UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA: CIÊNCIAS MÉDICAS

Efeito do treinamento de oito semanas com exercício físico aeróbico intervalado de alta intensidade comparado ao exercício aeróbico contínuo na função endotelial, no estresse oxidativo, controle glicêmico e incidência de hipoglicemia em pacientes com diabetes tipo 1.

Winston Isio Boff Pereira de Souza

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Tese de Doutorado submetida como requisito parcial para obtenção do título de Doutor em Medicina: Ciências Médicas, da Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Medicina: Ciências Médicas.

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“Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas Graças a Deus, não sou o que era antes”.
(Marthin Luther King)

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RESUMO

A disfunção endotelial é precursora da aterosclerose no Diabetes Mellitus tipo 1 (DM1) e se caracteriza essencialmente por uma disfunção na ação do óxido nítrico (NO) no endotélio vascular, em geral causado pela hiperglicemia crônica associada ao estresse oxidativo. O exercício físico aeróbico tem sido utilizado como um método de intervenção eficaz e capaz em reduzir o risco cardiovascular através da melhora da função vascular. O exercício provoca aumento do fluxo vascular e do estresse de cisalhamento que, em decorrência, ativa a sintase do óxido nítrico (eNOS), promovendo aumento da síntese de NO. O treinamento intervalado de alta intensidade (High intensity Interval Training) (HIIT) esta sendo comparado ao exercício aeróbico continuo (EAC) em pacientes com DM1 durante 8 semanas. Devido à maior intensidade de esforço o HIIT pode apresentar vantagens em relação ao exercício contínuo em parâmetros de função endotelial e condicionamento físico em adultos com DM1 que apresentem disfunção endotelial. Trabalhamos com a hipótese que o treinamento HIIT seria mais eficaz do que o EAC para melhorar a função endotelial nos DM1 que apresentassem disfunção endotelial. Em um ensaio clinico randomizado aberto, 27 pacientes com DM1 com idade média de 23.5±6 anos; índice de massa corporal médio de 23.37±2.3 kg/m2 foram selecionados com base na função endotelial determinada pelo percentual de dilatação mediada pelo fluxo (FMD) e randomizados em 3 grupos para avaliação do treinamento por 8 semanas com o protocolo HIIT, protocolo contínuo (CONT) e um grupo controle sem exercício. Foram realizadas 3 sessões semanais em ciclo-ergômetro por 8 semanas. O treinamento foi dividido em 3 fases, com duração variável de 30 a 40min. A fase 3 diferenciou os treinamentos pela intensidade, onde o protocolo HIIT tinha 5 períodos de 1 minuto com intensidade máxima de 85% do VO_{2max} retornando a base de 50% do VO_{2max}, enquanto o EAC a intensidade foi moderada contínua sempre a 50% do VO_{2max}. RESULTADOS: 27 pacientes completaram o estudo. Após 8 semanas de treinamento, o protocolo HIIT promoveu um aumento de 1,96x na FMD basal o qual foi maior comparado ao protocolo CONT e aos controles os quais não alteraram a %FMD. O % de pacientes com DE foi reduzido significativamente no grupo HIIT, mas não se alterou nos grupos EAC e Controles. O consumo máximo de oxigênio (VO_{2max}) aumentou significativamente em ambos os grupos HIIT e CONT, mas foi maior no grupo HIIT, não havendo alteração no grupo controle ($p>0.05$). Ambos os grupos, HIIT e EAC melhoraram o controle metabólico, determinado pela hemoglobina glicada (A1c) em relação aos controles, porém não diferiram entre si. Não houve alterações significativas nas medidas de estresse oxidativo nos 3 grupos estudados. A glicemia capilar foi verificada apenas nos grupos HIIT e EAC, antes e a cada cinco minutos cada sessão de exercício físico. A reposição de Carboidrato foi realizada sempre que a glicemia capilar estivesse ≥100mg/dl consecutivamente. Quanto à glicemia capilar, observou-se somente na fase 3 redução estatisticamente significativa entre os grupos. No protocolo AEC ocorreu significativamente mais eventos de hipoglicemia comparado com o protocolo HIIT. CONCLUSÕES: A partir dos resultados obtidos, pode-se perceber efeito favorável e seguro do HIIT no controle metabólico no DM1. O treinamento de 8 semanas com o protocolo HIIT promove um aumento significativo da vasodilatação endotélio dependente em pacientes com DM1, superior ao treinamento convencional, reverte a disfunção endotelial de e promove melhor condicionamento físico em pacientes com DM1

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LISTA DE ABREVIATURAS E SIGLAS

8-OHDG	8-Oxo-2'-deoxyguanosine
AcH	Acetilcolina
BH4	Tetrahidrobiopterina
DM	Diabetes Mellitus
DM1	Diabetes Mellitus Tipo 1
DM2	Diabetes Mellitus Tipo 2
EAC	Exercício aeróbio continuo
EAI	Exercício aeróbio intervalado
eNOS	Óxido Nítrico Sintase Endotelial
E-SELECTINA	Molécula de adesão à célula vascular
EURODIAB	Prospective Complications Study
FADH2	Flavin adenine dinucleotide phosphate-oxidase
FMD	Flow Mediated Dilation
g	Gramas
GC	Grupo controle sem exercício
GcA	Guanilatociclase
GE	Grupo exercício
GMP	Guanosina monofosfato
GMPc	Guanosina monofosfato cíclico
HbA1c	Hemoglobina Glicosilada
HDL	Lipoproteínas de alta densidade
HIIT	High intensity Interval Training
ICAM-1	Concentração sérica de moléculas de adesão Intercelular 1
IL-6	Interleucina-6
IMC	Índice de massa corporal
LDL	Lipoproteínas de baixa densidade
LNMMA	NG Monometil – L - Arginina
MDA	Malonaldeído
mg.dl	Miligramas decilitros
ml.kg.min.	Mililitros por quilograma de peso por minuto = mililitros de oxigênio absorvidos por quilograma de peso corporal no espaço de tempo de 1 minuto
MTT	Sal de tetrazólio
NADH2	Dinucleotídeo de adenina e nicotinamida fosfato
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase
NFKB	Fator nuclear kB
NO	Óxido Nitrico
PCR	Proteína C Reativa
ROS	Espécies reativas de oxigênio
T-BARS	Substâncias reativas ao ácido tiobarbitúrico
TIOIS	Composto organossulfurado
TNF- α	Fator de necrose tumoral
Ul/ml	Unidades Internacionais por mililitro
Unid.kg	Unidades quilogramas
VCAM -1	Molécula vascular da célula de adesão-1
VO_{2max}	Capacidade máxima do corpo de um indivíduo em transportar e metabolizar oxigênio durante um exercício físico.
vWf	vonWillebrand

SUMÁRIO

1. INTRODUÇÃO

A resposta endotelial a diferentes tipos de estresses tem sido motivo de estudos, tanto em indivíduos aparentemente saudáveis quanto em portadores de diabetes mellitus (DM). A alteração da função endotelial precede o desenvolvimento das alterações ateroscleróticas morfológicas e pode contribuir para o desenvolvimento da lesão e das complicações clínicas do DM e ao Infarto Agudo do Miocárdio. Com o objetivo de encontrar a melhor terapêutica para esta disfunção, a investigação clínica, por meio do exercício físico tem sido utilizado como um método de intervenção eficaz. Nesse mesmo raciocínio, um estudo especula que um dos prováveis mecanismos da disfunção endotelial exposto ao exercício está associado com a melhora da vasodilatação (cisalhamento vascular) e ao aumento da produção de óxido nítrico (NO) [(1)].

Os efeitos benéficos do exercício físico sobre a função vascular estão vinculados: ao fluxo elevado (implicando em adaptações crônicas do sistema vasodilatador); do estresse sobre o endotélio (não somente pelo fluxo elevado, mas também pelas alterações hemodinâmicas, frequência cardíaca e pressão arterial, e efeitos metabólicos da vascularidade); efeitos metabólicos gerais; e aumento sustentável sobre a síntese de NO [(2)]. Um estudo envolvendo 51 indivíduos, para avaliar as diferenças na função endotelial independente do endotélio, 18 Diabetes Mellitus Tipo 1 (DM1), 18 Diabetes Mellitus Tipo 2 (DM2) e 15 controles, verificou-se que, nos pacientes com DM, a função independente do endotelial estava prejudicada nos dois grupos com Diabetes, DM1 (23±17%), no DM2 (20±13%) o que não aconteceu no grupo controle (66±39%) [(3)]. O exercício físico realizado durante quatro meses mostrou melhoras nas variáveis pré e pós a realização no VO_2 máximo (28,1±1,2 vs. 35,7±2,8 $ml.kg^{-1}$ min^{-1}), na função endotelial (7,7±1,4 vs. 9,9±1,0%) e nas doses basais de insulina (0,62±0,07vs.0,51±0,05 unid. kg^{-1}. dia^{-1}), benefícios que poderiam induzir redução da morbimortalidade para doença micro e macrovascular nesta população de risco. Além disto, esses autores comprovaram a importância da continuidade do exercício, já que verificaram que oito semanas de destreinamento nesses mesmos pacientes com DM1 induziram o retorno basal dos efeitos benéficos do condicionamento físico sobre a função endotelial (9,9±1,0 vs.7,3±0,4 %) [(4)].

O fato de que no DM a função endotelial estar anormal faz do exercício físico um importante aliado no tratamento dos indivíduos com está propriedade. Em outra pesquisa com 15 pacientes com DM2, divididos em dois grupos: grupo exercício, participando de 8 semanas de treinamento de exercício aeróbico e outro grupo controle sem exercício, os desfechos evidenciaram que houve melhora da função endotelial no grupo exercício pré e pós a realização do treinamento, sendo de 1,7±0,5% vs. 5,0±0,4% (p <0,001) [2].

O exercício físico aeróbico influencia beneficamente o papel do NO derivado do endotélio na função vascular. Embora o exercício físico tenha demonstrado benefícios em relação à disfunção endotelial ainda temos uma grande lacuna a preencher. Faltam estudos que abordem os efeitos do exercício em diferentes condições patológicas, assim como, em diferentes tipos e intensidade. No entanto, o tema é relativamente novo e de extrema relevância para pessoas com DM1.

Em revisão da literatura, foi encontrado apenas três estudos abrangendo exercícios intermitentes a longo prazo e DM1 em humanos e uma meta análise [5, 6, 7, 8].

Nesse contexto, o treinmento HIIT provocou um aumento do VO_{2max} e adaptações musculares mitocondrias da mesma magnitude que o treinamento EAC, entretanto, com um volume total de exercício 90% menor que o treinamento de *endurance* [8]. Essas adaptações fisiológicas ao treinamento, sugere que a melhora da função endotelial está relacionada com a intensidade do exercicio.

2. REVISÃO DA LITERATURA

2.1 Estratégias de busca e seleção de informações

O objetivo desta revisão de literatura é localizar e selecionar informações da literatura científica atualizada acerca do DM1 e respostas primárias e secundárias relacionadas ao treinamento Intervalado de alta intensidade e/ou treinamento aeróbico contínuo de intensidade moderada sobre a Função Endotelial/Estresse Oxidativo nesta população. A estratégia de busca envolveu as seguintes bases de dados: periódicos CAPES, LILACS, SciELO, PubMed advanced , em inglês, no período de 1960 a 2016. Foram realizadas buscas através dos termos “Diabetes mellitus type1”, “Endothelium Function”, “Continuous Exercise”, “High-intensity interval trining”, “Oxidative stress” e suas combinações apresentadas na Figura 1.

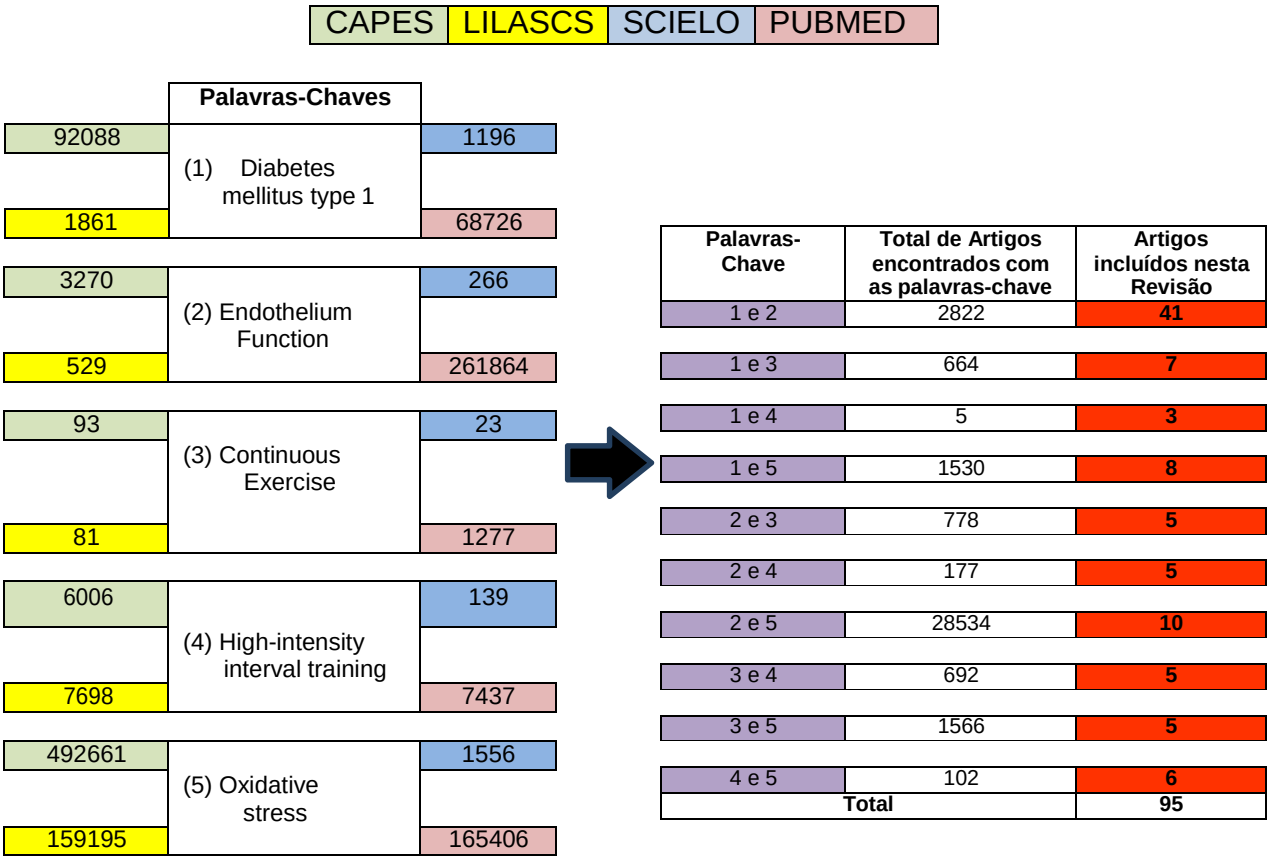

Figura 1. Estratégia de busca de referências bibliográficas sobre as bases que fundamentam os objetivos deste estudo. Caixas em laranja indicam os artigos finais que foram incluídos na revisão de acordo com os critérios de inclusão, tendo DM1 como fator de estudo ao Treinamento HIIT e Treinamento EAC sobre a Função Endotelial/Estresse Oxidativo como desfechos. Este é o resultado da busca da combinação das palavras-chave.(Boff W.; 2016)

2.2 Diabetes Mellitus Tipo 1

O Diabetes Mellitus (DM) é uma doença crônica caracterizada por hiperglicemia secundária a distúrbios na secreção e/ou ação da insulina [11,12]. O DM pode ser classificado em Diabetes Mellitus Tipo 1 (DM1), Diabetes Mellitus Tipo 2 (DM2), Diabetes Mellitus gestacional e outros casos específicos de diabetes [13].

O DM1 caracteriza-se pela deficiência total da secreção de insulina, resultante da destruição das células β-pancreáticas por processo autoimune e é responsável por 5 a 10% das formas de diabetes [11,13]. O DM1 é a forma mais frequente de diabetes na infância e adolescência e a sua incidência vem aumentando rapidamente, afetando cada vez mais crianças e adolescentes em idades mais precoces, provavelmente em virtude do aparecimento de novos fatores ambientais agressores [4]. Estima-se que, a cada ano, 65.000 novos casos de DM1 sejam diagnosticados em crianças abaixo de 15 anos [14].

Em um estudo, avaliando 4.370 adolescentes com diagnóstico de DM analisados no período de 1999-2002, 71% destes apresentavam DM1 e 29% DM2 e dos 1496 indivíduos controles (sem diagnóstico de DM), 11% já apresentavam alteração na glicemia de jejum (glicemias entre 100-125 mg/dl) [15].

As doenças micro e macrovasculares são a principal causa de morbimortalidade em pacientes com DM [16,17,18]. No DM1, o risco de morte por doença cardiovascular é 3 a 6 vezes maior quando comparado a indivíduos sem diabetes. No entanto, os fatores de risco tradicionais para doença coronária não explicam a totalidade deste excesso de risco [19,20].

A relação entre DM1 e doença cardiovascular é bem conhecida [21] e tem sido atribuída à associação entre hiperglicemia crônica, disfunção endotelial e inflamação crônica [22,23]. Em estudos clássicos e demostrado que a hiperglicemia crônica é um importante preditor de complicações micro e macrovasculares [16,17,24].

O endotélio vascular forma a camada celular que está em contato direto com o lúmen vascular e é separado da camada muscular lisa pela membrana basal. Seu papel é a manutenção da homeostase da vasculatura por meio da síntese de substâncias vasoativas que modulam o tônus vascular, inibem a agregação plaquetária e a proliferação das células musculares lisas vasculares. O NO tem papel fundamental neste equilíbrio [25].

Na célula endotelial, o NO é sintetizado a partir da L-arginina pelo óxido nítrico sintase endotelial (eNOS), na presença de oxigênio, e (tetrahidrobiopterina) BH4. Rapidamente, o NO se difunde do endotélio para a camada de células musculares lisas e plaquetas, onde ativa a guanilatociclase (Gca) com consequente produção de GMP cíclico (GMPc). A presença do GMPc promove relaxamento vascular e inibição da agregação plaquetária. A meia-vida do NO é de apenas alguns segundos, sendo rapidamente oxidado a nitrato antes de ser excretado na urina [26].

A ativação do eNOS é determinada por fatores como o estresse de cisalhamento (*shear stress*) causado pelo fluxo sanguíneo na parede luminal, pelo estiramento da parede vascular e pela baixa tensão de oxigênio na parede vascular [27,28], cujo sinal é modulado pela acetilcolina (Ach), bradicinina e inibidores da cálcio-ATPase. Por outro lado, a secreção basal de NO é inibida pela NG-monometil-L-arginina (L-NMMA), um inibidor específico de eNOS [29,30].

2.3 Disfunção Endotelial no Diabetes Mellitus Tipo 1

Em situações patológicas, como no DM, pode ocorrer o desacoplamento do eNOS, uma situação em que a transferência de elétrons na cadeia oxidativa não se completa adequadamente. Os elétrons vazam e são captados pelo oxigênio molecular, gerando radicais livres, como o superóxido. O desacoplamento do eNOS é, portanto, um importante mecanismo inicial de disfunção endotelial [31,32].

A disfunção endotelial é caracterizada pela perda das propriedades do endotélio, isto é, alteração na síntese de proteínas, aumento do tônus vascular, aumento da permeabilidade vascular e aquisição de atividade pró-trombótica e antifibrinolítica. Os principais determinantes são: a diminuição da disponibilidade de NO e a preponderância de fatores vasoconstritores liberados pelo endotélio, em detrimento aos fatores vasodilatadores [33]. A disfunção endotelial gera, portanto, alteração no perfil antiaterogênico, promovendo migração e proliferação de células musculares lisas, agregação plaquetária, oxidação do LDL, adesão de monócitos e plaquetas e síntese de citocinas inflamatórias, contribuindo para a aterogênese [34].

A análise do grau de disfunção endotelial em pacientes portadores de risco para o desenvolvimento de doença cardiovascular tornou-se um instrumento de avaliação de aterosclerose precoce [35]. O endotélio vascular é considerado um

órgão endócrino que regula o tônus vascular, homeostase e processo proliferativo fibroinflamatório local [36]. Sabe-se que alterações no endotélio vascular ocorrem de forma bastante precoce, principalmente em certos grupos de indivíduos, como crianças com baixo peso ao nascerem [37], com doenças vasculares, como Doença de Kavasaki [38], portadores de DM1[39,40], hipercolesterolemia familiar [41] e em jovens com sobrepeso [42] e obesidade [43].

A função endotelial no DM1 é modulada pelo grau de hiperglicemia [44], pela duração do diabetes [45,46], pelas concentrações séricas de insulina [47] e pela presença de complicações crônicas, especialmente neuropatia autonômica [48] e microalbuminúria [49].

A função endotelial pode ser investigada de maneira invasiva ou não-invasiva, com várias técnicas, em diferentes leitos vasculares e por diversos estímulos farmacológicos ou mecânicos. Em relação a primeira técnica, as artérias coronárias podem ser avaliadas em resposta à infusão de acetilcolina, da bradicinina ou pela indução de estresse de cisalhamento, com a utilização de angiografia biplanar quantitativa para mensuração das mudanças do diâmetro vascular [50]. Outra técnica invasiva é a pletismografia de oclusão venosa para mensuração do fluxo sanguíneo do antebraço em resposta à infusão de acetilcolina na artéria braquial [51]. A natureza invasiva destas técnicas, no entanto, envolvendo a canulação de uma artéria e a infusão de drogas vasoativas [52] torna inviável a sua ampla utilização na prática clínica. Assim, as técnicas que envolvem procedimentos não-invasivos ou com infusões de drogas a baixas concentrações e com efeitos somente em pequenos segmentos vêm sendo cada vez mais utilizadas.

A avaliação do grau de disfunção endotelial através de métodos não invasivos como a ecografia da artéria braquial para medida de dilatação mediada por fluxo e das artérias carótidas para análise do grau de espessamento das camadas íntima e média tornaram-se métodos importantes de análise de aterosclerose subclínica em crianças nos últimos anos [53]. Os dois métodos correlacionam-se inversamente, ou seja, quanto menor a dilatação da artéria braquial, maior o espessamento das camadas íntima e média carotídea [54].

A medida da resposta vasodilatadora ao aumento do fluxo de sangue na artéria braquial pode ser realizada, tanto em adultos quanto em crianças, por estímulo mecânico (isquemia temporária com aumento da pressão local com conseqüente aumento do fluxo sanguíneo e liberação de óxido nítrico (vasodilatação

endotélio-dependente) como por farmacológico com utilização de nitratos (vasodilatação endotélio-independente))[55]. Um percentual de vasodilatação inferior ao esperado é indicativo de disfunção endotelial [56,57].

A associação entre hemoglobina glicosilada (HbA1c) e dilatação mediada por fluxo foi estudada em número relativamente pequeno de pacientes com diabetes e é mal definida. Em um estudo [58], envolvendo 19 pacientes com DM1 normo e microalbuminúricos, observaram uma correlação positiva entre dilatação mediada por fluxo e HbA1c, (r=0,53, p=0,002). Ao contrário, de outro estudo transversal [59], o aumento da HbA1c parece ter impacto na dilatação mediada por fluxo, os pacientes com HbA1c>6% apresentaram significativo comprometimento da função endotelial, quando comparados aos pacientes com HbA1c < 6% sendo, HbA1c (r = -0,41; p = 0,032). .

Em outro trabalho, avaliando 11 pacientes com DM2 normoalbuminúricos, com bom controle glicêmico (HbA1c média 6,5%), observou-se uma correlação negativa entre a HbA1c e a dilatação mediada por fluxo (r= -0,37, p=0,0028). Quando comparados pela função endotelial, sete estavam com disfunção e quatro sem disfunção endotelial, a HbA1c foi maior no grupo com disfunção endotelial, 6,84 (0,24%) *versus* 6,18 (0,14%), p=0,004, respectivamente. Considera-se disfunção endotelial quando o aumento da dilatação for inferior a 8%. [60]. A principal explicação para estas discrepâncias deve-se ao fato de que a HbA1c não reflete as variações agudas da glicemia, que ocorrem no diabetes, embora seja um fator de risco para as complicações micro e macrovasculares do diabetes [61]. A glicemia de jejum, por sua vez, correlaciona-se com a redução percentual da dilatação mediada por fluxo [46]. O efeito da variação glicêmica foi estudado [62] em pacientes com DM1 normoalbuminúricos, em que foi avaliado o impacto da deterioração aguda do controle metabólico na disfunção endotelial. Todos os pacientes foram avaliados com dilatação mediada por fluxo e marcadores sorológicos de função endotelial após 48 horas de bom ou mau controle metabólico, após randomização. Este último induzido após redução da dose de insulina em 20% a 30% e liberação da dieta, por período de três semanas. A média da glicemia no período de bom controle foi 113 mg/dl comparada a 286 mg/dl no período de mau controle. Ambas as vasodilatações endotélio-dependente e endotélio-independente foram significativamente menores no período de piora do controle glicêmico em relação ao período de bom controle glicêmico. Estes resultados indicam que, no DM1, a função endotelial sofre impacto

significativo da variação aguda da glicemia, porém pode ser revertida com a melhora do controle glicêmico.

O efeito da glicemia pós-prandial na função endotelial tem sido pouco estudado no DM1. Ocorreu em um estudo [63] o impacto da glicemia pós-prandial e da hipertrigliceridemia pós-prandial na função endotelial de pacientes com DM2, e observaram que marcadores sorológicos de disfunção endotelial, como a concentração sérica de moléculas de adesão Intercelular 1 (ICAM-1), E-selecina, e marcadores de estresse oxidativo, como a nitrotirosina, aumentavam agudamente após a ingestão de 75 g de glicose oral. Este efeito, por sua vez, era ainda maior quando o estudo era realizado adicionalmente com uma sobrecarga lipídica [63], indicando que tanto a hiperglicemia aguda, como a lipemia aguda, interferem marcadamente na função endotelial no DM. Em outro estudo, os mesmos autores [64] observaram que após uma sobrecarga oral de glicose, a redução da capacidade de dilatação vascular mediada pelo endotélio acentua-se até a segunda hora, mas retorna ao basal ao completar quatro horas da sobrecarga de glicose. Com a sobrecarga lipídica, entretanto, a dilatação mediada por fluxo permanece alterada até quatro horas após. Estes dados sugerem que o efeito da glicemia pós-prandial é independente do efeito da lipemia pós-prandial e os autores destacam que ambas são mediadas pelo aumento do estresse oxidativo.

O tempo de diabetes é um determinante importante para a presença de disfunção endotelial no DM1. Em pacientes com mais de 10 anos de doença, a disfunção endotelial é um achado relativamente comum.

Entre cinco e dez anos de DM1, a disfunção endotelial ocorre mais ocasionalmente. Em um estudo, envolvendo trinta e um adolescentes com pelo menos um ano de DM1 (média 6,8 anos) e mau controle glicêmico (HbA1c 8,6%) comparados a indivíduos sem DM apresentaram comprometimento da vasodilatação endotélio-dependente [46]. Neste estudo não houve diferença na vasodilatação endotélio- independente nem na espessura da camada íntima- média da carótida[46]. A duração do diabetes apresentou correlação inversa com a dilatação endotélio-dependente ($r=–0,39$, $p=0,02$). Estes dados sugerem que a disfunção endotelial pode ocorrer na primeira década de DM1 e é mais precoce do que o aumento da espessura da camada intima-média das carótidas.

Outros dados indicam que a disfunção endotelial pode ser ainda mais precoce, ocorrendo antes de cinco anos do início da doença, inclusive precedendo a

microalbuminúria. Reforçando essa precocidade [55] compararam crianças de 11 anos de idade, com quatro anos de DM1, não-obesas, com mau controle (HbA1c= 9,8%) e crianças sem DM, em relação à dilatação mediada por fluxo e à espessura íntima-média da carótida e evidenciaram a presença de disfunção endotelial em 36% dos casos das crianças com DM1 [55]. Sendo que, a espessura íntima-média da carótida foi maior nas crianças com DM1. Os autores concluíram que disfunção endotelial é comum em crianças com DM1 nos primeiros anos da doença e pode ser um preditor para o desenvolvimento de aterosclerose prematura.

Em contrapartida, outro estudo avaliando adolescentes com 3 anos de DM1, com mau controle glicêmico (HbA1c =9,35%), não encontraram diferença na dilatação mediada por fluxo entre pacientes e controles [58]. Os dados dos estudos referidos anteriormente permitem inferir que a disfunção endotelial começa a surgir entre três e cinco anos após o início do DM1.

Os mecanismos pelo qual o DM1 leva à disfunção endotelial são complexos e parcialmente compreendidos. Embora a presença concomitante de hipertensão e dislipidemia possa contribuir diretamente para a disfunção endotelial, uma combinação de diversos mecanismos diretamente relacionados são determinantes para o desenvolvimento desta condição.

Tem sido sugerido que a disfunção endotelial induzida pela hiperglicemia seja mediada por radicais livres derivados do metabolismo do ácido araquidônico [54]. Em células endoteliais de aorta humana, a exposição prolongada a altas concentrações de glicose aumenta a expressão gênica do eNOS e a liberação de NO [65]. Contudo, ocorre aumento concomitante do radical superóxido, um potente oxidante. Estes ânions inativam o NO e levam à produção de peroxinitrito, um ativador da peroxidação lipídica e da produção de prostanóides.

Segundo Brownlee [66], a hiperglicemia leva à produção de superóxido nas células endoteliais em nível mitocondrial e está implicada na gênese das complicações do DM. O ânion superóxido liga-se ao NO, prejudicando a sua ação no endotélio [67]. Além disso, a produção aumentada de superóxido ativa a proteína quinase C, que por sua vez induz a síntese da enzima NADPH oxidase, que também contribui para produção de superóxido. A hiperglicemia também favorece, por meio da ativação do fator nuclear kB (NFkB), aumento da expressão do eNOS com geração de NO [68]. As superproduções de superóxido e de NO favorecem a formação de peroxinitrito, que apresenta ação citotóxica, interferindo no cofator para

produção de NO, tetrahidrobiopterina, promovendo também produção de superóxido, em vez de NO [69].

O manejo da disfunção endotelial no DM1 requer bom controle glicêmico, com ênfase, principalmente nas glicemias pós-prandiais [70]. Níveis adequados de pressão arterial, manejo da dislipidemia, suspensão do tabagismo, dieta pobre em gorduras e estímulo à prática da atividade física também são importantes [69,70]. O benefício do uso de agentes antioxidantes, como as vitaminas C e E, é controverso [71,72]. Colesterol HDL baixo, níveis elevados de apo-proteína B e lipoproteína(a), aumento do IMC, sedentarismo e história familiar de doença coronariana precoce correlacionam-se com disfunção endotelial [70], enquanto níveis de LDL, em idades entre 3 a 23 anos e sexo [70,71] parecem em crianças não interferir significativamente nesta análise. Em pacientes com DM1, os níveis de hemoglobina glicosilada correlacionam-se positivamente com o grau de disfunção endotelial [72].

A dosagem de marcadores inflamatórios em crianças e adolescentes em risco de desenvolver doença cardiovascular precoce tem evoluído muito nos últimos anos. O aumento sérico da proteína C reativa é sabidamente um predictor de evento cardiovascular em adultos, associando-se com disfunção endotelial [73]. Em crianças e adolescentes vários estudos já demonstraram esta relação [74,75,76]. A concentração plasmática de outros marcadores endoteliais como do Fator de Von Willembrand, de P-selectina e trombomodulina na sua forma solúvel também estão aumentados [77].

Considerando-se o aumento significativo da prevalência de DM1 em crianças e adolescentes e o risco aumentado de desenvolver doença cardiovascular precoce nesta população, torna-se importante avaliar o grau de disfunção endotelial para tomar medidas preventivas precoces como o combate à obesidade, à dislipidemia, ao sedentarismo e, quem sabe, obtermos uma reversão da disfunção endotelial através de mudanças de estilo de vida.

2.4 Exercício Físico Aeróbico Contínuo

O exercício físico aeróbico atua aumentando a biodisponibilidade do NO derivado do endotélio na função vascular. Em crianças com DM1, 30 minutos de treinamento aeróbico, duas vezes por semana, durante 18 semanas aumenta a dilatação mediada por fluxo em 65% [78]. Em adultos com DM1, 60 minutos de treinamento aeróbico, 2 vezes por semana, melhora a dilatação mediada por fluxo

em mais de 50% após 24 semanas [79]. Em estudo transversal, adolescentes com DM1 que fizeram mais de 60 minutos diários de atividade física moderada a vigorosa têm maior fluxo de dilatação mediada que os pacientes inativos com diabetes [80]. Muitos outros estudos também mostram melhora da resposta vasodilatadora em DM2 sem doença arterial coronariana, com o treinamento aeróbico / resistência aeróbia e misturado com 8 a 12 semanas de duração [81].

Embora o exercício físico tenha demonstrado benefícios em relação a função endotelial, ainda faltam estudos que abordem os efeitos de diferentes intensidade de exercício. A resposta da adaptação do sistema endotelial pode estar interligada ao sistema neuromuscular. O treinamento físico é caracterizado por modificações na capacidade de ativação e morfologia da musculatura utilizada. O ganho de força nas primeiras semanas de treino é atribuído às adaptações neurais [82], enquanto que adaptações morfológicas se alteram significativamente após 8 semanas de treinamento. Essas adaptações neuromusculares do músculo do paciente com DM1 submetidos a diferentes tipos de treinamentos, HIIT e ou EAC são de grande relevância fisiológica e motivacional para à adesão da pratica do exercício físico e podem ter impactos diferentes na função endotelial.

2.5 Exercício Físico Aeróbico Intervalado

O EAI consiste na alternância de diferentes intensidades de exercício, permitindo que o indivíduo alcance intensidades maiores em pouco tempo as quais não seria possível de sustentar forma persistente por período prolongado. Desta forma, o endotélio pode ser beneficiado por alterações mais intensas de fluxo o que poderia ter benefício adicional além do exercício moderado na função endotelial [83]. O HIIT por períodos superiores a 4-6 semanas leva a melhor performance nos exercícios, a maior oxidação das gorduras e aumento da capacidade aeróbica. Em um estudo [84] foram avaliados 43 idosos com DM2 sedentários de ambos os sexos**,** que foram submetidos a dois métodos de treino diferentes. Divididos em 3 grupos, o grupo (A) realizou treino aeróbico contínuo, o grupo (B) realizou treino intervalado de alta intensidade e o grupo (C) não realizou treinamento físico. Ao final de 12 semanas de treinamento, foi possível verificar uma melhora na função endotelial no percentual de 1,3% no grupo A, de 2% no grupo B e de 0,3% no grupo C. o estudo

foi realizado sem controle dietético, significativamente melhor, portanto no exercício intervalado.

Sessões com exercícios intervalados de alta intensidade também reduzem o risco de hipoglicemia durante e após o esforço, quando em comparação com o exercício aeróbico contínuo em DM1[85,86,87].Por estas razões, um programa de treinamento com a combinação alternado de exercícios envolvendo HIIT poderá provocar melhoras na saúde dos DM1, e ainda sim, envolver um menor tempo total nas sessões de treinamento quando em comparação ao treinamento concorrente tradicional.

2.6 Estresse Oxidativo no Diabetes Mellitus Tipo 1

A literatura científica sugere que o estresse oxidativo tenha papel central na patogênese das complicações do diabetes [88]. O estresse oxidativo é um estado de desequilíbrio entre a produção de espécies reativas de oxigênio (ROS) e a capacidade antioxidante endógena [89] e o seu papel como determinante principal do início e da progressão das complicações cardiovasculares associadas ao DM tem sido alvo de grande interesse. Mecanismos bioquímicos têm sido propostos para explicar as anormalidades estruturais e funcionais associadas com a exposição prolongada dos tecidos vasculares à hiperglicemia com indícios de que a capacidade antioxidante endógena esteja prejudicada nos indivíduos com DM1, dificultando a remoção dos radicais livres [90].

A disfunção endotelial e a inflamação crônica estão implicadas na patogênese da doença aterotrombótica cardiovascular em indivíduos com ou sem diabetes, independentemente da presença de fatores de risco, como hipertensão, dislipidemia, tabagismo. O primeiro estudo a demonstrá-la avaliou pacientes com doença coronariana leve, não obstrutiva e sem diabetes, os quais eram submetidos à avaliação da reatividade vascular por meio da administração intracoronária de acetilcolina, adenosina e nitroglicerina, seguido de ultra-som intravascular. Os pacientes eram estudados longitudinalmente para avaliação de desfechos cardiovasculares [91]. Neste estudo, os pacientes foram divididos de acordo com a gravidade da disfunção endotelial e acompanhados por 28 meses em média. Ao final do seguimento, 14% dos pacientes com disfunção endotelial grave apresentaram

eventos cardiovasculares (p<0,05), enquanto nenhuns dos pacientes com disfunção endotelial leve ou ausente tiveram desfechos cardíacos.

O valor preditivo para mortalidade cardiovascular de marcadores de disfunção endotelial como o fator de vonWillebrand (vWf) foi avaliado no estudo HOORN [92]. Este estudo foi uma coorte populacional de 2.484 indivíduos caucasianos com 50 a 70 anos de idade, entre 1989 e 1992, em que 27% apresentavam DM2 e 27% apresentavam intolerância à glicose [92]. Após cinco anos de acompanhamento, 58 indivíduos evoluíram a óbito. Quando se avaliou o vWf comparando os níveis encontrados no tercil superior (>1,56 UI/ml) em relação aos dois terços inferiores (<1,56 UI/ml), o risco de mortalidade cardiovascular em pacientes com diabetes foi de 2,30 (IC95% 0,80-6,64) após ajustes para idade, sexo, tolerância à glicose, enquanto em pacientes sem diabetes o risco foi de 4,10 (IC 95%: 0,96-17,54). Em todos os indivíduos agrupados, o risco relativo para mortalidade por todas as causas associado ao vWf no tercil superior foi de 2,03 (IC95%: 1,19-3,47). Estes resultados sugerem que o vWf, como marcador de disfunção endotelial, seja um predictor independente de morte cardiovascular [92].

Em pacientes com DM1, o vWf está, em geral, aumentado em comparação com indivíduos saudáveis [93].Este aumento é maior na presença de micro e macroalbuminúrias em relação aos pacientes normoalbuminúricos e correlaciona-se positivamente com a proteína C reativa (r = 0,44, p<0,0005), indicando associação entre disfunção endotelial e inflamação vascular [94]. Os mecanismos pelos quais o risco cardiovascular está associado a níveis elevados de vWf não são bem conhecidos, mas refletem disfunção endotelial generalizada e um estado pró-trombótico[95], o que representa risco maior de desenvolvimento de doença aterosclerótica [96]. Como o vWf também pode ser originado de plaquetas, a medida combinada de vWf e de ativador de plasminogênio tecidual (AP-t)pode ser um índice mais específico e sensível de alteração da célula endotelial [96].

Outros marcadores plasmáticos de disfunção endotelial incluem o AP-t, SE-selectina, molécula de adesão à célula vascular, molécula de adesão intracelular e fator de crescimento endotelial vascular [63].

Marcadores inflamatórios, como o necrose tumoral alfa (TNF-α), interleucina-6 (IL-6) e proteína C reativa ultra-sensível (PCR), estão positivamente associados ao risco de doença vascular em indivíduos sem diabetes [98]. Em pacientes com DM1, marcadores inflamatórios, como a PCR, IL-6 e TNF-α, também são importantes

determinantes de inflamação. No estudo (*EURODIAB Prospective Complications Study)* [22], 348 pacientes obtido de 543 participantes foram analisados os marcadores inflamatórios como PCR, TNF-α, IL-6 em indivíduos com DM1 e indivíduos sem diabetes e comparados com os níveis de marcadores de disfunção endotelial, como a E-seletina e a molécula vascular da célula de adesão-1 (VCAM-1). As medidas de inflamação estiveram diretamente associadas com o tempo de DM, controle glicêmico, perfil lipídico, pressão sistólica e com marcadores de disfunção endotelial [22]. Já a PCR apresenta-se como preditor de todas as causas de mortalidade cardiovascular, estando relacionada com outros fatores de risco [92].

Um estudo [99] avaliando 24 pacientes DM1 entre 2 a 12 anos durante a primeira semana de diagnostico clínico do diabetes, quando o controle metabólico já estava restaurado, demonstraram concentrações elevadas de malonaldeído (MDA) plasmático, produto final da oxidação de ácidos graxos poliinsaturados, em relação ao grupo-controle ($p<0,0001$), sugerindo que radicais livres do oxigênio possam exercer seus efeitos tóxicos em estágios iniciais da doença, mantendo-se elevados no curso dela, ao serem comparados com um grupo de 30 DM1 com mais tempo de doença e sem complicações. Demonstraram ainda baixos níveis de glutationa peroxidase em DM1 recém-diagnosticado em relação aos controles ($p<0,0001$), com progressivo declínio no curso da doença. Estes mesmos autores não encontraram correlação entre estes achados e os parâmetros de controle glicêmico e lipídico. Outro estudo [100] avaliando 38 pacientes DM1, com idade média de 16,1±10,3 anos desde o diagnóstico, não demonstraram diferença significativa nas variáveis de peroxidação lipídica e MDA em relação ao grupo-controle. Neste grupo, a capacidade antioxidante total do plasma (quantificada por quimioluminescência) foi 16% menor no grupo com DM1 ($p<0,0005$), a despeito do simultâneo aumento nos níveis de tocoferol ($p<0,05$), sem correlação com o controle glicêmico. Foi [101] demonstrado, em DM1 com media de 5,5±4,4 anos de doença, correlação direta entre aumento de 8-OhdG (8-hidroxi-2'-deoxiguanosina), outro marcador de estresse oxidativo, com o controle glicêmico e a presença de microalbuminúria, achados confirmados em outros estudos [99,102], avaliando um grupo de DM1 pré-púberes, com menos de cinco anos de diagnóstic9o, não observou diferença estatisticamente significante nos marcadores do estresse oxidativo em relação aos controles, com parâmetros bioquímicos similares entre os dois grupos. Na avaliação do estresse oxidativo em pacientes com DM1 [103], com 2,62±2,24 anos de doença, sem

comorbidades associadas e em tratamento intensivo do DM1 (aplicação de múltiplas doses de insulina), observou-se diferença estatisticamente significante na produção de ROS por granulócitos de DM1 em relação aos não DM1 ($p<0,05$), quantificados por quimioluminescência dependente de luminol (o método se fundamenta na amplificação pelo luminol da luminescência natural emitida pelas espécies reativas de oxigênio). O status antioxidante do plasma foi avaliado pela redução direta do MTT (sal de tetrazólio: [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) pelo plasma [104,105], ensaio utilizado na medida de viabilidade e proliferação celular, que se fundamenta na redução do MTT por meio de redutases, que agem como doadora de elétrons na redução do MTT. Esta redução, descrita inicialmente como fenômeno intracelular, envolve NADH2 e FADH2 e é primariamente uma medida da taxa de produção de NADH na hiperglicemia. Não foi observada diferença significativa na capacidade de redução direta do MTT pelo plasma entre DM1 e não-diabéticos ($p>0,05$), caracterizando manutenção do poder antioxidante do plasma neste grupo. Nenhuma correlação entre controle glicêmico e lipídico e os parâmetros avaliados foi encontrada.

3. MARCO TEÓRICO

O marco teórico esquemático apresentando a problemática da pesquisa. Boa parte dos pacientes com DM1 apresentam hiperglicemia, ocasionando um estresse oxidativo, desenvolvendo um quadro que pode progredir, em função da permanência dos quadros anteriores, a disfunção endotelial. Surgindo complicações oriundas desse processo, a inflamação, ocasionando aterosclerose e aumentando as chances de ocorrer um infarto agudo do miocárdio. Para estudos científicos, não existe ainda um modelo experimental que apresente o mesmo quadro que o observado em pacientes com DM1 e função endotelial. Ademais, pacientes com DM1 tendem a desenvolver disfunção endotelial e não podem ser diagnosticados com sintomas clínicos e sim por técnicas que, em sua maioria, de alto custo. Entretanto, o exercício físico tem sido utilizado como um método de intervenção terapêutica para prevenir e reverter esta disfunção, mas não é qualquer tipo de exercício que atua positivamente neste sentido. Nossa hipótese que defendemos é se o exercício aeróbico intervalado de alta intensidade tem a capacidade de melhorar a função endotelial em comparação ao exercício continuo de intensidade moderada em pacientes com DM1.

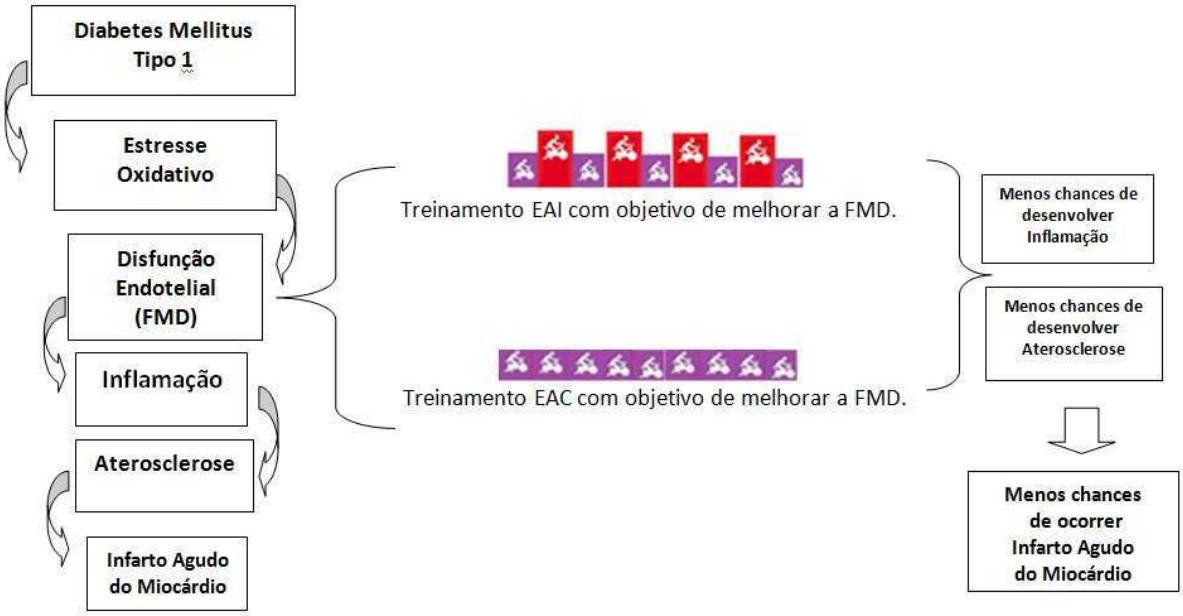

Figura 2 (Boff W.;2016)

4. JUSTIFICATIVA

A disfunção endotelial precede ao surgimento da aterosclerose e da doença cardiovascular no paciente com DM1. O exercício físico tem um importante impacto na função endotelial em pacientes com fatores de risco cardiovasculares, podendo ser uma ferramenta terapêutica importante na prevenção de doença cardiovascular. Estamos propondo realizar o exercício físico como forma avaliar o efeito de 2 protocolos de treinamento cuja característica é o treinamento com intensidades intervaladas alternando intensidades maiores e menores em comparação com um treinamento continuo com cargas menores em pacientes com diabetes. A ideia é que expondo o paciente a intensidades maiores intervaladamente o benefício do exercício na função endotelial e no estresse oxidativo e na inflamação vascular poderia ser maior. Como objetivos secundários também vão avaliar as mudanças no controle glicêmico relacionadas aos dois tipos de exercício.

5. OBJETIVOS

5.1 Objetivo Principal

Determinar o efeito de 8 semanas de ***HIIT*** comparado ao **EAC** e a um ***grupo controle sem exercício (C),*** na função endotelial medida por FMD (*Flow Mediated Dilation*) em pacientes com diabetes tipo 1.

5.2 Objetivos Secundários:

Determinar o efeito do treinamento com 8 semanas por ***HIIT*** comparado ao **EAC** no:

estresse oxidativo, substâncias reativas ao ácido tiobarbitúrico (T-BARS) e composto organossulfurado (TIOIS);

controle glicêmico (HbA1c);

excursão da glicemia capilar durante o exercício físico de pacientes com DM1.

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Superior effects of high-intensity interval vs. moderate-intensity continuous training on endothelial function and cardiorespiratory fitness in patients with type 1 diabetes: a randomized controlled trial

Superior effects of high-intensity interval *vs.* moderate-intensity continuous training on endothelial function and cardiorespiratory fitness in patients with type 1 diabetes: a randomized controlled trial

Running title: Exercise and endothelial function in diabetes

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ABSTRACT

Aims/hypothesis: To compare the effect of high-intensity interval training (HIIT) with moderate-intensity continuous training (MCT) on endothelial function, biochemical markers, oxidative stress and clinical fitness in patients with type 1 diabetes.

Methods: Thirty-six type 1 diabetic patients (mean age 23.5±6 years) were randomized into 3 groups: HIIT, MCT, and sedentary (CON). Exercise was performed in cycle ergometers during 40 min, 3 times/week, for 8 weeks at 50-85% maximal heart rate (HR_{max}) in HIIT and 50% HR_{max} in MCT. Endothelial function was measured by flow-mediated dilation (endothelium-dependent vasodilation [EDVD]), and smooth-muscle function by nitroglycerin-mediated dilation (endothelium-independent vasodilation [EIVD]). Peak oxygen consumption (VO_{2peak}) and oxidative stress markers were determined before and after training. Endothelial dysfunction was defined as an increase <8% in vascular diameter after cuff release. The trial is registered at ClinicalTrials.gov, identifier: NCT03451201.

Results: Twenty-seven patients completed the 8-week protocol, 9 in each group (3 random dropouts per group). Mean baseline EDVD was similar in all groups. After training, mean absolute EDVD response improved from baseline in HIIT: +5.5±5.4%, (p=0.0059), but remained unchanged in MCT: 0.2±4.1% (p=0.8593) and in CON: -2.6±6.4% (p=0.2635). EDVD increase was greater in HIIT *vs.* MCT (p=0.0074) and CON (p=0.0042) (ANOVA with Bonferroni). Baseline VO_{2peak} was similar in all groups (p=0.96). VO_{2peak} increased 17.6% from baseline after HIIT (p=0.0001), but only 3% after MCT (p=0.055); no change was detected in CON (p=0.63). EIVD was unchanged in all groups (p=0.18). Glycemic control was similar in all groups.

Conclusions/interpretation: In patients with type 1 diabetes without microvascular complications, 8-week HIIT produced greater improvement in endothelial function and physical fitness than MCT at similar glycemic control.

Keywords: high-intensity interval training; endothelium; flow-mediated dilation, endothelial dysfunction; diabetes mellitus, type 1; exercise; cardiorespiratory fitness

Abbreviations

CON	sedentary control group
DTNB	dithiobis (2-nitrobenzoic acid)
EDVD	endothelium-dependent vasodilation
EIVD	endothelium-independent vasodilation
FBG	fasting blood glucose
HIIT	high-intensity interval training
hs-CRP	high-sensitivity C-reactive protein
MCT	moderate-intensity continuous training
TBARS	thiobarbituric acid-reactive substances
T-SH	thiol group concentrations
TWS	tangential wall stress
UAC	urinary albumin concentration
VO_{2max}	maximal oxygen consumption during exercise
VO_{2peak}	peak oxygen consumption

Research in context

What is already known about this subject?

- Some studies show that exercise can improve endothelial function in young patients with type 1 diabetes, but results are not robust.
- Exercise intensity may be an important determinant of endothelial improvement.
- Meta-analyses show that high-intensity exercise training is better than continuous exercise training to improve fitness in type 2 diabetes.

What is the key question?

- Is high-intensity exercise training more effective than continuous exercise training in improving endothelial function and physical fitness in young type 1 diabetes?

What are the new findings?

- High-intensity exercise training for 8 weeks markedly improves endothelial function compared with continuous exercise training in young adults with non-complicated type 1 diabetes.
- High-intensity exercise training markedly improves while continuous exercise training only mildly improves physical fitness in young adults with type 1 diabetes.
- Improvements in endothelial function are not affected by changes in glycemic control.

How might this impact on clinical practice in the foreseeable future?

- High-intensity exercise training may be preferred to continuous exercise training as an exercise training prescription for young type 1 diabetes due to potential benefits on cardiovascular function.
- The impact of high-intensity exercise training on cardiovascular prevention should be investigated in long-term studies.

INTRODUCTION

Micro- and macrovascular complications are the main causes of morbidity and mortality in patients with type 1 diabetes [1, 2]. Endothelial dysfunction is supposed to precede atherosclerosis and microvascular disease [3]. The natural course of endothelial dysfunction in type 1 diabetes is linked to additional effects on chronic hyperglycemia, oxidative stress and subclinical endothelial inflammation, leading to accelerated development of atherosclerosis [4-6]. We previously demonstrated that long-term, rather than short-term, poor glycemic control is associated with endothelial dysfunction development in recently diagnosed adolescents with type 1 diabetes [7]. When poor glycemic control occurs in the first few years after type 1 diabetes onset, there is a greater impact of endothelial dysfunction, indicating an effect of metabolic memory [7].

In children and adolescents with type 1 diabetes, 30 min of aerobic training for 18 weeks significantly increased flow-mediated dilation (FMD) [8]. In adults with type 1 diabetes, aerobic training significantly enhanced FMD after 24 weeks of training [9]. In a cross-sectional study, adolescents with type 1 diabetes performing more than 60 min of daily moderate to vigorous exercise had greater FMD than sedentary patients [10]. Improvements in endothelium-dependent vasodilator response is also seen in type 2 diabetes without coronary artery disease, when patients are subjected to combined aerobic and resistance training [11].

Intensity changes during exercise seems to be an important determinant of effects on endothelial function. Studies in different populations, including type 2 diabetes, arterial hypertension, heart failure, obesity and metabolic syndrome have demonstrated that high-intensity interval training (HIIT) (i.e., high-intensity efforts interspersed with recovery period at lower intensity) can increase endothelium-dependent dilation more effectively than traditional moderate-intensity continuous training (MCT) [12-16]. In addition, HIIT is

associated with greater improvement in physical fitness performance (VO_{2max}) than MCT. A meta-analysis involving 10 studies demonstrated that HIIT exercise provided a better physical conditioning compared to MCT in subjects with established cardiovascular disease, metabolic syndrome and obesity [17]. Another recent meta-analysis found that HIIT was better than MCT in increasing VO_{2max} in type 2 diabetes [18].

So far, HIIT has not been tested against MCT in patients with type 1 diabetes. Our hypothesis was that if the patient is subjected to a greater exercise intensity as in HIIT, FMD and cardiorespiratory fitness will increase more than in MCT. Therefore, the main objective of this randomized controlled trial was to compare the effects of 8-week HIIT and MCT on endothelial function and cardiorespiratory fitness in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Design

A randomized, parallel-group clinical trial with 3 arms and a 1:1:1 allocation ratio. The eligibility criteria are shown in Figure 1. No changes were made to the methods after trial commencement.

Eligibility criteria

We searched for patients with type 1 diabetes above 18 years of age attending at the Institute for Children with Diabetes (ICD), who were included in ICD database from January 1, 2015, to January 1, 2016.

We recruited subjects of both genders, regularly attending clinic visits, who were physically inactive or not involved in exercise training programs in the previous 6 months and were interested in starting an exercise training program. We excluded smokers, pregnant women, patients with known co-morbidities not related to diabetes, patients taking drugs

other than insulin and those who presented with severe diabetes-related complications, such as: loss of renal function (serum creatinine above 132.60 µmol/L), moderate to severe retinopathy or blindness, suspected or confirmed coronary artery disease, severe peripheral neuropathy, foot ulcers or history of foot ulcers and any suspected or confirmed clinical autonomic neuropathy. Patients who met the eligibility criteria were invited to visit the research center.

Intervention

The intervention group was submitted to the HIIT protocol. We included an exercise control group (MCT) and a sedentary control group (CON). Training sessions were performed in the ESEFID training center under supervision of part of the team in the afternoon period.

As a general recommendation, patients in HIIT and MCT groups exercised on cycle ergometers 3 times a week, for 8 weeks. Heart rate was monitored during the whole exercise sessions using heart rate monitors (Polar® FT4, Polar Electro Oy, Kempele, Finland). All exercise sessions were supervised and adherence was monitored by group. Capillary blood glucose was measured every 5 min during all exercise training sessions and oral 10 g glucose gels were given whenever blood glucose was ≤5.55 mmol/L and a 20% decrease in insulin basal dose was recommended to all patients in the morning of every training day to minimize the risk of hypoglycemia. In addition, patients were recommended not to exercise at the peak of insulin action.

High-intensity interval training (HIIT) protocol

HIIT protocol was divided into 3 phases according to Mitranun et al (12) (Figure 2): phase 1: weeks 1-2, phase 2: weeks 3-4, phase 3: weeks 5-8. In phase 1, participants warmed

up for 5 min, increasing intensity gradually to reach 50% of maximal heart rate. It was maintained for 20 min and then followed by a recovery period of 5 min. In phase 2, there was a 5-min warm up to reach 50% maximum heart rate (HR_{max}), and it was followed by a 1-min sprint at 80% HR_{max}, then slowing down to 50% HR_{max} for 5 min. This procedure was repeated 3 more times and then followed by a recovery phase of 5 min. In phase 3, the protocol was longer and more intense. After a 5-min warm-up to 60% HR_{max}, patients performed six 1-min sprints at 85% HR_{max}, followed by 4-min slow-down intervals at 50%. The whole session lasted 40 min.

Moderate-intensity continuous training (MCT) protocol

MCT protocol was also divided into 3 phases as described previously (12). In phase 1, training was identical as in HIIT. In phase 2, participants exercised to attain 50% HR_{max} in 5 min and then increased intensity to 60% HR_{max} for 20 min, ending with 5 min to recover, totaling 30 min. In phase 3, participants attained 50% HR_{max} in 5 min and exercised constantly at 65% HR_{max} for 30 min, recovering in 5 min and totaling 40 min.

Sedentary control group (CON)

Control patients were only asked to follow general lifestyle recommendations, including to walk at least 3 times a week for a minimum of 30 min. This group was not supervised. No other exercise specifications were given.

Primary outcome

Flow-mediated dilation (FMD)

We pre-specified the difference between post- and pre-training percentage FMD as the primary outcome. FMD was determined as follows. Within 2 weeks of the first visit,

patients were assessed for pre-training endothelial function through brachial artery ultrasound in the left arm. The examination was performed by an experienced member, blinded to the results of the study, using the technique according to Corretti et al [19], which was previously described by our group [7]. Tests were performed in the non-invasive cardiovascular methods unit of Hospital de Clínicas de Porto Alegre (HCPA). Briefly, patients were studied in the morning, after the usual dose of basal insulin and a 200-kcal low-fat standard meal for breakfast. Arterial blood pressure was measured by the auscultatory technique, using an aneroid sphygmomanometer, at room temperature (22-24°C). All measurements were performed using-high resolution ultrasound equipment (EnVisor CHD, Philips, Bothell, USA) with a high-frequency transducer (3-12 MHz, L12-3 Philips) to obtain longitudinal images of the brachial artery. The ultrasound images were obtained with two-dimensional mode, color and spectral Doppler. The simultaneous electrocardiography (ECG) was recorded. To minimize operational errors, both transducer and arm positions were maintained throughout the procedure. Images were recorded with the patients at rest for 30 min. Endothelium-dependent vasodilation (EDVD) and endothelium-independent vasodilation (EIVD) were determined respectively by FMD and nitroglycerin vasodilation. Measurements were done at multiple vascular sites using the measurement system of the same equipment. Arterial diameter measurements were done off-line, at the end of diastole, at the peak of the R wave in the ECG.

After recording of baseline images, the brachial artery was occluded for 5 min with a pressure cuff positioned on the arm and inflated to 50 mmHg above systolic blood pressure for 4 min. The EDVD response was recorded between 45 and 60 s after cuff release. After 10 min resting, baseline images were repeated and then 0.4 mg of sublingual nitroglycerin spray (Natispray Trinitrite, Procter & Gamble Pharmaceutics, Paris-Cochin, France) was used to

evaluate EIVD 4 min after the spray. EDVD and EIVD were expressed as percent change in brachial artery diameter before and after cuff release or nitroglycerin administration, respectively. Endothelial dysfunction was considered when EDVD was less than 8% in relation to baseline [20, 21]. Smooth muscle dysfunction was considered by the same criteria after nitrate use for EIVD [20, 21].

Secondary outcome

Maximal oxygen consumption (VO_{2peak})

We pre-specified the difference between post- and pre-training VO_{2peak} values during a maximum load test. Briefly, all patients were submitted to cardiorespiratory fitness assessment one day before starting the training protocols, which was repeated within 48 h after the last training session was completed. An incremental maximal cycle ergometer (Cybex, Medway, USA) test was conducted to determine peak oxygen uptake (baseline VO_{2peak}), using the breath-by-breath method in an open circuit spirometry (Quark CPET, Cosmed, Rome, Italy). After a 3-min warm-up period, cycling at 50 W, the workload was increased by 25 W every min until fatigue. The heart rate was measured during the test, which was interrupted when cadence was <60 rpm. VO_{2peak} was defined as the highest mean value achieved within the last 15 s prior to exhaustion as described in Moser et al [22].

Sample size calculation

The sample size, using FMD as the primary outcome, was calculated according to the study of Mitranun et al [12] in type 2 diabetes, in which the difference between pre- and post-training values of FMD was around 2%. Considering a standard deviation of 1.1%, alpha = 0.05 and beta = 0.8, the minimal number of patients in each group was 8. We anticipated possible dropouts and decided to include 12 patients in each group.

Randomization

The randomization process was in blocks, according to FMD results before training. Baseline FMD results were ranked in decreasing order in blocks of 3, so that the first 3 patients with corresponding higher FMD results formed the first block, in a 1-2-3 sequence, respectively, Group 1 = HIIT, Group 2 = MCT, Group 3 = CON. The following block followed an inverse sequence (3-2-1) and then consecutively. This process ensured that baseline FMD was similar between the 3 groups before training intervention. For technical reasons, the intervention was not blinded, since sedentary control patients knew that they would not exercise. However, the investigator responsible for FMD determination was blinded for the rest of the study. All further evaluations were performed before and after the exercise training interventions by the same investigators.

Biochemical assays

Blood and urine samples were collected after 12 h fasting. Patients were asked to avoid exercise in the 48 h before blood and urine collection. Blood samples were routinely centrifuged for 15 min and serum and plasma were stored at -80ºC. HbA1c was determined by immunoturbidimetry (Certified Self-Analysis of the National Glycohemoglobin Standardization Program-Cobas Integra 400, Roche, Basel, Switzerland). Plasma glucose was

evaluated by the glucose-peroxidase method using enzymatic colorimetric reactions. Serum total cholesterol, high-density lipoprotein cholesterol (HDL-c) and triglyceride concentrations were also measured by the colorimetric enzyme method (Modular, Roche, Mannheim, Germany). LDLc was estimated by the Friedewald equation. Creatinine was measured by the method of Jaffe (Modular; Roche) and high-sensitivity C-reactive protein (hs-CRP) by nephelometry (BN II; Dade-Behring, Deerfield, IL, USA). Albuminuria was determined in a single urine sample obtained in morning using the immunoturbidimetric method: Url-Pack Bayer® MAlb Kit, Cobas Mira® Roche (AlbUCobas) [23].

Oxidative stress parameters

Total thiol group concentrations (T-SH) were assessed by reaction with [5,5′-dithiobis (2-nitrobenzoic acid); DTNB] [24], and reading at 412 nm. Levels of plasma thiobarbituric acid-reactive substances (TBARS) were evaluated as previously described [25], determined spectrophotometrically at 532 nm.

Statistics

Data distribution was evaluated by the Shapiro-Wilk test. ANOVA with Bonferroni/Dunn post-test was used to study FMD, NTG and VO_{2peak}. The differences between pre and post values for EDVD, EIVD and VO_{2peak} were referred to as DELTA. ANOVA with Bonferroni was used to make comparisons of DELTA between groups. The chi-square test was used for qualitative variables. Pearson's correlation coefficient was used to study the association between VO_{2peak} and FMD. Statistical tests were performed with the standard software package Statistical Analysis System (SAS) version QC (GraphPad, USA) and StatView (Abacus, USA).

Ethics and approvals

This clinical trial was registered at ClinicalTrials.gov Identifier: NCT03451201. This study protocol was approved by the HCPA ethics board, and the reported investigations were carried out in accordance with the principles of the Declaration of Helsinki. All participants provided oral and written consent prior to inclusion in the study. Those who agreed to participate were registered for further evaluation at HCPA and School of Physical Education, Physiotherapy and Dance (ESEFID).

RESULTS

The study randomized 36 patients with type 1 diabetes. During follow-up, 12 dropped out, 6 due to health problems not related to the study and 3 canceled consent for personal reasons. At the end, 27 patients completed the study, 9 in each group (Figure 1). All patients were analyzed in their original randomized groups. No interim analysis was performed. Patients were recruited from January 2015 to January 2016. The last follow-up visit was in May 2016. The trial ended due to the end of the protocol.

Baseline clinical and biochemical characteristics of patients are shown in Table 1. At baseline, the HIIT group showed slightly lower systolic and diastolic blood pressure values than the other groups. All other variables were similar between groups.

Changes in metabolic, oxidative stress, endothelial function and cardiovascular parameters between groups before and after training are shown in Table 2. Lipid profile, urinary albumin excretion, hs-CRP and oxidative stress measures did not differ between groups before and after training.

At baseline, the percentage of patients with endothelial dysfunction (%ED) was similar in all groups ($p = 0.60$), as well as the baseline mean EDVD (Table 2). After training, %ED was significantly lower in HIIT (22.2%) *vs.* MCT (88.8%) ($p = 0.044$) and *vs.* CON

88.8% (p = 0.0184) (Table 2). After training, EDVD increased from baseline in HIIT (p = 0.0059) and was significantly greater in relation to MCT (p = 0.0074) and CON (p = 0.0042) (Figure 3). No increase in EDVD was seen in MCT or CON. EIVD was unchanged between pre- and post-training in all groups.

Although systolic blood pressure (SBP) values were in the normal range within groups before and after training, SBP increased 7.4% in HIIT (p = 0.0378), while it was unchanged in MCT (p = 0.58) and CON (p = 0.08). Maximal heart rate was increased in HIIT in relation to CON ($p < 0.05$). There was no change in maximal heart rate between MCT and CON (Table 2).

VO_{2peak} was similar at baseline between groups (p = 0.96) and increased 17.6% from baseline after HIIT training (p = 0.0001) but only 3% in MCT (p = 0.055), with no change in CON (p = 0.63). There was a trend for a greater increase in VO_{2peak} after training in HIIT compared to MCT (p = 0.055) (Table 2).

We found a positive correlation (r=0.337, p=0.007) between the delta of VO_{2peak} and the delta of FMD, indicating that a better cardiorespiratory fitness was associated with an improvement in endothelial function (Figure 4).

After training, HbA1c was not significantly changed compared to baseline values in any of the protocol groups. No serious hypoglycemic episodes occurred. No patient had muscular injury or cardiovascular symptoms. All supervised exercise sessions were completed.

DISCUSSION

This randomized clinical trial examined the effects of HIIT in relation to MCT on endothelial function of young adults, with non-complicated type 1 diabetes. The study showed that 8 weeks of HIIT training markedly improved vascular function, by increasing

EDVD 2-fold from baseline, significantly more than MCT during a similar period of training, which was not dependent on improvements in glycemic control. Moreover, HIIT produced a robust improvement in physical fitness from baseline, while there was only a mild improvement in MCT. The strong positive correlation observed between the improvement in FMD and improvement in VO_{2peak} indicated that these variables are interdependent, and that changing intensity during exercise is an important determinant to improve physical fitness and vascular improvement in young patients with type 1 diabetes.

Exercise can improve endothelial function in both type 1 and type 2 diabetes when compared to non-exercising controls. Three studies have previously evaluated FMD in type 1 diabetes using different protocols. In a non-controlled study, Seeger et al [8] observed that, after performing 30-min sessions of aerobic training twice a week for 18 weeks, children and adolescents with type 1 diabetes showed a 65% increase in FMD, compared to non-exercising controls. Fuchsjager et al [9] observed that training sessions with aerobic exercise for 24 weeks improved FMD by more than 50%, in adults with non-complicated type 1 diabetes, while no change was observed in non-exercising individuals. In a cross-sectional study [10] including children and adolescents with type 1 diabetes, there was an association between FMD and exercising, and endothelial function was enhanced in patients who engaged in more than 60 min/day of moderate-to-vigorous physical activity.

Although exercise training effects have been studied in type 1 diabetes, this is, to the best of our knowledge, the first study comparing HIIT and MCT in a head-to-head randomized clinical trial. The effect of short intervals during exercise sessions was studied, however, in type 2 diabetes. Interval and continuous exercise training were compared in an open label clinical trial in relation to microvascular reactivity. Mitranun et al [12] randomized 45 patients with type 2 diabetes to perform exercise sessions with similar energy expenditure, walking on a treadmill for 30 and 40 min per day for 12 weeks. They observed that both

continuous and interval exercise training were effective in improving FMD from baseline, but there was a greater improvement in FMD in the group that performed intensity exercise intervals than in those who exercised in the continuous training group (37 *vs.* 27% increase, $p < 0.05$, respectively). In the present study, the differences in FMD caused by interval training were even more robust than those observed in type 2 diabetes by Mitranun et al [12]. We found that there was an almost doubling of FMD from baseline in HIIT (97% increase) with no change in MCT.

It is well known that acute exercise can enhance endothelial function compared to non-exercising controls in different clinical conditions. A meta-analysis indicated that all exercise modalities can enhance endothelial function [26]. Exercise can enhance endothelial function basically through 4 mechanisms: 1) by increasing nitric oxide (NO) bioavailability, which occurs secondarily to enhanced expression/stabilization of endothelial nitric oxide synthase enzyme (eNOS) and/or reduced inactivation/degradation of NO by free-radicals [27]; 2) increasing expression of antioxidant enzymes, superoxide dismutase, glutathione peroxidase and catalases thus enhancing antioxidant capacity [28] as well as reducing the expression of oxidant enzymes, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [29]; 3) reducing the expression of pro-inflammatory molecules such as interleukins, adhesion molecules [30]; and finally 4) increasing the number of endothelial progenitor cells (EPCs), which are important determinants of vascular endothelium regeneration and angiogenesis [31].

The mechanisms by which interval training could exert effects on endothelial function are still speculative. It is plausible, however, that shear stress may be increased after exercise [32] and that it could induce eNOS phosphorylation [33]. In a pilot trial, Dopheide et al [32] evaluated mean tangential wall stress (TWS) in the femoral artery, a surrogate marker of shear stress, in 40 patients with known peripheral artery disease, who were compared to

healthy individuals before and after supervised exercise training. Patients were asked to walk 60 min per day, at least 3-5 days a week. The intensity was limited by claudication, and they should rest for intervals of up to 5 min, repeating the same distance at lower intensity. There was a significant increase in TWS in relation to controls, indicating that intermittent exercise training may increase shear stress. Moreover, a recent study [33] demonstrated that repeated muscle contraction can induce eNOS phosphorylation in humans by increasing arterial shear stress. Casey et al [33] studied seven young males who performed 20 bouts of rhythmic forearm exercise at 20% maximal (3 min each) separated by 3 min of rest, over a 2 h period. Fresh endothelial cells were then obtained 2 h after exercise. They observed that protein expression and phosphorylation of eNOS was increased. This was the first evidence in humans that muscle contraction-induced increases in conduit arterial shear could lead to *in vivo* posttranslational modification of eNOS activity in endothelial cells.

Endothelial dysfunction in type 1 diabetes is known to be caused by chronic hyperglycemia and increased oxidative stress, also worsened by early vascular rigidity [3]. In the present study, however, exercise training did not change oxidative stress markers such as TBARS or T-SH levels, not supporting the hypothesis that a reduction in oxidative stress was critical for short-term improvements in endothelial function.

The present study had some limitations to be considered. Since we studied young patients with type 1 diabetes without established diabetic complications, extrapolating these results to a group with more advanced disease is limited. Long-term effects of exercise on endothelial function are also unknown. On the other hand, there were some important strengths to be considered. 1) We used randomization in blocks considering FMD, which favored very similar values at baseline, minimizing selection bias. 2) We had a very high adherence level, with minimal random dropouts occurring similarly in all groups, 3) FMD

measurements were performed by a single highly-trained blinded examiner, increasing accuracy.

CONCLUSION

In young adults with type 1 diabetes without complications and in moderate glycemic control, HIIT is superior to MCT in improving endothelial dysfunction and physical fitness during an 8-week training period. The effect on endothelial function was closely related to improvement of physical fitness and did not depend on glycemic control changes. Thus, HIIT can be recommended as a useful non-pharmacological alternative to improve vascular function in patients with type 1 diabetes. Long-term studies to examine the efficacy of HIIT in preventing micro- and macrovascular disease are still required.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

Author Contributions

WB conceived and executed the study, including the supervised exercise sessions; AMVS performed all ultrasound examinations and obtained flow-mediated dilation data; JF and JRK collected the data and organized the database at the Exercise Research Laboratory; ARO

implemented the study design in the Exercise Research Laboratory, supervised the exercise protocol, wrote and revised part of the manuscript; BT and MP organized the database and logistics at the Institute for Children with Diabetes (ICD) and revised the manuscript; MCB was the mentor of the study, designed and organized the logistics at Hospital de Clínicas de Porto Alegre (HCPA), raised the funds for the study, wrote, revised and submitted the manuscript.

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TABLES

Table 1. Baseline characteristics of patients.

	HIIT (*n* = 9)	**MCT (*n* = 9)**	**CON (*n* = 9)**	***p***
Age (yr)	26.1±7.8	23.7±5.8	20.8±2.6	0.18
Male/Female	3/6	5/4	4/5	0.63
Duration of type 1 diabetes (years)	9.1±2.9	10.4±2.8	9.7±2.7	0.64
Total daily insulin dose (UI/kg)	0.48±0.09	0.56±0.22	0.47±0.11	0.43
BMI (kg/m^2)	23.2±2.4	24.1±2.0	22.7±2.6	0.56
Systolic BP (mmHg)	108.3±7.9[ab]	120.5±8.8	116.5±7.5	0.03
Diastolic BP (mmHg)	71.1±8.2[c]	78.8±7.8	79.6±6.5	0.05
Fasting plasma glucose (mmol/L)	11.49±4.05	8.66±2.94	11.32±6.16	0.36
HbA_1c (%)	8.2±1.3	8.4±0.9	8.8±2.3	0.67
Total cholesterol (mmol/L)	4.77±0.77	4.57±0.84	5.33±1.73	0.38
LDL cholesterol (mmol/L)	2.87±0.70	2.31±0.57	3.16±1.37	0.64
HDL-c (mmol/L)	1.53±0.31	1.47±0.63	1.57±0.38	0.89
Triglycerides (mmol/L)	0.78±0.34	1.70±0.21	1.29±0.87	0.35
Serum creatinine (µmol/L)	51.85±9.91	59.48±9.15	50.33±11.44	0.82
Mean UAC (mg/L)	12.6 (3.0-41.0)	30.4 (3.3-184)	30.5 (3.0-142)	0.58
Microalbuminuria (%)	1/9 (11.1)	2/9 (22.2)	2/9 (22.2)	0.78
Endothelial dysfunction (%)	5/9 (55.5)	7/9 (77.7)	6/9 (66.6)	0.60

BMI, body mass index; BP, blood pressure; $HbA1_C$, glycated hemoglobin; HDL-c, high-density lipoprotein cholesterol; UAC, urinary albumin concentration.
Microalbuminuria was defined as UAC>30mg/g. Data are expressed as mean ± standard deviation, except for UAC.
ANOVA was used in parametric variables.
p = p for trend.
Male/female and % of microalbuminuria and endothelial dysfunction comparison were done using chi-square; UAC was analyzed using Kruskal-Wallis.
[a] $p = 0.022$ *vs.* MCT; [b] $p = 0.03$ *vs.* CON; [c] $p = 0.027$ *vs.* CON (ANOVA and Fisher).

Table 2. Metabolic, oxidative stress, endothelial function and cardiovascular parameters before (PRE) and after (POST) 8 week-training period in HIIT, MCT, and CON

	HIIT (*n* = 9)			MCT (*n* = 9)			CON (*n* = 9)		
Variables	**PRE**	**POST**	**DELTA**	**PRE**	**POST**	**DELTA**	**PRE**	**POST**	**DELTA**
Metabolic parameters									
Weight (kg)	64.1±7.3	61.7±9.3	-2.36±4.6	71.4±11.61	70.7±10.9	-0.7±2.0	65.6±9.5	63.3±6.5	0.03±0.9
FBG (mmol/L)	11.53±4.0z8	11.44±6.12	-0.08±5.76	8.67±2.96	9.70±3.15	1.02±4.17	11.34±6.20	11.73±6.95	-0.06±9.20
HbA1c (%)	8.2±1.3	8.0±1.0	-0.2±0.6	8.4±0.9	8.1±0.9	-0.3±0.3	8.8±2.3	9.2±2.4	0.4±0.8
TC (mmol/L)	4.77±0.77	4.69±0.93	-0.08±0.57	4.56±0.85	4.04±1.37	-0.01±1.45	5.34±1.74	5.54±2.05	0.21±0.98
LDL-c (mmol/L)	2.87±0.70	2.80±0.80	0.09±0.56	2.31±0.57	1.81±1.24	-0.51±1.32	3.16±1.37	3.16±1.40	0.00±0.75
HDL-c (mmol/L)	1.53±0.31	1.54±0.42	0.01±0.18	1.47±0.63	1.37±0.51	-0.08±0.20	1.57±0.38	1.62±0.55	0.05±0.27
TG (mmol/L)	0.78±0.34	0.80±0.28	0.02±0.25	1.70±0.21	1.86±0.16	0.16±0.92	1.29±0.87	1.64±1.53	0.32±0.71
hs-CRP (nmol/L)	18.10±20.95	19.05±15.24	0.95±9.52	31.43±23.81	37.14±31.43	5.71±24.76	47.62±43.81	102.86±147.62	55.24±153.34
Oxidative Stress									
TBARS (µM of MDA/L)	2.00±1.41	2.31±1.60	0.31±0.45	2.18±0.59	2.68±1.54	0.49±1.72	2.40±1.76	2.35±1.67	-0.04±2.63
T-SH (nmol/mg of GSH)	79.59±15.26	88.37±12.18	8.77±17.23	95.04±28.30	96.26±14.04	1.22±25.01	95.35±29.76	91.31±21.71	-4.03±32.59
Endothelial dysfunction									

Variables	HIIT (*n* = 9)			MCT (*n* = 9)			CON (*n* = 9)		
	PRE	**POST**	**DELTA**	**PRE**	**POST**	**DELTA**	**PRE**	**POST**	**DELTA**
EDVD									
Mean FMD (%)	5.7±5.0	11.2±5.4[ced]	5.5±4.4[ab]	5.2±3.3	5.4±3.3	0.24±4.0	7.6±7.4	5.0±3.3	-2.6±6.4
% with ED	5/9 (55.5)	2/9 (22.2)[cd]	-	7/9 (77.7)	8/9 (88,8)	-	6/9 (66.6)	8/9 (88.8)	-
EIVD									
Mean NTG (%)	24.1±7.3	22.5±5.3	-1.5±5.4	18.0±4.2	16.3±4.7	-1.7±3.9	26.3±6.7	18.6±7.7	-4.3±6.2
% with SMD	0	0	-	0	0	-	0	0	-
Cardiovascular parameters									
Systolic BP (mmHg)	108.3±7.9	116.1±9.2	7.7±9.3[a]	120.5±8.8	118.2±7.8	-1.6±8.6	116.5±7.5	120.5±7.2	4.0±5.5
Diastolic BP (mmHg)	71.1±8.2	78.8±8.9	7.7±9.0	78.8±7.8	81.6±8.2	2.7±8.7	79.6±6.5	80.6±7.6	1.0±7.4
Resting HR (bpm)	76.5±11.7	74.4±8.7	-2.1±12.5	73.2±4.7	76.0±8.4	2.1±8.3	77.7±10.5	84.1±7.5	6.3±8.5
Max HR_{peak} (bpm)	180.4±14	189.0±16	8.51±13.9[d]	179.2±16	178.3±14.9	-0.88±5.32	183.2±15	184.3±16	0.55±4.12
VO_{2peak} (ml/kg/min)	34±6.3	40.1±4.3	6.08±2.58[ae]	33±8.2	36±8.8	3.04±4.03[d]	33.2±10	32.7±10	-0.34±2.78

BP, blood pressure; CON, non-exercising controls; FBG, fasting blood glucose; HbA1c, Hemoglobin A1c; HDL-c, high-density lipoprotein cholesterol; HIIT, high intensity interval training; HR, heart rate; hs-CRP, high sensitivity C-reactive protein; LDL-c, low density lipoprotein cholesterol; Max HR_{peak}, maximal heart rate; MCT, moderate continuous training; TBARS, plasma thiobarbituric acid-reactive substances; TC,total cholesterol; TG, triglycerides; T-SH, total thiol group concentrations; VO_{2max}, maximal oxygen consumption during exercise. EDVD, endothelial dependent vasodilation; Mean FMD, mean flow-mediated dilation; % with ED, percent of patients with endothelial dysfunction; EIVD, endothelial independent vasodilation; Mean NTG, mean of nitroglycerin-mediated dilation; % with SMD, percent of patients with smooth muscle dysfunction. All variables were tested by ANOVA and Fisher; except in % with ED and % with EIVD where chi-square was used. Data are expressed as mean and standard deviation. PRE, corresponds to value obtained immediately before first training session; POST, correspond to values obtained immediately after the last exercise session; DELTA, corresponds to the mean of difference between post and pre values.

[a] $p < 0.05$ *vs.* MCT; [b] $p < 0.05$ *vs.* CON; [c] $p < 0.01$ *vs.* MCT; [d] $p < 0.01$ *vs.* CON; [e] $p < 0.01$ *vs* baseline-HIIT

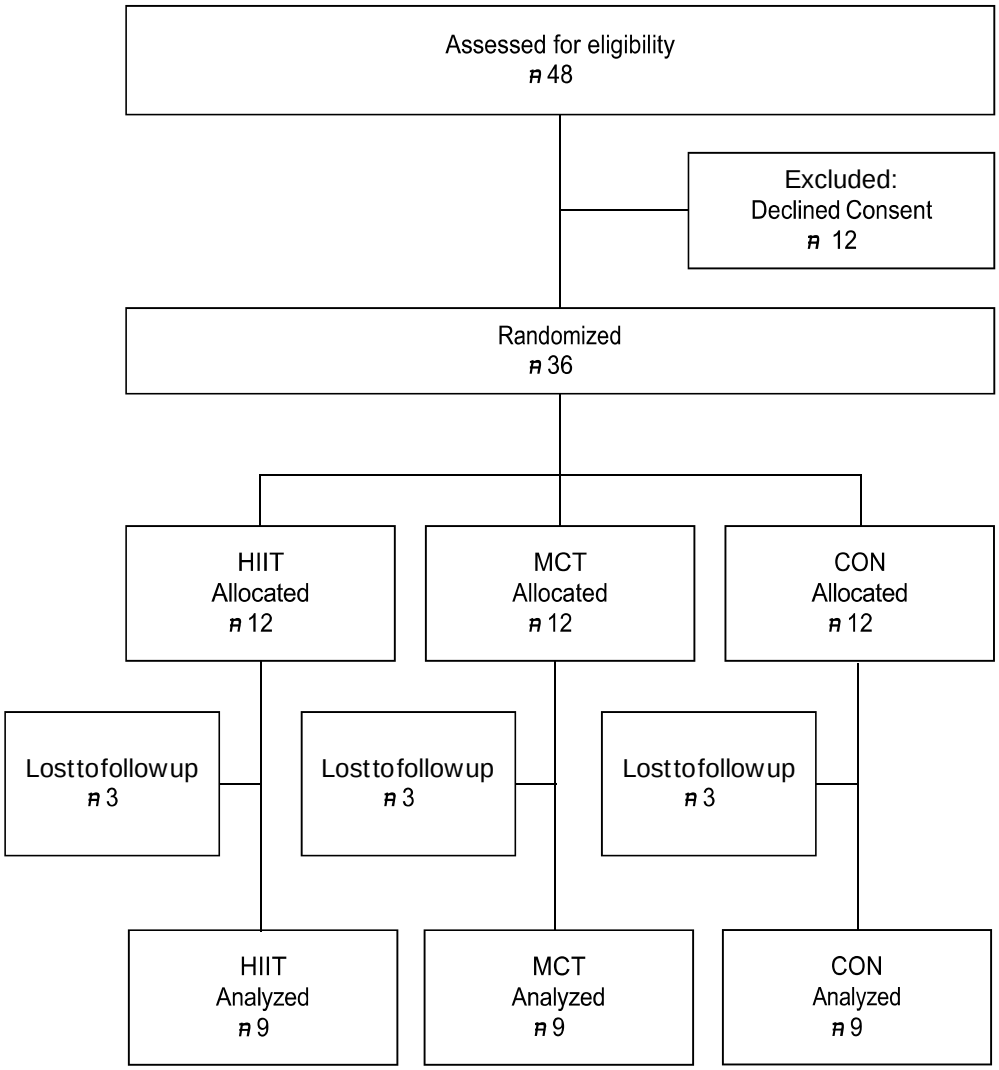
FIGURE LEGENDS

Figure 1. Flow diagram of inclusion of patients in the study.

Figure 2. Description of the exercise training protocols (high-intensity interval training - HIIT) and moderate-intensity continuous training - MCT) divided into phases according to exercise intensity related to percent of maximal heart rate obtained in the maximal exercise test, according to protocol of Mitranun et al (12).

Figure 3. A. Endothelium-dependent vasodilation, expressed as flow-mediated dilation (FMD) before and after 8 weeks of training with exercise protocols: high-intensity interval training (HIIT), moderate continuous training (MCT) and non-exercising patients (CON). B Differences between post- and pre-training values (DELTA) of FMD.

Figure 4. Correlation between the increment in flow-mediated dilation (DELTA-FMD) and the increment in peak oxygen consumption (DELTA-VO_{2peak}) with 8-week training in all patients with type 1 diabetes who exercised.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Excluded:
Declined Consent
n 12
Randomized
n 36
HIIT
Allocated
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MCT
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CON
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n 12
Losttofollowup
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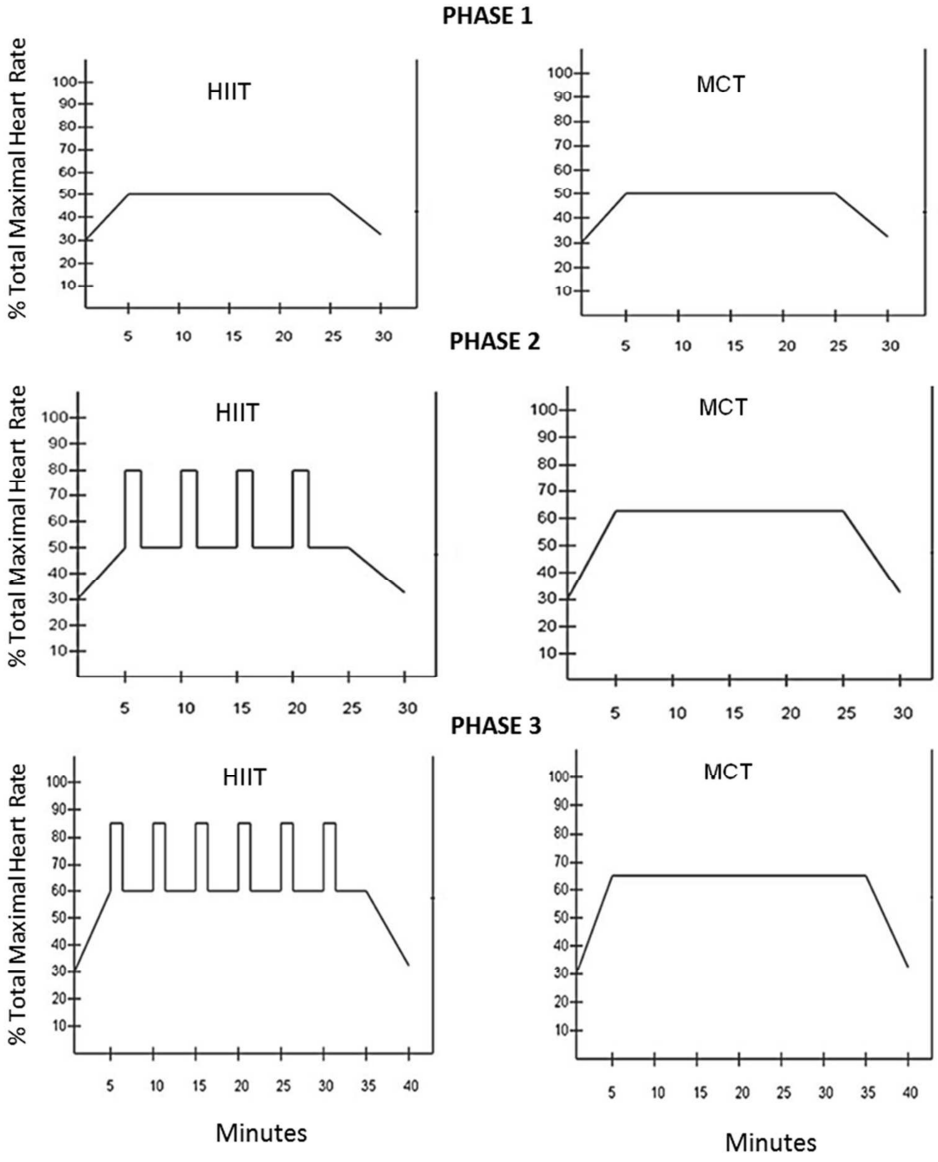

Figure 2. Description of the exercise training protocols (high-intensity interval training -HIIT) and moderate-intensity continuous training - MCT) divided into phases according to exercise intensity related to percent of maximal heart rate obtained in the maximal exercise test, according to protocol of Mitranun et al (12).

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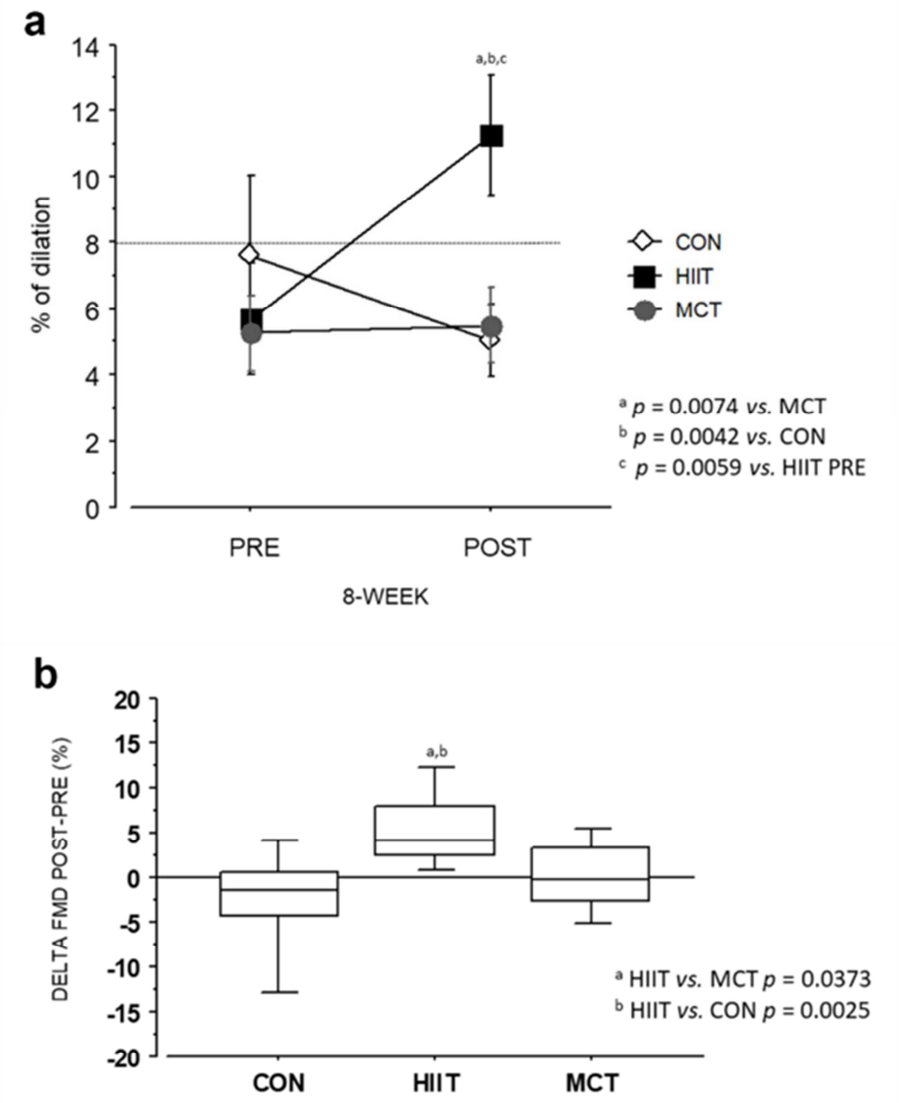

Figure 3. A. Endothelium-dependent vasodilation, expressed as flow-mediated dilation (FMD) before and after 8 weeks of training with exercise protocols: high-intensity interval training (HIIT), moderate continuous training (MCT) and non-exercising patients (CON). B Differences between post- and pre-training values (DELTA) of FMD.

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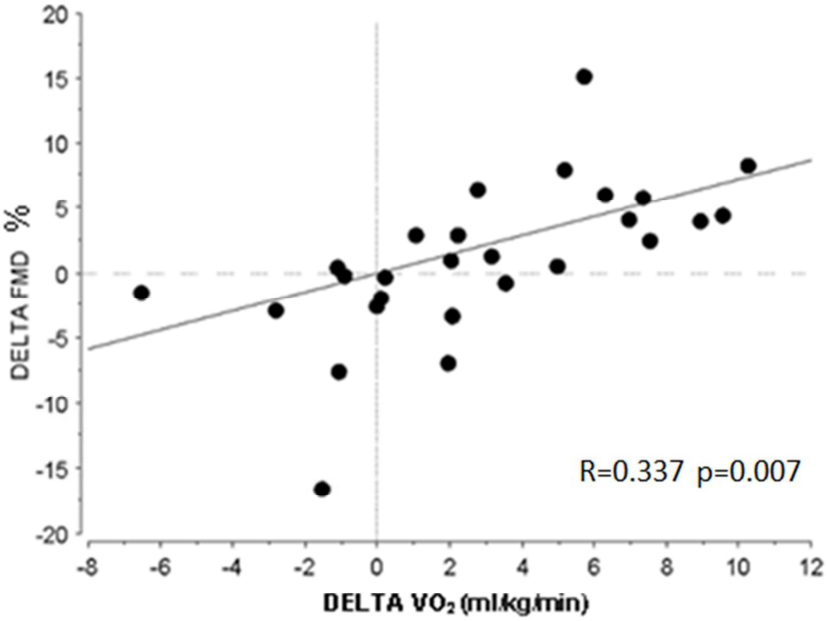

Figure 4. Correlation between the increment in flow-mediated dilation (DELTA-FMD) and the increment in peak oxygen consumption (DELTA-VO2peak) with 8-week training in all patients with type 1 diabetes who exercised.

99x80mm (300 x 300 DPI)

High Intensity Interval Exercise Training (HIIT) compared with Continuous Moderate Exercise Training in Glycemic Control, Glucose Excursion and Incidence of Hypoglycemia during exercise in Type 1 Diabetes.

Running Title:
Exercise and endothelial function in T1DM

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Abstract

The use of the high intensity interval training protocol (HIIT) is still underutilized in type 1 diabetes mellitus (T1DM) due to fear of hypoglycemia as a consequence of increased intensity. The present study aimed to study the differences between 8 weeks of HIIT and CONT in the glucose excursion, the rate of hypoglycemic episodes and the potential to improve HbA1c. This is a subset of a randomized open-label trial in which the HIIT and CONT training protocols were studied in sedentary T1DM patients with no chronic complications. In this study, 18 patients were studied in this reanalysis, 9 in the HIIT protocol and 9 in the CONT training. All patients exercised 3 times per week on cycle ergometer for 8 weeks, each session for 30 to 40 minutes. Patients in the HIIT and CONT groups performed 3 exercise sessions per week for 8 weeks. The HIIT and CONT protocols were divided into 3 phases. Phase 1 (initial training) included 6 30-min exercise sessions during weeks 1 and 2 with moderate intensity in both groups. Phase 2 (intermediate training) included 30 during weeks 3-4, where HIIT, but not CONT, included 4 seizures of 1 to 80% of maximal heart rate (HRmax). Phase 3 (intense phase) included 40 min exercise sessions during weeks 5 to 8, where HIIT but not CONT included 6 seizures of 1 min at 85% (HRmax). All exercise sessions were supervised and adherence monitored by the researchers. Capillary glycemia was measured every 5 min in all sessions. Oral glucose gels of 10 g were offered to patients whenever glucose reached 100 mg / dl or less. The degree of hypoglycemia was classified when the glucose levels were <80mg / dl, <60mg / dl or <50mg / dl verified by means of finger blood samples. HbA1c was determined before and after 8 weeks of formation. For each patient, in each exercise during exercise, the average of 6 or 12 glucose was used according to the exercise phase. We used ANOVA for repeated measures to analyze the excursions of glucose during exercise and chi-square for the glycemic episodes. Results: Mean blood glucose during exercise sessions was similar in the two groups at the beginning of phases 1, 2 and 3. The blood glucose capillary excursion was similar between HIIT and CONT at all times of exercise during the phases 1 and 2. In phase 3, there was an interaction between the glucose curves between HIIT and CONT ($p = 0.0012$), which was reduced more strongly in CONT than in HIIT, respectively, in 35 min: 112 ± 61 vs .134 ± 62 mg / dl ($p = 0.0384$) E at 40 ': 103 ± 82 Vs. 130 ± 84 mg / dl (0.0270). In stage 1, hypoglycemic episodes <80mg / dl were more incident in HIIT than in CONT: 9.28% vs. 4.98% ($p = 0.0299$), but episodes <60mg / dl and <50mg / dl were similar in both groups. In phase 2 there were no differences in the incidence of hypoglycemia between groups. In stage 3, however, there was a significant increase in the episodes <80mg / dl and <60mg / dl in the CONT compared to HIIT, respectively 10.5% vs. 7.5% ($p = 0.0301$) and 1.4 versus 0.5% ($p = 0.0535$). Both training protocols reduced HbA1c levels. We conclude that HIIT can be safer and more efficient than the currently prescribed CONT protocol and should be recommended for uncomplicated T1DM.

INTRODUCTION

Exercise is a fundamental tool in the treatment of type 1 diabetes mellitus (T1DM), due to its effect on reducing hyperglycemia as well mortality, preventing chronic complications, thus improving general health. In a metanalysis of prospective cohorts, including 5.859 individuals with diabetes [(1)] it was observed that physical activity was associated to a lesser risk of cardiovascular disease and total mortality. Compared with sedentary individuals, a reduced risk of mortality was observed in moderately active individuals. The greater the level of physical activity, the smaller the risk of mortality [(1)].

Improving glycemic control is a key feature of the exercise role in T1DM treatment. Metanalysis indicate that continuous aerobic training (CONT) can reduce 0.7% HbA1c in patients with diabetes who exercise, compared to those who do not exercise [(2,3)]. Currently it is recommended that T1DM should do aerobic exercise at moderate intensity for at least 150 minutes per week [(4)]. Exercise is also associated to reduced mortality and cardiovascular disease in diabetes [(5)].

High intensity interval training (HIIT) has recently emerged as an interesting option for improving physical conditioning and endothelial function in diabetes(6) Hypoglycemia, however, is one of the main limitations for exercise in T1DM, beside to its association to intensity and duration of exercise. It is reasonable to consider that the more intense or longer the exercise the higher would be the risk of hypoglycemia(7). This premise however does not consider the point that counter-regulatory hormones could attenuate the trend for hypoglycemia in high-intensity exercise protocols. As HIIT was found to promote several health benefits on other populations (8), it is reasonable to compare its safety regarding hypoglycemia against the currently recommended continuous moderate-intensity protocol for T1DM patients. The present study evaluates the glucose excursion and the incidence of hypoglycemia episodes during workouts related to 8 weeks of HIIT or CONT training protocols in T1DM patients.

MATERIAL AND METHODS

Study design and Patients

This is a sub-analysis of the original randomized opened clinical trial designed to compare flow mediated dilation (FMD) before and after a 8 week period either with high intensity interval training (HIIT) or aerobic continuous moderate exercise (CONT) in T1DM patients without severe chronic complications. Originally patients were divided into 3 groups: HIIT, CONT and controls. The present sub analysis re analized the data of HIIT and CONT groups only.

Recruited participants were T1DM patients above 18 years old with duration of 5 to 12 years, without known diabetes complications, regularly consulting at Instituto da Criança com Diabetes (ICD). All participants signed an informed consent based on Helsinki declaration. We excluded patients who were engaged in alternative exercise training programs in the last 6 months, smokers, pregnant women, any those with known co-morbidities not related to diabetes. We also excluded patients with severe diabetes-related complications that would contra-indicate intensive exercise training such as severe loss of renal function (serum creatinine above 1.5 mg/dl), confirmed coronary artery disease, severe neuropathy with foot ulcer or amputation and suspected or known clinical of autonomic neuropathy. Patients using other medications and those who refused to sign informed consent than insulin were also excluded. The study design was approved by the local Ethics Comitee in Research of Hospital

de Clinicas de Porto Alegre (HCPA), Escola de Educação Física, Fisioterapia e Dança (ESEFID) and Instituto da Criança com Diabetes and Grupo Hospitalar Conceição (ICD-GHC). Patients were selected by convenience from the general registry of ICD. Those who qualified for the eligibility criteria were invited by phone to a consultation at ICD and the informed consent was signed. Those who agreed to participate in the study were booked for further evaluations in the HCPA and ESEF.

Protocol

Training Process

All patients exercised in the afternoon, at least 2h from the last insulin dose. All patients were recommended to reduce insulin dose in 20% in day of training. All patients exercised in cycle ergometer at room temperature, with 3 sessions a week, along 8 weeks, in a total of 24 sessions. Both HIIT and CONT training protocols were divided into 3 phases. Phase 1 was performed in weeks 1 and 2, Phase 2 in weeks 3 to 4 and Phase 3 from weeks 4 to 8. Exercise sessions lasted 30 minutes in phases 1 and 2 and 40 minutes in phase 3. Heart rate was monitored during the exercise sessions using heart rate monitors (Polar® FT4, Polar Electro Oy, Kempele, Finland) and the maximal heart rate attained during maximal effort test was obtained. All exercise sessions were supervised and adherence monitored by the investigators.

High Intensity Interval Training (HIIT) Protocol

In phase 1, participants warmed up gradually to attain 50% of the maximal heart rate (HR_{max}) during 5 min, staying in stable intensity at 50% of HR_{max} during 20 min with 5 more min of recovering in the end. In phase 2, patients exercised more intensively. First there was a 5 min warm up to attain a heart rate of 50% of the HR_{max}. During the following 20 min, patients exercised at 50% of HR_{max}. However, there were 4 bouts of 1 at 80% of HR_{max} each 4 min. At the end, there was a recovering period of 5 min until stopping. In phase 3, participants warm up to 60% of HR_{max} for the first 5 min. Then they exercised at 50% of HR_{max}, however, there were 6 bouts of 1 at 85% HR_{max} each 4 min, with 5 min for recovering in the end. The whole sessions lasted 40 minutes(9)

Continuous Moderate Protocol (CONT)

CONT protocol was also divided into 3 phases. In phase 1, training was identical to described for HIIT. In phase 2, in the first 5 min, participants attained 50% of the HR_{max} and increased the intensity to 60% of HR_{max} during the following 20 min continuously, without increments. At the end, there was a 5 min recovering period, totalizing 30 min. In phase 3, participants attained 50% of HR_{max} in 5 min and exercised constantly at 65% of HRmax during 30 min, followed by a recovering lasting 5 min in the end, totalizing 40 min.

Glucose determinations and carbohydrate supplementation

Capillary blood glucose was measured every 5 min during each exercise session, in all 24 exercise sessions. Oral 10g glucose gels were taken, under supervision, whenever blood glucose was 100mg/dl or less, without interrupting the exercise session. The glucose reposition protocol is shown in figure 1. Before exercise session, patients were asked to reduce insulin dose in 20% in the training day to minimize the risk of hypoglycemia. In the same manner, patients were recommended not to exercise in the peak of insulin action.

Hypoglycemia

Hypoglycemia degree was classified when glucose levels below 80mg/dl (mild), 60mg/dl (moderate) or 50mg/dl (severe). As glucose was measured every 5 min, we consider a hypoglycemic episode if a low blood glucose at the above mentioned cut-offs was found independently of the previous or following values, even if episodes were sequential. By this criteria, in each exercise session it would be possible to present from 0 to 9 episodes depending on the phase.

Statistical Methods

Variable normality was checked by the Shapiro-Wilk method. ANOVA for repeated measures with Fisher post test were used to study glucose excursion. Chi squared was used for studying hypoglycemic episodes. All statistics were performed with a standard software package Statistical Analysis System (SAS) version QC (GraphPad, USA

RESULTS

We analyzed data of 18 patients who participate in the original study. Each patient exercised in a maximal of 24 sessions during the whole training period. Adhesion to training was similar in HIIT and in CONT, respectively: 90,2%+/-4,6 vs 92,4%+/-3,47; p=0,37.

Baseline clinical and biochemical characteristics of patients are demonstrated in table 1. We observed that T1DM patients in HIIT group presented a slight increased systolic and diastolic blood pressure, although all measures were in the normal range and no patient were considered as having hypertension. All other variables were similar between groups.

A total of 3.129 glucose measurements were obtained, being 1537 in HIIT group and 1592 in CONT group (Table 5).

Glucose excursion

Mean fasting glucose was similar between HIIT and CONT in all Phases of the study. In phase 1 and 2, glucose fell similarly in HIIT and CONT with no interaction between groups (table 4, Figure 1). In phase 3, however, there was a significant interaction between groups (p=0.0012) with HIIT presenting higher glucose values than CONT at times 35 and 40 min, respectively: HIIT vs. CONT: 103,13±8,02mg/dl vs. 130,76±8,02mg/dl (p=0,0270) and (HIIT vs CONT 134,26±6,71mg/dl vs 112,82±6,71mg/dl (p=0,0384).

Mild hypoglycemic episodes (<80mg/dl)

There were 251 light hypoglycemic episodes, corresponding to 8.02% of glucose measurements. Considering all phases together, light episodes were similar in both HIIT and CONT (7.48% vs8.54% p=0.27). However, in the more intense and longer phase 3, HIIT group presented significant lower number of episodes (7.56%, vs. 10.56% respectively; p=0.031). Curiously, in phase 1, where protocols were completely similar, HIIT group presented an increased number of light hypoglycemic episodes 9.28% vs 4.98% (table 5). In phase 1, HIIT participants presented a slight higher frequency of light hypoglycemia <80mg/dl than CONT patients ((9.28% vs 4.98%; p=0.029). In phase 2, there were no differences between in light hypoglycemic episodes between groups (Table 5, Figure 2).

Moderate Hypoglycemic episodes (<60mg/dl)

There were of 27 moderate episodes corresponding to 0.86% of total measurements. HIIT presented lower frequency of moderate hypoglycemic episodes, being 7 episodes in a total of 1.537 measurements (0.45%), while CONT presented 20 episodes in 1.592 measurements (1.25%) p=0.015. The greater part of these episodes in CONT occurred in the more prolonged phase 3, where HIIT presented a trend to smaller frequency 0.47% vs.

CONT 1.36% p=0.053. There were no differences in frequency of moderate episodes in phases 1 and 2 (Table 5).

Severe Hypoglycemic episodes (<50mg/dl)

There was a total of 10 severe episodes, being 2 of 1.537 measurements (0.13%) in HIIT and 8 of 1592 (0.50%) in CONT, p=0.065. There were no differences between groups in separate phases (Table 5).

Glucose Supplementation during exercise

Oral glucose supplementation was given in 10g amounts whenever blood glucose levels were below 100mg, at intervals of 5 min, if needed. Considering all phases, HIIT needed 331 doses in 1537 glucose measurements (21.5%) and CONT needed (22.4%) there were no differences between groups. However, in phase 3, HIIT demanded less reposition of glucose in relation to CONT, respectively 20,0% vs 26,8% p=0.002, while, in phase 2 HIIT demanded more glucose than CONT 23.2% vs 15.3% p=0.006. There were no differences in phase 1 (Table 6).

DISCUSSION

The present study showed that in T1DM patients without complications, the fall in blood glucose during exercise is similar between HIIT and CONT in the low intensity and shorter phases 1 and 2, but significantly less pronounced in the HIIT than in the CONT protocol in the more intense and prolonged phase of training (phase 3). In phase 3, despite starting from similar initial glucose values, blood glucose were significantly lower in CONT at the end of exercise period than in HIIT, at 35 and 40 min. Moreover, HIIT protocol was associated with significantly less frequent mild episodes of hypoglycemic in phase 3, and significantly less frequent moderate hypoglycemic episodes considering all phases together. Finally, in phase 3, HIIT promoted significantly less need for glucose reposition than CONT. These results indicate that, in T1DM without complications, HIIT is less prone to hypoglycemia than CONT, especially when exercise is in the more intense and prolonged. This suggests that HIIT may be an excellent alternative for CONT, not only for being safer, but also for allowing extra benefit such as a better physical conditioning and improvements in endothelial function, described in the original study.

Studies comparing specifically HIIT protocol with CONT protocol are few. In general, there are studies comparing the effect of variate modalities of exercise in glycemic control. In a small non-randomized clinical trial (6), a 0.96% reduction in HbA1c in 11 T1DM patients submitted to a circuit with 5 exercise modalities during 12 weeks, but not all studied show significant reductions of A1c in T1DM (7). In respect of the incidence of hypoglycemia compared with two protocols of different intensities, literature is also scarce. To our knowledge, this seems to be the first study comparing the chronic performance of HIIT and CONT protocols on glycaemia during workouts in T1DM patients, encompassing hypoglycemia frequency and glucose excursion during exercise.

Glucose excursion curve was less pronounced in HIIT than in CONT at the end of phase 3, where patients exercised most intensively. This is an apparent paradoxical finding, once the more intensive exercise would be expected to decrease more blood glucose than less intense exercises. However, a study using euglycemic hyperiinsulinemic clamp in patients with T1DM (8), submitted to different exercise intensities, showed an antagonic glycemic

response, in which lower decline glycaemia occurs when they compared high and low intensity exercises. They included 9 T1DM patients with age 21 and mean of 11 years of diabetes duration, with mean HbA1c 7,9%. The mean glucose infusion during clamp to maintain constant blood glucose levels increased progressively when the intensity of exercise reached 50% of maximal but fell progressively above 50% not being necessary to continue when the intensity reached 80%. At the same time, the catecholamine levels were higher when intensity reached 80%. The authors concluded that the increasing in exercise intensity promotes an inverted U curve with less fall in blood glucose when the exercise is more intense. This is likely to be related to the counter regulatory hormones released in the more intense exercise. These data suggest that, when the adrenergic response to hypoglycemia is preserved, as in T1DM patients without complications, HIIT protocol seems to be safe. However, in patients who counter regulatory mechanism are impaired, especially in cases of hypoglycemia unawareness, the utilization of high intensity exercise protocols need to be evaluated with caution and may even be avoided.

The present study have limitations. The main limitation is that this is a sub-analysis from the original study, and was not designed for the referred outcomes, and, by this way the sample size was not calculated a priori. However, as the results attained statistical significance, the beta error lost relevance and data could be considered reliable. Second, we did not controll patients food ingestion and exercises were done at least 2h after a meal. It is possible that differences in food composition may have interfered in glucose values. To minimize this effect, we used the mean of many glucose determinations at different days for each point of exercise glucose excursion. This reduced substantially the biological variability of glycemia during exercise and increased the power of the study. The main strength of the study was the very large number of glucose measurements and the low rate of missing values. Besides that, the study attained a very high adhesion rate, which also increases power.

In conclusion, in patients with T1DM with no diabetes related-complications, HIIT is clearly less prone to promote hypoglycemia than CONT, especially in the more intense and prolonged phases of the training protocol. Thus HIIT can be suggested as a safer alternative for exercise in T1DM patients, especially those who desire to perform high-intensity workouts.

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Table 1 Oral glucose supplementation before and during exercise in patients with DM1

Glycemia (mg/dL)	Oral glucose 10 g
< 80	Ingestion of 10g of glucose. Do not start the exercise.
80 a 100	Ingestion of 10g of glucose before starting and during: and reset every 5 min if the glucose level persists.
>100 e < 250	Start the exercise without supplementation.
> 300 e Ketonuria (-)	Start exercise without glucose replacement.
> 300 e Ketonuria (+)	Postpone the onset of exercise until normalization of ketone levels and water intake. Refer to physician.

Table 2 General clinical and biochemical characteristics of groups before training.

	HIIT (n=9)	CONT (n=9)	*P*
Age (yr)	26.1±7.8	23.7±5,8	ns
Male/female	3/7	5/4	ns
Duration of diabetes (years)	9.1±2.9	10.4±2.8	ns
Daily insulin dosage	30.6±5.7	30.0±9.8	ns
BMI (kg/m^2)	23.2±2.4	24,1±2,0	ns
Systolic BP (mmHg)	108.33±7.91	120.55±8.82	0.0417*
Diastolic BP (mmHg)	71.11±8.21	78.89±7.82	0.0246*
Fasting Blood Glucose (mg/dl)	207±73	156±53	ns
HbA1c (%)	8.1±1.3	8.3±0.9	ns
Total cholesterol (mg/dl)	184±29.6	176.3±32.6	ns
HDL cholesterol (mg/dl)	59±12	56.7±24.5	ns
Triglycerides (mg/dl)	68.6±30.4	150.8±18.7	ns
Serum Creatinine (mg/dl)	0.68±0.13	0.78±0.12	ns
UAC (mg/L)	12.6(3.0-41.0)	30.4(3.3-184)	ns

The data are described in mean and DP
P = HIIT vs CONTROL
The comparison between men and women was performed in Chi-square - p value = total interaction
UAC: urinary albumin concentration FBG = fasting glucose
UAC was analyzed using the Kruskall-Wallis method.
Body mass index (BMI)

Table 3

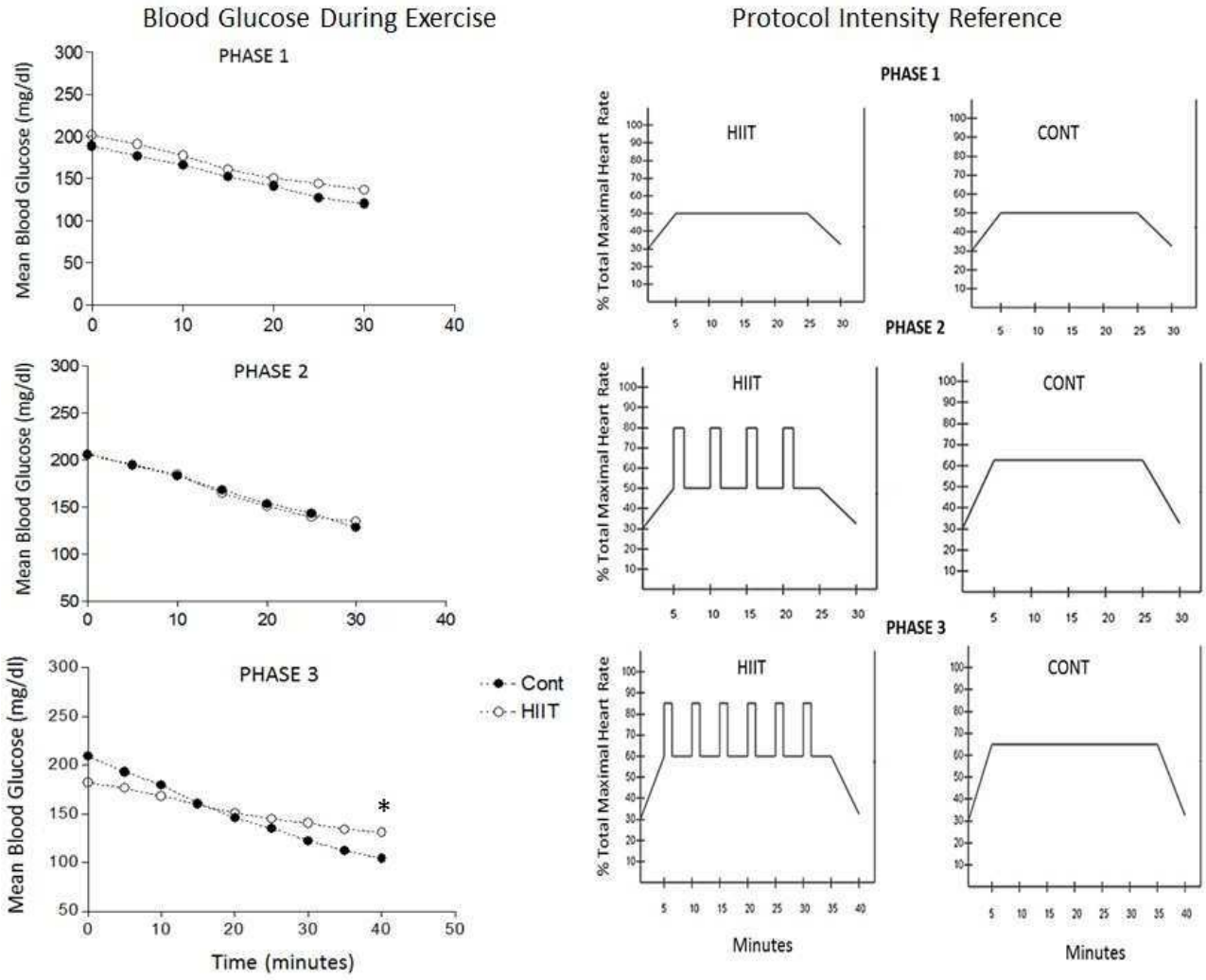
Weight and Glucose Control Changes (delta) before (pre) and after (post) 8 week-training period between HIIT and CONTprotocols in patients with Type 1 Diabetes.

	HIIT (n=9)				CONT (n=9)			
	Pre	**Post**	**Delta**	**% Change**	**Pre**	**Post**	**Delta**	**% Change**
Weight (kg)	64.0±7.6	61.7±9.2	-2.36±4.63	-3,72±7.12	71.3±11.6	70.7±10.9	-0.68±2.02	-0.79±2.72
TDI (UI/kg/day)	0,67±0.11	0,55±0,11	-0,141±0,08	-20 ,0±9.17	0,82±0,21	0,68±0,18	-0,141,±0,11	-16.86±12. 04
FBG (mg/dl)	207±73	206±110	-1,55±103,85	-	156±53	17 4±55	18,33±75	-
HbA1c (%)	8,1±1,3	7,9±0,9	-0.22±0.58	-2.07±5.87	8,3 ±0.9	8,0±0.8	-0.32±0.29	-3.76±3.46
$VO_{2\ max\ (ml/kg/min)}$	34±6,3	40,1±4,3	6,08±2,58	-	33±8,2	36±8,8	3,04±4,03	-

TDI: Total Daily Insulin Dose corrected by weight; FBG: Fasting Plasma Glucose; HbA1c: Glycated Haemoglobin A_{1c}; Delta: The difference between Post and Pre training values; HIIT; High Intensity Interval Training; CONT: Continuous moderate Training. Data are Mean and Standard Deviation.

Figure 1

Glucose excursion during HIIT and CONT protocols in Type 1 Diabetes patients in different phases.



Data are the mean of blood glucose samples obtained every 5 minutes during exercise in 3 different phases of training in both HIIT and CONT protocols groups. Each patient data corresponds to the mean of individual training set measurements of glucose in different days during phase 1, 2 and 3.

Table 4. Mean glucose data values from all training sets.

	HIIT	**CONT**	*p*
Phase 1			
	Mean±SD	Mean±SD	
0	201±51	188±25	*ns*
5	190±50	176±29	*ns*
10	177±45	166±29	*ns*
15	160±45	152±30	*ns*
20	150±44	141±22	*ns*
25	143±41	127±25	*ns*
30	136±40	120±17	*ns*
Phase 2			
0	206±59	205±50	*ns*
5	194±56	194±44	*ns*
10	184±53	182±41	*ns*
15	164±46	167±43	*ns*
20	150±43	153±39	*ns*
25	138±43	143±36	*ns*
30	134±40	127±31	*ns*
Phase 3			
0	182±1	208±1	*ns*
5	176±1	195±1	*ns*
10	168±9	180±9	*ns*
15	159±8	161±8	*ns*
20	150±7	146±7	*ns*
25	144±7	135±7	*ns*
30	140±7	122±7	*ns*
35	134±6	112±6*	*0.0384*
40	130±8	103±8*	*0.0270*

Table 5.Percent of hypoglycemic episodes defined at 3 different cut-offs in each phase of the training in the 2 different protocols. .

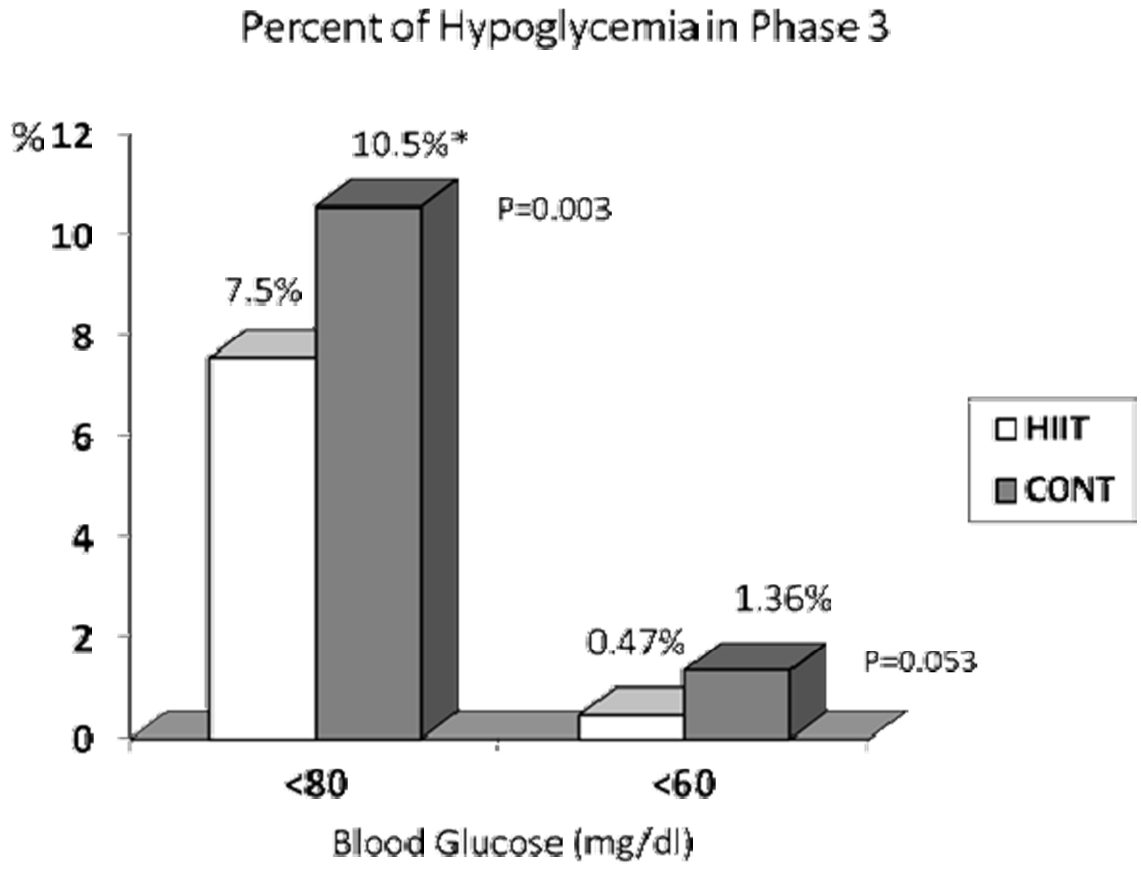
	HIIT			CONT			X^2	p
Glucose Cutt off	**n**	**Total**	**%**	**n**	**total**	**%**		
Phase1								
<50	0	334	0%	2	341	0.58%	*ns*	*ns*
<60	2	334	0.59%	4	341	1.17%	*0.631*	*0.426*
<80	31	334	9.28%	17	341	4.98%	*4.715*	*0.029*
Phase2								
<50	0	357	0	2	371	0.53%	*ns*	*ns*
<60	1	357	0.28%	4	371	1.07%	*1.698*	*0.192*
<80	20	357	5.60%	26	371	7.00%	*0.607*	*0.435*
Phase3								
<50	2	846	0.23%	4	880	0.45%	*0.592*	*0.441*
<60	4	846	0.47%	12	880	1.36%	*3.727*	*0.053*
<80	64	846	7.56%	93	880	10.56%	*4.705*	*0.030*

Intensity Interval Training; CONT: Continuous Moderate Training;
n is the number of hypoglycemic episodes; % is the percent of hypoglycemic episodes
Total is the total of glucose measurements. Statistics were Chi Squared.

Table 6. Glucose replacement during exercise training. Each unit replacement consisted in a 10g of liquid oral glucose gel. Glucose replacement was offered during exercise whenever blood glucose was 100mg/dl or less.

	HIIT				CONT					
PHASE	Glucose doses n	Total Glucose (g)	Total of Blood Glucose samples	%	Glucose Doses n	Total Glucose (g)	Total of samples	%	X2	p
1	78	780	334	23.3	64	640	341	18.7	*2.13*	*0.1439*
2	83	830	357	23.2	57	570	371	15.3	*7.28*	*0.0069*
3	170	1.700	846	20.0	236	2.360	880	26.8	*10.83*	*0.0009*

Figure 2. Percent of hypoglycemia episodes below 80mg/dl and below 60mg/dl of glucose from the total of blood glucose sample measurements during phase 3.



9. CONSIDERAÇÕES FINAIS

As mudanças de estilo de vida representam uma grande dificuldade para as pessoas, especialmente quando se trata de seguir uma rotina exercícios físicos. Em particular, as pessoas com DM1 devem, diariamente, tomar decisões para controlar sua doença, e estas decisões têm um maior impacto sobre os sistemas cardiovascular e metabólico. Como tal, o bom controle do DM1 tem um papel crucial clinicamente no bem estar geral do paciente. O estudo atual, demonstrou que o treinamento intervalado de alta intensidade melhorou a função endotelial (mediada pelo fluxo), a aptidão física cardiorrespiratória e menos episódios de complicações agudas ao exercício como a hipoglicemia. Esses resultados indicam que o treinamento aeróbio de alta intensidade pode ser recomendado com segurança para pacientes com DM1. E pode ocorrer menos complicações agudas e crônicas vasculares dos quais muitos DM1 muitas vezes sofrem.

10. PERSPECTIVAS FUTURAS

Como perspectivas futuras de aplicação do EAI, sugere-se um maior tempo de intervenção nas fases dois e três para efeitos mais expressivos.

Acreditamos que intervenções direcionadas à associação do exercício físico e controle nutricional e do estresse possam trazer melhores benefícios a curto e longo prazo para esta população.