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Orientador: Prof. Dr. Heitor Moreno Junior

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Farmacologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção de título de Doutora em Farmacologia.

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Dedico

*Aos **meus queridos pais,***

meus maiores exemplos de vida.

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"O nascimento do pensamento é igual ao nascimento de uma criança: tudo começa com um ato de amor. Uma semente há de ser depositada no ventre vazio. E a semente do pensamento é o sonho. Por isso os educadores, antes de serem especialistas em ferramentas do saber, deveriam ser especialistas em amor: intérpretes de sonhos"

Rubem Alves

Introdução: A Hipertensão arterial resistente (HAR) é uma doença multifatorial e poligênica, frequentemente associada à obesidade, e definida como níveis pressóricos acima das metas recomendadas, embora o uso de 3 ou mais fármacos anti-hipertensivos de diferentes classes em doses otimizadas e incluindo, se possível, um diurético. Estudos prévios demonstraram que níveis reduzidos de adiponectina, um hormônio produzido pelo tecido adiposo, foram associados à resistência ao tratamento anti-hipertensivo. Além disso, os polimorfismos de nucleotídeo único (SNPs) rs266729 e rs1501299 no gene da adiponectina *ADIPOQ* foram relacionados a risco de doenças cardiovasculares. O presente estudo avaliou a associação entre os dois SNPs mais estudados (-11377C/G e +276G/T) e os níveis de adiponectina nos pacientes resistentes. **Desenho de estudo e Métodos:** Este estudo do tipo transversal incluiu 109 pacientes com HAR genotipados para ambos os polimorfismos genéticos pelo método de reação em cadeia da polimerase (PCR) em tempo real com sondas fluorescentes. As características clínicas e bioquímicas foram comparadas entre os sujeitos homozigotos CC (n=56) *vs*. portadores do alelo G (n=53) para o SNP -11377C/G e homozigotos GG (n=49) *vs*. portadores do alelo T (n=60) para o SNP +276G/T. Os níveis plasmáticos de adiponectina foram determinados por ELISA. **Resultados**: Os níveis de PA de consultório e da MAPA, assim como os marcadores de lesão em órgãos-alvo, foram semelhantes nos subgrupos genotípicos estudados. Os níveis de adiponectina foram significativamente maiores em CC comparados aos portadores do alelo G (CC = 7,0 (4,0-10,2) *vs.* alelo G = 5,5 (2,5-7,9), p = 0,04) e reduzidos em GG quando comparados aos portadores do alelo T (GG = 5,3 (2,3-7,7) *vs*. alelo T = 7,1 (3,6-10,5), p = 0,04). As análises de regressão linear múltipla revelaram que os alelos menos frequentes G (coeficiente beta = -0,14, SE = 0,07, p = 0,03) e T (coeficiente beta = 0,12, SE = 0,06, p = 0,04), ajustados para as variáveis MAPA sistólica, índice de massa corporal, idade, gênero, raça e presença de diabetes tipo 2, foram preditores independentes dos níveis de adiponectina. **Conclusão:** Os SNPs -1377C/G e +276G/T no gene *ADIPOQ* foram associados aos níveis de adiponectina em hipertensos resistentes.

Palavras-chave: Hipertensão arterial refratária, obesidade, adiponectina, polimorfismos genéticos.

Background: Resistant hypertension (RHTN) is a multifactorial and polygenic disease, frequently associated with obesity, and defined as high blood pressure (BP) maintained in spite of concurrent use of three or more antihypertensive drugs of different classes, combined at optimal doses and including a diuretic. Previous studies demonstrated that low plasma adiponectin levels, a hormone produced by the adipose tissue, were associated with RHTN. Indeed, single-nucleotide polymorphisms (SNPs) rs266729 and rs1501299 in adiponectin coding gene *ADIPOQ* were associated with hypertension and risk of cardiovascular disease. This study evaluated the association between two of the most widely studied SNPs (-11377C/G and +276G/T) and adiponectin plasma levels in RHTN subjects.

Design and Methods: This study comprised 109 RH patients genotyped for both polymorphisms by Real-time Polymerase Chain Reaction (PCR) method using fluorescent probes. We conducted a cross-sectional design comparing clinical and laboratorial characteristics of CC homozygous (n=56) *vs*. G allele carriers (n=53) for -11377C/G and GG homozygous (n=49) *vs*. T allele carriers (n=60) for +276G/T. Adiponectin levels were measured by enzyme-linked immunoassay.

Results: Office and ambulatory BP measurements were similar among genotypes subgroups in both SNPs as well as the markers of target organ damage. Adiponectin levels were significantly higher in CC compared to G-carrier for -11377C/G (CC = 7.0 (4.0-10.2) *vs.* G allele = 5.5 (2.5-7.9), p=0.04) and lower in GG compared to T-carrier for +276G/T (GG = 5.3 (2.3-7.7) *vs.* T allele = 7.1 (3.6-10.5), p=0.04). Adjusting for systolic ambulatory BP, body mass index, age, gender, race and presence of type 2 diabetes, multiple linear regression revealed that the minor alleles G (beta coefficient = -0.14, SE = 0.07, p = 0.03) and T (beta coefficient = 0.12, SE = 0.06, p = 0.04) were independent predictors of adiponectin levels.

Conclusion: The -1377C/G and +276G/T polymorphisms in *ADIPOQ* were associated with adiponectin levels in RH subjects.

Keywords: Refractory hypertension; obesity; adiponectin; polymorphisms.

AGT	Angiotensinogênio
DM2	Diabetes *mellitus* tipo 2
ELISA	*Enzyme-linked immunosorbent assay*
HA	Hipertensão arterial
HAR	Hipertensão arterial resistente
HARC	Hipertensão arterial resistente controlada
HARNC	Hipertensão arterial resistente não controlada
HDL-c	Colesterol de lipoproteína de alta densidade
HVE	Hipertrofia ventricular esquerda
IM	Infarto do miocárdio
IC	Insuficiência cardíaca
IECA	Inibidores da enzima conversora de angiotensina
IMC	Índice de massa corporal
IMVE	Índice de massa ventricular esquerda
LDL-c	Colesterol de lipoproteína de baixa densidade
MAPA	Monitoração ambulatorial da pressão arterial
PA	Pressão arterial
RNA	Ácido ribonucleico
SNS	Sistema nervoso simpático
SRAA	Sistema renina angiotensina aldosterona
TNF-α	Fator de necrose tumoral alfa
VOP	Velocidade de onda de pulso

1 INTRODUÇÃO

1.1 Considerações Gerais

A hipertensão arterial resistente (HAR) persiste como uma condição sem definição universalmente aceita, sendo ainda a real prevalência também muito variável (1). De acordo com o *Seventh Report of the Joint National Committee* (JNC VII) (2) e a *European Society of Hypertension / European Society of Cardiology* (ESH/ESC) (3), a HAR é considerada aquela em que, após duas consultas consecutivas, os valores pressóricos mantêm-se acima da meta, a despeito de tratamento não farmacológico e farmacológico otimizado tríplice instituído, incluindo diurético, em pacientes que tiveram adesão plena ao tratamento dietético e medicamentoso. Recentemente, a *American Heart Association* (AHA), em seu *"Scientific Statement"* especificamente sobre HAR incluiu à definição pacientes que apresentem pressão arterial (PA) controlada, mas que necessitam de quatro ou mais classes de fármacos anti-hipertensivos (4). As metas pressóricas são as mesmas estabelecidas para a população em geral de hipertensos <140/90 mmHg e <130/80 mmHg em pacientes hipertensos com diabetes tipo 2 (DM2) ou doença renal crônica (2). A realização da monitoração ambulatorial da pressão arterial (MAPA) é mandatória para o diagnóstico de certeza da HAR, pois permite excluir os pacientes considerados pseudorresistentes (hipertensão do jaleco branco) (5, 6).

Obesidade e HAR são duas doenças complexas que estão intimamente relacionadas nos diversos sistemas cujos mecanismos fisiopatológicos permanecem ainda pouco definidos. Entre eles estão hiperativação do sistema renina angiotensina aldosterona (SRAA) e do sistema nervoso simpático (SNS), disfunção endotelial (7), lesões em órgãos-alvo incluindo rigidez arterial (8), hipertrofia ventricular esquerda (HVE) (9) e microalbuminúria (10) e alterações de adipocitocinas inflamatórias (11). Além disso, a hiperatividade do SRAA na obesidade tem sido alvo importante na ligação entre tecido adiposo visceral e HAR (12, 13), uma vez que evidências apontam elevados níveis plasmáticos de aldosterona como um dos fatores associados à patogênese da HAR em indivíduos obesos (14, 15). Nesse contexto, a produção pelo tecido adiposo de ácidos graxos estimula a secreção de aldosterona e esta, por sua vez, através de receptores mineralocorticoides,

promove adipogênese (16) e aumento do estado inflamatório adiposo, com consequente redução dos níveis de adiponectina (17). Por fim, a concentração de aldosterona tem sido considerada relevante por mediar a falta de controle pressórico e também alterações fisiopatológicas nos sistemas renal, cardiovascular e nervoso central (18).

A adiponectina é uma adipocitocina com propriedades anti-inflamatória, antiaterogênica e sensibilizante à insulina (19), correlacionada negativamente com índice de massa corpórea (IMC) (20) e com possível atuação em regular citocinas pró-inflamatórias em adipócitos (21). Estudos transversais demonstraram que a hipoadiponectinemia é um fator de risco independente para HA (22-24). Além disso, estudo prospectivo revelou a hipoadiponectinemia como preditora do desenvolvimento de HA em normotensos, independente das covariáveis gênero, idade, IMC, PA, resistência à insulina e proteína C reativa (25). Níveis reduzidos de adiponectina plasmática foram correlacionados a lesões em órgãos-alvo em prejuízo da resposta vasodilatora mediada pelo endotélio (26, 27), maior progressão da HVE (28) e preditora independente da rigidez arterial em hipertensos (29, 30). Finalmente, a adiponectina plasmática foi associada a lesões em órgãos-alvo e concentração de aldosterona em hipertensos resistentes (31, 32).

Diante da forte associação entre obesidade e HAR (33, 34) e estudos prévios que potencialmente sugerem a participação da adiponectina na fisiopatologia da HAR (31, 32), o estudo que se segue como capítulo I (submetido) visa identificar marcadores genéticos no gene que codifica a adiponectina (*ADIPOQ*) que podem estar relacionados à resistência à terapia anti-hipertensiva. O estudo que se segue como capítulo II desta tese de doutorado (publicado) visou identificar os níveis plasmáticos da adiponectina e as lesões em órgãos-alvo na HAR

1.2 Hipertensão Arterial Resistente – HAR

A hipertensão arterial essencial (HA) representa a doença crônica mais comum no mundo ocidental com uma prevalência estimada na população adulta maior que 25% (35), e é considerada o maior fator de risco para doenças cardiovasculares (36). Apesar da grande disponibilidade de

fármacos efetivos para o tratamento da HA, o relatório do *National Health Nutrition Education Survey* revelou que somente 27% da população americana de adultos hipertensos têm a pressão controlada (<140/90 mmHg), o restante não atinge as metas recomendadas pelo consenso (2, 36). Embora a baixa adesão (37) e/ou regimes de tratamento inadequados, medidas incorretas da PA e outras causas de pseudo-hipertensão possam explicar em parte esse insucesso no controle da PA, há um percentual ainda significativo de pacientes que, mesmo excluídos esses fatores, apresentam real dificuldade do controle pressórico. Portanto, a complexa etiologia e os numerosos fatores contribuintes para a patogênese da HA podem variar substancialmente entre os indivíduos, comprometendo a eficácia dos regimes terapêuticos. Dessa forma, esses diversos mecanismos etiológicos podem resultar em grandes dificuldades no controle da PA, mesmo quando administrados múltiplos fármacos com comprovada aderência, proporcionando resistência ao tratamento anti-hipertensivo (38, 39).

1.2.1 Definição

A HAR é definida, segundo a *American Heart Association,* como a PA que mantém acima das metas recomendadas (≥140/90mmHg), apesar do uso concomitante de 3 fármacos anti-hipertensivos de diferentes classes em doses otimizadas, sendo, idealmente, um deles diurético (HARNC). Além disso, os pacientes cuja PA está controlada com o uso de 4 ou mais anti-hipertensivos são considerados resistentes ao tratamento (HARC). Embora a definição seja arbitrária em termos de número de medicações necessárias ao tratamento, a HAR é atribuída à identificação dos pacientes que apresentam alto risco cardiovascular (4).

O fenótipo do hipertenso resistente tem características comuns consistentemente demonstradas (em estudos nacionais e internacionais): idade mais avançada (>55 anos), sexo feminino, afrodescendência, obesidade, diabete melito, nefropatia crônica, síndrome metabólica, aumento de ingestão de sal, maiores níveis pressóricos iniciais e sedentarismo (40-43). No entanto, o subgrupo de resistentes não controlados parece apresentar um pior prognóstico, sugerindo que que talvez haja uma gradação do risco, mesmo dentro do grupo de hipertensos resistentes.

Recentemente, demonstramos que os subgrupos (resistentes controlados - HARC e resistentes não controlados - HARNC) são bastante distintos em várias características, principalmente quanto ao excesso de aldosterona plasmática, rigidez arterial, HVE, microalbuminúria e adipocitocinas plasmáticas (31-33, 44).

Existe, portando, controvérsia se todos estes pacientes deveriam ser colocados em conjunto, sendo uma nova terminologia proposta, na qual deveriam ser separados em dois subgrupos: resistentes e refratários (45-47). Essa nova classificação ainda está em discussão, não sendo totalmente aceita (48), existindo, contudo, dados novos consistentes de que esses grupos seriam distintos e deveriam ser separados (49). No entanto, a simples divisão em pacientes controlados e não controlados já é consagrada e torna grupos reconhecidamente diferentes. Uma análise epidemiológica com pacientes diagnosticados para HAR, segundo a AHA, divididos em dois grupos de acordo com os níveis pressóricos, os controlados (com, no mínimo 4 fármacos) e os não controlados, mostrou que no grupo não controlado os níveis pressóricos (PAS e PAD), pressão de pulso, IMC, atividade de renina plasmática, concentração de aldosterona plasmática e relação aldosterona/renina são significativamente maiores quando comparados ao grupo controlado (33). Além disso, apresentou maior espessura íntima-média, velocidade de onda de pulso (VOP), HVE (33, 50), disfunção endotelial e menor queda noturna dos níveis pressóricos (51). Com isso, foi demonstrado que existem diferenças importantes entre os indivíduos controlados e não controlados, o que aumenta ainda mais a morbimortalidade do grupo que apresenta não controle da PA.

1.2.2 Prevalência

A prevalência da HAR ainda é incerta e varia conforme a especialização do centro e o tipo de análise epidemiológica. Estudos populacionais observacionais estimaram indiretamente a prevalência da HAR entre 15-18% na população geral de hipertensos (52, 53). Esses estudos, normalmente, não submeteram os pacientes a uma avaliação sistematizada para o diagnóstico de HAR e exclusão de causas secundárias ou removíveis e de pseudorresistência pela avaliação de pressão ambulatorial da PA (54, 55). O estudo de Framingham mostrou 52% de pacientes sem

controle adequado de PA (56). Entretanto, esses altos índices estão acrescidos de má aderência e tratamento inadequado. O estudo *Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial* (ALLHAT), no qual havia diversidade étnica importante e o tratamento dos pacientes era mais adequado e controlado, após 5 anos de seguimento aproximadamente 50% dos indivíduos necessitavam de 3 ou mais classes de fármacos para o tratamento (57). Contudo, esse valor deve estar superestimado, devido aos regimes terapêuticos restritos permitidos neste estudo, além das principais associações terapêuticas utilizadas, sendo que o uso de diuréticos tiazídicos, inibidores da ECA (IECA) e bloqueadores de canais de cálcio não foram encorajados (40). Uma análise dos pacientes do *National Health and Nutrition Examination Survey*, entre 2003 e 2008, classificou em hipertensos resistentes 12,8% dos indivíduos tratados com HA (52). Talvez, o estudo mais criterioso para tal fim seja o da Universidade do Alabama (Birmingham) (58), onde os pacientes foram encaminhados como pacientes com hipertensão de difícil controle e acompanhados por pelo menos 6 meses antes de considerados verdadeiros hipertensos resistentes. Destes, 9,5% permaneceram refratários ao tratamento (58). Outros estudos corroboram estes dados, mostrando uma prevalência aceita, hoje, entre 5 e 15% (59), demonstrando, inclusive, um aumento na prevalência ao longo das duas últimas décadas, de 5,5% para 8,5% e, então, 11,8% entre 2005 e 2008 (41, 58, 60). Por fim, estudo recente brasileiro estimaram uma prevalência menor de aproximadamente 3,0-4,5% de hipertensos (61).

1.2.3 Diagnóstico e Pseudorresistência

Segundo o 1º Posicionamento brasileiro sobre a HAR, o diagnóstico recai inicialmente no afastamento da pseudorresistência como causa. A pseudorresistência é descrita como o aparente não controle pressórico que, na verdade, é devido a medidas inadequadas da PA, escolha terapêutica, tanto de fármacos quanto de dosagens inapropriadas (40), falta de aderência ao tratamento farmacológico e não farmacológico e hipertensão do jaleco branco (62). Portanto, é de extrema importância para qualquer avaliação diagnóstica definir a presença de pseudorresistência entre os indivíduos considerados hipertensos resistentes. A hipertensão do jaleco branco é excluída pela

realização da MAPA ou MRPA (63). Conforme comprovação prévia, uma intensiva monitorização de aderência ao tratamento, ajuda na identificação dos pacientes hipertensos "verdadeiramente" resistentes (64). As causas removíveis incluem, de forma geral, ingestão elevada de sal e álcool ou de outras substâncias exógenas que dificultam o controle pressórico (anti-inflamatórios não hormonais, corticosteroides, contraceptivos orais, simpatomiméticos, quimioterápicos, antidepressivos, imunodepressores, descongestionantes nasais, anorexígenos e cocaína). Por fim, deve-se pesquisar causas secundárias de HAR, cujas principais então listadas na tabela 1.

Tabela 1: Principais causas de hipertensão secundária

Frequentes:
Apneia Obstrutiva do Sono
Doença Renal Parenquimatosa
Estenose de Artéria Renal
Hiperaldosteronismo Primário
Raras:
Feocromocitoma
Doença de Cushing
Hiperparatireoidismo
Coarctação de Aorta
Tumor Intracraniano

Adaptado de Calhoun et al (40)

1.2.4 Fisiopatologia

A fisiopatologia da HAR é multifatorial (65), o que reflete pior prognóstico quando comparada a HA. Ainda os mecanismos de resistência ao tratamento não são totalmente conhecidos podendo ser modulados por diversos fatores interligados: hiperativação do SNS e sistema renina-

angiotensina-aldosterona (SRAA) (66-68); excesso de aldosterona (18, 69); expansão volêmica (70), disfunção endotelial (50, 71, 72); e elevação da resistência vascular periférica (73). Recentemente, apneia obstrutiva do sono (74, 75), resistência à insulina (76) e alterações na função de adipocitocinas inflamatórias (77) também têm sido associados à patogenia da doença (Figura 1).

Figura 1: Fisiopatologia da HAR relacionando os principais mecanismos envolvidos (Modificado de *Tsioufis, Kordalis et al.*)(78). Condições como apneia obstrutiva do sono (AOS), obesidade e excesso de aldosterona são fatores desencadeantes de inflamação, resistência insulínica e disfunção endotelial. Como consequência, há hiperativação do SNS e SRAA que possuem a propriedade de hiperativação recíproca, podendo incorrer em HAR.

1.2.5 Tratamento

1.2.5.1 Não Farmacológico

O tratamento da HAR requer sempre o uso de fármacos anti-hipertensivos, conforme sua própria definição. Contudo são de suma importância as recomendações não farmacológicas

adjuvantes (40). Pacientes com HAR devem ser orientados quanto à importância da redução de sal na dieta, perda de peso, prática de exercícios físicos regulares e diminuição no consumo de bebidas alcoólicas (40, 79).

Apesar da dieta hipossódica reduzir os níveis pressóricos moderadamente, os pacientes com HAR são particularmente sal-sensíveis. Em estudo prévio, um consumo diário de até 2,5g de sal por dia reduziu em 23,0/9,0 mmHg a PA (80), mostrando a importância da redução da ingestão de sal, mesmo sendo uma meta de difícil alcance. É recomendado pela Organização Mundial de Saúde um consumo diário de 1,2g de sal por dia (79).

O consumo de álcool está intimamente ligado à elevação da PA. Homens que consomem mais de 4 doses de álcool por dia têm 50% mais chance de apresentar valores pressóricos fora das metas (56). A abstinência de álcool em grandes consumidores reduziram os níveis de PAS e PAD em 7,2 e 6,6 mmHg, respectivamente, além da prevalência de HA entre os participantes ter reduzido de 42% para 12% (81). O consumo alcoólico se possível deve ser ≤20g de etanol por dia ou abstinência total (79).

A redução de peso diminui a PA de forma significativa devido redução da retenção hídrica, da apneia obstrutiva do sono e da estimulação do SNS (40, 82, 83). Pacientes com IMC ≥30 apresentam chance 50% maior de apresentar PA não controlada em comparação a pessoas com IMC normal (33, 56). Com isso, a perda de peso deve sempre ser estimulada entre os indivíduos com HAR (59). Existe um benefício claro entre atividade física e redução da PA (40, 84). A prática de exercícios físicos regulares, além de reduzir os níveis pressóricos, também melhora o perfil metabólico, tanto com exercícios de resistência quanto aeróbicos (84). Assim, estes pacientes dever ser encorajados a realizar atividade física leve a moderada (79).

1.2.5.2 Farmacológico

A terapêutica na HAR não apresenta estratégia definida. Preconiza-se atingir a meta pressórica de PA de consultório ou MRPA de 130/80mmHg ou 125/75mmHg na MAPA (85).

O esquema anti-hipertensivo deve almejar o bloqueio de todos os possíveis mecanismos envolvidos na HAR. Logo, o bloqueio do SRAA, seja utilizando um IECA, antagonista do receptor de angiotensina II, associado a um antagonista dos canais de cálcio dihidropiridínico e um diurético tiazídico, é considerada a melhor combinação tripla, eficaz, sinérgica e tolerada (86).

Em relação ao uso de um quarto fármaco, não há consenso sobre sua escolha, tendo em vista que faltam estudos comparativos entre as classes demostrando superioridade em potência anti-hipertensiva e proteção cardiovascular (59). Segundo o estudo *Anglo-Scandinavian Cardiac Outcomes Trial* (ASCOT), a adição de espironolactona, um antagonista de receptores mineralocorticoides, ao esquema tríplice tradicional apresentaram quedas de 21,9 mmHg de PAS e 9,5 mmHg da PAD após 1,3 ano de tratamento (87). Outro estudo realizado em 2009, com pacientes hipertensos resistentes controlados e não controlados também mostrou resultados semelhantes (88). Esta redução é independente da presença de hiperaldosteronismo primário e não relacionadas aos níveis plasmáticos ou urinários basais iniciais de aldosterona, atividade plasmática de renina ou relação aldosterona/renina (89, 90).

A escolha de fármacos que sejam adicionados ao esquema quádruplo deve ser individualizada (79) de acordo com a experiência de cada médico e de outras comorbidades que o paciente possa ter. Inibidores adrenérgicos, incluindo beta e alfa-bloqueadores e inibidores de ação central, vasodilatadores e inibidores de renina, também são muito importantes como opções terapêuticas aos pacientes com HAR (59). Entretanto, seu uso está mais condicionado a situações especiais, como a necessidade de redução de frequência cardíaca, em relação ao uso de betabloqueadores. Além disso, apresentam maior incidência de efeitos adversos (40, 79).

Recentemente, uma nova classe de fármaco foi testada no tratamento anti-hipertensivo: os inibidores de fosfodiesterase-5 (91, 92). No entanto, apesar de algum grau de sucesso ter sido demonstrado, estudos maiores ainda são necessários para conclusões mais representativas.

1.2.5.3 Outros Tratamentos

Existem ainda, novas modalidades terapêuticas que estão em desenvolvimento para ajudarem o tratamento farmacológico no controle pressórico dos pacientes resistentes.

A estimulação dos barorreceptores presentes no seio carotídeo estimula o sistema nervoso parassimpático, propiciando, além de bradicardia, aumento na excreção renal de NaCl e água. A hipervolemia é um dos principais mecanismos envolvidos na resistência da HA destes pacientes, sendo que o aumento da diurese é um alvo terapêutico importante a ser atingido. As tentativas de reduzir a hiperatividade do SNS para reduzir os níveis pressóricos arteriais remontam ao início do século 20 (93). A estimulação tanto aguda quanto crônica do seio carotídeo para a redução da HA começou a mostrar resultados positivos nos anos 1960. No entanto, na ocasião, os aparelhos responsáveis pela estimulação eram grandes e com baterias de pouca duração (94). Nos dias de hoje, essa terapia está novamente em desenvolvimento, com técnicas novas de implantação, que reduzem seus efeitos colaterais e aparelhos mais modernos, menores e de duração maior. Estudos recentes têm demonstrado benefício da estimulação crônica como adjuvante ao tratamento da HAR, inclusive com redução do número de fármacos administrados diariamente (95, 96).

Outro tratamento que vem ganhando destaque é a desnervação simpática renal. Os nervos simpáticos renais contribuem para o surgimento e manutenção da HA, por meio de liberação de renina, retenção de sódio e aumento da volemia (97). Entre os anos 1920 e 1930 a simpatectomia radical era um tratamento utilizado para reduzir a PA em hipertensos graves. Contudo, este procedimento apresentava altos índices de complicações (98). Recentemente, novas técnicas intervencionistas com bloqueio da ação simpática eferente por radiofrequência têm mostrado redução significativa da PA em HA, sem maiores complicações descritas (99). No entanto, ainda faltam estudos que mostrem reprodutibilidade dos resultados (59).

1.2.6 Condições clínicas associadas

1.2.6.1 Lesões em órgãos-alvo

1.2.6.1.1 Rigidez Arterial

A rigidez arterial é determinada pela estrutura da parede arterial e pelas condições da parede, em especial da camada média. Esta rigidez arterial em hipertensos é praticamente atribuível a alterações estruturais na parede deste vaso (100). A complacência da parede vascular depende da contribuição do colágeno e elastina (quantidades diminuídas de elastina ou quebra delas), que conferem integridade estrutural e elasticidade ao vaso. Além de mudanças estruturais, a rigidez arterial também é afetada pelas células endoteliais da musculatura lisa. O tônus vascular pode ser modificado por deposição de cálcio e mediadores parácrinos como angiotensina II, endotelina, estresse oxidativo e óxido nítrico (101, 102). Além do próprio processo de envelhecimento das grandes artérias que resulta em menor complacência (103), doenças como o DM2 (104), HA (105), doença renal crônica e o tabagismo (106) participam como aceleradores desse processo.

A rigidez arterial pode ser avaliada de maneira não invasiva pela estimação da velocidade de onda de pulso (VOP) por um método simples e reprodutível (107, 108) de tonometria de aplanação. A VOP é obtida através dos dados das artérias carótida e femoral, que é o padrão-ouro para avaliação da rigidez, sendo que o valor considerado como preditor de lesão em órgão-alvo é de 10m/s (109).

Estudos epidemiológicos demonstraram que o aumento da VOP está associado a aumento de risco da morbidade e mortalidade por doença cardiovascular (110-112). Além disso, estudo recente, avaliando a rigidez arterial nos subgrupos da HAR, demonstrou maior VOP nos pacientes não controlados em relação aos controlados (33).

1.2.6.1.2 Hipertrofia Ventricular Esquerda

A HVE é a resposta cardíaca à sobrecarga pressórica e/ou volumétrica crônica, e sua prevalência e incidência elevam-se de acordo com a progressão de níveis de PA (113). Essa adaptação está associada à maior morbidade e mortalidade dos seus portadores. É possível que o

mecanismo adaptativo esteja acompanhado de alterações intrínsecas dos miócitos cardíacos ou de outras células miocárdicas, predispondo a um déficit contrátil e instabilidade elétrica do coração.

Com relação ao aumento da massa do VE, os resultados do *Framingham Heart Study* demonstraram de forma inequívoca o valor prognóstico da detecção de HVE na estratificação de risco para doença cardiovascular, morbidade e mortalidade (114, 115). A HVE é determinada pela análise da massa ventricular esquerda (MVE), sendo corrigida pela superfície corporal, obtendo desta forma, o índice de massa ventricular esquerda (IMVE), considerado normal até 95g/m^2 para o sexo feminino e até 115 g/m^2 para o sexo masculino (116).

O desenvolvimento de HVE é multifatorial, e está relacionada à HA, intolerância à glicose, perfil lipídico e tabagismo (117). Estudos epidemiológicos têm implicado HVE como fator de risco para o infarto do miocárdio (IM), insuficiência cardíaca (IC) e morte súbita (118). Estudo englobando HAR demonstrou incidência de HVE nos pacientes portadores de resistência aos fármacos anti-hipertensivos em relação aos hipertensos controlados e ao grupo controle de normotensos (88). Além disso, mais recentemente foi evidenciado que o subgrupo não controlado da HAR apresentou maior IMVE comparado aos indivíduos HAR controlados (33).

1.2.6.1.3 Microalbuminúria

Microalbuminúria é geralmente definida como uma taxa de excreção urinária de albumina entre 30 a 299 mg/dia (119). Embora muitas vezes visto como um sinal de doença renal precoce, a MA interage com vários fatores de risco convencionais vasculares e é também um marcador independente de disfunção endotelial (120). Inicialmente foi identificada como um marcador de doença renal (121), e atualmente, tem sido considerada como um fator de risco de morbidade e mortalidade cardiovascular (122). Estudos demonstraram que fatores de risco cardiovasculares como HA, metabolismo da glicose e dislipidemia, aumentam o risco de desenvolvimento de microalbuminúria (123-125).

1.2.6.2 Aldosterona

A hiperativação do SRAA tem sido extensivamente abordada devido ao seu potencial envolvimento na fisiopatologia da HAR associada à obesidade, sendo que o bloqueio desse sistema pode ser uma estratégia terapêutica benéfica para ambas condições (12).

A aldosterona tem importante papel sobre a volemia, função renal e vasos arteriais, principalmente quanto à indução de disfunção endotelial, inflamação, fibrose e rigidez vascular. O conjunto dessas alterações promove mudanças funcionais e estruturais de pequenas e grandes artérias, levando, consequentemente, ao aumento da PA, frequentemente resistente às classes de anti-hipertensivos atualmente utilizadas (33, 88). Por outro lado, a própria utilização de diuréticos, inibidores da enzima conversora e antagonistas dos receptores de angiotensina II pode causar aumento da aldosterona plasmática (escape da aldosterona) que contribui para a resistência ao tratamento anti-hipertensivo (126, 127). Ainda, há fortes evidências que consideram o SRAA intrinsecamente ligado à obesidade, resistência à insulina e dislipidemia (15). Níveis elevados de aldosterona contribuem diretamente para a patogênese da resistência à insulina e disfunção endotelial, processos estes que contribuem para os efeitos de remodelamento renal e cardiovascular. O excesso de aldosterona estimula vias clássicas de retenção sódica e expansão volêmica e também as não clássicas (ações não genômicas) envolvendo inflamação e estresse oxidativo, o que contribuiu para aceleração do desenvolvimento da HAR (18). Além disso, recentemente tem sido considerado um *crosstalk* entre essas vias clássicas e não clássicas potencializando as ações da aldosterona (128, 129).

Estudos recentes demonstram que pacientes hipertensos frequentemente exibem prejuízo na sinalização metabólica de insulina, dislipidemia, microalbuminúria e obesidade (130). Relatos demonstram a possível interação da aldosterona em receptores mineralocorticoides com a função de promover adipogênese e aumento da infiltração de macrófagos (16), caracterizada pela inflamação do tecido adiposo e expressão modificada de adipocitocinas inflamatórias. Além disso, o bloqueio do receptor mineralocorticoide reduziu a expressão de fatores pró-inflamatórios e aumentou a

expressão de adiponectina no coração e no tecido adiposo de camundongos obesos e diabéticos (17) (Figura 2).

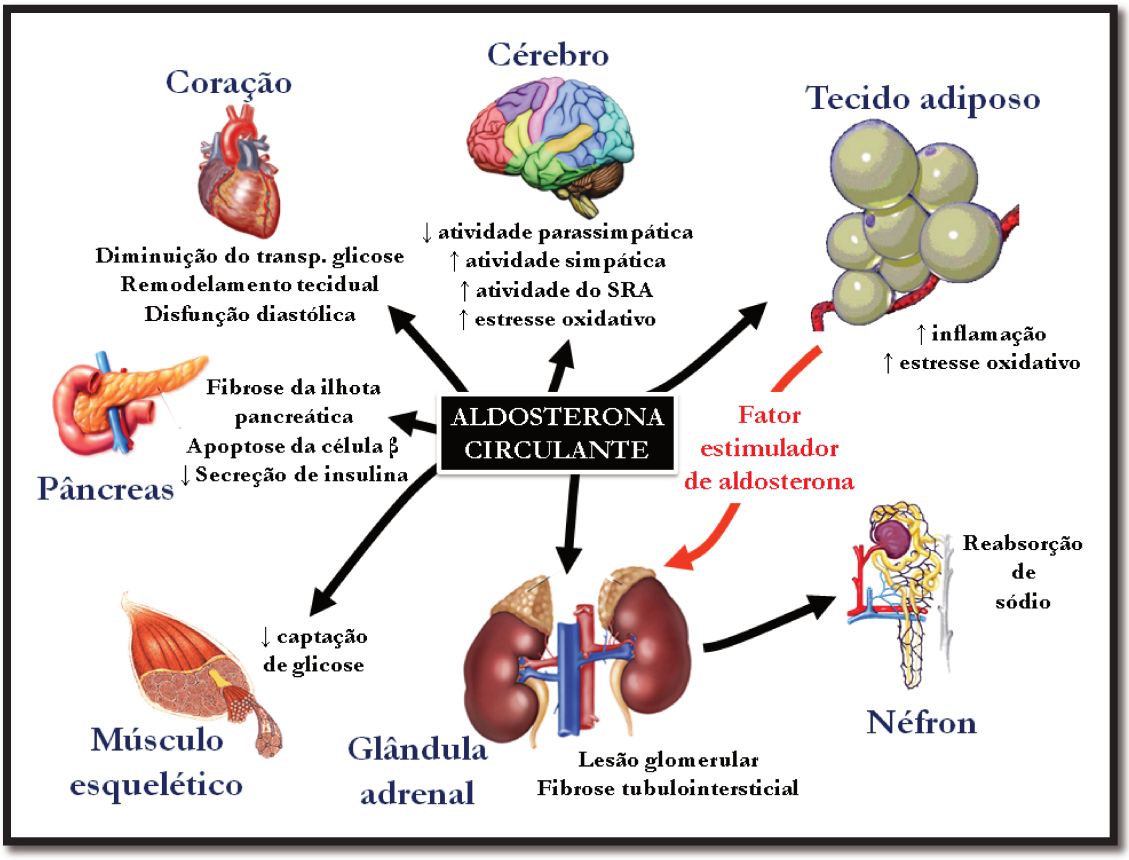

Figura 2: Ações da aldosterona plasmática nos mais diversos sistemas (Modificado de *Sowers, Whaley-Connell et al.*)(18). O TA produz um fator estimulador de aldosterona que ao agir sobre as glândulas adrenais promove o aumento da aldosterona circulante. Além de promover a reabsorção de sódio nos túbulos coletores, o aumento da aldosterona circulante está associado com várias alterações no organismo. Entre elas: o aumento da atividade do SNS, a HVE, disfunção endotelial, dano glomerular, além de processos inflamatórios e de estresse oxidativo. Em relação ao TA, a aldosterona, em receptores mineralocorticoides, promove adipogênese, inflamação e expressão alterada de adipocitocinas.

Finalmente, o tecido adiposo foi reconhecido com a capacidade de secretar hormônios, denominados adipocitocinas, consolidando a ideia de um órgão endócrino (131), que medeia inflamação sistêmica, estresse oxidativo e resistência à insulina (132).

1.2.6.3 Obesidade e resistência à insulina

A obesidade aumentou de forma alarmante, tornando-se um grave problema de saúde pública em razão do aumento expressivo da morbidade e mortalidade cardiovasculares, secundárias

a progressão e o agravamento da doença aterosclerótica (133, 134). Estudos experimentais e clínicos têm demonstrado que o excesso de peso eleva a PA. Os mecanismos fisiopatológicos da HA associada à obesidade são complexos. O aumento da atividade simpática, a ativação do SRAA, a resistência à ação da leptina, a alteração de fatores pró-inflamatórios e de coagulação, a disfunção endotelial e os fatores hemodinâmicos relacionam-se entre si de forma direta ou indireta (62, 135). Também a menor ação protetora da adiponectina parece explicar a relação obesidade e HA (136). Sabe-se que a obesidade está associada a aumento do débito cardíaco, resistência vascular periférica e fluxo sanguíneo regional, que promovem expansão do volume extracelular e alteração da função renal, manifestada por alteração da curva pressão/natriurese e retenção renal de sódio. Inicialmente, por um aumento da reabsorção tubular na fase inicial da obesidade e, posteriormente, secundária à lesão glomerular com perda de função (137, 138). Outro importante fator é a atividade de renina plasmática que está aumentada em obesos, independente da retenção de sódio e aumento do volume extracelular. O papel da angiotensina é reforçado pela eficácia observada no tratamento de jovens obesos hipertensos com IECA (139). Além disso, foi descrito um fator derivado do adipócito, como um derivado do ácido graxo livre, que aumenta a liberação de um estimulador hepático de síntese de aldosterona (140). Pacientes obesos com HA de difícil controle também exibem maior grau de resistência à insulina e obesidade e quando comparados a hipertensos bem controlados pareados por idade, sexo e IMC (141).

Finalmente, as adipocitocinas, secretadas ativamente pelo tecido adiposo, possuem ampla diversidade estrutural e funcional, podendo compreender desde proteínas relacionadas ao sistema imune, da via alternativa de complemento, fatores de crescimento, regulação de PA, coagulação sanguínea, angiogênese e homeostase glicêmica, entre outras (131).

1.2.6.4 Adiponectina e Desfechos Clínicos Cardiovasculares

A adiponectina foi identificada em meados de 1990 como uma proteína plasmática relativamente abundante produzida pelo tecido adiposo. O gene da adiponectina humana se localiza no cromossomo 3q27, um sítio associado com suscetibilidade ao DM2 (142).

Estudos demonstraram que a adiponectina está correlacionada negativamente com a porcentagem de gordura corpórea, distribuição de gordura central, insulina plasmática em jejum, tolerância oral à glicose e com fatores de risco cardiovascular associados à obesidade, incluindo PA sistólica e diastólica, colesterol total, triglicerídeos, LDL colesterol e ácido úrico. Além disso, houve correlação positiva com níveis de HDL colesterol (143). Níveis reduzidos são observados na presença de obesidade, doença arterial coronariana, HA e resistência à insulina (144) e podem refletir aumento de risco cardiovascular e inflamação (145). Estudos atuais sugerem que a atividade biológica da adiponectina se deve em grande parte aos multímeros de elevado peso molecular (146), tendo a mesma o valor prognóstico para eventos cardiovasculares em pacientes com doença coronariana (147).

Na vasculatura, os níveis de adiponectina estão fortemente ligados à função endotelial (148), principalmente por alterar efeitos vasculares mediados por citocinas, como TNF-α, suprimir geração de espécies reativas ao oxigênio e possibilitar a geração de óxido nítrico endotelial. Desta forma, a adiponectina tem propriedades anti-inflamatórias, antiaterogênicas e antiproliferativas nos vasos. Estudos prévios sugerem que a adiponectina é um fator de proteção contra HA através do mecanismo dependente do endotélio, como demonstrado em camundongos deficientes da proteína com resposta vasodilatadora prejudicada induzida pela acetilcolina (26). Ainda assim, em relação ao sistema vascular, a adiponectina se associou inversamente à rigidez arterial em pacientes hipertensos (29, 30, 149). Essa associação pode ser explicada pelo fato da adiponectina estimular a atividade da sintase endotelial para produção de NO (25) e inibir a proliferação da musculatura lisa vascular (150) e de componentes da matriz extracelular, como colágeno e fibronectina (151).

No estudo *Health Professionals Follow-up Study*, com 18.225 pacientes, o nível plasmático de adiponectina correlacionou-se com risco diminuído de IM (152). Em outro estudo com desfecho semelhante, níveis plasmáticos baixos de adiponectina foram associados com IM em indivíduos abaixo de 60 anos, independentemente de história prévia de HA, dislipidemia (HDL colesterol reduzido), tabagismo e IMC elevado (153).

A adiponectina parece ser negativamente regulada por ativação do SNS. Agonistas beta-adrenérgicos, via estimulação AMPc, inibem a expressão gênica, produção e secreção de adiponectina em camundongos e em modelos *in vitro*. Também inibem a expressão gênica de adiponectina no tecido adiposo visceral humano *in vitro* (154).

A liberação de adiponectina na circulação está associada com severidade de sintomas e mortalidade na IC (155, 156). Níveis elevados, por sua vez, refletem uma tentativa de atenuar estados pró-inflamatórios e demonstram um balanço entre vias prejudiciais e protetoras na disfunção sistólica do ventrículo esquerdo e IC (145). Desta forma, a adiponectina também influencia o remodelamento cardíaco inibindo o processo de hipertrofia cardíaca (157).

Estudos transversais relataram a hipoadiponectinemia como sendo um fator de risco independente para HA e como marcador de predisposição de HA em homens (24, 158). Em estudo prospectivo com duração de 5 anos, a hipoadiponectinemia foi relacionada como preditora do desenvolvimento da HA em não diabéticos, independente das variáveis idade, parâmetros de obesidade, PA média, resistência à insulina e proteína C reativa de alta sensibilidade (25) (Figura 3).

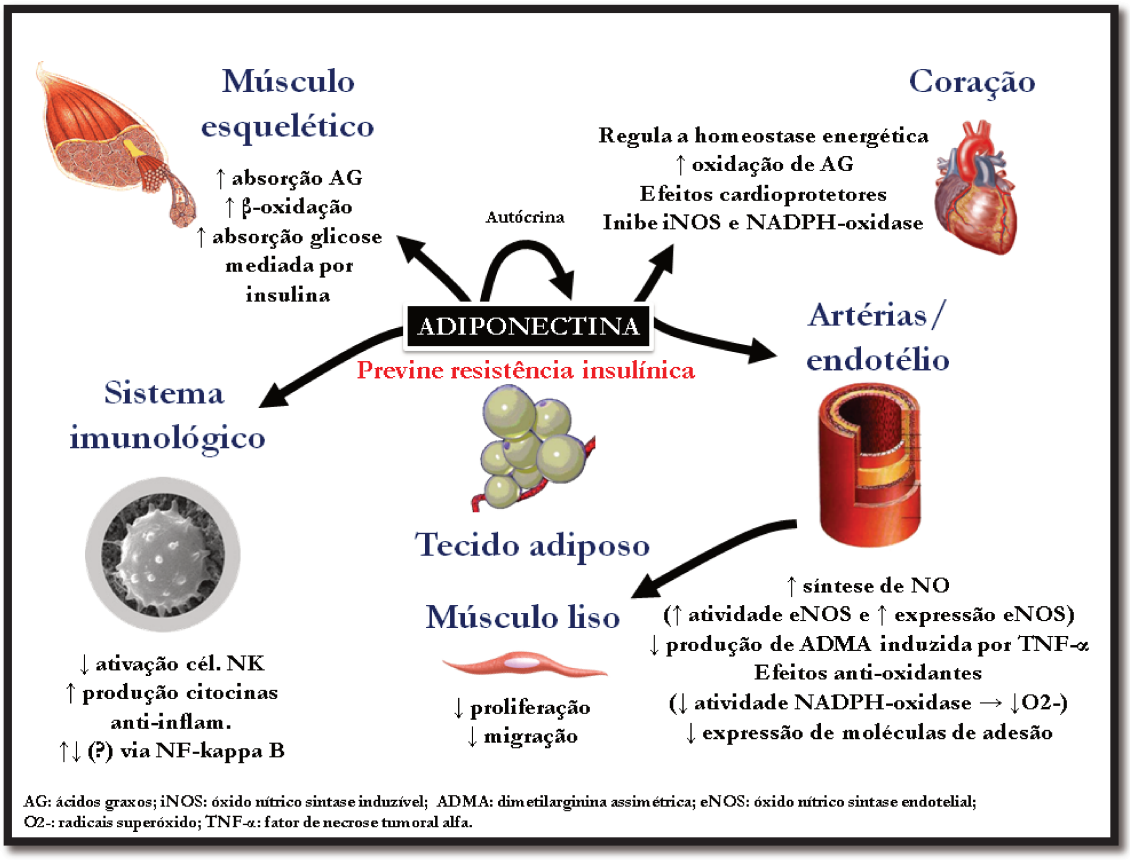

Figura 3: A relação entre adiponectina e os sistemas fisiológicos (Modificado de *Antoniades, Antonopoulos et al.)*(159). A adiponectina, uma adipocitocina com propriedades anti-inflamatória e antiaterogênica, é correlacionada negativamente com doenças cardiovasculares (HA, disfunção endotelial e rigidez vascular) e outras condições patológicas como DM 2 e resistência insulínica.

1.2.7 Aspectos genéticos

Além dos fatores ambientais convencionais, há fatores genéticos que podem influenciar os mecanismos de controle da PA. A herança na HA é complexa, assumida como poligênica e heterogênea, com contribuição importante do ambiente (multifatorial). Assim, as doenças poligênicas continuam a representar desafio considerável para a investigação genética e as tentativas de esclarecê-las estão apenas em seu início (160, 161).

A identificação de variações de genes (alelos) que contribuem para o desenvolvimento de HA é complicada pelo fato de dois fenótipos que determinam PA, o débito cardíaco e a resistência periférica, estarem controlados por fenótipos intermediários, incluindo atividade do SNS, SRAA,

sistemas renais calicreína-cininas e os fatores endoteliais, os quais por sua vez influenciam outros fatores intermediários como a excreção de sódio, a reatividade vascular e a contratilidade miocárdica (162). Desta forma, existem muito genes que podem participar no desenvolvimento da HA.

A localização de regiões no DNA responsáveis por determinado fenótipo é facilitada pela identificação de marcadores polimórficos ou sequências de DNA em localizações específicas no genoma que podem variar individualmente, definindo diferentes alelos. O termo "polimorfismo" é utilizado para referir-se a uma série de variações genéticas estáveis presentes ao menos em 1% da população. Os polimorfismos genéticos ocorrem devido à substituição de uma base nitrogenada (nucleotídeo), inserção ou deleção de sequências de DNA e finalmente por variações do número de repetições. O tipo mais comum de polimorfismo é o chamado polimorfismo de nucleotídeo único (*Single Nucleotide Polymorphism* - SNP). O polimorfismo de nucleotídeo único é resultado da substituição de um único par de bases da sequência de DNA, sendo o tipo mais comum de variação genética interindividual. Estima-se que um polimorfismo em um único nucleotídeo (pontual) ocorre em cada 1000 pares de bases. O genoma completo possui 3 a 10 milhões de polimorfismos de base única, destes, aproximadamente 1-1,5 milhões foram caracterizados pelo consórcio internacional de detecção de polimorfismos de base única (163).

Muitas destas alterações estão presentes em sequências de DNA que não são codificadores, as quais correspondem a 90% do total do DNA. Assim, apenas uma minoria dos polimorfismos pontuais ocorre em regiões codificadoras dos genes. Os polimorfismos de nucleotídeo único presentes nas sequências de DNA que são codificadores (chamados "*coding SNP*") podem produzir alterações se o códon for modificado, ocasionando uma substituição de um aminoácido, resultando em uma modificação qualitativa na estrutura da proteína (e na sua função) ou alteração da expressão da proteína (164, 165). Por outro lado, alguns polimorfismos posicionados tanto em sequências reguladoras como nos íntrons, podem afetar a transcrição aumentando-a ou diminuindo-a. Devido à

redundância do código genético, determinados polimorfismos não resultam em qualquer efeito (166).

Uma vez que a HAR é considerada um fenótipo extremo, é razoável predizer que fatores genéticos podem ter papel importante na determinação de resistência à terapia anti-hipertensiva. Porém, os estudos genéticos envolvendo hipertensos resistentes ainda são limitados (4).

O estudo *Genetics of Hypertension Associated Treatment* (GenHAT), um sub estudo complementar ao ALLHAT, considerou 78 polimorfismos genéticos como potenciais candidatos ao desenvolvimento de HAR e doença cardiovascular. Houve 2 variantes genéticas localizadas no gene do angiotensinogênio (AGT M235T rs699 e AGT-6G rs5051), que demonstraram significativa associação entre os participantes brancos após ajuste de variáveis de confusão e múltiplas análises. Além disso, foram observadas sugestivas associações envolvendo as variações genéticas da metaloproteinase de matriz 3 (5A/6A rs3025058) e do fator de necrose tumoral alfa – TNF-α (G/A rs361525) (167), substratos fisiopatológicos amplamente estudados em HA (168, 169).

Estudo recente do nosso laboratório avaliou o polimorfismo genético -344C/T *CYP11B2* (rs1799998) que codifica a aldosterona sintase (enzima responsável pelo passo final na síntese de aldosterona nas células da zona glomerulosa). Foi demonstrado que a concentração de aldosterona estava menor em portadores do alelo C quando comparados aos homozigotos TT. Além disso, quando os pacientes (CC/CT vs. TT) foram divididos em uso de espironolactona ou não, os indivíduos TT em uso de espironolactona apresentaram maior concentração de aldosterona plasmática em comparação com portadores do alelo C em uso da medicação e também em comparação aos indivíduos TT sem o uso do fármaco. Por fim, o genótipo TT e o uso de espironolactona foram preditores dos níveis de aldosterona nos hipertensos resistentes. Esses achados fortemente sugerem que o escape da aldosterona ocorre em resposta ao tratamento com minerolocorticoide em indivíduos homozigotos TT para esse polimorfismo [170].

Esses estudos genéticos, embora ainda escassos, tem contribuído para o melhor entendimento das bases genéticas que envolvem a HAR. Desta forma, estudos futuros nesta área são promissores e devem sem incentivados, pois podem auxiliar na abordagem terapêutica dos indivíduos resistentes.

1.2.7.1 Polimorfismos genéticos da adiponectina

O gene que codificam a adiponectina podem apresentar SNPs clinicamente relevantes para a resistência anti-hipertensiva. O gene codificador da adiponectina, *ADIPOQ*, está localizado no cromossomo 3q27, e representa o maior lócus gênico associado a síndrome metabólica (142) e DM2 (170). O gene *ADIPOQ* apresentou variações genéticas que estão associadas a alteração nas concentrações de adiponectina (171-173). Entre os polimorfismos clinicamente relevantes e amplamente estudados na literatura estão os SNPs -11377C>G (rs266729) e o +276G>T (rs1501299).

O -11377C>G foi correlacionado com baixos níveis de adiponectina circulante em indivíduos com menos de 60 anos (174). O alelo G foi associado a menores níveis de adiponectina e à maior chance de ocorrência de HA após ajuste de covariáveis (175). O -11377C>G também foi associado com doença cardiovascular (174); IM (176) e doença arterial coronariana (177, 178).

O polimorfismo +276G>T do gene *ADIPOQ* associou a presença do alelo G com a presença de HA e síndrome metabólica (179). Ainda, o alelo T foi associado a maior concentração sérica de adiponectina em pacientes com DM 2 (180). Em um estudo prospectivo com homens diabéticos o genótipo TT foi associado a baixo risco de doença cardiovascular e a maiores concentrações de adiponectina (181). Ainda, este polimorfismo foi associado a redução de risco de IM e na presença de diabetes. Particularmente, o genótipo TT demonstrou ser protetor para doença coronariana (178), apresentando, aproximadamente, metade de risco frente a qualquer doença cardiovascular ou associado ao diabetes, em comparação com portadores do alelo G (182).

É desconhecida, entretanto, a associação dos polimorfismos genéticos no gene *ADIPOQ* com os níveis de adiponectina, assim como, com as principais características que estão frequentemente associadas à falta de controle da PA. Também é desconhecida se os subgrupos hipertensos resistentes controlados e não controlados apresentam diferentes variantes genéticas, que poderiam explicar o distinto perfil fenotípico desses subgrupos.

2 JUSTIFICATIVA DO ESTUDO

A adiponectina plasmática tem sido investigada extensivamente em doenças cardiovasculares, resistência insulínica/diabetes *mellitus* tipo 2 e obesidade. Entretanto, pouco se conhece sobre a relação entre essa adipocitocina e a resistência ao tratamento farmacológico em pacientes hipertensos. Além disso, é desconhecida a influência de variações genéticas, como os SNPs no gene que codifica a adiponectina *ADIPOQ*, sobre os níveis de adiponectina em pacientes com hipertensão resistente.

A identificação de possíveis associações entre os polimorfismos genéticos e os níveis de adiponectina plasmática, e também algumas características clínicas frequentemente encontradas em pacientes com HAR, deverá contribuir para o melhor entendimento dos mecanismos fisiopatológicos relacionados à resistência farmacológica.

Os mesmos aspectos serão estudados nos subgrupos HARNC e HARC para estabelecermos se os mesmos apresentam diferenças genotípicas que potencialmente expliquem os achados distintos encontrados por nosso grupo em trabalhos anteriores, bem como, a própria natureza da resistência pressórica a fármacos.

3 OBJETIVOS

1. Avaliar a associação dos dois SNPs amplamente estudados, rs266729 e rs1501299, com os níveis plasmáticos de adiponectina total em hipertensos resistentes e nos subgrupos HARC e HARNC;

2. Identificar possíveis associações dos dois SNPs com as características fenotípicas frequentemente associadas a dificuldade de controle da PA em hipertensos resistentes.

4 CAPÍTULO I

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PROF. DR. JULIO LICINIO

EDITOR

THE PHARMACOGENOMICS JOURNAL

Dear Professor Julio Licinio,

Please find enclosed our original manuscript "**Adiponectin polymorphisms -11377C/G and +276G/T in resistant hypertension**". The paper hereby submitted follows some important previous publications of our group in resistant hypertension and we would like to publish it in The Pharmacogenomics Journal due to its clinical relevance.

All authors have read and approved the submission of the manuscript; the manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language, except as an abstract.

All authors have declared no financial or other relationships that might lead to a conflict of interest.

Yours sincerely,

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Title: Adiponectin polymorphisms -11377C/G and +276G/T are associated with adiponectin levels in resistant hypertension

Short title: Adiponectin polymorphisms in RHTN

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ABSTRACT

Background: Resistant hypertension (RHTN) is a multifactorial and polygenic disease, frequently associated with obesity. Previous studies demonstrated that low adiponectin levels, a hormone produced by the adipose tissue, were associated with RHTN. Single-nucleotide polymorphisms (SNPs) -11377C/G (rs266729) and +276G/T (rs1501299) in adiponectin gene *(ADIPOQ)* were associated with risk of cardiovascular disease. This study evaluated the association between two SNPs -11377C/G and +276G/T and adiponectin plasma levels in RHTN subjects. **Methods and Results:** This study comprised 109 RHTN patients genotyped for both polymorphisms by allelic discrimination assay using fluorescent probes. Adiponectin levels were measured by ELISA. We compare clinical and laboratorial characteristics of CC homozygous *vs.* G-allele carriers for -11377C/G and GG homozygous *vs.* T-allele carriers for +276G/T. Office and ambulatory BP measurements were similar among genotypes subgroups in both SNPs as well as the markers of target damage. Adiponectin levels were significantly higher in CC compared to G-carrier for -11377C/G (CC = 7.0 (4.0-10.2) *vs*. G allele = 5.5 (2.5-7.9), p=0.04) and lower in GG compared to T-carrier for +276G/T (GG = 5.3 (2.3-7.7) *vs*. T allele = 7.1 (3.6-10.5), p=0.04). Adjusting for systolic ambulatory BP, body mass index, age, gender, race and presence of type 2 diabetes, multiple linear regression revealed that the minor alleles G (beta coefficient = -0.14, SE = 0.07, p = 0.03) and T (beta coefficient = 0.12, SE = 0.06, p = 0.04) were independent predictors of adiponectin levels. **Conclusion:** The -1377C/G and +276G/T in *ADIPOQ* were associated with adiponectin levels in RHTN subjects.

Keywords: Refractory hypertension; blood pressure control; obesity; adipokines; polymorphisms.

INTRODUCTION

Resistant hypertension (RHTN) is a multifactorial disease defined as uncontrolled blood pressure (BP) despite the use of ≥3 antihypertensive agents from different classes or controlled BP with the use of ≥4 agents[1]. The mechanisms of resistance to antihypertensive treatment are not fully elucidated, but have been associated to hyperactivity of renin-angiotensin-aldosterone and sympathetic systems, endothelial dysfunction[2, 3], arterial stiffness[4], left ventricular hypertrophy[5], microalbuminuria[6] and deregulation of inflammatory adipokines [7]. In this context, a relationship between obesity and RHTN has been recognized since deregulation of adipokines may be relevant as a complicating factor in the lack of BP control [8] .

Low circulating levels of adiponectin – an adipocyte-derived hormone – are found in hypertensive patients[9], supporting an aetiological role of adiponectin in hypertension. Hypoadiponectinaemia may predispose to hypertension via several mechanisms such as sympathetic activation, insulin resistance, increased circulating fatty acid levels via reduced fatty acid oxidation, vascular inflammation and impaired endothelium-dependent vasodilation[9-11].

ADIPOQ – the gene encoding adiponectin – is located at 3q27 chromosome region and has been linked to presence of single nucleotide polymorphisms (SNPs) that modulate circulating concentration of this adipokine[12, 13]. Moreover, *ADIPOQ* has been associated with several features of the metabolic syndrome[14], diabetes type 2 and cardiovascular disease[15]. Among the SNPs, rs266729 (-11377C/G) is located in the promoter region and the presence of minor allele has been associated with down-regulation of adiponectin[10] whereas rs1501299 (+276G/T), situated in the intron 2 region, have opposite effects on up-regulating adiponectin levels[16].

We hypothesize that genetic variants may affect the levels of adiponectin, which in turn may contribute to resistance of antihypertensive therapy. This study was designed to evaluate the impact of *ADIPOQ* rs266729 and rs1501299 polymorphisms on plasma adiponectin levels in resistant hypertensive subjects, as well as the associations between these SNPs and other clinical features frequently associated with RHTN.

METHODS

Study Population

A cross-sectional study was conducted with 109 subjects diagnosed for "true" RHTN from the Outpatient Clinic specialized in Resistant

Hypertension of the University of Campinas (Campinas, Brazil). Resistant hypertension (RHTN) was defined according to *American Heart Association Statement* as blood pressure (BP) levels that remain above goal (≥140/90 mmHg) in spite of the concurrent use of three or more antihypertensive drugs of different classes. Ideally, one of the agents should be a diuretic, and all agents should be prescribed at optimal doses. Also the definition includes controlled BP using four or more antihypertensive medications [1].

All subjects were submitted to a 6-month period clinic follow-up for screening and exclusion of secondary causes of hypertension (pheochromocytoma, coarctation of the aorta, Conn's or Cushing's syndrome, renal artery stenosis). Patients with pseudoresistance hypertension, including lack of BP control secondary to poor medication adherence[17] as well as patients with white coat hypertension were properly identified and excluded by pill counts and ambulatory BP monitoring (ABPM), respectively. Inclusion criteria were subjects > 35 years. We excluded patients with symptomatic ischemic heart disease, impaired renal function, liver disease and history of stroke, myocardial infarction and peripheral vascular diseases.

We calculated a minimum sample size of 50 RHTN subjects per subgroup (CC *vs.* G-allele carriers for -11377C/G and GG *vs.* T-allele carriers for +276G/T) to detect a difference of mean at least 2.5 µg/mL (standard deviation of 5.0 µg/mL, power of 75%) on adiponectin levels – our primary outcome – among both studied polymorphisms.

This study was approved by the Research Ethics Committee of the Faculty of Medical Sciences, University of Campinas (Campinas, Brazil), and all participants were informed about the investigative nature of this study and gave written informed consent form before enrolling in the study (approval n. 222/2011).

Blood Pressure measurements

Office systolic BP (SBP) and diastolic BP (DBP) were evaluated at approximately 8:00 a.m in the right arm using a validated digital sphygmomanometer (HEM-907XL, OMRON Healthcare Inc., Bannockburn, IL, USA) in the sitting position after a 10-minute rest. Measurements were assessed by a trained health professional three consecutive times with a 3-minute interval between measurements.

Twenty-four hour ABPM was taken using automatic oscillometric monitor (Spacelabs 90207, Spacelabs Inc, Redmon, WA). The patients were instructed to keep normal daily activities and to

take notes of the 24 hour-time period activities in a personal diary.

Echocardiography

The left ventricular (LV) dimensions were measured using two-dimensional targeted M-mode echocardiography according to the American Society of Echocardiography (ASE) recommendations [18]. The diastolic and systolic LV diameters, as well as the interventricular septal and LV posterior wall thicknesses at the end of diastole, were determined using the features of the QRS wave. The LV mass index (LVMI) was calculated by dividing the LV mass by the body surface area. Two blinded independent investigators evaluated the echocardiographic measurements using a cardio-vascular ultrasound machine (Siemens Acuson CV70, Munich, Bavaria, Germany) with a multi-frequency sector transducer (2-4 MHz). The intraobserver and interobserver coefficients of variation were less than 9.5% for the LVMI.

Pulse Wave Velocity Assessment

Pulse wave velocity (PWV) is a noninvasive and reproducible method to determine arterial stiffness measured by Sphygmocor System (Artcor, Sidney, Australia). With patients in a supine position we determined pulse waves transcutaneously using the right common carotid and femoral arteries. PWV measurement was calculated from the distance traveled by the pulse waves between the supra-sternal notch and the femoral recording site minus the distance from the supra-sternal notch to the carotid recording site, divided by the transit time (distance in meters/Δtime in seconds) [19]. Three consecutive readings were obtained and we used the average of PWV values in the analyses, when differences were not greater than 0.5m/s.

Biochemical Measurements

Blood samples for the measurement of aldosterone and adiponectin plasma levels were collected at 08:00 hours after overnight fasting with the patients in the seated position. Aldosterone was measured by radioimmunoassay (RIA) (Immunotech SAS, Marseille, France) and total adiponectin levels were measured using enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Inc., Minneapolis, USA), both according to the manufacturer's instructions. The inter-assay and intra-assay coefficients of variance for the adiponectin ELISA kit were less than 5.5%.

-11377C/G and +276G/T ADIPOQ Genotyping

Venous blood samples were collected in tubes containing EDTA after overnight fasting. Genomic DNA was extracted using the Illustra blood genomicPrep Mini Spin Kit (GE Healthcare,

Buckinghamshire, UK). *ADIPOQ* genotypes -11377C/G and +276G/T were determined using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). In brief, polymerase chain reaction (PCR) was conducted with 10 ng of DNA followed by the steps: (1) 10 minutes at 95°C; (2) 40 cycles of DNA denaturation at 90°C for 15 seconds; and (3) annealing/extension at 60°C for 1 minute. Fluorescence signals were detected using StepOnePlus Real Time PCR System (Applied Biosystems) and analyzed with manufacturer's software.

Statistical Analyses

Continuous variables were expressed as mean and standard deviation or median (1st, 3rd quartiles), according to data distribution, assessed by the Kolmogorov–Smirnov test. Clinical data of the genotype subgroups (CC *vs.* G allele carriers (rs266729) and GG *vs.* T allele carriers (rs1501299)) were compared using the Student's t-test or the Mann Whitney test. Categorical variables were presented in frequencies and/or percentages and compared by chi-square test. Multiple linear regression analyses were performed by SigmaPlot program version 12.5 (Systat Software, Inc., USA) for both studied polymorphisms to evaluate the effect of minor allele on log adiponectin levels, adjusted for age, gender, race, ambulatory systolic BP, body mass index and presence of type 2 diabetes. Hardy-Weinberg equilibrium was evaluated using chi-square test. Haplotypes were estimated by PHASE program version 2.1 [20] and analyzed using Kruskal-Wallis test followed by a Dunn's post-hoc test. The level of significance accepted was 0.05.

RESULTS

Table 1 shows alleles, genotypes and haplotypes frequencies among resistant hypertensive patients. For both polymorphisms, no deviation from Hardy-Weinberg equilibrium was found ($p>0.05$). We did not found statistical differences in genotype, allele and haplotype frequencies for the -11377C/G and +276G/T *ADIPOQ* polymorphism between controlled and uncontrolled RHTN subgroups.

General characteristics of resistant hypertensive subjects according to -11377C/G and +276G/T *ADIPOQ* genotypes are shown in table 2 and table 3, respectively. Genotype subgroups CC *vs.* G carriers demonstrated differences among gender, race, and aldosterone levels; however, no difference was observed analysing GG *vs.* T carriers subgroups. Indeed, the use of antihypertensive drugs was similar between genotypes studied.

We found reduced levels of adiponectin in G allele carriers compared to CC genotype for the -11377C/G *ADIPOQ* polymorphism (figure 1A). Among uncontrolled RHTN subgroup, the G allele carriers had lower levels of adiponectin compared to CC genotype (figure 1B), but no difference was found in the controlled RHTN subgroup. For the +276G/T *ADIPOQ* polymorphism we observed higher adiponectin levels in T allele carries than in subjects with GG genotype (figure 2A). Moreover, we observed that T allele carriers of the controlled RHTN subgroup showed increased adiponectin levels compared with GG genotype subjects; however, this was not found in uncontrolled resistant hypertensive subjects (figure 2B).

Multiple regression analyses indicated that minor allele for both studied polymorphisms was predictors of log-adiponectin levels in RHTN subjects (-11377C/G, table 4 and +276G/T, table 5). Additionally, in another regression model, the presence of TT genotype for +276G/T *ADIPOQ* polymorphism – in addition to BMI and age – was also predictor of log-adiponectin levels in resistant hypertensive patients, revealing an additive effect (beta coefficient=0.151, SE=0.06; p=0.02).

Finally, none of the haplotypes reached statistical significance. Adiponectin levels were not statistically different among haplotypes, although declining trend has been observed (CT: 8.1±5.8 *vs.* CG: 7.2±5.5 *vs*. GG: 6.4±4.7 *vs*. GT: 4.9±3.7 µg/mL, p>0.05). Additionally, regression analysis indicated that presence of CT haplotype was not considered predictor of log-adiponectin levels (beta-coefficient=0.08, SE=0.05; p>0.05).

DISCUSSION

Previous studies have suggested that adiponectin has an important role in pathophysiology of RHTN [8, 11], which justifies the search of its potential genetic markers near *ADIPOQ* that may be implicated in resistance to antihypertensive therapy. This current study showed the association between two of the most widely studied SNPs (-11377C/G and +276G/T) and adiponectin plasma levels in RHTN subjects. Also, those genetic variations seem to affect differently the circulating levels of adiponectin in controlled and uncontrolled subgroups of RHTN.

Studies have suggested that minor allele of -11377C/G SNP is associated with lower levels of adiponectin in hypertensive and type 2 diabetic subjects [10, 21]. Minor G allele was significantly associated with higher odds of hypertension and also those patients required greater number of anti-hypertensive medication to control BP [10]. The mechanism of adiponectin levels variation lacks complete explanation. The presence of the minor

allele G has shown to destroy the binding site of transcriptional stimulatory protein (SP1). Thus, the presence of allele G of SNP −11377C/G in the genome results in loss of SP1 binding [22] and consequently influence adiponectin gene regulation and expression. In addition, altering DNA-binding promoter activity leads to lower basal and inducible promoter *ADIPOQ* activity in mice 3T3-L1 adipocytes [23],.

Similarly with our findings, some studies have revealed association between the presence of minor allele of +276G/T SNP and increased adiponectin levels in cardiovascular diseases such as hypertension and coronary artery disease [14, 24]. Moreover, a reduction of coronary artery disease risk was observed with recessive genetic model (TT *vs.* GT+GG: Odds Ratio= 0.81, p=0.01) [24]. However, there are conflicting results, mainly due to differences in studied population and ethnicity among the studies [25, 26]. There is no data on functional study of this intronic SNP, although transcription enhancers have been reported in adiponectin gene introns [27]. On the other hand, it has been suggested that this SNP is in linkage disequilibrium (LD) with other genetic variations, such as *ADIPOQ* +45 T/G, not evaluated in our study, which might explain the up-regulation of adiponectin [28]. The +45T/G SNP may alter RNA splicing or stability and thus expression of adiponectin[29].

The two studied SNPs seem to influence adiponectin levels differently among controlled and uncontrolled subgroups of RHTN. Although we did not find differences in allele, genotype, neither in haplotype frequencies, those findings require special attention since recent studies raised evidences that some features are different among RHTN subgroups [30-32]. Therefore, those aspects may have important implications because negative findings in clinical studies may be due to the inclusion of both controlled and uncontrolled individuals, which can decrease the chance of obtaining accurate associations between polymorphisms and RHTN [33].

We found increased aldosterone levels in G allele carriers compared to CC genotype subgroup for -11377C/G SNP. It has been demonstrated that detection of aldosterone excess (an increased level of aldosterone without diagnosis of primary hyperaldosteronism) is clinically important for RHTN [34] once aldosterone may be responsible for: (i) the lack of BP control in RHTN and (ii) causing maladaptive changes associated with cardiovascular abnormalities [3]. Moreover, studies have indicated that mineralocorticoid receptor (MR) antagonism rises as a therapeutic strategy to

downregulate proinflammatory adipokines [35, 36], and particularly to increase the expression of adiponectin [37]. Recent findings have shown that higher aldosterone and lower adiponectin levels may play a role on mediating the resistance to antihypertensive treatment [8, 11]. Finally, both polymorphisms were not associated with the other clinical features frequently associated with RHTN such as left ventricular hypertrophy (assessed by echocardiography), arterial stiffness (pulse wave velocity) and microalbuminuria (early marker of renal damage). This absence of associations does not necessarily indicate a lack of effect of *ADIPOQ* polymorphisms. It is well known that hypertension comprehends heritable characteristics and originate from the interactions of multiple genes, environmental factors and behavior [38]. This might explain the lack of the aforementioned associations in our resistant hypertension-based study.

Estimation of haplotypes has provided greater statistical power to detect disease susceptibility if they are directly responsible for the phenotype variation; or if they are in much higher LD with the functional polymorphism than individual markers [39]. However, we did not observe significant differences between haplotypes subgroups formed by clustering the two polymorphisms assessed in this current study. In addition, studies have estimated that these two SNPs are in weak pairwise LD with one another [12, 40], which suggests that rs266729 and rs1501299 represent distinct association signals and thus, adiponectin levels seem to be influenced by both regulatory segments as an independent effect.

It should be mentioned some limitations. Although the sample size examined in the present work is smaller than those included in other large-scale studies, the power calculation suggests that our sample had sufficient power to detect the primary outcome – adiponectin levels – with genetic effects in RHTN. On the other hand, the lack of association in the analyses of controlled and uncontrolled subgroups may be attributed to low statistical power to identify true association. A study has demonstrated no significant sex interaction between genetic variants and adiponectin level or hypertension [10]. Although we found difference on gender frequency – also on race distribution – between genotype subgroups in -11377C/G SNP, those findings did not affect our results because we performed the multiple regression analysis including gender and race as potential confounding factors. Some studies have reported that antihypertensive drugs interfering with renin-angiotensin system (angiotensin-converting enzyme inhibitors and angiotensin

receptor blockers) can improve adiponectin profile in hypertensive individuals [41, 42]. MR blockade improves expression of adiponectin and reduces expression of proinflammatory factors reversing obesity-related changes [37]. Although that, those possible sources of interferences did not affect our findings since genotype subgroups have similar proportion of antihypertensive agents use. Furthermore, RHTN subjects cannot be assessed withdrawing the antihypertensive drugs due to ethical concerns. Finally, as we could not control dietary trends and physical activity levels, those conditions may have influenced adiponectin levels.

In conclusion, this study supported the significant role of *ADIPOQ* variations, -11377 C/G and +276G/T, on regulation of adiponectin levels in RHTN individuals, with potential impact to affect the controlled and uncontrolled subgroups differently. To our knowledge, as this is the first report of *ADIPOQ* polymorphisms in resistant hypertensive subjects, our findings require confirmation using a larger RHTN population.

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Disclosures: None.

REFERENCES

1. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, et al. Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Hypertension. 2008; 51:1403-1419.

2. Wang ZV and Scherer PE. Adiponectin, cardiovascular function, and hypertension. Hypertension. 2008; 51:8-14.

3. Sowers JR, Whaley-Connell A, and Epstein M. Narrative review: the emerging clinical implications of the role of aldosterone in the metabolic syndrome and resistant hypertension. Ann Intern Med. 2009; 150:776-783.

4. Sutton-Tyrrell K, Newman A, Simonsick EM, Havlik R, Pahor M, Lakatta E, et al. Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. Hypertension. 2001; 38:429-433.

5. Salles GF, Fiszman R, Cardoso CR, and Muxfeldt ES. Relation of left ventricular hypertrophy with systemic inflammation and endothelial damage in resistant hypertension. Hypertension. 2007; 50:723-728.

6. Salles GF, Cardoso CR, Pereira VS, Fiszman R, and Muxfeldt ES. Prognostic significance of a reduced glomerular filtration rate and interaction with microalbuminuria in resistant hypertension: a cohort study. J Hypertens. 2011; 29:2014-2023.

7. Kurukulasuriya LR, Stas S, Lastra G, Manrique C, and Sowers JR. Hypertension in obesity. Med Clin North Am. 2011; 95:903-917.

8. Sabbatini AR, Faria AP, Barbaro NR, Gordo WM, Modolo RG, Pinho C, et al. Deregulation of adipokines related to target organ damage on resistant hypertension. J Hum Hypertens. 2013.

9. Chow WS, Cheung BM, Tso AW, Xu A, Wat NM, Fong CH, et al. Hypoadiponectinemia as a predictor for the development of hypertension: a 5-year prospective study. Hypertension. 2007; 49:1455-1461.

10. Ong KL, Li M, Tso AW, Xu A, Cherny SS, Sham PC, et al. Association of genetic variants in the adiponectin gene with adiponectin level and hypertension in Hong Kong Chinese. Eur J Endocrinol. 2010; 163:251-257.

11. de Faria AP, Demacq C, Figueiredo VN, Moraes CH, Santos RC, Sabbatini AR, et al. Hypoadiponectinemia and aldosterone excess are associated with lack of blood pressure control in subjects with resistant hypertension. Hypertens Res. 2013; 36:1067-1072.

12. Hivert MF, Manning AK, McAteer JB, Florez JC, Dupuis J, Fox CS, et al. Common variants in the adiponectin gene (ADIPOQ) associated with plasma adiponectin levels, type 2 diabetes, and diabetes-related quantitative traits: the Framingham Offspring Study. Diabetes. 2008; 57:3353-3359.

13. Heid IM, Wagner SA, Gohlke H, Iglseder B, Mueller JC, Cip P, et al. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes. 2006; 55:375-384.

14. Leu HB, Chung CM, Lin SJ, Jong YS, Pan WH, and Chen JW. Adiponectin gene polymorphism is selectively associated with the concomitant presence of metabolic syndrome and essential hypertension. PLoS One. 2011; 6:e19999.

15. Sun K, Li Y, Wei C, Tong Y, Zheng H, and Guo Y. Recessive protective effect of ADIPOQ rs1501299 on cardiovascular diseases with type 2 diabetes: a meta-analysis. Mol Cell Endocrinol. 2012; 349:162-169.

16. Chiodini BD, Specchia C, Gori F, Barlera S, D'Orazio A, Pietri S, et al. Adiponectin gene polymorphisms and their effect on the risk of myocardial infarction and type 2 diabetes: an association study in an Italian population. Ther Adv Cardiovasc Dis. 2010; 4:223-230.

17. de Souza WA, Sabha M, de Faveri Favero F, Bergsten-Mendes G, Yugar-Toledo JC, and Moreno H. Intensive monitoring of adherence to treatment helps to identify "true" resistant hypertension. J Clin Hypertens (Greenwich). 2009; 11:183-191.

18. Sahn DJ, DeMaria A, Kisslo J, and Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation. 1978; 58:1072-1083.

19. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006; 27:2588-2605.

20. Stephens M, Smith NJ, and Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 2001; 68:978-989.

21. Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, et al. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet. 2002; 11:2607-2614.

22. Zhang D, Ma J, Brismar K, Efendic S, and Gu HF. A single nucleotide polymorphism alters the sequence of SP1 binding site in the adiponectin promoter region and is associated with diabetic nephropathy among type 1 diabetic patients in the Genetics of Kidneys in Diabetes Study. J Diabetes Complications. 2009; 23:265-272.

23. Laumen H, Saningong AD, Heid IM, Hess J, Herder C, Claussnitzer M, et al. Functional characterization of promoter variants of the adiponectin gene complemented by epidemiological data. Diabetes. 2009; 58:984-991.

24. Yang Y, Zhang F, Ding R, Wang Y, Lei H, and Hu D. Association of ADIPOQ gene polymorphisms and coronary artery disease risk: a meta-analysis based on 12 465 subjects. Thromb Res. 2012; 130:58-64.

25. Xi B, He D, Wang Q, Xue J, Liu M, and Li J. Common polymorphisms (rs2241766 and rs1501299) in the ADIPOQ gene are not associated with hypertension susceptibility among the Chinese. Mol Biol Rep. 2012; 39:8771-8775.

26. Zhao T and Zhao J. Genetic effects of adiponectin on blood lipids and blood pressure. Clin Endocrinol (Oxf). 2011; 74:214-222.

27. Qiao L, Maclean PS, Schaack J, Orlicky DJ, Darimont C, Pagliassotti M, et al. C/EBPalpha regulates human adiponectin gene transcription through an intronic enhancer. Diabetes. 2005; 54:1744-1754.

28. Melistas L, Mantzoros CS, Kontogianni M, Antonopoulou S, Ordovas JM, and Yiannakouris N. Association of the +45T>G and +276G>T polymorphisms in the adiponectin gene with insulin

resistance in nondiabetic Greek women. Eur J Endocrinol. 2009; 161:845-852.

29. Yang WS, Tsou PL, Lee WJ, Tseng DL, Chen CL, Peng CC, et al. Allele-specific differential expression of a common adiponectin gene polymorphism related to obesity. J Mol Med (Berl). 2003; 81:428-434.

30. Martins LC, Figueiredo VN, Quinaglia T, Boer-Martins L, Yugar-Toledo JC, Martin JF, et al. Characteristics of resistant hypertension: ageing, body mass index, hyperaldosteronism, cardiac hypertrophy and vascular stiffness. J Hum Hypertens. 2011; 25:532-538.

31. Quinaglia T, Martins LC, Figueiredo VN, Santos RC, Yugar-Toledo JC, Martin JF, et al. Non-dipping pattern relates to endothelial dysfunction in patients with uncontrolled resistant hypertension. J Hum Hypertens. 2011; 25:656-664.

32. de Haro Moraes C, Figueiredo VN, de Faria AP, Barbaro NR, Sabbatini AR, Quinaglia T, et al. High-circulating leptin levels are associated with increased blood pressure in uncontrolled resistant hypertension. J Hum Hypertens. 2013; 27:225-230.

33. Faria AP, Sabbatini AR, Coca A, and Moreno H. Phenotypic characteristics of resistant hypertension in the Brazilian population. Arq Bras Cardiol. 2013; 100:579-582.

34. Gaddam KK, Nishizaka MK, Pratt-Ubunama MN, Pimenta E, Aban I, Oparil S, et al. Characterization of resistant hypertension: association between resistant hypertension, aldosterone, and persistent intravascular volume expansion. Arch Intern Med. 2008; 168:1159-1164.

35. da Silva AA, do Carmo J, Dubinion J, and Hall JE. The role of the sympathetic nervous system in obesity-related hypertension. Curr Hypertens Rep. 2009; 11:206-211.

36. Fang C, Lei J, Zhou SX, Zhang YL, Yuan GY, and Wang JF. Association of higher resistin levels with inflammatory activation and endothelial dysfunction in patients with essential hypertension. Chin Med J (Engl). 2013; 126:646-649.

37. Guo C, Ricchiuti V, Lian BQ, Yao TM, Coutinho P, Romero JR, et al. Mineralocorticoid receptor blockade reverses obesity-related changes in expression of adiponectin, peroxisome proliferator-activated receptor-gamma, and proinflammatory adipokines. Circulation. 2008; 117:2253-2261.

38. Kunes J and Zicha J. The interaction of genetic and environmental factors in the etiology of hypertension. Physiol Res. 2009; 58 Suppl 2:S33-41.

39. Bader JS. The relative power of SNPs and haplotype as genetic markers for association tests. Pharmacogenomics. 2001; 2:11-24.

40. Pollin TI, Tanner K, O'Connell J R, Ott SH, Damcott CM, Shuldiner AR, et al. Linkage of plasma adiponectin levels to 3q27 explained by association with variation in the APM1 gene. Diabetes. 2005; 54:268-274.

41. Fontana V, de Faria AP, Oliveira-Paula GH, Silva PS, Biagi C, Tanus-Santos JE, et al. Effects of Angiotensin-Converting Enzyme Inhibition on Leptin and Adiponectin Levels in Essential Hypertension. Basic Clin Pharmacol Toxicol. 2014.

42. Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, et al. Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. Hypertension. 2003; 42:76-81.

TABLES

Table 1. Allele, genotype and haplotype frequencies for -11377C/G (rs266729) and +276G/T (rs1501299) *ADIPOQ* polymorphisms in total RHTN subjects and RHTN subgroups

	RHTN (n=109)	**CRHTN (n =37)**	**UCRHTN (n =72)**	**p-value**
Allele frequencies				
ADIPOQ -11377 (C/G)	0.71/0.29	0.65/0.35	0.74/0.26	0.38
ADIPOQ +276 (G/T)	0.67/0.33	0.69/0.31	0.65/0.35	0.67
Genotype frequencies				
ADIPOQ-11377 (CC/CG/GG)	0.51/0.39/0.10	0.40/0.49/0.11	0.57/0.33/0.10	0.24
ADIPOQ+276 (GG/GT/TT)	0.45/0.43/0.12	0.46/0.46/0.08	0.44/0.42/0.14	0.67
Haplotype frequencies				
ADIPOQ -11377, +276 (CT/CG/GG/GT)	0.30/0.41/0.25/0.04 (N=218)	0.28/0.37/0.32/0.03 (N=74)	0.31/0.43/0.22/0.04 (N=144)	0.41

Table 2. General characteristics of resistant hypertensive subjects according to genotype subgroups (CC vs. CG/GG) for *ADIPOQ* -11377C/G (rs266729)

	CC (n=56)	G allele carriers (n=53)	p-value
Clinical data			
Age (years)	59±10	60±11	0.46
Female gender, n (%)	33 (59)	42 (79)	0.02
White race, n (%)	23 (41)	34 (64)	0.02
BMI (kg/m²)	31±6	32±6	0.84
Blood Pressure, mmHg			
office SBP	145 (134-158)	145 (128-160)	0.63
office DBP	83 (74-92)	82 (76-90)	0.62
office PP	60 (55-72)	59 (49-69)	0.44
ABPM SBP	132±18	131±20	0.80
ABPM DBP	75 (69-90)	75 (69-82)	0.58
ABPM PP	52 (46-56)	52 (44-61)	0.76
LVMI (g/m^2)	115±32	114±35	0.91
PWV (m/s)	10.0±2.3	9.6±2.2	0.37
Biochemical data			
Microalbuminuria (mg/g)	35±57	25±32	0.17
HbA1C (%)	6.4 (5.8-8.1)	6.4 (6.0-7.5)	0.61
Glucose (mg/mL)	100 (88-133)	110 (91-128)	0.70
Urea (mg/mL)	38±16	37±15	0.71
Creatinine (mg/mL)	0.9 (0.8-1.3)	0.9 (0.7-1.1)	0.21
Creat Clear (mL/min/1,73m^2)	83±32	85±31	0.82
Cholesterol (mg/mL)	200±46	199±39	0.93
HDL-c (mg/mL)	44 (39-52)	45 (39-55)	0.60
LDL-c (mg/mL)	120±39	117±33	0.70
Triglycerides (mg/mL)	141 (95-185)	149 (104-217)	0.63
Aldosterone (pg/mL)	53.3 (28.0-113.0)	87.7 (56.8-176.3)	0.03
Renin (pg/mL)	26 (11-48)	33 (17-42)	0.64
AntiHT drugs			
Number of classes	4.3±1	4.5±1	0.42
Diuretics, n (%)	56 (100)	53 (100)	1
Spironolactone, n (%)	22 (39)	25 (47)	0.44
ACEIs, n (%)	19 (34)	23 (43)	0.33
ARBs, n (%)	29 (51)	27 (51)	1

CCBs, n (%)	44 (79)	46 (87)	0.32
Beta-blockers, n (%)	33 (59)	38 (72)	0.23
Others, n (%)	10 (18)	06 (11)	0.42

Values are expressed as mean ± standard deviation or median (1st, 3rd quartiles), according to data distribution. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; ABPM: ambulatory blood pressure monitoring; LVMI; left ventricular mass index; PWV: pulse wave velocity; HbA1C: glycated hemoglobin; Creat Clear: creatinine clearance; LDL and HDL: low- and high-density lipoproteins, respectively; antiHT: antihypertensive drugs; ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CCBs: calcium channel blockers.

Table 3. General characteristics of resistant hypertensive subjects according to genotype subgroups (GG vs. GT/TT) for *ADIPOQ* +276G/T (rs1501299)

	GG (n=49)	T allele cariers (n=60)	p-value
Clinical data			
Age (years)	59±11	60±10	0.64
Female gender, n (%)	32 (65%)	43 (72%)	0.54
White race, n (%)	28 (57%)	29 (48%)	0.44
BMI (kg/m²)	31±6	32±6	0.58
Blood Pressure, mmHg			
office SBP	147±23	147±22	0,99
office DBP	82 (74-92)	83 (76-91)	0,76
office PP	60 (52-69)	60 (50-73)	0,88
ABPM SBP	131 (119-145)	125 (117-143)	0,28
ABPM DBP	77 (69-88)	74 (67-83)	0,39
ABPM PP	54 (46-60)	50 (45-58)	0,37
LVMI (g/m^2)	109 (88-136)	109 (90-136)	0.85
PWV (m/s)	10.0±2.3	9.5±2.6	0.33
Biochemical data			
Microalbuminuria (mg/g)	8.9 (6-31)	9.3 (6-34)	0.81
HbA1C (%)	6.4 (5.9-8.1)	6.5 (5.8-7.7)	0.59
Glucose (mg/mL)	108 (91-138)	106 (90-128)	0.57
Urea (mg/mL)	40±16	36±15	0.26
Creatinine (mg/mL)	1.0 (0.8-1.3)	0.9 (0.7-1.1)	0.07
Creat Clear (mL/min/1,73m^2)	82±33	90±39	0.37
Cholesterol (mg/mL)	203±48	197±38	0.52
HDL-c (mg/mL)	44 (37-52)	46 (40-57)	0.14
LDL-c (mg/mL)	123±41	115±32	0.32
Triglycerides (mg/mL)	150 (116-191)	133 (78-226)	0.21
Aldosterone (pg/mL)	72 (38-127)	89 (38-155)	0.81
Renin (pg/mL)	35 (13-44)	31 (13-73)	0.82
AntiHT drugs			
Number of classes	4.4±1	4.5±1	0.44
Diuretics, n (%)	49 (100)	60 (100)	1
Spironolactone, n (%)	21 (43)	25 (42)	1
ACEIs, n (%)	16 (33)	26 (43)	0.32
ARBs, n (%)	24 (49)	32 (53)	0.70
CCBs, n (%)	40 (82)	50 (83)	1

Beta-blockers, n (%)	30 (61)	41 (68)	0.54
Others, n (%)	08 (16)	08 (13)	0.79

Values are expressed as mean ± standard deviation or median (1st, 3rd quartiles), according to data distribution. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; ABPM: ambulatory blood pressure monitoring; LVMI; left ventricular mass index; PWV: pulse wave velocity; HbA1C: glycated hemoglobin; Creat Clear: creatinine clearance; LDL and HDL: low- and high-density lipoproteins, respectively; antiHT: antihypertensive drugs; ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CCBs: calcium channel blockers.

Tabela 4. Multiple linear regression for log-transformed adiponectin levels in total RHTN

	β	**SE**	**p-value**
Allele G carriers (rs266729)	-0.14	0.07	0.03
ABPM SBP (mmHg)	-0.003	0.002	0.13
BMI (kg/m2)	-0.01	0.006	0.04
Age (anos)	0.01	0.003	0.02
Gender	-0.12	0.07	0.08
Race	-0.006	0.07	0.93
Type 2 Diabetes	-0.03	0.07	0.64

BMI: body mass index; SBP: systolic blood pressure; ABPM: ambulatory blood pressure monitoring.

Tabela 5. Multiple linear regression for log-transformed adiponectin levels in total RHTN

	β	**SE**	**p-value**
Allele T carriers (rs1501299)	0.12	0.06	0.04
ABPM SBP (mmHg)	-0.002	0.002	0.21
BMI (kg/m2)	-0.01	0.006	0.01
Age (anos)	0.01	0.003	0.02
Gender	-0.04	0.06	0.53
Race	0.03	0.06	0.64
Type 2 Diabetes	-0.06	0.06	0.36

BMI: body mass index; SBP: systolic blood pressure; ABPM: ambulatory blood pressure monitoring.

FIGURES

Figure 1. Plasma levels of adiponectin according to genotype subgroups (CC vs. CG/GG) for *ADIPOQ* -11377C/G (rs266729) in total RHTN subjects (7.0 (4.0-10.2) *vs.* 5.5 (2.5-7.9) µg/mL, respectively, $p<0.05$; figure 1A) and subgroups CRHTN (5.6 (2.4-10.1) *vs.* 6.2 (2.9-9.6) µg/mL, respectively, $p>0.05$) and UCRHTN (7.1 (4.4-10.7) *vs.* 5.1 (2.4-8.4) µg/mL, respectively, $p<0.05$), figure 1B.

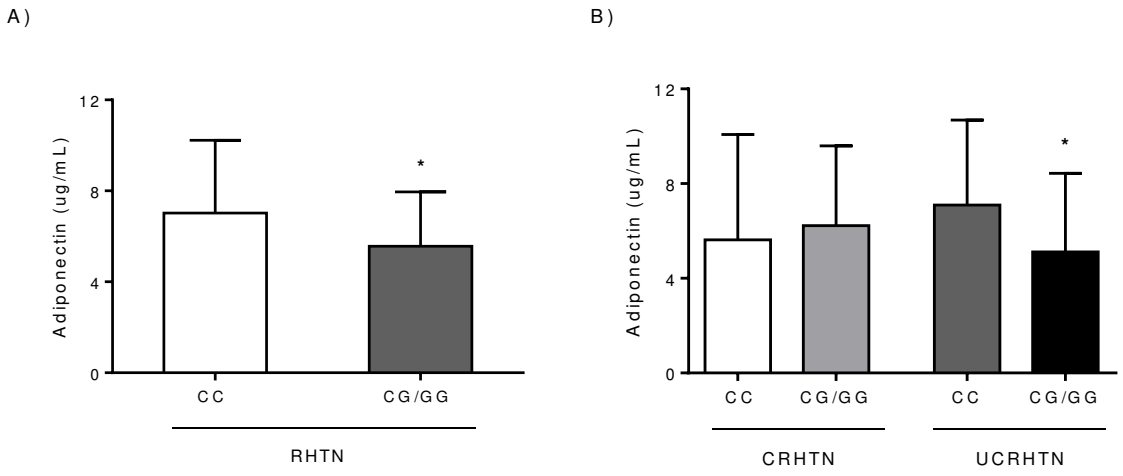
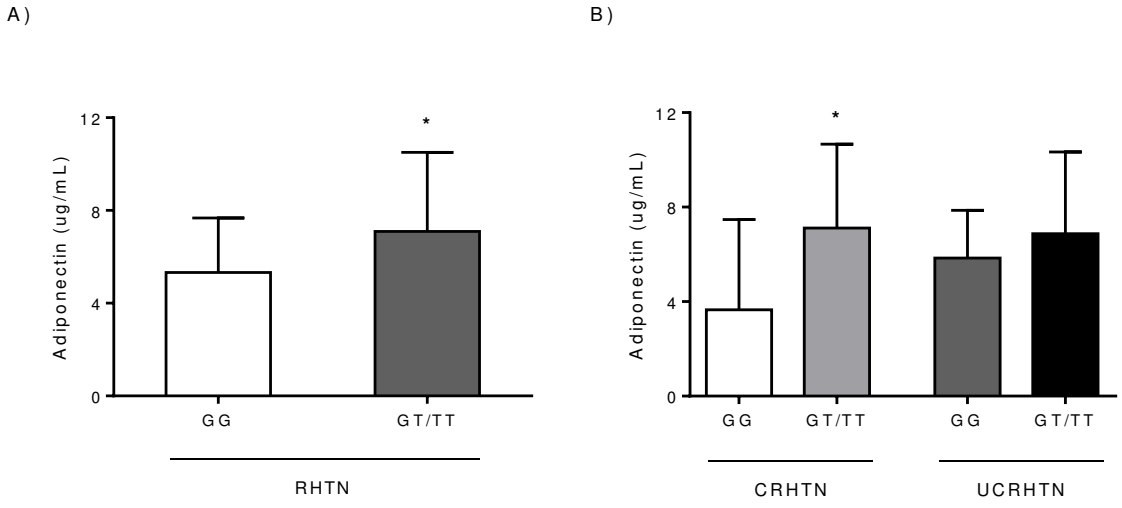


Figure 2. Plasma levels of adiponectin according to genotype subgroups (GG vs. GT/TT) for *ADIPOQ* +276G/T (rs1501299) in total RHTN subjects (5.3 (2.3-7.7) *vs.* 7.1 (3.6-10.5) µg/mL, p=0.04; figure 1A) and subgroups CRHTN (3.7 (1.7-7.5) *vs.* 7.2 (3.8-10.7) µg/mL, respectively, p<0.05) and UCRHTN (5.8 (3.5-7.9) *vs*. 6.9 (2.7-10.3) µg/mL, respectively, p>0.05), figure 2B.



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ORIGINAL ARTICLE

Hypoadiponectinemia and aldosterone excess are associated with lack of blood pressure control in subjects with resistant hypertension

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Obesity, arterial stiffness and high aldosterone levels can interact to cause resistant hypertension (RHTN). Lower adiponectin (APN) levels may be significantly associated with hypertension. However, the importance of hypoadiponectinemia as a complicating factor in the lack of blood pressure (BP) control in individuals with RHTN has not been demonstrated. Ninety-six RHTN patients were classified into uncontrolled (UCRHTN, $n=44$) and controlled (CRHTN, $n=52$) subgroups. Their APN and aldosterone levels, office and ambulatory BP (ABPM) measurements, endothelium-dependent brachial artery responses (flow-mediated dilation (FMD)), left ventricular mass index (LVMI) and pulse wave velocity (PWV) were evaluated. The UCRHTN subgroup had increased aldosterone levels, as well as higher LVMI and PWV. In addition, lower APN levels and impaired FMD response were found in this subgroup. The brachial and ABPM pulse pressures were inversely associated with the APN levels ($r=-0.45$, $P=0.002$; $r=-0.33$, $P=0.03$, respectively), as were the aldosterone levels and the PWV ($r=-0.38$, $P=0.01$; $r=-0.36$, $P=0.02$, respectively) in UCRHTN patients. The PWV was only significantly influenced by the APN level in the UCRHTN subgroup in the multivariate regression analysis. None of the correlations mentioned above were observed in the CRHTN subgroup. Hypoadiponectinemia and high aldosterone levels may therefore be implicated in resistance to antihypertensive therapy related to arterial stiffness.
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Keywords: adiponectin; aldosterone; arterial stiffness; obesity; resistant hypertension

INTRODUCTION

Resistant hypertension (RHTN) is a condition in which the blood pressure (BP) remains above the target level (140/90 mm Hg) despite the concurrent use of three or more antihypertensive drugs of different classes. Ideally, one of these agents should be a diuretic, and all agents should be prescribed at optimal doses.[1] This revised definition includes a subgroup of resistant hypertensive patients whose BP is controlled using four or more antihypertensive medications; these patients are referred to as having controlled RHTN (CRHTN).[1] This designation may be useful for better categorizing RHTN with regard to etiology and prognosis, but we recently demonstrated that some important clinical and mechanistic findings differ between CRHTN and uncontrolled RHTN (UCRHTN) subjects.[2,3] In addition, emerging data suggest that obesity, arterial stiffness, cardiac hypertrophy, high plasma aldosterone levels and endothelial dysfunction are not only associated with RHTN but may also interact to have an important role in causing RHTN.[2,4] Moreover, vascular stiffness, as assessed by the pulse wave velocity (PWV), has been shown to be correlated with RHTN.[5]

Putative mechanisms of obesity-related hypertension include increased sympathetic activity, hyperstimulation of the renin–angiotensin–aldosterone system, impaired endothelial function and reduced urinary sodium excretion.[6] These mechanisms underlie the hypothesis that obesity and RHTN are linked to excess circulating aldosterone, which has a significant role in the pathogenesis of metabolic syndrome and RHTN.[7] A growing body of evidence indicates that cardiovascular and renal abnormalities associated with insulin resistance are mediated in part by aldosterone, acting on the mineralocorticoid receptor. Furthermore, the detection of hyperaldosteronism is important not only to treat cardiometabolic disease but also to indicate the state of 'aldosterone excess,' which is an increased level of aldosterone without hyperaldosteronism. Aldosterone excess has detrimental metabolic effects that contribute to metabolic syndrome and endothelial dysfunction, as well as to the

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development of RHTN, cardiovascular disease and chronic kidney disease.[7]

Adipose tissue is currently considered to be a large endocrine gland that participates in the regulation of diverse biological functions.[8] The communication between adipose tissue and other biological systems is accomplished by the expression of a large number of bioactive mediators that are collectively called adipokines.[8] Adiponectin (APN) is one of the most important adipokines. Previous evidence suggests that lower plasma APN concentrations are significantly associated with hypertension;[9–11] however, the importance of hypoadiponectinemia as a complicating factor for the lack of BP control has not been evaluated in individuals with RHTN.

This study sought to determine the plasma APN levels and its association with the lack of BP control in RHTN patients. The associations between the APN levels and other clinical features frequently associated with this condition were also evaluated.

METHODS

This cross-sectional study was approved by the Research Ethics Committee of the Faculty of Medical Sciences, University of Campinas (Campinas, Brazil), and all participants gave written informed consent form before enrolling in the study (approval no. 222/2011).

Patient population

Ninety-six RHTN subjects regularly evaluated at the Outpatient Resistant Hypertension Clinic of the University of Campinas (Campinas, Brazil) who complied with the pharmacological treatment regimen for RHTN were recruited to participate in this observational study. Patients were classified in two groups—the UCRHTN ($n=44$) and CRHTN ($n=52$) groups—and were matched for age, sex and body mass index (BMI). The diagnosis of RHTN requires a good office BP measurement technique and ambulatory BP monitoring (ABPM) to confirm persistently elevated BP levels.[1] White coat hypertension was excluded by ABPM.[1] Patients with pseudoresistance, including a lack of BP control secondary to poor medication adherence, as well as patients with secondary forms of hypertension, were properly identified and excluded.[12] All individuals were regularly assessed during the first 6 months for drug therapy optimization. ABPM was used not only to exclude pseudoresistance to antihypertensive treatment but also as an auxiliary method to characterize UCRHTN and CRHTN patients.

The exclusion criteria were the presence of type 1 and type 2 diabetes, acute or moderate-severe renal or liver dysfunction, noncompliance with the pharmacological treatment regimen, obesity (BMI $\geqslant 30\,kg\,m^{-2}$), heart failure (ejection fraction $<50\%$), valvular heart disease, cardiomyopathies, primary hyperaldosteronism (aldosterone–plasma renin activity ratio (ARR) $>20\,ng\,dl^{-1}$ per $ng\,ml^{-1}\,h^{-1}$), sleep apnea (classified as 'high risk' by the Berlin sleep questionnaire), cardiac arrhythmias, aortic disease (Marfan's syndrome, coarctation of the aorta, aneurysms or aortic surgery), a clinical history of coronary artery disease or proven coronary artery disease by coronary angiography or noninvasive tests, previous stroke, peripheral vascular disease, familial hyperlipidemia, pregnancy or oral contraceptive use, connective tissue disorders, neurological problems, malignancies, psychiatric diseases, smoking, alcohol use and drug abuse.

Nonpharmacological therapies were optimized, including a salt-restricted diet, which was monitored by measuring urinary sodium excretion (<100 mEq per 24 h).

Office BP measurements

BP was assessed at ~0800 hours. Each subject's BP (SBP—systolic BP; DBP- diastolic BP and PP—pulse pressure) was measured three times using a digital sphygmomanometer (Omron HEM-711DLX, OMRON Healthcare, Bannockburn, IL, USA) on the right upper arm in the sitting position after a 10-minute rest.

Ambulatory BP monitoring

The 24-hour ABPM was taken using a Spacelabs 90217 ambulatory BP monitor (Spacelabs, Redmond, WA, USA).[13] Patients were instructed to perform normal daily activities and to note their sleep period in a personal diary.

Echocardiography

Measurements of the left ventricular (LV) dimensions were performed according to the American Society of Echocardiography recommendations[14] using two-dimensional targeted M-mode echocardiography. The diastolic LV and systolic LV diameters, as well as the interventricular septal (IVS) and LV posterior wall thicknesses at the end of diastole, were measured using the features of the QRS wave assessed by electrocardiography. The LV mass index (LVMI) was calculated by dividing the LV mass by the body surface area. The echocardiographic measurements were evaluated by two-blinded independent investigators using a cardiovascular ultrasound machine (Siemens Acuson CV70, Munich, Bavaria, Germany) with a multifrequency sector transducer (2–4 MHz). The intraobserver and interobserver Ok! coefficients of variation were $<5.5\%$ for the diastolic LV diameter, systolic LV diameter, interventricular septal and LV posterior wall thicknesses, and $<9.5\%$ for the LVMI.

Endothelial function

Brachial artery dilation was measured using a linear vascular transducer (7–12 MHz, Toshiba Powervision 6000, Tokyo, Japan) coupled with computer-assisted analysis software and automated brachial analyzer software (Brachial Analyzer, Medical Imaging Applications, Coralville, IA, USA). The endothelium-dependent brachial artery responses (flow-mediated dilation, FMD) and endothelium-independent brachial artery responses (glyceryl-trinitrate mediated) were determined in accordance with current guidelines.[15] All studies were initiated at 0800 hours after overnight fasting, and the subjects were placed in a supine position in a quiet, air-conditioned room (22–24 °C). Changes in the brachial artery diameter in both the endothelium-dependent and endothelium-independent analyses were expressed as a percentage change relative to the vessel diameter immediately before cuff inflation and drug administration, respectively. The vascular function was assessed by only one experienced blinded examiner, and there was no significant intraobserver measurement variability.

Aortic PWV measurement

The aortic PWV was measured using the velocity method with the patients in a supine position using a previously validated Complior SP system and software (Artech-Medical, Paris, France).[16] Waveforms were obtained transcutaneously using the right common carotid and femoral arteries simultaneously during a minimum period of 10–15 s. The time delay (t) between the two waveforms was measured, and the distance (D) covered by the waves was measured directly between the femoral recording site and the suprasternal notch minus the distance to the carotid recording site. The PWV was calculated as D (m)/t (sec). Three consecutive readings were obtained, and the PWV is reported as the mean. The values were corrected to account for the mean arterial pressure.

Laboratory assessment

Baseline blood samples for the measurement of the plasma aldosterone concentration (PAC), plasma renin activity and plasma APN level were collected at 0800 hours after overnight fasting. During this time, the volunteers rested in a supine position for 8 h, followed by 1 h in an upright position in an air-conditioned room (22–24 °C). The PAC and total plasma APN levels were measured using enzyme-linked immunosorbent assays (ELISA; DRG International, NJ, USA; and Quantikine Human total adiponectin/Acrp30 Immunoassay DRP 300, R&D Systems, Minneapolis, MN, USA, respectively) according to the manufacturer's instructions. Plasma renin activity radioimmunoassay was performed using standard technique (Mayo Clinic Laboratories, Rochester, MN, USA). The inter-assay and intra-assay coefficients of variance were $<8.5\%$ for the PAC ELISA kit and $<5.5\%$ for the APN ELISA kit.

Statistical analysis

The data are expressed as the mean and and standard erron of the mean (s.e.m) or median (1st and 3rd quartiles). The normality of the distributions was assessed using the Kolmogorov–Smirnov test. Significant differences between the study groups were identified using Student's *t*-test or the Mann–Whitney test for independent samples and Fisher's test for categorical variables. Pearson's or Spearman's correlation test was used for the correlation analyses. Multiple regression analysis was used to predict the PWV from the age, aldosterone concentration and APN level, and to predict the FMD from the aldosterone and APN plasma level, with the aim of identifying the relative effects of these variables on the PWV and FMD. The level of significance accepted was 0.05.

RESULTS

Table 1 lists the general characteristics of the study groups. No significant differences were observed between the UCRHTN and CRHTN subgroups with respect to age, sex and BMI. The mean ages of patients were 57 ± 1.6 and 59 ± 1.5 years in the UCRHTN (26 female/18 male) and CRHTN (35 female/17 male) groups, respectively, and all patients were overweight ($25.0 \leqslant BMI \leqslant 29.9$). As expected, higher office and ABPM SBPs and DBPs, as well as PPs, were found in UCRHTN individuals. Although both subgroups had LV hypertrophy, the LVMI was higher in the UCRHTN patients than the CRHTN patients. In addition, the UCRHTN subgroup had higher aortic PWV and more severe impairment in the FMD test than the CRHTN subgroup. The NTG responses in the UCRHTN and CRHTN subgroups were similar.

The biochemical test results did not show differences between the UCRHTN and CRHTN subgroups except with respect to the PAC, ARR and APN levels (Table 2). The PAC and ARR levels were higher and the APN levels were lower in UCRHTN than in CRHTN patients.

UCRHTN subjects were taking a mean of 4.9 classes of antihypertensive drugs daily, and their drug distribution was diuretics (100%), spironolactone (31.8%), β-blockers (52.3%), angiotensin-converting enzyme inhibitors (27.3%), angiotensin receptor blockers (77.3%), calcium channel blockers (65.9%) and centrally acting antihypertensive drugs (11.4%). The CRHTN subgroup was taking a mean of 4.5 drugs daily, with the prescribed medications being diuretics (100%), spironolactone (23.1%), β-blockers (46.2%), angiotensin-converting enzyme inhibitors (32.7%), angiotensin receptor blockers (34.6%), calcium channel blockers (28.8%) and centrally acting antihypertensive drugs (3.8%).

Correlation analysis for the UCRHTN subjects indicated that the plasma APN levels were inversely associated with the aldosterone concentration and the PWV ($r = -0.38$, $P = 0.01$; and $r = -0.36$, $P = 0.02$, respectively) (Figure 1). Furthermore, the plasma APN levels were inversely correlated with the office (Figure 2a) and ABPM SBPs ($r = -0.33$, $P = 0.03$; and $r = -0.42$, $P = 0.01$, respectively), as well as the brachial (Figure 2b) and ABPM PPs ($r = -0.45$, $P = 0.002$; and $r = -0.33$, $P = 0.03$, respectively) in UCRHTN patients. However, the plasma APN levels were not associated with the office (Figure 2c) and ABPM DBPs, the LVMI, the FMD or the ARR in the same group. The aldosterone concentrations were correlated with the PWV ($r = 0.43$; $P = 0.003$) in uncontrolled patients but not in the controlled patients. Multivariate linear regression analysis showed that the PWV was only significantly influenced by the APN level in UCRHTN patients (Table 3). The APN and aldosterone levels did not influence the FMD test results in the same subgroup (data not shown). Finally, the plasma APN levels were not correlated with any parameter in CRHTN subjects, in contrast to the findings for the UCRHTN subgroup.

Table 1 General characteristics of the RHTN subgroups

	UCRHTN (n = 44)	*CRHTN (n = 52)*	*P-value*
Gender (F/M)	26/18	35/17	0.52
Age (years)	57 ± 1.6	59 ± 1.5	0.20
BMI (kg m^{-2})	27.9 ± 0.2	28.2 ± 0.1	0.40
Office SBP (mm Hg)	157.8 (143.1, 169.1)	132.9 (126.0, 137.4)	<0.0001
Office DBP (mm Hg)	94.3 (92.9, 100.2)	79.3 (74.1, 84.1)	<0.0001
Office PP (mm Hg)	59.7 (57.7, 69.1)	54.8 (52.4, 58.6)	<0.0001
ABPM SBP (mm Hg)	136.5 (135.0, 139.0)	119.0 (111.3, 126.8)	<0.0001
ABPM DBP mm Hg)	85.0 (83.0, 88.8)	70.0 (64.3, 75.0)	<0.0001
ABPM PP (mm Hg)	52.0 (50.0, 54.0)	49.0 (42.3, 54.0)	<0.001
LVMI (g m^{-2})	142.2 ± 6.0	122.9 ± 4.3	0.02
FMD (%)	6.6 (6.3, 7.0)	7.3 (6.8, 7.8)	<0.01
NTG (%)	22.5 ± 1.0	23.5 ± 1.3	0.61
PWV (m/s)	12.0 ± 0.3	9.2 ± 0.2	<0.0001

Abbreviations: ABPM, ambulatory blood pressure monitoring measurements; BMI, body mass index; CRHTN, controlled resistant hypertension sub group; DBP, diastolic blood pressure; F, female; FMD, flow-mediated dilation; LVMI, left ventricular mass index; M, male; NTG, nitroglycerin; PP, pulse pressure; PWV, pulse wave velocity; SBP, systolic blood pressure; UCRHTN, uncontrolled resistant hypertension sub group.
Values are expressed as means ± s.e.m. or median (1st, 3rd quartiles).

Table 2 Biochemical parameters of RHTN subgroups

	UCRHTN (n = 44)	*CRHTN (n = 52)*	*P-value*
Glucose (mg dl^{-1})	99.7 ± 1.4	98.8 ± 1.5	0.88
Cholesterol (mg dl^{-1})	193.3 ± 4.6	189.2 ± 4.3	0.35
HDL-c (mg dl^{-1})	47.1 ± 0.9	47.8 ± 1.1	0.72
LDL-c (mg dl^{-1})	117.8 ± 3.5	113.3 ± 3.7	0.33
Triglycerides (mg dl^{-1})	144.2 ± 10.9	139.8 ± 6.8	0.29
Creatinine (mg dl^{-1})	0.97 ± 0.03	0.94 ± 0.03	0.38
Creatinine clearance (ml per min per 1.73 m^2)	98.6 ± 1.8	100.1 ± 1.7	0.21
Urea (mg dl^{-1})	36.2 ± 1.5	37.8 ± 1.3	0.39
Microalbuminuria (mg g^{-1})	43.4 ± 9.8	30.8 ± 7.9	0.14
Sodium (mEq l^{-1})	141.0 ± 0.3	140.8 ± 0.3	0.76
Potassium (mEq l^{-1})	4.2 ± 0.06	4.3 ± 0.05	0.17
Urinary sodium excretion	98.7 ± 1.4	95.7 ± 2.7	0.71
PRA (ng ml^{-1} h^{-1})	3.7 ± 0.3	4.3 ± 0.3	0.19
PAC (ng dl^{-1})	12.6 ± 1.4	8.9 ± 0.8	0.02
ARR (ng dl^{-1} per ng ml^{-1} h^{-1})	4.8 ± 1.2	2.7 ± 0.3	0.04
APN (μg ml^{-1})	6.9 ± 0.7	9.5 ± 0.8	0.01

Abbreviations: APN, plasma adiponectin level; ARR, aldosterone–renin ratio; CRHTN, controlled resistant hypertension sub group; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAC, plasma aldosterone concentration; PRA, plasma renin activity; RHTN, resistant hypertension; UCRHTN, uncontrolled resistant hypertension sub group.
Values are expressed as means ± s.e.m.

DISCUSSION

To the best of our knowledge, this is the first study to analyze the plasma APN levels in RHTN patients. We found that a lower plasma APN level, a higher PAC, increased vascular stiffness and LV hypertrophy, and greater impairment of endothelial function are characteristics that distinguish patients with UCRHTN from those with CRHTN. Taken together, these results, except the APN levels, support the results of previous studies using RHTN subgroups.[2,3] Poorly controlled hypertension undoubtedly leads to progressive vascular and heart damage.[3] In addition, the APN levels were inversely associated with the PWV, PAC and office and ABPM pulse, and SBPs in the UCRHTN subgroup but not in the CRHTN subgroup. Finally, the APN level was predictor of the PWV in patients

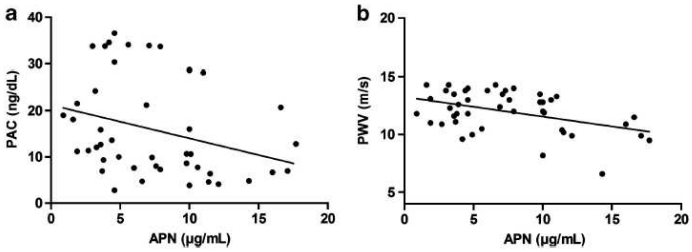

Figure 1 Correlations between the plasma APN level and the PAC ($r = -0.38$; $P = 0.01$) (**a**); and between the plasma APN level and the PWV ($r = -0.36$; $P = 0.02$ (**b**) in UCRHTN patients.

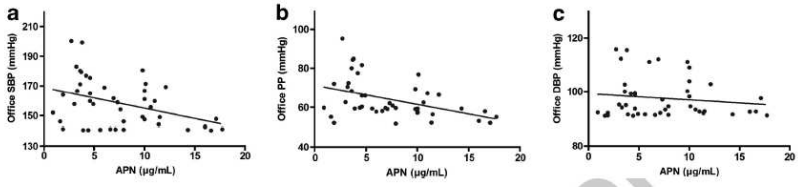

Figure 2 Correlations between the plasma APN level and the office SBP ($r = -0.33$; $P = 0.03$) (**a**); office PP ($r = -0.45$; $P = 0.002$) (**b**); and office DBP ($r = -0.05$; $P = 0.73$) (**c**) in UCRHTN patients.

Table 3 Multivariate linear regression analysis of PWV with age, aldosterone and adiponectin in UCRHTN subgroup

	β	*SE*	*P-value*
APN (µgml ^{1})	0.16	0.05	0.01
PAC (ngdl ^{1})	0.01	0.03	0.57
Age (years)	0.02	0.02	0.45

Abbreviations: APN, plasma adiponectin level; PAC, plasma aldosterone concentration; PWV, pulse wave velocity; UCRHTN, uncontrolled resistant hypertension subgroup.
$R^2 = 0.20$; adjusted $R^2 = 0.14$; β, beta coefficient.

with UCRHTN. These findings suggest that there are several important differences in pathophysiology that may underlie the development of RHTN with respect to subgroups.

Hypoadiponectinemia can be related to hypertension via multiple mechanisms, such as insulin resistance, sympathetic activation, increased circulating fatty acid levels, impaired endothelium-dependent vasodilation and vascular inflammation.[10] In turn, we showed that the APN level was inversely correlated with the office SBP and PPs in UCRHTN patients but not with the office diastolic pressure. In addition, the APN level was inversely associated with the systolic and pulse ABPM pressures. The observed correlations between the APN level and the SBP and PP can be explained by the fact that both BP components increase in response to increases in vascular resistance and large-artery stiffness. Therefore, cardiovascular risk may be more closely related to the pulsatile stress caused by large-artery stiffness during systole than to the steady-state stress due to small-vessel resistance during diastole.[17,18]

Our results revealed a significant inverse relationship between the APN levels and the PWV in UCRHTN subjects. Recent studies confirm that the APN level is inversely associated with arterial stiffness in hypertensive patients.[19,20] Different mechanisms have been suggested to explain this association. APN may stimulate the activity of endothelial nitric oxide synthase,[21] and also it is associated with reduced vascular smooth muscle cell proliferation.[22] APN inhibits almost all pathological conditions involved in vascular disease and exerts its multiple pleiotropic effects by its direct actions on several vascular cell types.[23]

We found that the APN level was not associated with vascular function or the LVMI. Previous studies have found that hypoadiponectinemia was associated with impaired endothelium-dependent vasorelaxation.[24] In addition, the APN level was found to be inversely correlated with the LVMI in a cross-sectional study.[25] We hypothesized that the plasma APN level may be associated with endothelial dysfunction and LV hypertrophy in the early stages of hypertension. Endothelial dysfunction should be considered to be restricted to the early phase in the pathogenesis of cardiovascular disease. Moreover, several studies have established an effect of endothelial dysfunction on arterial stiffening, and the reduced bioavailability of nitric oxide impairs vascular smooth muscle relaxation, causing progressive arterial stiffening.[26] The APN level might be helpful as a marker of endothelial dysfunction and might be useful in assessing the early stages of atherosclerosis.[24] Conversely, the correlation between endothelial function and arterial stiffness might be reduced in advanced stages of hypertension, as observed in the RHTN group.[27] This pattern may explain the poor correlation between the APN level and the brachial FMD in both the UCRHTN and CRHTN subgroups (Figure 3).

Accumulating evidence indicates that increased levels of aldosterone are related to the pathogenesis of RHTN in obese individuals and mediate several maladaptive changes.[7] The PAC and BP both fall when patients successfully lose weight.[28] The APN level was inversely associated with the aldosterone concentration, which in turn was positively associated with arterial stiffness. Similarly, higher leptin levels have been shown to be associated with increased aldosterone and BP levels in UCRHTN patients when compared with CRHTN and well-controlled hypertensive patients.[29] Li *et al.*[30] demonstrated that aldosterone decreases APN expression in adipocytes, and this effect may be one of the primary mechanisms by which aldosterone is

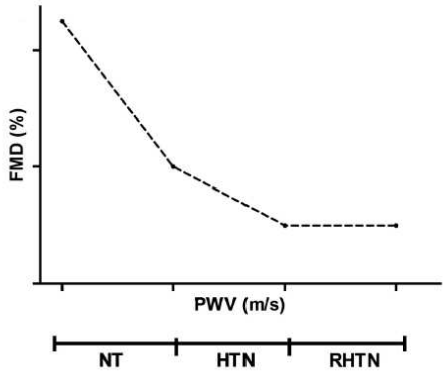

Figure 3 Progression from the early to severe stages of hypertension and difficult-to-control high BP levels are characterized by increased arterial rigidity and impaired endothelial function. Therefore, the correlation between endothelial dysfunction and arterial rigidity might be reduced or even absent in the advanced stages of hypertension (NT, normotensive patients; HTN, hypertensive patients; RHTN, resistant hypertensive patients).

related to metabolic and cardiovascular disorders. High aldosterone concentration results in the dysregulation of proinflammatory cytokines and the APN mRNA levels in adipocytes through the activation of mineralocorticoid receptors.[31] With respect to arterial stiffness, aldosterone promotes collagen deposition, enhancing vascular remodeling and impairing arterial elasticity.[32]

Weight loss is one possible way to increase the plasma APN level, and significant reductions in the body weight due to important lifestyle changes (almost 14% reduction in the BMI)[33] modify circulating the APN level.

The management of RHTN should include nonpharmacological approaches aimed to reduce the amount of adipose tissue by rigorous lifestyle changes; the early administration of intensive pharmacological therapies, primarily those targeting the renin–angiotensin–aldosterone system and mineralocorticoid receptors; and the optimization of the treatments of RHTN-related disorders, such as diabetes and dyslipidemia.

The main limitation of this study was the small number of UCRHTN and CRHTN patients enrolled. This study did not evaluate the identity of the multimeric APN species present in the circulation,[34] and further research is needed to determine whether any specific multimeric species is more closely associated with the variables assessed in this study. The 24-hour urinary aldosterone excretion rate test was not performed, although this assay could help to assess patients with changes in aldosterone physiology. As previously reported in Fasshauer *et al*,[35] the APN levels were higher in women than in men (data not shown). For this reason, the UCRHTN and CRHTN groups were carefully sex-matched in this study to eliminate the effects of this bias. Some pharmacological aspects of our results should be taken into account. Although antihypertensive drugs can influence the APN level,[31,36,37] these possible sources of interferences did not affect our findings. The UCRHTN subjects had reduced APN levels even though they used a greater number of antihypertensive agents. Furthermore, the lack of standard antihypertensive therapy was because of the use of individualized care. Resistant hypertensive subjects could not be assessed withdrawing the antihypertensive drugs—and trying to exclude the influence of these medications on the adiponectin levels—due to ethical concerns.

Because this study was cross-sectional, causal inferences cannot be made. However, our findings support a possible link between hypoadiponectinemia and vascular disease in RHTN condition.

In summary, we demonstrated that the PP, PAC and PWV were inversely associated with the plasma APN level only in UCRHTN patients. In addition to the involvement of a higher BP and PAC, lower APN levels and increased arterial stiffness can contribute to the greater resistance to antihypertensive treatment in the uncontrolled group and expose this group to increased cardiovascular risk. These outcomes have important implications for preventing and treating RHTN, with an intensive approach to lifestyle changes being important. The findings of the current study need to be confirmed in prospective clinical assessments using a larger RHTN population.

CONFLICT OF INTEREST

Leandro Boer-Martins and Caroline Demacq are employees of Novartis Biociências S.A. (Brazil). All authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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1 Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, White A, Cushman WC, White W, Sica D, Ferdinand K, Giles TD, Falkner B, Carey RM. Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Hypertension* 2008; **51**: 1403–1419.
2 Quinaglia T, Martins LC, Figueiredo VN, Santos RC, Yugar-Toledo JC, Martin JF, Demacq C, Pimenta E, Calhoun DA, Moreno H Jr. Non-dipping pattern relates to endothelial dysfunction in patients with uncontrolled resistant hypertension. *J Hum Hypertens* 2011; **25**: 656–664.
3 Martins LC, Figueiredo VN, Quinaglia T, Boer-Martins L, Yugar-Toledo JC, Martin JF, Demacq C, Pimenta E, Calhoun DA, Moreno H Jr. Characteristics of resistant hypertension: ageing, body mass index, hyperaldosteronism, cardiac hypertrophy and vascular stiffness. *J Hum Hypertens* 2011; **25**: 532–538.
4 Yugar-Toledo JC, Tanus-Santos JE, Sabha M, Sousa MG, Cittadino M, Tacito LH, Moreno H Jr. Uncontrolled hypertension, uncompensated type II diabetes, and smoking have different patterns of vascular dysfunction. *Chest* 2004; **125**: 823–830.
5 Pabuccu T, Baris N, Ozpelit E, Akdeniz B, Guneri S. The relationship between resistant hypertension and arterial stiffness. *Clin Exp Hypertens* 2011; **34**: 57–62.
6 Kotchen TA, Grim CE, Kotchen JM, Krishnaswami S, Yang H, Hoffmann RG, McGinley EL. Altered relationship of blood pressure to adiposity in hypertension. *Am J Hypertens* 2008; **21**: 284–289.
7 Sowers JR, Whaley-Connell A, Epstein M. Narrative review: the emerging clinical implications of the role of aldosterone in the metabolic syndrome and resistant hypertension. *Ann Intern Med* 2009; **150**: 776–783.
8 Frayn KN, Karpe F, Fielding BA, Macdonald IA, Coppack SW. Integrative physiology of human adipose tissue. *Int J Obes Relat Metab Disord* 2003; **27**: 875–888.
9 Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, Fu Y, Motone M, Yamamoto K, Matsuo A, Ohashi K, Kihara S, Funahashi T, Rakugi H, Matsuzawa Y, Ogihara T. Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension* 2004; **43**: 1318–1323.
10 Chow WS, Cheung BM, Tso AW, Xu A, Wat NM, Fong CH, Ong LH, Tam S, Tan KC, Janus ED, Lam TH, Lam KS. Hypoadiponectinemia as a predictor for the development of hypertension: a 5-year prospective study. *Hypertension* 2007; **49**: 1455–1461.
11 Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa Y. Decreased plasma adiponectin concentration in patients with essential hypertension. *Am J Hypertens* 2003; **16**: 72–75.
12 de Souza WA, Sabha M, de Faveri Favero F, Bergsten-Mendes G, Yugar-Toledo JC, Moreno H. Intensive monitoring of adherence to treatment helps to identify 'true' resistant hypertension. *J Clin Hypertens* 2009; **11**: 183–191.
13 Groppelli A, Omboni S, Parati G, Mancia G. Evaluation of noninvasive blood pressure monitoring devices Spacelabs 90202 and 90207 versus resting and ambulatory 24-hour intra-arterial blood pressure. *Hypertension* 1992; **20**: 227–232.
14 Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978; **58**: 1072–1083.

15 Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; **39**: 257–265.
16 Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R, Levy BI. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension* 1995; **26**: 485–490.
17 Christensen KL. Reducing pulse pressure in hypertension may normalize small artery structure. *Hypertension* 1991; **18**: 722–727.
18 Elzinga G, Westerhof N. Pressure and flow generated by the left ventricle against different impedances. *Circ Res* 1973; **32**: 178–186.
19 Youn JC, Kim C, Park S, Lee SH, Kang SM, Choi D, Son NH, Shin DJ, Jang Y. Adiponectin and progression of arterial stiffness in hypertensive patients. *Int J Cardiol* 2011; **163**: 316–319.
20 Mahmud A, Feely J. Adiponectin and arterial stiffness. *Am J Hypertens* 2005; **18**: 1543–1548.
21 Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 2003; **278**: 45021–45026.
22 Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem* 2002; **277**: 37487–37491.
23 Zhu W, Cheng KK, Vanhoutte PM, Lam KS, Xu A. Vascular effects of adiponectin: molecular mechanisms and potential therapeutic intervention. *Clin Sci (Lond)* 2008; **114**: 361–374.
24 Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, Kumada M, Ohashi K, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Nagasawa A, Kobayashi H, Hiraoka H, Komai N, Kaibe M, Rakugi H, Ogihara T, Matsuzawa Y. Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension* 2003; **42**: 231–234.
25 Hong SJ, Park CG, Seo HS, Oh DJ, Ro YM. Associations among plasma adiponectin, hypertension, left ventricular diastolic function and left ventricular mass index. *Blood Press* 2004; **13**: 236–242.
26 Wilkinson IB, MacCallum H, Cockcroft JR, Webb DJ. Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity *in vivo*. *Br J Clin Pharmacol* 2002; **53**: 189–192.
27 Figueiredo VN, Yugar-Toledo JC, Martins LC, Martins LB, de Faria AP, de Haro Moraes C, Sierra C, Coca A, Moreno H. Vascular stiffness and endothelial dysfunction: correlations at different levels of blood pressure. *Blood Press* 2012; **21**: 31–38.
28 Tuck ML, Sowers J, Dornfeld L, Kledzik G, Maxwell M. The effect of weight reduction on blood pressure, plasma renin activity, and plasma aldosterone levels in obese patients. *N Engl J Med* 1981; **304**: 930–933.
29 de Haro Moraes C, Figueiredo VN, de Faria AP, Barbaro NR, Sabbatini AR, Quinaglia T, Ferreira-Melo SE, Martins LC, Demacq C, Junior HM. High-circulating leptin levels are associated with increased blood pressure in uncontrolled resistant hypertension. *J Hum Hypertens* 2012; **27**: 225–230.
30 Li P, Zhang XN, Pan CM, Sun F, Zhu DL, Song HD, Chen MD. Aldosterone perturbs adiponectin and PAI-1 expression and secretion in 3T3-L1 adipocytes. *Horm Metab Res* 2011; **43**: 464–469.
31 Guo C, Ricchiuti V, Lian BQ, Yao TM, Coutinho P, Romero JR, Li J, Williams GH, Adler GK. Mineralocorticoid receptor blockade reverses obesity-related changes in expression of adiponectin, peroxisome proliferator-activated receptor-gamma, and proinflammatory adipokines. *Circulation* 2008; **117**: 2253–2261.
32 Brilla CG, Matsubara LS, Weber KT. Antifibrotic effects of spironolactone in preventing myocardial fibrosis in systemic arterial hypertension. *Am J Cardiol* 1993; **71**: 12A–16A.
33 Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, Giugliano D. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* 2003; **289**: 1799–1804.
34 Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. *J Biol Chem* 2003; **278**: 9073–9085.
35 Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 2002; **290**: 1084–1089.
36 Yamada S, Ano N, Toda K, Kitaoka A, Shiono K, Inoue G, Atsuda K, Irie J. Telmisartan but not candesartan affects adiponectin expression *in vivo* and *in vitro*. *Hypertens Res* 2008; **31**: 601–606.
37 Yilmaz MI, Sonmez A, Caglar K, Celik T, Yenicesu M, Eyileten T, Acikel C, Oguz Y, Yavuz I, Vural A. Effect of antihypertensive agents on plasma adiponectin levels in hypertensive patients with metabolic syndrome. *Nephrology* 2007; **12**: 147–153.

6 CONCLUSÕES

1. Os SNPs -11377C/G (rs266729) e +276G>T (rs1501299) foram associados aos níveis plasmáticos de adiponectina total em pacientes com HAR;

2. Os dois SNPs parecem influenciar diferentemente os níveis de adiponectina nos subgrupos HARC e HARNC. No entanto, não houve diferenças nas frequências genotípicas, alélicas e haplotípicas entre os subgrupos;

3. Por fim, os portadores do alelo menos frequente nos dois polimorfismos genéticos estudados foram preditores independentes para os níveis de adiponectina, sugerindo a participação dessa adipocitocina na resistência à terapia anti-hipertensiva.

7 REFERÊNCIAS

1. Alderman MH, Budner N, Cohen H, Lamport B, and Ooi WL, Prevalence of drug resistant hypertension. Hypertension. 1988;11(3 Pt 2):II71-5.
2. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., et al., The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA. 2003;289(19):2560-72.
3. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al., 2013 ESH/ESC Guidelines for themanagement of arterial hypertension The Task Force for the management ofarterial hypertension of the European Society ofHypertension (ESH) and of the European Society of Cardiology (ESC). Journal of Hypertension. 2013;31(7):1281-357.
4. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, et al., Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Hypertension. 2008;51(6):1403-19.
5. Brown MA, Buddle ML, and Martin A, Is resistant hypertension really resistant? Am J Hypertens. 2001;14(12):1263-9.
6. Muxfeldt ES, Bloch KV, Nogueira AR, and Salles GF, Twenty-four hour ambulatory blood pressure monitoring pattern of resistant hypertension. Blood Press Monit. 2003;8(5):181-5.
7. Wang ZV and Scherer PE, Adiponectin, cardiovascular function, and hypertension. Hypertension. 2008;51(1):8-14.
8. Sutton-Tyrrell K, Newman A, Simonsick EM, Havlik R, Pahor M, Lakatta E, et al., Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. Hypertension. 2001;38(3):429-33.
9. Salles GF, Fiszman R, Cardoso CR, and Muxfeldt ES, Relation of left ventricular hypertrophy with systemic inflammation and endothelial damage in resistant hypertension. Hypertension. 2007;50(4):723-8.
10. Salles GF, Cardoso CR, Pereira VS, Fiszman R, and Muxfeldt ES, Prognostic significance of a reduced glomerular filtration rate and interaction with microalbuminuria in resistant hypertension: a cohort study. J Hypertens. 2011;29(10):2014-23.
11. Kurukulasuriya LR, Stas S, Lastra G, Manrique C, and Sowers JR, Hypertension in obesity. Med Clin North Am. 2011;95(5):903-17.
12. Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, et al., Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. FASEB J. 2001;15(14):2727-9.
13. Cooper R, McFarlane-Anderson N, Bennett FI, Wilks R, Puras A, Tewksbury D, et al., ACE, angiotensinogen and obesity: a potential pathway leading to hypertension. J Hum Hypertens. 1997;11(2):107-11.
14. Chaudhary K, Buddineni JP, Nistala R, and Whaley-Connell A, Resistant hypertension in the high-risk metabolic patient. Curr Diab Rep. 2011;11(1):41-6.
15. Whaley-Connell A, Johnson MS, and Sowers JR, Aldosterone: role in the cardiometabolic syndrome and resistant hypertension. Prog Cardiovasc Dis. 2010;52(5):401-9.
16. Caprio M, Feve B, Claes A, Viengchareun S, Lombes M, and Zennaro MC, Pivotal role of the mineralocorticoid receptor in corticosteroid-induced adipogenesis. FASEB J. 2007;21(9):2185-94.

17. Guo C, Ricchiuti V, Lian BQ, Yao TM, Coutinho P, Romero JR, et al., Mineralocorticoid receptor blockade reverses obesity-related changes in expression of adiponectin, peroxisome proliferator-activated receptor-gamma, and proinflammatory adipokines. Circulation. 2008;117(17):2253-61.
18. Sowers JR, Whaley-Connell A, and Epstein M, Narrative review: the emerging clinical implications of the role of aldosterone in the metabolic syndrome and resistant hypertension. Ann Intern Med. 2009;150(11):776-83.
19. Kadowaki T and Yamauchi T, Adiponectin and adiponectin receptors. Endocr Rev. 2005;26(3):439-51.
20. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, et al., Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia. 2003;46(4):459-69.
21. Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, et al., Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. Biochem Biophys Res Commun. 2003;301(4):1045-50.
22. Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, and Matsuzawa Y, Decreased plasma adiponectin concentration in patients with essential hypertension. Am J Hypertens. 2003;16(1):72-5.
23. Francischetti EA, Celoria BM, Duarte SF, da Silva EG, Santos IJ, Cabello PH, et al., Hypoadiponectinemia is associated with blood pressure increase in obese insulin-resistant individuals. Metabolism. 2007;56(11):1464-9.
24. Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, et al., Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension. 2004;43(6):1318-23.
25. Chow WS, Cheung BM, Tso AW, Xu A, Wat NM, Fong CH, et al., Hypoadiponectinemia as a predictor for the development of hypertension: a 5-year prospective study. Hypertension. 2007;49(6):1455-61.
26. Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, et al., Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension. 2003;42(3):231-4.
27. Tan KC, Xu A, Chow WS, Lam MC, Ai VH, Tam SC, et al., Hypoadiponectinemia is associated with impaired endothelium-dependent vasodilation. J Clin Endocrinol Metab. 2004;89(2):765-9.
28. Hong SJ, Park CG, Seo HS, Oh DJ, and Ro YM, Associations among plasma adiponectin, hypertension, left ventricular diastolic function and left ventricular mass index. Blood Press. 2004;13(4):236-42.
29. Mahmud A and Feely J, Adiponectin and arterial stiffness. Am J Hypertens. 2005;18(12 Pt 1):1543-8.
30. Youn JC, Kim C, Park S, Lee SH, Kang SM, Choi D, et al., Adiponectin and progression of arterial stiffness in hypertensive patients. Int J Cardiol. 2011.
31. de Faria AP, Demacq C, Figueiredo VN, Moraes CH, Santos RC, Sabbatini AR, et al., Hypoadiponectinemia and aldosterone excess are associated with lack of blood pressure control in subjects with resistant hypertension. Hypertens Res. 2013;36(12):1067-72.

32. Sabbatini AR, Faria AP, Barbaro NR, Gordo WM, Modolo RG, Pinho C, et al., Deregulation of adipokines related to target organ damage on resistant hypertension. J Hum Hypertens. 2014;28(6):388-92.
33. Martins LC, Figueiredo VN, Quinaglia T, Boer-Martins L, Yugar-Toledo JC, Martin JF, et al., Characteristics of resistant hypertension: ageing, body mass index, hyperaldosteronism, cardiac hypertrophy and vascular stiffness. J Hum Hypertens. 2011;25(9):532-8.
34. Quinaglia T, Martins LC, Figueiredo VN, Santos RC, Yugar-Toledo JC, Martin JF, et al., Non-dipping pattern relates to endothelial dysfunction in patients with uncontrolled resistant hypertension. J Hum Hypertens. 25(11):656-64.
35. Wolf-Maier K, Cooper RS, Banegas JR, Giampaoli S, Hense HW, Joffres M, et al., Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. JAMA. 2003;289(18):2363-9.
36. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., et al., Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003;42(6):1206-52.
37. de Souza WA, Yugar-Toledo JC, Bergsten-Mendes G, Sabha M, and Moreno H, Jr., Effect of pharmaceutical care on blood pressure control and health-related quality of life in patients with resistant hypertension. Am J Health Syst Pharm. 2007;64(18):1955-61.
38. Sarafidis PA, Epidemiology of resistant hypertension. J Clin Hypertens (Greenwich). 2011;13(7):523-8.
39. Williams B, Resistant hypertension: an unmet treatment need. Lancet. 2009;374(9699):1396-8.
40. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, et al., Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Circulation. 2008;117(25):e510-26.
41. de la Sierra A, Segura J, Banegas JR, Gorostidi M, de la Cruz JJ, Armario P, et al., Clinical features of 8295 patients with resistant hypertension classified on the basis of ambulatory blood pressure monitoring. Hypertension. 2011;57(5):898-902.
42. McAdam-Marx C, Ye X, Sung JC, Brixner DI, and Kahler KH, Results of a retrospective, observational pilot study using electronic medical records to assess the prevalence and characteristics of patients with resistant hypertension in an ambulatory care setting. Clin Ther. 2009;31(5):1116-23.
43. Faria AP, Sabbatini AR, Coca A, and Moreno-Junior H, Phenotypic characteristics of resistant hypertension in the Brazilian population. Arq Bras Cardiol. 2013.
44. de Haro Moraes C, Figueiredo VN, de Faria AP, Barbaro NR, Sabbatini AR, Quinaglia T, et al., High-circulating leptin levels are associated with increased blood pressure in uncontrolled resistant hypertension. J Hum Hypertens. 2013;27(4):225-30.
45. Moreno H, Jr. and Coca A, Resistant and refractory hypertension: reflections on pathophysiology and terminology. Blood Press. 2012;21(4):209-10.
46. Townsend RR, Refractory or resistant hypertension. J Clin Hypertens (Greenwich). 2002;4(1):61.
47. Moreno H and Calhoun DA, True resistant hypertension: definition and prevalence. J Hypertens. 2012;30(11):2241-2; author reply 2-3.

48. Ram CV and Silverstein RL, "Refractory" resistant hypertension: new terminology for an old problem. J Clin Hypertens (Greenwich). 2012;14(1):5-6.
49. Calhoun DA, Booth JN, Oparil S, Irvin MR, Shimbo D, Lackland DT, et al., Refractory Hypertension Determination of Prevalence, Risk Factors, and Comorbidities in a Large, Population-Based Cohort. Hypertension. 2014;63(3):451-8.
50. Figueiredo VN, Yugar-Toledo JC, Martins LC, Martins LB, de Faria AP, de Haro Moraes C, et al., Vascular stiffness and endothelial dysfunction: Correlations at different levels of blood pressure. Blood Press. 2012;21(1):31-8.
51. Quinaglia T, Martins LC, Figueiredo VN, Santos RC, Yugar-Toledo JC, Martin JF, et al., Non-dipping pattern relates to endothelial dysfunction in patients with uncontrolled resistant hypertension. J Hum Hypertens. 2011;25(11):656-64.
52. Persell SD, Prevalence of resistant hypertension in the United States, 2003-2008. Hypertension. 2011;57(6):1076-80.
53. Pimenta E and Calhoun DA, Resistant hypertension: incidence, prevalence, and prognosis. Circulation. 2012;125(13):1594-6.
54. Yakovlevitch M and Black HR, Resistant hypertension in a tertiary care clinic. Arch Intern Med. 1991;151(9):1786-92.
55. Garg JP, Elliott WJ, Folker A, Izhar M, and Black HR, Resistant hypertension revisited: a comparison of two university-based cohorts. Am J Hypertens. 2005;18(5 Pt 1):619-26.
56. Lloyd-Jones DM, Evans JC, Larson MG, O'Donnell CJ, Roccella EJ, and Levy D, Differential control of systolic and diastolic blood pressure : factors associated with lack of blood pressure control in the community. Hypertension. 2000;36(4):594-9.
57. Hollenberg NK, The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic. Curr Hypertens Rep. 2003;5(3):183-5.
58. Acelajado MC, Pisoni R, Dudenbostel T, Dell'Italia LJ, Cartmill F, Zhang B, et al., Refractory hypertension: definition, prevalence, and patient characteristics. J Clin Hypertens (Greenwich). 2012;14(1):7-12.
59. de Souza WS, Alessi A, Cordeiro A, da Rocha Nogueira A, Feitosa A, Amodeo C, et al., First Brazilian position on resistant hypertension. Arq Bras Cardiol. 2012;99(1):576-85.
60. Egan BM, Zhao Y, Axon RN, Brzezinski WA, and Ferdinand KC, Uncontrolled and apparent treatment resistant hypertension in the United States, 1988 to 2008. Circulation. 2011;124(9):1046-58.
61. Massierer D, Oliveira AC, Steinhorst AM, Gus M, Ascoli AM, Goncalves SC, et al., Prevalence of resistant hypertension in non-elderly adults: prospective study in a clinical setting. Arq Bras Cardiol. 2012;99(1):630-5.
62. Pimenta E, Calhoun DA, and Oparil S, Mechanisms and treatment of resistant hypertension. Arq Bras Cardiol. 2007;88(6):683-92.
63. White WB, Ambulatory blood pressure monitoring as an investigative tool for characterizing resistant hypertension and its rational treatment. J Clin Hypertens (Greenwich). 2007;9(1 Suppl 1):25-30.

64. de Souza WA, Sabha M, de Faveri Favero F, Bergsten-Mendes G, Yugar-Toledo JC, and Moreno H, Intensive monitoring of adherence to treatment helps to identify "true" resistant hypertension. J Clin Hypertens (Greenwich). 2009;11(4):183-91.
65. Park J and Campese V, Clinical characteristics of resistant hypertension: the importance of compliance and the role of diagnostic evaluation in delineating pathogenesis. J Clin Hypertens (Greenwich). 2007;9(1 Suppl 1):7-12.
66. DiBona GF, Nervous kidney. Interaction between renal sympathetic nerves and the renin-angiotensin system in the control of renal function. Hypertension. 2000;36(6):1083-8.
67. Ferrario CM, Gildenberg PL, and McCubbin JW, Cardiovascular effects of angiotensin mediated by the central nervous system. Circ Res. 1972;30(3):257-62.
68. Zimmerman BG, Sybertz EJ, and Wong PC, Interaction between sympathetic and renin-angiotensin system. J Hypertens. 1984;2(6):581-7.
69. Tsioufis C, Tsiachris D, Dimitriadis K, Stougiannos P, Missovoulos P, Kakkavas A, et al., Myocardial and aortic stiffening in the early course of primary aldosteronism. Clin Cardiol. 2008;31(9):431-6.
70. Gaddam KK, Nishizaka MK, Pratt-Ubunama MN, Pimenta E, Aban I, Oparil S, et al., Characterization of resistant hypertension: association between resistant hypertension, aldosterone, and persistent intravascular volume expansion. Arch Intern Med. 2008;168(11):1159-64.
71. Thomopoulos C, Tsioufis C, Dimitriadis K, Tsiachris D, Tousoulis D, Manolis A, et al., Obstructive sleep apnoea syndrome is associated with enhanced sub-clinical inflammation and asymmetric dimethyl-arginine levels in hypertensives. J Hum Hypertens. 2009;23(1):65-7.
72. Yugar-Toledo JC, Tanus-Santos JE, Sabha M, Sousa MG, Cittadino M, Tacito LH, et al., Uncontrolled hypertension, uncompensated type II diabetes, and smoking have different patterns of vascular dysfunction. Chest. 2004;125(3):823-30.
73. Taler SJ, Textor SC, and Augustine JE, Resistant hypertension: comparing hemodynamic management to specialist care. Hypertension. 2002;39(5):982-8.
74. Parati G, Ochoa JE, Bilo G, Mattaliano P, Salvi P, Kario K, et al., Obstructive sleep apnea syndrome as a cause of resistant hypertension. Hypertens Res. 2014.
75. Dudenbostel T and Calhoun DA, Resistant hypertension, obstructive sleep apnoea and aldosterone. J Hum Hypertens. 2012;26(5):281-7.
76. Esler M, Straznicky N, Eikelis N, Masuo K, Lambert G, and Lambert E, Mechanisms of sympathetic activation in obesity-related hypertension. Hypertension. 2006;48(5):787-96.
77. Rahmouni K and Morgan DA, Hypothalamic arcuate nucleus mediates the sympathetic and arterial pressure responses to leptin. Hypertension. 2007;49(3):647-52.
78. Tsioufis C, Kordalis A, Flessas D, Anastasopoulos I, Tsiachris D, Papademetriou V, et al., Pathophysiology of resistant hypertension: the role of sympathetic nervous system. Int J Hypertens. 2011;2011:642416.
79. VI Diretrizes Brasileiras de Hipertensão. Arq Bras Cardiol. 2010;95(1 Suppl):1-51.
80. Pimenta E, Gaddam KK, Oparil S, Aban I, Husain S, Dell'Italia LJ, et al., Effects of dietary sodium reduction on blood pressure in subjects with resistant hypertension: results from a randomized trial. Hypertension. 2009;54(3):475-81.

81. Aguilera MT, de la Sierra A, Coca A, Estruch R, Fernandez-Sola J, and Urbano-Marquez A, Effect of alcohol abstinence on blood pressure: assessment by 24-hour ambulatory blood pressure monitoring. Hypertension. 1999;33(2):653-7.
82. Sander GE and Giles TD, Resistant hypertension: concepts and approach to management. Curr Hypertens Rep. 2011;13(5):347-55.
83. Pedrosa RP, Drager LF, Gonzaga CC, Sousa MG, de Paula LK, Amaro AC, et al., Obstructive sleep apnea: the most common secondary cause of hypertension associated with resistant hypertension. Hypertension. 2011;58(5):811-7.
84. Cornelissen VA, Fagard RH, Coeckelberghs E, and Vanhees L, Impact of resistance training on blood pressure and other cardiovascular risk factors: a meta-analysis of randomized, controlled trials. Hypertension. 2011;58(5):950-8.
85. Salles GF, Cardoso CR, and Muxfeldt ES, Prognostic influence of office and ambulatory blood pressures in resistant hypertension. Arch Intern Med. 2008;168(21):2340-6.
86. Mancia G, Laurent S, Agabiti-Rosei E, Ambrosioni E, Burnier M, Caulfield MJ, et al., Reappraisal of European guidelines on hypertension management: a European Society of Hypertension Task Force document. Blood Press. 2009;18(6):308-47.
87. Chapman N, Dobson J, Wilson S, Dahlof B, Sever PS, Wedel H, et al., Effect of spironolactone on blood pressure in subjects with resistant hypertension. Hypertension. 2007;49(4):839-45.
88. Ubaid-Girioli S, Adriana de Souza L, Yugar-Toledo JC, Martins LC, Ferreira-Melo S, Coelho OR, et al., Aldosterone excess or escape: Treating resistant hypertension. J Clin Hypertens (Greenwich). 2009;11(5):245-52.
89. Nishizaka MK, Zaman MA, and Calhoun DA, Efficacy of low-dose spironolactone in subjects with resistant hypertension. Am J Hypertens. 2003;16(11 Pt 1):925-30.
90. Zannad F, Aldosterone antagonist therapy in resistant hypertension. J Hypertens. 2007;25(4):747-50.
91. Oliver JJ, Hughes VE, Dear JW, and Webb DJ, Clinical potential of combined organic nitrate and phosphodiesterase type 5 inhibitor in treatment-resistant hypertension. Hypertension. 2010;56(1):62-7.
92. Oliver JJ, Melville VP, and Webb DJ, Effect of regular phosphodiesterase type 5 inhibition in hypertension. Hypertension. 2006;48(4):622-7.
93. Consolim-Colombo FM, De Angelis K, and Irigoyen MC, Terapia de Estimulação dos Barorreceptores. Revista Brasileira de Hipertensão. 2011;18(4):149-52.
94. Schwartz SI, Griffith LS, Neistadt A, and Hagfors N, Chronic carotid sinus nerve stimulation in the treatment of essential hypertension. Am J Surg. 1967;114(1):5-15.
95. Bisognano JD, Bakris G, Nadim MK, Sanchez L, Kroon AA, Schafer J, et al., Baroreflex activation therapy lowers blood pressure in patients with resistant hypertension: results from the double-blind, randomized, placebo-controlled rheos pivotal trial. J Am Coll Cardiol. 2011;58(7):765-73.
96. Wustmann K, Kucera JP, Scheffers I, Mohaupt M, Kroon AA, de Leeuw PW, et al., Effects of chronic baroreceptor stimulation on the autonomic cardiovascular regulation in patients with drug-resistant arterial hypertension. Hypertension. 2009;54(3):530-6.
97. Brito TM and Bortolotto LA, Denervação Renal no Tratamento de Hipertensão Arterial Resistente. Revista Brasileira de Hipertensão. 2011;18(4):145-8.

98. Morrissey DM, Brookes VS, and Cooke WT, Sympathectomy in the treatment of hypertension; review of 122 cases. Lancet. 1953;1(6757):403-8.
99. Dibona GF, Sympathetic Nervous System and Hypertension. Hypertension. 2013.
100. Stewart AD, Jiang B, Millasseau SC, Ritter JM, and Chowienczyk PJ, Acute reduction of blood pressure by nitroglycerin does not normalize large artery stiffness in essential hypertension. Hypertension. 2006;48(3):404-10.
101. Safar ME and Lacolley P, Disturbance of macro- and microcirculation: relations with pulse pressure and cardiac organ damage. Am J Physiol Heart Circ Physiol. 2007;293(1):H1-7.
102. Zieman SJ, Melenovsky V, and Kass DA, Mechanisms, pathophysiology, and therapy of arterial stiffness. Arterioscler Thromb Vasc Biol. 2005;25(5):932-43.
103. O'Rourke MF, Blazek JV, Morreels CL, Jr., and Krovetz LJ, Pressure wave transmission along the human aorta. Changes with age and in arterial degenerative disease. Circ Res. 1968;23(4):567-79.
104. Aronson D, Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. J Hypertens. 2003;21(1):3-12.
105. Benetos A, Adamopoulos C, Bureau JM, Temmar M, Labat C, Bean K, et al., Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. Circulation. 2002;105(10):1202-7.
106. Sigrist M, Bungay P, Taal MW, and McIntyre CW, Vascular calcification and cardiovascular function in chronic kidney disease. Nephrol Dial Transplant. 2006;21(3):707-14.
107. Asmar RG, Brunel PC, Pannier BM, Lacolley PJ, and Safar ME, Arterial distensibility and ambulatory blood pressure monitoring in essential hypertension. Am J Cardiol. 1988;61(13):1066-70.
108. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, et al., Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension. 1995;26(3):485-90.
109. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al., Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012;30(3):445-8.
110. Laurent S, Katsahian S, Fassot C, Tropeano AI, Gautier I, Laloux B, et al., Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. Stroke. 2003;34(5):1203-6.
111. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, and London GM, Impact of aortic stiffness on survival in end-stage renal disease. Circulation. 1999;99(18):2434-9.
112. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al., Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. 2001;37(5):1236-41.
113. Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS, et al., ACC/AHA 2002 guideline update for the management of patients with chronic stable angina--summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Chronic Stable Angina). Circulation. 2003;107(1):149-58.

114. Levy D, Murabito JM, Anderson KM, Christiansen JC, and Castelli WP, Echocardiographic left ventricular hypertrophy: clinical characteristics. The Framingham Heart Study. Clin Exp Hypertens A. 1992;14(1-2):85-97.
115. de Simone G, Devereux RB, Chinali M, Roman MJ, Lee ET, Resnick HE, et al., Metabolic syndrome and left ventricular hypertrophy in the prediction of cardiovascular events: the Strong Heart Study. Nutr Metab Cardiovasc Dis. 2009;19(2):98-104.
116. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al., Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr. 2005;18(12):1440-63.
117. Kannel WB and Cobb J, Left ventricular hypertrophy and mortality--results from the Framingham Study. Cardiology. 1992;81(4-5):291-8.
118. Kannel WB, Coronary risk factors: an overview. 1995:1809-92.
119. Brosius FC, 3rd, Hostetter TH, Kelepouris E, Mitsnefes MM, Moe SM, Moore MA, et al., Detection of chronic kidney disease in patients with or at increased risk of cardiovascular disease: a science advisory from the American Heart Association Kidney and Cardiovascular Disease Council; the Councils on High Blood Pressure Research, Cardiovascular Disease in the Young, and Epidemiology and Prevention; and the Quality of Care and Outcomes Research Interdisciplinary Working Group: Developed in Collaboration With the National Kidney Foundation. Hypertension. 2006;48(4):751-5.
120. Ovbiagele B, Microalbuminuria: risk factor and potential therapeutic target for stroke? J Neurol Sci. 2008;271(1-2):21-8.
121. Mogensen CE, Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med. 1984;310(6):356-60.
122. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, et al., Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. Circulation. 2002;106(14):1777-82.
123. Ravid M, Brosh D, Ravid-Safran D, Levy Z, and Rachmani R, Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. Arch Intern Med. 1998;158(9):998-1004.
124. Pascual JM, Rodilla E, Gonzalez C, Perez-Hoyos S, and Redon J, Long-term impact of systolic blood pressure and glycemia on the development of microalbuminuria in essential hypertension. Hypertension. 2005;45(6):1125-30.
125. Pascual JM, Rodilla E, Miralles A, Gonzalez C, and Redon J, Determinants of urinary albumin excretion reduction in essential hypertension: A long-term follow-up study. J Hypertens. 2006;24(11):2277-84.
126. Christ M, Grimm W, and Maisch B, [Significance of aldosterone antagonist therapy]. Internist (Berl). 2004;45(3):347-54.
127. Ubaid-Girioli S, Ferreira-Melo SE, Souza LA, Nogueira EA, Yugar-Toledo JC, Coca A, et al., Aldosterone escape with diuretic or angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker combination therapy in patients with mild to moderate hypertension. J Clin Hypertens (Greenwich). 2007;9(10):770-4.

128. Williams JS, Evolving research in nongenomic actions of aldosterone. Curr Opin Endocrinol Diabetes Obes. 2013;20(3):198-203.
129. Grossmann C, Krug AW, Freudinger R, Mildenberger S, Voelker K, and Gekle M, Aldosterone-induced EGFR expression: interaction between the human mineralocorticoid receptor and the human EGFR promoter. Am J Physiol Endocrinol Metab. 2007;292(6):E1790-800.
130. Manrique C, Lastra G, Whaley-Connell A, and Sowers JR, Hypertension and the cardiometabolic syndrome. J Clin Hypertens (Greenwich). 2005;7(8):471-6.
131. Fonseca-Alaniz MH, Takada J, Alonso-Vale MI, and Lima FB, Adipose tissue as an endocrine organ: from theory to practice. J Pediatr (Rio J). 2007;83(5 Suppl):S192-203.
132. Ehrhart-Bornstein M, Arakelyan K, Krug AW, Scherbaum WA, and Bornstein SR, Fat cells may be the obesity-hypertension link: human adipogenic factors stimulate aldosterone secretion from adrenocortical cells. Endocr Res. 2004;30(4):865-70.
133. Hubert HB, Feinleib M, McNamara PM, and Castelli WP, Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation. 1983;67(5):968-77.
134. Mahamat A, Richard F, Arveiler D, Bongard V, Yarnell J, Ducimetiere P, et al., Body mass index, hypertension and 5-year coronary heart disease incidence in middle aged men: the PRIME study. J Hypertens. 2003;21(3):519-24.
135. Narkiewicz K, Diagnosis and management of hypertension in obesity. Obes Rev. 2006;7(2):155-62.
136. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, and Tobe K, Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 2006;116(7):1784-92.
137. Hall JE, Mechanisms of abnormal renal sodium handling in obesity hypertension. Am J Hypertens. 1997;10(5 Pt 2):49S-55S.
138. Hall JE, Hildebrandt DA, and Kuo J, Obesity hypertension: role of leptin and sympathetic nervous system. Am J Hypertens. 2001;14(6 Pt 2):103S-15S.
139. Rocchini AP, Obesity hypertension. Am J Hypertens. 2002;15(2 Pt 2):50S-2S.
140. Goodfriend TL, Egan BM, and Kelley DE, Aldosterone in obesity. Endocr Res. 1998;24(3-4):789-96.
141. Hall JE, Summers RL, Brands MW, Keen H, and Alonso-Galicia M, Resistance to metabolic actions of insulin and its role in hypertension. Am J Hypertens. 1994;7(8):772-88.
142. Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, et al., Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci U S A. 2000;97(26):14478-83.
143. Matsuzawa Y, Funahashi T, Kihara S, and Shimomura I, Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol. 2004;24(1):29-33.
144. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al., Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol. 2000;20(6):1595-9.

145. Biolo A, Shibata R, Ouchi N, Kihara S, Sonoda M, Walsh K, et al., Determinants of adiponectin levels in patients with chronic systolic heart failure. Am J Cardiol. 2010;105(8):1147-52.
146. Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, et al., Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. J Biol Chem. 2003;278(11):9073-85.
147. Inoue T, Kotooka N, Morooka T, Komoda H, Uchida T, Aso Y, et al., High molecular weight adiponectin as a predictor of long-term clinical outcome in patients with coronary artery disease. Am J Cardiol. 2007;100(4):569-74.
148. Goldstein BJ and Scalia R, Adiponectin: A novel adipokine linking adipocytes and vascular function. J Clin Endocrinol Metab. 2004;89(6):2563-8.
149. Tsioufis C, Dimitriadis K, Selima M, Thomopoulos C, Mihas C, Skiadas I, et al., Low-grade inflammation and hypoadiponectinaemia have an additive detrimental effect on aortic stiffness in essential hypertensive patients. Eur Heart J. 2007;28(9):1162-9.
150. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, et al., Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. Circulation. 2002;105(24):2893-8.
151. Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, et al., An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res. 2000;32(2):47-50.
152. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, and Rimm EB, Plasma adiponectin levels and risk of myocardial infarction in men. JAMA. 2004;291(14):1730-7.
153. Persson J, Lindberg K, Gustafsson TP, Eriksson P, Paulsson-Berne G, and Lundman P, Low plasma adiponectin concentration is associated with myocardial infarction in young individuals. J Intern Med. 2010;268(2):194-205.
154. Delporte ML, Funahashi T, Takahashi M, Matsuzawa Y, and Brichard SM, Pre- and post-translational negative effect of beta-adrenoceptor agonists on adiponectin secretion: in vitro and in vivo studies. Biochem J. 2002;367(Pt 3):677-85.
155. Takano H, Obata JE, Kodama Y, Kitta Y, Nakamura T, Mende A, et al., Adiponectin is released from the heart in patients with heart failure. Int J Cardiol. 2009;132(2):221-6.
156. Kistorp C, Faber J, Galatius S, Gustafsson F, Frystyk J, Flyvbjerg A, et al., Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. Circulation. 2005;112(12):1756-62.
157. Shibata R, Ouchi N, Ito M, Kihara S, Shiojima I, Pimentel DR, et al., Adiponectin-mediated modulation of hypertrophic signals in the heart. Nat Med. 2004;10(12):1384-9.
158. Kazumi T, Kawaguchi A, Sakai K, Hirano T, and Yoshino G, Young men with high-normal blood pressure have lower serum adiponectin, smaller LDL size, and higher elevated heart rate than those with optimal blood pressure. Diabetes Care. 2002;25(6):971-6.
159. Antoniades C, Antonopoulos AS, Tousoulis D, and Stefanadis C, Adiponectin: from obesity to cardiovascular disease. Obes Rev. 2009;10(3):269-79.
160. Harrap SB, Hypertension: genes versus environment. Lancet. 1994;344(8916):169-71.

161. Kim HS, Krege JH, Kluckman KD, Hagaman JR, Hodgin JB, Best CF, et al., Genetic control of blood pressure and the angiotensinogen locus. Proc Natl Acad Sci U S A. 1995;92(7):2735-9.
162. Williams JD and Coles GA, Proteinuria--a direct cause of renal morbidity? Kidney Int. 1994;45(2):443-50.
163. Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, et al., A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature. 2001;409(6822):928-33.
164. Sunyaev S, Ramensky V, Koch I, Lathe W, 3rd, Kondrashov AS, and Bork P, Prediction of deleterious human alleles. Hum Mol Genet. 2001;10(6):591-7.
165. Ng PC and Henikoff S, Accounting for human polymorphisms predicted to affect protein function. Genome Res. 2002;12(3):436-46.
166. Risch NJ, Searching for genetic determinants in the new millennium. Nature. 2000;405(6788):847-56.
167. Lynch AI, Irvin MR, Davis BR, Ford CE, Eckfeldt JH, and Arnett DK, Genetic and Adverse Health Outcome Associations with Treatment Resistant Hypertension in GenHAT. Int J Hypertens. 2013;2013:578578.
168. Sherva R, Ford CE, Eckfeldt JH, Davis BR, Boerwinkle E, and Arnett DK, Pharmacogenetic effect of the stromelysin (MMP3) polymorphism on stroke risk in relation to antihypertensive treatment: the genetics of hypertension associated treatment study. Stroke. 2011;42(2):330-5.
169. Lee SJ, Kim WJ, and Moon SK, TNF-alpha regulates vascular smooth muscle cell responses in genetic hypertension. Int Immunopharmacol. 2009;9(7-8):837-43.
170. Vionnet N, Hani EH, Dupont S, Gallina S, Francke S, Dotte S, et al., Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am J Hum Genet. 2000;67(6):1470-80.
171. Patel S, Flyvbjerg A, Kozakova M, Frystyk J, Ibrahim IM, Petrie JR, et al., Variation in the ADIPOQ gene promoter is associated with carotid intima media thickness independent of plasma adiponectin levels in healthy subjects. Eur Heart J. 2008;29(3):386-93.
172. Hivert MF, Manning AK, McAteer JB, Florez JC, Dupuis J, Fox CS, et al., Common variants in the adiponectin gene (ADIPOQ) associated with plasma adiponectin levels, type 2 diabetes, and diabetes-related quantitative traits: the Framingham Offspring Study. Diabetes. 2008;57(12):3353-9.
173. Heid IM, Wagner SA, Gohlke H, Iglseder B, Mueller JC, Cip P, et al., Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes. 2006;55(2):375-84.
174. Hoefle G, Muendlein A, Saely CH, Risch L, Rein P, Koch L, et al., The -11377 C>G promoter variant of the adiponectin gene, prevalence of coronary atherosclerosis, and incidence of vascular events in men. Thromb Haemost. 2007;97(3):451-7.
175. Ong KL, Li M, Tso AW, Xu A, Cherny SS, Sham PC, et al., Association of genetic variants in the adiponectin gene with adiponectin level and hypertension in Hong Kong Chinese. Eur J Endocrinol. 2010;163(2):251-7.

176. Gable DR, Matin J, Whittall R, Cakmak H, Li KW, Cooper J, et al., Common adiponectin gene variants show different effects on risk of cardiovascular disease and type 2 diabetes in European subjects. Ann Hum Genet. 2007;71(Pt 4):453-66.
177. Zhou L, Xi B, Wei Y, Pan H, Yang W, Shen W, et al., Association between adiponectin gene polymorphisms and coronary artery disease across different populations. Thromb Res. 2012;130(1):52-7.
178. Yang Y, Zhang F, Ding R, Wang Y, Lei H, and Hu D, Association of ADIPOQ gene polymorphisms and coronary artery disease risk: a meta-analysis based on 12 465 subjects. Thromb Res. 2012;130(1):58-64.
179. Leu HB, Chung CM, Lin SJ, Jong YS, Pan WH, and Chen JW, Adiponectin gene polymorphism is selectively associated with the concomitant presence of metabolic syndrome and essential hypertension. PLoS One. 2011;6(5):e19999.
180. Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, et al., Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet. 2002;11(21):2607-14.
181. Qi L, Li T, Rimm E, Zhang C, Rifai N, Hunter D, et al., The +276 polymorphism of the APM1 gene, plasma adiponectin concentration, and cardiovascular risk in diabetic men. Diabetes. 2005;54(5):1607-10.
182. Chiodini BD, Specchia C, Gori F, Barlera S, D'Orazio A, Pietri S, et al., Adiponectin gene polymorphisms and their effect on the risk of myocardial infarction and type 2 diabetes: an association study in an Italian population. Ther Adv Cardiovasc Dis. 2010.