclerosis.

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Noninvasive Measurement of Aortic Compliance as a Marker of Increased Risk of Coronary Artery Disease

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Purpose of the Study: Advanced atherosclerosis typically results in reductions in aortic compliance (AC). This study examines the potential of using noninvasive measures of AC using impedance cardiography as a marker of advanced coronary artery disease (CAD) in patients presenting to the emergency department (ED) with acute chest pain.

Methods: AC was calculated from noninvasive measurements of stroke volume and pulse pressure using impedance cardiography and sphygmomanometry in a convenience sample of patients presenting with acute chest pain and in whom a recent cardiac catheterization had been performed. The determined AC for each patient (corrected for age and mean arterial pressure) was compared to the graded measure of the degree of coronary disease found by catheterization (mild, moderate, severe). The sensitivity of the measured AC in predicting CAD was calculated using a cutoff level comparable to the normal average AC found in patients without disease (1.5 ml/mmHg).

Results: In 42 patients studied, a stepwise correlation was found between the graded degree of CAD and the determined compliance. In those patients with minimal or moderate CAD, mean compliance was 1.0 ml/mmHg as compared to a significantly lower value of 0.59 ml/mmHg found for patients with more severe disease ($p < 0.01$). In all patients with CAD, 95 % of the AC values fell below the 1.5 ml/mmHg cutoff.

Conclusion: Noninvasively determined AC measures might potentially be useful as a marker of CAD in patients presenting to the ED with acute chest pain.

Brachial and aortic blood pressure in a population sample: should we classify the subjects according to their central rather than peripheral pressure?

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Purpose of the Study: To address the question if one should classify hypertension according to brachial systolic BP (BSBP) or to aortic systolic BP (ASBP).

Methods: Healthy subjects 25–65 yrs.of age were randomly selected and examined within the framework of a population survey. In 290 subjects (143 males and 147 females), radial pulse wave analysis, using the Sphygmocor system, was performed. This noninvasive method allows an estimate of the ASBP; repeated validation studies against invasive BP measurements showed good agreement.

Results: BSBP 120 mmHg (= upper limit of optimal BP) and 140 mmHg (= lower limit of hypertension) correspond to ASBP 107 and 125 mmHg, respectively, in males; and to 108 and 130 mmHg, respectively, in females (with similar percentile distribution). The subjects were divided into three groups according to BSBP (< 120, 120–139, > 140 mmHg) as well as into three groups according to the corresponding limits of ASBP. 10.5 % of males were classified to a lower category of BSBP than of ASBP, and 7 % to a higher category of BSBP than of ASBP.In females, the values were 9.4 % and 6.8 %, respectively. When the analysis was performed separately for younger [25–45] and older subjects (46–65 years), the highest disagreement between the two classes was found in younger females (a total of 21.8 %).

Conclusion: There exists a discrepancy between the classifications according to the BSBP and estimated ASBP; the latter is probably more closely related to the incidence of future cardiovascular events. When classifying according to BSBP instead of ASBP, we may underestimate the vascular risk associated with hypertension.

Insulin-resistance is an independent predictor of subclinical macrovascular involvement in mild Type 2 diabetes

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Purpose of the Study: In Type 2 Diabetes (DM), macrovascular complications seem to depend mainly on lipid abnormalities. Insulin-resistance (IR) provides a unifying hypothesis accounting for the various metabolic fingerprints. To assess the role of IR in the early stage of large artery involvement in DM.

Methods: 19 non-obese non-hypertensive pts with DM under hypoglycemic therapy and 10 age- and sex-matched normal controls (NL) were studied. BMI, BP and total cholesterol (TC) were similar in both groups. Insulin sensitivity was assessed by a 2-hr euglycemic hyperinsulinemic clamp (40 mU/min/m2).Carotid artery (CA) compliance was determined by continuous and simultaneous recording of CA diameter (Wall Tracking) and finger arterial pressure,and was expressed as the area under the diameter/pressure curve (AUC) over a given pressure range (70–130 mmHg).

Results: Insulin-stimulated glucose uptake (M: µmol/min/per kg of free fatty mass) was 46 ± 10 in NL, and 33 ± 13 in DM ($p < 0.005$). Within DM pts, 11 were insulin-sensitive (IS: M-value within 2SD of the mean of NL) and 8 were insulin-resistant (M in IS vs IR pts: 41 ± 9 vs 21 ± 3, $p < 0.001$). IR pts had a lower AUC than IS and NL (0.315 ± 0.104, 0.481 ± 0.107 and 0.518 ± 0.188 (mm2/

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ORIGINAL ARTICLE

Mild hyperhomocysteinaemia is associated with increased aortic stiffness in general population

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Total homocysteine (tHcy) level was identified as a strong and independent predictor of cardiovascular events. We investigated the association between tHcy and mechanical properties of large arteries in a random, general population-based sample of 251 subjects (mean age 48 years). Large artery properties, such as aortic and peripheral (lower-limb) pulse wave velocity (PWV), and augmentation index of radial artery were measured using semi-automatic Sphygmocor® device. Aortic PWV (APWV) positively correlated with tHcy ($r=0.28$, $P<0.0001$), and a significant increasing trend of APWV was found by tHcy quartiles ($P=0.0003$ by ANOVA). This association remained significant after adjustment for conventional cardiovascular risk factors (age, gender, smoking, overweight, hypertension, dyslipidaemia and impaired glucose metabolism) and for usual homocysteine confounders (folate, B_{12}, renal function). Subjects with mild hyperhomocysteinaemia (i.e. with tHcy $\geqslant 15\,\mu$mol/l) had 2.74 times higher risk of having their APWV over 8.42 m/s (i.e. in the top quartile). No such association was found either for PWV measured at lower extremity or for radial augmentation index. In conclusion, in our series of subjects from general population, we found a strong and independent relationship between homocysteine concentration and APWV, a parameter of stiffness of central arteries.
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Keywords: homocysteine; pulse wave velocity; augmentation index; aortic; peripheral; vascular diseases

Introduction

Numerous prospective studies identified homocysteine as a strong predictor of vascular diseases. Patients with plasma total homocysteine (tHcy) of 15–19.9 μmol/l had about 2.5 times higher mortality and those with tHcy more than 20 μmol/l about six times higher mortality than patients with tHcy $<$ 9 μmol/l.[1] There are several potential vascular consequences of elevated tHcy. Besides well-evident effect on coagulation, oxidative status and endothelial dysfunction,[2] several experimental studies reported that elevated tHcy can induce smooth muscle proliferation, collagen synthesis and deterioration of the elastic structures of the vessel wall,[3–6] a phenomenon resulting in increased stiffness of vessel wall.

Recent studies identified increased large artery stiffness as independent predictor of cardiovascular outcomes. Laurent *et al.*[7] reported that a 5 m/s increase of carotid-femoral pulse-wave velocity (a marker of aortic stiffness) was associated in hypertensive patients with 2.35 times higher risk of cardiovascular death. Similar relationship between aortic stiffness and mortality were found in subjects older than 70 years[8] and in patients with end-stage renal disease.[9]

The aim of our study was to establish whether or not there exists an association between homocysteine concentrations and arterial stiffness; we further asked whether this association is independent of other cardiovascular risk factors and parameters known to influence the homocysteine concentrations.

Methods

Subjects

The study population consisted from random general population sample. A survey of risk factors was undertaken in 2000–2001 as a part of Czech post-MONICA study.[10] One per cent of residents of City of Pilsen, aged 25–65 years, was selected (stratified by age and sex) from the General Health Insurance Registry. A total of 1009 subjects, 488 males and 522 females, mean age 48.1, responded to the survey.

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A random 25% subsample, in which arterial properties were examined, was evaluated in the present study.

Examinations and materials

All study procedures were done according to Good Clinical Practice regulation and were approved by the Local Ethical Committee. The Informed consent was obtained from all subjects and all personal data were stored under the provisions of the Czech Data Protection Act. All responders were interviewed and examined by standardized methods using WHO Monica standard manual. Methods of interview were described in details elsewhere.[10] Briefly, information on personal and demographic characteristics, personal and family history of coronary heart disease, lifestyle and current pharmacotherapy were obtained at interview. Following standardized examinations were performed: height and weight in light indoor clothes without shoes by DETECTO 20 (Webb City, USA) scale and measuring stick. Blood pressure (BP) was measured three times in the sitting position on the right arm using standard mercury sphygmomanometers to the nearest 2 mmHg; appropriate cuff size was used.

Large artery properties were measured using the semi-automatic Sphygmocor device (AtCor Medical Ltd, Australia); the methods of measurement were described in detail in our previous report.[11] The first part of this examination was the registration of radial pulse wave which was used to evaluate wave reflection; the parameter showing wave reflection is augmentation index (AIx), defined as the ratio of pulse pressure at the peaks of secondary to primary wave. Immediately before the radial pulse wave registration, an additional BP reading was obtained using the Omron 705CP oscillometric device. The probability of observer bias is minimized with this type of device. The second part of the examination was pulse wave velocity (PWV) measurement assessing arterial stiffness. We used the same device and measured it with the patient in supine position in the aorta, that is, between carotid and femoral arteries (aortic PWV, APWV), and in the lower extremity, that is, between femoral and dorsalis pedis/tibialis posterior arteries (peripheral PWV, PWV). Consecutive registrations of the pulse waves are ECG gated and thus, the time shift between the appearance of wave at the first and the second sites can be calculated. The distance between the two sites was measured on the body surface; to determine APWV, we measured the distance from the jugular fossa to the pulsation of the femoral artery in the groin subtracting, from this distance, the distance from the jugular fossa to carotid pulsation as the pulse runs here in the direction opposite to that in the aorta. The average of measurements over a period of 8 s (nine to ten cardiac cycles) was calculated after the exclusion of extreme values. The methods were shown to have good reproducibility.[12,13]

Venous blood samples were drawn in fasting state, and frozen serum samples, stored at −80°C, were used for biochemical laboratory analyses in the series. The laboratory examinations, including assessment of total (TCHOL) and HDL cholesterol (HDL), triglycerides (TG) were provided by the central laboratories of survey (Institute of Clinical and Experimental Medicine, Prague) using Cobas Mira analyser (Basel, Switzerland) and commercially available kits of the same provenience. Glucose levels (GLU) were analysed by enzymatic method in the same laboratory using LACHEMA (Brno, Czech Republic) standard kits. LDL cholesterol (LDL) was calculated by Friedewald Equation that is, LDL=TCHOL−HDL−(TG/5). Serum tHcy, plasma folate and B_{12} vitamin (B_{12}) concentrations were estimated from aliquots series, stored at −80°C, by commercial FPIA kits and automatic analysers (AxSYM, Abbott Laboratories, Wiesbaden, Germany, for tHcy, and ACCESS Beckman Coulter, Fullerton, USA, for folate and B_{12}). Variation for all these measurements was less than 1.5%.

Data analysis

The study was designed as a cross-sectional analysis. Statistical analysis of the data was done using STATA 6. Power calculation was done using standard deviations, ascertained in our previous studies. The minimal sample size, necessary to detect the differences in measured variables at 5% significance and 90% power, was 107 subjects. The significance of differences among specific groups was evaluated by Fishers χ^2-test for categorical variables and by analysis of variance (ANOVA) or Mann–Whitney U test for continuous variables. Multiple logistic regression was used to ascertain the association between tHcy and large artery properties after adjustment for potential confounders.

Results

A total of 251 subjects, aged 48.1±0.69 years (mean±s.e.m.), 126 males and 125 females, were evaluated in the present study. Baseline characteristics of study sample as a whole and variables sorted by tHcy quartiles are given in Table 1. Statistically significant increasing trends were found in age, systolic blood pressure, use of lipid-lowering drugs and APWV across tHcy quartiles, while there were decreasing trends in folate and B_{12} vitamin concentrations. Similar statistical differences were found, when only lowest and highest quartile were compared; moreover, there was a higher BMI and lower proportion of females in the highest quartile.

Table 1 Basic characteristics of the study population and reported treatment in total sample and by total homocysteine (tHcy) quartiles (First: ⩽8.9; second: 9–10.6; third 10.7–12.9 and fourth: ⩾13.0 μmol/l)

	Total sample	*Homocysteine quartiles*					
		First	*Second*	*Third*	*Fourth*	*P[a] for trend*	*P[b] for 1st versus 4th quartile*
n	251	64	69	56	62	—	—
Age (years)	48.1±0.69	43.6±1.27	48.7±1.20	50.4±1.49	49.3±1.53	<0.02	<0.005
Female gender (%)	49.8	57.8	49.3	53.6	38.7	0.17*	<0.04*
Body mass index (kg/m2)	26.5±0.25	26.9±0.54	26.3±0.42	26.7±0.47	27.5±0.58	0.26	<0.04
Current smoking (%)	33.1	28.1	31.9	28.6	42.9	0.26*	0.08*
Total homocysteine (μmol/l)	11.5±0.27	7.89±0.09	9.89±0.06	11.61±0.09	16.9±0.64	<0.0001	<0.0001
Serum folate (ng/ml)	6.22±0.19	7.06±0.38	6.93±0.44	5.93±0.36	4.80±0.26	<0.0001	<0.0001
Serum B_{12} (pg/ml)	323.6±9.52	373.9±19.8	339.9±18.1	302.2±23.3	271.6±11.5	<0.0001	<0.0001
Peripheral augmentation index	71.6±1.34	67.4±2.33	72.4±2.40	75.2±2.82	69.1±3.19	0.40	0.64
Peripheral pulse wave velocity (m/s)	13.3±0.57	11.21±0.41	14.0±1.19	14.5±1.62	13.4±1.21	0.96	0.40
Aortic pulse wave velocity (m/s)	7.56±0.15	6.81±0.18	7.19±0.20	7.51±0.29	8.71±0.44	<0.001	<0.0001
Systolic blood pressure (mmHg)	125.8±1.02	120.1±1.90	126.3±1.63	127.7±2.17	128.8±2.33	<0.02	<0.009
Diastolic blood pressure (mmHg)	80.3±0.61	79.6±1.21	80.3±1.08	79.6±1.24	81.6±1.45	0.6	0.29
Heart rate (beats/min)	70.3±0.61	69.5±0.99	68.6±1.06	70.5±1.28	72.8±1.60	0.82	0.36
Pulse pressure (mmHg)	45.5±0.75	41.1±1.26	45.6±1.22	47.2±1.69	47.2±1.66	<0.005	<0.002
Antihypertensive treatment (%)	*18.3*	*10.9*	*17.4*	*23.2*	*22.6*	*0.26**	*0.08**
Total cholesterol (mmol/l)	5.88±0.07	5.74±0.15	6.06±0.15	5.85±0.16	5.86±0.15	0.73	0.41
Triglycerides (mmol/l)	1.72±0.06	1.63±0.12	1.67±0.12	1.80±0.12	1.80±0.13	0.4	0.27
HDL cholesterol (mmol/l)	1.42±0.02	1.40±0.04	1.47±0.04	1.45±0.05	1.39±0.05	0.5	0.62
Lipid-lowering treatment	*6.0*	*0.0*	*5.8*	*1.8*	*16.1*	*<0.02**	*<0.001**
Fasting glucose (mmol/l)	5.55±0.08	5.31±0.07	5.51±0.13	5.68±0.24	5.69±0.15	0.2	0.59
Antidiabetic treatment	*1.2*	*0.0*	*2.9*	*1.8*	*0.0*	*0.34**	—

[a]Kruskall–Wallis ANOVA or [b]Mann–Whitney *U* test for continuous variables, Pearson's χ^2-test for categorical* ones.

The relation between APWV and tHcy in a continuous manner is shown in Figure 1. A statistically significant positive association was found between these two variables, Spearman's correlation coefficient was 0.28 (with *P*-value 0.000005).

Associations between APWV and categorized cardiovascular risk factors are given in Table 2. Subjects with mild hyperhomocysteinaemia (i.e. with tHcy ⩾15 μmol/l) had 2.74 times higher risk of having their APWV over 8.42 m/s. Out of other cardiovascular risk factors, age ⩾50 years, hypertension and overweight significantly and independently entered the regression (model A). The association between increased APWV and homocysteinaemia remained significant after adjustment for factors known to influence the tHcy levels such as age, gender, current smoking, folate, B_{12} concentrations and treatment with fibrates (Table 2, model B). No significant relation was found between tHcy concentrations and PPWV or radial AIx.

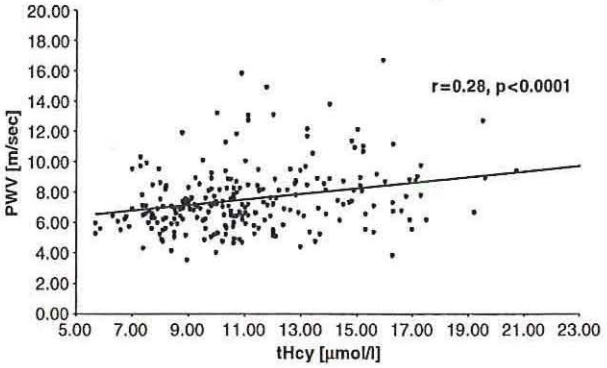

Figure 1 Correlation between total homocysteine concentration and aortic pulse wave velocity (Spearman's correlation coefficient).

Discussion

We found in our study that elevated homocysteine was significantly associated with increased central arterial stiffness, measured by APWV. Relative risk of increased APWV (over 8.42 m/s, i.e., in the top quartile) was nearly three times higher for subjects with homocysteine ⩾15 μmol/l (i.e. with mild hyperhomocysteinaemia according to usual definition); this association remained significant after adjustment for other cardiovascular risk factors or parameters, known to influence the homocysteine concentrations. These findings are in agreement with some already published data. Bortolotto *et al.*[14] reported that homocysteine strongly and independently correlate with APWV in patients with hypertension. Sutton-Tyrrel *et al.*[15] reported an independent relation between elevated homocysteine and isolated systolic hypertension, a clinical manifestation of increased aortic stiffness, in older adults.

Table 2 Multivariate association between increased aortic stiffness (PWV over 8.42 m/s as dependent variable), hyperhomocysteinaemia and categorized conventional cardiovascular risk factors (model A) or usual homocysteine confounders (model B) as independent variables (multiple logistic regression, odds ratios and 95% confidence intervals)

Independent variables	*Model A*	*Model B*
Mild hyperhomocysteinaemia[a]	**2.74 (1.16–6.49)**	**3.14 (1.32–7.47)**
Age ⩾50 years	**5.05 (2.35–10.7)**	**8.72 (4.16–18.27)**
Male gender	1.05 (0.53–2.08)	1.33 (0.68–2.61)
Current smoking	1.76 (0.84–3.70)	1.69 (0.82–3.49)
Overweight or obesity[b]	**2.16 (1.00–4.69)**	—
Hypertension[c]	**2.39 (1.17–4.87)**	—
Hypercholesterolaemia[d]	0.83 (0.32–2.19)	—
Increased fasting glucose or diabetes[e]	2.31 (0.77–7.00)	—
Low folate[f]	—	1.04 (0.50–2.16)
Low vitamin B_{12}[g]	—	0.49 (0.24–1.02)
Fibrate treatment[h]	—	1.27 (0.56–2.85)

[a]Serum total homocysteine ⩾15 μmol/l.
[b]BMI > 25 kg/m².
[c]Systolic blood pressure ⩾140 mmHg and/or diastolic blood pressure ⩾90 mmHg, and/or treatment with antihypertensives.
[d]Total cholesterol ⩾5 and/or LDL cholesterol ⩾3 mmol/l and/or treatment with lipid-lowering drugs.
[e]Fasting glucose >6.5 mmol/l and/or treatment with antidiabetic drugs.
[f]Plasma folate ⩽4.48 ng/ml (i.e. bottom tertile).
[g]Plasma B_{12} vitamin ⩽248 pg/ml (i.e. bottom tertile).
[h]Reported treatment with any fibrate, with exception of gemfibrozil.
Statistically significant association in bold.

It is evident, that aortic stiffness is strongly related mainly to age, because of age-dependent changes in elastin and collagen content in the vessel wall. However, homocysteine may probably accelerate this process. It was reported, that homocysteine gradually increased collagen synthesis in vascular smooth muscle cells culture (VSMC) and was accompanied by an excessive accumulation of insoluble collagen in the cell layer, indicating the impaired collagen degradation.[4,5] Charpiot *et al.*[6] reported, that dietary-induced hyperhomocysteinaemia in minipig decreased, subsequently, the elastin content and increased metalloproteinase-dependent elastolysis in abdominal aorta and coronary arteries. The link between collagen/elastin metabolism and homocysteine could be a hypothetical explanation of differential effect on central and peripheral artery stiffness. Central arteries (such as aorta) contain relatively more elastin and less collagen than peripheral arteries. Thus, aorta could be more dependent than the peripheral arteries on any imbalance in elastin and collagen synthesis/degradation, namely in subjects free of manifest vascular disease.

Two studies reported that aortic stiffness could also be increased by acute rise in homocysteine concentration. Nestel *et al.*[16] found that oral methionine load, causing a threefold increase in tHcy concentration, caused increase of stiffness in the central arterial system by about 22%; the stiffness was measured at baseline and 5 h after the methionine load. Similarly, Davis *et al.*[17] reported that acute tHcy elevation significantly increased pulse pressure (as a marker of central arterial stiffness) by about 4 mmHg, measured 8 h after methionine load. There is a hypothetical explanation of this acute effect of homocysteine from experimental studies. Neves *et al.*[18] found in an experimental study that treatment with angiotensin-II increased arterial stiffness of mesenteric artery significantly more in hyperhomocysteinaemic (methylenetetrahydrofolate reductase deficient), than in control normohomocysteinaemic (wild-type) mice. Another experimental study[19] found in rats that elevated homocysteine increased extremely the responsiveness of its aortic vascular smooth muscle cells to angiontensin-II. Thus, homocysteine may probably induce vascular reactivity in conjunction with other vasoactive agents, such as angiotensin-II.

Various methods can be used for the evaluation of arterial wall properties. In some studies, Doppler ultasound technique is used for the measurement of PWV: it is obtained by dividing the length of a particular arterial segment by the 'foot-to-foot' transit time. It has been shown that APWV measured with this method predicts strongly and independently cardiovascular mortality in patients with end-stage renal disease.[20] Magnetic resonance imaging is a method enabling to study biophysical properties of aortic segments in details (distensibility, pulse and flow wave velocities). This method has been used for the studies in special diseases where arterial function is likely to be altered, such as juvenile arthritis[21] or Marfan syndrome.[22] For the purpose of a population-based study, however, our sphygmometric method is the most suitable as it is relatively simple, fast, well reproducible and inexpensive. A large arterial segment, such as the aorta or lower extremity arteries, is studied as a whole and therefore, the result of a measurement is not much influenced, for example, by the presence of a local atherosclerotic lesion.

In conclusion, in our study we found a strong and independent relationship between total serum homocysteine concentrations and APWV, as a

What is known about topic
- Mild hyperhomocysteinaemia is associated with increased risk of cardiovascular disease
- The well-established mechanisms for this association are the effects of homocysteine on coagulation, oxidative status and endothelial function
- Some data indicate that elevated homocysteine level may induce smooth muscle cell proliferation, collagen synthesis and deterioration of elastic structures of the arterial wall; these changes could result in increased arterial stiffness

What this study adds
- Total homocysteine level is positively associated with stiffness of aortic wall in subjects selected from general population aged 25–65 years
- This association is independent of other risk factors for cardiovascular disease
- There is no relationship between homocysteine and stiffness of peripheral large arteries

parameter of central arterial stiffness. On the other hand, owing to cross-sectional design of our study, we have no direct evidence for cause–effect interaction. The causality of elevated homocysteine in increased aortic stiffness needs to be confirmed by interventional prospective study with folate, which represents a standard treatment approach in mild hyperhomocysteinaemia.

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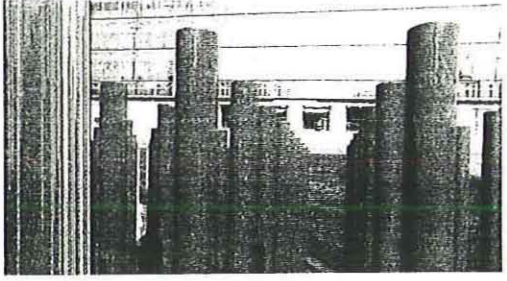
Příloha 8

European Society of Hypertension



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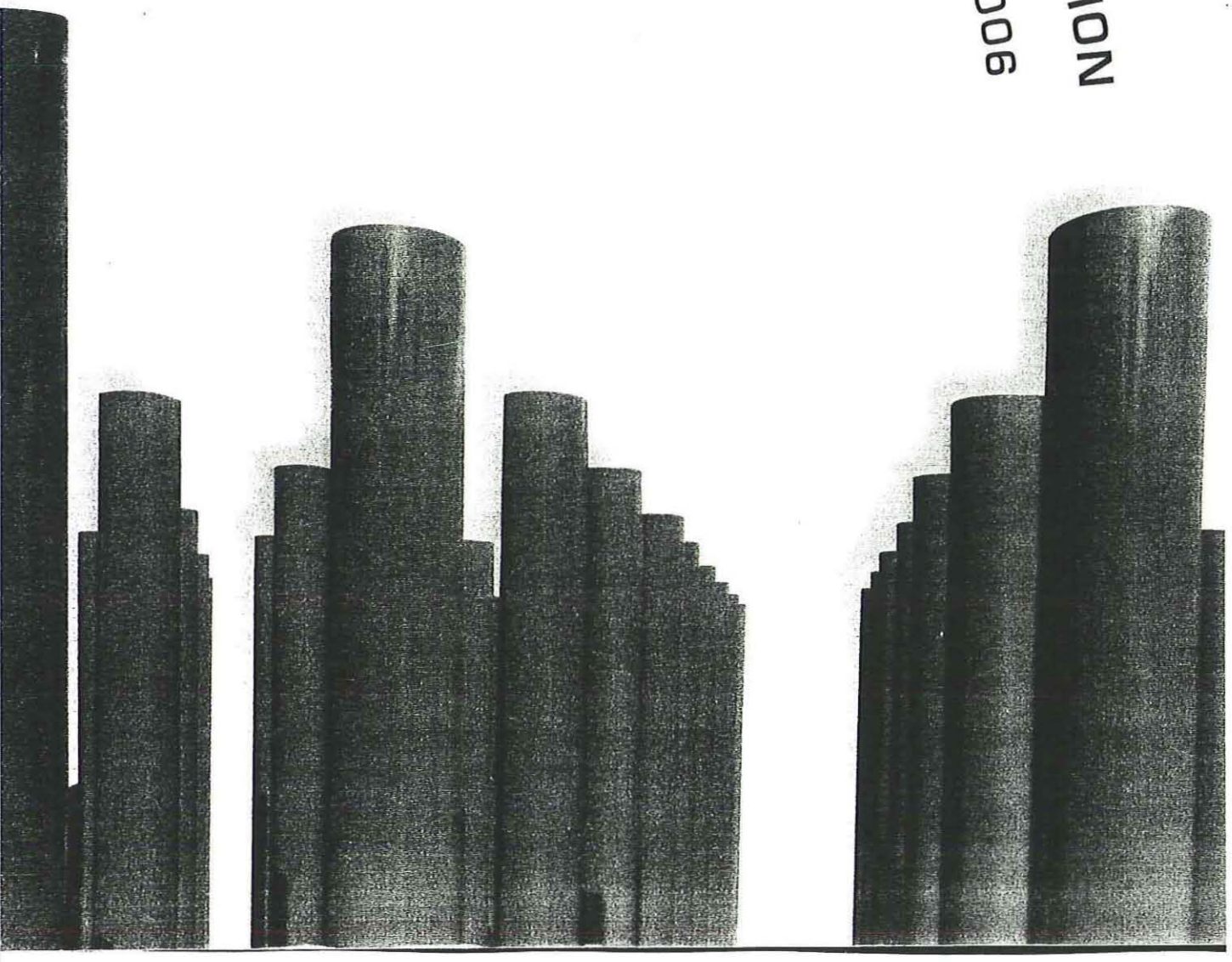

blood pressure, increased heart rate, normalized SS, and was associated with the restoration of a positive relationship between FMDμm and DSS ($P = 0.010$), but FMDμm and FMDμm/DSSPa10−1 were unchanged. Long-term increase in WBV and SS induced by improvement of anemia was associated with an increase in FMDμm ($P < 0.01$), as well as an increase in FMDμm and FMDμm/DSSPa10−1 ($p < 0.01$).

Conclusion: In ESRD, in response to local SS, the FMD is impaired but improved by correction of anemia and increase in WBV, thereby implying that in ESRD anemia-related low WBV can affect the endothelial-mediated flow dilation.

9C.3 HYPERTENSIVE MEN WITH ERECTILE DYSFUNCTION HAVE INCREASED AORTIC STIFFNESS COMPARED TO HYPERTENSIVES WITH NORMAL ERECTILE FUNCTION

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Background and Aims: Erectile dysfunction may be an early manifestation of generalized vascular disease. The association of erectile dysfunction with aortic stiffness and wave reflections in hypertensive patients without clinical atherosclerosis has not been defined yet.

Methods: We studied 51 (aged 56 ± 10 years) never treated hypertensive men with no clinical atherosclerosis who suffered from non-psychogenic and non-hormonal erectile dysfunction. A control group of 51 never treated hypertensive men with normal erectile function who were matched for age, body mass index, systolic and diastolic blood pressure, heart rate and traditional risk factors for cardiovascular disease were enrolled. Erectile dysfunction diagnosis and score were evaluated according to the International Index of Erectile Function questionnaire. Carotid-femoral Pulse Wave Velocity was measured as an index of aortic stiffness using Complior® and Augmentation Index as a measure of wave reflections using SphygmoCor®.

Results: Pulse Wave Velocity was higher in patients with erectile dysfunction than in the control group (8.1 ± 1.2 vs 7.6 ± 1.1 m/s, $P < 0.05$); Augmentation Index did not differ (26.3 ± 10.5 vs 26.7 ± 7.7%, P = NS).

Conclusions: Aortic elastic properties but not wave reflections are impaired in never treated hypertensive men with erectile dysfunction compared to hypertensives without erectile dysfunction. This finding suggests that hypertensive patients with erectile dysfunction may be at greater cardiovascular risk.

9C.4 ASSOCIATION BETWEEN FREE THYROXINE, AORTIC RIGIDITY AND GENETIC POLYMORHISM OF ANGIOTENSIN II TYPE 1 RECEPTOR IN A POPULATION SAMPLE

O. Mayer, J. Filipovský, M. Pesta, M. Dolejsova, J. Hrbkova. *2nd Dept. of Internal Medicine, Charles University, Medical Faculty, Plzen, Czech Republic*

Background: Thyroid hormones showed a direct proliferative effect on cardiovascular system, beside others also by modulating the expression of renin–angiotensin axis. The aim of our study was to establish, whether even mild changes in free thyroxine (fT4) may influence the aortic rigidity and whether this relation could be modified by polymorphism of angiotensin II type 1 receptor (AGTR1).

Methods: 249 subjects (m121, f128, mean age 48.03±0.70) was selected from population-based postMONICA study. Aortic pulse wave velocity (APWV), as a measure of aortic rigidity, was estimated using Sphygmocor device, AGTR1 polymorphism (A1166C mutation) by PCR. The whole sample was stratified according to fT4 quintiles into following strata: optimal (11.7−14.59 pmol/l, 2nd to 4th quintile), low-normal (9−11.6 pmol/l, 1st quintile) and high-normal (14.60−22 pmol/l, top quintile).

Results: see table.

fT4 strata	Optimal	Low-normal (p1)	High-normal (p2)
wild type of AGTR1:			
Age [years]	47.7 ± 1.29	49.0 ± 2.17 (0.45)	51.34 ± 1.91 (0.10)
Systolic BP [mmHg]	122.6 ± 1.72	128.4 ± 3.4 (0.12*)	130.6 ± 3.21 (0.07*)
Diastolic BP[mmHg]	79.2 ± 1.06	81.4 ± 1.93 (0.43*)	81.5 ± 1.57 (0.50*)
APWV [m/s]	7.38 ± 0.24	7.87 ± 0.41 (0.40*)	8.06 ± 0.75 (0.57*)
homo- or heterozygote for A1166C:			
Age	46.0 ± 1.31	49.6 ± 1.98 (0.17)	49.4 ± 2.68 (0.26)
Systolic BP	123.5 ± 1.82	128.7 ± 3.19(0.33*)	133.5 ± 4.27 (0.09*)
Diastolic BP	79.3 ± 1.14	82.4 ± 2.50 (0.34*)	81.3 ± 2.85 (0.89*)
APWV	7.26 ± 0.20	7.46 ± 0.42 (0.88*)	8.63 ± 0.72 (<0.04)

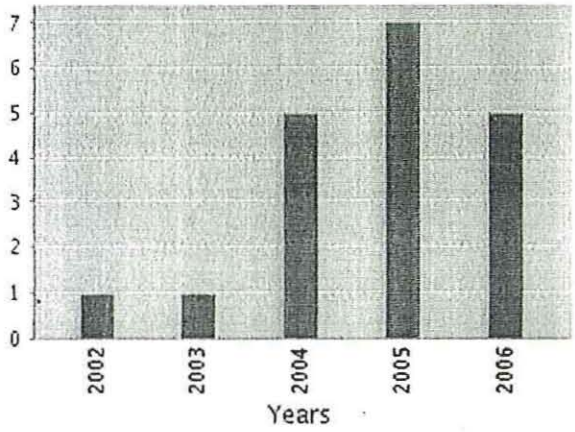
[mean ± SEM; optimal vs. low-normal (p1) or high-normal (p2) fT4, *p value adjusted for age and gender]

Conclusion: In our sample of general population we found that high-normal fT4 was associated with increased APWV, however reached statistical significance only in patient with A1166C mutation of AGTR1.

Study supported by grant IGA-MZ 7534−3.

Příloha 9

Published Items in Each Year



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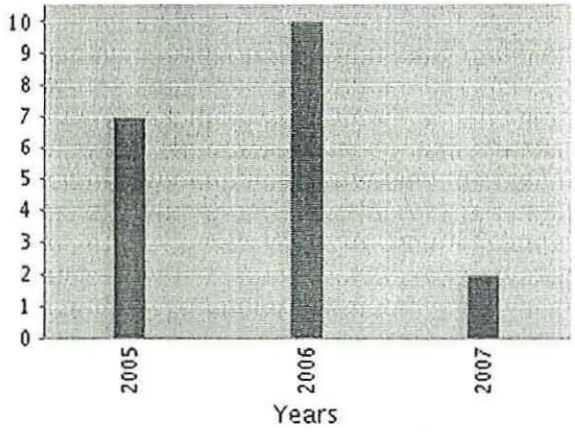

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		2003	2004	2005	2006	2007	Total	Average Citations per Year
		0	**0**	**7**	**10**	**2**	**19**	**3.80**
1.	**Author(s):** Wojciechowska, W; Staessen, JA; Stolarz, K; et al. **Group Author(s):** European Project Genes Hypertensio **Title:** Association of peripheral and central arterial wave reflections with the CYP11B2-344C allele and sodium excretion **Source:** JOURNAL OF HYPERTENSION, 22 (12): 2311-2319 DEC 2004 **ISSN:** 0263-6352		0	3	7	1	11	3.67
2.	**Author(s):** Cwynar, M; Staessen, JA; Ticha, M; et al. **Group Author(s):** European Project Genes Hypertensi **Title:** Epistatic interaction between alpha- and gamma-adducin influences peripheral and central pulse pressures in white Europeans **Source:** JOURNAL OF HYPERTENSION, 23 (5): 961-969 MAY 2005 **ISSN:** 0263-6352			3	1	1	5	1.67
3.	**Author(s):** Wojciechowska, W; Staessen, JA; Nawrot, T; et al. **Group Author(s):** European Project Genes **Title:** Reference values in white Europeans for the arterial pulse wave recorded by means of the SphygmoCor device **Source:** HYPERTENSION RESEARCH, 29 (7):				1	0	1	0.50

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4.	**Author(s):** Mayer, O; Filipovsky, J; Dolejsova, M; et al. **Title:** Mild hyperhomocysteinaemia is associated with increased aortic stiffness in general population **Source:** JOURNAL OF HUMAN HYPERTENSION, 20 (4): 267-271 APR 2006 **ISSN:** 0950-9240			1	0	1	0.50
5.	**Author(s):** Cwynar, M; Staessen, JA; Ticha, M; et al. **Title:** Epistatic interaction between alpha and gamma-adducin influences peripheral and central pulse pressures in White Europeans **Source:** JOURNAL OF HYPERTENSION, 22: S72-S73 Suppl. 2 JUN 2004 **ISSN:** 0263-6352	0	1	0	0	1	0.25
6.	**Author(s):** Filipovsky, J; Mayer, O; Pesta, M; et al. **Title:** The relationship of angiotensine II receptor polymorphism with stiffness of aorta and arteries of lower extremity **Source:** JOURNAL OF HYPERTENSION, 24: S74-S74 Suppl. 4 JUN 2006 **ISSN:** 0263-6352			0	0	0	0.00
7.	**Author(s):** Tikhonoff, V; Stolarz, K; Brand, E; et al. **Title:** The metabolic syndrome in relation to three candidate genes in 6 European populations **Source:** JOURNAL OF HYPERTENSION, 24: S145-S145 Suppl. 4 JUN 2006 **ISSN:** 0263-6352			0	0	0	0.00
8.	**Author(s):** Tikhonoff, V; Kuznetsova, T; Brand-Herrmann, SM; et al. **Title:** Serum HDL cholesterol in relation to genetic variation in PPAR-gamma2, GNB3 and alcohol intake **Source:** JOURNAL OF HYPERTENSION, 24: S392-S392 Suppl. 4 JUN 2006 **ISSN:** 0263-6352			0	0	0	0.00
9.	**Author(s):** Kucerova, J; Staessen, JA; Kuznetsova, T; et al. **Title:** The heritability of arterial properties **Source:** HYPERTENSION, 46 (4): 904-904 OCT 2005 **ISSN:** 0194-911X		0	0	0	0	0.00
10.	**Author(s):** Kucerova, J; Staessen, JA; Cwynar, M; et al. **Title:** Arterial characteristics in normotensive offspring with parental history of hypertension **Source:** JOURNAL OF HYPERTENSION, 23: S35-S35 Suppl. 2 JUN 2005 **ISSN:** 0263-6352		0	0	0	0	0.00

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