UFCSPA
Universidade Federal de Ciências da Saúde de Porto Alegre

Histórico familiar de hipertensão compromete o equilíbrio simpato-vagal, mas não a função endotelial de jovens jogadores de futebol

Autor: Walter Oliveira de Vargas

Orientador: Dra. Katya Rigatto

Biblioteca Paulo Lacerda de Azevedo

2016

Fisiologia e Patogênese

Tese de Doutorado

Walter Oliveira de Vargas

Histórico familiar de hipertensão compromete o equilíbrio simpato-vagal, mas não a função endotelial de jovens jogadores de futebol

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, como requisito parcial para a obtenção do título de Doutor em Ciências da Saúde.

Orientador: Profa. Katya Vianna Rigatto

Porto Alegre
2016

Catalogação na Publicação

Oliveira de Vargas, Walter
Histórico familiar de hipertensão compromete o equilíbrio simpato-vagal, mas não a função endotelial de jovens jogadores de futebol / Walter Oliveira de Vargas. -- 2016.
119 f. : 30 cm.

Tese (doutorado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em Ciências da Saúde, 2016.

Orientador(a): Katya Vianna Rigatto.

1. Exercício físico aeróbio. 2. Pressão arterial. 3. variabilidade da frequência cardíaca. 4. função endotelial. 5. histórico familiar de hipertensão. I. Título.

Sistema de Geração de Ficha Catalográfica da UFCSPA com os dados fornecidos pelo(a) autor(a).

Pois que aproveitaria ao homem ganhar todo o mundo e perder a sua alma?

Marcos

AGRADECIMENTOS

Agradeço a *UFCSPA*, ao Programa Pós-Graduação em Ciências da Saúde e a todos os professores que contribuíram para a realização deste trabalho, através de seus ensinamentos.

Agradeço aos meus pais, (*dona*) Rosa Maria Rejane de Oliveira pela educação, carinho, amor incondicional e auxílio em todos os momentos e (*seu*) Walter Diniz de Vargas pelo amor que depositou e deposita em mim todos os dias. Agradeço aos meus irmãos, *Déa*, *Juno* e *Gordo* por serem a referência que sempre levarei para toda a vida e por cuidarem do irmão mais novo com tanto amor. Agradeço aos sobrinhos e afilhados, *Caroline, Arthur, Camili, Luara, Gabriely, Iago, Eduardo e Vicente* pelos momentos de carinho e descontração que também foram fundamentais.

Agradeço a minha segunda família, *Jaime, Aida, Daiana, Maiara, Rafael e Matheus*, por serem pessoas tão maravilhosas, que me ajudaram e compreenderam os momentos difíceis que passamos nos últimos quatro anos, em função dos nossos estudos.

Agradeço de forma especial à minha orientadora, Professora *Katya Vianna Rigatto* por esses sete anos de convivência, troca e aprendizado. Desde 2009 quando fiz meu primeiro contato para ingressar no Mestrado sempre foi uma pessoa transparente. Soube extrair de mim o melhor, foi incansável frente aos desafios. Poder trabalhar com ela foi um privilégio. Obrigado também pela amizade, confiança e principalmente por ser um exemplo de competência.

Gostaria de agradecer aos colegas do grupo de estudos em Fisiologia Translacional, *Ana, Gabriela, Thalis, Bárbara, Fabrício, Jéssica, Carmem, Felipe, Taís, Marcelo, Victória e Ricardo* por todo o auxílio, dúvidas e questionamentos que fizeram deste um trabalho melhor, mais sólido. Também aos demais colegas do PPG em Ciências da Saúde.

Gostaria de agradecer aos colegas do grupo de estudos em Hipertensão Experimental do InCor - HC-FMUSP, em especial as professoras *Valéria Hong e Maria Cláudia* por todo o auxílio no meu treinamento e por terem me acolhido junto ao seus colegas de grupo. Sem dúvida vocês fizeram a diferença na elaboração deste trabalho.

Gostaria de agradecer ao *Esporte Clube Novo Hamburgo* e aos seus atletas por terem colaborado para a elaboração deste trabalho.

Gostaria de agradecer a todos os meus *Amigos*. Não me atrevo a nomear todos, pois certamente cometeria alguma injustiça esquecendo alguém fundamental em minha vida. Sem vocês e nossos momentos de descontração, não conseguiria chegar até aqui. Muito obrigado!

Gostaria de agradecer de forma especial a minha esposa, *Tássia Silveira Furlanetto*, por todo amor, carinho, compreensão, por me ajudar todos os dias, me tornando uma pessoa melhor. Mais ainda, tu me deste o maior presente que eu poderia ganhar, a nossa filha *Fernanda*. Te amo!

Por último, mas não menos importante, gostaria de agradecer a *Deus*. Obrigado por ter me concedido o dom da vida, me permitido chegar até aqui, vivendo experiências inesquecíveis e perdoando meus equívocos. Obrigado ainda por ter colocado em meu caminho pessoas tão especiais.

RESUMO

A história familiar de hipertensão impõe risco consistente para muitas doenças crônicas que são acompanhadas da hipertensão primária. Além disso, a variabilidade da frequência cardíaca (VFC) e a vasodilatação mediada pelo fluxo (VMF) são geralmente prejudicadas durante a hipertensão. O exercício físico aeróbio surge como a principal intervenção não farmacológica para o controle da hipertensão primária, uma vez que melhora a função endotelial, aumenta a modulação parassimpática e diminui simpática cardíaca e aumenta o VO_{2max}. A principal modalidade de exercício físico associada a melhora na função cardiovascular e redução dos valores de pressão arterial (PA) é o exercício de predominância aeróbia, como o futebol. Por outro lado, não está estabelecido na literatura se em indivíduos saudáveis, com história familiar de hipertensão, tem também uma deficiência precoce nesses parâmetros. Assim, nosso objetivo foi comparar a modulação simpática e parassimpática, a função endotelial e o consumo máximo de oxigênio (VO_{2max}) de jovens atletas, separados de acordo com a pressão arterial (PA) dos seus pais, para estudar a influência da ascendência genética nestes parâmetros. Também, verificar a possível associação entre a função endotelial com o *Yo-Yo intermittent recovery (Yo-Yo IR) test*, um importante teste de campo para avaliar atletas de esportes intermitentes. MÉTODOS: Quarenta e seis jovens jogadores de futebol do sexo masculino (18±2 anos) foram divididos em quatro grupos: 1- pai e mãe normotensos (FM-N); 2-apenas pai hipertenso (F-H); 3-apenas mãe hipertensa (M-H); 4-pai e sua mãe hipertensos (FM-H). Foi medido PA (mmHg), a VMF (%), a VFC e VO_{2max} (ml/min/kg) de todos os atletas. RESULTADOS: O desvio padrão do intervalo RR normais (SDNN, ms; FM-N= 314±185; FM-H= 182.4± 57.8; P=0,049), a raiz quadrada média da soma dos quadrados das diferenças entre os intervalos N-N adjacentes (RMSSD, ms; FM-N= 248±134; FM-H= 87±51; P=0,028),o índice triangular da VFC (FM-N= 35.7±5.4; FM-H= 18.2±3.9; P=0,002), o número de diferenças de intervalo de intervalos sucessivos NN maiores do que 50 ms (NN50, contagem; FM-N= 367±83.4; FM-H= 229±55; P=0,000), a proporção derivado dividindo NN50 pelo número total de intervalos NN (pNN50, %; FM-N= 32.4±6.2; FM-H= 21.1±5.3; P=0,000) e os componentes de baixa (LF, ms^2; FM-N= 50.9±8.9; FM-H= 64.6±12; P=0,019) e alta frequência (HF, ms^2; FM-N= 49±8.9; FM-H= 35.3±12; P=0,019) em unidades normalizadas (%) foram significativamente mais baixos no FM-H quando

comparados ao grupo FM-N, enquanto que a relação LF/HF (ms^2) foi significativamente maior (P=0,029). Ainda, verificou-se uma associação positiva e significativa entre o *Yo-Yo IR* e a função endotelial (r= 081; P=0,001). Contudo, não houve diferença significativa entre os grupos em relação à idade (anos), peso (kg), altura (cm), PAS e PAD (mmHg). Também não houve diferença significativa entre todos os grupos em todas as medidas alcançadas a partir da avaliação da função endotelial, como VMF, diâmetro basal da artéria braquial, diâmetro na hiperemia reativa, antes ou depois vasodilatação mediada por nitroglicerina. CONCLUSÃO: em jovens jogadores de futebol do sexo masculino, a história familiar de hipertensão é potencialmente importante quando ambos os pais são hipertensos, promovendo um prejuízo no controle simpato-vagal, o que parece preceder o dano endotelial. Também, o *Yo-Yo IR* mostrou uma associação com a função endotelial, mostrando uma interação entre a aptidão física específica e a função vascular em jovens jogadores de futebol.

PALAVRAS-CHAVE: Pressão arterial, variabilidade da frequência cardíaca, função endotelial, exercício físico aeróbio, histórico familiar de hipertensão, *Yo-Yo intermittent recovery test.*

ABSTRACT

Family history of hypertension requires consistent risk for many chronic diseases that are accompanied by primary hypertension. In addition, the heart rate variability (HRV), and the flow-mediated dilatation (FMD) are generally impaired during hypertension. The aerobic exercise emerges as the primary non-pharmacological intervention to control the primary pressure, it improves endothelial function and increases cardiac sympathetic and parasympathetic modulation decreases and increases maximum oxygen consumption (VO_{2max}). The main mode of exercise associated with improvements in cardiovascular function and reduce blood pressure (BP) is the exercise of aerobic predominance, like football. On the other hand, is not established in the literature in healthy subjects with a family history of hypertension, also has an early deficiency in these parameters. So, our goal was to compare the sympathetic and parasympathetic modulation, endothelial function and VO_{2max} of young athletes, separated according to the blood pressure (BP) of their parents, to study the influence of genetic ancestry in these parameters. Also, check the association between endothelial function with the Yo-Yo intermittent recovery (Yo-Yo IR) test, an important field test to evaluate athletes intermittent sports. METHODS: Forty-six young male soccer players (18 ± 2 years) were divided into four groups: 1- father and mother normotensive (FM-N); 2-hypertensive only father (F-H); 3-hypertensive only mother (M-H); 4-father and mother with hypertension (FM-H). It was measured pressure (mmHg), the FMD (%), HRV and VO_{2max} (ml / min / kg) of all athletes. RESULTS: standard deviation of the normal RR intervals (SDNN, ms; FM-N = 314 ± 185; FM-H = 182.4 ± 57.8; p = 0.049), the mean square root of the sum of squares of differences between adjacent NN intervals (RMSSD ms; FM-N = 248 ± 134; FM -H = 87 ± 51; p = 0.028), the triangular index of HRV (FM-N = 35.7 ± 5.4; FM-H = 18.2 ± 3.9; P = 0.002), the number NN successive intervals interval differences larger than 50 ms (NN50, counting; FM-N = 367 ± 83.4; FM H = 229 ± 55; p = 0.000), the ratio derived by dividing NN50 by total number of NN intervals (pNN50,%, FM-N = 32.4 ± 6.2, FM-H = 21.1 ± 5.3; P = 0.000) and lower components (LF, ms^2, FM-N = 50.9 ± 8.9; FM- H = 64.6 ± 12; p = 0.019) and high frequency (HF ms^2, FM N = 49 ± 8.9; FM-H = 35.3 ± 12; p = 0.019) in normalized units (%) were significantly lower in the FM-H when compared with the FM-N group, while the LF / HF ratio (ms^2) was significantly higher (P = 0.029). Still, there was a positive

and significant association between the Yo-Yo IR and endothelial function (r = 081, P = 0.001). However, there was no significant difference between groups with respect to age (years), weight (kg), height (cm), SBP and DBP (mmHg). There was also no significant difference between the groups in all measures reached from the assessment of endothelial function, as FMD, basal diameter of the brachial artery diameter in reactive hyperemia before or after nitroglycerin-mediated vasodilation. CONCLUSION: in young male football players, family history of hypertension is potentially important when both parents are hypertensive, promoting a loss in the sympathetic-vagal control, which seems to precede endothelial damage. Also, the Yo-Yo IR showed an association with endothelial function, showing an interaction between the specific physical fitness and vascular function in young soccer players.

KEYWORDS: Blood pressure, heart rate variability, endothelial function, aerobic exercise, Family history of hypertension, Yo-Yo intermittent recovery test.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

AR	Modelo auto regressivo
Ca^{2+}	Cálcio
FC	Frequência cardíaca
HF	Frequência alta
LF	Frequência baixa
LF/HF	Balanço simpato-vagal ou balanço autonômico
NO	Óxido nítrico
NOS	Óxido nítrico sintase
NOSe	Óxido nítrico sintase endotelial
PA	Pressão arterial
PAD	Pressão arterial diastólica
PAS	Pressão arterial sistólica
NN50	Número de intervalos sucessivos diferentes maiores do que 50ms nos intervalos R-R
pNN50	Proporção derivado dividindo NN50 pelo número total de intervalos R-R
RMSSD	Raiz quadrada média da soma dos quadrados das diferenças entre os intervalos N-N adjacentes
SDNN	Desvio padrão dos intervalos N-N
UM	Unidades normalizadas
VFC	Variabilidade da frequência cardíaca
VLF	Frequência muito baixa
VMF	Vasodilatação mediada por fluxo
$VO_{2máx}$	Consumo máximo de oxigênio
Yo-Yo IR	*Yo-Yo Intermittent Recovery*

SUMÁRIO

Capítulo I – Revisão da Literatura

1. REVISÃO DE LITERATURA

1.1 Pressão arterial

A elevação sustentada da pressão arterial (PA) é um importante fator de risco associado ao aumento em todas as causas de mortalidade por doença cardiovascular. (CIOLAC, GUIMARAES et al., 2008). As doenças cardiovasculares constituem uma das principais causas de morte em todo o mundo. Só em 2003, cerca de 16,7 milhões de pessoas morreram por doenças cardiovasculares em todo o mundo e estima-se que esse número aumente para 23,3 milhões em 2030. A relação entre a PA e o risco de eventos cardiovasculares é contínua e independente de outros fatores de risco (CARDOSO et al., 2012). As últimas orientações para o tratamento da hipertensão primária estabeleceram que os valores desejáveis de PA sistólica (PAS) e diastólica (PAD) são <120 e 80 mmHg, respectivamente (GUIDELINES, 2013). Os eventos cardiovasculares, como infarto do miocárdio e acidente vascular cerebral podem facilmente ocorrer com pressões abaixo de 139/89 mm Hg, um limite ainda considerado normal (GUIDELINES, 2013; LAZDAM et al., 2012). Este fato indica a importância de manter a PA em valores mais baixos possíveis.

Contudo, o aumento nos valores da PA não foi reconhecido como uma ameaça à saúde até a década de sessenta, isso porque se acreditava que um aumento na PA seria uma compensação importante e mecanismo essencial para manter a perfusão sanguínea adequada em indivíduos com idade avançada (FREIS, 1974). Desde então, significativos avanços foram alcançados no tratamento e controle da PA, mas, mesmo

assim, a hipertensão primária continua a ser um grave problema de saúde pública até os dias atuais (GUIDELINES, 2013) e seu tratamento ainda é um desafio.

Em condições normais, a PA deve ser mantida em uma estreita faixa de variação, permitindo uma adequada perfusão tecidual. A manutenção dos níveis pressóricos dentro de uma faixa de normalidade depende de variações do débito cardíaco, da resistência periférica ou de ambos. Um grande número de substâncias e sistemas fisiológicos que interagem de maneira complexa, e com redundância de controle, estão envolvidos não só na manutenção, como na variação momento-a-momento da PA. Esses sistemas regulam o calibre e a reatividade vascular, a distribuição de fluido dentro e fora dos vasos e o débito cardíaco nas mais diversas situações fisiológicas (KRIEGER et al. 2001).

Atualmente, em se tratando de mecanismos de controle da PA, o papel do endotélio vascular e o controle neural (VARGAS et al., 2014), bem como a ascendência genética, tem-se destacado (SANDOO et al., 2010).

Para melhor entender o envolvimento do endotélio, sistema nervoso simpático e parassimpático e a história familiar no controle da PA, algumas considerações serão feitas envolvendo essas importantes participações.

1.2 O endotélio vascular

A capacidade de responder a estímulos físicos e químicos confere aos vasos sanguíneos capacidade de autorregular o tônus, ajustar o fluxo e a distribuição do sangue em resposta às diversas mudanças que podem ocorrer em nível tecidual

(CORRETTI et al., 2002). O controle e a distribuição regional do fluxo sanguíneo é um mecanismo de regulação essencial do endotélio vascular mediado pela contração e relaxamento das células musculares lisas dos vasos de resistência (DELLA ROCCA & PEPINE, 2010). Muitos vasos sanguíneos respondem com dilatação a um aumento no fluxo, ou mais precisamente ao estresse de cisalhamento (CORRETTI et al., 2002). Essa resposta é extremamente importante, pois vai determinar a capacidade circulatória para cada órgão ou tecido.

Por esta razão, inicialmente considerado apenas como uma camada de células entre o sangue e a parede dos vasos, o endotélio é hoje considerado fundamental na regulação do sistema cardiovascular (DELLA ROCCA & PEPINE, 2010; SANDOO et al., 2010). As células endoteliais desempenham papel fundamental na manutenção da função vascular, relaxamento, inibição da resposta inflamatória e antitrombótica (DELLA ROCCA & PEPINE, 2010). Isso se deve ao fato do endotélio controlar o tônus da musculatura lisa vascular pela produção de mediadores que podem produzir vasodilatação ou vasoconstrição (MAIORANA et al., 2003). Este controle é conseguido através da síntese e metabolismo de inúmeras substâncias em resposta a estímulos químicos e mecânicos como o estresse de cisalhamento (DELLA ROCCA & PEPINE, 2010). A produção equilibrada destes fatores vasoativos é ateroprotetora, enquanto que em um endotélio danificado, a sua produção está prejudicada. O desequilíbrio resultante leva à disfunção endotelial, que é um indicador precoce de aterosclerose (SANDOO et al.; 2010).

As células musculares lisas vasculares interagem com células endoteliais para regular a homeostase da parede vascular por meio das pontes mioendoteliais. Estas ligam diretamente as células endoteliais às células musculares lisas, afetando o crescimento celular, a migração, diferenciação e função, além de regulação parácrina.

Muitos desses efeitos são mediados pelo óxido nítrico (NO), sintetizado no organismo a partir do aminoácido L-arginina pela enzima NO sintase (NOS) em resposta a estímulos químicos ou físicos (DELLA ROCCA & PEPINE, 2010).

A inatividade da enzima NOS endotelial (NOSe) está vinculada à proteína caveolina e esta localiza-se em pequenas invaginações na membrana celular chamadas cavéolas. Quando as concentrações intracelulares de cálcio (Ca^{2+}) aumentam, a NOSe se desprende da caveolina e é ativada (Figura 1). Após a depleção dos estoques de Ca^{2+}, um sinal é enviado aos receptores de membrana para abrirem os canais de Ca^{2+} permitindo que o Ca^{2+} extracelular entre na célula. Em seguida, a NOSe converte L-arginina em NO. Esta via de produção de NO é representada abaixo (Figura 1) (CORRETTI et al., 2002) e demonstra claramente a importância do NO no processo de relaxamento vascular derivado do endotélio. Este conjunto de respostas desencadeia o que chamamos de vasodilatação mediada por fluxo (VMF) (CORRETTI et al., 2002).

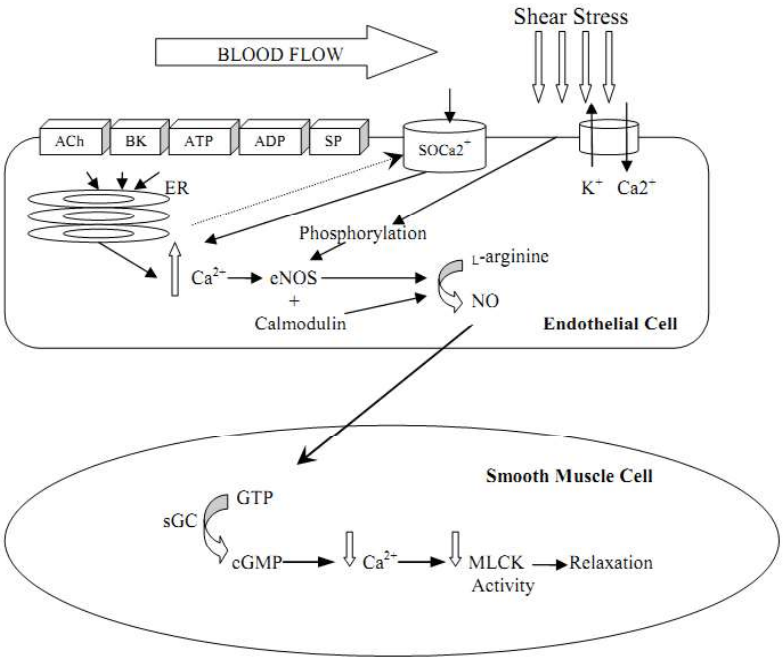


Figura 1 – Ação e produção de NO na célula muscular lisa vascular. ACh= acetilcolina; BK= bradicinina; ATP= adenosina trifosfato; ADP= adenosina difosfato; SP= substância P; SOCa2+ = reserva operacional dos canais de Ca2+; ER= retículo endoplasmático; NO= óxido nítrico; sGC= guanilato ciclase solúvel; cGMP= guanosina 3',5 monofosfato cíclica; MLCK = miosina quinase da cadeia leve. *Quando as reservas de Ca^{2+} do retículo endoplasmático são depletadas, um sinal é enviado para o canal $SOCa^{2+}$ que permite que o Ca^{2+} extracelular entre na célula endotelial (CORRETTI et al., 2002).

Uma vez sintetizado, o NO se difunde através da célula endotelial para o músculo liso adjacente, onde se liga a enzima guanilato ciclase solúvel. Esta enzima, quando ativada, em última instância promove o relaxamento do músculo liso.

Muitos consideram o padrão de referência para testes de função endotelial arterial a avaliação em laboratório por cateterismo com a administração local de acetilcolina. Contudo, estes procedimentos laboratoriais de cateterismo são invasivos e portanto de maior risco ao paciente; e ainda apresentam alto custo financeiro, não sendo adequado para realização em grandes populações. Algumas alternativas mais seguras têm sido propostas na literatura, como a ecocardiografia e a tomografia por emissão de pósitrons, e até ressonância magnética tem sido utilizada para este fim. No entanto, devido à disfunção endotelial ser comum a várias doenças, técnicas menos invasivas e de menor custo financeiro se fazem necessárias (DELLA ROCCA & PEPINE, 2010).

Nesse sentido, uma técnica não-invasiva tem evoluído para avaliar a VMF na artéria braquial. Este fluxo é dependente da função endotelial (CORRETTI et al., 2002), demonstrando a capacidade de formar NO. A utilização da ultra-sonografia da artéria braquial (Figura 2) é uma técnica não-invasiva amplamente utilizada para avaliar esta função. Ela mede a VMF relacionada à isquemia de pequenos vasos na mão e antebraço (DELLA ROCCA & PEPINE, 2010). Uma das limitações da técnica é a necessidade de

um forte treinamento dos pesquisadores ao obter os resultados e minimizar a variabilidade dos dados (CORRETTI et al., 2002; DELLA ROCCA & PEPINE, 2010).

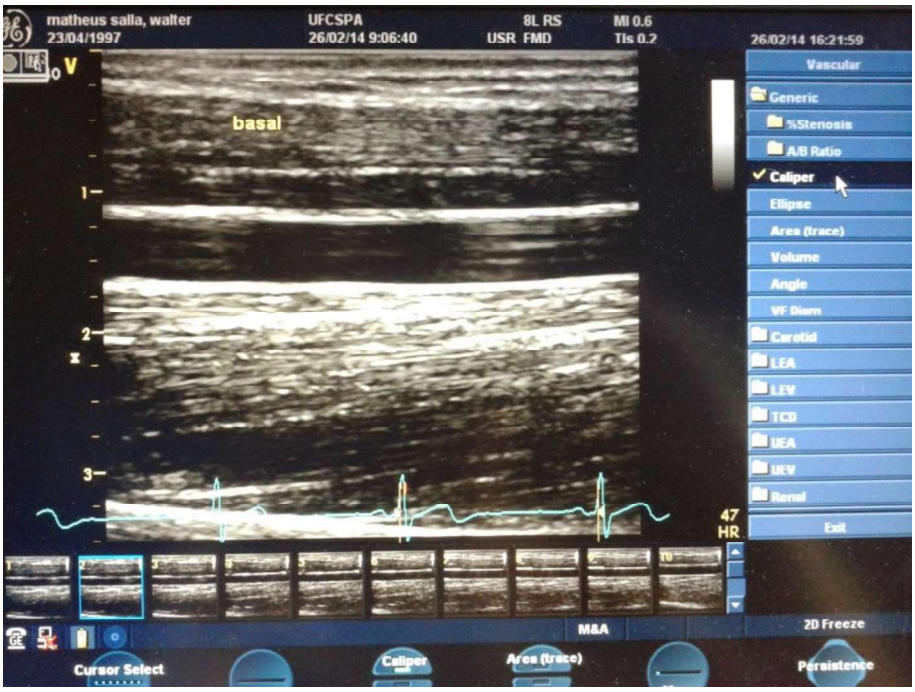


Figura 2: Imagem de ultra-som da artéria braquial.

Em suma, se acreditava que a maioria das complicações cardiovasculares estava mais relacionada a alterações na estrutura e função vascular, e a disfunção endotelial podia, então, ser considerada como uma das mais importantes causas fisiopatológicas que atuavam no desenvolvimento das doenças cardiovasculares (TADDEI et al. 1995).

Além disso, nas últimas décadas houve o reconhecimento de uma associação positiva e significativa entre o funcionamento do sistema nervoso simpático e parassimpático e a mortalidade por doença cardiovascular (TASK FORCE, 1996). Sendo assim, neste trabalho será abordada a associação entre esses dois sistemas, dentre outros fatores que interferem fortemente na regulação da PA, na tentativa de elucidar a fisiopatologia da hipertensão primária.

1.3 Balanço Simpato-vagal

Evidências experimentais mostraram uma associação entre arritmias letais e sinais de aumento, mesmo que pequeno da atividade simpática ou redução parassimpática para o controle do sistema cardiovascular. Isso tem incentivado o desenvolvimento de marcadores quantitativos da atividade autonômica e sua relação com parâmetros de saúde cardiovascular (TASK FORCE, 1996).

As oscilações que ocorrem entre os intervalos R-R (figura 3), intervalo entre dois pontos R do eletrocardiograma ou entre dois ciclos cardíacos, tem sido descrita como a variabilidade da frequência cardíaca (VFC) (LAHIRI, KANNANKERIL et al., 2008).

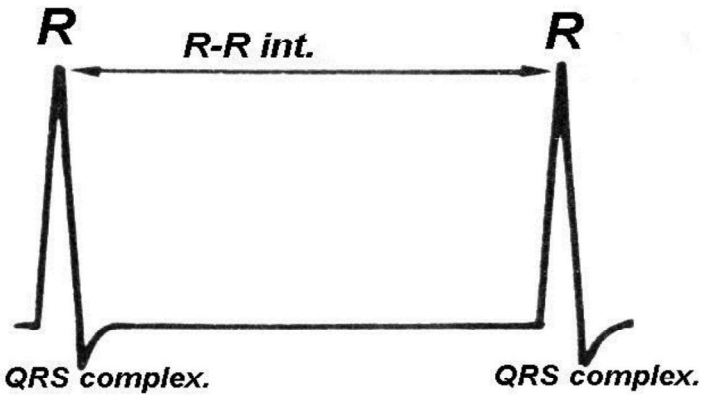


Figura 3: representação do intervalo R-R no eletrocardiograma

A análise da VFC é um método não-invasivo valioso no estudo da função simpática e parassimpática cardiovascular. Ela tem sido frequentemente utilizada na análise de sinais fisiológicos em diferentes condições, mesmo na fase pré-clínica. Na medicina esportiva, a VFC tem sido considerada útil, tanto para avaliar o estado físico, quanto para identificar o limiar anaeróbio de atletas (TASK FORCE, 1996). Diversos

estudos mostraram que há uma relação entre a VFC e o avanço da idade, sugerindo um declínio dependente da idade. Tal afirmação tem aplicação clínica por apresentar correlação inversa com o risco de morte por doenças cardiovasculares, ou seja, quanto maior a VFC em repouso menor o risco de morte (FURLAN et al., 1993).

Variações nos intervalos R-R podem ser avaliadas por diversos métodos, entre eles o método no *domínio do tempo*. Este método avalia a frequência cardíaca (FC) em qualquer ponto do tempo ou dos intervalos R-R, com base em cálculos estatísticos. Muitas medidas podem ser realizadas a partir do método no *domínio do tempo* e estas estão intimamente relacionadas, as medidas do desvio padrão dos intervalos R-R (SDNN), o índice triangular da VFC, a raiz quadrada média da soma dos quadrados das diferenças entre os intervalos N-N adjacentes (RMSSD), o número de intervalos sucessivos diferentes maiores do que 50ms nos intervalos R-R (NN50) e a proporção derivado dividindo NN50 pelo número total de intervalos R-R (pNN50) (TASK FORCE, 1996). As medidas no método do *domínio do tempo*, SDNN e índice triangular da VFC são medidas para uma estimativa geral da VFC, enquanto as medidas RMSSD, NN50 e pNN50 representam uma estimativa de curto prazo, comumente utilizadas para estimar a atividade parassimpática para o controle do coração. Todas estas medidas de curto prazo são altamente correlacionadas (TASK FORCE, 1996).

Por outro lado, os métodos no *domínio da frequência* fornecem uma análise precisa e mais específica. Esse método utiliza a análise espectral de uma sequência de intervalos R-R e fornece informações sobre como o poder (variância) é distribuído. Eles decompõem o ciclo cardíaco em diferentes ondas elementares de amplitude e frequência, em que gravações de poucos minutos (2 a 5 minutos), produzem até três picos de faixas de frequência. A frequência muito baixa (0,003 a 0,04 Hz - VLF), frequência baixa (0,04 a 0,15 Hz - LF) e frequência alta (0,15 a 0,4 Hz - HF), onde os

poderes LF e HF são frequentemente relatados em unidades normalizadas (un), relativa ou fracionada, que representam o valor relativo de cada poder na mesma proporção da potência total. Isto é feito para minimizar o efeito das mudanças no poder total sobre o valor da HF e componentes LF. O componente HF é considerado como a modulação da descarga do nervo vago, representando a atividade parassimpática, enquanto o componente LF representa a atividade simpática. A proporção de LF absoluta sobre o poder HF tem sido usada como um índice do balanço simpato-vagal (LF/HF) (TASK FORCE, 1996).

A mais comum das estimativas espectrais utiliza o algoritmo da transformação rápida de Fourier, para dados não paramétricos, e o modelo auto regressivo (AR) para dados paramétricos. Ambos permitem identificar os três componentes espectrais: LF, HF e VLF (FURLAN et al., 1993; TASK FORCE, 1996).

Tanto a função endotelial, como o sistema nervoso simpático e parassimpático são de fundamental importância na regulação da PA, de tal maneira que alguns estudos tem demonstrado uma íntima associação entre estes a VMF e a VFC (WEHRENS ; HAMPTON ; SKENE , 2012). Apesar disso, um dos principais fatores que irão interferir nas respostas pressóricas, sem dúvida, é a ascendência genética. Este último, cada vez mais tem se mostrado muito importante para adoção de estratégias na saúde pública.

1.4 Histórico familiar de hipertensão

A etiologia da hipertensão primária ainda é desconhecida, apenas 5% dos pacientes hipertensos tem uma causa definida (BRIONES & TOUYZ, 2009) para a

doença. No entanto, é sabido que a elevação da PA pode ser devida a um conjunto de interações complexas que envolvem diversos sistemas fisiológicos e ainda pode estar associada a estímulos ambientais, obesidade, fumo, álcool e consumo excessivo de sal (GUIDELINES, 2013). Neste contexto, a história familiar de hipertensão emergiu como um importante preditor de risco a ser considerado para a criação de estratégias de prevenção (TOZAWA, OSHIRO et al., 2001; MARTINEZ-AGUAYO e FARDELLA, 2009). Na verdade, orientações profissionais já incluem o histórico familiar como potencial risco à saúde (LIU et al., 2014). No entanto, até o momento a obtenção de resultados na literatura não tem considerado o histórico familiar de hipertensão para selecionar os indivíduos controles em seus estudos sobre a fisiopatologia do sistema cardiovascular. Nenhum estudo teve como foco identificar se a ascendência genética influenciaria, em conjunto, o balanço simpato-vagal, o VO_{2max} e a função endotelial em jovens atletas, quando a PA dos atletas se encontra dentro dos limites de normalidade.

Estudos com gêmeos têm mostrado que até metade da variação nos valores da PA é hereditária, isso por que certo número de genes e suas mutações têm sido descritas por serem responsáveis na regulação da PA em famílias portadoras dessas variantes genéticas (MARTINEZ-AGUAYO e FARDELLA, 2009). Evidências sugerem que 66% e 60% das variações na PAS e PAD, respectivamente, são devidas a ascendência genética (ORG, EYHERAMENDY et al., 2009), reforçando a importância de se observar o histórico familiar para hipertensão no momento de selecionar controles adequados para estudos que visem monitorar a modulação autonômica para o coração.

Devido à contribuição dos fatores genéticos na predisposição para o desenvolvimento das doenças cardiovasculares, pesquisadores tem se buscado o desenvolvimento de uma abordagem mais personalizada para o tratamento da hipertensão primária (BYRD, 2016). Dados da literatura têm mostrado que indivíduos

normotensos com história familiar de hipertensão possuem uma diminuição na modulação parassimpática, que são acompanhados por desequilíbrio simpato-vagal e redução da VFC. Esse desequilíbrio também está associado com o aumento da modulação simpática (LUCINI et al., 2002) . A diminuição da modulação simpática ajuda a prevenir o risco de morte prematura e deve ser considerada como um objetivo de tratamento da doença cardiovascular (GUIDELINES, 2013).

Assim, fica claro que aumentos na PA podem envolver também fatores ambientais e hereditários, sendo classificada como uma doença multifatorial e complexa (MARTINEZ-AGUAYO e FARDELLA, 2009). Entre essas causas multifatoriais, é preciso esclarecer quais são os mecanismos que diferenciam os indivíduos com histórico familiar para a hipertensão daqueles indivíduos que não apresentam esta predisposição genética. Sabemos que, potencialmente, somos candidatos a nos tornar hipertensos com a idade, no entanto, não está claro na literatura quais os componentes que podem estar envolvidos no início do processo hipertensivo. Este conhecimento poderá favorecer o desenvolvimento de estratégias específicas para prevenir a hipertensão naqueles indivíduos que conhecidamente apresentam maior chance para se tornar hipertensos. Além disso, pretendemos chamar a atenção para o fato de que, mesmo tendo PA dentro de limites considerados fisiológicos, é possível que o balanço simpato-vagal já não seja representativo dos limites desejáveis pra este balanço, especialmente se o indivíduo possui histórico genético de hipertensão.

1.5 Exercício

O exercício físico representa uma excelente alternativa para estudos que visem elucidar fenômenos fisiológicos. Quando praticado regularmente é considerado

fundamental na manutenção da PA e na saúde do sistema cardiovascular (CORNELISSEN; FAGARD, 2005; FAGARD; CORNELISSEN, 2007; BRIONES & TOUYZ, 2009). Diversos estudos mostraram melhora na regulação cardiovascular pelo sistema nervoso simpático e parassimpático, como resultado de treinamento físico regular. Entre as alterações provocadas pelo exercício regular podemos citar o aumento da modulação parassimpática e a redução da simpática no repouso (VARGAS et al., 2014; IELLAMO et al., 2002; FURLAN et al., 1993).

Isso é confirmado no estudo de Iellano e colaboradores (2002), que mostrou em indivíduos jovens e saudáveis, que há um aumento na modulação parassimpática cardíaca relacionada ao aumento no consumo máximo de oxigênio (VO_{2max}) (IELLAMO et al., 2002). Por outro lado, dados do nosso laboratório obtidos em atletas do futebol normotensos demonstraram que um aumento de 10 mmHg na PA média é suficiente para alterar o equilíbrio autonômico sem, no entanto, ser detectada alteração no VO_{2max} e na função endotelial (VARGAS et al., 2014). Embora não seja possível concluir se a PA ou o balanço simpato-vagal são uma causa ou uma consequência, este resultado indica que a alteração no balanço simpato-vagal precede possíveis alterações no VO_{2max} e na função endotelial naquela amostra.

Não está claro na literatura se o balanço simpato-vagal está alterado em indivíduos saudáveis, normotensos, fisicamente ativos, porém com histórico familiar de hipertensão. Até então, não sabemos se ocorrem diferenças na modulação simpática e parassimpática, na função endotelial e/ou na aptidão física de indivíduos filhos de pais hipertensos

Assim, nosso estudo foi desenhado para investigar a modulação simpática e parassimpática, a função endotelial e o VO_{2max} de jovens atletas, separados de acordo com a PA de seus pais. Nosso objetivo foi identificar a influência de sua ascendência

genética nesses parâmetros. A ideia foi detectar se atletas normotensos têm diferenças de controle do sistema cardiovascular, e, portanto, na variabilidade da frequência cardíaca capaz de comprometer o VO_{2max}, o que poderia comprometer também o seu desempenho.

Além disso, sempre que estudar a modulação simpato-vagal para um determinado sistema, é nossa intenção chamar a atenção para a importância de observar questões genéticas que podem influenciar as respostas fisiológicas deste sistema. Também é nosso objetivo pontuar a importância da adoção de um estilo de vida saudável, especialmente quando a história família está associada com a hipertensão primária. Possivelmente, o exercício seja decisivo para manter a pressão arterial dentro dos limites de normalidade em situações onde nada se pode fazer para mudar, como a ascendência genética que trazemos dos nossos pais.

REFERÊNCIAS

2013 ESH/ESC Guidelines for the management of arterial hypertension. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of Hypertension 2013; 31:1281–1357.

BANGSBO J, IAIA FM, KRUSTRUP P. The Yo-Yo intermittent recovery test: a useful tool for evaluation of physical performance in intermittent sports. Sports Med 2008;38:37–51.

BRIONES AM, TOUYZ RM. Moderate exercise decreases inflammation and oxidative stress in hypertension: but what are the mechanisms? Hypertension 2009;54(6):1206-8.

BYRD JB. Personalized medicine and treatment approaches in hypertension: current perspectives. Integr Blood Press Control. 2016 Apr 6;9:59-67.

CARDOSO CR, LEITE NC, MUXFELDT ES, SALLES GF. Thresholds of ambulatory blood pressure associated with chronic complications in type 2 diabetes. Am J Hypertens. 2012, Jan;25(1):82-8.

CIOLAC, E. G. et al. Acute aerobic exercise reduces 24-h ambulatory blood pressure levels in long-term-treated hypertensive patients. Clinics (Sao Paulo) 2008; 63, 6:753-8.

CORNELISSEN, V. A.; FAGARD, R. H. Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. Hypertension 2005;46, 4:667-75.

CORRETTI MC, ANDERSON TJ, BENJAMIN EJ, CELERMAJER D, CHARBONNEAU F, CREAGER MA, DEANFIELD J, DREXLER H, GERHARD-HERMAN M, HERRINGTON D, VALLANCE P, VITA J, VOGELET R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–265.

DELLA ROCCA, D. G.; PEPINE, C. J. Endothelium as a predictor of adverse outcomes. Clin Cardiol 2010; 33, 12:730-2.

FAGARD, R. H.; CORNELISSEN, V. A. Effect of exercise on blood pressure control in hypertensive patients. Eur J Cardiovasc Prev Rehabil 2007;14,1:12-7.

FURLAN R, PIAZZA S, DELL'ORTO S, GENTILE E, CERUTTI S, PAGANI M, MALLIANI A. Early and late effects of exercise and athletic training on neural mechanisms controlling heart rate. Cardiovasc Res. 1993;27(3):482-8.

FREIS, E. D. The Veterans Administration cooperative study on antihypertensive agents. Implications for stroke prevention. Stroke 1974; 5, 1:76-7.

Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J 1996;17:354-381.

KRIEGER EM, DA SILVA GJ, NEGRÃO CE. Effects of exercise training on baroreflex control of the cardiovascular system. Ann N Y Acad Sci. 2001;940:338-47.

LAHIRI, M. K.; KANNANKERIL, P. J.; GOLDBERGER, J. J. Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. J Am Coll Cardiol 2008;51,18:1725-33.

LAZDAM M, LEWANDOWSKI AJ, KYLINTIREAS I, CUNNINGTON C, DIESCH J, FRANCIS J, TREVITT C, NEUBAUER S, SINGHAL A, LEESON P. Impaired endothelial responses in apparently healthy young people associated with subclinical variation in blood pressure and cardiovascular phenotype. Am J Hypertens 2012;25:46–53.

LIU J, SEKINE M, TATSUSE T, HAMANISHI S, FUJIMURA Y, ZHENG X. Family History of Hypertension and the Risk of Overweight in Japanese Children: Results From the Toyama Birth Cohort Study. J Epidemiol 2014;24(4):304-311.

LUCINI D, MELA GS, MALLIANI A, PAGANI M. Variability in Humans: Insights From Spectral Analysis of Beat-by-Beat Cardiovascular Impairment in Cardiac Autonomic Regulation Preceding Arterial Hypertension. Circulation 2002;106:2673-2679.

MAIORANA, A. et al. Exercise and the nitric oxide vasodilator system. Sports Med, v. 33, n. 14, p. 1013-35, 2003.

MARTINEZ-AGUAYO A, FARDELLA C. Genetics of hypertensive syndrome. Horm Res. 2009;71(5):253-9.

ORG E, EYHERAMENDY S, et al. Genome-wide scan identifies CDH13 as a novel susceptibility locus contributing to blood pressure determination in two European populations. Hum Mol Genet 2009;8:2288-96.

SANDOO, A. et al. The endothelium and its role in regulating vascular tone. Open Cardiovasc Med J 2010;4:302-12.

TADDEI S, VIRDIS A, GHIADONI L, SALVETTI A. Endothelial function and arterial hypertension. Ann Ital Med Int. 1995 Oct;10.

TOZAWA M, OSHIRO S, ISEKI C, SESOKO S, HIGASHIUESATO Y, TANA T, IKEMIYA Y, ISEKI K, FUKIYAMA K. Family history of hypertension and blood pressure in a screened cohort. Hypertens Res 2001;24(2):93-8.

VARGAS W, DIPP T, PLENTZ RDM, RIGATTO K. Higher mean blood pressure is associated with autonomic imbalance but not with endothelial dysfunction in young soccer players. Am J Hypertens 2014;27(4):508-13.

WEHRENS SM, HAMPTON SM, SKENE DJ. Heart rate variability and endothelial function after sleep deprivation and recovery sleep among male shift and non-shift workers. Scand J Work Environ Health. 2012 Mar;38(2):171-81.

2. OBJETIVOS

2.1 Objetivo Geral

O objetivo do presente estudo foi analisar a influência do histórico familiar de hipertensão sobre a função cardiovascular e seus mecanismos de controle em jovens jogadores de futebol.

2.1 Objetivos Específicos

Verificar a influência do histórico familiar de hipertensão na:

- Função endotelial arterial;
- Atividade do sistema nervoso simpático e parassimpático;
- Consumo máximo de oxigênio.

3. HIPÓTESE

O histórico familiar de hipertensão primária está associado a uma diminuição na função endotelial, a variabilidade da frequência cardíaca e no consumo máximo de oxigênio de jovens atletas de futebol.

Capítulo II – Artigo em Inglês

Family history of hypertension impairs the autonomic balance, but not the endothelial function in young soccer players

Autores: Walter Vargas & Katya Rigatto

Artigo formatação *Autonomic Neuroscience: basic and clinic* – Fator de impacto: 1.562

Family history of hypertension impairs the autonomic balance, but not the endothelial function in young soccer players

Walter Vargas[1], Katya Rigatto[1]

[1]Pós-graduação em Ciências da Saúde, Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil

Short Title: Parents blood pressure and young men autonomic impairment

Corresponding Author: Katya Rigatto

DCBS, Universidade Federal de Ciências da Saúde de Porto Alegre.

Sarmento Leite, 245, Porto Alegre, RS, Brazil. Zip Code: 90050-170

Telephone: 55 (51) 3303-8753 - Fax Number: 55 (51) 3303-8810

E-mail: krigatto@gmail.com

Disclosure: No conflicts of interest.

Key Words: autonomic nervous system; heart rate variability; endothelial function; hypertension; blood pressure, family history of hypertension

ABSTRACT

BACKGROUND: Family history of hypertension imposes consistent risk for many chronic diseases that are accompanied of hypertension. Furthermore, heart rate variability (HRV) and flow-mediated dilation (FMD) are usually impaired during hypertension. On the other hand, it is not established in the literature whether in healthy subjects, with family history of hypertension, have also a precocious impairment in those parameters. Thus, our objective was to compare the autonomic modulation, the endothelial function and the maximum oxygen uptake (VO_{2max}) of young athletes, separated according to their parents blood pressure (BP) history, to study the influence of the genetic "baggage" in those parameters. **METHODS:** Forty-six young male soccer players (18±2 years) were divided into four groups: 1-normotensive father and mother (FM-N); 2-only father was hypertensive (F-H); 3-only mother was hypertensive (M-H); 4-father and mother were hypertensive (FM-H). It was measured BP, FMD, HRV and VO_{2max}. **RESULTS:** The standard deviation of normal RR intervals (SDNN; FM-N= 314±185; FM-H= 182.4± 57.8), the square root of the mean squared differences in successive RR intervals (RMSSD; FM-N= 248±134; FM-H= 87±51), the number of interval differences of successive NN intervals greater than 50ms (NN50; FM-N= 367±83.4; FM-H= 229±55), the proportion derived by dividing NN50 by the total number of NN intervals (pNN50; FM-N= 32.4±6.2; FM-H= 21.1±5.3) and the high (HF; FM-N= 49±8.9; FM-H= 35.3±12) and low-frequency (LF; FM-N= 50.9±8.9; FM-H= 64.6±12) components, in normalized units (%) were significantly lower in the FM-H than in the FM-N group ($P<0.05$), while the LF/HF ratio (ms^2) was significantly higher ($P<0.05$). **CONCLUSIONS:** In young male soccer players, the family history of hypertension is potentially important to impair the autonomic balance, specially when both parents are hypertensive. In this case, there is a decrease in the sympathetic-vagal control which seems to precede the endothelial damage.

1. INTRODUCTION

Cardiovascular diseases represent one of the major causes of death in the world. Only in 2003 about 16.7 million people died from cardiovascular diseases worldwide and it is estimated that this number will increase to 23.3 million in 2030[1]. The association between blood pressure (BP) and the risk of cardiovascular events is continuous and independent of other risk factors[2]. The last Guidelines for the management of arterial hypertension established that the optimal values of systolic (SBP) and diastolic (DBP) are <120 and 80 mmHg, respectively[2]. Cardiovascular events such as sudden coronary death, myocardial infarction and stroke might easily occur at pressure even below 139/89mmHg, a threshold considered normal BP[3,4]. This fact indicates the importance of keeping the BP at lower values.

In this context, family history of hypertension emerges as an important predictor of risk to be considered for creating prevention strategies. In fact, professional guidelines already include the genetic family history to assess health risks[5]. Evidence suggests that the variation of 66% in SBP and 60% in DPB are due to genetic "baggage"[6].

Data from the literature has shown that normotensive subjects with family history of hypertension have lower parasympathetic modulation to the heart and also heart rate variability (HRV). These findings are accompanied by sympathovagal imbalance[7]. Moreover, it has been postulated that this imbalance is associated to an increased sympathetic participation that could be used as a marker for monitoring the cardiovascular system[8]. A decreased sympathetic modulation helps preventing the risk of premature death[9] and should be a goal for treating cardiovascular system disease.

On the other hand, in healthy young subjects, there is consistent evidence that enhanced parasympathetic activity is associated with an increase in maximal oxygen uptake (VO_{2max})[10]. However, data from our laboratory have shown in a normotensive group of young soccer players that a difference of 10mmHg in mean BP is enough to change the autonomic balance, without changing VO_{2max} and endothelial function[11]. Although it is not possible to conclude whether BP or autonomic balance is the cause or the consequence, this result indicates that the alteration in autonomic balance probably precedes the VO_{2max} or endothelial.

Thus, our study was designed to compare the autonomic modulation, the endothelial function and the VO_{2max} of young athletes grouped according to the parents BP history. The goal was to access the influence of the genetic "baggage" in those parameters and whether normotensive athletes have differences in the cardiovascular system control that could compromise their performance. Additionally, it is our intention to drive the attention to the importance to prevent cardiovascular diseases and also to find out which system is the first to be compromised in normotensive subjects with a family history associated to hypertension.

2. MATERIAL AND METHODS

The Ethics Committee of the *Universidade Federal de Ciências da Saúde de Porto Alegre* (UFCSPA) approved the study (CEP/UFCSPA protocol number 562.572). It was performed in forty-six young male soccer players (18±2 years): anthropometric and BP measurements, autonomic nervous system and endothelial function evaluation, exercise tests. All players had at least 2 years of prior soccer-specific training and living in the club accommodations to avoid significant differences in life style. Moreover, all meals were provided assuring similar diet and nutrients.

2.1 Study design

Before data collection, the athletes were fully informed about the tests to be performed and provided written informed consent to participate. The data were collected during the soccer preseason, when the athletes were training but not participating in a competition.

The athletes were instructed to attend the Laboratory of Physiotherapy/UFCSPA at 7am and to fast beforehand. The BP and HR were measured followed by arterial endothelial function in the brachial artery. To avoid an excess of measurements in a single day, the anthropometric data (height, weight, age, body fat percentage, and time of training) and VO_{2max} were collected one week later. The athletes were grouped according to their family history of hypertension: 1- normotensive father and mother (FM-N), with 14 athletes; 2- only father was hypertensive (F-H), with 11 athletes; 3- only mother was hypertensive (M-H), with 10 athletes; and 4- father and mother were hypertensive (FM-H), with 11 athletes. Following the guidelines for this evaluation[12],

the athletes BP were measured as well as their parents. The hypertensive states of the athlete's parents was previously been done by a physician. 53.3% were taking anti-hypertensive drugs and 3.3% were not treating their state. Individuals who showed changes in BP values were advised to seek medical attention.

2.2 Blood pressure measurement

The athletes were kept in a quiet environment for at least 5 minutes before BP measurements. It was used an auscultatory method, with their feet on the ground, right arms supported at heart level and the BP cuff covering at least 80% of the upper arm. To confirm the data, the BP measurement was repeated at least twice at 2-minute interval. When a difference of more than 6 mmHg was detected in two successive measurements, the measurements were repeated until the difference was less than 4 mmHg. For each athlete, an average of two measurements was used to obtain SBP[12].

2.3 Heart Rate Variability

A Polar model RS800CX heart-rate monitor (Polar Electro Öy, Kempele, Finland), was used to collect heart rate (HR) data at a sampling frequency of 1000Hz. For the evaluation of HRV, the athletes were instructed to lie quietly on a stretcher in the supine position. After 10 minutes, still in the supine position, the HR signal was recorded for 10 minutes follow by additional 10 minutes with the athlete standing in front of the stretcher[12]. The signal was automatically stored as an RR interval and analyzed with Kubios HRV software version 2.0 (University of Kuopio, Kuopio, Finland). A 1,000-Hz sampling rate was chosen to provide a temporal resolution of 1

ms for each RR interval, a standard deviation of normal RR intervals (SDNN, ms), the square root of the mean squared differences between consecutive RR intervals (RMSSD, ms), the number of interval differences of successive NN intervals greater than 50 ms (NN50, ms), and the proportion derived by dividing NN50 by the total number of NN intervals (pNN50; ms)[8].

An autoregressive method was used to determine HRV, on the basis of the spectral power integrated in two frequency bands: (i) a high-frequency (HF) band from 0.15–0.4 Hz; and (ii) a low-frequency (LF) band from 0.03–0.15 Hz. The results were expressed in absolute values (HFa and LFa, ms2) and their respective percentages (HFnu and LFnu, %). The LF/HF ratio (ms2) was calculated according to LFa and HFa[8].

2.4 Endothelial Function Assessment

Endothelial function was assessed noninvasively by means of a brachial artery ultrasound probe (GE Medical Systems, Vivid I Ultrasound, Israel) and Doppler ultrasonography, using an instrument equipped with a 7- 12-MHz high-resolution linear probe (L12-3, GE Medical Systems, Israel). The ultrasonography was performed in a silent, temperature-controlled room. At rest, the left brachial artery diameter was measured by B-mode ultrasound images to detect reactive hyperemia. Before BP cuff inflation, a resting scan was performed. After the resting measurement, the cuff was inflated for 5 min at 50 mmHg above SBP, to occlude the arterial flow. This procedure causes ischemia followed by vasodilation due to auto-regulatory mechanisms. After cuff deflation, a second continuous scan was recorded from 30–120 seconds. The same experienced sonographer performed and analyzed all ultrasound scans without knowing

the genetic history of each athlete. At a fixed position, the vessel diameter was measured offline with ultrasonic calipers at end-diastole and incident with the R wave on an electrocardiogram that it was continuously recorded. After an interval of 10 seconds and during the period within 30–180 seconds, the dilatation was obtained by the difference from baseline. After the release of the sphygmomanometer cuff, the flow-mediated dilation (FMD, %) indicates the increase in blood flow[13].

2.5 Maximal oxygen uptake

The Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1) was used to infer VO_{2max}. The athletes performed 2×20-minute shuttle runs at increasing speeds, interspersed with a 10-second period of active recovery. The test was controlled by audio signals from a compact-disc player and ended when the athlete was unable to maintain the speed for the test. The distance traveled at that point was the result of the test, as described by Bangsbo et al.[14]. The indirect measurement of VO_{2max} was calculated as follow:

VO_{2max} (ml/min/kg) = IR1 distance (meters) × 0.0084 + 36.4 [14]

2.6 Statistical analysis

The data normality and equality were assessed through the Shapiro–Wilkand Levene tests. The one-way ANOVA was used to compare groups, follow by Tukey's post hoc test. The association between variables was assessed through Pearson correlations.

All analyses were done with SPSS software version 10.0 (SPSS Inc., Chicago, IL). The data are presented as means±SD or, when stated, means±SE. The difference was considered statistically significant when $P<0.05$. To detect a minimum 30% difference between groups with a minimum probability of a type I error of 5% ($\alpha = 0.05$) and a probability of type II error of 20% ($\beta = 0.2$), the minimum number of individuals each group was estimated at 10.

3. RESULTS

3.1 Anthropometric, SBP, DBP and maximal oxygen uptake measurements

There was no significant difference among groups regarding to age (years; FM-N=17.8±0.8; F-H =17.7±1.0; M-H=17.6±0.8; FM-H=17.5±0.7), weight (kg; FM-N=69.4±3.2; F-H=67.4±3.1; M-H=69.6±4; FM-H=70.6±4), height (cm; FM-N=175.1±5.3; F-H=172.2±4.7; M-H=177.5±7.3; FM-H=175.9±5.5)

Moreover, VO_{2max} (ml/min/kg), indicating that physical fitness was similar among groups, and SBP and DBP (mmHg) were not different among groups (Table 1a).

3.2 Heart rate and time-domain and frequency-domain measurements of resting heart-rate variation

The HRV in the time domain examined in the study was significantly lower in the FM-H than in the FM-N group (Table 2). The spectral analysis, from frequency-domain method, HFnu, was significantly lower in the FM-H than in the FM-N group and LFnu and LF/HF were significantly higher in the FM-H than in the FM-N group.

3.3 Endothelial function assessment

There was no significant difference among groups in FMD or in baseline brachial artery diameter upon reactive hyperemia, either before or after nitroglycerin-mediated vasodilatation (Table 3; $P<0.05$).

4. DISCUSSION

The most important observation of this study was that, in young soccer players, independently of FMD, SBP, DBP or VO_{2max}, the family history hypertension was crucial to the autonomic modulation, even without significant difference in BP among athletes.

According to literature data, the prevalence of hypertension appears to be from about 30% to 45% of the general population[12]. In our study, we found a prevalence of 53.3% for the parents of athletes (Table1b), values above the world average. We believe that socio-economic factors can explain the difference found in our sample.

Our results provide, for the first time, evidence that family history of hypertension might be crucial to the progressive imbalance of autonomic regulation in healthy young athletes with normal BP. To our knowledge, this is the first study that shows the possible precocious involvement of the autonomic modulation in the hypertensive process. At least partially, it is reasonable to believe that our results point out toward to the fact that the first change in the hypertensive process has as a target the sympathetic and parasympathetic system. These conclusions are in agreement with Vargas et al.[11] who also showed in athletes that a small increase in BP induces a change in the autonomic nervous system without changing the endothelial function or VO_{2max}.

Considering that the autonomic regulation can be assessed with a non-invasive approach to evaluate the HRV in the time and frequency domain[8], it could be useful to detect its impairment and provide the physicians with valuable information to evaluate the treatment efficiency or even to prevent diseases. Despite of the enormous impact of the decrease in HRV over the cardiovascular risk, we did not find any research in the

literature showing an association between family history of hypertension and these parameters in healthy subjects. We believe that our results may drive the attention to a method that is easy, has low cost and that it can show data associated to a significant cardiovascular risk, such as HRV. They will contribute not only to prevent hypertension in subjects who are at genetic risk, but also to open up a new possibility of monitoring hypertensive patients.

In our study, the FM-H group showed higher LFnu and LF/HF ratio than did the FM-N group. In addition, in the FM-H group, the HFnu, in the frequency-domain, and SDNN, RMSSD, NN50, pNN50 and HRV triangular index, in the time-domain, were significantly lower than those in the FM-N group. These results indicate that family history of hypertension is accompanied by an increase in cardiac sympathetic modulation and a decrease in parasympathetic modulation, regardless of the normal BP of each soccer player.

Moreover, Tozawa et al[16] seek to determine whether the family history of hypertension is quantitatively associated with the prevalence of hypertension in a screened cohort. They have concluded that the increasing number of family members with hypertension has an association with an increased prevalence of higher BP, independent of conventional risk factors for hypertension. These findings are in agreement with ours, since, we also found a significant difference in autonomic modulation only when both parents were hypertensive, emphasizing the importance of genetic background for the HRV, which is a predictor of cardiovascular risk.

There is no doubt that physical exercise is associated with beneficial effects on BP. Because exercise is a healthy method to control cardiovascular diseases[3], we have chosen to study only athletes. Our results emphasize the importance of the genetic

background. Healthy and young athletes, who had hypertensive father and mother, presented a significant increase of LF/HF ratio, as well as a reduction in HRV.

There is also consistent evidence showing that enhanced parasympathetic modulation is associated with an increase in VO_{2max} in healthy young subjects[10]. However, in the present study we have found no difference in VO_{2max}, BP and endothelial function among all groups. This is probably due to the fact that, being young athletes and eating similar diet and nutrients, our groups had a high physical performance, attenuating the differences.

However, we did find a significant difference in cardiac autonomic modulation between the FM-H and FM-N but we did find athletes with a desirable physical fitness that improves VO_{2max}, endothelial function that, ultimately, keeping the BP in normal values despite of family history of hypertension.

In agreement with Lucini et al[9], our results demonstrated that the autonomic changes possibly precede endothelial dysfunction. They showed that, in subjects with BP in the upper normotensive range, the HRV was impaired. These authors also reported that these changes might suggest that the disturbance in the autonomic regulation precedes the hypertensive state[9] as seen in the neurogenic hypertension.

A point of criticism of our method is the fact that we did not separate the groups according to the parents's type of hypertension. On the other hand, we do know that the probability to have only parents with neurogenic hypertension in the FM-H group is very low. Thus, it is reasonable to believe that, independently of the cause of hypertension, the endothelial function was preserved.

According to our results, strengthened by previous studies that also were looking for answers about the beginning of the arterial hypertensive process[9,11,16], it appears that the autonomic dysfunction precedes the endothelial dysfunction. Thus, it is a challenge

to discover a treatment for the sympathovagal imbalance and reduce the cardiovascular risk.

5. CONCLUSIONS

Although our study has the limitation to have a small sample size, the present study suggests that HRV, in the time and frequency domain, might provide a useful functional outcome to earlier assess the cardiovascular system control. This advantage is useful for healthy young people, as young soccer players, and also probably more important for sedentary people at risk. Doing exercise, more than treat the borderline hypertension, represents an alternative to prevent the increase in BP through strategies that treat the mechanisms by which normal BP, ultimately, become hypertension.

Acknowledgments: This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

1. Dabhadkar KC, Kulshreshtha A, Ali MK, Narayan KM. Prospects for a cardiovascular disease prevention polypill. *Annu Rev Public Health* 2011; 32: 23-38.

2. Cardoso CR, Leite NC, Muxfeldt ES, Salles GF. Thresholds of ambulatory blood pressure associated with chronic complications in type 2 diabetes. *Am J Hypertens* 2012;25:82–88.

3. 2013 ESH/ESC Guidelines for the management of arterial hypertension. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Journal of Hypertension* 2013; 31:1281–1357.

4. Lazdam M, Lewandowski AJ, Kylintireas I, Cunnington C, Diesch J, Francis J, Trevitt C, Neubauer S, Singhal A, Leeson P. Impaired endothelial responses in apparently healthy young people associated with subclinical variation in blood pressure and cardiovascular phenotype. *Am J Hypertens* 2012;25:46–53.

5. Liu J, Sekine M, Tatsuse T, Hamanishi S, Fujimura Y, Zheng X. Family History of Hypertension and the Risk of Overweight in Japanese Children: Results From the Toyama Birth Cohort Study. *J Epidemiol* 2014;24(4):304-311.

6. Org, E., S. Eyheramendy, et al. Genome-wide scan identifies CDH13 as a novel susceptibility locus contributing to blood pressure determination in two European populations. *Hum Mol Genet* 2009;8:2288-96.

7. Duprez DA. Cardiac autonomic imbalance in pre-hypertension and in a family history of hypertension. *J Am Coll Cardiol*. 2008 May 13;51(19):1902-3.

8. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur Heart J* 1996;17:354-381.

9. Lucini D, Mela GS, Malliani A, Pagani M. Variability in Humans: Insights From Spectral Analysis of Beat-by-Beat Cardiovascular Impairment in Cardiac Autonomic Regulation Preceding Arterial Hypertension. *Circulation* 2002;106:2673-2679.

10. Iellamo F, Legramante JM, Pigozzi F, Spataro A, Norbiato G, Lucini D, Pagani M. Conversion from vagal to sympathetic predominance with strenuous training in high-performance world class athletes. *Circulation* 2002;105:2719-2724.

11. Vargas W, Dipp T, Plentz RDM, Rigatto K. Higher mean blood pressure is associated with autonomic imbalance but not with endothelial dysfunction in young soccer players. *Am J Hypertens* 2014;27(4):508-13.

12. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, Lackland DT, LeFevre ML, MacKenzie TD, Ogedegbe O, Smith SC Jr, Svetkey LP, Taler SJ, Townsend RR, Wright JT Jr, Narva AS, Ortiz E. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8).. JAMA. 2014 Feb 5;311(5):507-20.

13. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogelet R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;39:257–265. Epub 2002/01/15.

14. Bangsbo J, Iaia FM, Krustrup P. The Yo-Yo intermittent recovery test: a useful tool for evaluation of physical performance in intermittent sports. *Sports Med* 2008;38:37–51.

15. Bertagnolli M, Schenkel PC, Campos C, Mostarda CT, Casarini DE, Belló-Klein A, Irigoyen CM, Rigatto K. Exercise training reduces sympathetic modulation on cardiovascular system and cardiac oxidative stress in spontaneously hypertensive rats. *Am J Hypertens* 2008;21:1188–1193.

16. Tozawa M, Oshiro S, Iseki C, Sesoko S, Higashiuesato Y, Tana T, Ikemiya Y, Iseki K, Fukiyama K. Family history of hypertension and blood pressure in a screened cohort. *Hypertens Res* 2001;24(2):93-8.

17. Byrd JB. Personalized medicine and treatment approaches in hypertension: current perspectives. *Integr Blood Press Control*. 2016 Apr 6;9:59-67.

Table 1a. Measurements of systolic and diastolic blood pressure and maximal oxygen uptake

	FM-N (n=14)	**F-H (n=11)**	**M-H (n=10)**	**FM-H (n=11)**
SBP (mmHg)	124±4.9	127±7	127±11.2	127±5.7
DBP (mmHg)	71±8.2	76±6.7	78±9.3	78±11.6
VO_{2max} (ml/kg/min)	53.5±2.5	52.3±2.9	53.4±1.1	51.4±1.6

Abbreviations: SBP. Systolic blood pressure; DBP. Diastolic blood pressure; VO_{2max}. Maximal oxygen uptake. Values are the mean ± SD.

Table 1b. Measurements of parent's systolic and diastolic blood

	FM-N (n=14)		F-H (n=11)		M-H (n=10)		FM-H (n=11)	
	Father	**Mother**	**Father**	**Mother**	**Father**	**Mother**	**Father**	**Mother**
SBP (mmHg)	130±17.3	124±5.4	147±13	124±3	128±5.5	150±11	148±8.4	147±8.7
DBP (mmHg)	86±6.5	85±4.3	96±6.7	83±3.4	85±2.4	93±7	97±7	92±6.7

Abbreviations: SBP. Systolic blood pressure; DBP. Diastolic blood pressure. Values are the mean ± SD.

Table 2. Heart rate and time-domain and frequency-domain measurements of resting heart-rate variation

	FM-N (n=14)	F-H (n=11)	M-H (n=10)	FM-H (n=11)
RMSSD (ms)	248±134	157±95	196±211	87±51*
NN50(count)	356±82	260±50	296±81.3	218.8±44*
pNN50 (%)	31.5±6.4	23.6±3.4	25.8±6.3	20.2±4.5*
HRV triangular index	26.6±7	21.9±6.1	20.8±7.4	17.2±2.5*
SDNN (ms)	317.4±163	235.6± 103.4	198.2± 97.6	174.4± 53.2*
HFa (ms)	24718±32307	19180± 27687	15243± 23814	7934± 9090
HFnu (%)	48.6±8.6	40.3±13	38.4±10.3	33.8±11.2*
LFa (ms)	28216± 34030	21920± 25985	8763± 13184	8944± 9918
LFnu (%)	51.4±8.6	59.7±13	61.6±10.3	66.2±11.2*
LF/HF (ms^2)	1.1±0.4	1.8±1.1	1.7±0.6	2.3±1.4*

Values are means ± SD. *A value of $P < 0.05$ was considered statistically significant when compared to the group FM-H.

Abbreviations: RMSSD, square root of the mean squared differences between consecutive RR intervals; NN50, the number of successive NN intervals greater than 50ms; pNN50, the proportion derived by dividing NN50 by the total number of NN intervals; HRV, heart rate variability; SDNN, standard deviation of normal RR intervals; HFa, absolute values of high-frequency component; nu, normalized units; LFa, absolute values of low-frequency component; LF/HF, ratio between low- and high-frequency power components.

Table 3. Brachial artery characteristics of athletes in supine position

	FM-N (n=14)	F-H (n=11)	M-H (n=10)	FM-H (n=11)
B-DIA (mm)	0.355±0.043	0.364±0.035	0.344±0.041	0.383±0.037
RH-DIA (mm)	0.387±0.042	0.387±0.028	0.366±0.042	0.402±0.045
FMD (%)	9.323±3.028	6.745±1.263	6.261±1.726	5.097±3.157
Before NTG (mm)	0.368±0.044	0.363±0.032	0.352±0.043	0.387±0.039
After NTG. (mm)	0.431±0.039	0.431±0.036	0.419±0.041	0.453±0.034
NTG (%)	17.639±7.086	18.920±3.991	19.472±6.456	17.678±7.503

Abbreviations: B-DIA. basal brachial artery diameter; FMD. flow-mediated dilation; NTG. brachial artery diameter with nitroglycerin; RH-DIA. brachial artery diameter with reactive hyperemia. Values are the mean ± SD.

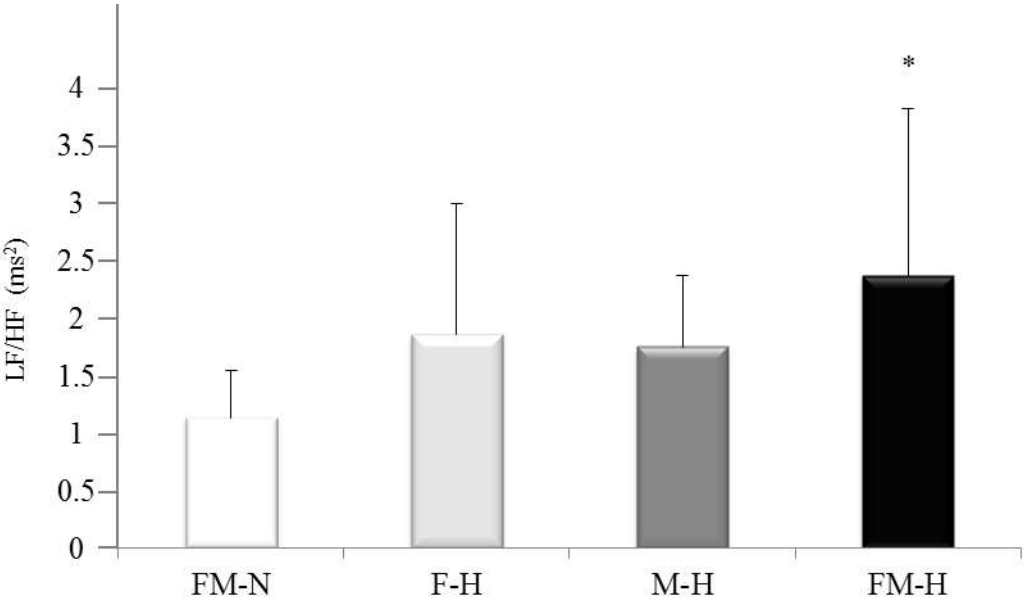


Figure 1. LF/HF= Ratio between low and high frequency power components, i.e. autonomic balance, of the FM-N, F-H, M-H and FM-H groups .* Difference between the FM-N and FM-H groups (P<0.005)

Capítulo III – Artigo em Inglês

Distance on the Yo-Yo IR1 is associated with FMD in young football players

Autores: Walter Vargas & Katya Rigatto

Artigo formatação The Journal of Sports Medicine and Physical Fitness – Fator de impacto: 0.972

Distance on the Yo-Yo IR1 is associated with FMD in young football players

Abstract

The Yo-Yo intermittent recovery test evaluates the individual capacity to carry out intermittent exercise leading to a maximal activation of the aerobic system. It has also been shown that aerobic exercise improves endothelial function. Moreover, studies indicate that there is a positive association between endothelial function and maximal oxygen uptake, but no data is available about the association with the Yo-Yo intermittent recovery test. Thus, the aim of the present study was to investigate, in young football players, whether the Yo-Yo intermittent recovery test level 1 could also be associated to the endothelial function. Thirteen young male football players (athletes) and eleven young male non-athletes (control) participated in this study. The endothelial function and Yo-Yo intermittent recovery test level 1 were measured in each group. Endothelial function and the distance on Yo-Yo intermittent recovery test level 1 were significantly higher ($P<0.002$ and $P<0.001$, respectively) in the athletes (FMD=11.5±3.8; Yo-YoIR1= 2009.2 ± 263.8) than in control group (FMD=4.9 ± 5.4; Yo-YoIR1= 1420 ± 423.8). The Pearson correlation between endothelial function and the distance on the Yo-Yo intermittent recovery test level 1 in the athletes group was $r = 0.81$ ($P<0.005$), while in the control group it was $r = 0.03$ ($P<0.005$). Our results indicate that there is a positive association between Yo-Yo intermittent recovery test level 1 and endothelial function in young football players. This association strengthens the Yo-Yo intermittent recovery test level 1 as an effective tool in evaluating the performance of intermittent sports athletes.

Keywords: Yo-Yo intermittent recovery test, endothelial function, flow-mediated dilation, football players

Introduction

Football is one of the most popular sports and is practiced worldwide. It is practiced by children and adolescents with different performances[1], including professional. On the other hand, there is a lack of studies showing the physiological effects of this sport in children and adolescents[2]. Moreover, there is no doubt that high values of maximum oxygen uptake (VO_{2max}) are necessary in high-performance athletes to manage the cardiovascular function[1].

Traditionally, the capacity of an athlete has been evaluated using continuous exercise tests, such as the VO_{2max} test. The VO_{2max} is considered the best measure of cardiovascular fitness and exercise capacity[3]. On the other hand, this method has been questioned because it does not show appreciable changes for the performance of athlete who practice intermittent exercise[4]. In fact, to evaluate this kind of exercise, the Yo-Yo intermittent recovery (IR) tests, one of the most extensively studied fitness tests in exercise physiology, may be recommended[1]. Due to its specificity and practicality many team sports have used this test, such as basketball[5], handball[6], as well as football[7]. In addition, the Yo-Yo IR tests are a more specific measurement of changing in performance for intermittent sports[1,8]. Thus, in order to examine changes in performance, the Yo-Yo IR test provides, with a simple protocol, important information of the ability of an individual to perform repeated intense exercise[8].

Bangsbo et al. [8], analyzing 141 subjects, found a positive association ($r = 0.70$; $p < 0.05$) between VO_{2max} and the Yo-Yo IR level 1 (IR1) test in football players. In addition, studies indicate that there is also a positive association between VO_{2max} and endothelial function in adolescents[9] and adults[10]. It has been shown that aerobic exercise improves endothelial function, measured by the flow-mediated dilation in the brachial

artery (FMD) in heart failure[11], coronary heart disease[12], hypertension[13] (Higashi et al., 1999) and in healthy subjects[10]. Revising extensively the literature, there is no doubt that aerobic exercise is essential for improving fitness and that exercise has favorable effects on endothelial function[14,15,16,17].

Endothelial cells play a key role in the control of vascular function, because they mediate vasodilation and vasoconstriction of the vascular smooth muscle cells[15,17]. The equilibrium between these vasoactive components is atheroprotective, whereas a damaged endothelium can provoke an imbalance in these vasoactive components and induce cardiovascular disease, such as atherosclerosis, hypertension, and heart failure[14].

In the 1990s, a technique that uses ultrasound was evolved to evaluate foot and mouth disease, which stimulates the release of nitric oxide (NO) with subsequent vasodilatation[18]. Vasodilatation can be quantified as an index of vasomotor function. However, there are technical and interpretive limitations[14], which can represent an important barrier for its use in the sports evaluation. Thus, it would be desirable to use a method, such as Yo-Yo IR1 to assess the endothelial and cardiorespiratory function, considering the importance of these parameters for sports performance. Therefore, the objective of this study was to investigate whether the Yo-Yo IR1 could also be associated with the endothelial function and, thus, provides a valorous information about football players performance with a simple, easy and cheap technic.

Methods

The Ethics Committee of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) approved the study (CEP/UFCSPA protocol number 562.572).

Subjects were informed about the tests to be performed in the study, after which they provided written informed consent prior to data collection. All study participants underwent three evaluations: evaluation of arterial endothelial function, Yo-Yo IR1 test and anthropometric measurements.

Participants

Thirteen young male football players (athletes; age 16.8 ± 0.8 years) and eleven young males (non-athletes; age 16.4 ± 0.7 years) participated in the study. All football players had at least three years prior specific training, while the control subjects did not practice sports competitively. The endothelial function, distance on Yo-Yo IR and anthropometric data were measured in both groups.

Measures

For arterial endothelial function evaluation, subjects were instructed to attend the Physiotherapy Laboratory of the University where the study was conducted at 7 a.m., fasting. One week later, anthropometric measurements – height, weight, age, body fat percentage and Yo-Yo IR1 – were performed at a football stadium.

Procedures

Assessment of endothelial function

Endothelial function was assessed noninvasively by means of a brachial artery ultrasound probe (GE Medical Systems, Vivid I Ultrasound, Israel) and Doppler

ultrasonography, using an instrument equipped with a 7- to 12-MHz high-resolution linear probe (L12-3, GE Medical Systems, Israel). The ultrasonography was performed in a silent, temperature-controlled laboratory room. At rest, the left brachial artery diameter was measured by B-mode ultrasound images to detect reactive hyperemia. Before blood pressure (BP) cuff inflation, a resting scan was performed. After the resting measurement, the cuff was inflated for 5 min at 50 mmHg above systolic BP (SBP), to occlude the arterial flow. This procedure causes ischemia followed by vasodilation due to auto regulatory mechanisms. After cuff deflation, a second continuous scan was recorded from 30–120 seconds. The same experienced sonographer performed and analyzed all ultrasound scans. The sonographer had no information about the subjects. At a fixed position, the vessel diameter was measured offline with ultrasonic calipers at end-diastole and incident with the R wave on an electrocardiogram that it was continuously recorded. After an interval of 10 seconds and during the period within 30–180 seconds, the dilatation was obtained by the difference from baseline. After the release of the sphygmomanometer cuff, the value of FMD (%) indicates the increase in blood flow[14].

Yo-Yo intermittent recovery test level 1

The subjects performed 2×20-meters shuttle runs at increasing speeds, interspersed with a 10-second period of active recovery. As described by Bangsbo and coworkers[8], the distance covered at this point was the result of the Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1).

Body fat percentage

We used the Faulkner protocol[19] (1968) to calculate the body fat percentage of individuals from both groups. The method uses measurements of four skinfolds: triceps, subscapular, abdominal and suprailiac to perform the calculation, as follows:

Body fat percentage = 5.783 + 0.153 (∑ of the four skinfolds)

Statistical analysis

The normality and equality of variance of the data were assessed through the Shapiro–Wilk and Levene tests. The data were compared through the use of the Student's "t"-test. All analyses were conducted with SPSS software version 10.0 (SPSS Inc., Chicago, IL). The association between variables was assessed through Pearson correlations. The data are presented as means ± SD. A value of $P < 0.05$ was considered statistically significant.

Results

Athletes and control group baseline characteristics are presented in Table 1. There were no significant differences between the two groups regarding age, body weight and height or body mass index. On the other hand, the body fat percentage was statistically different between groups ($P<0.001$).

Measurements of Yo-Yo IR1 and endothelial function

Distance covered on Yo-Yo IR (m) and endothelial function (%) were significantly higher ($P<0.001$ and $P<0.002$, respectively) in the athletes group (2009, SD 264 and 11.5, SD 3.8, respectively) than in the control group (1420, SD 424 and 4.9, SD 5.4, respectively – Table 2).

The Pearson correlation between the endothelial function and the distance on the Yo-Yo IR1 in the athletes group was r=0.81 (P<0.005; Figure 1). In contrast, for the control group, the Pearson correlation was r =0.03 (P<0.005; Figure 2).

(insert Figure 1 here)

Discussion

The main finding of this study is that there was a positive and significant association between endothelial function and the Yo-Yo IR1 in young football players. As expected, such association did not occur within subjects from the control group. Differently of VO_{2max}, the Yo-YoIR1 has high sensitivity to detect changes in athlete performance of intermittent sports, and according to our results, it could also be used as an important tool to predict endothelial function. Traditionally, a continuous incremental load exercise protocol has been used to evaluate the VO_{2max} of an athlete. However, this test has low sensitivity and it does not respond proportionally to the athlete's performance improvements in intermittent sports. Consequently, the relevance of these protocols has been questioned for those kind of sports[8].

Regarding to the well-known advantages of an intermittent protocol to measure a player's performances, our study provides the first evidence that the Yo-Yo IR1 is also related to endothelial function, a crucial measurement that can inform about cardiovascular function. Our results showed that the better the athlete's performance is in the Yo-Yo IR test, the better the FMD or arterial function are. The application of these results, in terms of sport, lies in the easiness of inferring the endothelial function without performing an additional measurement. Moreover, it aggregates valuable information about cardiovascular physiology. Importantly, it is crucial to consider that

an imbalance in the endothelial function leads to impairment in the cardiovascular function[20] and thus, probably reduces the athlete's performance.

In addition, because of the length of a match, football is mainly dependent upon aerobic metabolism. During a 90-minute game, the average work intensity is normally between 80–90% of maximal heart rate. This condition is close to the anaerobic threshold[7], increasing the capacity and efficiency of the cardiovascular system.

To our knowledge, there are no previous studies demonstrating an association between the Yo-Yo IR1 test and the FMD. However, some studies do have demonstrated a significant association between VO_{2max} and FMD[9,10]. Since our results showed an association between the Yo-Yo IR1 test and the FMD in athletes, but not in the control group, it demonstrates the specificity of the Yo-Yo IR1 test to evaluate athlete's performance with accuracy. Probably, in healthy subjects, but not trained ones, the FMD and the results of Yo-Yo IR1 test do not go along with the increase in cardiovascular demand.

Guiraud and coworkers[21] have published a non-systematic review exploring the effects of high-intensity interval training (HIIT) in patients with coronary artery disease. The authors reported an increase in arterial endothelial function in these patients. The HIIT is quite similar to the training performed by intermittent sports athletes, such as football players. This finding further validates our results because, in our study, only the athlete group showed an association between endothelial function and Yo-Yo IR1. According to our findings, the HIIT also improves both physical condition and endothelial function.

Finally, data recently published in a systematic review by Ashor et al.[22] also showed an association between the exercise modalities and endothelial function. They showed that the intensity of aerobic exercise and endothelial function are associated; for

every increase in intensity, there is an improvement in FMD. It is well established in the literature that Yo-Yo IR1 is associated to VO_{2max} (aerobic capacity). Thus, this finding further strengthens our results since it also confirms the association between Yo-Yo IR1 and FMD.

Due to the fact that it is not invasive, is used widely and allows repeated measurements over time, the FMD technique is attractive for the study of the effectiveness of various interventions. On the other hand, there are some technical and interpretive limitations to the use of this technique. It would require intense training to master the technique in order to minimize the data variability and accuracy[14]. In addition, ultrasound machines are expensive, while the Yo-Yo test is easy to apply and is low cost. Therefore, it has been important to demonstrate the association of the Yo-Yo IR1 with a parameter that reflects the cardiovascular function, as FMD.

In conclusion, the reason for considering the Yo-Yo IR1 test as a good alternative lies in the importance of minimizing the problems of performing the FMD technique and also in providing important information about athletes' cardiovascular physiology and performance. Future research is necessary to confirm that important association.

Acknowledgements

This study was performed in compliance with the laws of the Brazil. There is no conflict of interest. This study was supported by *Conselho Nacional de Desenvolvimento Cientifico e Tecnológico* (CNPq).

A special acknowledgement to *Esporte Clube Novo Hamburgo*.

Conflicts of interest

The authors declare that there were no conflicts of interest related to the study.

References

1- Stolen T, Chamari K, Castagna C, Wisløff U. Physiology of soccer: an update. *Sports Med* 2005; 35: 501-536.

2- Henderson B, Cook J, Kidgell DJ, Gastin PB. Game and training load differences in elite junior australian football. *J Sports Sci Med* 2015; Aug 11;14(3):494-500.

3- Lear SA, Brozic A, Myers JN, Ignaszewski A. Exercise stress testing. An overview of current guidelines. *Sports Med* 1999; May 27;5:285-312.

4- Mohr M, Krustrup P, Bangsbo J. Fatigue in soccer: a brief review. *J Sports Sci* 2005; 23: 593-599.

5- Castagna C, Impellizzeri FM, Rampinini E, D'Ottavio S, Manzi V. The Yo-Yo intermittent recovery test in basketball players. *J Sci Med Sport* 2008; 11: 202-208.

6- Hermassi S, Aouadi R, Khalifa R, van den Tillaar R, Shephard RJ, Chelly MS. Relationships between the Yo-Yo intermittent recovery test and anaerobic performance tests in adolescent handball players. *J Hum Kinet* 2014; 42: 197-205.

7- Rampini E, Sassi A, Azzalin A, Castagna C, Menaspa P, Carlomagno D, Impellizzeri Fm. Physiological determinants of Yo-Yo intermittent recovery tests in male soccer players. *Eur J Appl Physiol* 2010; 108: 401–409.

8- Bangsbo J, Iaia FM, Krustrup P. The Yo-Yo intermittent recovery test: a useful tool for evaluation of physical performance in intermittent sports. *Sports Med* 2008; 38: 37-51.

9- Pahkala K, Heinonen OJ, Lagström H, Hakala P, Simell O, Viikari JS, Rönnemaa T, Hernelahti M, Sillanmäki L, Raitakari OT. Vascular endothelial function and leisure-time physical activity in adolescents. *Circulation* 2008; 118: 2353-2359.

10- Buscemi S, Canino B, Batsis JA, Buscemi C, Calandrino V, Mattina A, Arnone M, Caimi G, Cerasola G, Verga S. Relationships between maximal oxygen uptake and endothelial function in healthy male adults: a preliminary study. *Acta Diabetol* 2013 50: 135-141.

11- Hornig B, Maier V, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. *Circulation* 1996; 93: 210–214.

12- Gokce N, Vita JA, Bader DS, Sherman DL, Hunter LM, Holbrook M, O'Malley C, Keaney JF Jr, Balady GJ. Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. *Am J Cardiol* 2002; 90: 124–127.

13- Higashi Y, Sasaki S, Kurisu S, Yoshimizu A, Sasaki N, Matsuura H et al (1999) Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. *Circulation* 100:1194–1202.

14- Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogelet R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39:257–265.

15- Della Rocca, DG, Pepine CJ. Endothelium as a predictor of adverse outcomes. *Clin Cardiol* 2010; 33: 730-732.

16- Fagard RH, Cornelissen VA. Effect of exercise on blood pressure control in hypertensive patients. *Eur J Cardiovasc Prev R* 2007;14:12–17.

17- Sandoo A, van Zanten JJ, Metsios GS, Carroll D, Kitas GD. The endothelium and its role in regulating vascular tone. *Open Cardiovasc Med J* 2010; 4: 302-12.

18- Laurent S, Lacolley P, Brunel P, Laloux B, Pannier B, Safar M. Flow-dependent vasodilation of brachial artery in essential hypertension. *Am J Physiol* 1990;258: 1004–11.

19- Faulkner JA. Physiology of swimming and diving. In: H. Falls. *Exercise Physiology*, Baltimore: Academic Press, 1968.

20- Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N, Sone H. Cardiorespiratory fitness as a

quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women. *JAMA* 2009; 301: 2024–2035.

21- Guiraud T, Nigam A, Gremeaux V, Meyer P, Juneau M, Bosquet L. High-intensity interval training in cardiac rehabilitation. *Sports Med* 2012; 42: 587-605.

22- Ashor AW, Lara J, Siervo M, Celis-Morales C, Oggioni C, Jakovljevic DG, Mathers JC. Exercise modalities and endothelial function: a systematic review and dose-response meta-analysis of randomized controlled trials. *Sports Med* 2015; 44: 279-296.

Table 1

Physical characteristics of the subjects

	Means ± SD		**P**
	Athletes Group	Non-athletes Group	
Weight (kg)	70.3 ± 3.7	74.5± 5.6	0.133
Height (cm)	174.7 ± 5.7	170.1 ± 8.1	0.493
Body mass index	23.43 ± 1.59	25.76 ± 2.33	0.286
Body fat percentage (%)	7.9 ± 0.9	13.7 ± 2.5	0.001

P<0.05 was considered statistically significant. Values are the mean ± SD.

Table 2

Physical fitness and endothelial function

	Means ± SD		**P**
	Athletes Group	Non-athletes Group	
Yo-Yo IR1 (m)	2009.2 ± 263.8	1420 ± 423.8	0.001
FMD (%)	11.5 ± 3.8	4.9 ± 5.4	0.002

P<0.05 was considered statistically significant. Values are the mean ± SD.

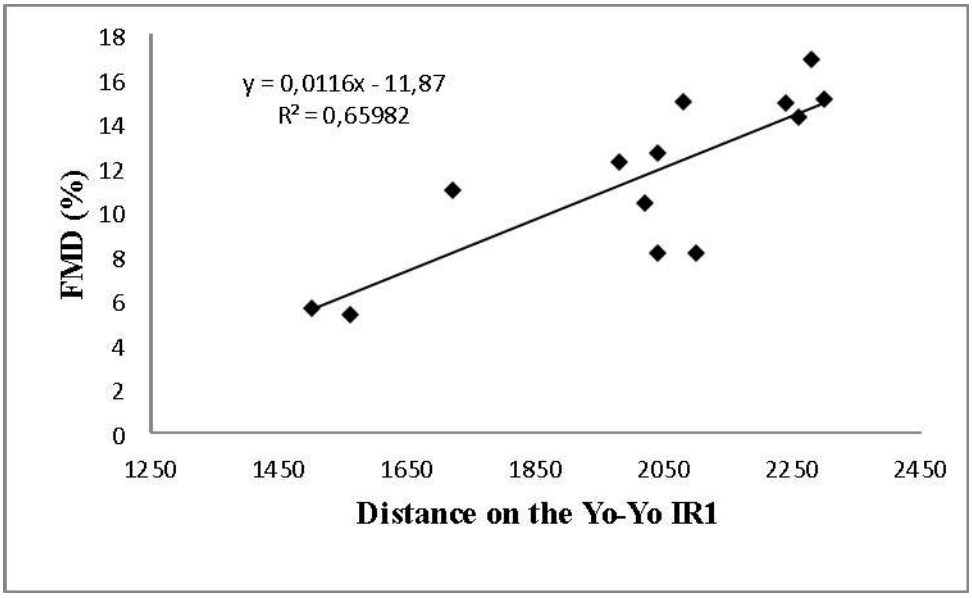


Figure 1

Correlation and trendline between FMD and the distance on the Yo-Yo IR1 on athletes group

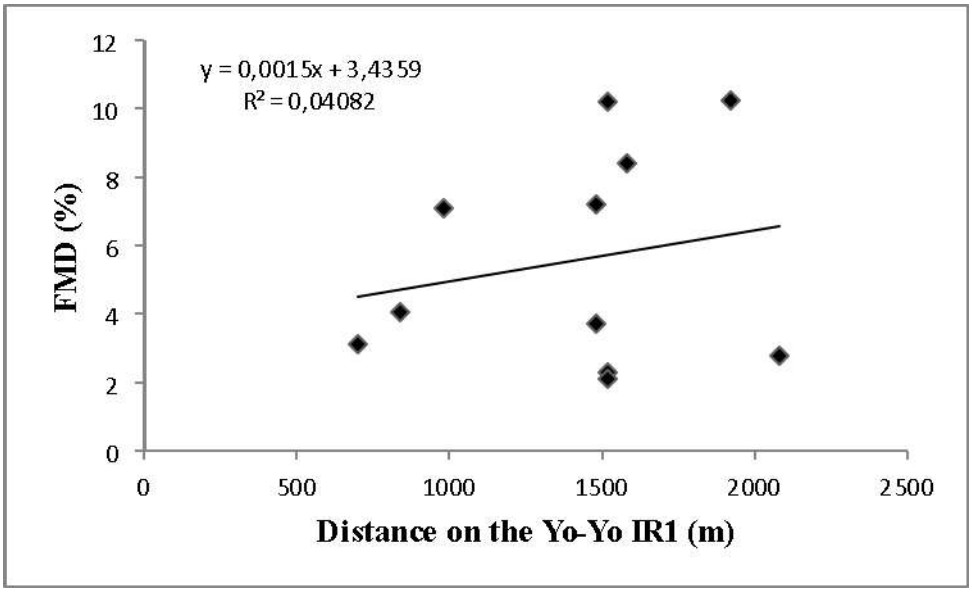


Figure 2

Correlation and trendline between FMD and the distance on the Yo-Yo on non-athletes group

ANEXOS

ANEXO 1
TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

COREN n°:_____ Porto Alegre,_____de 20____

1- Você está sendo convidado a participar do projeto de pesquisa sobre "Histórico familiar de hipertensão compromete o equilíbrio autonômico, mas não a função endotelial de jovens jogadores de futebol**".** Você deverá comparecer ao Laboratório de Investigação Clínica do Instituto de Cardiologia do RS, às 7 horas e 30 minutos, em jejum.

2- O objetivo principal deste trabalho é identificar se a ascendência genética influencia a função endotelial arterial de indivíduos normotensos, jovens atletas de equipes de futebol profissional. O sangue será coletado com os atletas em jejum, por pessoal treinado, colocado em tubos plásticos para realização posterior das medidas bioquímicas.

3- Antes de assinar o consentimento para a sua participação, por favor, leia (ou escute) com atenção as informações abaixo. Aproveite a oportunidade para esclarecer todas as dúvidas com o pesquisador que lhe apresentou o estudo. Queremos deixar claro que, caso você decida não participar, não haverá nenhum prejuízo ao seu treinamento.

4- Se você aceitar participar deste estudo, pesquisadores da área da saúde lhe farão perguntas sobre seus hábitos de vida antes da consulta.

5- Além de lhe fazer perguntas, verificaremos a sua pressão e coletaremos sangue, que inclui uma picada de agulha, causando um pequeno desconforto. Essas informações serão utilizadas para o nosso estudo. Pela sua participação no estudo, você não receberá nenhum valor em dinheiro, mas terá a garantia de que todas as despesas necessárias à realização da pesquisa não serão de sua responsabilidade.

6- Os procedimentos são simples, não lhe causarão dor e garantimos a você que o seu nome, ou o de qualquer participante, não será de forma alguma revelado. Ainda assim, você poderá retirar seu consentimento a qualquer momento, sem nenhum prejuízo tendo direito de receber todas as informações que desejar.

7- Qualquer dúvida você poderá contatar o Comitê de Ética da Universidade Federal de Ciências da Saúde e do Instituto de Cardiologia que aprovou esse estudo, pelo telefone 3223-7393 ramal 26 com Maria Lúcia; Você ainda pode conversar com um dos pesquisadores:

- Pesquisadora Katya Rigatto, telefone: (51) 3303-8753.
- Pesquisador Walter Vargas, telefone: (51) 3037-6521.
- Comitê de Ética em Pesquisa da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA): (51) 3303-9000.

Assino o presente documento, em duas vias de igual teor, ficando uma em minha posse.

Nome do atleta:_____

_____

Assinatura do atleta

Nome do responsável legal:_____

_____

Assinatura do responsável legal

Sugestões/Reclamações/Contribuições que o paciente julgue necessárias:

_____
_____

Data:_____/_____/_____Nome do responsável: _____

_____

Assinatura do pesquisador responsável – Katya V. Rigatto

ANEXO 2

Autonomic Neuroscience: basic and clinic: Author information

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.

To find out more, please visit the Preparation section below.

Introduction

This is an international journal with broad coverage of all aspects of the autonomic nervous system in man and animals. The main areas of interest include the innervation of blood vessels and viscera, autonomic ganglia, efferent and afferent autonomic pathways, and autonomic nuclei and pathways in the central nervous system.

The Editors will consider papers that deal with any aspect of the autonomic nervous system, including structure, physiology, pharmacology, biochemistry, development, evolution, ageing, behavioural aspects, integrative role and influence on emotional and physical states of the body. Interdisciplinary studies will be encouraged. Studies dealing with human pathology will be also welcome.

Types of Article

The journal publishes full-length articles of original research; Short Communications; Rapid communications; Review articles; Clinical Reports and Letters to the Editor.

Review Articles

These are exhaustive reviews on a specific topic in autonomic neuroscience. Authors should endeavor to make their reviews understandable to neuroscientists of other disciplines. Review submissions are typically 8,000 word, but this is flexible.

Mini-Review Articles

These focus on recent aspects of a field or to provide an overview to an emerging field or technique. Mini-review submissions are typically 4,500 words, but this is flexible.

Rapid communications

Reports on exciting new results within the scope of the journal can be submitted for publication in the rapid communications section. A rapid communication should not exceed 700 words and should contain at most one simple table or figure. A maximum of 8 references may be used. The manuscript should be arranged in the following order: title (not exceeding 100 characters including spaces between words); surname(s) of author(s), preceded by one name spelled out in full; name and address of the establishment where the work was done (all on 1 page); abstract (max. 75 words) and keywords (indexing terms, max. 3 items); text without subheadings; acknowledgement(s); references; figure legend and figure or table. Name, full postal address, telephone, fax numbers and e-mail address of the author to whom correspondence is to be sent should be mentioned on the title page. Rapid communications have priority at the editorial office and publisher.

Short communications

Short communications should be prepared as rapid communications but should not exceed four pages in print (approx. 2000-3000 words including abstract, captions and references). A maximum of 2 illustrations (figures and tables) is allowed. An abstract of not more than 100 words should be provided and 3-6 keywords should be listed immediately below the abstract.

Clinical reports

Clinical reports should be prepared as Short communications

Page charges

This journal has no page charges.

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Human and animal rights

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If there are no conflicts of interest then please state this: 'Conflicts of interest: none'. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see 'Multiple, redundant or concurrent publication' section of our ethics policy for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the

same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck.

Changes to authorship

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors after the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies

Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some funding bodies will reimburse the author for the Open Access Publication Fee. Details of existing agreements are available online.

Open access

This journal offers authors a choice in publishing their research:

Open access

• Articles are freely available to both subscribers and the wider public with permitted reuse.

• An open access publication fee is payable by authors or on their behalf, e.g. by their research funder or institution.

Subscription

• Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs.

• No open access publication fee payable by authors.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)

Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The open access publication fee for this journal is USD 2500, excluding taxes. Learn more about Elsevier's pricing policy: External link http://www.elsevier.com/openaccesspricing.

Green open access

Authors can share their research in a variety of different ways and Elsevier has a number of green open access options available. We recommend authors see our green open access page for further information. Authors can also self-archive their manuscripts immediately and enable public access from their institution's repository after an embargo period. This is the version that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and in editor-author communications. Embargo period: For subscription articles, an appropriate amount of time is needed for journals to deliver value to subscribing customers before an article becomes freely available to the public. This is the embargo period and it begins from the date the article is formally published online in its final and fully citable form.

This journal has an embargo period of 12 months.

Elsevier Publishing Campus

The Elsevier Publishing Campus (www.publishingcampus.com) is an online platform offering free lectures, interactive training and professional advice to support you in publishing your research. The College of Skills training offers modules on how to prepare, write and structure your article and explains how editors will look at your paper when it is submitted for publication. Use these resources, and more, to ensure that your submission will be the best that you can make it.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Submit your article

Please submit your article via External link http://ees.elsevier.com/autneu/.

Referees

Please submit the names and institutional e-mail addresses of several potential referees. For more details, visit our Support site. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.

If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.

Divide the article into clearly defined sections.

Figures and tables embedded in text

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file.

REVISED SUBMISSIONS

Use of word processing software

Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Experimental

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

• Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.

• Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

The abstract should summarize the results obtained and the major conclusions in such a way that a reader not familiar with the particular area of work can understand the implications of the work. It should not exceed one twentieth of the length of the manuscript.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file

types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.

Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images and in accordance with all technical requirements: Illustration Service.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). You can view example Highlights on our information site.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Artwork

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.

• Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.

• Number the illustrations according to their sequence in the text.

• Use a logical naming convention for your artwork files.

• Indicate per figure if it is a single, 1.5 or 2-column fitting image.

• For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.

• Please note that individual figure files larger than 10 MB must be provided in separate source files.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.

TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.

TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.

TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.

• Supply files that are too low in resolution.

• Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Illustration services

Elsevier's WebShop offers Illustration Services to authors preparing to submit a manuscript but concerned about the quality of the images accompanying their article. Elsevier's expert illustrators can produce scientific, technical and medical-style images, as well as a full range of charts, tables and graphs. Image 'polishing' is also available, where our illustrators take your image(s) and improve them to a professional standard. Please visit the website to find out more.

Figure captions

Ensure that each illustration has a caption. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be

sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

References

Citations in the text should be given in parentheses at the appropriate place by author(s) name(s) followed by the year in chronological order according to the Harvard system (Paintal, 1973; Birdsall et al., 1980). With more than two authors, name only the first followed by "et al." (Birdsall et al., 1980). When two or more papers by the same author(s) appear in one year, distinguish them by a, b, etc. after the date.

The Reference List

The reference list should be submitted in double spacing. It should be arranged in alphabetical order of the first author's name. If the first author's name appears more than once, the order is as follows: (1) single author: chronological sequence; (2) author and co-author: alphabetically according to co-author; (3) author and more than one co-author: chronological sequence (as in the text these will be referred to as "et al."). Reference must be complete including, in this order: author's name, initials, year of publication, title of article, title of the journal, volume, first and last page number of the article cited. Title abbreviations should conform to those adopted by List of Serial Title Word Abbreviations (available from International Serial data System, 20 Rue Bachaumont, 75002 Paris, France, ISBN 2-904938-02-8).

Examples: Paintal, A.S., 1973. Vagal sensory receptors and their reflex effects. Physiol. Rev. 53, 159-227. Birdsall, N.J.M., Hulme, B.C., Hamner, R., Stockton, J.R., 1980. Subclasses of muscarinic receptors. In: Yamamura, H.I., Olsen, R.W., Usdin, E. (Eds.), Psychopharmacology and Biochemistry of Transmitters and Receptors. Elsevier, Amsterdam, pp. 97-100. Leiblich, I., 1982. Genetics of the Brain. Elsevier, Amsterdam, 492 pp.

Unpublished experiments may be mentioned only in the text. They must not be included in the list of References. Papers which have been accepted for publication but which have not appeared may be quoted in the reference list as "in press". Personal communications may be used only when written authorization from the investigator is submitted with the manuscript. They must not be included in the list of references. All references listed should be referred to in the text and vice versa.

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

A DOI can be used to cite and link to electronic articles where an article is in-press and full citation details are not yet known, but the article is available online. A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, http://dx.doi.org/10.1029/2001JB000884i. Please note the format of such citations should be in the same style as all other references in the paper.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software

Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley and Zotero, as well as EndNote. Using the word processor plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:

External link http://open.mendeley.com/use-citation-style/autonomic-neuroscience-basic-and-clinical

When preparing your manuscript, you will then be able to select this style using the Mendeley plug-ins for Microsoft Word or LibreOffice.

Reference formatting

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style

Text: All citations in the text should refer to:

1. Single author: the author's name (without initials, unless there is ambiguity) and the year of publication;

2. Two authors: both authors' names and the year of publication;

3. Three or more authors: first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.

Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Reference to a website:

Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13.03.03).

Journal abbreviations source

Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video data

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 150 MB. Video and animation files supplied will be published

online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary material

Supplementary material can support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Please note that such items are published online exactly as they are submitted; there is no typesetting involved (supplementary data supplied as an Excel file or as a PowerPoint slide will appear as such online). Please submit the material together with the article and supply a concise and descriptive caption for each file. If you wish to make any changes to supplementary data during any stage of the process, then please make sure to provide an updated file, and do not annotate any corrections on a previous version. Please also make sure to switch off the 'Track Changes' option in any Microsoft Office files as these will appear in the published supplementary file(s). For more detailed instructions please visit our artwork instruction pages.

Database linking

Elsevier encourages authors to connect articles with external databases, giving readers access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). More information and a full list of supported databases.

AudioSlides

The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Interactive plots

This journal enables you to show an Interactive Plot with your article by simply submitting a data file. Full instructions.

Manuscript Preparation

Manuscript Preparation

Manuscripts mailed to the editorial office will not be processed. Registration is required if using the system for the first time. After accessing the web site click on the "Register" link located towards the middle of the top of the screen. Detailed instructions are provided by clicking on the "Tutorial for Authors" link in the grey Author Information box in the upper right corner of the screen. The author should select a set of classifications from a list, and a category designation for their manuscript (original article, letter to the editor, short communication, etc.). Details of five reviewers that are required with a submission can be provided in the comments box or at a later stage when uploading the files for submission.

Authors may send queries concerning the submission process, manuscript status, or journal procedures to the Editorial Office (autneu@elsevier.com). Once the uploading is done, the system automatically generates an electronic (PDF) proof, which is then used for reviewing. All correspondence, including the Editor's decision and request for revisions, will be by e-mail.

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

• E-mail address

• Full postal address

All necessary files have been uploaded, and contain:

• Keywords

• All figure captions

• All tables (including title, description, footnotes)

Further considerations

• Manuscript has been 'spell-checked' and 'grammar-checked'

• All references mentioned in the Reference list are cited in the text, and vice versa

• Permission has been obtained for use of copyrighted material from other sources (including the Internet)

Printed version of figures (if applicable) in color or black-and-white

• Indicate clearly whether or not color or black-and-white in print is required.

For any further information please visit our Support Center.

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Webshop. Corresponding authors who have published their

article open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

Track your submitted article

Track your accepted article

You are also welcome to contact the Elsevier Contact Center.

ANEXO 3

The Journal of Sports Medicine and Physical Fitness : INSTRUCTIONS TO AUTHORS

The Journal of Sports Medicine and Physical Fitness publishes scientific papers relating to the area of the applied physiology, preventive medicine, sports medicine and traumatology, sports psychology. Manuscripts may be submitted in the form of editorials, original articles, review articles, case reports, special articles and letters to the Editor.

Manuscripts are expected to comply with the instructions to authors which conform to the Uniform Requirements for Manuscripts Submitted to Biomedical Editors by the International Committee of Medical Journal Editors (http://www.icmje.org).

Articles not conforming to international standards will not be considered for acceptance.

Submission of manuscripts

Papers should be submitted directly to the online Editorial Office at the Edizioni Minerva Medica website: http://www.minervamedicaonlinesubmission.it

Duplicate or multiple publication

Submission of the manuscript means that the paper is original and has not yet been totally or partially published, is not currently under evaluation elsewhere, and, if accepted, will not be published elsewhere either wholly or in part.

Splitting the data concerning one study in more than one publication could be acceptable if authors justify the choice with good reasons both in the cover letter and in the manuscript. Authors should state the new scientific contribution of their manuscript as compared to any previously published article derived from the same study. Relevant previously published articles should be included in the cover letter of the currently submitted article.

Permissions to reproduce previously published material

Material (such as illustrations) taken from other publications must be accompanied by the publisher's permission.

Copyright

The Authors agree to transfer the ownership of copyright to The Journal of Sports Medicine and Physical Fitness in the event the manuscript is published.

Ethics committee approval

All articles dealing with original human or animal data must include a statement on ethics approval at the beginning of the methods section, clearly indicating that the study has been approved by the ethics committee. This paragraph must contain the following information: the identification details of the ethics committee; the name of the chairperson of the ethics

committee; the protocol number that was attributed by the ethics committee and the date of approval by the ethics committee.

The journal adheres to the principles set forth in the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/index.html) and states that all reported research concerning human beings should be conducted in accordance with such principles. The journal also adheres to the International Guiding Principles for Biomedical Research Involving Animals (http://www.cioms.ch/index.php/12-newsflash/227-cioms-and-iclas-release-the-new-international-guiding-principles-for-biomedical-research-involving-animals) recommended by the WHO and requires that all research on animals be conducted in accordance with these principles.

Patient consent

Authors should include at the beginning of the methods section of their manuscript a statement clearly indicating that patients have given their informed consent for participation in the research study.

Every precaution must be taken to protect the privacy of patients. Authors should obtain permission from the patients for the publication of photographs or other material that might identify them. If necessary the Editors may request a copy of such permission.

Conflicts of interest

Authors must disclose possible conflicts of interest including financial agreements or consultant relationships with organizations involved in the research. All conflicts of interest must be declared both in the authors' statement form and in the manuscript file. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript.

Authorship and contributorship

All persons and organizations that have participated to the study must be listed in the byline of the article (authors) or in the notes (contributors). The manuscript should be approved by all co-authors, if any, as well as, tacitly or explicitly, by the responsible authorities of the institution where the work was carried out. Authors and contributors must meet the criteria for authorship and contributorship established by the Uniform Requirements for Manuscripts Submitted to Biomedical Editors by the International Committee of Medical Journal Editors (http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html).

Authors' statement

Papers must be accompanied by the authors' statement (http://www.minervamedica.it/en/journals/sports-med-physical-fitness/index.php) relative to copyright, originality, authorship, ethics and conflicts of interest, signed by all authors.

Disclaimer

The Publisher, Editors, and Editorial Board cannot be held responsible for the opinions and contents of publications contained in this journal.

The authors implicitly agree to their paper being peer-reviewed. All manuscripts will be reviewed by Editorial Board members who reserve the right to reject the manuscript without entering the review process in the case that the topic, the format or ethical aspects are inappropriate. Once accepted, all manuscripts are subjected to copy editing. If modifications to the manuscript are requested, the corresponding author should send to the online Editorial Office the revised manuscript under two separate files, one file containing the revised clean version and another containing both a letter with point-by-point responses to the reviewers' comments and the revised version with corrections highlighted.

Correction of proofs should be limited to typographical errors. Substantial changes in content (changes of title and authorship, new results and corrected values, changes in figures and tables) are subject to editorial review. Changes that do not conform to the journal's style are not accepted. Corrected proofs must be sent back within 3 working days to the online Editorial Office of Journal of The Journal of Sports Medicine and Physical Fitness. In case of delay, the editorial staff of the journal may correct the proofs on the basis of the original manuscript.

Publication of manuscripts is free of charge. Colour figures, linguistic revision, and excessive alterations to proofs will be charged to the authors. Authors will receive instructions on how to order reprints and a copy of the manuscript in PDF.

For further information about publication terms please contact the Editorial Office of The Journal of Sports Medicine and Physical Fitness, Edizioni Minerva Medica, Corso Bramante 83-85, 10126 Torino, Italy – Phone +39-011-678282 – Fax +39-011-674502

E-mail: journals6.dept@minervamedica.it.

ARTICLE TYPES

Instructions for the most frequent types of articles submitted to the journal.

Editorials. Commissioned by the Editor in Chief or the Managing Editor, editorials deal with a subject of topical interest about which the author expresses his/her personal opinion. No more than 1000 words (3 typed, double-spaced pages) and up to 15 references will be accepted.

Original articles. These should be original contributions to the subject. The text should be 3000-5500 words (8 to 16 typed, double-spaced pages) not including references, tables, figures. No more than 50 references will be accepted. The article must be subdivided into the following sections: introduction, materials (patients) and methods, results, discussion, conclusions. The introduction should describe the theoretical background, the aim of the study and the hypothesis to be tested. The materials and methods section should describe in a logical sequence how the study was designed and carried out, how the data were analyzed (what hypothesis was tested, what type of study was carried out, how randomization was done, how the subjects were recruited and chosen, provide accurate details of the main features of treatment, of the materials used, of drug dosages, of unusual equipments, of the statistical method ...). In the results section the answers to the questions posed in the introduction should be given. The results should be reported fully, clearly and concisely supported, if necessary, by figures, graphs and tables. The discussion section should sum up the main results, critically analyze the methods used, compare the results obtained with other published data and discuss the implica-tions of the results. The conclusions should briefly sum up the significance of the study and its future implications. For

randomised controlled trials it is suggested to the authors to follow the guidelines reported by the CONSORT statement (http://www.consort-statement.org).

Review articles. These articles are commissioned by the Editor in Chief or the Managing Editor. They should discuss a topic of current interest, outline current knowledge of the subject, analyze different opinions regarding the problem discussed, be up-to-date on the latest data in the literature. Systematic reviews and meta-analyses must be subdivided into the following sections: introduction, evidence acquisition, evidence synthesis, conclusions. For systematic reviews and meta-analyses it is suggested to the authors to follow the guidelines reported by the PRISMA statement (http://www.prisma-statement.org). The text should be 6000-12000 words (17 to 34 typed, double-spaced pages) not including references, tables, figures. No more than 100 references will be accepted.

Case reports. These give a description of particularly interesting cases. The text should be 2000-3000 words (6 to 8 typed, double-spaced pages) not including references, tables, figures. No more than 30 references will be accepted. The article must be subdivided into the following sections: introduction, case report or clinical series, discussion, conclusions. It is suggested to the authors to follow the guidelines reported by the CARE statement (http://www.care-statement.org).

Special articles. These are articles on the history of medicine, health care delivery, ethics, economic policy and law concerning sports medicine. The text should be 3000-7000 words (8 to 20 typed, double-spaced pages) not including references, tables, figures. No more than 50 references will be accepted.

Letters to the Editor. These may refer to articles already published in the journal or to a subject of topical interest that the authors wish to present to readers in a concise form. The text should be 500-1000 words (1 to 3 typed, double-spaced pages) not including references, tables, figures. No more than 5 references will be accepted.

Guidelines. These are documents drawn up by special committees or authoritative sources.

The number of figures and tables should be appropriate for the type and length of the paper.

PREPARATION OF MANUSCRIPTS

Text file

Manuscripts must be drafted according to the template for each type of paper (editorial, original article, review, case report, special article, letter to the Editor).

The formats accepted are Word (.DOC) and RFT. The text file must contain title, authors' details, abstract, key words, text, references, notes and titles of tables and figures. Tables and figures should be submitted as separate files. The file should not contain active hyperlinks.

Title and authors' details

Short title, with no abbreviations. First name in full, middle name's initial, surname of the authors. Collective name, if any, as last author. Corresponding author marked with an asterisk. Affiliation (section, department and institution) of each author. Name, address, e-mail of the corresponding author.

Abstract and key words

Articles should include an abstract of between 200 and 250 words. For original articles, the abstract should be structured as follows: background (what is already known about the subject and what the study intends to examine), methods (experimental design, patients and interventions), results (what was found), conclusions (meaning of the study). For systematic reviews and meta-analyses, the abstract should be structured as follows: introduction, evidence acquisition, evidence synthesis, conclusions. Key words should refer to the terms from Medical Subject Headings (MeSH) of MEDLINE/PubMed. No abstracts are required for editorials or letters to the Editor.

Text

Identify methodologies, equipment (give name and address of manufacturer in brackets) and procedures in sufficient detail to allow other researchers to reproduce results. Specify well-known methods including statistical procedures; mention and provide a brief description of published methods which are not yet well known; describe new or modified methods at length; justify their use and evaluate their limits. For each drug generic name, dosage and administration routes should be given. Brand names for drugs should be given in brackets. Units of measurement, symbols and abbreviations must conform to international standards. Measurements of length, height, weight and volume should be given in metric units (meter, kilogram, liter) or their decimal multiples. Temperatures must be expressed in degrees Celsius. Blood pressure must be expressed in millimeters of mercury. All clinical chemistry measurements should be expressed in metric units using the International System of Units (SI). The use of unusual symbols or abbreviations is strongly discouraged. The first time an abbreviation appears in the text, it should be preceded by the words for which it stands.

References

It is expected that all cited references will have been read by the authors. The references must contain only the authors cited in the text, be numbered in Arabic numerals and consecutively as they are cited. Bibliographical entries in the text should be quoted using superscripted Arabic numerals. References must be set out in the standard format approved by the International Committee of Medical Journal Editors (http://www.icmje.org).

Journals

Each entry must specify the author's surname and initials (list all authors when there are six or fewer; when there are seven or more, list only the first six and then "et al."), the article's original title, the name of the Journal (according to the abbreviations used by MEDLINE/PubMed), the year of publication, the volume number and the number of the first and last pages. When citing references, please follow the rules for international standard punctuation carefully.

Examples:

– Standard article.

Sutherland DE, Simmons RL, Howard RJ. Intracapsular technique of transplant nephrectomy. Surg Gynecol Obstet 1978;146:951-2.

– Organization as author

International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Ann Int Med 1988;108:258-65.

– Issue with supplement

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1 Suppl 2):89-97.

Books and monographs

For occasional publications, the names of authors, title, edition, place, publisher and year of publication must be given.

Examples:

– Books by one or more authors

Rossi G. Manual of Otorhinolaryngology. Turin: Edizioni Minerva Medica; 1987.

– Chapter from book

De Meester TR. Gastroesophageal reflux disease. In: Moody FG, Carey LC, Scott Jones R, Ketly KA, Nahrwold DL, Skinner DB, editors. Surgical treatment of digestive diseases. Chicago: Year Book Medical Publishers; 1986. p. 132-58.

– Congress proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

Electronic material

– Standard journal article on the Internet

Kaul S, Diamond GA. Good enough: a primer on the analysis and interpretation of noninferiority trials. Ann Intern Med [Internet]. 2006 Jul 4 [cited 2007 Jan 4];145(1):62-9. Available from:

http://www.annals.org/cgi/reprint/145/1/62.pdf

– Standard citation to a book on CD-ROM or DVD

Kacmarek RM. Advanced respiratory care [CD-ROM]. Version 3.0. Philadelphia: Lippincott Williams & Wilkins; ©2000. 1 CD-ROM: sound, color, 4 3/4 in.

– Standard citation to a homepage

AMA: helping doctors help patients [Internet]. Chicago: American Medical Association; ©1995-2007 [cited 2007 Feb 22]. Available from: http://www.ama-assn.org/.

Footnotes and endnotes of Word must not be used in the preparation of references.

References first cited in a table or figure legend should be numbered so that they will be in sequence with references cited in the text taking into consideration the point where the table or figure is first mentioned. Therefore, those references should not be listed at the end of the reference section but consecutively as they are cited.

Notes

Authors' contribution statement; list of the members of the collective name (author's name in full, middle name's initial in capital letters and surname, with relevant affiliation); contributors' names; mention of any funding, research contracts; conflicts of interest; dates of any congress where the paper has already been presented; acknowledgements.

Titles of tables and figures

Titles of tables and figures should be included both in the text file and in the file of tables and figures.

File of tables

Each table should be submitted as a separate file. Formats accepted are Word (.DOC) and RTF. Each table should be created with the Table menu of the word processing software of the operating system employed, by selecting the number of rows and columns needed. Tabulations are not allowed. Each table must be typed correctly and prepared graphically in keeping with the page layout of the journal, numbered in Roman numerals and accompanied by the relevant title. Notes should be inserted at the foot of the table and not in the title. Tables should be referenced in the text sequentially.

File of figures

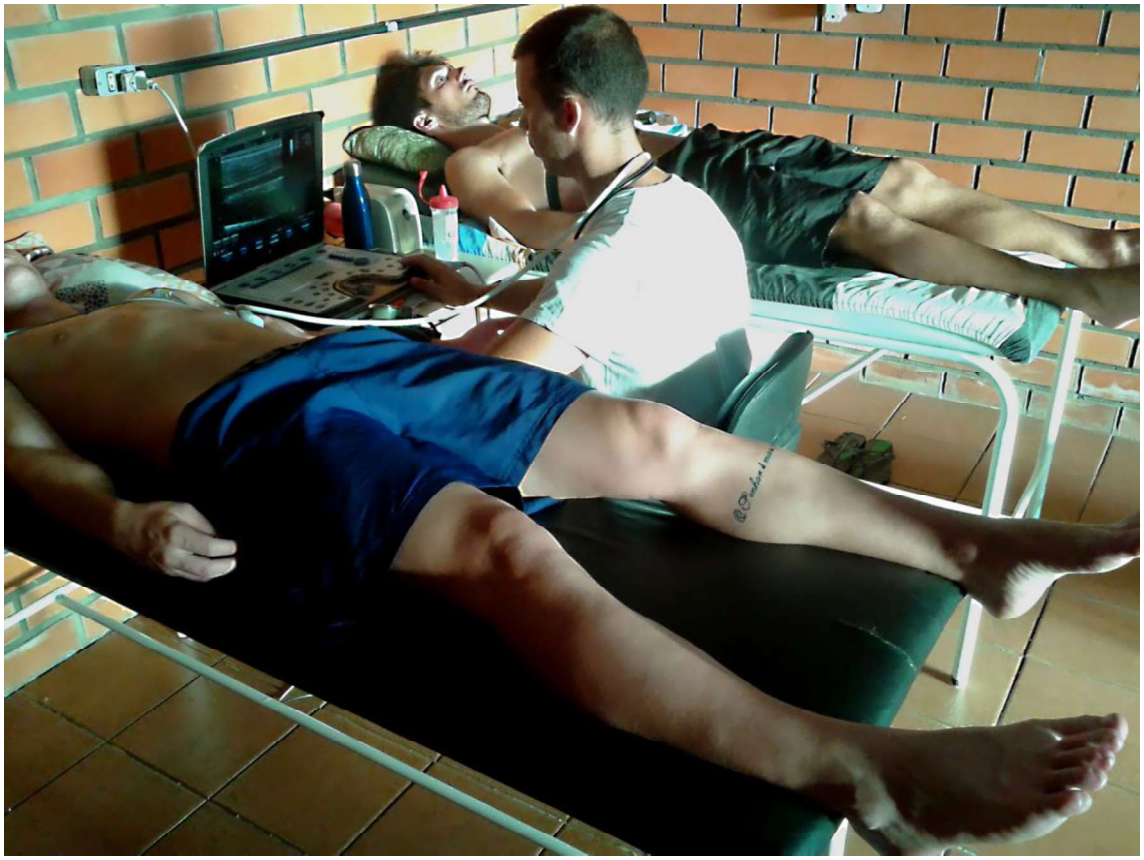
Each figure should be submitted as a separate file. Formats accepted: JPEG set at 300 dpi resolution preferred; other formats accepted are TIFF and PDF (high quality). Figures should be numbered in Arabic numerals and accompanied by the relevant title. Figures should be referenced in the text sequentially.

Reproductions should be limited to the part that is essential to the paper.

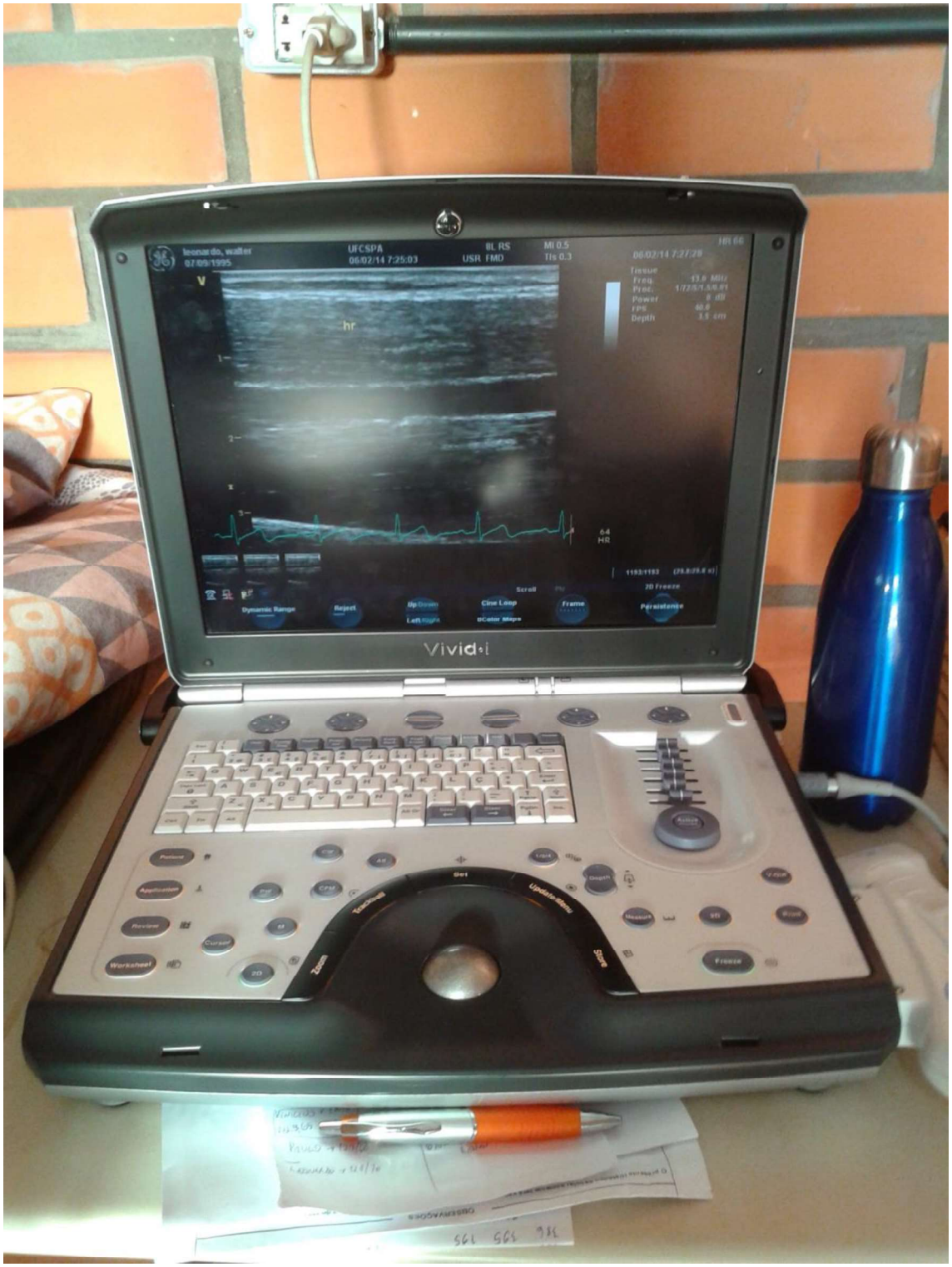
Histological photographs should always be accompanied by the magnification ratio and the staining method.

If figures are in color, it should always be specified whether color or black and white reproduction is required.

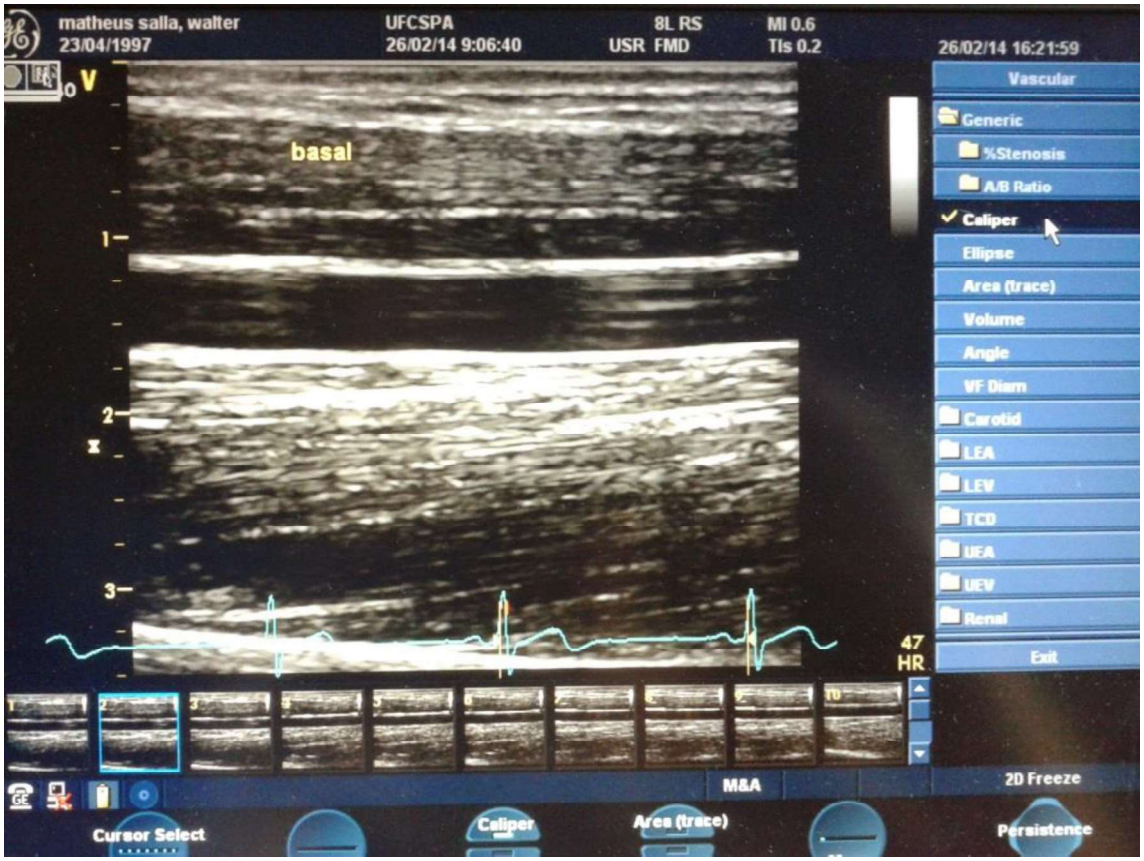
ANEXO 4



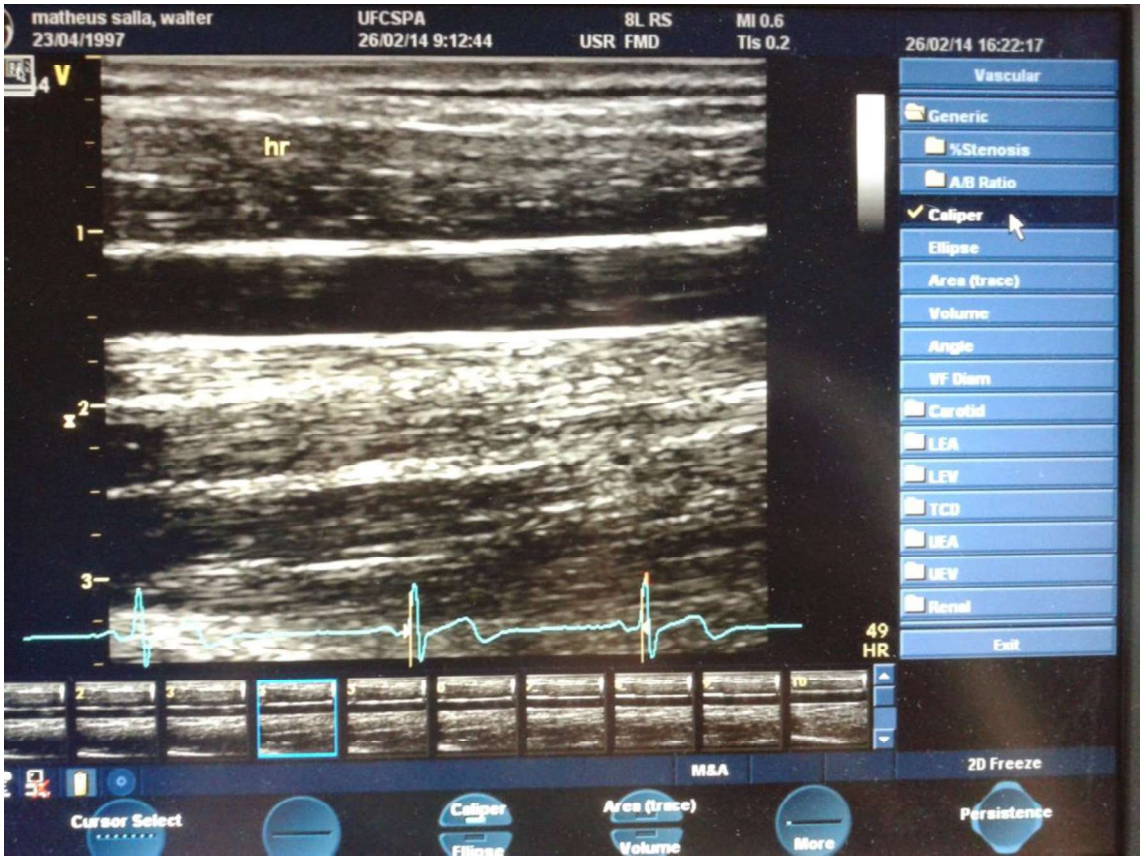
Laboratório de fisiologia do Esporte Clube Novo Hamburgo



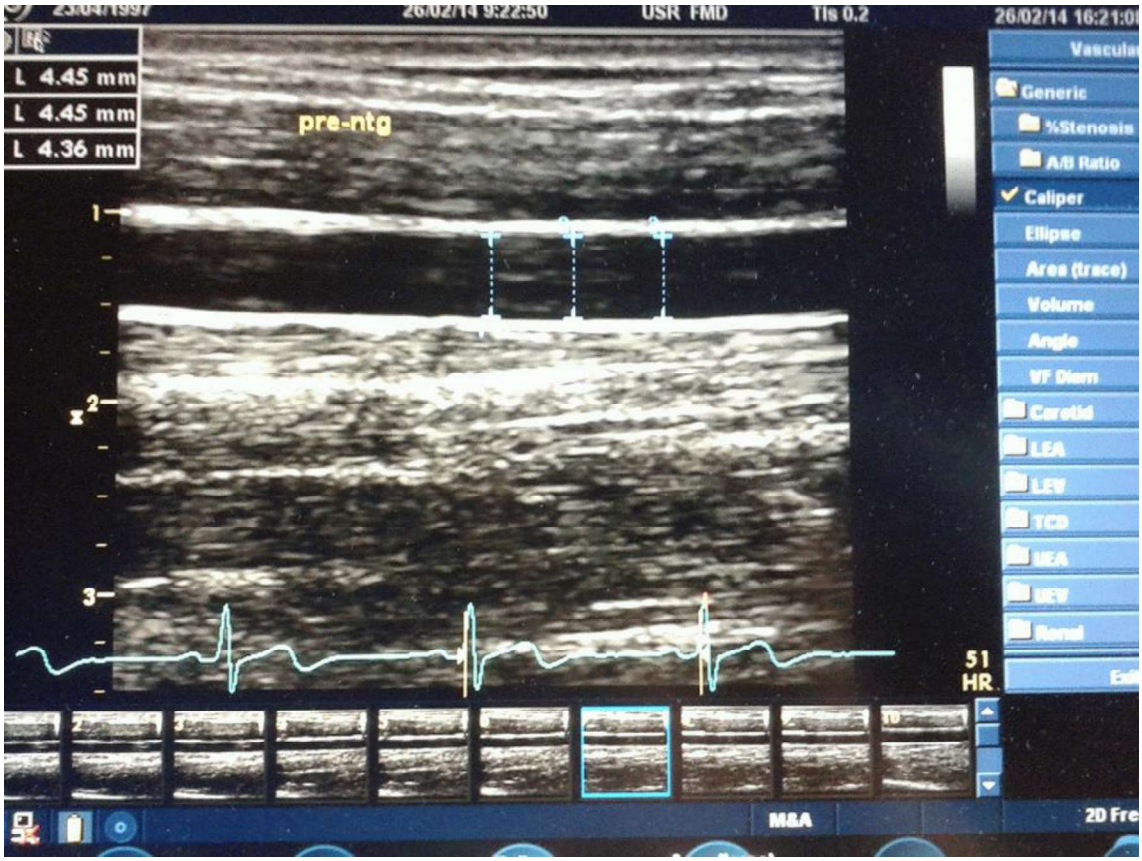
Aparelho de Ultrasound (GE Medical Systems, Vivid I Ultrasound, Israel)



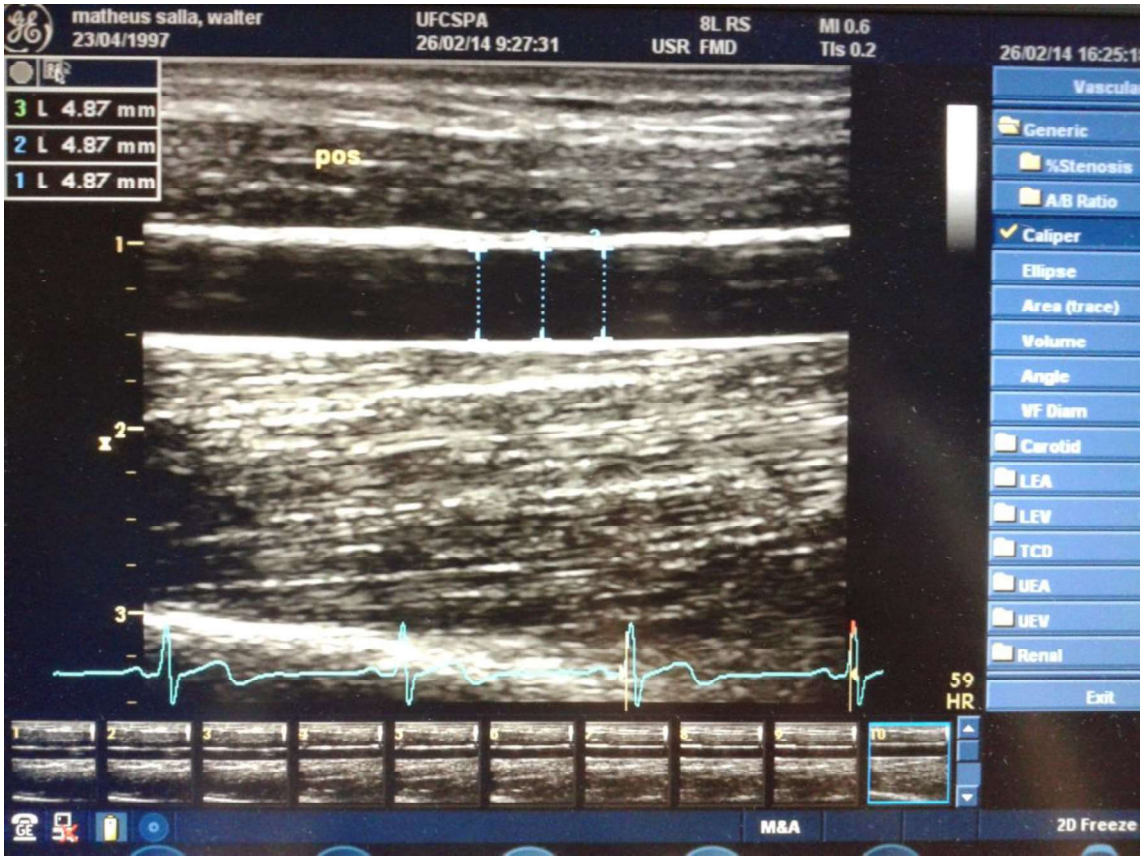
Ultrassonografia da artéria braquial, utilizando o método de vasodilatação mediada por fluxo (imagem basal da artéria braquial)



Ultrassonografia da artéria braquial, utilizando o método de vasodilatação mediada por fluxo (imagem da hiperemia reativa, após a desobstrução da artéria braquial)



Ultrassonografia da artéria braquial, utilizando o método de vasodilatação mediada por fluxo (imagem pré-nitroglicerina sublingual)



Ultrassonografia da artéria braquial, utilizando o método de vasodilatação mediada por fluxo (imagem pós-nitroglicerina sublingual)

Teste de esforço máximo: *Yo-Yo Intermittent Recovery test* nível 1

Frequencímetro Polar RS800CX, utilizado para captar o sinal de frequência cardíaca para posterior análise da variabilidade da frequência cardíaca

ANEXO 5