LEANDRO DE MATTOS BOER MARTINS

VARIABILIDADE DA FUNÇÃO AUTONÔMICA EM PACIENTES COM HIPERTENSÃO ARTERIAL RESISTENTE

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VARIABILIDADE DA FUNÇÃO AUTONÔMICA EM PACIENTES COM HIPERTENSÃO ARTERIAL RESISTENTE

Leandro de Mattos Boer Martins

Tese de Doutorado apresentada à Pós-Graduação da Faculdade de Ciências Médicas da Universidade Estadual de Campinas - UNICAMP para obtenção do título de Doutor em Farmacologia. Sob orientação do Prof. Dr. Heitor Moreno Jr.

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Tudo o que fizerem,
seja em palavra ou em ação,
façam-no em nome do Senhor Jesus,
dando por meio dele graças a Deus Pai.
Colossenses 3.17

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

AOS	Apneia obstrutiva do sono
ARA	Antagonista do receptor de angiotensina
DM	Diabetes *mellitus*
DM2	Diabetes *mellitus* tipo 2
DRC	Doença renal crônica
eNOS	Óxido nítrico sintase
HAR	Hipertensão arterial resistente
HTN	Hipertensão arterial
IMC	Índice de massa corporal
LR	Receptor de leptina
LRa	Forma curta do receptor de leptina
NA	Noradrenalina
NO	Óxido nítrico
NF-kβ	Fator nuclear kappa-beta
RNAm	Ácido ribonucléico mensageiro
SNA	Sistema nervoso autônomo
SNP	Sistema nervoso parassimpático
SNS	Sistema nervoso simpático
SRAA	Sistema renina-angiotensina-aldosterona
TNF-α	Fator de necrose tumoral alfa
VFC	Variabilidade da freqüência cardíaca

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RESUMO

Considerando a forte associação entre a atividade do sistema nervoso autônomo, a obesidade e a resistência insulínica na hipertensão arterial resistente (HAR), esta pesquisa teve a finalidade de identificar a associação entre a função do sistema nervoso autonômico e importantes hormônios relacionados à síndrome cardiometabólica como adiponectina, leptina e aldosterona. Vinte e cinco pacientes portadores de hipertensão arterial resistente foram divididos em dois grupos: com (DM2) e sem diabetes *mellitus* tipo 2 (NDM2). Ambos os grupos foram avaliados em relação à variabilidade da frequência cardíaca (VFC) pelo sistema Holter de 24 horas, nos domínios do tempo e da frequência, e aos hormônios plasmáticos adiponectina, leptina e aldosterona. A análise dos resultados demonstrou maior disfunção autonômica e hipoadiponectinemia no subgrupo DM2 em relação ao subgrupo NDM2, correlação positiva entre VFC no domínio do tempo e a adiponectina no total de pacientes, ruptura do ritmo circadiano de ambos os grupos (tônus simpático aumentado no período noturno e diminuído no período diurno; tônus parassimpático aumentado no período diurno e diminuído no período noturno) e correlação positiva entre a banda de baixa de frequência em unidades normalizadas (LFnu) e aldosterona, e correlação negativa entre a banda de alta frequência em unidades normalizadas (HFnu) e aldosterona no total de pacientes e em ambos os grupos. O grupo DM2 obteve maiores valores de leptina e índice de massa corporal. Entretanto, não houve correlação entre a VFC e leptina em ambos os grupos. Desta forma, identificou-se ruptura do ritmo circadiano e a associação entre o balanço autonômico e os níveis de adiponectina e aldosterona plasmática na HAR com e sem diabetes tipo 2.

Palavras-chave: variabilidade da frequência cardíaca, aldosterona, adiponectina, leptina, hipertensão arterial resistente, sistema nervoso simpático, sistema nervoso parassimpático, ritmo circadiano.

ABSTRACT

Considering the strong association between the autonomic nervous system activity, obesity and insulin resistance in resistant hypertension (RH), this research aimed to identify the association of the autonomic nervous system function and important hormones related to the cardiometabolic syndrome such as adiponectin, leptin and aldosterone. Twenty five RH patients were divided into two groups: with (T2D) and without type-2 diabetes (NT2D). Both groups were evaluated regarding the heart rate variability (HRV) by the Holter system in 24 hours, in time and frequency domains, and the plasma hormones adiponectin, leptin and aldosterone. The analysis of the results demonstrated greater autonomic dysfunction and hypoadiponectinemia in T2D subgroup compared to the NT2D subgroup, positive correlation between HRV in time domain and adiponectin in all patients, circadian disruption in both groups (increased sympathetic drive during nighttime and decreased during daytime; increased parasympathetic drive during daytime and decreased during nighttime) and positive correlation between the low frequency band in normalized units (LFnu) and aldosterone, and negative correlation between the high frequency band in normalized units (HFnu) and aldosterone in all patients and both subgroups. The T2D subgroup had higher levels of leptin and body mass index. However, there was no correlation between HRV and leptin in both groups. Thereby, it was found circadian disruption and the relationship between autonomic balance and plasma adiponectin and aldosterone in RH with or without type 2 diabetes.

Key words: heart rate variability, aldosterone, adiponectin, leptin, resistant hypertension, sympathetic nervous system, parasympathetic nervous system, circadian rhythm.

1. INTRODUÇÃO

1.1 Aspectos preliminares

O conceito de que a origem da hipertensão essencial depende grandemente das alterações da homeostase do controle adrenérgico da pressão arterial foi desenvolvido há algumas décadas e recebeu continuamente evidências robustas de estudos experimentais e clínicos [1, 2].

Nos últimos anos, novas descobertas em relação ao papel do sistema nervoso simpático (SNS) na fisiopatologia da hipertensão arterial foram feitas. Entre elas, incluem-se a ativação simpática no desenvolvimento da hipertensão arterial (HTN) não apenas nos estágios iniciais da doença, mas na sua manutenção e agravamento com o surgimento de leões de órgão-alvo tais como hipertrofia ventricular esquerda, disfunção diastólica do ventrículo esquerdo e enrijecimento e remodelamento arteriolares [3-7].

Adicionalmente, foram descobertas fortes associações entre o SNS, as alterações hemodinâmicas (aumento da resistência vascular periférica, diminuição da distensibilidade arterial e redução da perfusão tecidual) e metabólicas (resistência insulínica, obesidade e dislipidemia), frequentemente encontradas no paciente hipertenso [8-13].

Logo, diante do acúmulo de evidências do comportamento da atividade simpática na doença hipertensiva associada a várias outras condições mórbidas como insuficiência cardíaca, obesidade e síndrome metabólica, o conceito de "reforço neuroadrenérgico" surgiu na literatura científica [1, 2]. Este conceito visa

demonstrar que a hiperativação simpática prepondera na doença hipertensiva com complicações clínicas [1, 2].

Diante da forte associação entre a atividade do sistema nervoso autônomo (SNA), a obesidade e a resistência insulínica na hipertensão resistente (HAR), os estudos que se seguem como capítulos desta tese de doutorado (modelo alternativo) visam à identificação da associação entre a atividade do sistema nervoso simpático e importantes hormônios relacionados à síndrome cardiometabólica como adiponectina, leptina e aldosterona.

1.2 A hipertensão arterial resistente e o sistema nervoso autônomo

A hipertensão resistente (HAR) é uma doença hipertensiva com complicações clínicas em que se figura o "reforço neuroadrenérgico" [14, 15]. O diagnóstico de HAR pode ser estabelecido pela pressão arterial que permanece acima das metas apesar do uso concomitante de três agentes anti-hipertensivos de diferentes classes farmacológicas. Idealmente, um destes três agentes deve ser um diurético e todos os fármacos devem estar com suas doses plenas. Ademais, o diagnóstico de HAR também é estendido aos pacientes cuja pressão arterial foi controlada com quatro ou mais medicações [14].

De acordo com as diretrizes da *American Heart Association* para HAR [14], baseando-se em dados demográficos e resultados dos estudos de Framingham e ALLHAT, os maiores preditores de ausência de controle pressórico são a idade

avançada, pressão arterial demasiadamente elevada na avaliação inicial, obesidade, excesso de ingestão de sódio, diabetes *mellitus* (DM) e doença renal crônica (DRC). O envelhecimento e sua interface com a ativação do SNS estão bem documentados [16-20]. Vários estudos demonstraram que toda a atividade nervosa simpática está aumentada com o envelhecimento e alguns índices de atividade simpática, especialmente a atividade simpática muscular, estão mais relacionadas à pressão arterial nas idades avançadas [16, 21].

Além do envelhecimento, obesidade, excesso de aldosterona, DM e apneia obstrutiva do sono (AOS) cobrem uma grande área do mosaico de características dos pacientes hipertensos resistentes [14, 22]. Em coortes com pacientes com HAR, a média do índice de massa corporal (IMC) foi maior do que 32 km/m², a prevalência de hiperaldosteronismo foi de aproximadamente 20% e os casos de AOS diagnosticados ou suspeitos foram de alta prevalência [23, 24]. Ademais, entre os pacientes com HAR, o hiperaldosteronismo foi mais prevalente nos pacientes com diagnóstico de AOS confirmado do que nos pacientes com baixo risco desta comorbidade. Os dados existentes suportam que a AOS, o excesso de aldosterona e a obesidade são comorbidades frequentes nos pacientes hipertensos resistentes e influenciam a atividade simpática (Figura 1).

Com relação à importância da resistência insulínica na HAR, estudos clínicos têm indicado que o controle adequado da pressão arterial em hipertensos diabéticos é obtido com a variação média de 2,8 a 4,2 fármacos anti-hipertensivos prescritos [25]. A intensidade com que a resistência insulínica diretamente contribui

para o desenvolvimento da HTN ou se simplesmente está associada a ela ainda não está completamente determinada [14]. Os efeitos fisiopatológicos atribuídos à resistência insulínica que podem contribuir para a piora da HTN incluem o aumento da atividade simpática, a proliferação das células musculares lisas dos vasos e o aumento da retenção de sódio [14].

O prognóstico dos pacientes com HAR comparado aos pacientes com HTN de mais fácil controle não foi avaliado profundamente até o momento. Presumivelmente, o prognóstico é menos favorável, visto que estes pacientes possuem um histórico prolongado de HTN não controlada somada a outros fatores de risco cardiovascular como diabetes, AOS, hipertrofia do ventrículo esquerdo e doença renal crônica. O grau de redução dos riscos cardiovasculares ao tratamento da HAR também é desconhecido [14].

A estratégia terapêutica na HAR visa ao bloqueio de todos os mecanismos possíveis para a elevação da pressão arterial. A terapia combinada com diurético permanece como “pedra angular” do tratamento atual [22]. Ademais, há consenso de que o tratamento da HAR necessitará da administração de três ou mais fármacos [26].

Estudos clínicos têm sugerido que a adição de espironolactona ou eplerenone aos regimes terapêuticos de pacientes hipertensos resistentes proporciona reduções pressóricas significativas [14, 26, 27]. Também é digno de nota que as reduções pressóricas foram similares em pacientes com e sem hiperaldosteronismo primário e que não foram preditas pelos níveis basais

plasmáticos ou urinários (24 horas) da aldosterona, atividade plasmática de renina ou relação aldosterona: renina [28].

Com relação às opções terapêuticas para a hiperatividade do SNS, além do bloqueio periférico dos receptores adrenérgicos com o uso de alfa e beta-bloqueadores, faz-se necessário mencionar as emergentes evidências da atividade simpato-inibitória dos antagonistas dos receptores de angiotensina (ARA) e mineralocorticóide [29-31].

Agentes de ação central são efetivos como anti-hipertensivos na HAR, mas possuem alta incidência de eventos adversos e falta de evidências de desfecho cardiovascular primário [14]. Entretanto, resultados promissores têm sido alcançados com recentes estudos intervencionistas de inibição simpática através da ativação de barorreceptores carotídeos com estímulos elétricos [32] e desnervação simpática renal seletiva [33].

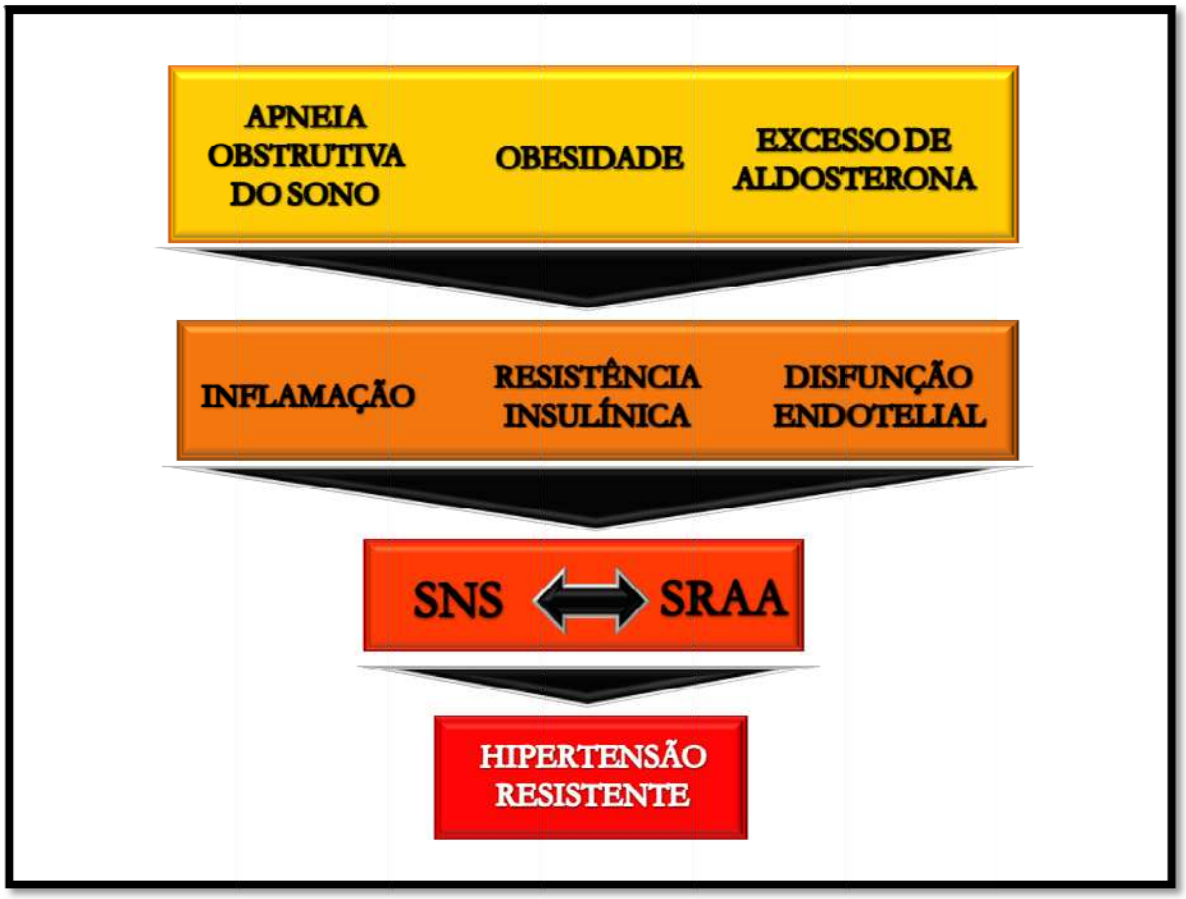

Figura 1: Fisiopatologia da HAR relacionada à hiperatividade simpática (Modificado de Tsioufis, Kordalis *et al.*) [22]. Condições como AOS, obesidade e excesso de aldosterona são fatores desencadeantes de inflamação, resistência insulínica e disfunção endotelial. Como conseqüência, há hiperativação do SNS e sistema renina-angiotensina-aldosterona (SRAA) que possuem, por suas vezes, a propriedade de hiperativação recíproca podendo incorrer em HAR.

1.3 A avaliação do sistema nervoso autônomo em pacientes hipertensos

Os métodos clínicos para avaliação regional da função do SNS em pacientes hipertensos são três: a eletrofisiologia (utilizando-se a microneurografia), a neuroquímica [utilizando-se a dosagem da diluição plasmática de isótopos de noradrenalina (NA) em veias de órgãos específicos como o coração e o rim] e a

utilização da eletrocardiografia ambulatorial, como o sistema Holter, para avaliação da variabilidade da freqüência cardíaca [2] (Figura 2).

A microneurografia é uma técnica que permite o estudo dos disparos nervosos em fibras simpáticas subcutâneas distribuídas na pele e no músculo esquelético. A técnica envolve a inserção de eletrodos de tungstênio posicionando-os sobre as fibras simpáticas, mais comumente, os nervos fibular ou mediano. Registros na forma de *bursts* da atividade nervosa autonômica, sincronizados com os batimentos cardíacos, são gerados [34].

A dosagem das taxas de NA liberada pode ser avaliada clinicamente utilizando-se medidas radioisotópicas de noradrenalina plasmática nos órgãos dos indivíduos. Como os métodos microneurográficos são incapazes de avaliarem a atividade dos nervos simpáticos dos órgãos internos, a dosagem de NA orgânica regional pode ser utilizada. Através da infusão de NA tritiada e as dosagens locais do plasma do seio coronário e das veias renais, é possível estimar o grau de liberação do neurotransmissor no coração e nos rins [35-37].

Embora a investigação clínica tenha aumentado o nosso conhecimento sobre a função do SNS e seu envolvimento na fisiopatologia de várias doenças cardiovasculares, incluindo a HTN, não há nenhuma recomendação para a estimativa da atividade adrenérgica na HAR. Apesar das avaliações da função adrenérgica feitas com a microneurografia da atividade simpática muscular e as medidas de NA plasmática em órgãos específicos terem sido realizadas em estudos clínicos, nenhuma técnica pode ser classificada como “padrão ouro” e as

metodologias descritas acima são basicamente utilizadas como ferramentas de investigação clínica para estudos científicos [38]. A freqüência cardíaca em repouso e ao estímulo são reguladas não apenas pelo sistema nervoso simpático, mas pelo sistema nervoso parassimpático (SNP), que é também dependente dos receptores cardíacos adrenérgicos. Ademais, a freqüência cardíaca em repouso na posição supina denota limitada correlação com outros índices da atividade simpática como a NA plasmática e atividade simpática muscular [39]. Com relação à excreção urinária de catecolaminas em 24 horas, a inabilidade de avaliação dinâmica da atividade do SNS e a dificuldade de se determinar se a origem das catecolaminas é sistêmica ou renal são dignas de nota [40]. Especificamente com relação aos níveis de NA plasmática, constituem-se limitações substancias à análise e interpretação dos dados a sua baixa reprodutibilidade, baixa sensibilidade e a incapacidade de discriminação se os níveis de NA são decorrentes do aumento da secreção ou diminuição do *clearance* dos níveis elevados de neurotransmissores circulantes [38, 41].

A análise do poder espectral da freqüência cardíaca é comumente aplicada como alternativa não-invasiva do estudo da atividade simpática do coração [42, 43]. A variabilidade da freqüência cardíaca (VFC) pode ser usada para avaliação dos distúrbios autonômicos, doenças e mortalidade geral [44]. As medidas da VFC nos domínios do tempo e da freqüência têm sido utilizadas com sucesso para avaliação do tônus simpático e parassimpático [45]. Embora haja ainda diferenças nos valores de referência da VFC em diversos estudos, há consenso de que menores valores destes índices referentes à função vagal estão

associados, prospectivamente, à morte e incapacidade [46]. A atividade parassimpática e a VFC estão associadas à disfunção imunológica e inflamação que estão, direta ou indiretamente, relacionadas à grande gama de doenças cardiovasculares e à diabetes [47, 48].

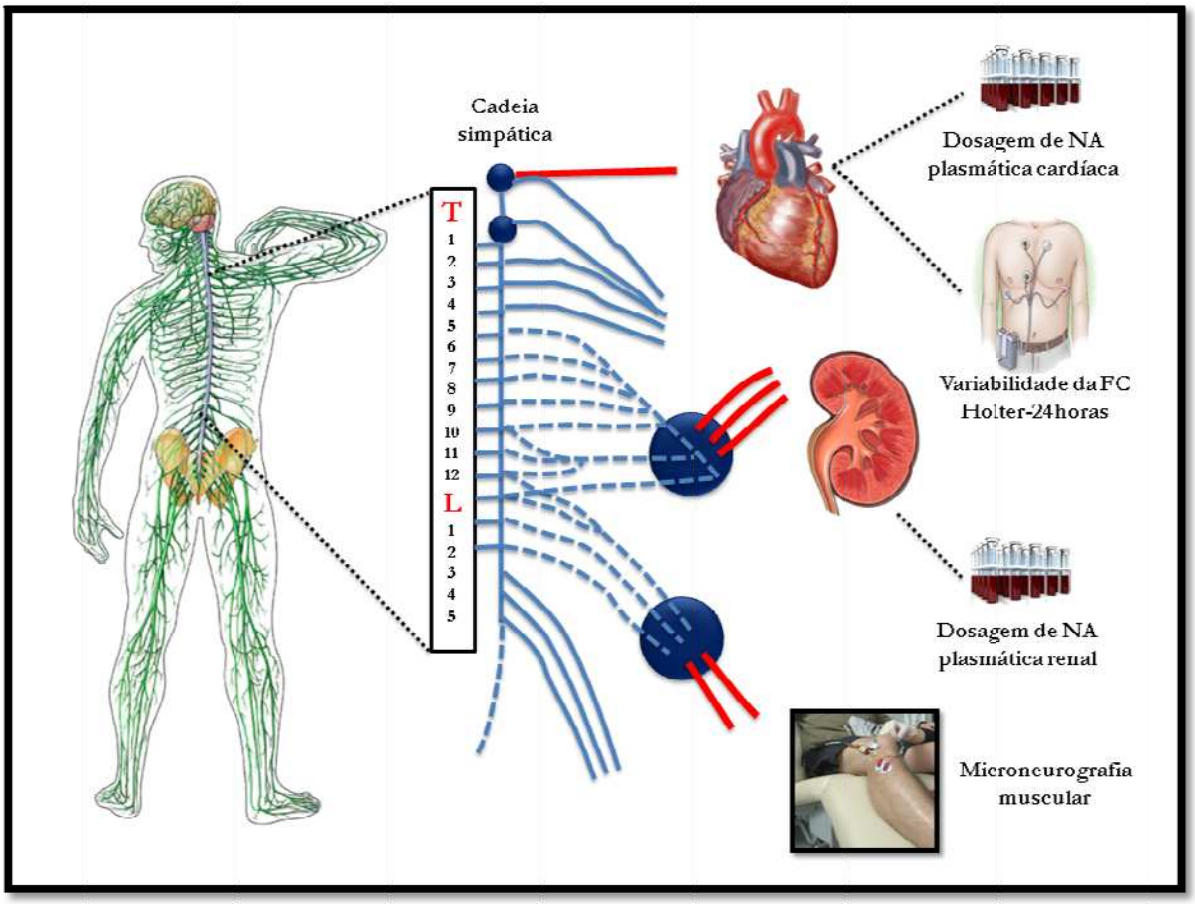

Figura 2: Métodos para avaliação da atividade simpática em pacientes hipertensos (Modificado de Esler) [2]. A dosagem plasmática de noradrenalina em órgãos como o coração e rim, a microneurografia do nervo fibular e a análise da variabilidade da freqüência cardíaca pela eletrocardiografia ambulatorial (Sistema Holter) são métodos utilizados para avaliação do sistema nervoso simpático em pacientes hipertensos.

1.3 O ritmo circadiano e o cosmo

A atividade do sistema nervoso autônomo não se encontra, porém, dissociada do meio ambiente [49]. A harmonia entre o cosmo e a vida no planeta Terra demanda a existência de um relógio biológico sincronizado com os ciclos regulares de nosso planeta no Sistema Solar [49]. O relógio biológico localizado essencialmente no sistema nervoso central no núcleo hipotalâmico supraquiasmático integra-se, por mecanismos ainda não bem compreendidos, com o relógio biológico de cada célula periférica de todo o organismo [50]. Esta adequação não é exclusiva dos seres humanos e o comportamento do relógio biológico de roedores, insetos, plantas, algas, fungos e até mesmo bactérias também está programado para ciclos de 24 horas [51-58].

A fisiologia cardiovascular e a homeostase do organismo estão relacionadas ao ritmo circadiano em ciclos endógenos com duração de aproximadamente 24 horas [59], adaptando-se perfeitamente à média do dia solar [60]. A freqüência cardíaca, pressão arterial, função endotelial e a secreção de hormônios relacionados ao metabolismo glicídico e lipídico demonstram variações durante o dia [61].

Entretanto, o estilo de vida ocidental tem sido modificado intensamente nas últimas décadas levando os seres humanos à ruptura do ritmo circadiano por três comportamentos preocupantes: a hiperalimentação, principalmente com alimentos gordurosos, o sedentarismo e o encurtamento do período de sono,

principalmente pela disponibilidade de luz artificial e a necessidade de agendamento de atividades laborais e recreativas durante o período noturno [62].

Esses comportamentos são influenciadores epigenéticos conhecidos como *zeitgebers* -"guardadores do tempo" – que têm a propriedade de promover o *reset* dos relógios biológicos podendo levar à dessincronização do ritmo circadiano que é conhecida como "ruptura do ritmo circadiano" [63] (Figura 3).

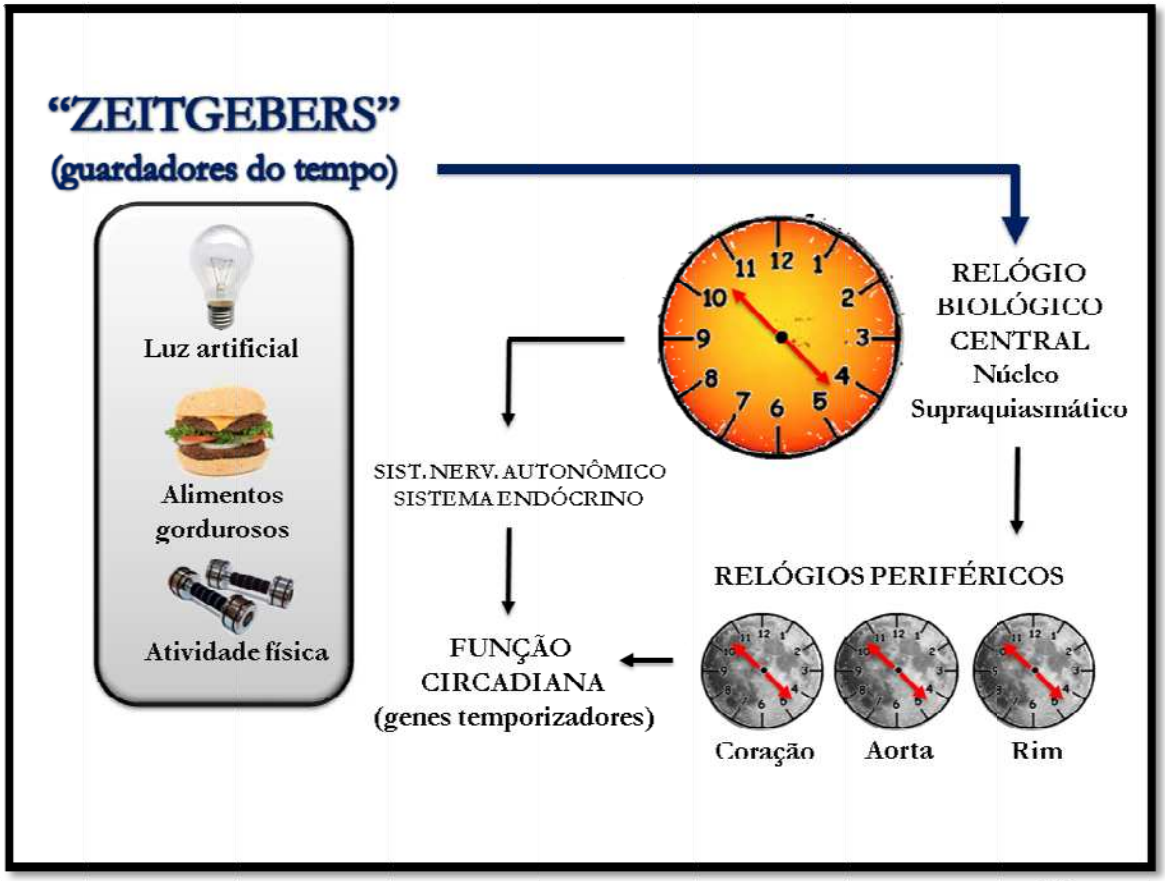

Figura 3: A ruptura do ritmo circadiano (Modificado de Maury, Ramsey *et al.*) [50]. Fatores conhecidos como *zeitgebers* (luz artificial, alimentos gordurosos e atividade física) influenciam o relógio biológico central localizado no núcleo supraquiasmático do hipotálamo reprogramando-o (*reset*) para a função diurna. As células periféricas também possuem um relógio biológico que se harmoniza com o relógio biológico central. Quando não há harmonização entre os relógios biológicos (central e periférico), há ruptura do ritmo circadiano.

1.4 A ruptura do ritmo circadiano e suas conseqüências fisiopatológicas

A ruptura do ritmo circadiano tem conseqüências fisiopatológicas de curto e longo prazo [50]. O aumento do apetite e diminuição do metabolismo da glicose e lípides com o distúrbio da secreção de adipocitocinas, vasopressina, aldosterona e outros reguladores da sensação de saciedade têm promovido o aumento do IMC e a deterioração do perfil metabólico laboratorial a níveis que se traduzem em maior risco cardiovascular acumulado, como o desenvolvimento de HTN, resistência insulínica e diabetes *mellitus* tipo 2 (DM2) [64-68] (Figura 4).

Desta forma, a compreensão de como o SNA desarmoniza-se com os relógios biológicos por meio de adipocitocinas e outros hormônios é fundamental para o aprofundamento do conhecimento da cronofarmacologia.

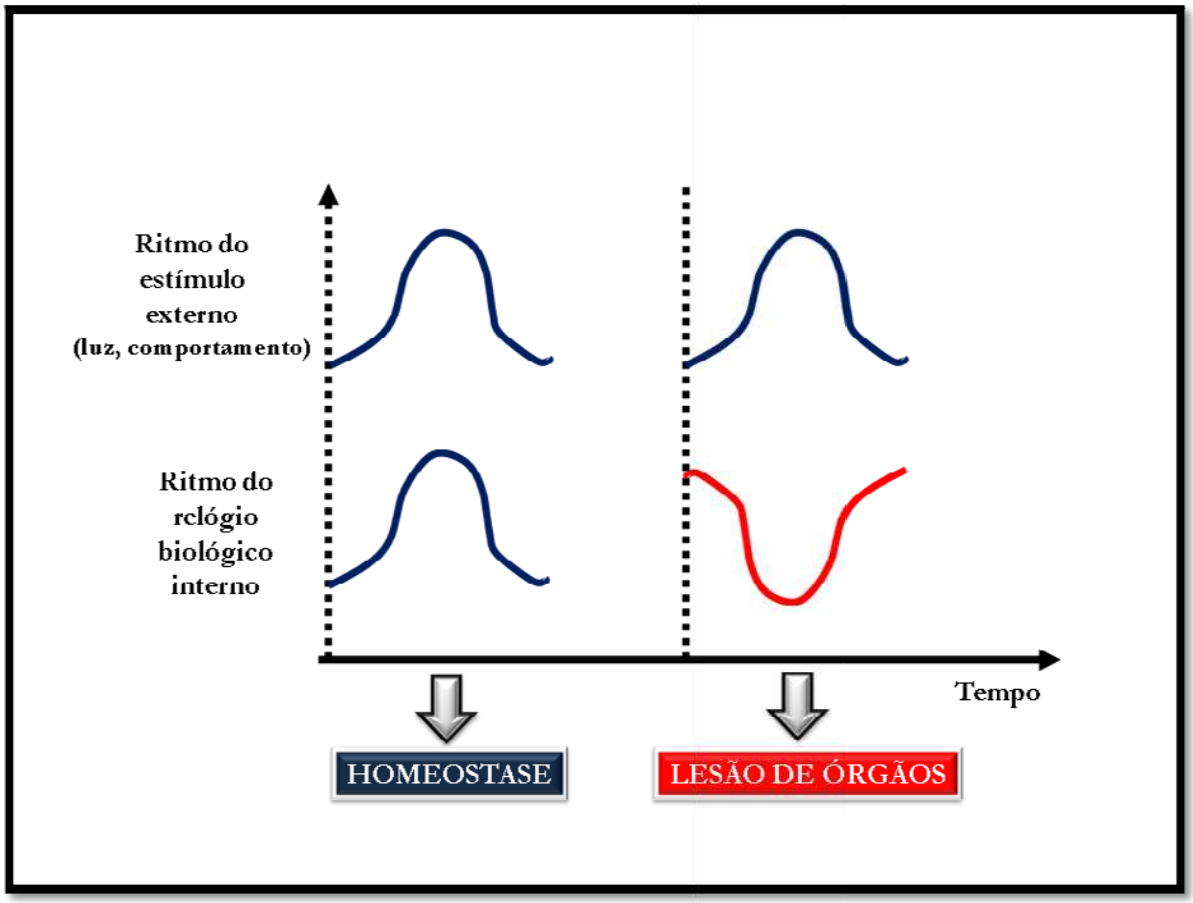

Figura 4: Ruptura do ritmo circadiano e complicações cardiovasculares (Modificado de Maury, Ramsey *et al.*) [50]. O sincronismo entre os ritmos do estímulo externo e do relógio biológico interno garantem a homeostase. Entretanto, na ocorrência da dessincronização destes ritmos há maior propensão de lesão de órgãos-alvo cardiovasculares.

1.5 O sistema nervoso autonômico e a adiponectina na doença hipertensiva

A atividade do sistema nervoso autônomo possui várias interfaces com o sistema endócrino através de importantes hormônios como as adipocitocinas (adiponectina e leptina).

A adiponectina é um hormônio produzido pelos adipócitos [69]. A adiponectina circulante existe no plasma humano nas formas de alto peso molecular monomérica, oligomérica e multimérica, e os efeitos biológicos de cada

uma destas isoformas permanecem desconhecidos [70]. Entretanto, sugere-se que a adiponectina de alto peso molecular tenha importância através dos receptores AdipoR1 e AdipoR2 [71] por sensibilização à insulina e efeitos vasoprotetores [70]. Não está bem estabelecido se a adiponectina melhora a captação de glicose mediada por insulina pelo músculo esquelético e supressão da produção hepática de glicose [72]. Adicionalmente, por promover oxidação de ácidos graxos, a adiponectina diminui o conteúdo de triglicérides tecidual no músculo esquelético e melhora a resistência insulínica [72, 73] (Figura 4). Em pacientes com DM2, baixos níveis de adiponectina estão associados com resistência insulínica e têm sido considerados um independente fator preditor de DM2 [74, 75].

Devido à sua ação no metabolismo de carboidratos, lípides e a homeostase energética geral [76], a adiponectina parece ter um importante papel na homeostase vascular humana. As evidências sugerem que a adiponectina afeta importantes mecanismos inflamatórios envolvidos na doença cardiovascular e especialmente a aterogênese. Demonstrou-se que a adiponectina interfere nas sinalizações intracelulares do fator nuclear kappa-β (NF-kβ) suprimindo, portanto, a expressão de moléculas de adesão [77]. Ademais, o fator de necrose tumoral alfa (TNF-α) - a molécula que inicia e organiza as mudanças inflamatórias no tecido vascular e cuja expressão é controlada pelo NF-kβ - parece ter relação recíproca com a adiponectina (Figura 4). Ratos sem expressão de adiponectina demonstram altos níveis de ácido ribonucléico mensageiro (RNAm) de TNF-α no tecido adiposo e altos níveis de TNF-α plasmática [78].

Também se demonstrou que a adiponectina possui a habilidade de regular negativamente a ativação de células *natural killers* (NK) induzidas pelo IL-2 e de inibir a síntese de citocinas inflamatórias derivadas do endotélio [79]. A adiponectina induziu a produção de mediadores anti-inflamatórios IL-10 e IL-1A em células inflamatórios e prejudicou a produção de inteferon-γ, outro alvo genético do NF-kβ [79] (Figura 4).

A adiponectina também possui efeitos sobre o endotélio vascular. Estudos *in vitro* demonstraram que a adiponectina estimula diretamente a produção endotelial de óxido nítrico pelas vias dependentes de PI-3, que aumentam a atividade da óxido nítrico sintase (eNOS) ativando sua AMPK induzida pela fosforilação [80]. Outros estudos em cultura de células demonstraram a habilidade da adiponectina na diminuição da produção induzida por TNF-α de dimetilarginina assimétrica - um análogo de L-arginina que inibe a formação de óxido nítrico (NO) e, portanto, pode prejudicar a função vascular [81]. Esta ativação e *up-regulation* da eNOS poderia explicar algumas das propriedades vasoprotetoras da adiponectina. Aparte dos efeitos benéficos sobre a eNOS, a adiponectina melhora o estado redox do endotélio pela supressão do NADPH-oxidase derivado da geração de superóxido [82]. As evidências também sugerem que a adiponectina de alto peso molecular suprime a apoptose das células endoteliais e promove a regeneração vascular e angiogênese [83, 84] (Figura 4).

A hiperativação do sistema nervoso simpático está associada à hipoadiponectinemia [85]. Há evidências de que a expressão de RNAm da

adiponectina foi inibida pela estimulação β-adrenérgica via proteína quinase A nos adipócitos 3T3L1 [86] e que a estimulação β-adrenérgica provocou o *down-regulation* do RNAm de tecidos viscerais e subcutâneos de roedores [87]. Baseando-se nestas observações experimentais, é possível postular que em paciente com resistência insulínica substancial hiperativação simpática pode reduzir a expressão gênica de adiponectina [85]. Entretanto, ainda não está claro se a concentração baixa de adiponectina, como a observada na prática clínica, é a causa ou o resultado da hiperatividade simpática. Em relação à função vagal, a sensibilidade barorreflexa foi menor em pacientes com hipoadiponectinemia do que em pacientes com normoadiponectinemia e foi, positivamente, correlacionada com a adiponectina plasmática. Devido à alta interação entre atividade simpática e vagal, é incerto se os baixos valores de sensibilidade barorreflexa observados em pacientes hipoadiponectinêmicos refletem, relativamente, a atividade vagal suprimida em resposta à hiperativação simpática [88].

Entretanto, apesar das evidências expostas acima, ainda há escassez de dados sobre a relação entre adiponectinemia, obesidade, DM2 e a função do SNA cardíaco na HAR.

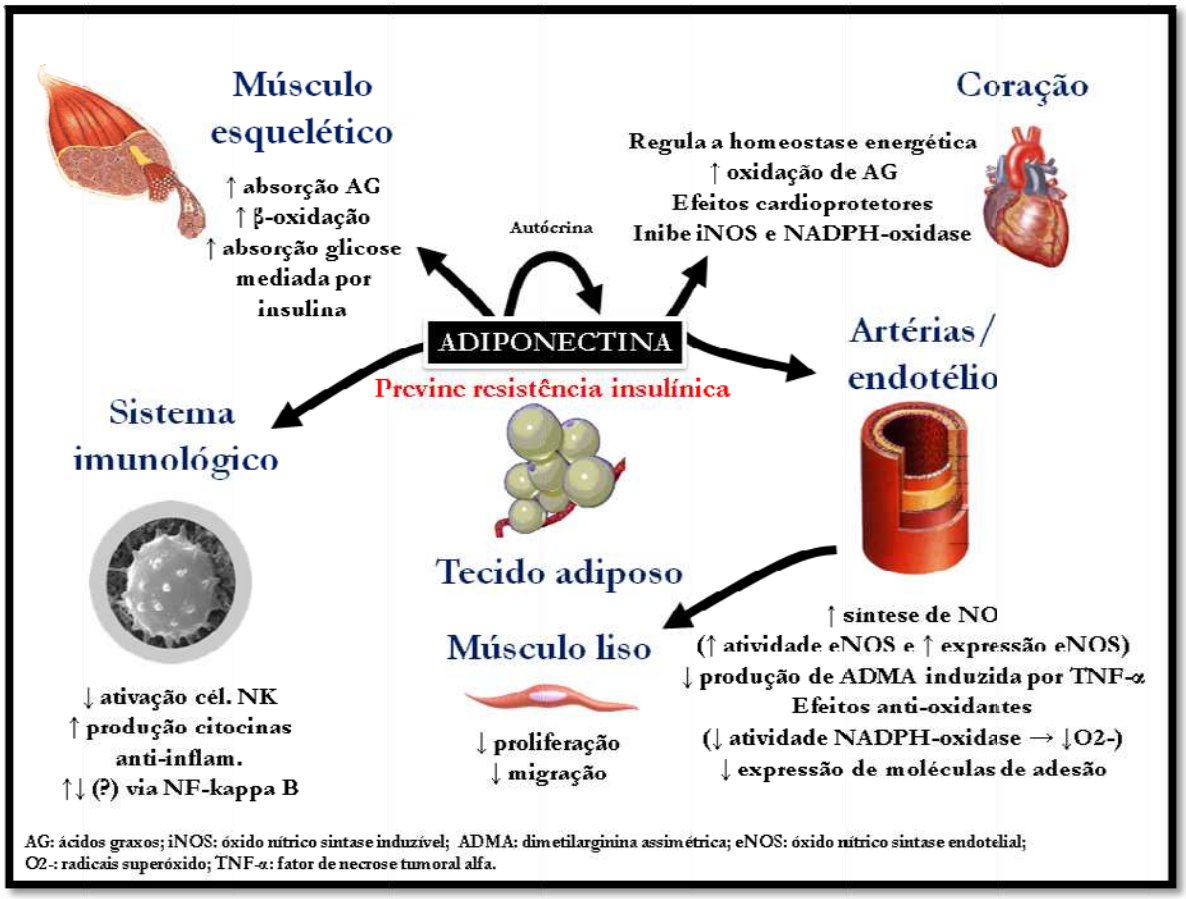

Figura 5: A relação entre adiponectina e os sistemas fisiológicos (Modificado de Antoniades, Antonopoulos *et al.*) [74]. A adiponectina produzida no tecido adiposo, além do seu efeito autócrino, colabora com a homeostase do músculo esquelético, sistema imunológico, vasos sanguíneos e miocárdio.

1.6 O sistema nervoso autonômico e a leptina na doença hipertensiva

Estudos iniciais com a leptina demonstraram que ela regula o apetite e aumenta o gasto energético pela ativação do sistema nervoso simpático termogênico do tecido adiposo marrom [89]. Outros efeitos benéficos como o aumento da excreção de sódio, manutenção do tônus vascular e até mesmo reparo do miocárdio têm sido associados com os efeitos da leptina em seus níveis normais plasmáticos [90]. Entretanto, altos níveis plasmáticos de leptina têm sido

associados com glomeruloesclerose, aumento do tônus vascular, diminuição da excreção de sódio, aumento da atividade do SNS, hipertrofia miocárdica e lesão vascular [90] (Figura 6).

A descoberta da leptina, hormônio produzido proporcionalmente ao grau de adiposidade, também trouxe novos conhecimentos sobre os mecanismos fisiopatológicos da obesidade e doenças associadas [91]. Foi demonstrado que a leptina ativa o SNS renal levando ao aumento da pressão arterial [89]. A leptina atravessa a barreira hematoencefálica pela via saturada do sistema de transporte mediada do receptor [92]. A forma curta do receptor de leptina (LRa), altamente expressada na microvasculatura cerebral, é tida como uma das principais transportadoras [92]. Entretanto, outros fatores modulam o transporte de leptina através da membrana hematoencefálica, e na obesidade, a eficiência da captação de leptina pelo cérebro está reduzida [92]. Altos níveis de triglicérides, os quais inibem o transporte de leptina através da barreira hematoencefálica, podem, parcialmente, explicar a redução da eficiência do transporte de leptina na obesidade [92].

A leptina se liga a vários receptores em várias regiões do sistema nervoso central, incluindo o hipotálamo e o sistema nervoso, onde ativa as vias que diminuem o apetite e aumentam a atividade do sistema nervoso simpático e gasto energético [93]. Evidências de que a leptina é um potente controlador do balanço energético provêm de estudos em ratos e seres humanos que demonstraram que mutações do gene da leptina ou de seu receptor (LR) causavam intenso quadro de

obesidade [94]. Entretanto, mutações do gene da leptina em seres humanos são raras e há poucas evidências de que as mutações do LR contribuam significativamente para o ganho de peso na maioria das pessoas obesas [95]. Todavia, a obesidade pode causar “resistência” aos efeitos anorexígenos da leptina, talvez analogamente à resistência induzida por obesidade dos efeitos metabólicos da insulina [95].

Injeções agudas de leptina possuem pouco efeito sobre a pressão arterial apesar da ativação do SNS, provavelmente devido ao efeito vasodilator da produção de NO que também é estimulada pela leptina [89]. Igualmente, os aumentos da atividade do SNS mediados pela leptina podem não ser tão intensos ao ponto de provocarem vasoconstrição periférica e aumento agudo da pressão arterial [89].

Entretanto, aumentos crônicos da leptina plasmática, comparáveis àqueles encontrados na obesidade grau III, podem aumentar a pressão arterial. Os aumentos da pressão arterial mediados pela leptina ocorrem no período de alguns dias e são completamente abolidos pelo bloqueio α e β-adrenérgico, indicando que eles são mediados pela ativação do SNS [89]. O fato de que o aumento da pressão arterial ocorre vagarosamente com a hiperleptinemia sugere que não há um massivo aumento da atividade do SNS capaz de causar intensa vasoconstrição periférica; ao invés, o aumento da atividade adrenérgica aumenta a pressão arterial por mecanismos mais lentos, incluindo os seus efeitos renais [89].

Os efeitos hipertensivos da leptina em animais magros são modestos, mas ocorrem apesar da diminuição da alimentação e perda de peso que deveriam, por outro lado, tender à redução da pressão arterial [89]. Ademais, os efeitos hipertensivos crônicos da leptina são exacerbados pela redução da síntese de NO, como ocorre frequentemente em indivíduos obesos com disfunção endotelial. Logo, o aumento da leptina, comparável com o achado em indivíduos obesos, pode aumentar a pressão arterial por ativação adrenérgica especialmente quando a síntese de NO está prejudicada [89].

Outras evidências possíveis para a ligação entre a leptina, obesidade e HTN provêm da observação de que a deficiência de leptina está presente em ratos não-hipertensos apesar da obesidade grave, resistência insulínica e dislipidemia [96]. Resultados similares foram encontrados em crianças obesas com mutações genéticas da leptina que apresentavam pressão arterial normal apesar da presença de obesidade e outras características da síndrome metabólica, incluindo a resistência insulínica e dislipidemia [97]. Essas crianças tiveram a atividade do SNS diminuída ao invés de aumentada, assim como hipotensão postural e resposta atenuada do SRAA na posição ereta [97].

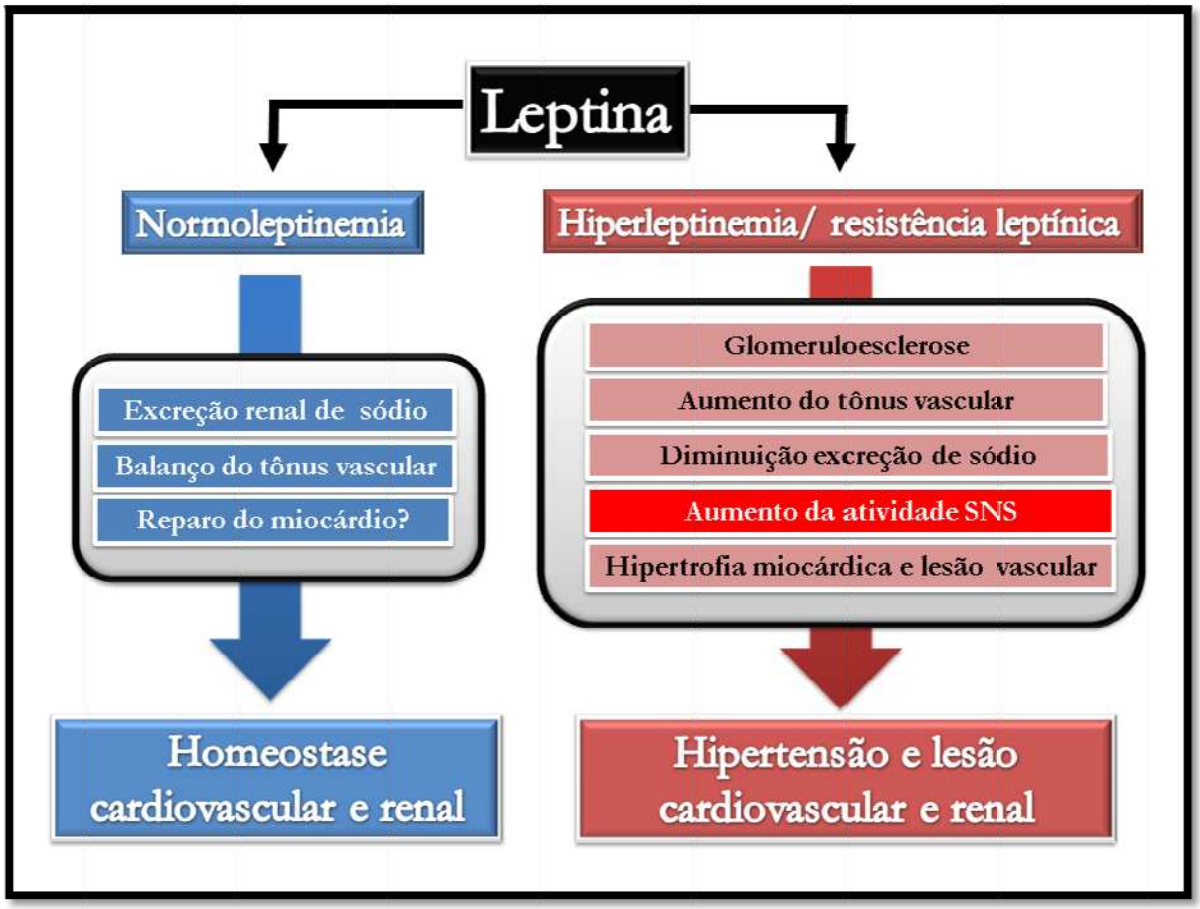

Figura 6: Os níveis plasmáticos de leptina e seus efeitos biológicos (Modificado de Kshatriya, Liu *et al.*) [90]. Níveis normais de leptina promovem excreção renal de sódio, balanço do tônus vascular e, possivelmente, reparo do miocárdio, cooperando para a homeostase cardiovascular e renal. Níveis elevados de leptina com resistência leptínica podem provocar glomeruloesclerose, aumento do tônus vascular, diminuição da excreção de sódio, aumento da atividade do sistema nervoso simpático, hipertrofia miocárdica e lesão vascular, predispondo à hipertensão e lesão cardiovascular e renal.

1.7 O sistema nervoso autonômico e a aldosterona na doença hipertensiva

A aldosterona, a proteína conhecida há mais tempo entre os três hormônios abordados nesta revisão, figura entre os principais fatores causadores de distúrbios cardiovasculares [98]. Os efeitos clássicos da aldosterona estão relacionados ao balanço hidroeletrolítico de sódio e potássio levando à expansão

do volume intravascular e hipocalemia [22]. Entretanto, a aldosterona também promove mudanças mal adaptadas renais, cardiovasculares e do sistema nervoso central levando à hiperativação simpática [98]. Há evidências recentes de que o tecido adiposo produz um fator lipofílico que estimula a secreção de aldosterona [99]. Em contrapartida, há também evidências de que tanto a aldosterona como os glicocorticóides podem interagir via receptores de mineralocorticóides na promoção da adipogênese e aumento da infiltração macrofagocitária no tecido adiposo [99, 100]. Desta forma, há nítida interação de coestimulação entre o tecido adiposo, o córtex adrenal e a aldosterona: adipócitos aumentam a produção de aldosterona e glicocorticóides e estes hormônios, por sua vez, promovem a adipogênese, inflamação do tecido adiposo [101] (Figura 7).

Evidências recentes sugerem que o aumento não genômico da sinalização dos receptores de mineralocorticóide, em resposta aos elevados níveis de aldosterona, está envolvido na fisiopatologia da resistência insulínica e outros componentes da síndrome cardiometabólica [98] (Figura 7). De fato, os receptores de mineralocorticóide possuem alta afinidade tanto pela aldosterona como pelas 11-β-hidróxiglicocorticóides, os quais estão frequentemente elevados nos estados clínicos de obesidade central como a síndrome cardiometabólica [98]. A enzima 11-β-hidróxi-esteroide desidrogenase, que previne a sinalização dos glicocorticóides através do receptor de mineralocorticóides, está presente em níveis mais baixos em tecidos cardiovasculares e metabólicos como o músculo esquelético, fígado e tecido adiposo, permitindo, portanto, que tanto a aldosterona como os 11-β-hidróxiglicocorticóides atuem através do receptor de mineralocorticóide na

sinalização insulínica e subseqüente remodelamento tecidual mal adaptado [102, 103]. Este fato é de significativa importância visto que as concentrações de glicocorticóides são, geralmente, bem maiores que a de aldosterona [98].

Outros mecanismos de resistência insulínica mediada pela aldosterona têm sido sugeridos incluindo seus efeitos negativos sobre a função da célula β pancreática e a estimulação da gliconeogênese hepática. Neste contexto, a hipocalemia tem sido demonstrada como atuante direto na função da célula β pancreática [23, 98, 104].

A resistência insulínica, por sua vez, parece possuir forte influência sobre a HAR através da disfunção endotelial [101]. Na resistência insulínica, há diminuição da estimulação da bioatividade do NO com diminuída ativação da NO sintase (e aumento da destruição de NO), diminuição da vasodilatação e prejuízo do transporte de substratos para a produção de NO [98]. As evidências demonstram que níveis elevados de aldosterona contribuem para a diminuição da sinalização metabólica insulínica no tecido vascular [105-108]. O aumento da produção de NO tem importante papel na sinalização metabólica insulínica mediada pela aldosterona e angiotensina II. A hiperativação do SRAA, que gera espécies reativas de oxigênio, promove a ativação de quinases de serina redox-sensíveis, as quais promovem a fosforilação da serina dos substratos tipo 1 dos receptores de insulina. Este aumento de fosforilação da serina nestes receptores reduz a ligação da fosfoinositol 3-quinase resultando na diminuição da proteína quinase B (Akt) e ativação atípica da proteína quinase da fosforilação da NO

sintase endotelial [105-108]. Como resultado, indivíduos com resistência à insulina, obesidade e níveis elevados de aldosterona são mais propensos à disfunção endotelial e subseqüente desenvolvimento de HTN [101] (Figura 7).

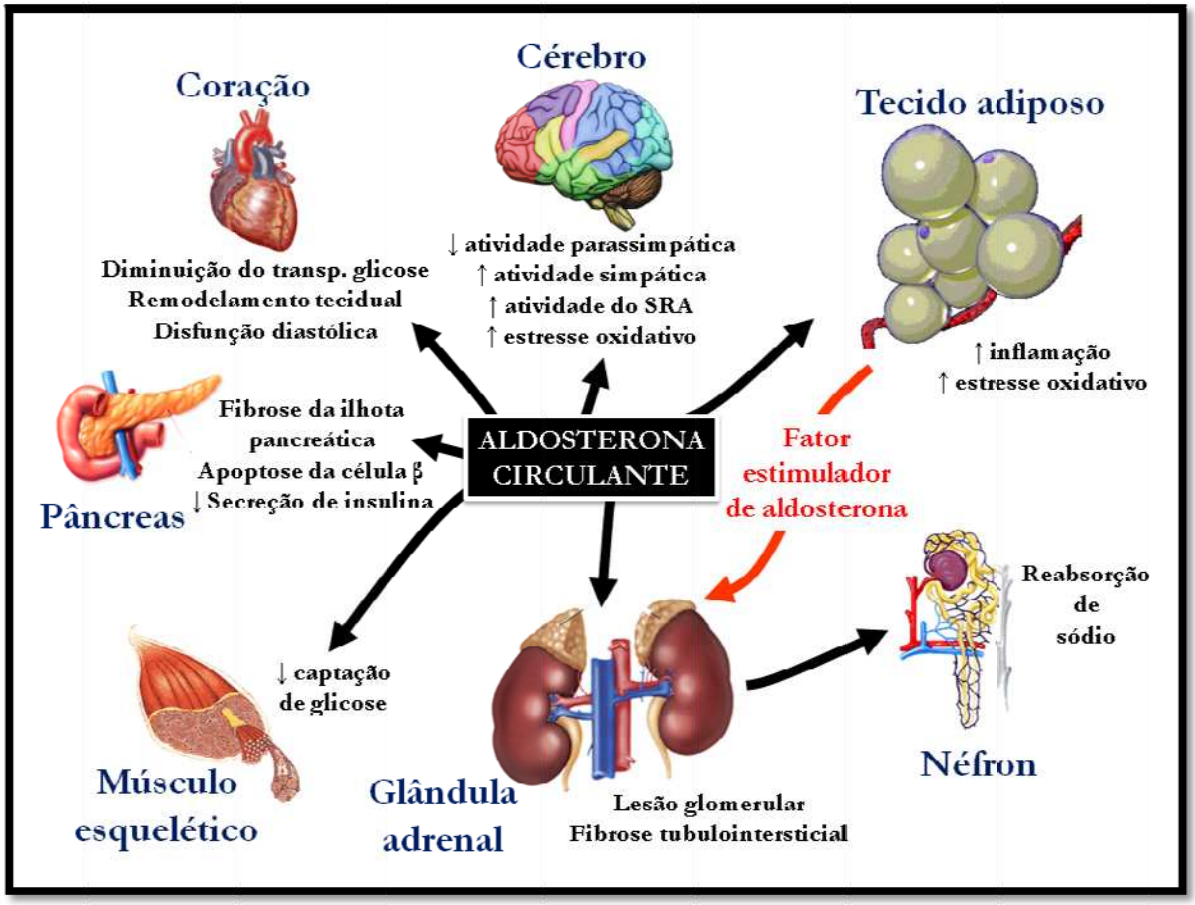

Figura 7: O fator estimulador da secreção de aldosterona (Modificado de Sowers, Whaley-Connell *et al.*) [98]. O tecido adiposo produz um fator estimulador de aldosterona que ao agir sobre as glândulas adrenais promove o aumento da aldosterona circulante. Além de promover a reabsorção de sódio nos túbulos coletores, o aumento da aldosterona circulante está associado com várias alterações no organismo como o aumento da atividade do SNS, diminuição da atividade do SNP, disfunção diastólica miocárdica, apoptose das células beta pancreáticas, fibrose da ilhota pancreática, diminuição da captação de glicose pelo músculo esquelético e aumento do estresse oxidativo.

2. OBJETIVOS

2.1 Subestudo 1

O objetivo primário do primeiro estudo visou à averiguação das seguintes hipóteses:

- **Hipótese nula:** Não há diferença da VFC entre hipertensos resistentes com e sem DM2.
- **Hipótese alternativa:** Há diferença da VFC entre os hipertensos resistentes com e sem DM2.

Os objetivos secundários do estudo compreenderam: avaliação dos índices de adiponectina e suas correlações com a VFC em pacientes hipertensos resistentes com ou sem DM2.

2.2 Subestudo 2

O objetivo primário do segundo estudo visou à averiguação das seguintes hipóteses:

- **Hipótese nula:** Não há correlação entre a leptina e aldosterona plasmáticas com a atividade SNS através do estudo da VFC no domínio do tempo.

- **Hipótese alternativa:** Há correlação entre a leptina e aldosterona plasmáticas com a atividade do SNS através do estudo da VFC no domínio do tempo.

3. CONCLUSÕES

3.1 Subestudo 1

1. Os pacientes hipertensos resistentes com DM2 possuem menores índices de VFC do que os pacientes hipertensos resistentes sem DM2.
2. Os índices de adiponectina correlacionaram-se com a VFC demonstrando associação entre hipoadiponectinemia e disfunção autonômica em pacientes hipertensos resistentes com ou sem DM2.
3. Os pacientes hipertensos resistentes que participaram deste estudo demonstram padrão de ruptura do ritmo circadiano à análise da VFC no domínio da freqüência (período diurno: tônus parassimpático maior do que o tônus simpático; período noturno: tônus simpático maior do que o tônus parassimpático).

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3.2 Subestudo 2

1. A aldosterona correlacionou-se com a atividade do SNS à análise da VFC no domínio da freqüência em pacientes hipertensos resistentes com e sem DM2.

2. A leptina plasmática não se correlacionou com a atividade do SNS à análise da VFC no domínio da freqüência em pacientes hipertensos resistentes com e sem DM2.

(Trabalho submetido à revista Arquivos Brasileiros de Cardiologia no dia 1 de novembro de 2011)

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CARDIO VASCULAR DIABETOLOGY

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Relationship of autonomic imbalance and circadian disruption with obesity and type 2 diabetes in resistant hypertensive patients

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Abstract

Background: Hypertension, diabetes and obesity are not isolated findings, but a series of interacting interactive physiologic derangements. Taking into account genetic background and lifestyle behavior, AI (autonomic imbalance) could be a common root for RHTN (resistant hypertension) or RHTN plus type 2 diabetes (T2D) comorbidity development. Moreover, circadian disruption can lead to metabolic and vasomotor impairments such as obesity, insulin resistance and resistant hypertension. In order to better understand the triggered emergence of obesity and T2D comorbidity in resistant hypertension, we investigated the pattern of autonomic activity in the circadian rhythm in RHTN with and without type 2 diabetes (T2D), and its relationship with serum adiponectin concentration.

Methods: Twenty five RHTN patients (15 non-T2D and 10 T2D, 15 males, 10 females; age range 34 to 70 years) were evaluated using the following parameters: BMI (body mass index), biochemical analysis, serum adiponectinemia, echocardiogram and ambulatory electrocardiograph heart rate variability (HRV) in time and frequency domains stratified into three periods: 24 hour, day time and night time.

Results: Both groups demonstrated similar characteristics despite of the laboratory analysis concerning T2D like fasting glucose, HbA1c levels and hypertriglyceridemia. Both groups also revealed disruption of the circadian rhythm: inverted sympathetic and parasympathetic tones during day (parasympathetic > sympathetic tone) and night periods (sympathetic > parasympathetic tone). T2D group had increased BMI and serum triglyceride levels (mean 33.7 ± 4.0 *vs* 26.6 ± 3.7 kg/m^2 - p = 0.00; 254.8 ± 226.4 *vs* 108.6 ± 48.7 mg/dL - p = 0.04), lower levels of adiponectin (6729.7 ± 3381.5 *vs* 10911.5 ± 5554.0 ng/mL - p = 0.04) and greater autonomic imbalance evaluated by HRV parameters in time domain compared to non-T2D RHTN patients. Total patients had HRV correlated positively with serum adiponectin (r = 0.37 [95% CI -0.04 - 1.00] p = 0.03), negatively with HbA1c levels (r = -0.58 [95% CI -1.00 - -0.3] p = 0.00) and also adiponectin correlated negatively with HbA1c levels (r = -0.40 [95% CI -1.00 - -0.07] p = 0.02).

Conclusion: Type 2 diabetes comorbidity is associated with greater autonomic imbalance, lower adiponectin levels and greater BMI in RHTN patients. Similar circadian disruption was also found in both groups indicating the importance of lifestyle behavior in the genesis of RHTN.

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Background

Hypertension, diabetes and obesity are not isolated findings, but a series of interactive physiologic derangements [1]. For instance, it is well known that obesity and diabetes mellitus are factors associated with resistance to antihypertensive drugs. An understanding of interactions among these pathophysiologic pathways can assist in choosing treatment and thereby improving total cardiovascular risk management [1].

Autonomic imbalance, characterized by a hyperactive sympathetic system and a hypoactive parasympathetic system, is associated with various pathological conditions [2,3]. Over time, excessive energy demands on the system can lead to premature aging and diseases [2,3]. Therefore, autonomic imbalance may be a final common pathway to increased morbidity and mortality from a host of conditions and diseases, including cardiovascular disease [4,5].

Heart rate variability (HRV) may be used to assess autonomic imbalances, diseases and mortality [6]. Measures of heart rate variability (HRV) in both time and frequency domains have been used successfully to index vagal activity [7]. Nevertheless, while there are some differences among HRV parameters found in many studies, the consensus is that lower values of these indices of vagal function are associated prospectively with death and disability [8]. Parasympathetic activity and HRV have been associated to immune dysfunction and inflammation, which have been implicated in a wide range of conditions including CVD and diabetes [2,3].

There is a pathogenic link between autonomic imbalance and insulin resistance and hypertension onset [9-14]. In addition to genetic background and environment, AI (autonomic imbalance) could be a common root of HTN (hypertension) or HTN plus T2D (type 2 diabetes) comorbidity development. T2D comorbidity can be added to HTN by decreased energy dissipation, gaining weight and then insulin resistance [15]. It is known that a chronic increase in sympathetic outflow has been reported to decrease β-adrenergic responsiveness itself, by a down-regulation of β-adrenergic receptors, which are known to mediate energy expenditure either at rest or after food intake [16].

These obesity-related disorders including metabolic syndrome, diabetes, atherosclerosis, hypertension, and coronary artery disease are associated with dysregulated adipokine(s) expression such as adiponectin [17].

Adiponectin is a hormone that is produced by adipocytes [18]. In patients with type 2 diabetes mellitus, low plasma adiponectin levels are associated with insulin resistance and have also been shown to be an independent predictor of type 2 diabetes mellitus [19]. In addition, sympathetic nervous overactivity is associated with hypoadiponectinemia [20,21]. However, there is still limited information on the relationship between plasma adiponectin, obesity, T2D and cardiac autonomic nervous function, especially in resistant hypertension (RHTN).

In order to better understand the triggered emergence of obesity and T2D comorbidity in resistant hypertension, we investigated the pattern of autonomic activity in the circadian rhythm in this population with and without type 2 diabetes (T2D) and its relationship with serum adiponectin concentration.

Methods

Twenty-five (25) RHTN subjects [22] [15 non-T2D and 10 T2D, 15 (60%) females and 10 (40%) males], regularly followed in the ambulatory service of cardiovascular clinical pharmacology, complying with pharmacological prescription for HTN and T2D, were recruited to participate in this transversal study. The diagnosis of resistant hypertension required a good office blood pressure measurement technique and ambulatory blood pressure monitoring (ABPM) to confirm persistently elevated blood pressure levels [23]. Pseudoresistance cases, including lack of blood pressure control secondary to poor medication adherence, were properly observed and excluded [24]. White coat hypertension (WCH) was excluded by ABPM [23]. Regarding obstructive sleep apnea (OSA), only patients classified as "low risk" by Berlin sleep questionnaire were enrolled [25]. Resistant hypertension include patients whose blood pressure is uncontrolled with use of more than three medications or patients whose blood pressure is controlled, but required four or more medications to achieve blood pressure goals [23]. All subjects provided written informed consent and the study was approved by the local ethics committee.

The exclusion criteria comprised: acute or moderate-severe renal dysfunction, non-complied pharmacological prescription, use of beta-blockers within the last six months, severe obesity (body mass index ≥ 35 kg/m^2), heart failure (ejection fraction < 50%), valvular heart disease, cardiomyopathies, primary hyperaldosteronism [aldosterone:PRA ratio > 20 ng per 100 mL per ng.ml (-1)h(-1)], sleep apnea (classified as "high risk" by the Berlin sleep questionnaire), atrial fibrillation, sick sinus syndrome, supraventricular and ventricular tachycardias, aortic disease (Marfan's syndrome, coarctation of the aorta, aneurysms or aortic surgery, etc), history of coronary artery disease or proven coronary artery disease by coronary angiography or noninvasive tests, familial hyperlipidemia, asthma or chronic obstructive lung disease, pregnancy or oral contraceptive use, connective tissue disorders, neurological problems, malignancies, psychiatric diseases, other than T2D endocrinological diseases, smoking, alcohol use and drug abuse.

Blood pressure measurements

Blood pressure was assessed by considering the orientations of the last guideline on hypertension of the European Society of Cardiology [26]. Blood pressure (SBP - systolic blood pressure/DBP - diastolic blood pressure) was measured three times for each subject using a digital sphygmomanometer (Omron HEM-711DLX) on the right upper arm in the sitting position after a 10-minute rest. The average of two consecutive measurements was used with a variation lower than 5 mmHg.

Laboratory analysis

All subjects underwent the following laboratory tests: hemogram, serum fasting glucose, glycolized hemoglobin (HbA1c), serum urea and creatinine, serum total cholesterol, serum LDL-cholesterol fraction, serum HDL-cholesterol fraction, serum triglycerides, serum uric acid, serum sodium and potassium, and plasma adiponectin levels (Quantikine® Human total adiponectin/Acrp30 Immunoassay DRP 300, R&D Systems, Inc., Minneapolis, USA).

Echocardiographic examination

All subjects were submitted to standard transthoracic echocardiography in the left lateral decubitus position by using a Vivid 7 Pro machine with a 2.5 Mhz probe (General Electric, Florida, USA). Standard transthoracic views were used to determine end-diastolic and end-systolic volumes, stroke volume index, left ventricular ejection fraction (LVEF), transmitral E and A waves velocities, E/A ratio, tissue doppler velocity of the mitral annulus and left ventricular mass index (LVMI). The left ventricular diastolic dysfunction (LVDD) was assessed by the Omnen SR and Nishimura RA algorithm[27]. The echocardiographic examination was performed by only one experienced cardiologist examiner. There was no intra or inter-observer measurement variability.

Heart rate variability

Heart rate variability (HRV) parameters were derived from the recording of 24-hour Holter monitoring and analyzed in time and frequency domains. Measures were stratified into three time periods for time domain: 24 hour period (24 h), day time period (DT), 1 p.m. to 5 p.m. and night time period (NT), from 2 a.m to 6 a.m. Frequency domain measures were stratified into two periods of one hour each at 3 a.m. (night time period - NT) and 3 p.m. (day time period - DT). A three-channel, 24-hour Holter recording was obtained from each subject using the Cardio light digital 24-hour recorder device and the CardioSmart Institutional CS 550 software (Cardio Sistema Comércio e Indústria Ltda, São Paulo, SP, Brazil).

Time domain HRV parameters included the following measures [6,28]:

- rMSSD (ms): Square root of the mean of the squares of differences between successive RR intervals.
- SDNN (ms): Standard deviation of all normal RR intervals in 24-hour Holter recording.
- SDANN (ms): Standard deviation of RR intervals means in all 5-minute segments of 24-hour recording.
- pNN50 (%): Percentage of differences between successive RR intervals that are greater than 50 ms.

Frequency domain measures were calculated using the Fast Fourier Transform (FFT) to break down the time series to its underlying periodic function. Frequency domain HRV parameters included the following measures [6,28]:

- Low frequency (LF) and high frequency (HF) measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the very low frequency (VLF) component. Normalized LF (LF nu) was calculated as LF power in normalized units LF/(total power-VLF) × 100, and normalized HF (HF nu) as HF power in normalized units HF/(total power-VLF) × 100. Low frequency (LF) and high frequency (HF). LF nu and HF nu denote the energy in the heart period power spectrum between 0.04 and 0.15 Hz (which is due to the joint action of the vagal and sympathetic components on the heart, with a predominance of the sympathetic ones) and 0.15 and 0.40 Hz (which corresponds to the respiratory modulation and is an indicator of the performance of the vagus nerve on the heart), respectively. "Day time" and "night time" were established at 3:00 p.m. and 3:00 a.m., respectively, in order to collect HRV data during wake and sleep periods.

Statistical analysis

Data were expressed as mean (μ) and standard deviation (SD) or mean (μ) and standard error of the mean (SEM) for HRV measures. Unpaired groups were compared using Mann-Whitney U test while correlation analysis were performed using Spearman's rank test. Fisher's exact test was used to determine whether a certain group had significantly different proportion of a particular characteristic. The level of statistical significance accepted was less than 0.05. All data were entered into a spreadsheet program (MS Excel Microsoft Corp,

Table 1 General characteristics of study groups

Characteristic/Variable	Non-T2D group (n = 15)	T2D group (n = 10)	*p*-value
Gender	60% (female)/40% (male)	60% (female)/40% (male)	1.00
Age (year)	54.7 ± 10.0 [49.2 - 60.3]	54.9 ± 8.7 [48.7 - 61.1]	0.89
Hemoglobin (g/dL)	13.6 ± 1.1 [13.0 - 14.3]	13.6 ± 1.4 [12.6 - 14.6]	0.97
Hematocrit (%)	41.1 ± 2.7 [39.6 - 42.7]	40.4 ± 4.0 [37.5 - 43.3]	0.56
Body mass index (kg/m^2)*	26.6 ± 3.7 [24.5 - 28.7]	33,7 ± 4,0 [30.8 - 36.6]	**0.00**
Fasting glucose (mg/dL)*	92.9 ± 9.2 [87.9 -98.0]	167.8 ± 64.0 [117.8 - 258.2]	**0.00**
HbA1c (%)*	5.8 ± 0.3 [5.6 - 6.0]	9.3 ± 2.1 [7.7 - 10.8]	**0.00**
Serum adiponectin (ng/mL)*	10911.5 ± 5554.0 [7835.7 - 13987.2]	6729.7 ± 3381.5 [4310.8 - 9148.7]	**0.04**
Serum urea (mg/dL)	34.2 ± 12.8 [27.1 - 41.3]	38.0 ± 7.8 [32.4 - 43.6]	0.46
Serum creatinine (mg/dL)	0.9 ± 0.2 [0.8 - 1.0]	0,9 ± 0.1 [0.7 - 1.0]	0.93
Total cholesterol (mg/dL)	193.7 ± 48.8 [166.7 - 220.8]	193.5 ± 33.2 [169.8 - 217.2]	0.36
LDL-cholesterol (mg/dL)	119.4 ± 47.0 [93.4 - 145.4]	104.3 ± 31.1 [82.0 - 126.6]	0.84
HDL-cholesterol (mg/dL)	50.8 ± 17.0 [41.4 - 60.2]	42.9 ± 11.0 [35.0 - 50.8]	0.21
Serum triglycerides (mg/dL)*	108.6 ± 48.7 [81.6 -135.6]	254.8 ± 226.4 [92.9 - 416.7]	**0.04**
Uric Acid (mg/dL)	5.3 ± 1.1 [4.7 - 6.0]	5.7 ± 1.2 [4.8 - 6.5]	0.60
Na (mEq/L)	140.1 ± 2.2 [138.9 - 141.3]	138.8 ± 2.0 [137.3 - 140.3]	0.17
K (mEq/L)	4.3 ± 0.5 [4.0 -4.6]	4.2 ± 0.4 [3.8 - 4.5]	0.49
UACR (mg/g)	11.3 ± 13.2 [4.0 - 18.7]	138.2 ± 229.8 [-26.2 - 302.6]	0.17
Office SBP (mmHg)	154.3 ± 21.6 [142.3 -166.2]	156.5 ± 30.6 [134.7 - 178.4]	0.93
Office DBP (mmHg)	92.9 ± 10.8 [86.9 - 98.8]	90.1 ± 18.2 [77.0 - 103.1]	0.33

The values are expressed as means ± standard deviation; [95% confidence interval]; (*) Statistical significance (p < 0.05); Non-T2D: non type 2 diabetes resistant hypertension; T2D: type 2 diabetes resistant hypertension.

Phoenix, Arizona, USA) for statistical analysis. Analytical statistics were performed by Analyse-it version 2.21 Excel 12+ (Analyse-it Software Ltd., Leeds, UK), a statistical add-in program for Excel (MS Excel Microsoft Corp, Phoenix, Arizona, USA).

Results

The general characteristics of the study groups are listed in table 1. No statistical differences were observed between the non-T2D and T2D subgroups with respect to age and gender. The mean ages were 54.7 and 54.9 in non-T2D and T2D patients, respectively. Women made up equally 40% of the patients in these groups. Both groups demonstrated similar characteristics despite of the laboratory analysis concerning T2D diagnosis like fasting glucose (167.8 ± 9.2 vs 92.9 ± 9.2 mg/dL - p < 0.0001) and HbA1c levels (9.3 ± 2.1 vs 5.8 ± 0.3% - p < 0.0001) (Table 1). However, the T2D group showed a greater BMI and higher serum triglyceride levels than the non-T2D group (33.7 ± 4.0 vs 26.6 ± 3.7 kg/m^2, - p = 0.0002; 254.8 ± 226.4 vs 108.6 ± 48.7 mg/dL - p = 0.041) (Table 1). Concerning HRV parameters, the following evaluations were reduced in T2D: 24 hour-SDNN (89.1 ± 19.9 *vs* 122.9 ± 39.5 ms; p = 0.0009), Day time SDNN (58.2 ± 13.6 *vs* 78.5 ± 24.9 ms; p = 0.03), 24 hour-SDANN (79.8 ± 17.1 *vs* 122.9 ± 39.5 ms; p = 0.0012), Day time SDANN (47.8 ± 3.5 *vs* 65.5 ± 6.5 ms; p = 0.03), Day time rMSSD (13.8 ± 1.9 *vs* 19.8 ± 2.2; p = 0.05), 24 hour-pNN50 (1.8 ± 2.1 vs 5.3 ± 6.4%; p = 0.047), Day time pNN50 (0.5 ± 0.5 *vs* 2.6 ± 2.9%; p = 0.035) and Night time pNN50 (4.2 ± 1.6 *vs* 12.4 ± 4.7; p = 0.04) (Figure 1). Although the remaining HRV parameters in time domain have demonstrated greater autonomic imbalance in the T2D group, they did not achieve statistically significance (p > 0.05) (Figure 1).

Frequency domain parameters demonstrated inverted pattern of tone intensity for both branches of the autonomic system during day and night periods in both groups. Regarding the non-T2D group, LF nu during day and night were 22.5 ± 3.2 *vs* 51.0 ± 7.1 (p = 0.00) and HF nu were 77.5 ± 3.2 *vs* 48.9 ± 7.1 (p = 0.00), respectively. For the same group, HF nu during day and night were 77.5 ± 3.2 *vs* 48.9 ± 7.1 (p = 0.00), respectively. Regarding the T2D group, LF nu during day and night were 25.7 ± 7.1 *vs* 47.1 ± 7.3 (p = 0.01) and HF nu were 74.2 ± 7.1 *vs* 52.8 ± 7.3 (p = 0.01), respectively (Figure 2). For the same group, HF nu during day and night were 74.2 ± 7.1 *vs* 52.8 ± 7.3 (p = 0.01), respectively (Figure 2). There were no differences between non-T2D and T2D groups in frequency domain parameters (Table 2).

Total patients (non-T2D and T2D groups) had HRV correlated positively with serum adiponectin (r = 0.37 [95% CI -0.04 - 1.00] p = 0.03) and negatively with HbA1c levels (r = -0.58 [95% CI -1.00 - -0.3] p = 0.00). Total patients had also adiponectin correlated negatively with HbA1c levels (r = -0.40 [95% CI -1.00 - -0.07] p = 0.02) (Figure 3).

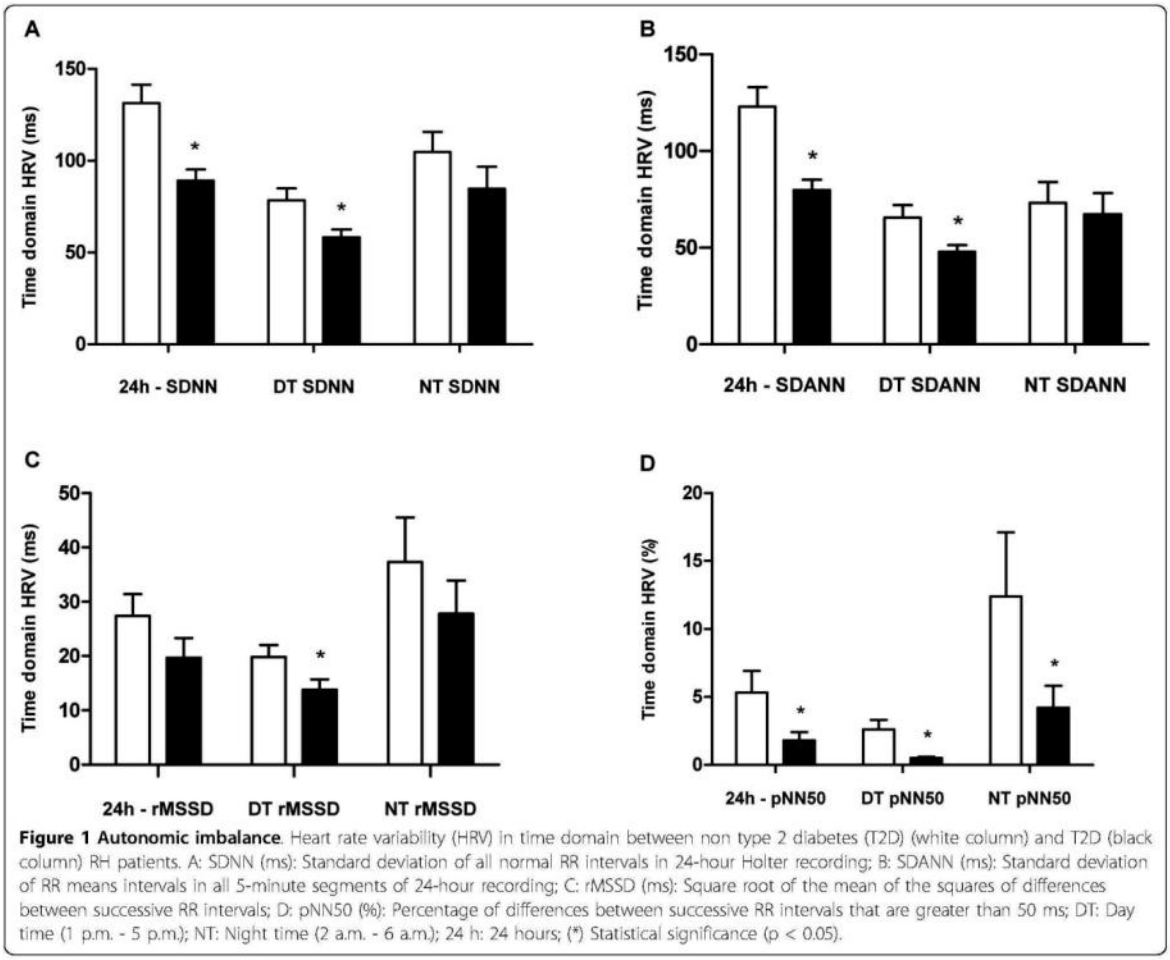

Figure 1 Autonomic imbalance. Heart rate variability (HRV) in time domain between non type 2 diabetes (T2D) (white column) and T2D (black column) RH patients. A: SDNN (ms): Standard deviation of all normal RR intervals in 24-hour Holter recording; B: SDANN (ms): Standard deviation of RR means intervals in all 5-minute segments of 24-hour recording; C: rMSSD (ms): Square root of the mean of the squares of differences between successive RR intervals; D: pNN50 (%): Percentage of differences between successive RR intervals that are greater than 50 ms; DT: Day time (1 p.m. - 5 p.m.); NT: Night time (2 a.m. - 6 a.m.); 24 h: 24 hours; (*) Statistical significance ($p < 0.05$).

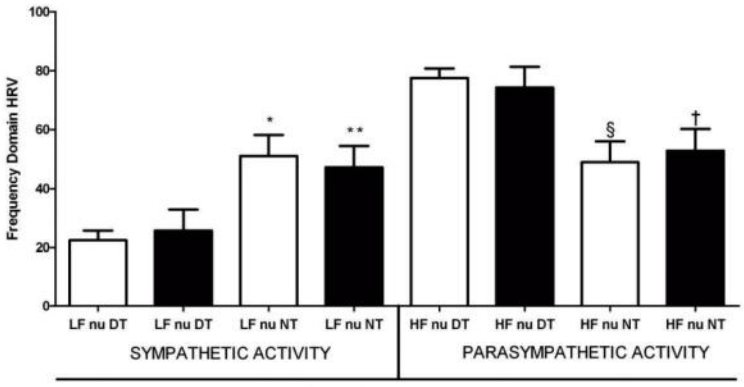

Figure 2 LF nu: Circadian disruption. Heart rate variability (HRV) in frequency domain between non type 2 diabetes (T2D) (white column) and T2D (black column) RH patients. LF nu: Low frequency in normalized units; HF nu: High frequency in normalized units; DT: Day time (3 p.m.); NT: Night time (3 a.m.); Statistical significance: (*) $p < 0.05$ *vs* LF nu DT non-T2D (**) $p < 0.05$ *vs* LF nu DT T2D; (§) $p < 0.05$ *vs* HF nu DT non-T2D; (†) $p < 0.05$ *vs* HF nu DT T2D.

Table 2 Heart rate variability (HRV) in time and frequency domain between non-T2D and T2D resistant hypertension patients

HRV variable	Non-T2D group (n = 15)	T2D group (n = 10)	*p*-value
24 hour-SDNN *	131.5 ± 9.9 [110 - 152,9]	89.1 ± 6.2 [74.9 - 103.3]	**0.00**
Day time SDNN *	78.5 ± 6.4 [64.7 - 92.3]	58.2 ± 4.3 [48.5 - 67.9]	**0.03**
Night time SDNN	104.7 ± 11.1 [80.8 -128.5]	84.7 ± 12.0 [57.6 - 111.8]	0.21
24 hour-SDANN *	122.9 ± 10.2 [101.0 - 144.8]	79.8 ± 5.4 [67.6 - 92.0]	**0.00**
Day time SDANN *	65.5 ± 6.5 [51.5 - 79.4]	47.8 ± 3.5 [39.7 - 55.9]	**0.03**
Night time SDANN	73.2 ± 10.7 [50.1 - 96.3]	67.3 ± 11.0 [42.3 - 92.3]	0.80
24 hour-pNN50 *	5.3 ± 1.6 [1.8 - 8.9]	1.8 ± 0.6 [0.3 - 3.3]	**0.05**
Day time pNN50 *	2.6 ± 0.7 [0.9 - 4.2]	0.5 ± 0.1 [0.1 - 0.8]	**0.04**
Night time pNN50 *	12.4 ± 4.7 [2.3 - 22.5]	4.2 ± 1.6 [0.5 - 7.9]	**0.04**
24 hour-rMSSD	27.4 ± 4.0 [18.7 - 36.1]	19.7 ± 3.6 [11.6 - 27.8]	0.17
Day time rMSSD *	19.8 ± 2.2 [14.9 - 24.7]	13.8 ± 1.9 [9.5 - 18.1]	**0.05**
Night time rMSSD	37.3 ± 8.2 [19.6 - 55.0]	27.8 ± 6.1 [14.0 - 41.6]	0.21
Day time LF nu	22.5 ± 3.2 [15.4 - 29.5]	25.7 ± 7.1 [9.6 - 41.8]	0.97
Night time LF nu	51.0 ± 7.1 [35.8 - 66.3]	47.1 ± 7.3 [30.5 - 63.8]	0.80
Day time HF nu	77.5 ± 3.2 [70.4 - 84.5]	74.2 ± 7.1 [58.1 - 90.3]	0.97
Night time HF nu	48.9 ± 7.1 [33.6 - 64.1]	52.8 ± 7.3 [36.2 - 69.4]	0.80

The values are expressed as means ± standard error of the mean; [95% confidence interval]; (*) Statistical significance ($p < 0.05$). SDNN (ms): Standard deviation of all normal RR intervals in 24-hour Holter recording; SDANN (ms): Standard deviation of means of RR intervals in all 5-minute segments of 24-hour recording; pNN50 (%): Percentage of differences between successive RR intervals that are greater than 50 ms; rMSSD (ms): Square root of the mean of the squares of differences between successive RR intervals; LF nu: Low frequency in normalized units from the power spectra of HRV by computer analysis using Fast Fourier Transformation (FFT); HF nu: High frequency in normalized units from the power spectra of HRV by computer analysis using FFT; Non-T2D: non type 2 diabetes resistant hypertension; T2D: type 2 diabetes resistant hypertension.

It has also been observed that the T2D group was taking more anti-hypertensive drugs than the non-T2D group (Table 3). In addition, the T2D group demonstrated greater prevalence of left ventricular diastolic dysfunction than the non-T2D group despite of similar left ventricle hypertrophy index (Table 4).

Discussion

Important findings

We found that the AI is more impaired in T2D RHTN patients than the non-T2D group. As expected, the T2D group had greater BMI and serum triglycerides than the non-T2D group. Considering the total of patients, an established negative correlation between HbA1c levels and serum adiponectin concentration was also observed. In addition to these well-known characteristics between non-T2D and T2D patients, considering the total patients, HRV correlated positively with adiponectin and negatively with HbA1c levels. Interestingly, despite of the higher AI in the T2D subgroup, both groups demonstrated similar inverted pattern of sympathetic and parasympathetic tones during day and night periods. As far as we know, this is the first time that AI and circadian disruption (CD) were evaluated in RHTN patients with or without T2D in order to better understand their synergistic role in obesity and T2D association with RHTN.

Historical aspects of autonomic dysfunction

Since the fifth decade, cardiovascular autonomic researchers have cogitated whether AI would be a cause or consequence of hypertension [29]. Subsequently, it was demonstrated that AI could lead not only to hypertension, but also to T2D [7]. Reasonable amount of data provide evidences for prediction hypertension or diabetes onset due autonomic imbalance evaluated by HRV parameters in time or frequency domain [9-14]. Autonomic imbalance causes, at first, increased insulin sensitivity and reduced energy dissipation [15]. Concomitantly, insulin resistance impairs the overall mean levels of cardiac autonomic modulation among persons with T2D [30].

Pathogenesis of autonomic dysfunction and metabolic disorders

The increased concentration of angiotensin II inhibits intracellular signaling to GLUT 4 expression on the surface of the cells leading to insulin resistance [31,32]. However, the observation that not all subjects follow this link suggests that other factors, including genetic predisposition and environment, can influence it at various levels [33-38].

A chronic increase in sympathetic outflow has been reported to decrease β-adrenergic responsiveness itself, through down-regulation of β-adrenergic receptors, which are known to mediate energy expenditure both at

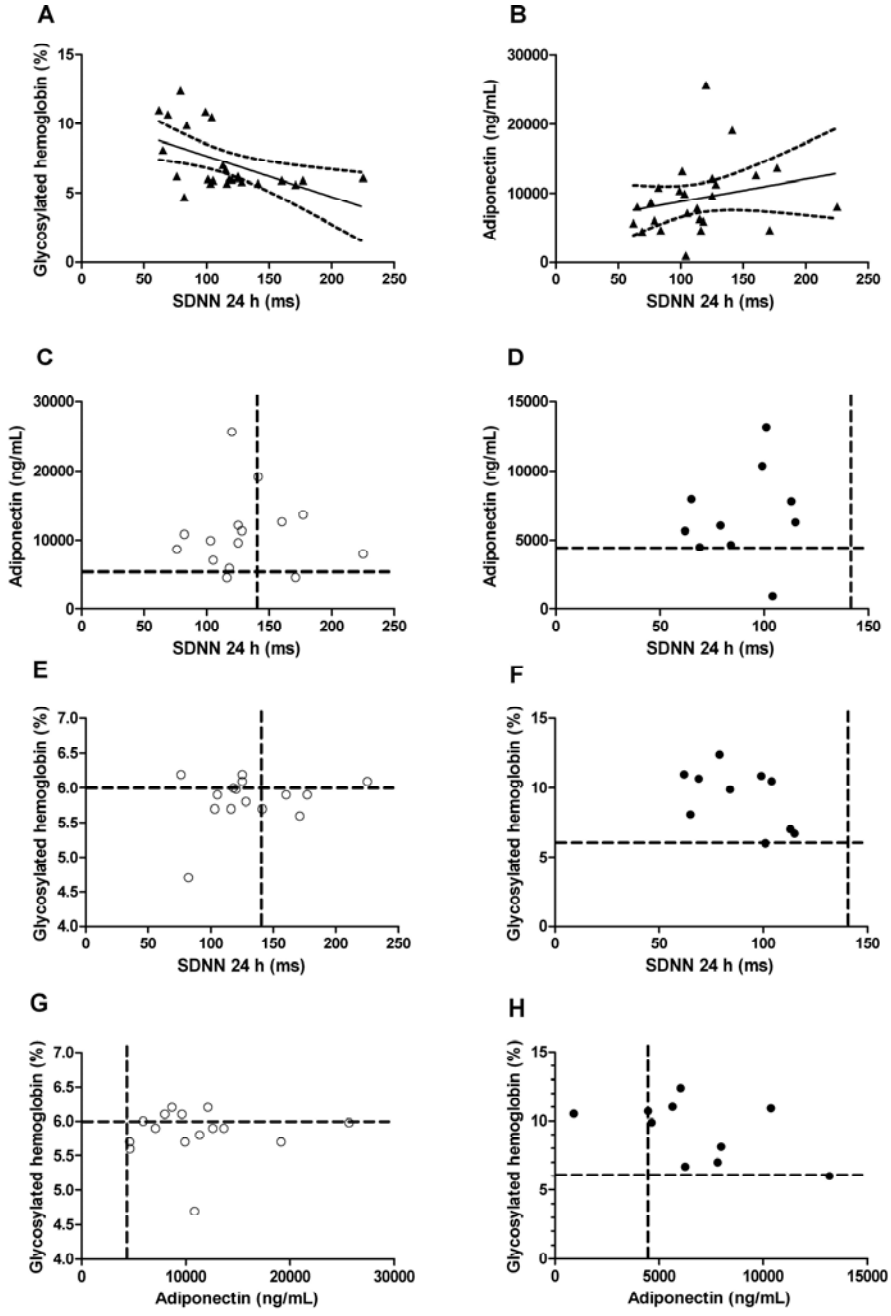

Figure 3 A: Correlation between HRV and glycosylated hemoglobin (HbA1c) levels of total RH patients (r = -58; [95% CI -1.0 - -0.3]; p = 0.00); B: Correlation between HRV and adiponectin of total RH patients (r = 0.37; [95% CI -0.04 - 1.0]; p = 0.03); C and D: Matrix plot of the distribution of values between HRV and adiponectin; E and F: Matrix plot of the distribution of values between HRV and HbA1c levels; G and H: Matrix plot of the distribution between HbA1c levels and adiponectin. Non-T2D group: white circles; T2D group: black circles. Parameters for the matrix plot in means: Adiponectin (5548 ng/mL - Healthy volunteers evaluated with plasma EDTA of Quantikine® lab test), SDNN 24 h (141 ms)[6] and HbA1c (6.0%).

Table 3 Anti-hypertensive (anti-HTN) drugs distribution

Characteristic/Variable	Non-T2D group (n = 15)	T2D group (n = 10)	*p*-value
Total anti-HTN drugs*	3.3 ± 0.5 [3.1 - 3.6]	4.1 ± 0.7 [3.6 - 4.6]	**0.02**
Thiazide diuretic	100% (15)	100% (10)	-
Aldosterone receptor inhibitor	26.6% (4)	50% (5)	0.40
Angiotensin converting enzyme inhibitor	66.6% (10)	50% (5)	0.68
Angiotensin receptor blocker	40% (6)	70% (7)	0.23
Calcium channel blocker	86.6% (13)	100% (10)	0.50
Centrally acting anti-hypertensive drug	6.6% (1)	40% (4)	0.12

The values are expressed as means ± standard deviation; [95% confidence interval]; (*) Statistical significance ($p < 0.05$). Non-T2D: non type 2 diabetes resistant hypertension; T2D: type 2 diabetes resistant hypertension.

rest and after food intake [16]. This mechanism could result in a reduced ability to dissipate energy leading to weight gain. At first glance, it appears to be in contrast to the previous reports in rats, where the down-regulation of β-adrenergic receptors, following chronic catecholamine stimulation, has been associated with increased insulin sensitivity [39]. Moreover, the hypothalamus is a regulatory center of satiety and of the autonomic nervous system (ANS). Therefore, abnormalities in the hypothalamus may cause obesity and autonomic dysfunction. This may explain the alterations observed in the HRV indices [40].

Corroborating the mechanism above, the correlation between high sympathetic activity and hypoadiponectinemia was previously established [21]. Since we evaluated RHTN patients and hypoadiponectinemia is also associated with sympathetic activation and severity of OSA [41], only true RHTN patients classified as "low risk" (Berlin sleep questionnaire) of OSA diagnosis were included in this study. We have found positive correlation between HRV and adiponectinemia and it is properly presumable to us that this adipocytokine may play an important role in the development of obesity and T2D in RHTN by the AI patient status. Recently, an interventional clinical trial with prolonged insulin-glucose infusion was performed with healthy human subjects in order to evaluate the effect of insulin on adiponectin multimers and nuclear factor-kappaB (NF-kB) activity in human endothelial cells [42]. Hyperinsulinemic induction significantly decreased high molecular weight and total adiponectin levels but increased NF-kB activity in serum treated microvascular endothelial cells [42].

Lifestyle and circadian disruption

Regarding the contribution of environment in the genesis of resistant hypertension, obesity and T2D, the circadian disruption seems to be an important complicating factor. Disrupted circadian rhythms caused by disturbed sleep-wake cycles, inactivity during the active period, enhanced activity during the rest period and high food consumption might lead to attenuated feeding rhythms, disrupted metabolism and obesity [43]. This lifestyle may cause high parasympathetic output to the viscera leading to obesity, hyperinsulinemia, and hyperlipidemia, or high sympathetic output to the muscle and heart leading to vasoconstriction and hypertension [44]. In addition to the impairment of AI, circadian disruption was found in both groups. Since the genetic background allied to the disruption of the circadian rhythm favors HTN and metabolic disorders, our results are in accordance with the literature: findings in murine models show the strong link between genetic background and circadian rhythm disruption in determining the severity of metabolic disorders [43].

Hypoadiponectinemia and metabolic disorders

Diabetic patients had greater BMI and lower adiponectin values compared to non-T2D patients. It was also notable that the diabetic group had greater BMI and higher serum triglyceride levels than the non-diabetic group. The association between BMI and hypertriglyceridemia

Table 4 Echocardiographic parameters of study groups

Characteristic/Variable	Non-T2D group (n = 15)	T2D group (n = 10)	*p*-value
LVEF (%)	70.3 ± 7.3 [66.3 -74.4]	66.5 ± 21.7 [51.0 - 82.0]	0.28
LVMI (g/m^2)	134.1 ± 30.9 [116.9 - 151.2]	148.4 ± 31.6 [125.8 - 171.0]	0.31
LVDD	Present in 60%	Present in 100%	0.05

The values are expressed as means ± standard deviation; [95% confidence interval]; Non-T2D: non type 2 diabetes resistant hypertension; T2D: type 2 diabetes resistant hypertension; LVEF: left ventricle ejection fraction; LVMI: left ventricle mass index; LVDD: left ventricle diastolic dysfunction (assessed by the Omnen SR and Nishimura RA algorithm [27]).

is also in line with the scientific literature [45]. In addition, hypoadiponectinemia *per se* may not be the main issue in metabolic alterations associated with obesity. A harmonized pattern of rhythmic expression of adiponectin by visceral and subcutaneous abdominal adipose tissue seems to be crucial to body fat distribution homeostasis as well as to prevent metabolic alterations associated with obesity [46].

Based on the above considerations, the imbalance of autonomic function represents a primary defect leading to RHTN and insulin resistance. Moreover, circadian disruption caused by behavior and genetic background disturbs the balance between sympathetic and parasympathetic branches during day and night periods. In our opinion, a higher degree of AI underlies obesity and T2D onset in RHTN compared to non-T2D RHTN (Figure 4).

Severity of hypertension and autonomic imbalance

However, the significant worsing in AI found in the T2D group is of interest because we considered the null hypothesis of no difference between groups due the severity of hypertension such in RHTN. At first, we presumed that AI could be different in mild to moderate stages of hypertension whilst in RHTN this difference would be strongly attenuated. Since the degree of impairment in cardiac autonomic control is proportional to the severity of hypertension, and not necessarily impaired in mild long-lasting essential hypertension [47], T2D comorbidity would be determinant of AI in mild to moderate hypertension, but not so decisive for AI in RHTN. In fact, Mussalo H et al [47] compared HRV between severe and mild hypertension. However, the severe group was not stratified into non-T2D and T2D subgroups. Once more, in accordance with the clinical trials of our laboratory [48], RHTN proved to have a plural complexity. The AI gap between both groups demonstrated that RHTN *per se* is not an end stage of hypertension disease. Especially in RHTN without T2D, there is still a favorable residual autonomic function which needs to be preserved or enhanced.

Pharmacologic perspectives for RHTN and T2D treatment

It is important to point out that in cases of T2D comorbidity, a better awareness of specific characteristics of diabetes such as decreased energy expenditure, lipolysis and insulin sensitivity can guide doctors to avoid drugs which can enhance these phenomena such as conventional or non-vasodilating beta-blockers [49]. However, considerable heterogeneity exists within the β-blocker class. Perhaps most important is a variation in adrenergic receptor selectivity; nevertheless, there is also variability in lipophilicity, intrinsic sympathomimetic activity (ISA), membrane-stabilizing action, and vasodilating properties [50]. Carvedilol, for instance, is a highly non-selective lipophilic β-blocker with α_1-blocking activity and no ISA [51]. Its pharmacologic properties may be associated with a more comprehensive treatment of AI since they can improve insulin sensitivity in T2D patients [52-54]. This mechanism, although very intriguing, needs further support from more complete (possibly longitudinal) studies and opens a new and stimulating field towards pharmacology intervention focusing the improvement of autonomic imbalance in T2D patients in order to optimize glucose metabolism in addition to BP control in RHTN patients.

Non-pharmacologic treatment of RHTN in real-life settings

There are different methods to measure the circadian rhythm: sleep diaries, polysomnographic recording, actimetry, chronotype identification, body temperature, pre-sleep measures of melatonin secretion, cortisol

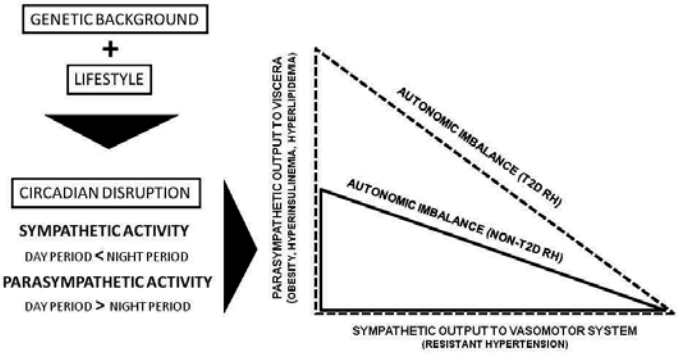

Figure 4 Circadian disruption and autonomic imbalance and their relationship with vasomotor and visceral disturbances in non-T2D and T2D resistant hypertensive patients. The circadian disruption maximizes the autonomic imbalance in the development of obesity and T2D in RH.

secretion and activity of clock genes [55]. However, the approach of circadian rhythm may not need all these methods, but simple oriented questions regarding day-night behavior. In addition to the well-known lifestyle factors to be assessed by anamnesis such as obesity, dietary salt intake and alcohol consumption [22], we endorse that the approach of the chronotype of RHTN patients should also be encouraged by all hypertension guidelines.

The circadian disruption found in this study also reinforces the need for lifestyle change in RHTN. Even in asymptomatic obese adults, abnormalities in circadian blood pressure variability and endothelial function exhibited unfavorable cardiometabolic profiles such as elevated high-sensitivity C-reactive protein, fibrinogen, fasting serum glucose and cardiac risk ratios (Total Cholesterol:HDL-cholesterol and LDL-cholesterol:HDL-cholesterol ratios) [56]. A more comprehensive anamnesis over feeding behavior, physical inactivity during the day, hyperactivity during the night (or repose) and duration of sleepiness may help physicians and patients think about strategies to avoid the circadian disruption.

Limitations of the study

Our main limitation was the recruitment of "true" RHTN patients and then the small sample size is noteworthy. Despite of the small sample size, it was possible to identify greater AI in the T2D subgroup. We assumed that the small sample size of the T2D group is a result of the prevalent exclusion criteria of moderate to severe renal dysfunction, history of coronary artery disease and β-blocker already in use in this population.

General findings and future perspectives

We concluded that, in spite of circadian disruption, even in RHTN patients there is a residual autonomic function compared to T2D patients. Furthermore, it is clear that the evaluation of BMI, adiponectin and HRV of RHTN patients can reveal the risk of T2D association or future development in this high risk population. However, an appropriate risk matrix to evaluate this prognostic information will demand a longitudinal clinical study with significant greater sample size of RHTN patients.

Conclusion

Type 2 diabetes comorbidity is associated with greater autonomic imbalance, lower adiponectin levels and greater BMI in RHTN patients. Moreover, similar circadian disruption was also found in both groups indicating the importance of lifestyle behavior in the genesis of RHTN. A better comprehension of the patterns of autonomic imbalance and circadian disruption in RHTN is one more parameter to guide clinicians toward a holistic treatment of hypertension and diabetes.

List of Abbreviations Used

AI: autonomic imbalance; HTN: hypertension; T2D: type 2 diabetes; HRV: heart rate variability; BMI: body mass index; RHTN: resistant hypertension; SBP: systolic blood pressure; DBP: diastolic blood pressure; EDTA: ethylenediamine tetraacetic acid; LVEF: left ventricle ejection fraction; LVMI: left ventricle mass index; DT: day time; NT: night time; rMSSD: Square root of the mean of the squares of differences between successive RR intervals; SDNN: Standard deviation of all normal RR intervals in 24-hour Holter recording; SDANN: Standard deviation of RR intervals means in all 5-minute segments of 24-hour recording; pNN50: Percentage of differences between successive RR intervals that are greater than 50 ms; FFT: Fast Fourier Transformation; LF nu: low frequency in normalized units; HF nu: high frequency in normalized units; VLF: very low frequency; SD: standard deviation; SEM: standard error of the mean; ANS: autonomic nervous system; OSA: obstructive sleep apnea; ISA: intrinsic sympathomimetic activity; NF-kB: nuclear factor-kappaB.

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Authors' contributions

LBM, LCM and HMJ contributed to the design, analysis, and interpretation of this study. VNF and CD contributed to the collection and critical analysis of clinical and laboratory data. FPSC and MJF contributed to critical analysis of heart rate variability data. FCB contributed to the writing of this manuscript and critical review of the version for submission. All authors have read and approved the final manuscript.

Competing interests

LBM and CD are employees of Novartis Biociências S.A. (Brazil).

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Capítulo II

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1st November 2011

PROF. DR. LUIZ FELIPE MOREIRA
EDITOR
ARQUIVOS BRASILEIROS DE CARDIOLOGIA

Dear Prof. Luiz Felipe Moreira,

Please find enclosed our original manuscript "**Relationship of aldosterone and high sympathetic activity in resistant hypertension with or without type 2 diabetes".** The paper hereby submitted follows some important publications of our group in resistant hypertension. This group of patients has already been described in our recent article for Cardiovascular Diabetology (Boer-Martins, et al; 10:24), where we have shown that type 2 diabetes comorbidity is associated with greater autonomic imbalance, lower adiponectin levels and greater BMI. In the present article, we examined the relationship between sympathetic overactivity and plasma leptin and aldosterone levels in resistant hypertension (RHTN), comparing the groups with and without T2D.

We would like to publish it in the ***Arquivos Brasileiros de Cardiologia*** due its relevance for the threatening pandemic obesity-hypertension and its *continuum* and related comorbidities.

This manuscript is original and is not under consideration by any other journal.

All authors have approved the submission of the present study in this journal and there are no financial or other relationships that might lead to a conflict of interest.

Yours sincerely,

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Relationship of aldosterone and high sympathetic activity in resistant hypertension with or without type 2 diabetes

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Relationship of aldosterone and high sympathetic activity in resistant hypertension with or without type 2 diabetes

Associação da aldosterona e aumento da atividade simpática em hipertensos resistentes com ou sem diabetes tipo 2

Summarized title: Aldosterone in sympathetic activity

Total electronic word count: 4215

Keywords: leptin, aldosterone, sympathetic activity, heart rate variability, resistant hypertension.

ABSTRACT:
Study Basis: The finding of adipocyte-derived hormone leptin as an overstimulator of sympathetic activity brought a new perspective to the pathophysiological mechanisms of obesity-hypertension. **Objectives:** As aldosterone also increases sympathetic activity, we aimed to assess the relationship between sympathetic overactivity and plasma leptin and aldosterone levels in resistant hypertension (RHTN), comparing the groups with and without T2D. **Methods:** Twenty-five RHTN patients underwent ambulatory electrocardiography to analyze heart rate variability (HRV) in time and frequency domains, which were stratified into two periods: 24 hours and daytime (DT), comprising the records between 2:00 p.m to 6:00 p.m (time domain) and one hour at 3:00 p.m (frequency domain). **Results:** T2D group (n=10) had higher serum aldosterone and plasma leptin levels than the non-T2D (n=15) (26.0±11.5 *vs* 16.9±7.0 ng/dL – p=0.021; 81,368.7±47,086.1 *vs* 41,228.1±24,523.1 pg/mL – p=0.048, respectively). Both groups had aldosterone correlated with HRV in frequency domain. Non-T2D had aldosterone correlated with DT low frequency in normalized units (LF nu) (r=0.6 [0.12–0.85] p=0.018) and DT high frequency in normalized units (HF nu) (r=-0.6 [-0.85- -0.12] p=0.018). Type-2-diabetes group had aldosterone correlated with DT LF nu (r=0.72 [0.16–0.93] p=0.019) and DT HF nu (r=-0.72 [-0.93- -0.16] p=0.019). However, despite of the importance of leptin in sympathetic overactivity in hypertension, leptin did not correlate with HRV. **Conclusion:** Aldosterone seems to overdrive sympathetic activity in RHTN with and without T2D. This information combined with the clinical efficacy of mineralocorticoid receptor blocker in RHTN reinforces that aldosterone is a major player to be a therapeutic target in RHTN.

RESUMO

Racional: A descoberta da leptina como um estimulador da atividade simpática trouxe uma nova perspectiva para os mecanismos fisiopatológicos da obesidade-hipertensão. **Objetivos:** Avaliamos a relação entre a atividade simpática aumentada e as concentrações plasmáticas de leptina e aldosterona em hipertensos resistentes (HR), comparando os grupos com e sem diabetes tipo 2 (DT2). **Métodos:** Vinte e cinco pacientes HR foram avaliados por eletrocardiografia ambulatorial para análise da variabilidade da frequência cardíaca (VFC) nos domínios do tempo e frequência, os quais foram estratificados em dois períodos: 24 horas e período diurno (D), compreendendo as medidas entre 14 e 18h (domínio do tempo) e uma hora às 15h (domínio da frequência). **Resultados:** O grupo DT2 (n=10) apresentou maiores concentrações de aldosterona e leptina que o non-DT2 (n=15) (26.0±11.5 *vs* 16.9±7.0 ng/dL – p=0.021; 81,368.7±47,086.1 *vs* 41,228.1±24,523.1 pg/mL – p=0.048, respectivamente). Houve correlação entre aldosterona e VFC no domínio da frequência em ambos os grupos. Não-DT2 apresentaram a aldosterona correlacionada com D baixa frequência em unidades normalizadas (LFnu) (r=0.6 [0.12–0.85] p=0.018) e D alta frequência em unidades normalizadas (HFnu) (r=-0.6 [-0.85- -0.12] p=0.018). No grupo com diabetes, a aldosterona correlacionou-se com DLFnu (r=0.72 [0.16–0.93] p=0.019) e DHFnu (r=-0.72 [-0.93- -0.16] p=0.019). Apesar da importância da leptina na atividade simpática aumentada na hipertensão, não houve correlação com VFC. **Conclusão:** A aldosterona parece estimular a atividade simpática em HR com ou sem DT2. Esta informação combinada com a eficácia clínica dos bloqueadores de receptor mineralocorticóide em HR reforça a aldosterona como alvo terapêutico relevantes em HR.

INTRODUCTION

Hypertension, diabetes and obesity comprise a series of interactive physiologic disorders (1). For instance, it is well known that obesity and diabetes mellitus are factors associated with resistance to antihypertensive drugs. Therefore, a better knowledge on the interactions among these pathophysiologic pathways can aid treatment choice and thereby improving total cardiovascular risk management (1).

The discovery of leptin brought new insights to the pathophysiological mechanisms of obesity and associated diseases (2). Initial studies of leptin showed that it regulates appetite and enhances energy expenditure by activating sympathetic nerve activity to thermogenic brown adipose tissue (3). It was also demonstrated that leptin causes sympathetic excitation to the kidney that, in turn, increases arterial pressure (3).

It is also well-known the high prevalence of hyperaldosteronism in resistant hypertension (RHTN) (4). Moreover, primary aldosteronism patients showed significantly higher levels of leptin than essential hypertension (HTN) subjects (5). However, a growing body of evidence suggests that aldosterone contributes broadly to the development and severity of HTN separately from the presence of classically defined primary aldosteronism (6). In addition, aldosterone promotes RHTN by mediating maladaptive changes in the renal, cardiovascular and central nervous systems (7). Sympathetic nervous system (SNS) activation seems to be a basic component of the adverse impact of aldosterone excess in the central nervous system (8).

Heart rate variability (HRV) may be used to assess autonomic imbalances, diseases and mortality (9). Measures of heart rate variability (HRV) in time and frequency domains have been used successfully to index sympathetic and vagal activity (10). Nevertheless, while there are some differences among HRV parameters found in many studies, the consensus is that lower values of these indices of vagal function are prospectively associated with death and disability (11).

Considering the significant influences that leptin and aldosterone exerts on the pathophysiology of RHTN (8, 12), we aimed to access the relationship between sympathetic activity and these two hormones in RHTN with and without type 2 diabetes (T2D).

METHODS

We included twenty-five (25) RHTN subjects (13), 15 non-T2D and 10 T2D, regularly followed in the Ambulatory Service of Cardiovascular Clinical Pharmacology, complying with pharmacological prescription for HTN and T2D (taking 80% to 120% of the prescribed daily dose). This group of patients has already been recently described by our group (14).

An accurate office blood pressure measurement technique and ambulatory blood pressure monitoring (ABPM) were applied to diagnosis resistant hypertension (15). We excluded cases of pseudoresistance, including lack of blood pressure control secondary to poor medication adherence (16). White coat hypertension (WCH) was excluded by ABPM (15). Resistant hypertension included patients whose blood pressure was uncontrolled with the use of more than three medications or patients whose blood pressure was controlled, but required four or more drugs to achieve

blood pressure goals (15). All subjects provided written informed consent, and the study was approved by the local ethics committee.

The exclusion criteria comprised: acute or moderate-severe renal dysfunction (creatinine clearance < 40 mL/min/1,73 m²), non-complied pharmacological prescription, use of beta-blockers within the last six months, severe obesity (body mass index ≥ 35 kg/m²), heart failure (ejection fraction < 50%), valvular heart disease, cardiomyopathies, primary hyperaldosteronism [aldosterone:PRA ratio > 20 ng per 100 mL per ng.ml(-1)h(-1)], atrial fibrillation, sick sinus syndrome, supraventricular and ventricular tachycardias, aortic disease (Marfan´s syndrome, coarctation of the aorta, aneurysms or aortic surgery, history of coronary artery disease or proven coronary artery disease by coronary angiography or noninvasive tests, familial hyperlipidemia, asthma or chronic obstructive lung disease, pregnancy or oral contraceptive use, connective tissue disorders, neurological problems, oncological malignancies, psychiatric diseases, other than T2D endocrinological diseases, smoking, alcohol use and drug abuse.

Blood pressure measurements

Blood pressure was assessed by considering the orientations of the last guideline on hypertension of the European Society of Cardiology (17). The blood pressure (SBP – systolic blood pressure/ DBP – diastolic blood pressure) of each subject was measured three times, using a digital sphygmomanometer (Omron HEM-711DLX) on the right upper arm, in the sitting position, after a 10-minute rest. The average of two consecutive measurements was used, with a variation lower than 5 mmHg.

Laboratory analysis

All subjects underwent the following laboratory tests: hemogram, fasting serum glucose, glycolized hemoglobin (HbA1c), urea and creatinine, total cholesterol, LDL-cholesterol fraction, HDL-cholesterol fraction, triglycerides, uric acid, sodium and potassium, aldosterone (collected during DT) and plasma leptin levels (collected during DT) using ethylenediaminetetraacetic acid (EDTA) as an anticoagulant (Quantikine® Human Leptin Immunoassay, Catalog Number DLP00, R&D Systems, Inc., Minneapolis, USA).

Heart rate variability

Heart rate variability (HRV) parameters were derived from the recording of 24-hour Holter monitoring and analyzed in time and frequency domains. Measures were stratified into two time periods for time domain: 24-hour period (24h), daytime period (DT), comprising the records from 1:00 p.m. to 5:00 p.m. Frequency domain measures were obtained from one hour records at 3:00 p.m. (daytime period – DT). A three-channel, 24-hour Holter recording was obtained from each subject, using the CardioLight digital 24-hour recorder device and the CardioSmart Institutional CS 550 software (Cardio Sistema Comércio e Indústria Ltda, São Paulo, SP, Brazil).

Time domain HRV parameters included the following measures (9, 18):

- rMSSD (ms): Square root of the mean squared differences between successive RR intervals.
- SDNN (ms): Standard deviation of all normal RR intervals in a 24-hour Holter recording.
- SDANN (ms): Standard deviation of mean RR interval in all 5-minute segments of a 24-hour recording.
- pNN50 (%): Percentage of differences between successive RR intervals greater than 50 ms.

Frequency domain measures were calculated using the fast Fourier transform (FFT) to break down the time series to its underlying periodic function. Frequency domain HRV parameters included the following measures (9, 18):

Low frequency (LF) and high frequency (HF) measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the very low frequency (VLF) component. Normalized LF (LF nu) was calculated as LF power in normalized units LF/(total power-VLF) x 100, and normalized HF (HF nu) as HF power in normalized units HF/(total power-VLF) x 100. Low frequency (LF) and high frequency (HF), LF nu and HF nu denote the energy in the heart period power spectrum between 0.04 and 0.15 Hz (which is due to the joint action of the vagal and sympathetic components on the heart, with a predominance of the sympathetic ones) and 0.15 and 0.40 Hz (which corresponds to the respiratory modulation and is an indicator of the performance of the vagus nerve on the heart), respectively. "Daytime" was established at 3:00 p.m. in order to collect HRV data during wake.

The LF nu/HF nu ratio reflects the global sympatho-vagal balance and can be used as a measure of this balance. In a normal adult under resting conditions, the ratio is generally between 1 and 2.

Statistical analysis

Data were expressed as mean (μ) and standard deviation (SD) or mean (μ) and standard error of the mean (SEM) for HRV measures according its correct use. Unpaired groups were compared using Mann-Whitney U test, while correlation analysis was performed using Spearman´s rank test. Fisher's exact test was used to determine whether a certain group had significantly different proportion of a particular characteristic. The level of statistical significance accepted was less than 0.05. All data were entered into a spreadsheet program (MS Excel Microsoft Corp, Phoenix, Arizona, USA) for statistical analysis. Analytical statistics were performed by Analyse-it version 2.21 Excel 12+ (Analyse-it Software Ltd., Leeds, UK), a statistical add-in program for Excel (MS Excel Microsoft Corp, Phoenix, Arizona, USA).

RESULTS

We found no differences in age and gender between the non-T2D and T2D subgroups (54.7 ± 10.0 *vs* 54.9 ± 8.7 years and equally 60% of female prevalence for both groups - $p > 0.05$). Both groups demonstrated similar characteristics despite of laboratory analysis concerning T2D diagnosis like fasting glucose (167.8 ± 9.2 *vs* 92.9 ± 9.2 mg/dL – $p < 0.0001$, T2D *vs* non-T2D) and HbA1c levels (9.3 ± 2.1 *vs* 5.8 ± 0.3 % - $p < 0.0001$, T2D *vs* non-T2D). However, the T2D group showed a greater body mass index (BMI) and higher serum triglyceride values than the non-T2D group (33.7 ± 4.0 *vs* 26.6 ± 3.7 kg/m², – $p=0.0002$; 190.2 ± 103.6 *vs* 108.6 ± 48.7 mg/dL – $p=0.041$). Similarly, plasma leptin (81,368.7 ± 47,086.1 *vs* 41,228.1 ± 24,523.1 pg/mL – $p=0.048$) and serum aldosterone (26.0 ± 11.5 *vs* 16.9 ± 7.0 ng/dL – $p=0.021$) were increased in the T2D group compared with the non-T2D.

Regarding the anti-hypertensive drugs distribution, the T2D group was taking more

antihypertensive drugs than the non-T2D group (4.1 ± 0.7 *vs* 3.3 ± 0.5 – p=0.02).

Concerning HRV parameters, the following evaluations were reduced in T2D when comparing with non-T2D: 24-hour-SDNN (89.1 ± 19.9 *vs* 122.9 ± 39.5 ms; p=0.0009), daytime SDNN (58.2 ± 13.6 *vs* 78.5 ± 24.9 ms; p=0.03), 24-hour-SDANN (79.8 ± 17.1 *vs* 122.9 ± 39.5 ms; p=0.0012), daytime SDANN (47.8 ± 3.5 *vs* 65.5 ± 6.5 ms; p=0.03), daytime rMSSD (13.8 ± 1.9 *vs* 19.8 ± 2.2; p=0.05), 24-hour-pNN50 (1.8 ± 2.1 *vs* 5.3 ± 6.4 %; p=0.047), daytime pNN50 (0.5 ± 0.5 *vs* 2.6 ± 2.9 %; p=0.035) (Fig. 1). Although the remaining HRV parameters in time domain tended to lower values in T2D group, they did not achieve statistically significance. There were no differences between non-T2D and T2D groups in frequency domain parameters (data no shown).

Considering total patients (non-T2D and T2D groups), BMI correlated positively with aldosterone (r=0.47 [0.09 – 0.73] p=0.018; Fig. 2a) and leptin (r=0.58 [0.24 – 0.8] p=0.002; Fig. 2b). In addition, total patients had HRV in frequency domain correlated with serum aldosterone. Aldosterone also correlated with LF nu/HF nu ratio (r=0.60 [0.28 – 0.81] p=0.001), LF nu (r=0.60 [0.28 - 0.81] p=0.001; Fig. 2c) and HF nu (r= -0.60 [-0.81 - -0.28] p=0.001; Fig. 2d). However, we found no correlation with plasma leptin (daytime LF nu/HF nu: r=-0.09 [-0.48 – 0.33] p=0.68; daytime LF nu: r=0.01 [-0.39 - 0.41] p=0.95; daytime HF nu: r=-0.01 [-0.41 -0.39] p=0.95).

Both groups (non-T2D and T2D) separately also had aldosterone correlated with HRV in frequency domain. Non-T2D had aldosterone correlated with daytime LF nu (r=0.60 [0.12 – 0.85] p=0.018; Fig. 2e) and daytime HF nu (r=-0.60 [-0.85 - -0.12] p=0.018; Fig. 2f). In addition, T2D group had aldosterone correlated with daytime LF nu (r=0.72 [0.16 – 0.93] p=0.019; Fig. 2g) and daytime HF nu (r=-0.72 [-0.93 - -0.16] p=0.019; Fig. 2h).

DISCUSSION

The main finding of our study was that serum aldosterone levels correlated positively with sympathetic activity in RHTN with and without T2D. Moreover, in spite of the importance of leptin in sympathetic overactivity in hypertension, we found no correlation between leptin and HRV.

We also found that the HRV is lower in T2D RHTN patients than in the non-T2D group. In addition, the group T2D showed higher correlations between aldosterone and HRV than the non-T2D. These results are in accordance with previous studies showing that aldosterone exerts a major influence on both sympathetic overactivity and disturbances of hepatic gluconeogenesis (7, 19-22).

The role of aldosterone in hypertension

Aldosterone mediates several maladaptive changes in the nervous and cardiovascular systems that promote hypertension in addition to cardiovascular disease (CVD) and chronic kidney disease (CKD) (23). Elevated levels of aldosterone, in association with obesity and insulin resistance, promote nongenomic inflammation and oxidative stress pathways that advance the development of resistant hypertension through a number of mechanisms(7). These actions potentiate the elevation of blood pressure that occurs from the classic effects of aldosterone to promote salt retention and volume expansion, causing severe hypertension resistant to treatment, unless a mineralocorticoid receptor (MR) antagonist such as spironolactone or epleronone is used as part of the therapeutic regimen (7, 24). Similarly to the heart, vasculature, pancreas, skeletal muscle and fat, high levels of circulating aldosterone can also overactivate local renin-angiotensin-aldosterone system in brain regions that contribute to increased sympathetic tone in hypertension (7, 21, 22). Supplementarily, aldosterone also causes disturbances in hepatic insulin metabolic signaling, contributing, in part, to increased hepatic gluconeogenesis (19, 20). Our results are aligned with this information due the correlation between serum aldosterone and sympathetic and parasympathetic activity during daytime.

Pharmacologic perspectives for RHTN treatment

Mineralocorticoid receptor blockade has been a therapeutic target for RHTN in several studies (25, 26). There is an agreement on its results toward safety and efficacy in RHTN with the MR blocker spironolactone. These data reinforce the importance of aldosterone in RHTN pathophysiology. Corroborating these evidences, this study demonstrated a strong correlation between serum aldosterone and sympathetic activity, which has a major influence on RHTN.

Therefore, the panorama of evidences indicates that the encouragement of the use of MR blockers in RHTN is evidence-oriented and may be supported by hypertension guidelines.

The role of leptin in hypertension

The evidence that increasing plasma leptin to levels similar to those found in obesity raises arterial pressure in non-obese rats is consistent with the hypothesis that leptin is an important link between obesity, sympathetic activity and hypertension (27). Since obesity plays a major role in contributing to human essential hypertension, it is not surprising that plasma leptin concentrations are often elevated in hypertensive patients, or that leptin and blood pressure are correlated (27). Illustrating this statement, it has been found that serum leptin levels were highly correlated with mean arterial pressure and BMI in male Japanese adolescents. Moreover, heart rate was also correlated with serum leptin even after

adjustment for age and BMI (28). It was also observed that systolic blood pressure correlated with plasma leptin after adjustment for BMI in hypertensive women and in nonhypertensive men, but not in hypertensive men (29). Most of the data suggest that the correlation between leptin and blood pressure in hypertensive men is related mainly to the correlation between adiposity and blood pressure. However, not all studies have demonstrated a close relationship between leptin and hypertension. For example, leptin gene polymorphisms were not linked to hypertension in African Americans (30).

Regarding our findings, although leptin correlated with BMI, we have not found a correlation between leptin and sympathetic activity in this group of resistant hypertensive patients.

Limitations of the study

Our main limitation was the recruitment of "true" RHTN patients and, subsequently, the small sample size. Despite of the small sample size, it was possible to identify lower HRV in the T2D subgroup. We assumed that the small sample size of the T2D group is a result of the prevalent exclusion criteria of moderate to severe renal dysfunction, history of coronary artery disease and the widespread β-blocker prescribing in this population in Brazil.

CONCLUSION

Aldosterone correlated positively with sympathetic activity in RHTN. This information summed with the recent clinical trial evidences, reinforces the effectiveness of MR blockers in resistant hypertension and points to aldosterone as a major player in pharmacologic therapeutic.

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CONFLICT OF INTERESTS

LBM and CD are employees of Novartis Biociências S.A. (Brazil).

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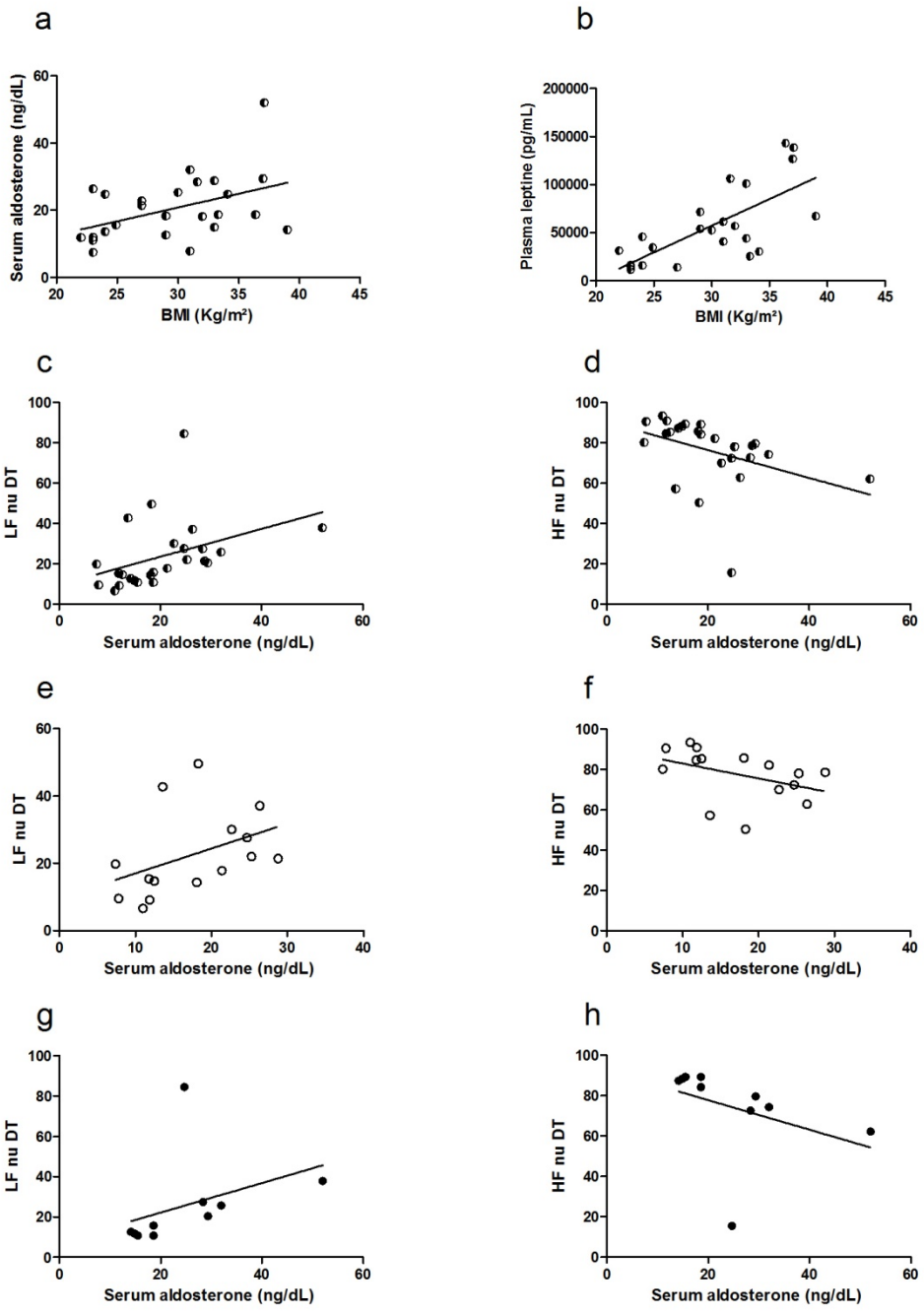
FIGURE LEGENDS

Figure 1: Autonomic imbalance. Heart rate variability (HRV) in time domain between non type 2 diabetes (non-T2D) (white column) and T2D (black column) RHTN patients. A: SDNN (ms): Standard deviation of all normal RR intervals in 24-hour Holter recording; B: SDANN (ms): Standard deviation of mean RR interval in all 5-minute segments of 24-hour recording; C: rMSSD (ms): Square root of the mean squared differences between successive RR intervals; D: pNN50 (%): Percentage of differences between successive RR intervals that are greater than 50 ms; DT: Daytime (1 p.m. – 5 p.m.); 24h: 24 hours; (*) Statistical significance ($p < 0.05$).

Figure 2: A and B: Correlations between BMI and the hormones aldosterone and leptin, respectively, in the all group of patients (black-white circle). C-H: Correlations between HRV (sympathetic and parasympathetic activities) and aldosterone in the all group of patients (black-white circle), non-T2D (white circle), and T2D (black circle). C: LF nu DT and aldosterone in all patients; D: HF nu DT and aldosterone in all patients; E: LF nu DT and aldosterone in non-T2D; F: HF nu DT and aldosterone in non-T2D; G- LF nu DT and aldosterone in T2D; H- HF nu DT and aldosterone in T2D. BMI: body mass index; HRV: heart rate variability; T2D: type 2 diabetes; LF: low frequency; DT: daytime; HF: high frequency.


a
Serum aldosterone (ng/dL)
BMI (Kg/m²)
b
Plasma leptine (pg/mL)
BMI (Kg/m²)
c
LF nu DT
Serum aldosterone (ng/dL)
d
HF nu DT
Serum aldosterone (ng/dL)
e
LF nu DT
Serum aldosterone (ng/dL)
f
HF nu DT
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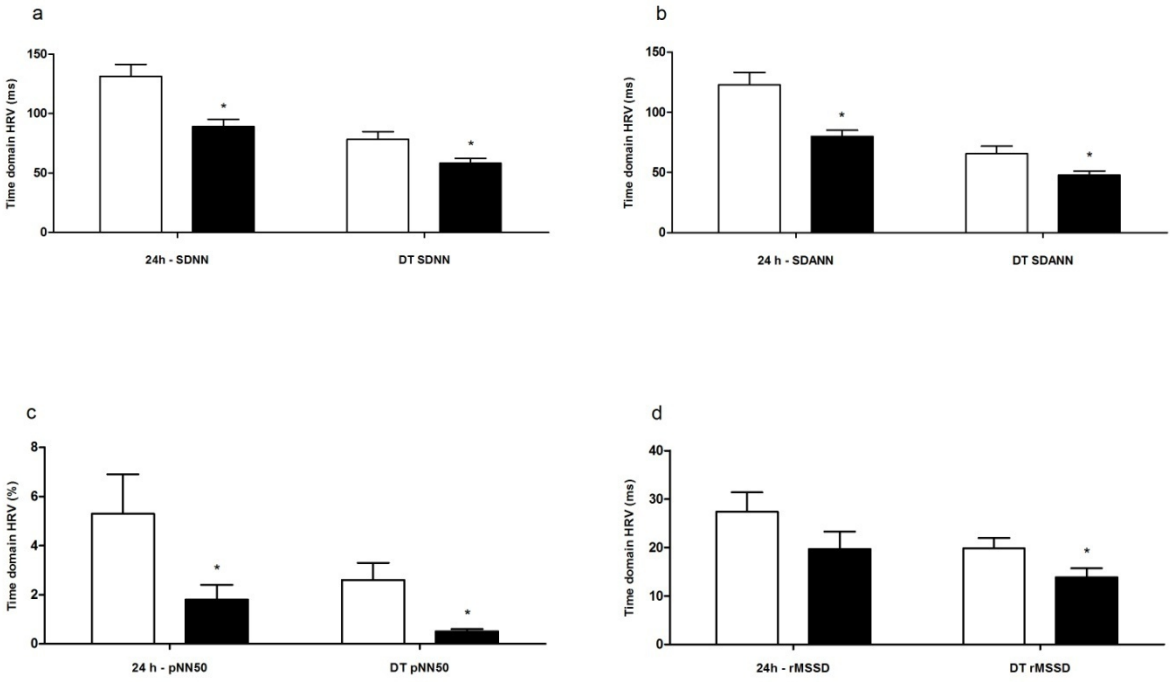
g
LF nu DT
Serum aldosterone (ng/dL)
h
HF nu DT
Serum aldosterone (ng/dL)

Figure 1


a
Time domain HRV (ms)
150
100
50
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*
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24h - SDNN
DT SDNN
b
Time domain HRV (ms)
150
100
50
0
*
*
24 h - SDANN
DT SDANN
c
Time domain HRV (%)
8
6
4
2
0
*
*
24 h - pNN50
DT pNN50
d
Time domain HRV (ms)
40
30
20
10
0
*
24h - rMSSD
DT rMSSD

Figure 2

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