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Wellington Santana da Silva Júnior

Atividade da dipeptidil peptidase 4 (DPP4) e sua associação com biomarcadores inflamatórios e reatividade microvascular em indivíduos com excesso de peso e sem diabetes

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Tese apresentada, como requisito parcial para obtenção do título de Doutor, ao Programa de Pós-Graduação em Fisiopatologia Clínica e Experimental, da Universidade do Estado do Rio de Janeiro.

Orientador: Prof. Dr. Luiz Guilherme Kraemer de Aguiar

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RESUMO

SILVA JÚNIOR, Wellington Santana da. *Atividade da dipeptidil peptidase 4 (DPP4) e sua associação com biomarcadores inflamatórios e reatividade microvascular em indivíduos com excesso de peso e sem diabetes.* 2017. 115 f. Tese (Doutorado em Fisiopatologia Clínica e Experimental) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2017.

Neste estudo transversal, a associação entre a atividade constitutiva da dipeptidil peptidase 4 (DPP4), biomarcadores inflamatórios e reatividade microvascular em indivíduos com excesso de peso e sem diabetes foi investigada. Quarenta indivíduos com índice de massa corporal (IMC) ≥25,0 kg/m² e ausência de diabetes foram avaliados. O fluxo sanguíneo microvascular e a vasomotricidade foram aferidos pela amplificação da luz por emissão estimulada de radiação (LASER) Doppler fluxometria. As seguintes variáveis foram analisadas no estado basal, 30 e 60 min após uma refeição padronizada: atividade da DPP4, glicemia, proteína C-reativa ultrassensível, fator alfa de necrose tumoral, interleucina-6 (IL-6), inibidor-1 do ativador do plasminogênio (PAI-1), molécula-1 de adesão intercelular emolécula-1 de adesão da célula vascular (VCAM-1). A atividade de DPP4 foi inversamente correlacionada com a VCAM-1 no basal ($P<0,05$) e a área sob a curva (AUC) da atividade da DPP4 foi inversamente associada à AUC do componente miogênico da vasomotricidade ($P<0,05$). Na análise de regressão múltipla, o modelo de avaliação da homeostase-adiponectina (HOMA-AD), IL-6, VCAM-1, PAI-1, fluxo sanguíneo e vasomotricidade influenciaram a atividade da DPP4 e explicaram quase 40% da sua variação. Quando HOMA-AD, VCAM-1 e fluxo sanguíneo foram, respectivamente, tomados como variáveis dependentes, a atividade da DPP4 exerceu um efeito significativo sobre todos eles. Em conclusão, a atividade constitutiva da DPP4 foi associada a marcadores de ativação pró-inflamatória endotelial e função microvascular e parece ter influenciado ou sido influenciada pela inflamação e fluxo sanguíneo microvascular em indivíduos com excesso de peso e sem diabetes.

Palavras-chave: Dipeptidil peptidase 4. Inflamação. Reatividade microvascular. Pré-diabetes. Disfunção endotelial.

ABSTRACT

SILVA JÚNIOR, Wellington Santana da. *Dipeptidyl peptidase 4 (DPP4) activity and its association with inflammatory biomarkers and microvascular reactivity in subjects with excessive weight and without diabetes.* 2017. 115 f. Tese (Doutoradoem Fisiopatologia Clínica e Experimental) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2017.

In this cross-sectional study, the association between constitutive dipeptidyl peptidase 4 (DPP4) activity, inflammatory biomarkers, and microvascular reactivity in subjects with excess body weight without diabetes were investigated. Forty subjects withbody mass index (BMI) ≥25.0 kg/m² and absence of diabetes were evaluated. Microvascular blood flow and vasomotion were assessed by light amplification by stimulated emission of radiation (LASER) Doppler flowmetry. The following variables were measured at baseline, 30 and 60 min after a standardized meal: DPP4 activity, blood glucose, ultra-sensitive C-reactive protein, tumor necrosis factor alpha, interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 (VCAM-1). DPP4 activity was inversely correlated to VCAM-1 at baseline ($P<0.05$) and DPP4 $activity_{AUC}$ was inversely correlated with the myogenic $component_{AUC}$ of vasomotion ($P<0.05$). In multiple regression analysis, homeostasis model assessment-adiponectin (HOMA-AD), IL-6, VCAM-1, PAI-1, blood flow, and vasomotion influenced DPP4 activity and explained almost 40% of the variance on it. When HOMA-AD, VCAM-1, and blood flow were respectively placed as dependent variables, DPP4 activity exerted a significant effect in all of them. In conclusion, constitutive DPP4 activity was associated with early markers of endothelial proinflammatory activation and microvascular function and may have an influence or even be influenced by inflammation and microvascular blood flow in subjects with excess body weight without diabetes.

Keywords: Dipeptidyl peptidase 4. Inflammation. Microvascular reactivity. Prediabetes. Endothelial dysfunction.

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LISTA DE ABREVIATURAS E SIGLAS

A1c	Hemoglobina glicada A1c
ADA	Adenosina deaminase
AGEs	*Advanced glycation end products*
ANOVA	*Analysis of variance*
AUC	*Area under the curve*
BioVasc	Laboratório de Pesquisas Clínicas e Experimentais em Biologia Vascular
BNP	*B-type natriuretic peptide*
CD26	*Cluster* de diferenciação 26
CPEs	Células progenitoras endoteliais
CV	Coeficiente de variação
DM	Diabetes mellitus
DM2	Diabetes mellitus tipo 2
DO	Densidade óptica
DPP4	Dipeptidil peptidase 4
EDHF	*Endothelium-derived hyperpolarizing factor*
EDTA	*Ethylenediamine tetraacetic acid*
ELISA	*Enzyme-linked immunosorbent assay*
FCM	Faculdade de Ciências Médicas
G-CSF	*Granulocyte colony-stimulating factor*
GIP	*Glucose-dependent insulinotropic peptide*
GJ	Glicemia em jejum
GLP-1	*Glucagon-like peptide-1*
GLP-2	*Glucagon-like peptide-2*
GM-CSF	*Granulocyte-macrophage colony-stimulating factor*
GRP	*Gastrin-releasing peptide*
HDL	*High-density lipoprotein*
HGP	*Hepatic glucose production*
HOMA-AD	*Homeostasis model assessment-adiponectin*
HOMA-IR	*Homeostasis model assessment to quantify insulin resistance*
HUPE	Hospital Universitário Pedro Ernesto

ICAM-1	*Intercellular adhesion molecule-1*
IJ	Insulinemia em jejum
IL-1β	Interleucina-1 beta
IL-6	Interleucina-6
IL-8	Interleucina-8
IMC	Índice de massa corporal
iNOS	*Inducible nitric oxide synthase*
IP-10	*Interferon gamma-induced protein-10*
LASER	*Light amplification by stimulated emission of radiation*
LabLip	Laboratório de Lípides
LDF	LASER Doppler fluxometria
LDL	*Low-density lipoprotein*
MDC	*Macrophage derived chemokine*
MHNI	Monitorização hemodinâmica não invasiva
MIG	*Monokine induced by interferon gamma*
NGT	*Normal glucose tolerance*
NO	*Nitric oxide*
NPY	Neuropeptídeo Y
PAI-1	*Plasminogen activator inhibitor-1*
PAM	Pressão arterial média
PCRus	Proteína C-reativa ultrassensível
Pré-DM	Pré-diabetes
PU	*Perfusion unity*
PYY	Peptídeo YY
QUICKI	*Quantitative insulin sensitivity check index*
RAGE	*Receptor for advanced glycation end products*
RANTES	*Regulated on activation, normal T-cell expressed and secreted*
RI	Resistência à insulina
RNA	*Ribonucleic acid*
SDF-1	*Stromal cell-derived factor 1*
SDF-1α	*Stromal cell-derived factor 1 alpha*
SHBG	*Sex hormone-binding globulin*
TCLE	Termo de consentimento livre e esclarecido

TG	Triglicerídeos
TG/HDL	Relação triglicerídeos/HDL colesterol
TNF-α	*Tumor necrosis factor alpha*
TTGO	Teste de tolerância à glicose oral
UERJ	Universidade do Estado do Rio de Janeiro
VCAM-1	*Vascular cell adhesion molecule-1*
vs.	Versus

LISTA DE SÍMBOLOS

β	Beta
α	Alfa
%	Porcentagem
<	Menor que
mg	Miligrama
/	Divisão
dL	Decilitro
g	Grama
cm	Centímetro
kg	Kilograma
=	Igual a
×	Multiplicação
–	Subtração
kcal	Kilocaloria
µM	Micromolar
mL	Mililitro
min	Minuto
pg	Picograma
ng	Nanograma
-	Menos (ou Subtração)
°C	Graus Celsius
mm	Milímetro
µL	Microlitro
nm	Nanômetro
mM	Milimolar
µUI	Microunidades internacionais
mmol	Milimol
µg	Micrograma
±	Mais ou menos
Hz	Hertz

mmHg	Milímetro de mercúrio
Δ	Delta
>	Maior que
≥	Maior ou igual a
pM	Picomolar

SUMÁRIO

INTRODUÇÃO

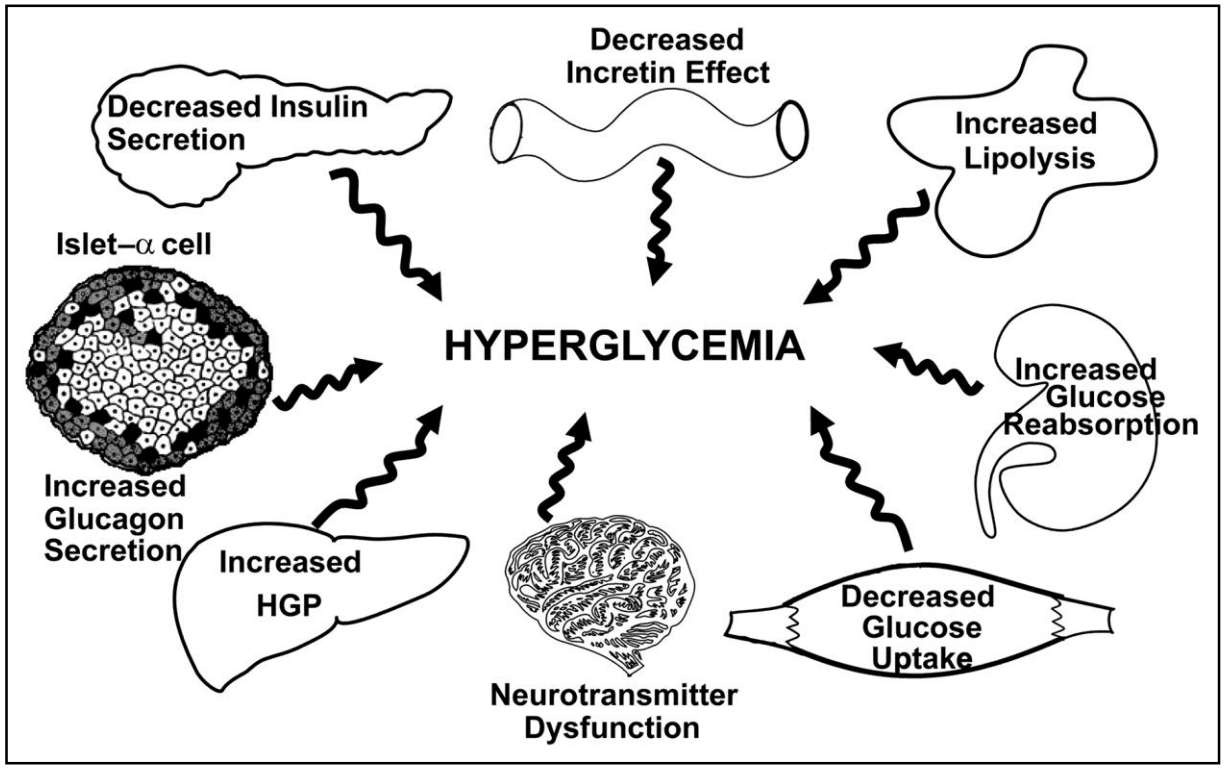
Existe um espectro contínuo de valores de glicose entre os considerados normais e aqueles que caracterizam o diabetes mellitus (DM) (1). Além disso, as complicações vasculares secundárias à hiperglicemia se iniciam precocemente durante a progressão do estado euglicêmico para o DM franco (1). Portanto, considera-se relevante a identificação dos indivíduos com pré-diabetes, ou seja, aqueles que não preenchem critérios para DM, mas que apresentam valores de glicemia em jejum (GJ), hemoglobina glicada A1c (A1c) e/ou glicemia de 2 horas durante o teste de tolerância à glicose oral (TTGO) suficientemente altos para que não sejam considerados normais (2).

Embora a hiperglicemia desencadeie efeitos adversos em cada elemento celular que compõe a parede vascular (3), os mecanismos responsáveis pela aterosclerose acelerada observada em indivíduos com DM não são completamente compreendidos (4). O óxido nítrico (NO, em inglês, *nitric oxide*) é o principal responsável pela manutenção da integridade vascular e outros mediadores, como o fator hiperpolarizante derivado do endotélio (EDHF, em inglês, *endothelium-derived hyperpolarizing factor*) e as prostaglandinas, também têm os seus papéis nesse contexto. A disfunção endotelial, ou seja, a redução na biodisponibilidade do NO no ambiente periendotelial, é um dos eventos mais precoces no desenvolvimento da aterosclerose (4). A ocorrência de comprometimento da função endotelial antes do desenvolvimento do DM sugere a existência de mecanismos fisiopatológicos comuns entre a piora da tolerância à glicose e a disfunção endotelial, reforçando a possibilidade de uma relação causal entre resistência à insulina (RI) e aterosclerose (5). A participação da inflamação nesses mecanismos, favorecendo o desenvolvimento das complicações vasculares relacionadas à hiperglicemia, também tem sido alvo de atenção especial (6). Cabe ressaltar que a aterosclerose é uma causa importante de morbimortalidade tanto em indivíduos com pré-diabetes quanto naqueles com DM (7-9).

A forma mais prevalente de DM, o diabetes mellitus tipo 2 (DM2), apresenta uma fisiopatologia complexa, que envolve elementos como a RI em tecido muscular e hepático, a disfunção da célula β pancreática, a deficiência/resistência incretínica e a hiperglucagonemia (Figura 1) (10). O estado de deficiência/resistência às incretinas reflete o comprometimento do efeito incretínico, definido como a amplificação da secreção de insulina em resposta a uma quantidade oral de glicose, quando comparada à resposta insulínica observada após a mesma quantidade de glicose por via endovenosa. Além disso, a deficiência/resistência incretínica

está associada ao comprometimento da supressão da secreção inadequada de glucagon e ao prejuízo ao retardo do esvaziamento gástrico (11).

Figura 1 – Fisiopatologia do diabetes mellitus tipo 2 (octeto ominoso)



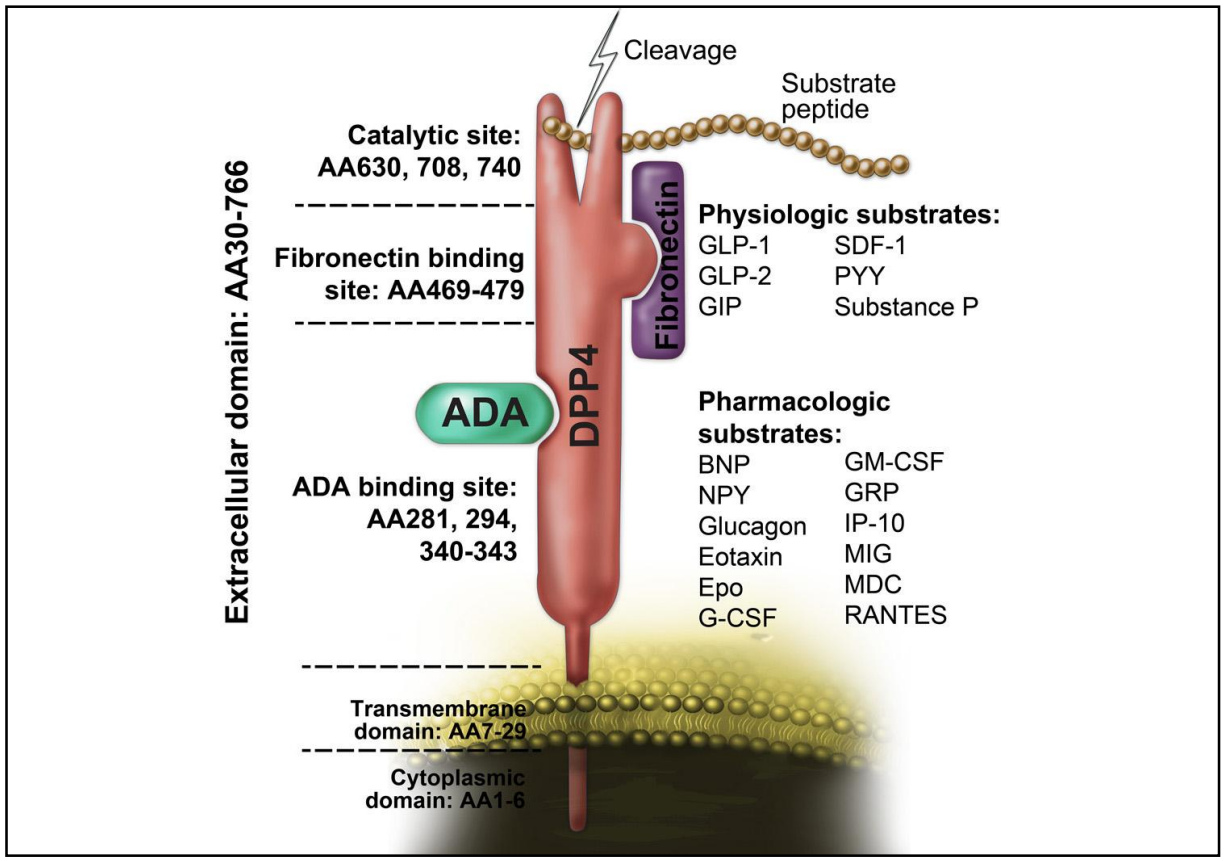
Legenda: HGP – produção hepática de glicose (em inglês, *hepatic glucose production*).
Fonte: Defronzo, 2009 (10).

Existem duas incretinas principais, ambas secretadas pelo trato gastrintestinal: o peptídeo insulinotrópico dependente de glicose (GIP, em inglês, *glucose-dependent insulinotropic peptide*) e o peptídeo-1 semelhante ao glucagon(GLP-1, em inglês, *glucagon-like peptide-1*) (12). Tanto o GIP quanto o GLP-1 apresentam meia-vida muito curta, pois são rapidamente degradados pela dipeptidil peptidase 4 (DPP4), uma enzima ubíqua encontrada em sua forma solúvel no plasma ou como componente transmembrana de muitas células (13), incluindo as células endoteliais (14).

A DPP4, também conhecida como proteína de ligação à adenosina deaminase ou *cluster* de diferenciação 26 (CD26), é uma serino exopeptidase que apresenta funções distintas, seja como protease regulatória ou como proteína de ligação (15). Imediatamente após a sua síntese, a DPP4 é incorporada à membrana plasmática de diversos tipos celulares,

dentre os quais se destacam as células endoteliais, epiteliais, os leucócitos e as células do tecido adiposo (16-18). Entretanto, sob certos estímulos, a DPP4 pode ser liberada da membrana, constituindo uma forma solúvel que também é dotada de atividade enzimática (16). Assim, ambas as formas da DPP4 são capazes de inativar oligopeptídeos que apresentam como penúltimo e último resíduos a prolina e a hidroxiprolina ou a alanina, respectivamente (19). Além das incretinas, a DPP4 parece regular fisiologicamente citocinas, quimiocinas e neuropeptídeos envolvidos em inflamação, imunidade e função vascular (20;21) (Figura 2).

Figura 2 – Estrutura e funções da DPP4



Legenda: ADA – adenosina deaminase; BNP – peptídeo natriurético tipo-B (em inglês, *B-type natriuretic peptide*); DPP4 – dipeptidil peptidase 4; GIP – peptídeo insulinotrópico dependente de glicose(em inglês, *glucose-dependent insulinotropic peptide*); GLP-1 – peptídeo-1 semelhante ao glucagon(em inglês, *glucagon-like peptide-1*); GLP-2 – peptídeo-2 semelhante ao glucagon(em inglês, *glucagon-like peptide-2*);G-CSF – fator estimulador de colônia de granulócito (em inglês, *granulocyte colony-stimulating factor*); GM-CSF – fator estimulador de colônia de macrófago (em inglês, *granulocyte-macrophage colony-stimulating factor*); GRP – peptídio liberador da gastrina (em inglês, *gastrin-releasing peptide*); IP-10 – proteína-10 induzida por interferon gama (em inglês, *interferon gamma-induced protein-10*); MDC – quimiocina derivada de macrófago (em inglês, *macrophage derived chemokine*); MIG – monocina induzida por interferon gama (em inglês, *monokine induced by interferon gamma*); NPY – neuropeptídeo Y; PYY – peptídeo YY; RANTES – quimiocina regulada na ativação, expressa e secretada por célula-T normal (em inglês, *regulated on activation, normal T-cell expressed and secreted*); SDF-1 – fator 1 derivado de célula estromal (em inglês, *stromal cell-derived factor 1*).

Fonte: Zhong, 2015 (21).

Como as incretinas são rapidamente degradadas pela DPP4, é razoável supor que um aumento na concentração e/ou atividade dessa enzima possa contribuir para o comprometimento do efeito incretínico observado nos pacientes com DM2 (22). De fato, nesse perfil de pacientes, a atividade de DPP4 se correlaciona positivamente com a piora do controle metabólico (22). Entretanto, os primeiros estudos avaliando a atividade da DPP4 em pacientes com DM2 evidenciaram que ela poderia estar tanto diminuída (23;24) quanto aumentada (22;25;26). Esses resultados discrepantes podem ter ocorrido em virtude do uso, pelos pacientes, de medicamentos antidiabéticos como a metformina e as tiazolidinedionas, capazes de promover a diminuição da atividade da DPP4 (27-29). Em um estudo comparando a atividade plasmática e os níveis séricos da DPP4 entre pacientes com DM2 e indivíduos saudáveis, valores significativamente aumentados dessas variáveis nos indivíduos com DM2 foram evidenciados apenas após os usuários da metformina e/ou das tiazolidinedionas terem sido excluídos da análise (30).

Pacientes com pré-diabetes também parecem apresentar um comprometimento do efeito incretínico. Quando comparados aos indivíduos euglicêmicos, aqueles com pré-diabetes exibem diminuição dos níveis de GLP-1 e níveis de GIP inalterados (31). Por isso, especula-se que a redução nos níveis de GLP-1 ou talvez uma maior resistência ao GIP possam contribuir para a redução da sensibilidade à glicose pela célula β e para o comprometimento da secreção insulínica evidenciada pelo aumento da glicemia de 2 horas durante o TTGO em alguns desses indivíduos (31;32).

Um estudo longitudinal com duração de quatro anos, avaliando indivíduos com diferentes graus de tolerância à glicose, constatou que a atividade da DPP4 no início do seguimento apresentava associação inversa com os níveis basais do GLP-1. Além disso, a atividade dessa enzima mostrou-se um preditor independente de risco para o desenvolvimento de pré-diabetes e DM2 (33). Assim, a hipótese de que as alterações incretínicas no pré-diabetes estejam diretamente relacionadas à DPP4 parece ser plausível. Essa hipótese adquire ainda maior relevância ao se considerar que as complicações vasculares relacionadas à hiperglicemia possam ser, em parte, secundárias ao comprometimento dos efeitos pleiotrópicos benéficos do GLP-1 evidenciados no sistema cardiovascular (34-38).

As células endoteliais dos compartimentos microvascular e macrovascular são provavelmente a principal fonte endógena da DPP4 (39). A atividade da DPP4 no sítio endotelial parece ser mais substancial do que a da forma circulante da enzima (39). É interessante ressaltar que a expressão da DPP4 pelo endotélio microvascular humano *in vitro*, bem como a sua atividade enzimática, encontram-se significativamente aumentadas após a

exposição crônica a altas concentrações de glicose (40). Entretanto, a hiperglicemia só é capaz de aumentar significativamente a atividade da DPP4 nas células endoteliais do compartimento microvascular (41).

A DPP4 é também considerada uma adipomiocina, secretada principalmente pelo tecido adiposo (18), mas também pelos miotubos humanos (42). Como adipocina, a DPP4 é liberada especialmente pelos adipócitos maduros e o seu nível sérico se correlaciona significativamente com o tamanho do adipócito (18). Além disso, há indícios de que a DPP4 seja um marcador de obesidade visceral, RI e síndrome metabólica (43). Já o papel da DPP4 como miocina ainda precisa ser elucidado mas, de forma semelhante à que ocorre para muitas adipomiocinas, a sua concentração tissular pode ser diferente do seu nível sérico e a possibilidade de efeitos autócrinos e endócrinos distintos deve ser considerada (44). Presume-se que as miocinas sejam essenciais para o metabolismo muscular durante o processo de contração. Em contrapartida, a elevação crônica das adipocinas pode induzir efeitos adversos, como a RI (44). Nesse sentido, existem evidências de que a DPP4 possa inibir a fosforilação da Akt induzida pela insulina em adipócitos e miócitos provenientes de músculo liso e esquelético, indicando um comprometimento da sinalização da insulina e da sua ação nos tecidos adiposo e muscular (18).

Sabe-se que o prejuízo à sinalização da insulina resulta na elevação da glicemia e tanto a hiperglicemia crônica quanto a RI estão associadas à formação dos produtos finais de glicação avançada (AGEs, em inglês, *advanced glycation end products*) (45). Os AGEs são formados a partir de reações não enzimáticas entre açúcares reduzidos e o grupamento amino de proteínas, lipídios e ácidos nucleicos (45). Existe uma correlação independente entre os níveis séricos de AGEs e a presença de RI mesmo em indivíduos sem DM (46). A interação entre os AGEs e o receptor para os produtos finais de glicação avançada (RAGE, em inglês, *receptor for advanced glycation end products*) desencadeia a geração de estresse oxidativo, deflagrando reações proliferativas, inflamatórias e fibróticas que comprometem a integridade estrutural e a funcionalidade de diversas proteínas (45). Assim, os AGEs parecem participar de mecanismos fisiopatológicos que reforçam a possibilidade de uma relação causal entre RI e disfunção endotelial. A participação ativa do eixo AGEs-RAGE na aterosclerose acelerada relacionada à hiperglicemia já foi demonstrada (47). Curiosamente, parece existir uma correlação independente entre os níveis séricos de AGEs e os níveis da enzima DPP4 (48). Os AGEs estimulam a expressão da DPP4 e a sua liberação (48), enquanto a DPP4 aumenta a expressão gênica do RAGE (49), sugerindo a existência de uma interação entre o eixo AGEs-

RAGE e a enzima DPP4 na patogênese das complicações vasculares associadas à hiperglicemia crônica (47).

Nos últimos anos, novas opções terapêuticas surgiram para os pacientes com DM2, visando o controle da hiperglicemia e a prevenção das complicações a ela associadas. Dentre essas opções, merecem destaque as gliptinas ou "inibidores da DPP4". Ao promover a inibição enzimática da DPP4, as gliptinas atenuam a deficiência/resistência incretínica e a hiperglucagonemia típicas da fisiopatologia do DM2 (50). Esses fármacos e os agonistas do receptor do GLP-1 compõem o grupo das terapias baseadas em incretinas (12). Diversas gliptinas foram lançadas no mercado e têm sido utilizadas no tratamento do DM2 (vildagliptina, sitagliptina, saxagliptina, linagliptina e alogliptina) (12). Todas demonstraram ser eficazes no controle glicêmico, com perfis de segurança e tolerabilidade bastante satisfatórios (51).

Estudos *in vivo* têm sugerido que a inibição da DPP4 apresenta efeitos independentes de GLP-1 capazes de atenuar a disfunção endotelial e a aterogênese (52-54). Como exposto previamente, além do GLP-1, a DPP4 também degrada o GIP, algumas citocinas e quimiocinas, como o fator 1 alfa derivado da célula estromal (SDF-1α, em inglês, *stromal cell-derived factor 1 alpha*), de modo que outros substratos podem ser responsáveis pela melhora na função endotelial associada à inibição farmacológica da DPP4 nos pacientes com DM2 (53). A inibição da DPP4 pelo uso de sitagliptina é capaz de aumentar o número de células progenitoras endoteliais, possivelmente pelo aumento concomitante do SDF-1α (54), e de inibir a expressão, mediada pelo fator alfa de necrose tumoral (TNF-α, em inglês, *tumor necrosis factor alpha*), do inibidor-1 do ativador do plasminogênio (PAI-1, em inglês, *plasminogen activator inhibitor-1*), da molécula-1 de adesão intercelular (ICAM-1, em inglês, *intercellular adhesion molecule-1*) e da molécula-1 de adesão da célula vascular (VCAM-1, em inglês, *vascular cell adhesion molecule-1*) (52). Outro inibidor da DPP4 (linagliptina) mostrou-se capaz de suprimir o estresse oxidativo e a aterogênese, bem como de melhorar a vasodilatação endotélio-dependente de forma glicose-independente, em camundongos deficientes da apolipoproteína-E, um modelo experimental de hipercolesterolemia e aterosclerose prematura (55). Todos esses efeitos conferem à inibição farmacológica da DPP4 propriedades relacionadas à melhora da disfunção endotelial e à ateroproteção por mecanismos parcialmente dependentes ou independentes de GLP-1 (52;55).

Estudos observacionais e experimentais também têm demonstrado a existência de um elo entre disfunção endotelial e inflamação (56-59) e existem evidências de uma clara conexão entre doenças metabólicas, como a obesidade e o DM2, e respostas inflamatórias de

baixo grau (60-64). O excesso de adiposidade é capaz de induzir um estado inflamatório crônico (62;63) que caracteristicamente se associa ao aumento dos níveis séricos da interleucina-6 (IL-6), um citocina pró-inflamatória, e da proteína C-reativa, um biomarcador pró-inflamatório induzido pela IL-6 (60;64). Tanto a IL-6 quanto a proteína C-reativa mostram-se preditoras do desenvolvimento de DM2 (60;61). Além disso, citocinas pró-inflamatórias como o TNF-α e a interleucina-1 beta (IL-1β) são capazes de promover o aumento da expressão das moléculas de adesão VCAM-1, ICAM-1 e E-selectina na superfície das células endoteliais *in vitro*(57) e de comprometer a dilatação venosa endotélio-dependente *in vivo*(58). Em culturas de células endoteliais, mediadores pró-inflamatórios foram capazes de induzir a expressão da óxido nítrico sintase induzível ou isoforma II (iNOS, em inglês, *inducible nitric oxide synthase*), responsável pela produção de grandes quantidades de NO com efeito citostático (não relacionado à vasodilatação), e de diminuir a expressão das enzimas do citocromo P450 relacionadas à síntese do EDHF (59), sabidamente associado ao relaxamento de células musculares lisas e à vasodilatação. Todos esses achados apontam para um papel da inflamação na mediação das relações entre obesidade, DM2 e disfunção endotelial que se manifesta por vias não necessariamente dependentes da hiperglicemia.

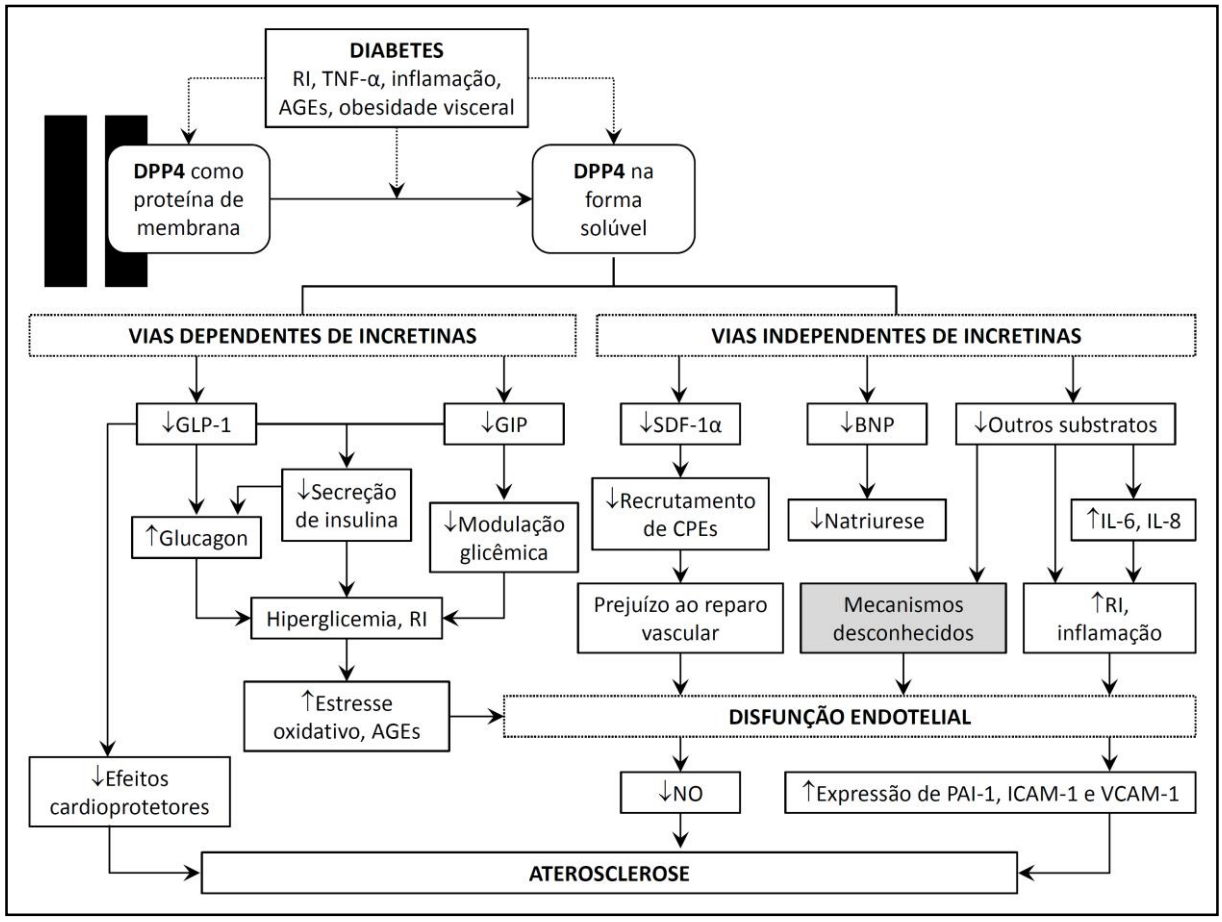
A DPP4 também parece exercer um papel importante em processos inflamatórios (65). Em pacientes com DM2, o uso de inibidores da DPP4 promove a diminuição dos níveis séricos de citocinas inflamatórias (66). Por outro lado, a própria RI e a inflamação crônica a ela associada podem aumentar a expressão e a liberação da DPP4 em vários tecidos (30). O aumento da RI, avaliada a partir da insulinemia em jejum e do modelo de avaliação da homeostase para quantificar a resistência à insulina (HOMA-IR, em inglês, *homeostasis model assessment to quantify insulin resistance*), está associado ao aumento na expressão da DPP4 em macrófagos do tecido adiposo visceral (67). Esses macrófagos, bem como os adipócitos do tecido adiposo visceral, são capazes de liberar a DPP4 quando estimulados pelo TNF-α (18).

Em um cenário inflamatório, a inibição da DPP4 está diretamente associada com proliferação *in vitro* e *in vivo* de células endoteliais (65), bem como com a migração dessas células e com a neovascularização (65;68). Assim, sugere-se que a inibição da DPP4 possa ser uma abordagem útil para a recuperação da proliferação de células endoteliais e o restabelecimento da circulação local após danos vasculares secundários à hiperglicemia (65).

Avaliadas em conjunto, essas evidências sugerem a existência de uma interação fisiopatológica entre DPP4, disfunção endotelial e inflamação, fatores que estão diretamente ligados à etiopatogenia e às manifestações clínicas do DM2. Entretanto, ainda são exíguos os

estudos avaliando a possível associação entre a atividade constitutiva da DPP4 (isto é, fora do contexto de inibição farmacológica da enzima) e marcadores de inflamação e de função endotelial, em especial aqueles testados na microcirculação cutânea. Além disso, considerando que os inibidores da DPP4 são utilizados para o tratamento dos pacientes com DM2, estudos avaliando a atividade da DPP4 em indivíduos sem diabetes também são escassos. Uma vez que os riscos associados à hiperglicemia, em especial ao tecido vascular, ocorrem em níveis glicêmicos menores do que os que atualmente definem o DM (1), a busca por evidências que corroborem a interação entre a atividade de DPP4, a disfunção endotelial e a inflamação já em estágios iniciais do comprometimento da tolerância à glicose mostra-se relevante. A associação da DPP4 com o diabetes, a RI e a aterosclerose está representada de maneira esquemática na Figura 3 e ilustra a importância deste estudo para o melhor entendimento dessas possíveis interações fisiopatológicas também em estágios iniciais do continuum da hiperglicemia.

Figura 3 – Diagrama esquemático ilustrando a associação da DPP4 com o diabetes, a resistência à insulina e a aterosclerose



Legenda: AGEs – produtos finais de glicação avançada(em inglês, *advanced glycation end products*); BNP – peptídeo natriurético tipo-B (em inglês, *B-type natriuretic peptide*); CPEs – células progenitoras endoteliais; DPP4 – dipeptidil peptidase 4; GIP – peptídeo insulinotrópico dependente de glicose (em inglês, *glucose-dependent insulinotropic peptide*); GLP-1 – peptídeo-1 semelhante ao glucagon (em inglês, *glucagon-like peptide-1*); ICAM-1 – molécula-1 de adesão intercelular(em inglês, *intercellular adhesion molecule-1*); IL-6 – interleucina-6; IL-8 – interleucina-8;NO – óxido nítrico (em inglês, *nitric oxide*); PAI-1 – inibidor-1 do ativador do plasminogênio(em inglês, *plasminogen activator inhibitor-1*); RI – resistência à insulina; SDF-1α – fator 1 alfa derivado de célula estromal (em inglês, *stromal cell-derived factor 1 alpha*); TNF-α – fator de necrose tumoral alfa (em inglês, *tumor necrosis factor alpha*); VCAM-1 – molécula-1 de adesão da célula vascular (em inglês, *vascular cell adhesion molecule-1*).
Fonte: O autor, 2015.

A hipótese principal trabalhada neste estudo foi de que a atividade constitutiva da DPP4 pudesse estar associada diretamente à inflamação e inversamente ao fluxo microvascular cutâneo e a um ou mais componentes da vasomotricidade mesmo na ausência de diabetes. Adicionalmente, investigou-se se a atividade constitutiva da DPP4 estaria diretamente associada não apenas com a massa adiposa e marcadores de RI, mas também com

a massa magra (Apêndice C), conforme evidenciado em um estudo envolvendo uma população distinta composta por indivíduos magros e saudáveis (69).

1 **OBJETIVOS**

1.1 **Geral**

Estudar a associaçãoda atividade constitutiva da DPP4 combiomarcadores inflamatórios e reatividade microvascular cutânea em indivíduos com excesso de peso e normoglicemia ou pré-diabetes.

1.2 **Específicos**

a) Comparar os indivíduos com normoglicemia e aqueles com pré-diabetes quanto à atividade da DPP4, os biomarcadores inflamatórios e a reatividade microvascular cutânea;
b) Verificar se a atividade constitutiva da DPP4 se correlaciona com parâmetros de composição corporal, medidas de adiposidade e marcadores de RI (Apêndice C);
c) Avaliar as relações da atividade da DPP4 com adipocinas e peptídeos intestinais no estado de jejum e até 60 min após a ingestão de uma refeição padronizada (Apêndice C).

2 **MATERIAL E MÉTODOS**

2.1 **Desenho do estudo**

Estudo transversal.

2.2 **População estudada**

Quarenta indivíduos de ambos os sexos, na faixa etária de 18 a 50 anos, com excesso de peso (sobrepeso e obesidade grau I) e ausência de diabetes (euglicêmicos/normotolerantes epessoas com pré-diabetes).

2.3 **Recrutamento**

Os sujeitos da pesquisa foram recrutados a partir de anúncios. Estes foram submetidos a uma fase de recrutamento antes de serem considerados aptos a participarem da segunda fase do estudo. Aqueles que atenderam aos critérios de seleção e que assinaram o Termo de Consentimento Livre e Esclarecido (TCLE) foram divididos em dois grupos: grupo doseuglicêmicos/normotolerantes ou grupo tolerância normal à glicose (NGT, em inglês, *normal glucose tolerance*) (n = 21) e grupo dos indivíduos com pré-diabetes (grupo Pré-DM)(n = 19).

O critério utilizado para definição de excesso de peso na fase de recrutamento foi o índice de massa corporal (IMC) variando de 25,0 à 34,9 kg/m². Os critérios da Associação Americana de Diabetes (2) foram adaptados para definição dos grupos NGT e Pré-DM. Dessa forma, caracterizou-se como euglicêmico/normotolerante o indivíduo com GJ <100 mg/dL e/ou glicemia de 2 horas durante o TTGO 75 g <140 mg/dL. A presença de pré-diabetes foi caracterizada por GJ variando de 100-125 mg/dL e/ou glicemia de 2 horas no TTGO 75 g de 140-199 mg/dL. A A1c não foi utilizada como critério para caracterização dos grupos.

2.3.1 Critérios de inclusão

a) Sexo: homens e mulheres;
b) Faixa etária: 18 a 50 anos;
c) IMC: 25,0 à 34,9 kg/m²;
d) Indivíduos euglicêmicos/normotolerantes ou com pré-diabetes, conforme os seguintes critérios:

   - Euglicêmicos/normotolerantes: GJ <100 mg/dL e glicemia de 2 horas durante o TTGO 75 g <140 mg/dL;

   - Indivíduos com pré-diabetes: GJ variando de 100-125 mg/dL e/ou glicemia de 2 horas no TTGO 75 g de 140-199 mg/dL.

2.3.2Critérios de exclusão

a) Diagnóstico de DM (diagnóstico de qualquer tipo de DM realizado durante a fase de recrutamento ou diagnóstico preexistente ou utilização de antidiabéticos orais ou injetáveis para o tratamento de outras condições clínicas constituiu critério de exclusão para as análises relativas ao objetivo geral deste estudo. Entretanto, para as análises referentes aos objetivos específicos, cujos resultados estão descritos no Apêndice C, incluiu-se um grupo de 10 pacientes com DM);
b) IMC <25 kg/m² ou >34,9 kg/m² durante a fase de recrutamento;
c) Doença aguda no momento da coleta das amostras, definida pela presença de mal estar moderado a grave, com ou sem febre;
d) Hipertensão arterial sistêmica não controlada, definida como pressão arterial sistólica ≥140 mmHg e/ou pressão arterial diastólica ≥90 mmHg;
e) Tabagismo;
f) Etilismo grave (homens que consomem acima de 510 g de álcool por semana e mulheres que consomem acima de 310 g por semana);

g) Doenças crônicas pulmonares, cardiovasculares, hepáticas e/ou renais em estágios avançados;
h) Hipertrigliceridemia acima de 400 mg/dL;
i) Gestação e amamentação;
j) Mulheres no climatério;
k) Indivíduos que tenham sido submetidos a procedimentos bariátricos;
l) Início de estatina ou mudança de dose há menos de 60 dias;
m) Uso de ácido acetilsalicílicoe/ou de fluconazol nos 10 dias que antecederam aos exames.

2.4**Fases do estudo**

2.4.1 Primeira fase (recrutamento)

a) Seleção dos sujeitos da pesquisa, apresentação e assinatura do TCLE e solicitação dos exames iniciais;
b) Avaliação clínico-antropométrica.

2.4.2Segunda fase

a) Avaliação antropométrica;
b) Monitorização hemodinâmica não invasiva (MHNI);
c) Coleta de sangue no basal e após estímulo com refeição padronizada;
d) Testes para avaliação da reatividade microvascularcutânea.

2.5 **Avaliação clínico-antropométrica**

Todos os voluntários responderam a um questionário visando a obtenção de dados clínicos e foram submetidos a um exame físico, incluindo a avaliação dos níveis pressóricos. Foram coletadas informações acerca das doenças crônicas preexistentes e dos medicamentos de uso regular.Em ambas asfases do estudo, altura e peso foram medidos com precisão de 0,5 cm e 0,1 kg utilizando-se uma balança eletrônica com estadiômetro modelo *Personal Line 180* (Filizola®, Brasil). Esses parâmetrosforamutilizados para o cálculo do IMC, utilizando-se a fórmula:

$$\text{IMC} = \text{peso em kilogramas}/(\text{altura em metros})^2 \quad (1)$$

2.6 **Monitorização hemodinâmica não invasiva**

Na segunda fase do estudo, os participantes compareceram ao Laboratório de Pesquisas Clínicas e Experimentais em Biologia Vascular (BioVasc) após um período mínimo de 8 horas de jejum. Todos foram previamente orientados a evitar o consumo de bebidas alcoólicas nas 48 horas que antecederam aos exames e também a evitar uso de medicamentos eventuais (analgésicos e anti-inflamatórios, por exemplo) no dia anterior aos exames. Antes da realização da MHNI, todos foram aclimatizados por 15 min em uma sala mantida a 22 ± 1 °C. A MHNI foi realizada de forma contínua, por 10 min, com o auxílio do aparelho Finometer PRO (Finapres Medical Systems®, Amsterdã, Holanda), para aquisição de dados sobre o comportamento dafrequência cardíaca e dos valores pressóricos de cada indivíduo.O Finometer PRO foi conectado aos participantes através de um manguito posicionado envolvendo o 3° quirodáctilo direito. Esse exame visou identificar pacientes com bradicardia ou taquicardia sustentadas (frequências cardíacas <60 ou >100 batimentos/min por mais de 1 min, respectivamente) ou com valores de pressão arterial média (PAM) <75 ou >105 mmHg, os quais impossibilitariam a avaliação da reatividade microvascular.

2.7 **Avaliação bioquímica**

Os testes bioquímicos foram realizados no Laboratório de Lípides (LabLip) da Policlínica Piquet Carneiro, no Laboratório de Hormônios do Hospital Universitário Pedro Ernesto da Universidade do Estado do Rio de Janeiro (HUPE-UERJ) e no Laboratório BioVasc. Um catéter intravenoso foi inserido na veia antecubital direita de cada participante e mantido no local durante todo o exame, a fim de minimizar eventuais desconfortos relacionados às coletas sanguíneas.No máximo 30 min após a coleta do sangue, cada tubo contendo ácido etilenodiamino tetra-acético (EDTA, em inglês, *ethylenediamine tetraacetic acid*) como anticoagulante foi centrifugado a 1300 g por 15 min à 4 °C. Ao término da centrifugação, as amostras de plasma foram clarificadas por filtração (filtro Millex com membrana de polietersulfona, 33 mm, Merck Millipore®, Tullagreen, Irlanda). Uma parte delas foi imediatamente encaminhada para análise, enquanto o restante foi incubado com inibidores específicos, processado em centrífuga refrigerada, aliquotado e armazenado à -80 °C. Os biomarcadores de inflamação foram analisados no aparelho Milliplex Analyzer (Millipore®, EUA), no BioVasc.

Todas as coletas foram efetuadas em três tempos: basal (após jejum mínimo de 8 horas e máximo de 14 horas), 30 e 60 min após ingestão de uma refeição padronizada. Ela foi composta por duas fatias de pão de forma, uma unidade de queijo Polenguinho® *light*, duas colheres de sopa de leite em pó desnatado e uma colher de sopa de achocolatado, perfazendo um total de 247 kcal (64,5% de carboidratos, 19,5% de proteínas e 16% de lipídeos). Todos foram orientados a ingeri-la em um tempo máximo de 5 min.

Para cada um dos tempos, foram efetuadas as seguintes dosagens em duplicata:

a) Basal: atividade plasmática da DPP4 (colorimetria; sensibilidade: 0,1 μM/mL/min; coeficiente de variação [CV] intraensaio: <3%); GJ (glicose oxidase); colesterol total (colesterol oxidase peroxidase); HDL colesterol e triglicerídeos (enzimático colorimétrico); proteína C-reativaultrassensível (PCRus) (turbidimetria); insulina (Luminex; Milliplex®, HMHMAG-34K; sensibilidade: 15 pmol/L; CV intraensaio: <10%; CV interensaio: <20%), IL-6 (ensaio de imunoabsorção enzimática[ELISA, em inglês, *enzyme-linked immunosorbent assay*]; R&D Systems®; HS600B; sensibilidade:0,11 pg/mL; CV intraensaio: <8%; CV interensaio: <10%) e TNF-α (ELISA; R&D Systems®; HSTA00E;

sensibilidade:0,049 pg/mL; CV intraensaio: <3%; CV interensaio: <7%) (Luminex; Milliplex®, HMHMAG-34K); PAI-1 (sensibilidade: 4,1 pg/mL; CV intraensaio: <5%; CV interensaio: <14%), adiponectina (sensibilidade: 11 pg/mL; CV intraensaio: <4%; CV interensaio: <10%) (ELISA; R&D Systems®; DRSN00); ICAM-1 (sensibilidade: 0,0019 ng/mL; CV intraensaio: <15%; CV interensaio: <20%) e VCAM-1 (sensibilidade: 0,024 ng/mL; CV intraensaio: <15%; CV interensaio: <20%) (Luminex; Milliplex®, HCVD2MAG-67K). A lipoproteína de baixa densidade (LDL, em inglês, *low-density lipoprotein*) foi calculada através da equação de Friedewald (70), que utiliza os valores de colesterol total, lipoproteína de alta densidade (HDL, em inglês, *high-density lipoprotein*) e triglicerídeos em mg/dL:

LDL = colesterol total – HDL – triglicerídeos/5 (2)

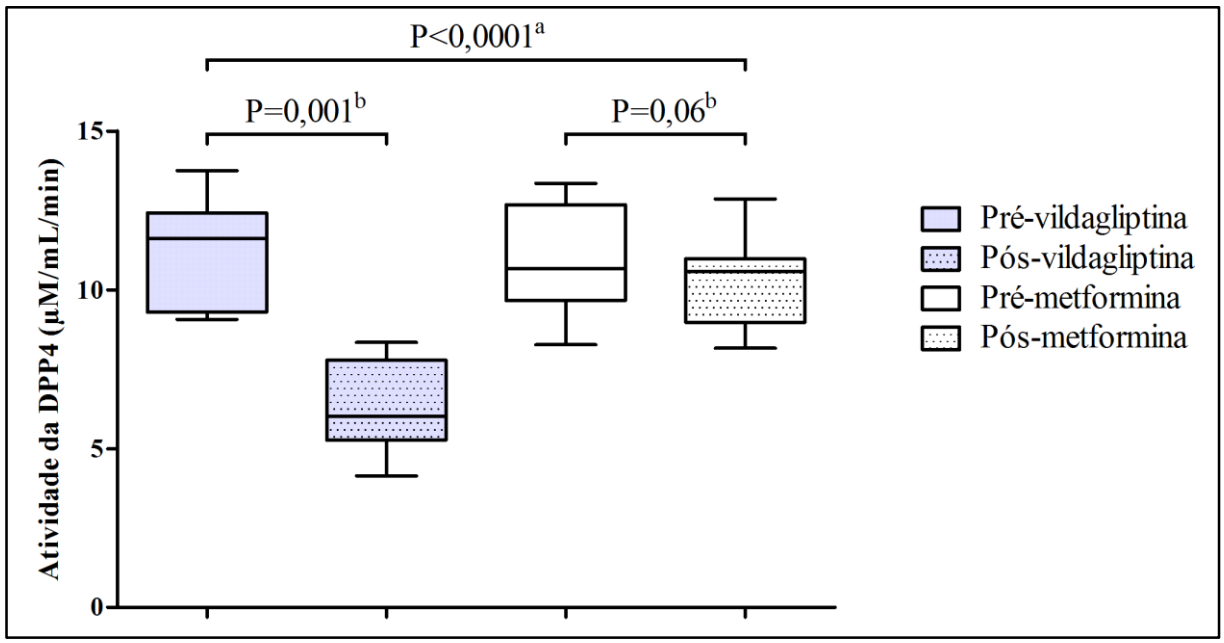
b) 30 e 60 min após refeição padronizada: atividade plasmática da DPP4, glicemia, PCRus, TNF-α, IL-6, PAI-1, ICAM-1 e VCAM-1.

2.7.1 Ensaio colorimétrico para avaliação da atividade da DPP4

Para avaliação da atividade enzimática da DPP4 por colorimetria, o substrato cromogênico utilizado foi aglicil-prolil-p-nitroanilida (Sigma-Aldrich®, Saint Louis, MO, EUA). Em uma placa de 96 cavidades, 10 µL de plasma foram adicionados à 180 µL de tampão Tris 50mM, com pH de 8,0. Em seguida, foram adicionados 10 µL daglicil-prolil-p-nitroanilida à solução e a primeira leitura de densidade óptica (DO) a 405 nm no leitor universal de microplacas *BioTek ELX800*(BioTek Instruments®, Winnoski, VT, EUA)foi realizada para avaliação da liberação de p-nitroanilina no início da reação enzimática. Efetuou-se ainda uma segunda leitura após 60 min de incubação da placa à 37 °C. A atividade da DPP4 nas amostras foi determinada a partir da comparação da DO de cada amostra com a DO dos pontos da curva-padrão de p-nitroanilina. Os resultados foram expressos em µM de p-nitroanilina/mL/min (71;72). Essa técnica foi testada no BioVasc, antes do início das análises deste estudo, em 22 voluntários com DM2 virgens de tratamento, no período basal e após

terem sido submetidos por 30 dias à utilização de um inibidor da DPP4 (vildagliptina, Novartis®) ou da metformina (Gráfico 1). Observou-se que o uso de vildagliptina por esses voluntários foi capaz de reduzir a atividade plasmática da DPP4 significativamente e de validar a técnica empregada.

Gráfico 1 – Validação da técnica de colorimetria para mensuração da atividade plasmática da DPP4



Legenda: [a]Análise de variância (ANOVA, em inglês, *analysis of variance*);[b]Teste de Wilcoxon. DPP4 – dipeptidil peptidase 4. Mensurações efetuadas antes e após 30 dias do uso dos antidiabéticos orais vildagliptina
(n = 11) ou metformina (n = 11).
Fonte: Gráfico gentilmente cedido pela Dr.ª Maria das Graças Coelho de Souza, Laboratório BioVasc, 2015.

2.8 **Índices para avaliação da resistência à insulina**

Considerando-se o objetivo geral deste estudo e as possíveis implicações da RI na atividade da DPP4 (18;30), o *homeostasis model assessment-adiponectin* (HOMA-AD) foi o índice escolhido para avaliação da RI, uma vez que o seu cálculo incorpora a adiponectina (73), uma citocina com propriedades anti-inflamatórias e antiaterogênicas (74).

Para o cálculo do HOMA-AD, utilizou-se a fórmula:

HOMA-AD = insulina (µUI/mL) × GJ (mg/dL)/adiponectina (µg/mL) (3)

Outros métodos indiretos para avaliação da RI também foram empregados para contemplar os objetivos secundários deste estudo (Apêndice C):

a) Insulinemia em jejum (IJ);
b) Área sob a curva (AUC, em inglês, *area under the curve*) da insulina plasmática e do peptídeo C;
c) Relação triglicerídeos/HDL colesterol (TG/HDL);
d) Níveis séricos de globulina ligadora de hormônio sexual (SHBG, em inglês, *sex hormone-binding globulin*) (75);
e) HOMA-IR (76);
f) Índice quantitativo de verificação da sensibilidade à insulina (QUICKI, do inglês, *quantitative insulin sensitivity check index*) (77).

Para cálculo do HOMA-IR e do QUICKI, um método indireto para avaliação da sensibilidade à insulina (77), foram utilizadas as seguintes fórmulas, respectivamente:

HOMA-IR = IJ (µUI/mL) × GJ (mmol/mL)/22,5 (4)

QUICKI = 1/[log(IJ em µUI/mL) + log(GJ em mg/dL)] (5)

2.9 **Avaliação da reatividade microvascular**

Após a aclimatação por 30 min em uma sala mantida a 22 ± 1 °C e a coleta de sangue para realização dos exames basais, os participantes do estudo foram submetidos à amplificação da luz por emissão estimulada de radiação(LASER, em inglês, *light amplification by stimulated emission of radiation*) Doppler fluxometria, um método não invasivo para avaliação da reatividade microvascular (78). A LASER Doppler fluxometria (LDF) de ponto único (composto por um transmissor e um receptor de fibra óptica) apresenta ótima acurácia para quantificar mudanças rápidas no fluxo de sangue (79). O exame foi realizado através do aparelho *PeriFlux System* (Perimed®, Estocolmo, Suécia), o qual apresenta um transmissor que emite um LASER de baixa potência (780 nm) capaz de penetrar a pele na profundidade de 0,4 a 1,0 mm. A luz do LASER é desviada pelas hemácias, o que

gera um desvio de frequência (efeito Doppler) proporcional à velocidade de deslocamento dessas células. Parte da radiação desviada, tanto pelas hemácias quanto pelos tecidos estáticos, é capturada e guiada por um receptor de fibra óptica, posicionado próximo ao transmissor que irradia o LASER, até um detector no aparelho, gerando um sinal que apresenta uma relação direta com o fluxo (80) e permite a avaliação da perfusão do tecido (81). O sinal é quantificado como o produto da velocidade média dos eritrócitos pela sua concentração, sendo expresso em uma unidade arbitrária denominada unidade de perfusão (PU, em inglês, *perfusion unity*) (79). Um protocolo previamente validado em nosso laboratório foi utilizado (81).

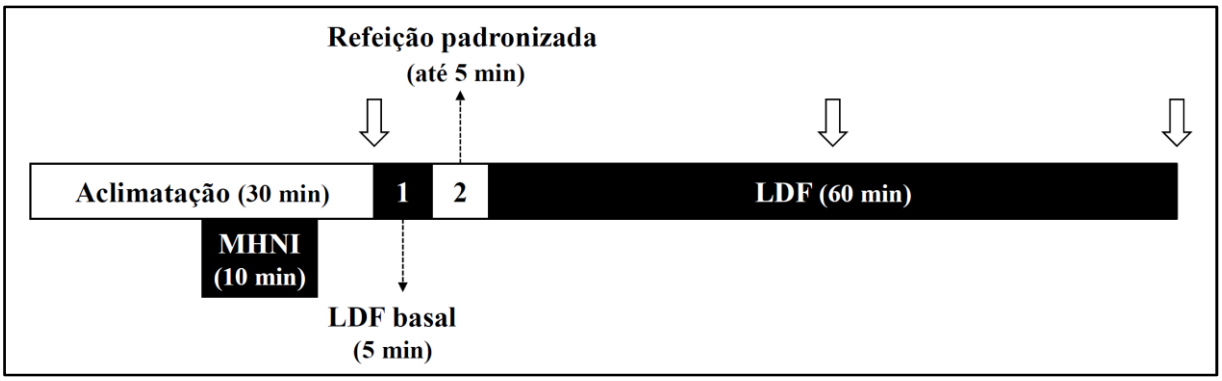
O programa *PeriSoft for Windows 2.5* (Perimed®, Estocolmo, Suécia) foi utilizado para a realização da análise espectral do sinal de fluxo, através da transformada rápida de Fourier. Esse procedimento visa à identificação dos diferentes componentes que contribuem para a vasomotricidade, uma vez que cada um deles está relacionado a uma respectiva oscilação de frequência do sinal de fluxo. Esses componentes podem ser definidos a partir de cinco intervalos de frequência no espectro de oscilações de 0,01 à 1,6 Hz: componente endotelial (oscilações de 0,01-0,02 Hz); neurogênico (0,02-0,06 Hz); miogênico (0,06-0,15 Hz); respiratório (0,15-0,4 Hz) e cardíaco (0,4-1,6 Hz) (82). As variações absolutas de amplitude de cada um desses intervalos, bem como seus valores normalizados, obtidos através da razão entre a amplitude média do respectivo intervalo de frequência e a amplitude média de todo o espectro de oscilações de 0,005 a 2,0 Hz, foram os parâmetros analisados (82).

Para obtenção dos dados utilizados na avaliação da vasomotricidade, a medição contínua do fluxo microvascular foi realizada no intervalo de tempo entre -5 e 60 min, tomando-se como referência a ingestão da refeição padronizada, que ocorreu em até 5 minutos (duração total do registro de fluxo microvascular de 65 minutos e duração total do exame de até 70 min).

2.10 **Desenho do estudo**

A Figura 4 ilustra o desenho do estudo, com a sequência cronológica das coletas sanguíneas para as análises laboratoriais e das técnicas utilizadas para a MHNI e avaliação da reatividade microvascular cutânea.

Figura 4 – Desenho do estudo



Legenda: MHNI – monitorização hemodinâmica não invasiva; LDF – LASER Doppler fluxometria. As setas brancas representam os tempos de coleta sanguínea para os exames laboratoriais.
Fonte: O autor, 2017.

2.11**Método estatístico e análise**

Os programas GraphPad Prism® 5 (GraphPad Software Inc., San Diego, CA, EUA) e STATISTICA® 7.0 (StatSoft Inc., Tulsa, OK, EUA) foram utilizados para as análises estatísticas. A distribuição gaussiana foi checada e os dados paramétricos e não paramétricos foram expressos como média±desvio-padrão e mediana [1º-3º quartis], respectivamente. O teste-t não pareado e o teste U foram utilizados para comparações entre os grupos NGT e Pré-DM. Visando otimizar os dados obtidos nos períodos pré- e pós-ingestão da refeição padronizada, foram determinadas, para cada indivíduo, além das AUC, as respectivas equações de regressão linear que modelam a relação entre as variáveis independentes (atividade da DPP4, biomarcadores inflamatórios ou componentes de reatividade microvascular) e a variável independente (tempo). As equações obtidas forneceram valores deinclinaçãoou "*slope*" e intercepto, os quais definem a relação linear entre a variável dependente (variável testada) e o tempo de coleta. O *slope* representa o grau de inclinação da linha de regressão, podendo ser caracterizado como positivo, negativo ou zero. Quanto maior a magnitude do *slope*, maior a taxa de mudança da variável dependente em função do tempo. O intercepto representa o ponto onde a linha de regressão cruza o eixo da variável dependente. Os valores médios de *slopes* e interceptos também foram comparados entre os grupos. Correlações lineares (testes de Pearson ou Spearman) entre a atividade da DPP4 e as demais variáveis testadas foram realizadas. Subsequentemente, análises de regressão múltipla foram empregadas para testar se algumas variáveis laboratoriais–microvasculares poderiam

influenciar a atividade plasmática da DPP4, ou serem influenciadas por ela. Essa mesma estratégia foi utilizada também para se avaliar a influência da combinação de variáveis de composição corporal–antropométricas–laboratoriais na atividade da DPP4 (Apêndice C). O programa G*Power 3.1.9.2 (Universität Kiel, Alemanha) foi utilizado para o cálculo do tamanho amostral, indicando que seriam necessários 39 indivíduos para garantir um poder estatístico de 0,95 (testes-t; ponto bisserial; bicaudado; tamanho de efeito: 0,51; probabilidade de erro tipo I: 0,05; poder estatístico:0,9500156). Valores de $P<0,05$ foram consideradosestatisticamente significativos.

2.12 **Aspectos éticos**

O protocolo do estudo foi aprovado pelo Comitê de Ética em Pesquisa do HUPE-UERJ, número de protocolo: CAAE 24360513.1.0000.5282 (Anexo), e todos os participantes assinaram o TCLE após a completa explicação do propósito do estudo e dos procedimentos utilizados. O estudo foi registrado no ClinicalTrials.gov (NCT03178019).

Ao final do período de investigação, os participantes que apresentaram alterações no exame clínico ou laboratorial foramencaminhados aos ambulatórios específicos para tratamento de suas condições patológicas.

3**RESULTADOS**

Quarenta indivíduos, com idade de 38,4±8,65 anos e IMC de 29,1±2,76 kg/m², foram incluídos no estudo e quase a metade (47%) deles foi composta por mulheres. No estado basal (pré-refeição padronizada), o grupo Pré-DM apresentou maior IMC, GJ, IJ, IL-6 e PAI-1, bem como tendência (P=0,06) a maior HOMA-AD, em comparação ao grupo NGT (Tabela 1). Não houve diferença na atividade da DPP4 entre os indivíduos euglicêmicos/normotolerantes e aqueles com pré-diabetes. Além disso, os grupos NGT e Pré-DM foram comparados em relação às AUC, às diferenças de 30 min e 60 min a partir do basal (deltas [Δ] 30-0 e 60-0, respectivamente) e aos *slopes* e interceptos de cada variável laboratorial. As seguintes diferenças foram observadas: $\text{Glicemia}_{\text{AUC}}$ (7157±749,6 vs. 6339±871,2; P<0,05) e $\text{PAI-1}_{\text{AUC}}$ (1652 [1217–2112] vs.1108 [656.8–1358]; P<0,01) foramambas maiores no grupo Pré-DM em comparação ao NGT (Tabela 2), enquanto o $\text{IL-6}_{\Delta 30\text{-}0}$ (-0,27 [-0,44–-0,15] vs. -0,15 [-0,26–-0,01]; P<0,05) e o $\text{PAI-1}_{\Delta 60\text{-}0}$ (-10,89±8.85 vs. -6.2±8.31; P<0,05) foram ambos menores no grupo Pré-DM (Tabela 3). Essas diferenças nos deltas evidenciam uma tendência à redução de ambos os marcadores inflamatórios após a ingestão da refeição padronizada, embora mais acentuada no grupo Pré-DM. Não houve outras diferenças nessa análise e os *slopes*,tanto da atividade da DPP4 quanto dos biomarcadores inflamatórios, também não diferiram entre os grupos. Por outro lado, foram evidenciadas diferenças no $\text{Glicemia}_{\text{intercepto}}$, $\text{PAI-1}_{\text{intercepto}}$e $\text{PCRus}_{\text{intercepto}}$ entre os grupos, sendo que o $\text{PCRus}_{\text{intercepto}}$ apresentou um padrão distinto dos demais, pois foi menor no grupo Pré-DM em comparação ao grupo NGT (Tabela 4). Dessa forma, de acordo com os dados no período basal e também com algumas variáveis testadas no período pós-prandial, os grupos NGT e Pré-DM, embora fenotipicamente muito próximos um do outro, exibiramalgumas particularidades, incluindo maiores níveis de mediadores de ativação endotelial de caráter pró-inflamatório ou pró-trombótico no grupo Pré-DM.

A reatividade microvascular também apresentou algumas diferenças entre os grupos. No estado basal, o componente endotelial da vasomotricidade foi significativamente menor no grupo Pré-DM em comparação ao grupo NGT (3,785 [3,103-5,607] vs. 5,780 [3,583-6,622]; P<0,05),mas não houve diferenças entre os grupos em relação ao fluxo microvascular e à velocidade de fluxo. O mesmo padrão foi evidenciado para as AUC dessas variáveis, uma vez que a $\text{componente endotelial}_{\text{AUC}}$ foi menor no grupo Pré-DM em comparação ao grupo NGT (269,4±98,44 vs. 335,4±97,12; P<0,05) e que não houve diferenças entre os grupos em

relação às fluxo microvascular$_{AUC}$ e velocidade de fluxo$_{AUC}$ (Tabela 2). Esses dados sugerem uma menor resposta vasomotora relacionada à atividade endotelial no grupo Pré-DM. Ademais, nem os *slopes*, nem os interceptos das variáveis de reatividade microvascular diferiram entre os grupos (Tabela 4).

Tabela 1 –Características clínicas e bioquímicas dos participantes no período basal (pré-refeição padronizada) e na fase de recrutamento

	Total (n = 40)	**Grupo NGT (n = 21)**	**Grupo Pré-DM (n = 19)**
Idade (anos)	38,4 ± 8,65	37,67 ± 8,26	39,21 ± 9,21
Mulheres [n, (%)]	19 (47,5%)	8 (38%)	11 (57%)
IMC (kg/m²)	29,11 ± 2,76	**27,59 ± 1,65**	**30,8 ± 2,8‡**
PAM (mmHg)	82,98 ± 8,37	83,8 ± 7,54	82,09 ± 9,34
Atividade da DPP4 (µM/mL/min)	13,47 ± 3,16	13,85 ± 2,74	13,05 ± 3,59
GJ (mg/dL)	95 [87,5–108]	**89 [82,5–93,5]**	**106 [96–111]‡**
Insulina (pmol/L)	48,38 [30,99–90,91]	**36,67 [27,03–57,3]**	**57,68 [37,53–94,18]***
HOMA-AD	53,41 [26,16–137,8]	41,76 [18,13–82,14]	61,92 [19,26–141,8]
Colesterol total (mg/dL)	204,2 ± 43,76	205,8 ± 45,99	202,5 ± 42,34
HDL (mg/dL)	51,72 ± 13,84	50,1 ± 10,68	53,53 ± 16,8
LDL (mg/dL)	125,95 ± 35,74	129,0 ± 36,11	122,6 ± 36,01
TG (mg/dL)	113 [87,25–176,3]	109 [89,5–175,5]	113 [86–174]
PCRus	0,21 [0,06–0,41]	0,22 [0,09–0,44]	0,17 [0,04–0,35]
IL-6 (pg/mL)	1,31 [1,04–1,91]	**1,19 [0,89–1,47]**	**1,41 [1,18–1,98]***
TNF-α (pg/ml)	0,9 ± 0,21	0,87 ± 0,15	0,92 ± 0,27
PAI-1 (pg/mL)	26,84 [19,86–36,32]	**23,86 [12,0–29,56]**	**31,78 [26,25–42,8]†**
ICAM-1 (ng/mL)	96 [72,7–113,5]	101 [80–109,5]	80 [70–115]
VCAM-1 (ng/mL)	535,5 [440,5–615,8]	527 [452,5–623,5]	566 [426–617]
Fase de recrutamento			
GJ (mg/dL)	99,5 [92,25–113]	**92 [85–96,5]**	**107 [100–113]‡**
TTGO 75 g – glicemia de 2 horas (mg/dL)	119,1 ± 29,93	**103,0 ± 21,51**	**137,0 ± 28,01‡**
Colesterol total (mg/dL)	199,47 ± 43,75	196,6 ± 40,46	202,6 ± 48,05
HDL (mg/dL)	54,75 ± 15,22	**48,76 ± 11,73**	**61,37 ± 16,16†**
LDL (mg/dL)	117,43 ± 38,87	123,2 ± 39,48	111,1 ± 38,21
TG (mg/dL)	119,5 [83–170,8]	118 [83–163,5]	140 [82–202]

Legenda: Dados expressos como média±desvio-padrão, mediana [1º–3º quartis] ou n (%). *P<0,05. †P<0,01. ‡P<0,001.NGT – normoglicemia; Pré-DM – pré-diabetes; IMC –índice de massa corporal; PAM – pressão arterial média; DPP4 – dipeptidil peptidase 4; GJ –glicemia em jejum; TTGO –teste de tolerância à glicose oral; HOMA-AD –modelo de avaliação da homeostase-adiponectina; TG – triglicerídeos; PCRus –proteína C-reativa ultrassensível; IL-6 – interleucina-6; TNF-α –fator alfa de necrose tumoral; PAI-1 –inibidor do ativador do plasminogênio tipo-1; ICAM-1 –molécula-1 de adesão intercelular; VCAM-1 –molécula-1 de adesão da célula vascular.

Fonte: O autor, 2017.

Tabela 2 – Análise comparativa das áreas sob a curva da atividade da DPP4 e das variáveis bioquímicas e de reatividade microvascular entre os grupos estudados

	Grupo NGT (n = 21)	**Grupo Pré-DM (n = 19)**
Atividade de DPP4	797,0 ± 159,2	753,4 ± 190,5
Glicemia	**6339 ± 871,2**	**7157 ± 749,6†**
PCRus	12,9 [5,85–29,1]	9,3 [2,4–20,25]
IL-6	63,21 [46,99–84,7]	76,83 [67,34–111,9]
TNF-α	50,22 ± 8,33	52,82 ± 16,0
PAI-1	**1108 [656,8–1358]**	**1652 [1217–2112]†**
ICAM-1	5520 [4350–5895]	4530 [4215–6450]
VCAM-1	29460 [26558–35018]	32055 [24990–34290]
Fluxo sanguíneo	44264 [36037–76862]	48189 [35912–55363]
Velocidade de fluxo	829,8 [691,6–1108]	780,9 [659,7–1043]
Componente de vasomotricidade		
Endotelial	**335,4 ± 97,12**	**269,4 ± 98,44***
Neurogênico	240,8 ± 65,51	206,3 ± 61,99
Miogênico	142,0 ± 20,57	141,7 ± 28,44
Respiratório	72,11 [68,85–80,69]	79,07 [69,91–82,67]
Cardiogênico	42,44 [38,67–44,38]	43,59 [40,28–45,45]

Legenda: Dados expressos como média±desvio-padrão ou mediana [1º–3º quartis]. *P<0,05. †P<0,01. NGT – normoglicemia; Pré-DM – pré-diabetes; DPP4 – dipeptidil peptidase 4; PCRus – proteína C-reativa ultrassensível; IL-6 – interleucina-6; TNF-α – fator alfa de necrose tumoral; PAI-1 – inibidor do ativador do plasminogênio tipo-1; ICAM-1 – molécula-1 de adesão intercelular; VCAM-1 – molécula-1 de adesão da célula vascular.

Fonte: O autor, 2017.

Tabela 3 – Análise comparativados deltas 30-0 e 60-0das variáveis bioquímicas e de reatividade microvascularentre os grupos Normoglicemia e Pré-diabetes

	Delta 30-0 (n = 40)		**Delta 60-0 (n = 40)**	
	Grupo NGT	***Grupo Pré-DM***	***Grupo NGT***	***Grupo Pré-DM***
Atividade da DPP4	-0,81 [-1,28–-0,16]	-0,44 [-1,14–0,03]	-0,71 [-1,2–-0,22]	-0,61 [-1,1–-0,15]
Glicemia	26,55 ± 15,49	20,66 ± 12,13	17,86 ± 26,5	18,2 ± 24,58
PCRus	-0,01 [-0,04–0,0]	-0,005 [-0,02–0,0]	-0,01 [-0,02–0,002]	-0,01 [-0,02–0,007]
IL-6	**-0,15 [-0,26–-0,01]**	**-0,27 [-0,44–-0,15]***	-0,08 [-0,28–0,03]	-0,15 [-0,39–0,004]
TNF-α	-0,04 [-0,07–-0,01]	-0,06 [-0,08–-0,03]	-0,05 [-0,09–-0,03]	-0,07 [-0,08–-0,03]
PAI-1	-5,41 ± 6,92	-7,11 ± 7,45	**-6,2 ± 8,31**	**-10,89 ± 8,85***
ICAM-1	-10,5 [-17,7–-0,5]	-5 [-12–-2]	-11 [-22,5–-1,75]	-7 [-18,75–1,75]
VCAM-1	-32,77 ± 38,28	-46,84 ± 30,15	-46,05 ± 53,72	-43,7 ± 42,09
Fluxo sanguíneo	530 [-3077–2982]	-1247 [-5811–2566]	663,6 [-4710–7837]	104,3 [-5275–2596]
Velocidade de fluxo	0,45 [-1,07–1,78]	-1,14 [-4,03–0,79]	0,49 [-053–4,6]	-1,16 [-4,77–1,38]
Componente da vasomotricidade				
Endotelial	0,003 ± 2,28	0,16 ± 1,67	-0,42 ± 2,23	0,44 ± 1,5
Neurogênico	0,27 ± 1,37	0,11 ± 1,19	0,15 ± 1,11	0,05 ± 1,41
Miogênico	0,03 [-0,22–0,29]	-0,01 [-0,28–0,2]	0,06 [-0,18–0,5]	-0,11 [-0,37–0,29]
Respiratório	0,02 ± 0,22	0,02 ± 0,18	**-0,06 ± 0,14**	**0,05 ± 0,19***
Cardiogênico	-0,01 ± 0,07	-0,003 ± 0,06	-0,0005 ± 0,07	-0,01 ± 0,06

Legenda: Dados expressos como média±desvio-padrão ou mediana [1º–3º quartis]. *P<0,05 para comparações no mesmo delta. NGT – normoglicemia; Pré-DM – pré-diabetes; DPP4 – dipeptidil peptidase 4; PCRus – proteína C-reativa ultrassensível; IL-6 – interleucina-6; TNF-α – fator alfa de necrose tumoral; PAI-1 – inibidor do ativador do plasminogênio tipo-1; ICAM-1 – molécula-1 de adesão intercelular; VCAM-1 – molécula-1 de adesão da célula vascular.

Fonte: O autor, 2017.

Tabela 4 – Análise comparativa dos interceptos das variáveis bioquímicas e de reatividade microvascular entre os grupos Normoglicemia e Pré-diabetes

	Grupo NGT (n = 21)	**Grupo Pré-DM (n = 19)**
Atividade da DPP4	13,7 ± 2,73	13,0 ± 3,51
Glicemia	**93,51 ± 9,87**	**108,5 ± 12,62‡**
PCRus	**0,24 [0,1–0,47]**	**0,13 [0,03–0,33]***
IL-6	1,16 [0,87–1,41]	1,34 [1,11–1,99]
TNF-α	0,87 ± 0,03	0,92 ± 0,06
PAI-1	**21,44 [11,68–28,87]**	**33,79 [25,44–43,46]†**
ICAM-1	100,2 [78,75–109,9]	78,67 [69,67–114,7]
VCAM-1	515,8 [458,3–617,2]	549,8 [426,7–604,3]
Fluxo sanguíneo	14160 [11480–23410]	16210 [12680–20570]
Velocidade de fluxo	12,92 [11,21–18,44]	14,61 [12,07–17,65]
Componente da vasomotricidade		
Endotelial	0,98 ± 0,45	0,75 ± 0,36
Neurogênico	0,6 [0,51–0,88]	0,48 [0,39–0,86]
Miogênico	0,37 [0,24–0,53]	0,34 [0,28–0,48]
Respiratório	0,19 [0,15–0,27]	0,19 [0,14–0,26]
Cardiogênico	0,1 [0,08–0,13]	0,1 [0,1–0,14]

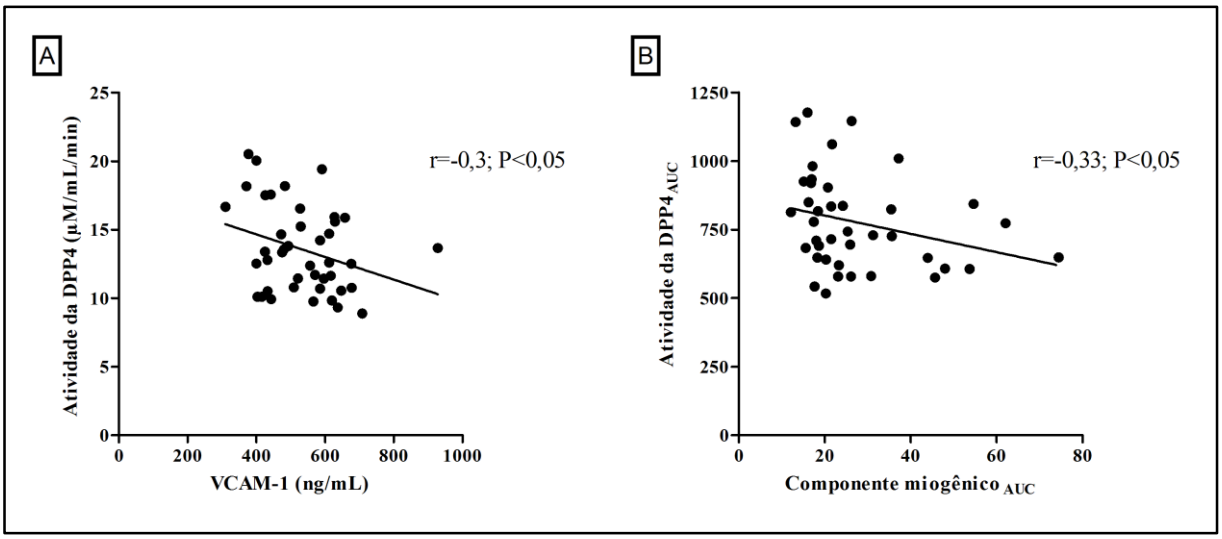
Legenda: Dados expressos como média±desvio-padrão ou mediana [1º–3º quartis]. *P<0,05. †P<0,01. ‡P<0,001. NGT – normoglicemia; Pré-DM – pré-diabetes; DPP4 – dipeptidil peptidase 4; PCRus – proteína C-reativa ultrassensível; IL-6 – interleucina-6; TNF-α – fator alfa de necrose tumoral; PAI-1 – inibidor do ativador do plasminogênio tipo-1; ICAM-1 – molécula-1 de adesão intercelular; VCAM-1 – molécula-1 de adesão da célula vascular.

Fonte: O autor, 2017.

A correlação da atividade constitutiva da DPP4 com biomarcadores inflamatórios e parâmetros de reatividade microvascular cutânea foi o o principal objetivo do presente estudo e toda a amostra foi utilizada para testá-la.Observou-se uma correlação linear inversa entre a atividade da DPP4 e a VCAM-1 (r=-0,30, P<0,05; Gráfico2A), um marcador de disfunção endotelial. Adicionalmente, a atividade basal da DPP4 (estado de jejum) foi diretamente correlacionada com o HOMA-AD (r=0,45, P<0,01) (Gráfico3), mas não houve correlações da atividade da DPP4 com PCR, IL-6, TNF-α, PAI-1 e ICAM-1. Considerando-se os dados do período pós-prandial, foievidenciada correlação linear entre o atividade da $DPP4_{\Delta 60-0}$ e o $PCR_{\Delta 60-0}$ (r=0,31, P<0,05). Também foi observada correlação inversa entre a atividade da DPP4 e os componentes neurogênico (r=-0,33, P<0,05) e miogênico (r=-0,39, P=0,01) da vasomotricidade no tempo 30 min pós-prandial. Não houve outras associações significativas entre a atividade da DPP4 e outros componentes da vasomotricidade.Ressalta-se que a

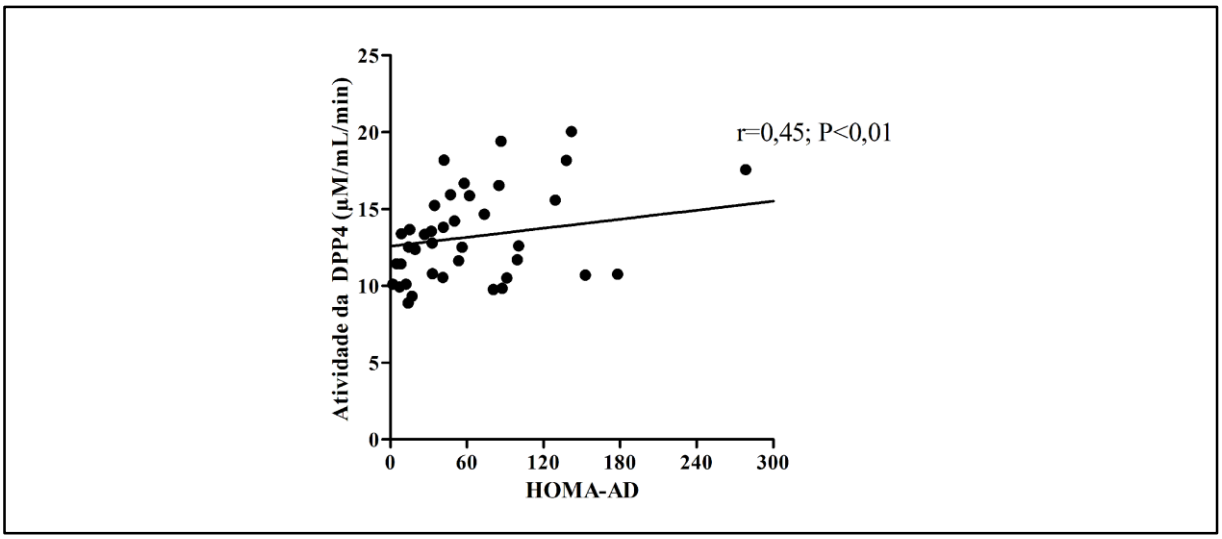
atividade da $DPP4_{AUC}$ apresentou uma correlação significativa com a componente $miogênico_{AUC}$ da vasomotricidade (r=-0,33, P<0,05; Gráfico2B).

Gráfico 2 –Correlações lineares entre a atividade da DPP4 e a VCAM -1 e entre a atividade de $DPP4_{AUC}$ e o componente miogênico da $vasomotricidade_{AUC}$



Legenda: DPP4 – dipeptidil peptidase 4; VCAM-1 – molécula-1 de adesão da célula vascular; AUC – área sob a curva.
Fonte: O autor, 2017.

Gráfico 3 – Correlação linear entre a atividade da DPP4 e o HOMA-AD



Legenda: DPP4 – dipeptidil peptidase 4; HOMA-AD – modelo de avaliação da homeostase-adiponectina.
Fonte: O autor, 2017.

Subsequentemente, testou-se se algumas variáveis laboratoriais–microvasculares poderiam influenciar a atividade da DPP4 ou serem influenciadas por ela (Tabela5). Para essa análise, optou-se por utilizar não apenas a atividade da DPP4, mas também o HOMA-AD, a VCAM-1 e o fluxo microvascular como variáveis dependentes nos modelos 1, 2, 3 e 4, respectivamente. Evidenciou-se que o HOMA-AD, a IL-6, o VCAM-1, o PAI-1, ofluxo microvascular e a vasomotricidadeinfluenciaram a atividade daDPP4 (modelo 1) e explicaram aproximadamente 40% da sua variância. HOMA-AD, VCAM-1 e fluxo microvascular foram as variáveis que exerceram maior efeito na atividade da DPP4. Tomando-se o HOMA-AD, a VCAM-1e o fluxo microvascular como variáveis dependentes (modelos 2, 3 e 4, respectivamente), é válidoressaltar que a atividade da DPP4 exerceu efeito significativo em todos esses modelos, sugerindo a existência de influências bidirecionais entre inflamação, reatividade microvascular e atividade constitutiva da DPP4nos estágios iniciais do espectro da tolerância à glicose nesses indivíduos com sobrepeso/obesidade.

Tabela 5 –Análises de regressão múltipla

Modelo	β	P-valor	R^2 ajustado	P-valor
1.			0,413	<0,001
HOMA-AD	**0,537**	**0,0003**		
VCAM-1	**-0,385**	**0,006**		
Fluxo sanguíneo	**0,422**	**0,006**		
Neurogênico	-0,195	0,172		
IL-6	-0,160	0,206		
2.			0,380	<0,001
Atividade da DPP4	**0,573**	**0,0002**		
Fluxo sanguíneo	**-0,370**	**0,012**		
VCAM-1	0,226	0,124		
PAI-1	0,148	0,278		
3.			0,226	<0,05
Fluxo sanguíneo	**0,468**	**0,004**		
Atividade da DPP4	**-0,504**	**0,005**		
HOMA-AD	0,300	0,103		
IL-6	-0,165	0,251		
4.			0,637	<0,001
Endotelial	**0,335**	**0,014**		
VCAM-1	0,186	0,113		
Atividade da DPP4	**0,262**	**0,043**		
HOMA-AD	-0,166	0,200		
IL-6	0,202	0,052		
Respiratório	**-0,977**	**0,00006**		
Cardiogênico	**1,060**	**0,00008**		
PAI-1	-0,154	0,155		

Legenda: Atividade da DPP4, HOMA-AD, VCAM-1, IL-6, PAI-1, fluxo sanguíneo e os componentes da vasomotricidade foram as variáveis envolvidas na análise. Atividade da DPP4, HOMA-AD, VCAM-1 e fluxo microvascular foram consideradas variáveis dependentes nos modelos 1, 2, 3, and 4, respectivamente. "Neurogênico", "endotelial", "respiratório" e "cardiogênico" referem-se aos respectivos componentes da vasomotricidade. HOMA-AD – modelo de avaliação da homeostase-adiponectina; VCAM-1 – molécula-1 de adesão da célula vascular; IL-6 – interleucina-6; DPP4 – dipeptidil peptidase 4; PAI-1 – inibidor do ativador do plasminogênio tipo-1.

Fonte: O autor, 2017.

4 **DISCUSSÃO**

Além das já conhecidas ações enzimáticas da DPP4 no metabolismo da glicose, essa enzima também é capaz de inativar uma série de peptídeos envolvidos em processos inflamatórios, imunológicos e relacionados à função vascular (20;83) e, em pacientes com DM2, a inibição farmacológica da DPP4 está associada à redução dos níveis de citocinas inflamatórias (66) e à atenuação da disfunção endotelial e da aterogênese (84;85). Neste trabalho, foi originalmente investigada a associação entre a atividade constitutiva da DPP4, biomarcadores inflamatórios e reatividade microvascular em pacientes com excesso de peso e normoglicemia ou pré-diabetes. Uma vez que a disfunção microvascular é considerada um processo sistêmico que ocorre de maneira semelhante nos múltiplos leitos vasculares dos mais variados tecidos pelo corpo (86), o teste da reatividade microvascular cutânea foi utilizado como um modelo para avaliação da função microvascular sistêmica.

Nesse sentido, foram observadas algumas importantes correlações entre a atividade da DPP4 e as variáveis avaliadas. O principal achado foi a associação da atividade da DPP4 com biomarcadores inflamatórios e parâmetros de função microvascular, notadamente as correlações entre a atividade da DPP4 e a VCAM-1 no estado basal e também entre a atividade da $DPP4_{AUC}$ e a componente $miogênico_{AUC}$ da vasomotricidade. Além disso, na análise de regressão múltipla, confirmou-se a hipótese da existência de uma interação entre atividade da DPP4, inflamação e função microvascular que ocorre precocemente no espectro da tolerância à glicose.

De maneira intrigante, constatou-se uma correlação inversa entre a atividade da DPP4 e a VCAM-1, um marcador precoce de inflamação vascular (87). Há evidência prévia de que a exposição de células endoteliais vasculares humanas à sitagliptina, um inibidor da DPP4, é capaz de inibir a síntese do ácido ribonucleico (RNA, em inglês, *ribonucleic acid*) mensageiro da VCAM-1 e a sua expressão, ambas induzidas pelo TNF-α (52). Essa aparente disparidade pode ser justificada pelo fato de que a medida da VCAM-1 solúvel não é necessariamente representativa da sua expressão na superfície celular (88). Em conjunto, esses achados sugerem a hipótese de que a atividade da DPP4 possa aumentar a expressão tecidual da VCAM-1 e reduzir a sua liberação, fazendo com que essa molécula de adesão permaneça aderida ao endotélio. Cabe destacar que, na análise de regressão múltipla, VCAM-1 e atividade da DPP4 exerceram efeitos significativos entre si (β=-0,385, $P<0,01$ para VCAM-1

como variável independente no modelo 1 e β=-0,504, P<0,01 para atividade da DPP4 como variável independente no modelo 3).

Em relação à função microvascular, identificou-se uma correlação negativa entre a atividade da $DPP4_{AUC}$ e a componente $miogênico_{AUC}$ da vasomotricidade. Esse achado está de acordo com um estudo experimental prévio envolvendo células musculares lisas vasculares humanas, que evidenciou a ativação direta e proeminente da via das proteínas quinases ativadas por mitógeno e da via do fator nuclear *kappa* B pela DPP4 solúvel, ambas capazes de promover alterações pró-aterogênicas nessas células, caracterizadas pelo aumento da proliferação e da inflamação (89). Esses autores especulam se a DPP4 solúvel poderia agir de maneira parácrina ou endócrina na parede vascular, potencialmente contribuindo para a inflamação nesse cenário (89). Vale ressaltar que a vasomotricidade *in vivo* é associada com oscilações rítimicas no diâmetro do vaso que modificam/redistribuem o fluxo sanguíneo (90). Esse dado está de acordo com os achados deste estudo, que evidenciaram que a DPP4 e o fluxo vascular exerceram um significativo efeito mútuo (β=0,422, P<0,01 para o fluxo microvascular como variável independente no modelo 1 e β=0,262, P<0,05 para a atividade da DPP4 como variável independente no modelo 4). Esses modelos explicaram quase 40% e 60% das variações na atividade da DPP4 e no fluxo sanguíneo, respectivamente, sugerindo que a atividade da DPP4 poderia ser capaz de influenciar ou de ser influenciada pelo fluxo microvascular.

Como esperado, IMC, GJ, IJ, IL-6 e PAI-1 foram significativamente maiores no grupo Pré-DM em comparação ao grupo NGT. Também foi evidenciada uma tendência a maior HOMA-AD no grupo Pré-DM. Embora não tenham havido diferenças entre os grupos no fluxo microvascular e na velocidade de fluxo, tanto o componente endotelial basal quanto a componente $endotelial_{AUC}$ da vasomotricidade foram significativamente menores no grupo Pré-DM, sugerindo um comprometimento do componente endotelial da vasomotricidade já no estado de pré-diabetes. Ressalta-se que não foram constatadas diferenças entre os grupos em relação à atividade da DPP4 basal e à atividade da $DPP4_{AUC}$ neste estudo. As informações acerca da atividade da DPP4 em indivíduos com pré-diabetes, em comparação aos indivíduos euglicêmicos/normotolerantes, são escassas e conflitantes (33;91). Há um trabalho que também não identificou diferenças nesse contexto (91) e outro que evidenciou maior atividade da DPP4 em pessoas com pré-diabetes (33). As diferenças no $IL\text{-}6_{\Delta 30\text{-}0}$ e no $PAI\text{-}1_{\Delta 60\text{-}0}$ entre os grupos NGT e Pré-DM sugerem uma tendência à redução de ambos os biomarcadores ao longo do tempo, embora em momentos diferentes após a refeição padronizada. Curiosamente, ambas as reduções foram mais pronunciadas no grupo Pré-DM.

A ausência de diferenças no atividade da $DPP4_{slope}$ e também nos *slopes* dos biomarcadores inflamatórios entre os grupos NGT e Pré-DM sugerem que a taxa de mudança de cada variável após a ingestão da refeição padronizada foi similar em ambos os grupos. Ademais, uma vez que a variável independente na regressão linear foi o tempo de coleta em função da ingestão da refeição padronizada, os interceptos foram similares aos respectivos valores basais de cada variável, contudo influenciados pelos valores obtidos nos tempos 30 e 60 min após a ingestão da refeição. Logo, os achados de maiores $Glicemia_{intercepto}$ e $PAI\text{-}1_{intercepto}$ no grupo Pré-DM em comparação ao grupo NGT refletem principalmente, mas não exclusivamente, as comparações da GJ e do PAI-1 entre esses dois grupos no estado basal.

Sabe-se que a ação de agonistas pró-inflamatórios e a estimulação biomecânica secundária à pertubação do fluxo vascular desencadeiam o processo de ativação endotelial (92). Esses estímulos bioquímicos e biomecânicos resultam em um programa coordenado de regulação genética no interior da célula endotelial, que incluem a síntese de moléculas de adesão, como a VCAM-1, e a expressão na membrana e secreção de quimiocinas e mediadores pró-trombóticos, como o PAI-1 (92). Esses eventos estimulam o recrutamento seletivo de monócitos e linfócitos T, que passam a ocupar o espaço subendotelial e à perpetuar o estado pró-inflamatório crônico que resulta na progressão da lesão aterosclerótica (92). Neste estudo, a atividade constitutiva da DPP4 mostrou-se capaz de interagir com biomarcadores inflamatórios e com o fluxo sanguíneo microvascular de indivíduos em estágios iniciais do espectro da tolerância à glicose, o que sugere que essa enzima possa realmente estar envolvida precocemente no processo aterosclerótico de pessoas com excesso de peso e sem diabetes.

Este trabalho tem pontos fortes e limitações. Enquanto a maioria absoluta dos estudos analisou a atividade da DPP4 sob inibição farmacológica e, consequentemente, envolveu apenas pacientes com DM2, neste estudo avaliou-se a atividade constitutiva da DPP4 em pacientes sem diabetes. Com isso, alterações precoces do processo aterosclerótico puderam ser investigadas. Além disso, os dados de fluxo sanguíneo e vasomotricidade foram registrados através da LDF, um método acurado para avaliação da reatividade microvascular cutânea. As limitações do estudo estão relacionadas ao seu desenho transversal, o que significa que ele serve apenas para testar associação, mas não para demonstrar causalidade direta, e ao seu pequeno tamanho amostral, limitando o poder das análises de subgrupo. Ressalta-se ainda que esses resultados não podem ser extrapolados para a população geral, uma vez que a amostra é limitada e envolve apenas indivíduos com sobrepeso/obesidade.

CONCLUSÃO

Em consonância com os estudos que indicam que a enzima DPP4 seja capaz de inativar diversos peptídeos relacionados à inflamação, imunidade e função vascular (20;83), este estudo fornece evidências de que a atividade constitutiva da DPP4 associa-se a marcadores precoces de ativação endotelial pró-inflamatória e de reatividade microvascular e de que ela possa influenciar ou ser influenciada pela inflamação e pelo fluxo sanguíneo em indivíduos com excesso de peso e sem diabetes.Além disso, constatou-se maiores níveis de mediadores pró-inflamatórios ou pró-trombóticos e menor resposta vasomotora relacionada à atividade endotelial nos indivíduos com pré-diabetes em comparação àqueles com normoglicemia, a despeito dosseus valores constitutivos semelhantes de atividadeda DPP4.

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APÊNDICE A –*Dipeptidyl peptidse 4: a new link between diabetes mellitus and atherosclerosis?* (Artigo publicado)

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Review Article

Dipeptidyl Peptidase 4: A New Link between Diabetes Mellitus and Atherosclerosis?

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Type 2 diabetes mellitus (T2DM) has become one of the most prevalent noncommunicable diseases in the past years. It is undoubtedly associated with atherosclerosis and increased risk for cardiovascular diseases. Incretins, which are intestinal peptides secreted during digestion, are able to increase insulin secretion and its impaired function and/or secretion is involved in the pathophysiology of T2DM. Dipeptidyl peptidase 4 (DPP4) is an ubiquitous enzyme that regulates incretins and consequently is related to the pathophysiology of T2DM. DPP4 is mainly secreted by endothelial cells and acts as a regulatory protease for cytokines, chemokines, and neuropeptides involved in inflammation, immunity, and vascular function. In T2DM, the activity of DPP4 seems to be increased and there are a growing number of *in vitro* and *in vivo* studies suggesting that this enzyme could be a new link between T2DM and atherosclerosis. Gliptins are a new class of pharmaceutical agents that acts by inhibiting DPP4. Thus, it is expected that gliptin represents a new pharmacological approach not only for reducing glycemic levels in T2DM, but also for the prevention and treatment of atherosclerotic cardiovascular disease in diabetic subjects. We aimed to review the evidences that reinforce the associations between DPP4, atherosclerosis, and T2DM.

1. Introduction

Atherosclerosis is the leading cause of death and an important cause of morbidity in patients with type 2 diabetes mellitus (T2DM) [1]. However, the mechanisms responsible for the accelerated atherosclerosis observed in T2DM are not yet fully understood [2]. Reduction in the bioavailability of nitric oxide (NO) in the periendothelial environment, which characterizes endothelial dysfunction, is the earliest event in the development of atherosclerosis [2]. Since the occurrence of endothelial dysfunction may be observed before the development of T2DM, it is suggested that these two entities, T2DM and atherosclerosis, may have common pathogenic mechanisms which enhances the possibility of a causal relationship between them [3]. Not only reduced endothelial NO bioavailability, but also inflammation has a role in the promotion of vascular damage in T2DM and has been receiving special attention [4]. Some recent findings add knowledge in these intricate mechanisms and relate the enzyme dipeptidyl peptidase 4 (DPP4) with them.

T2DM has a complex pathophysiology, mainly characterized by insulin resistance (IR) in fat, muscle, and liver tissues associated with pancreatic α and β cell dysfunctions [5, 6]. However, other factors play a role in

the development of T2DM. Among them, stands out the incretin deficiency/resistance [5]. Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are the main incretins secreted by the gastrointestinal tract soon after a meal ingestion [7]. Both are able to enhance insulin secretion in a glucose-dependent fashion while suppressing glucagon secretion [6], although GIP has a more complex relationship with glucagon. Actually, GIP acts as a hormone that stabilizes glucose in T2DM by increasing glucagon response during hypoglycemia, the secretion rate of insulin during hyperglycemia, and both mechanisms when fasting glucose levels are around 8 mmol/L [8].

The state of incretin deficiency/resistance reflects the impairment of the "incretin effect," defined as the amplification of insulin secretion in response to an oral glucose load when compared to the insulin response observed after the same glycemic levels achieved after intravenous glucose infusion [9]. Both GIP and GLP-1 have short half-lives, since they are rapidly degraded by DPP4, an ubiquitous enzyme found in soluble form in plasma or as a membrane component of many cells [10], including endothelial cells [11]. The findings of increased concentrations and activity of DPP4 in patients with diabetes [12–15] may justify, at least partially, the status of incretin deficiency/resistance related to T2DM.

In recent years, new drugs for the treatment of T2DM have emerged into the market, among which the gliptins stand out. These drugs act through the inhibition of DPP4; consequently they are able to ameliorate the incretin deficiency and to attenuate the hyperglucagonemia, two important aspects in the pathophysiology of the T2DM [6]. Gliptins and the GLP-1 receptors agonists comprise the group of incretin-based therapies for T2DM [7].

An important point to emphasize is the capacity of DPP4 to inactivate not only incretins, but also a number of cytokines, chemokines, and neuropeptides involved in inflammation, immunity, and vascular function [16]. Furthermore, the pharmacological inhibition of DPP4 is associated with attenuation of endothelial dysfunction and atherogenesis [17] and also with reduction of inflammatory markers [18]. Considering the higher concentrations and activity of DPP4 in patients with diabetes when compared to nondiabetic subjects [12–15], it is possible that DPP4 constitutes a new link between diabetes and atherosclerosis.

2. DPP4: A Regulatory Serine Exopeptidase

DPP4, also known as adenosine deaminase binding protein or cluster of differentiation 26 (CD26), is a serine exopeptidase able to inactivate various oligopeptides through the removal of N-terminal dipeptides. Its chemical structure remained relatively preserved over evolutionary process and it has been observed in very distinct species, including prokaryotes and eukaryotes organisms [19].

In humans, the DPP4 gene is located on chromosome 2q23, encoding a protein of 766 amino acids [19]. Immediately after its synthesis, DPP4 is incorporated into the plasma membrane of many cell types. It is a type II surface protein, which means that the greatest part of its structure, including the C-terminal domain, is in the extracellular portion [20]. However, under certain stimuli, like IR, tumor necrosis factor α (TNF-α), and chronic low-grade inflammation, DPP4 can be released from the membrane, constituting a soluble form [15, 20, 21].

DPP4 is widely expressed in many specialized cell types and has distinct functions independently of its form, anchored to the membrane or in soluble form. As a cell surface protein, it acts as a regulatory protease and participates in complex mechanisms such as cell-cell interaction and activation of transduction pathways of intracellular signals. The soluble form of DPP4 appears to be derived primarily from endothelial cells, epithelial cells, and leukocytes and, as previously mentioned, it is also endowed with enzymatic activity [20, 28].

DPP4 activation involves a dimerisation process with the formation of a homodimer. The activity of its monomeric form is not significant [29] which possibly explains the apparent dissociation observed between serum levels of DPP4 and its enzymatic activity in humans.

Like other serine proteases, this enzyme has no absolute specificity, although it has a better affinity for oligopeptides composed of proline, hydroxyproline, or alanine as the penultimate residue [19]. DPP4 has currently many known substrates (see Table 1).

Since DPP4 has a wide capacity to act in various peptides, it appears to regulate several physiological pathways involved not only in glucose homeostasis but also in inflammation, immunity, and vascular and cardiac functions [16, 30]. These properties reinforce the hypothesis that this enzyme may act on regulatory mechanisms of endothelial function and inflammatory processes by incretin-dependent and also incretin-independent pathways.

3. The Role of DPP4 in Diabetes Pathophysiology and Related Complications

Since incretin hormones are rapidly degraded by DPP4, it is reasonable to assume that an increase in DPP4 level and/or enzymatic activity may contribute to the impaired incretin effect observed in patients with T2DM [12]. Preliminary studies assessing DPP4 activity in patients with T2DM have shown contradictory results such as reduced [31, 32] or increased activity [12, 13, 30]. However, these disparate results may have occurred due to the use of drugs such as metformin and glitazones, which are both able to promote a decrease in DPP4 activity [33–35].

A more recent study [15] that compared serum levels and plasma activity of the DPP4 among patients with T2DM and healthy subjects showed significant higher levels and activity of the DPP4 in those with diabetes than in controls, but only after excluding patients treated with metformin and/or glitazones.

Interestingly, not only patients with T2DM but also those at prediabetes status appear to have an impairment of incretin effect. It has been shown that patients with prediabetes exhibit decreased GLP-1 and unaltered GIP levels, as compared to those with normal glucose tolerance [36]. Therefore, it is

Table 1: Dipeptidyl peptidase 4 (DPP4) substrates.

β-Casomorphin-2	GLP-1* and -2*	MCP
Aprotinin	Glucagon	MDC
Bradykinin	GRF	Morphiceptin
BNP*	GRP	Neuropeptide Y
CLIP	IGF-1	PACAP27
Chromogranin	IL-1β	PACAP38
Endomorphin-1	IL-2	Procalcitonin
Endomorphin-2	GCP-2 (CXCL6)	Peptide YY
Enterostatin	Mig (CXCL9)	PHM
Eotaxin (CCL11)	IP-10 (CXCL10)	RANTES (CCL5)
Monomeric fibrin (α-chain)	I-TAC (CXCL11)	Substance P*
GHRH	SDF-1α and -1β (CXCL12)*	Vasostatin I
GIP*	LD78β (CCL3L1)	VIP

BNP: B-type natriuretic peptide, formerly named brain natriuretic peptide; CLIP: corticotropin-like intermediate lobe peptide; GHRH: growth hormone-releasing hormone; GIP: glucose-dependent insulinotropic polypeptide; GLP-1: glucagon-like peptide 1; GLP-2: glucagon-like peptide 2; GRF: growth hormone-releasing factor; GRP: gastrin-releasing peptide; IGF-1: insulin-like growth factor 1; IL-1β: interleukin-1β; IL-2: interleukin-2; GCP-2: granulocyte chemotactic protein 2; IP-10: interferon γ-inducible protein 10; I-TAC: interferon γ-inducible T cell alpha chemoattractant; SDF-1α: stromal cell-derived factor 1α; SDF-1β: stromal cell-derived factor 1β; LD78β: isoform of macrophage inflammatory protein-1α (MIP-1α); MCP: monocyte chemotactic protein; MDC: macrophage-derived chemokine; PACAP27: pituitary adenylate cyclase-activating peptide 27; PACAP38: pituitary adenylate cyclase-activating peptide 38; PHM: peptide histidine methionine; RANTES: regulated on activation, normal T-cell expressed and secreted; VIP: vasoactive intestinal peptide. *Peptides whose endogenous levels of intact to cleaved forms are significantly different following genetic inactivation or chemical inhibition of DPP4 activity *in vivo*. Adapted from [20, 28, 30].

suggested that the reduction in GLP-1 levels and/or a greater GIP resistance may contribute to impairment in insulin secretion in patients with prediabetes [36, 37].

Regardless of the glucose tolerance status (normal glucose tolerance, prediabetes, or T2DM), a 4-year longitudinal study showed that baseline DPP4 activity and GLP-1 were negatively associated. Moreover, DPP4 activity was an independent predictor of risk for developing prediabetes (relative risk (RR): 2.77; 95% confidence interval (CI): 1.38–5.55; $P < 0.01$) and T2DM (RR: 5.10; 95% CI: 1.48–17.61; $P < 0.05$) after adjustment for confounding risk factors [38]. The hypothesis that the changes in incretins in prediabetes are directly related to DPP4 seems to be a plausible one. Considering the cardiovascular (CV) complications of diabetes, this hypothesis acquired even greater relevance since a number of studies provided evidence for the pleiotropic effects of GLP-1 on the CV system [39–43].

Advanced glycation end products (AGEs) are a well-known consequence of the chronic hyperglycemia related to uncontrolled diabetes. They are formed by nonenzymatic reaction between reducing sugars and amino groups of proteins, lipids, and nucleic acids. The interaction between AGE and its receptor (RAGE) elicits oxidative stress generation, thereby evoking proliferative, inflammatory, and fibrotic reactions, which impairs structural integrity and function of many proteins. An active participation of AGEs-RAGE axis in the accelerated atherosclerosis observed in diabetes was already denoted [44]. In respect of DPP4, it was demonstrated that levels of AGEs are independently correlated with the levels of this enzyme [45]. Curiously, AGEs enhance the expression of DPP4 and its release [45], while DPP4 increases RAGE gene expression [46], suggesting the existence of a cross talk between the AGEs-RAGE axis and DPP4 in the pathogenesis of diabetes-associated complications [44].

4. Interaction between DPP4 and Endothelium

Endothelial cells independent of their site, that is, microvascular or macrovascular compartments, are probably the main endogenous source of DPP4. Its activity at endothelial milieu appears to be more substantial than that of the circulating form [47]. Endothelial cells from microvascular compartment showed significant increased expression of DPP4, as well as enzymatic activity, after chronic exposure to high glucose concentrations *in vitro* [48]. Microcirculation is the site of tissue nutrition, of gas exchange, and also of removal of cellular excreta and, although DPP4 is present in all vascular beds, hyperglycemia is able to increase the DPP4 activity only from the endothelial cells at the microvascular compartment [49].

In vivo studies added important knowledge about the action of gliptins on atherosclerosis and, interestingly, have suggested that DPP4 inhibition has GLP-1-independent effects, possibly through regulation of other enzyme substrates, acting on attenuation of endothelial dysfunction and atherogenesis [17]. Among them, the chemokine stromal cell-derived factor 1α (SDF-1α) has received special attention. SDF-1α is highly expressed by the human bone marrow endothelium and it is implicated in the migration, proliferation, differentiation, and survival of many cell types, including human hematopoietic stem cells and progenitor cells [50, 51]. This chemokine has its own receptor, named CXCR4, which is a seven-transmembrane G-protein receptor widely expressed by a variety of cell types, including hematopoietic, endothelial, and stromal cells [51]. The SDF-1α-CXCR4 axis participates in the recruitment of endothelial progenitor cells (EPCs) from bone marrow to areas of vascular damage, constituting an important mechanism of vascular repair [52, 53]. There is a positive relationship between the number of EPCs and the improvement in vascular repair and, actually, EPCs are used as a marker to assess endothelial function. It was demonstrated that DPP4 inhibition with a gliptin (sitagliptin) increased the number of EPCs, possibly due to a concomitant increase on the levels of SDF-1α [52]. Furthermore, this mechanism may be also responsible for the observed improvement in endothelial function in patients with T2DM following pharmacological inhibition of DPP4 with other gliptins (vildagliptin) [54]. All these effects mediated by DPP4 inhibition may confer some properties to gliptins that are related to reduction of

endothelial damage and also to improvement in endothelial function, with possibly atheroprotective action.

5. DPP4 and Inflammation

DPP4 also seems to play an important role in low-grade inflammation [55] and particularly in the development of inflammatory reactions in patients with T2DM [15]. IR *per se* and the chronic low-grade inflammation present in T2DM may increase the expression and release of DPP4 from several tissues [15]. Indirect markers of IR, such as fasting insulin and homeostasis model assessment to quantify insulin resistance (HOMA-IR), were positively associated with DPP4 expression in visceral adipose tissue (VAT) macrophages [56]. These macrophages, as well as the visceral adipocytes, were able to release DPP4 when stimulated by TNF-α [56].

On the other hand, treatment of human vascular endothelial cells with sitagliptin is able to inhibit TNF-α induction of plasminogen activator inhibitor type-1 (PAI-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) mRNA and protein expression [17]. DPP4 inhibition is also able to decrease serum levels of inflammatory cytokines, such as interleukin-6 and interleukin-18, in patients with T2DM [18]. Taken together, these evidences suggest the existence of a pathophysiological interaction between DPP4, endothelial dysfunction, and inflammation, factors that are directly linked to the pathogenesis and clinical manifestations of T2DM and atherosclerosis.

6. DPP4 as an Adipokine

Adipose tissue (AT) is definitely an endocrine organ. It expresses and secretes several proteins, known as adipokines, as well as inflammatory cytokines [57, 58]. Adipokines and cytokines participate in the main pathophysiological mechanism linking obesity, IR, T2DM, and atherosclerotic disease [58, 59]. Recently, DPP4 was identified as a new adipokine, possibly linking AT to IR and the metabolic syndrome [21].

In a series of basic and clinical researches, including a proteomic profile of human adipocyte, it was demonstrated that (1) DPP4 is highly expressed in and released by adipocytes; (2) DPP4 inhibits insulin-stimulated Akt phosphorylation in muscle and adipocyte, therefore impeding insulin signaling, and this effect was totally reversed by a DPP4 inhibitor which strongly suggest its role in IR; (3) DPP4 levels are higher in obese as compared to lean subjects and its expression is increased in VAT of obese when compared to subcutaneous adipose tissue (SAT) of obese or lean subjects; (4) DPP4 concentration correlated with several biochemical parameters, such as insulin, leptin (directly), and adiponectin (inversely) [21]. To further refine these observations, Sell et al. [60] studied DPP4 expression and release by VAT and SAT in a cohort of 196 subjects before an open abdominal surgery, by collecting AT biopsies. These authors demonstrated a positive relationship between DPP4 expression and body mass index in both SAT and VAT, with VAT exhibiting higher expression. Furthermore, VAT released more DPP4 than SAT. Interestingly, DPP4 serum levels were higher in insulin resistant as compared to insulin sensitive subjects matched for BMI. Taken together, these data demonstrated that DPP4 is a new adipokine associated with increased visceral obesity, IR, and metabolic syndrome, which are all well-known risk factors for atherosclerotic disease.

Figure 1 provides a schematic diagram illustrating the above-mentioned associations between DPP4, T2DM, insulin resistance, and atherosclerosis.

7. Impact of DPP4 Inhibition on Atherosclerotic Cardiovascular Disease: Some Clinical Aspects

Several DPP4 inhibitors have been launched in the market and are now being used for the treatment of T2DM (vildagliptin, sitagliptin, saxagliptin, linagliptin, and alogliptin) [7]. All of them proved efficacy in glycemic control with impressive safety and tolerance profiles [26]. Gliptins can be used as monotherapy or in combination with other oral agents (in dual or triple therapy) and even with insulin [61]. A systematic review and meta-analyses showed similar efficacy and safety for gliptins as monotherapy or as combination therapy for T2DM [62].

As add-on therapy to metformin, DPP4 inhibitors reduced mean A1c by 0.5–1.1% compared with placebo [63–66]. Considering the glycemic control achieved by a combination of metformin with sulfonylureas or glitazones, gliptins provided comparable improvements [67–69], although with reduced risk of hypoglycemia and weight gain when compared to sulphonylureas and greater cost to the patients. However, in a recent retrospective analysis, gliptins in combination with metformin showed better metabolic control, lower rates of hypoglycemia, and even lower health costs in comparison to metformin and other oral agents in subjects with T2DM and renal impairment [70]. A study [71] comparing gliptins, sulphonylureas, insulin, and GLP-1 receptor agonists for use after metformin is ongoing and will possibly add knowledge about the most appropriate drugs for the treatment of T2DM.

Several meta-analyses of phase III randomized clinical trials (RCTs) have been published evaluating the impact of DPP4 inhibitors on CV outcomes. Frederich et al. [72] and Johansen et al. [73] showed, respectively, that saxagliptin and linagliptin were able to reduce CV outcomes (hazard ratio (HR) from 0.34 to 0.43) when compared to other agents, including placebo. No differences in CV events were observed by Schweizer et al. [74] and Engel et al. [75] when, respectively, vildagliptin and sitagliptin were compared to other oral drugs or placebo. Eighteen RCTs were analyzed together in a meta-analysis that included 8544 patients treated for at least 24 weeks with gliptins or other oral antidiabetic drugs. These investigators found that gliptins may possibly reduce risk of adverse CV events by observing a RR of 0.48 (95% CI: 0.31–0.75; $P = 0.001$) for any adverse CV event and a RR of 0.40 (95% CI: 0.18–0.88, $P = 0.02$) for nonfatal myocardial infarction (MI) or acute coronary syndrome in those treated with a DPP4 inhibitor [76]. Similar results were obtained by Monami et al. [61] in a meta-analysis enrolling

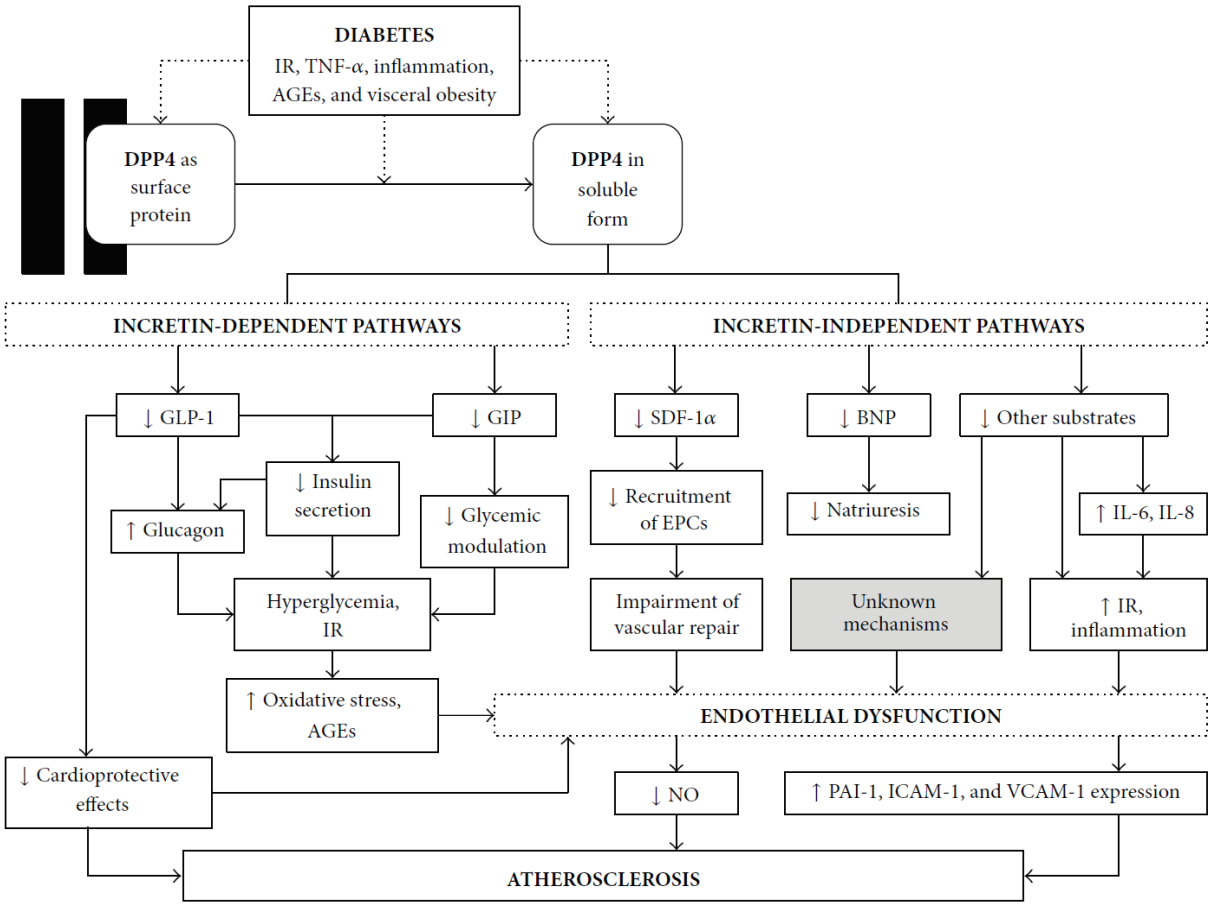

Figure 1: Schematic diagram illustrating the role of DPP4 and its associations with diabetes, insulin resistance, and atherosclerosis. AGEs: advanced glycation end products; BNP: B-type natriuretic peptide; DPP4: dipeptidyl peptidase 4; EPCs: endothelial progenitor cells; GIP: glucose-dependent insulinotropic polypeptide; GLP-1: glucagon-like peptide 1; ICAM-1: intercellular adhesion molecule-1; IL-6: interleukin-6; IL-8: interleukin-8; IR: insulin resistance; NO: nitric oxide; PAI-1: plasminogen activator inhibitor type-1; SDF-1α: stromal cell-derived factor 1α; TNF-α: tumor necrosis factor α; VCAM-1: vascular cell adhesion molecule-1.

almost 42000 patients with T2DM. They found that gliptins promoted a 29% reduction in major cardiovascular events (MACE), mostly due to reducing MI (<36%) and all-cause mortality (<40%). Individually, vildagliptin and saxagliptin were associated with less MACE [61].

Questions about the validity of these comparisons must be taken in account, since many pitfalls in primary composite endpoints and CV adjudication methods were noted [73]. It is also not clear how these potential benefits may be mediated, but possibly these drugs acted through the improvement in endothelial function, inflammation, and reduction of atherosclerosis [77]. Interestingly, an *in vivo* experiment recently demonstrated that vildagliptin or sitagliptin reduced MI size in rats in a glucose-dependent manner through GLP-1 receptor-protein kinase A pathway [78], while linagliptin attenuated neointima formation after vascular injury and *in vitro* vascular smooth muscle cells proliferation beyond the glucose-lowering effect [79].

Table 2 shows some characteristics of phase IV clinical trials evaluating the impact of long-term DPP4 inhibition on CV outcomes. In RCTs designed to demonstrate noninferiority, alogliptin and saxagliptin were neutral regarding MACE [22, 23]. In the EXAMINE trial [22], involving patients with T2DM who had recent hospitalization for acute coronary syndrome, MACE rates did not differ between those who used alogliptin compared to placebo (HR: 0.96; upper boundary of the one sided repeated confidence interval: 1.16; $P = 0.32$ for superiority; $P < 0.001$ for noninferiority) after a follow-up period greater than 40 months (median of 18 months). In the SAVOR-TIMI 53 trial [23], DPP4 inhibition with saxagliptin did not alter the rate of CV events (HR: 1.0; 95% CI: 0.89 to 1.12; $P = 0.99$ for superiority; $P < 0.001$ for noninferiority), although a higher heart failure hospitalization rate among saxagliptin users has been detected (HR: 1.27; 95% CI: 1.07–1.51; $P = 0.007$) during follow-up (median 2.1 years).

The increased risk of hospitalization for heart failure associated with the use of gliptins still requires further analysis. In a later study with SAVOR-TIMI 53 data [80], it was demonstrated that although the absolute risk of hospitalization for heart failure was highest among the 12.8% of patients who had a history of this condition, the relative risk

Table 2: Prospective, randomized, controlled trials involving DPP4 inhibitors (gliptins) and cardiovascular outcomes in diabetic patients.

Gliptin versus comparator	Study	Doses (mg/day)	Composite primary endpoints	Population
Alogliptin versus placebo [22]	*Examination of cardiovascular outcomes with alogliptin versus standard of care* (*EXAMINE*)*	6.25, 12.5, or 25	Nonfatal MI, nonfatal stroke, or CV death	Patients with T2DM recently hospitalized for an ACS ($n = 5380$)
Saxagliptin versus placebo [23]	*Saxagliptin assessment of vascular outcomes recorded in patients with diabetes mellitus – thrombolysis in myocardial infarction 53 trial* (*SAVOR-TIMI 53*)**	2.5 or 5	Nonfatal MI, nonfatal ischemic stroke, or CV death	High-risk CV patients with T2DM ($n = 16492$)
Linagliptin versus glimepiride [24]	*Cardiovascular outcome study of the DPP-4 inhibitor linagliptin* (*CAROLINA*)**¶	5	Nonfatal MI, nonfatal stroke, hospitalization for unstable angina, or CV death	High-risk CV patients with T2DM ($n = \sim6000$)
Sitagliptin versus placebo [25]	*Trial to evaluate cardiovascular outcomes after treatment with sitagliptin* (*TECOS*)***¶	50 or 100	Nonfatal MI, nonfatal stroke, or hospitalization for unstable angina	Patients with T2DM and previous CV disease ($n = \sim14000$)

ACS: acute coronary syndrome; CV: cardiovascular; MI: myocardial infarction; T2DM: type 2 diabetes mellitus. *Superiority trial. **Noninferiority and superiority trial. ***Noninferiority trial. ¶Ongoing study. This is adapted from [26, 27].

of hospitalization for the same cause among patients assigned to saxagliptin was similar regardless of the baseline history (HR: 1.21; 95% CI: 0.93–1.58 versus HR: 1.32; 95% CI: 1.04–1.65; $P = 0.68$ for interaction). Moreover, in a reanalysis of the EXAMINE trial [81], including patients with a history of heart failure and/or high baseline levels of N-terminal pro-B-type natriuretic peptide, there was no evidence of an increased risk of CV outcomes or the rate of hospitalization for heart failure among patients assigned to alogliptin compared to placebo. During follow-up, alogliptin did not induce the onset of heart failure in patients without this diagnosis, or worsening of symptoms in patients with this previous diagnosis [81].

Despite these evidences, in a recent meta-analysis of 94 RCTs enrolling 85224 patients, including data from SAVOR-TIMI 53 and EXAMINE trials, Savarese et al. [82] observed that gliptins did not affect all-cause and CV mortality, as well as stroke, both in short- (<29 weeks) and long-term (≥29 weeks) therapies. With respect to the risk of MI, they also noted that gliptins reduced this risk in short-term treatment (RR: 0.58; 95% CI: 0.36–0.94; $P = 0.02$), but it did not persist in the long-term. Furthermore, long-term treatment with gliptins was associated with a 15.8% increase in the risk of heart failure (RR: 1.15; CI: 1.01–1.32; $P = 0.03$). So, it is still not possible to rule out the existence of an interaction between DPP4 inhibition and heart failure. As mentioned above, B-type natriuretic peptide (BNP) and substance P are both substrates of the DPP4 enzyme and may have implications on the possible association between heart failure and gliptins use, since it is already known that BNP levels increased more than 100 times in patients with heart failure and substance P is able to increase sympathetic activity during combined inhibition of angiotensin-converting enzyme and DPP4 [30, 77, 83, 84].

Regarding the risk of MI, patients enrolled in RCTs assessing the CV safety of gliptins have some characteristics that could be responsible for the observed diversities in the obtained results. To investigate it, Dicembrini and Mannucci [85] performed a meta-analysis with RCTs designed for glycemic endpoints that had a duration of 52 weeks or longer. All RCTs were identified from Savarese et al. [82], except those studies with a CV endpoint. During a mean follow-up of 86.3 weeks, gliptins were associated with a significant reduction of MI (RR: 0.48; 95% CI: 0.31–0.73; $P = 0.001$) similar to that observed in short-term therapy by Savarese et al. [82]. The authors concluded that maybe gliptins have a protective effect only in earlier stages of the natural history of T2DM (i.e., in younger subjects with a short duration of disease and without an established CV disease) whereas this benefit is lost in older patients with already established CV disease [85]. Therefore, there seems to be a window of opportunity for gliptins to reduce CV outcomes in subjects with T2DM that must be further investigated with studies primarily aimed at CV outcomes. The CAROLINA [24] and TECOS [25] trials, involving, respectively, linagliptin and sitagliptin, are still in progress and will possibly provide additional important information about the impact of pharmacological inhibition of DPP4 on CV outcomes.

8. Conclusion

The activity of DPP4 seems to be increased in patients with T2DM and there are a fair number of *in vitro* and *in vivo* studies demonstrating that this enzyme is able to interact with proinflammatory pathways and to impair endothelial function through incretin-dependent and independent mechanisms, potentially providing a new link between T2DM and atherosclerosis. In this way, it has been demonstrated that DPP4 is a new adipokine associated with increased visceral obesity, IR, and metabolic syndrome, which is consistent with its possible link with atherosclerosis. Many studies

showed that DPP4 inhibition attenuated endothelial dysfunction, inflammation, and atherosclerotic process, but available phase IV studies did not associate the use of gliptins with reduced CV events in T2DM. In light of current evidence, we believe that further clinical studies with gliptins are warranted, especially those primarily aimed to investigate cardiovascular outcome.

Abbreviations

AGEs:	Advanced glycation end products
AT:	Adipose tissue
BMI:	Body mass index
BNP:	B-type natriuretic peptide
CD26:	Cluster of differentiation 26
CI:	Confidence interval
CV:	Cardiovascular
DPP4:	Dipeptidyl peptidase 4
EPCs:	Endothelial progenitor cells
GIP:	Glucose-dependent insulinotropic polypeptide
GLP-1:	Glucagon-like peptide 1
HR:	Hazard ratio
HOMA-IR:	Homeostasis model assessment to quantify insulin resistance
ICAM-1:	Intercellular adhesion molecule-1
IR:	Insulin resistance
MACE:	Major cardiovascular events
MI:	Myocardial infarction
NO:	Nitric oxide
PAI-1:	Plasminogen activator inhibitor type-1
RAGE:	Receptor for advanced glycation end products
RCTs:	Randomized clinical trials
RR:	Relative risk
SAT:	Subcutaneous adipose tissue
SDF-1α:	Stromal cell-derived factor 1α
T2DM:	Type 2 diabetes
TNF-α:	Tumor necrosis factor α
VAT:	Visceral adipose tissue
VCAM-1:	Vascular cell adhesion molecule-1.

Conflict of Interests

Wellington Santana da Silva Júnior has no conflict of interests to disclose. Amélio Fernando de Godoy-Matos has received honoraria for lectures, travel support, and consultancy services from pharmaceutical companies manufacturing diabetes treatments, including Novartis, Novo Nordisk, and Takeda. He was also a Principal Investigator for clinical trials involving GLP-1RA from Sanofi-Aventis. Luiz Guilherme Kraemer-Aguiar is a Principal Investigator of a clinical trial involving Vildagliptin from Novartis and receives a research grant from the National Council for Scientific and Technologic Development (CNPq) and from the Carlos Chagas Filho Foundation for Research Support in the State of Rio de Janeiro (FAPERJ).

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APÊNDICE B –*Constitutive DPP4 activity, inflammation and microvascular reactivity in people with excess body weight and without diabetes* (Artigo submetido)

Constitutive DPP4 activity, inflammation and microvascular reactivity in people with excess body weight and without diabetes

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ABSTRACT

Objective

In patients with diabetes, dipeptidyl peptidase 4 (DPP4) inhibition is associated with attenuation of inflammation and endothelial dysfunction. Here, we investigated the association between constitutive DPP4 activity, inflammatory biomarkers, and microvascular reactivity in subjects with excess body weight without diabetes.

Design

This was a cross-sectional study in which participants were subjected to a screening phase before being eligible to participate in the study.

Methods

Forty subjects of BMI≥25.0 kg/m² and absence of diabetes were evaluated. Microvascular blood flux and vasomotion were assessed by laser Doppler flowmetry. The following variables were measured at baseline, 30 and 60 min after a standardized meal: DPP4 activity, glucose, insulin, hs-CRP, TNF-α, IL-6, PAI-1, ICAM-1, and VCAM-1.

Results

DPP4 activity was inversely correlated to VCAM-1 at baseline ($P<0.05$) and DPP4 $activity_{AUC}$ was inversely correlated with the myogenic $component_{AUC}$ of vasomotion ($P<0.05$). In multiple regression analysis, HOMA-AD, IL-6, VCAM-1, PAI-1, blood flux, and vasomotion influenced DPP4 activity and explained almost 40% of the variance on it. When HOMA-AD, VCAM-1, and blood flux were respectively placed as dependent variables, DPP4 activity exerted a significant effect in all of them.

Conclusions

Constitutive DPP4 activity was associated with early markers of endothelial proinflammatory activation and microvascular function and may have an influence and even be influenced by

inflammation and microvascular blood flux in subjects with excess body weight without diabetes.

INTRODUCTION

Dipeptidyl peptidase 4 (DPP4) is a serine exopeptidase able to inactivate various oligopeptides composed of proline, hydroxyproline, or alanine as the penultimate residue [1]. Immediately after DPP4 synthesis, it is incorporated to the plasma membrane of many cell types [2]. However, under certain inflammatory stimuli, it can be released from the membrane as a soluble form also endowed with enzymatic activity, possibly derived primarily from endothelial cells, epithelial cells, leukocytes, and adipose tissue [2-4].

In recent years, DPP4 has received attention due to its ability to rapidly inactivate the main incretins secreted by the gastrointestinal tract: glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide [5]. It seems to be particularly relevant to people with type 2 diabetes, in which DPP4 activity correlates positively with worse metabolic control (14). However, DPP4 not only inactivate incretins, but also a number of cytokines, chemokines, and neuropeptides involved in inflammation, immunity, and vascular function [8,9]. Furthermore, evidence from *in vitro* and *in vivo* studies, including clinical ones in patients with type 2 diabetes and excess weight, suggested pharmacological inhibition of DPP4 was associated with reduction of inflammatory biomarkers [10-12] and also attenuation of endothelial dysfunction and atherogenesis [7,13,14], possibly through regulation of the DPP4 substrates [9].

There is a paucity of studies that associate the constitutive DPP4 activity (*i.e.*, outside the context of pharmacological inhibition of the enzyme) with markers of inflammation and endothelial function, specially tested on skin microcirculation. Moreover, once DPP4 inhibitors are used to treat type 2 diabetes, studies evaluating DPP4 activity that involves individuals without type 2 diabetes are even scarcer. We hypothesized that constitutive DPP4 activity might be directly associated to inflammation and inversely correlated with skin blood

flux and one or more components of vasomotion even in the absence of diabetes. Our aim was to investigate the association between constitutive DPP4 activity, inflammatory biomarkers, and skin microvascular reactivity in subjects with excess body weigth, normoglycemia and prediabetes.

SUBJECTS AND METHODS

Subjects

Subjects with overweight and obesity (n = 40) were recruited after clinical and laboratorial assessments. The study protocol was approved by the local Ethics Committee (CAAE: 24360513.1.0000.5282) and all participants provided their written informed consent after full explanation of the purpose and nature of all procedures used. No stipends were given to the participants. This study is registered in ClinicalTrials.gov (NCT03178019).

Subjects were subjected to a screening phase before being eligible to participate in the study, which comprised individual clinical history, physical examination, and measurements of weight and height to calculate BMI. Fasting plasma glucose (FPG), total cholesterol, high density lipoprotein, and triglycerides, with a calculated low-density lipoprotein, were assessed after 8-h overnight fast. A 2-h plasma glucose (PG) after a 75-g oral glucose tolerance test (OGTT) was also performed in all volunteers.

Inclusion criteria were: men and women aged between 18 and 50 years, body mass index (BMI) $\geq$ 25.0 kg/m² and the presence of different degrees of glucose tolerance, according to the American Diabetes Association (ADA) criteria [15]: (1) normoglycemia/normotolerance (NGT group): FPG <100 mg/dL and 2-h PG in the 75-g OGTT <140 mg/dL; (2) prediabetes (impaired fasting glucose and/or impared glucose intolerance; Pre-DM group): FPG 100

mg/dL to 125 mg/dL and/or 2-h PG in the 75-g OGTT 140 mg/dL to 199 mg/dL. Hemoglobin A1c was not used for the evaluation of the degree of glucose tolerance.

Exclusion criteria were: type 2 diabetes (diagnosis during recruitment period or pre-existing diagnosis or in use of any antidiabetic drugs); BMI <25.0 kg/m²; uncontrolled chronic diseases, such as arterial hypertension; smoking; severe alcoholism; moderate to severe chronic kidney disease, heart failure, chronic lung disease, and chronic liver disease; fasting serum triglycerides >400 mg/dL; fasting serum cholesterol >300 mg/dL; pregnancy and breastfeeding; women in the climacteric period; individuals who undergo bariatric surgery; acute disease at the time of sampling, defined as the presence of moderate to severe malaise, with or without fever; initiation of statin or change in its dose within 60 days; use of aspirin and/or fluconazole within 10 days prior to the exams.

Study design

This was a cross-sectional study in which participants were subjected to a screening phase before being eligible to participate in the study. After a 15 min acclimatization period, their blood pressure levels and behavior were evaluated during 10 min using the Finometer PRO (Finapres Medical Systems®, Amsterdam, Netherlands). Records of skin microvascular blood flux lasted up to 65 min. After the first 5 min, the participants ingested a standardized meal (247 kcal, 64.5% carbohydrates, 19.5% protein, 16.0% fat) during up to 5 min. Subjects were also subjected to three venous blood collections: baseline (fasting state), 30 and 60 min after the intake of the above-mentioned meal (Fig. 1).

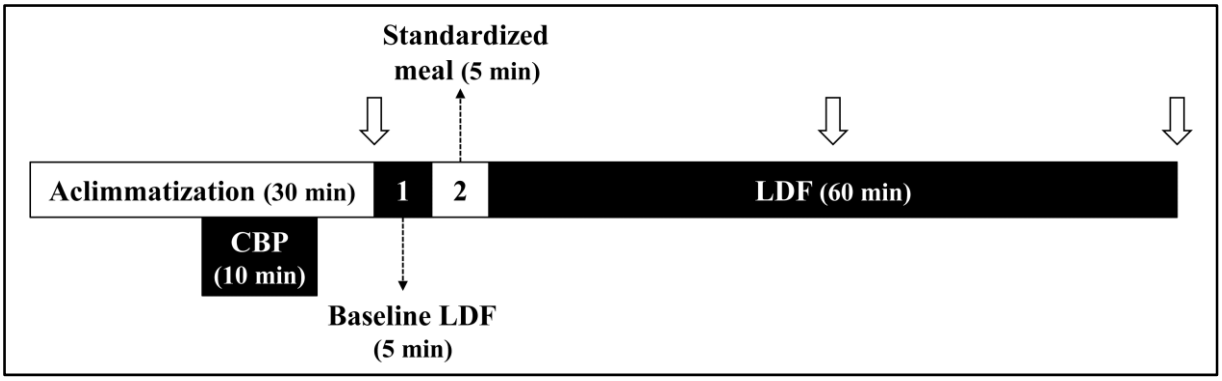

Figure 1 – Experimental design. Empty arrows represents venous blood collection at baseline and 30 and 60 min after a standardized meal. CBP: continuous blood pressure; LDF: laser Doppler flowmetry.

Laboratory analysis

On the morning of the exams, after an 8-h overnight fast, subjects were accommodated on the examination chair, in a temperature-controlled room (22 ± 1 °C). An intravenous catheter was inserted in the subject's right antecubital vein and kept in place during the exam for venous blood collections. Ethylenediamine tetraacetic acid (EDTA) tubes containing blood samples were centrifuged immediately after collections at 1.300 g for 15 min at 4 °C and plasma samples were clarified by filtration (Millex filter with polyethersulfone membrane, 33 mm, Merck Millipore®, Tullagreen, Ireland), aliquoted, and stored at -80 °C.

All laboratory measurements were performed in duplicate, as follows: (1) baseline: plasma DPP4 activity (colorimetry; sensibility: 0.1 μM/mL/min; intra-assay coefficient of variation [IACV]: <3%); PG (glucose oxidase); high-sensitivity C-reactive protein (hs-CRP) (turbidimetry); insulin (Luminex; Milliplex®, HMHMAG-34K; sensibility: 15 pmol/L; IACV: <10%; inter-assay coefficient of variation [IECV]: <20%); tumor necrosis factor-α (TNF-α [ELISA; R&D Systems®; HSTA00E; sensibility: 0.049 pg/mL; IACV: <3%; IECV: <7%]); interleukin-6 (IL-6 [ELISA; R&D Systems®; HS600B; sensibility: 0.11 pg/mL;

IACV: <8%; IECV: <10%]); plasminogen activator inhibitor-1 (PAI-1 [sensibility: 4.1 pg/mL; IACV: <5%; IECV: <14%]) and adiponectin (sensibility: 11 pg/mL; IACV: <4%; IECV: <10%) (Luminex; Milliplex®, HADK1MAG-61K); intercellular adhesion molecule-1 (ICAM-1 [sensibility: 0.0019 ng/mL; IACV: <15%; IECV: <20%]) and vascular cell adhesion protein-1 (VCAM-1 [sensibility: 0.024 ng/mL; IACV: <15%; IECV: <20%]) (Luminex; Milliplex®, HCVD2MAG-67K); (2) at 30 and 60 min after meal intake, additional samples for plasma DPP4 activity, PG, insulin, hs-CRP, TNF-α, IL-6, PAI-1, ICAM-1, and VCAM-1 were collected, prepared, and stored.

FPG, insulin, and adiponectin were used to calculate the homeostasis model assessment-adiponectin (HOMA-AD) as follows: HOMA-AD = insulin (μUI/mL) x FPG (mg/dL)/adiponectin (μg/mL) [16]. Considering the purpose of this study and the implications of insulin resistance (IR) on the DPP4 activity [4,17], we decided to use HOMA-AD among others IR indexes because to calculate it we need the levels of adiponectin [16], a cytokine with anti-inflammatory and anti-atherogenic properties [18].

Skin microvascular blood flux and vasomotion

Skin microvascular blood flux and vasomotion were assessed by single-point laser Doppler flowmetry (LDF). Single-point LDF (comprising one transmitting and one receiving optical fiber) is accurate to quantify fast changes in skin blood flux [19]. Briefly, LDF provide an index of skin perfusion by measuring the Doppler shift induced by coherent monochromatic light scattering by moving red blood cells. The signal is quantified as the product of average red blood cell velocity and concentration. Since it does not provide an exact measure of flow (mL/min), it is often referred as flux, expressed in arbitrary units (pefusion units - PU) [19].

After baseline blood sample collections and acclimatizing patients into a room temperature of 22 ± 1 ºC for 30 min, blood flux and vasomotion were recorded using PeriFlux System (Perimed®, Stockholm, Sweden). These records lasted for 65 min and the probe was positioned at the dorsal side of the left wrist, according to our previously published protocol [20].

To determine the contribution of different vasomotion frequency components to the variability of the flux signal, the obtained signals were submitted to spectral analysis through fast Fourier transform using PeriSoft for Windows 2.5 software (Perimed®, Stockholm, Sweden). Five frequency intervals previously defined [21] were assumed in the spectrum between 0.01 and 1.6 Hz: (1) endothelial (0.01–0.02 Hz); (2) neurogenic (0.02–0.06 Hz); (3) myogenic (0.06–0.15 Hz); (4) respiratory (0.15–0.4 Hz); and (5) cardiogenic (0.4–1.6 Hz) activities.Absolute amplitude variations within each frequency band were analyzed by bivariate analysis and their normalized values, defined as the interval of the mean amplitude divided by the total spectrum mean amplitude [21], were analyzed by univariate analysis.

Statistical analysis

We used GraphPad Prism® 5 (GraphPad Software Inc., San Diego, CA, USA) and STATISTICA® 7.0 (StatSoft Inc., Tulsa, OK, USA) for statistical analysis. Gaussian distribution was checked and parametric and non-parametric data are expressed as mean±SD and median [1st–3rd quartiles], respectively. We used unpaired *t*-test and U test accordding to data distribution to compare groups. In order to optimize data obtained during fasting plus post-prandial periods, we proceeded individual regression equations for modeling the relationship between the independent (time) and dependent variables (DPP4 activity, biomarkers of inflammation or components of microvascular function). These equations

found slopes and intercepts for each variable, which define the linear relationship between the dependent (variable tested) and independent (time of collection) variables. Slope represents the steepness of the regression line, which can be positive, negative or zero. The greater the magnitude of the slope, the greater the rate of change. Intercept is the point where this regression line crosses the axis of the dependent variable. Once the independent variable was the time after meal intake, it can be presumed that the intercept will be quite similar to baseline value obtained for each variable, although influenced by the subsequent values measured at 30 and 60 min after meal intake. Slopes and intercepts were also compared between groups. Linear correlations between DPP4 activity and the above-mentioned variables were conducted. In a step further, multiple regression analysis were also performed to test whether some laboratorial–microvascular variables could influence, or be influenced by,the plasma DPP4 activity. We used G*Power 3.1.9.2 (Universität Kiel, Germany) to calculate sample size of 39 patients with an actual power of 0.9500156 (*t* tests; point biserial; two-tailed; effect size of 0.51; α prob error of 0.05; and a power of 0.95). Values of $P<0.05$ were considered statistically significant.

RESULTS

Forty subjects, aged 38.4±8.65 years and BMI 29.1±2.76 kg/m², were included and almost half (47.5%) of them were women. At baseline, we noticed higher BMI, FPG, and basal levels of insulin, IL-6, and PAI-1 in the Pre-DM compared to NGT group (Table 1) and also a trend ($P=0.06$) towards a higher HOMA-AD in the former one. DPP4 activity had no difference between groups. Additionally, we have stretched data to investigate possible intergroup differences by calculating AUC, differences from baseline to 30 min and 60 min (deltas 30-0 and 60-0, respectively), and also slopes and intercepts of the variables. Some differences were

noticed, as follows: PG_{AUC} (7157±749.6 vs. 6339±871.2; P<0.05) and $PAI\text{-}1_{AUC}$ (1652 [1217–2112] vs. 1108 [656.8–1358]; P<0.01) were both higher in Pre-DM compared to NGT group, while $IL\text{-}6_{\Delta 30\text{-}0}$ (-0.27 [-0.44–-0.15] vs. -0.15 [-0.26–-0.01]; P<0.05) and $PAI\text{-}1_{\Delta 60\text{-}0}$ (-10.89±8.85 vs. -6.2±8.31; P<0.05) were both lower in Pre-DM group. These differences in deltas evidence a trend toward a reduction of both inflammatory biomarkers through the time, however more pronounced in the Pre-DM group. No other difference was observed in this analysis and the slopes for DPP4 activity and biomarkers of inflammation were not different between groups as well. On the other hand, we noticed $PG_{intercept}$ and $PAI\text{-}1_{intercept}$ were still different between groups, but another important inflammatory marker has now expressed a distint pattern, represented by a lower $hs\text{-}CRP_{intercept}$ in the Pre-DM compared to NGT group. Therefore, according to our baseline data and also to some tested variables during post-prandial period, NGT and Pre-DM groups, although very phenotypically close to each other, showed some fine-tune particularities, such as the expression of higher levels of proinflammatory and prothrombotic mediators of endothelial activation in the Pre-DM group. Microvascular reactivity also showed some differences between groups. At basal state, the endothelial component of vasomotion was significantly lower in Pre-DM compared to NGT group (3.785 [3.103-5.607] vs. 5.780 [3.583-6.622]; P<0.05), while no intergroup differences were observed in blood flux and flux velocity. The same pattern was evidenced for $endothelial\ component_{AUC}$ of vasomotion, which was also lower in Pre-DM compared to NGT group (269.4±98.44 vs. 335.4±97.12; P<0.05), while no intergroup differences were observed in $blood\ flux_{AUC}$ and $flux\ velocity_{AUC}$. These data points to a lower vasomotion response related to endothelial activity in the Pre-DM group. Neither the slopes nor the intercepts of the microvascular reactivity variables were different between groups.

Table 1. Clinical and biochemical characteristics of subjects at baseline.

	All groups (n = 40)	NGT group (n = 21)	Pre-DM group (n = 19)
Age (years)	38.4 ± 8.65	37.67 ± 8.26	39.21 ± 9.21
Female [n, (%)]	19 (47.5%)	8 (38%)	11 (57%)
BMI (kg/m²)	29.11 ± 2.76	**27.59 ± 1.65**	**30.8 ± 2.8‡**
MAP (mmHg)	82.98 ± 8.37	83.8 ± 7.54	82.09 ± 9.34
DPP4 activity (µM/mL/min)	13.47 ± 3.16	13.85 ± 2.74	13.05 ± 3.59
FPG (mg/dL)	95 [87.5–108]	**89 [82.5–93.5]**	**106 [96–111]‡**
Insulin (pmol/L)	48.38 [30.99–90.91]	**36.67 [27.03–57.3]**	**57.68 [37.53–94.18]***
HOMA-AD	53.41 [26.16–137.8]	41.76 [18.13–82.14]	61.92 [19.26–141.8]
Total cholesterol (mg/dL)	204.2 ± 43.76	205.8 ± 45.99	202.5 ± 42.34
HDL (mg/dL)	51.72 ± 13.84	50.1 ± 10.68	53.53 ± 16.8
LDL (mg/dL)	125.95 ± 35.74	129.0 ± 36.11	122.6 ± 36.01
TG (mg/dL)	113 [87.25–176.3]	109 [89.5–175.5]	113 [86–174]
hs-CRP	0.21 [0.06–0.41]	0.22 [0.09–0.44]	0.17 [0.04–0.35]
IL-6 (pg/mL)	1.31 [1.04–1.91]	**1.19 [0.89–1.47]**	**1.41 [1.18–1.98]***
TNF-α (pg/ml)	0.9 ± 0.21	0.87 ± 0.15	0.92 ± 0.27
PAI-1 (pg/mL)	26.84 [19.86–36.32]	**23.86 [12.0–29.56]**	**31.78 [26.25–42.8]†**
ICAM-1 (ng/mL)	96 [72.7–113.5]	101 [80–109.5]	80 [70–115]
VCAM-1 (ng/mL)	535.5 [440.5–615.8]	527 [452.5–623.5]	566 [426–617]
Screening phase			
FPG (mg/dL)	99.5 [92.25–113]	**92 [85–96.5]**	**107 [100–113]‡**
2-h PG in the 75-g OGTT (mg/dL)	119.1 ± 29.93	**103.0 ± 21.51**	**137.0 ± 28.01‡**
Total cholesterol (mg/dL)	199.47 ± 43.75	196.6 ± 40.46	202.6 ± 48.05
HDL (mg/dL)	54.75 ± 15.22	**48.76 ± 11.73**	**61.37 ± 16.16†**
LDL (mg/dL)	117.43 ± 38.87	123.2 ± 39.48	111.1 ± 38.21
TG (mg/dL)	119.5 [83–170.8]	118 [83–163.5]	140 [82–202]

Data are presented as mean ± SD, median [1st–3rd quartiles], or n (%). *P<0.05. †P<0.01. ‡P<0.001. NGT: normoglycemia; Pre-DM: prediabetes; BMI: body mass index; MAP: mean arterial pressure; DPP4: dipeptidyl peptidase 4; FPG: fasting plasma glucose; PG: plasma glucose; OGTT: oral glucose tolerance test; HOMA-AD: homeostasis model assessment to quantify insulin resistance-adiponectin; TG: triglycerides; hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; PAI-1: plasminogen activator inhibitor type-1; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1.

The correlation of endothelial function and DPP4 activity was our main objective and we gather the pooled sample to test it. Of interest, we observed an inverse linear correlation between DPP4 activity and VCAM-1 (r=-0.30, P<0.05; figure 2A), a marker of endothelial dysfunction. Additonally, data from basal levels at fasting state showed DPP4 activity was directly correlated to HOMA-AD (r=0.45, P<0.01), but no correlation among the former and

hs-CRP, IL-6, TNF-α, PAI-1, and ICAM-1 were found. By including data from post-prandial period, we observed a positive linear correlation between the DPP4 activity$_{\Delta 60\text{-}0}$ and hs-CRP$_{\Delta 60\text{-}0}$ (r=0.31, P<0.05). We also found an inverse correlation between DPP4 activity and the neurogenic (r=-0.33, P<0.05) and myogenic (r=-0.39, P=0.01) components of vasomotion at 30 min during post-prandial period, whereas there were no significant correlations between DPP4 activity and other components of vasomotion. Of note, DPP4 activity$_{AUC}$ had a significant correlation to the myogenic component$_{AUC}$ of vasomotion (r=-0.33, P<0.05; Figure 2B).

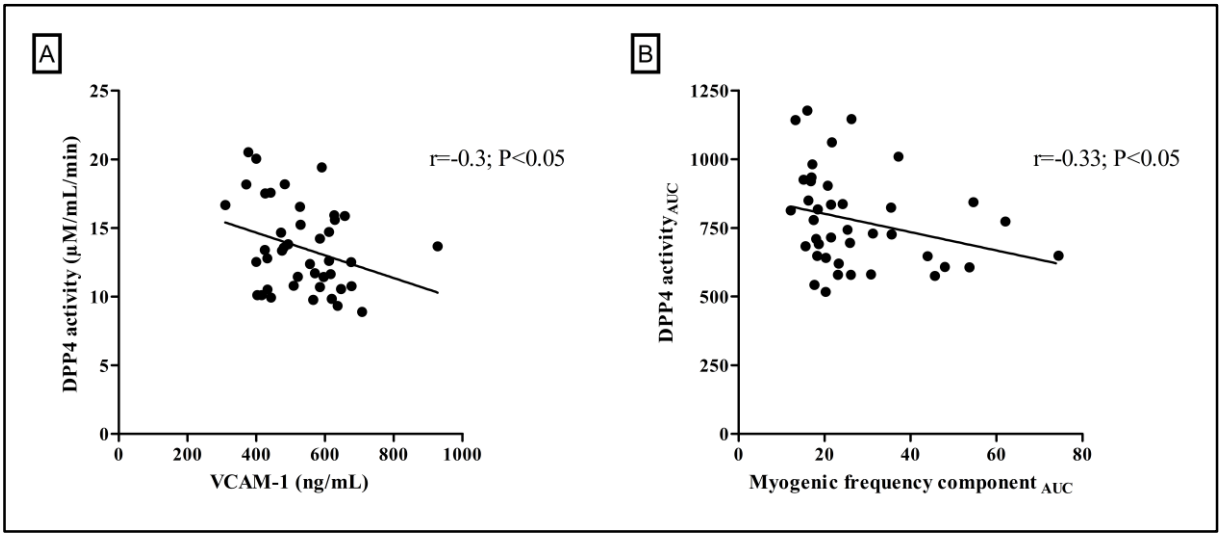

Figure 2 – Scatter plots demonstrating bivariate correlations in the pooled group. Correlations between (A) DPP4 activity and VCAM-1 and (B) DPP4 activity$_{AUC}$ and the myogenic frequency component$_{AUC}$ of vasomotion. DPP4: dipeptidyl peptidase 4; VCAM-1: vascular cell adhesion molecule-1; AUC: area under the curve.

In a step further, we tested whether some laboratorial–microvascular variables could influence, or even be influenced by, DPP4 activity (Table 2). To evaluate it, we opted to use not only DPP4 activity but also HOMA-AD, VCAM-1, and blood flux as dependent variables in models 1, 2, 3, and 4, respectively. Our results showed HOMA-AD, IL-6, VCAM-1, PAI-

1, blood flux, and vasomotion influenced DPP4 activity (model 1) and explained approximately 40% of the variance on it. HOMA-AD, VCAM-1, and blood flux exerted the greater effect on DPP4 activity. Considering HOMA-AD, VCAM-1, and blood flux as dependent variables, it is worth to point out that DPP4 activity exerted a significant effect in all these models, suggesting the existence of a bidirectional influence among inflammation, microvascular reactivity, and plasma DPP4 activity that seems to occur early in the spectrum of glucose tolerance in subjects with overweight/obesity.

Table 2. Multiple regression analysis of the pooled group.

Model	**B**	**P-value**	**Adjusted R^2**	**P-value**
1.			0.413	<0.001
HOMA-AD	**0.537**	**0.0003**		
VCAM-1	**-0.385**	**0.006**		
Blood flux	**0.422**	**0.006**		
Neurogenic	-0.195	0.172		
IL-6	-0.160	0.206		
2.			0.380	<0.001
DPP4 activity	**0.573**	**0.0002**		
Blood flux	**-0.370**	**0.012**		
VCAM-1	0.226	0.124		
PAI-1	0.148	0.278		
3.			0.226	<0.05
Blood flux	**0.468**	**0.004**		
DPP4 activity	**-0.504**	**0.005**		
HOMA-AD	0.300	0.103		
IL-6	-0.165	0.251		
4.			0.637	<0.001
Endothelial	**0.335**	**0.014**		
VCAM-1	0.186	0.113		
DPP4 activity	**0.262**	**0.043**		
HOMA-AD	-0.166	0.200		
IL-6	0.202	0.052		
Respiratory	**-0.977**	**0.00006**		
Cardiogenic	**1.060**	**0.00008**		
PAI-1	-0.154	0.155		

DPP4 activity, HOMA-AD, VCAM-1, and blood flux were considered as dependent variables in models 1, 2, 3, and 4, respectively. "Neurogenic", "endothelial", "respiratory", and "cardiogenic" refer to the respective component of vasomotion. HOMA-AD: homeostasis model assessment-adiponectin; VCAM-1: vascular cell adhesion molecule-1; IL-6: interleukin-6; DPP4: dipeptidyl peptidase 4; PAI-1: plasminogen activator inhibitor type-1.

DISCUSSION

Far beyond DPP4 enzyme´s known actions on glucose metabolism, this enzyme is able to inactivate a number of peptides involved in inflammation, immunity, and vascular function [8,9] and, in patients with type 2 diabetes, pharmacological inhibition of DPP4 is associated with reduction of circulating inflammatory cytokines [10] and also in attenuation of endothelial dysfunction and atherogenesis [13,14]. Here, we investigated the association between constitutive DPP4 activity, inflammatory biomarkers, and microvascular function in subjects with excess body weight and normoglycemia or prediabetes. To the best of our knowledge, it is the first study evaluating these relationships in subjects without diabetes. Since microvascular dysfunction is considered a systemic process that occurs in a similar manner in multiple tissue beds throughout the body [22], we used human skin microcirculation as a model of generalized microvascular function.

According to this, we have observed some important associations between DPP4 activity and the elected variables herein tested. Our main finding is that constitutive DPP4 activity were associated to inflammatory biomarkers and microvascular function, since we observed correlations between DPP4 activity and VCAM-1 at baseline, and also between DPP4 $activity_{AUC}$ and the myogenic $component_{AUC}$ of vasomotion. Futhermore, in multiple regression analysis, we confirmed our hypothesis that there is an interaction between DPP4 activity, inflammation, and microvascular function that occurs early in the spectrum of glucose tolerance.

Interestingly, we found an inverse correlation between baseline DPP4 activity and VCAM-1, an early marker of vascular inflammation [23]. It was previously demonstrated that treatment of human vascular endothelial cells with sitagliptin, a DPP4 inhibitor, is able to inhibit TNF-α induction of VCAM-1 mRNA and its expression [7]. This apparent disparity may be

explained by the fact that measurements of soluble VCAM-1 is not necessarily representative of its expression on the cell surface [24]. Additionally, in multiple regression analysis, VCAM-1 and DPP4 activity exerted a significant effect on each other (β=-0.385, $P<0.01$ to VCAM-1 as independent variable in model 1 and β=-0.504, $P<0.01$ to DPP4 activity as independent variable in model 3). Regarding microvascular function, we found a negative correlation between DPP4 $activity_{AUC}$ and the $myogenic\ component_{AUC}$ of vasomotion. This finding is in accordance with a previous experimental study involving human vascular smooth muscle cells (hVSMC), that shows soluble DPP4 directly and markedly activating the mitogen activated protein kinases and nuclear factor kappa B signaling pathways, leading to pro-atherogenic changes in hVSMC, characterized by increased proliferation and inflammation [25]. The authors also speculated that soluble DPP4 may act in a para- or endocrine fashion on the vascular wall, potentially contributing to inflammation in this setting [25]. Furthermore, it is noteworthy that vasomotion *in vivo* is associated with rhythmic oscillations in vessel diameter that modify/redistribute blood flux [26]. This finding is in agreement with our results, which evidenced that DPP4 activity and blood flux exerted a significant effect on each other (β=0.422, $P<0.01$ to skin blood flux as independent variable in model 1 and β=0.262, $P<0.05$ to DPP4 activity as independent variable in model 4). These models explained almost 40% and 60% of the variance of the DPP4 activity and of the blood flux, respectively, and in summary suggest DPP4 activity would influence and would be influenced by the blood flux.

As expected, BMI, FPG, insulin levels, IL-6, and PAI-1 were significantly higher in Pre-DM in compared to NGT group. We also evidenced a trend towards higher HOMA-AD in the Pre-DM group. Although there were no intergroup differences in blood flux and in flux velocity, the baseline and the $endothelial\ component_{AUC}$ of vasomotion were significative lower in Pre-DM group, suggesting an impairment of the endothelial component in vasomotion in subjects

that already have prediabetes. We highlight no significant differences were observed in baseline levels or in the AUC of DPP4 activity between Pre-DM and NGT groups. Information regard DPP4 activity in subjects with prediabetes, in comparison to normoglycemic subjects, are scarce and conflitant [27,28]. Although one study had results in accordance to our findings [27], another one evidenced higher DPP4 activity in subjects with prediabetes [28]. The differences in IL-$6_{\Delta 30\text{-}0}$ and in PAI-$1_{\Delta 60\text{-}0}$ between NGT and Pre-DM groups suggest a trend to reduction of both inflammatory biomarkers through time, however in different moments after the standardized meal intake. Curiously, both reductions were more pronounced in Pre-DM group. The absence of differences in DPP4 $activity_{slope}$ and also in the slopes of inflammatory biomarkersbetween NGT and Pre-DM groups suggests the rate of change in each variable after meal intake are similar in both groups. Moreover, as expected, the intercepts of each variable were quite similar to their respective baseline levels, although influenced by its values measured at 30 and 60 min after standardized meal intake. So, the findings of higher $PG_{intercept}$ and PAI-$1_{intercept}$ in the Pre-DM group in comparison to NGT group are reflections of the results of baseline between-groups comparisons of the FPG and PAI-1 levels.

It is already known that actions of proinflammatory agonists, as well as biomechanical stimulation by disturbed blood flow, leads to endothelial activation [29]. These biochemical and biomechanical stimuli result in a coordinated program of genetic regulation within the endothelial cell, which includes the cell surface expression of adhesion molecules, such as VCAM-1, and secreted and membrane-associated chemokines and prothrombotic mediators, like PAI-1 [29]. These events foster the selective recruitment of monocytes and T lymphocytes, which become resident in the subendothelial space and perpetuate a chronic proinflammatory state that results in atherosclerotic lesion progression [29]. In this study, we demonstrated that constitutive DPP4 activity interacts with inflammation and blood flux even

in early stages of the spectrum of glucose tolerance, which suggests this enzyme could be involved in early atherosclerotic process in subjects with excess body weight without diabetes.

Our study has strengths and limitations. Absolute majority of studies evaluate DPP4 activity under pharmacological inhibition and, consequently, they involve only subjects with type 2 diabetes.Moreover, we assessed skin blood flux and vasomotion by single-point LDF, an accurate method to evaluate microvascular function. Limitations of the study are associated with its cross-sectional design, implying it is explicitly correlational and cannot directly demonstrate causality, and with the small sample size, limiting the power of our subgroup analysis. Therefore, we highlight the results cannot be generalized to the general population, since the study sample is limited and involved only subjects with overweight/obesity.

In accordance with previous studies suggesting DPP4 is able to inactivate a number of peptides involved in inflammation, immunity, and vascular function [8,9], this study provides evidences that constitutive DPP4 activity was associated with early markers of endothelial proinflammatory activation and microvascular function and it seems to influence and also be influenced by inflammation and blood flux in subjects with excess body weight without diabetes.

DECLARATION OF INTEREST

The authors declared no conflict of interest.

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AUTHOR CONTRIBUTIONS

WSSJ researched and analysed data and wrote the manuscript. MGCS and JFNN researched data. EB reviewed the manuscript. LGKA designed the study, analysed data, contributed to the discussion, and reviewed/edited the manuscript. All authors had final approval of the submitted and published versions.

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APÊNDICE C –*Dipeptidyl peptidase 4 activity is related to body composition, measures of adiposity, and insulin resistance in subjects with excessive adiposity and different degrees of glucose tolerance* (Artigo submetido)

Dipeptidyl peptidase 4 activity is related to body composition, measures of adiposity, and insulin resistance in subjects with excessive adiposity and different degrees of glucose tolerance.

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ABSTRACT

Background

The enzyme dipeptidyl peptidase 4 (DPP4) has been recently recognized as an adipo-myokine. However, studies that associate its constitutive activity with body composition, anthropometry, and insulin resistance (IR) are very scarce and included only healthy people.

Methods

First, we investigated the relationships of constitutive DPP4 activity, body composition (assessed by bioelectrical impedance analysis), and measures of adiposity and IR in fifty-two subjects of both sexes, 18-50 years, and BMI ≥25.0 kg/m² who comprised three groups according to glucose tolerance. Additionally, we evaluated associations among DPP4 activity and adipokines, gut peptides, and biochemical variables at fasting and 30 and 60 min after a standardized meal intake.

Results

DPP4 activity was no different among the three groups. At fasting, pooled analysis showed it was direct correlated to measures of central adiposity, such as WC ($P<0.05$) and WHR ($P<0.01$), and to all measures of IR, but inversely related to indexes of general adiposity, such as fat mass percentage ($P<0.05$) and BAI ($P<0.001$). DPP4 activity was also associated to lean mass ($r=0.58$, $P<0.001$). After meal intake, DPP4 activity remained significantly associated to insulin, leptin, and resistin and, in multiple regression analysis, BAI, WHR, percent lean mass, HOMA-IR, and leptin influenced DPP4 activity and explained approximately 26% of the variance on it.

Conclusions

Constitutive DPP4 activity is positively associated to lean mass, central adiposity, and IR and negatively to general adiposity. Futhermore, it seems to be influenced by body composition

and IR and could be also viewed as an adipo-myokine in subjects with excessive adiposity and different stages of glucose tolerance.

Trial registration: ClinicalTrials.gov; ID: NCT03178019.

Keywords: Dipeptidyl peptidase 4; Body composition; Anthropometry; Adiposity; Insulin resistance; Bioelectrical impedance analysis.

BACKGROUND

Dipeptidyl Peptidase 4 (DPP4), also known as adenosine deaminase binding protein or cluster of differentiation 26 (CD26), is a serine protease widely expressed by many specialized cell types [1]. DPP4 inactivates various oligopeptides composed of proline, hydroxyproline, or alanine as the penultimate residue [2], including incretin hormones secreted by the gastrointestinal tract soon during post-prandial period: glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Once these incretins are able to enhance insulin secretion in a glucose dependent fashion [3], DPP4 could be considered strictly related to the pathophysiology of type 2 diabetes mellitus [4].

DPP4 is also an adipo-myokine, secreted mainly by adipose tissue [5], but also by human myotubes [6]. As an adipokine, DPP4 is mainly released by fully differentiated adipocytes and its serum levels significantly correlate to adipocyte size [5]. Therefore, it is suggested that it is a marker of visceral obesity, insulin resistance, and metabolic syndrome [7]. The role of DPP4 as a myokine still needs to be understood but, as for many adipo-myokines, their tissue concentrations may be divergent from their serum levels, and distinct auto- and endocrine effects need to be considered [8]. Hypothetically, myokines are essential for muscle metabolism during contraction. On the counterpart, chronic elevation of adipokines may induce adverse effects leading to insulin resistance [8]. There is already evidence that the addition of DPP4 to adipocyte, skeletal and smooth muscle cells inhibits insulin-stimulated Akt phosphorylation, impairing insulin signaling and its action in muscle and fat tissues [5].

Despite of the above mentioned knowledge, a paucity of studies that associate constitutive DPP4 activity with body composition, measures of adiposity, and also with insulin resistance markers is still the rule. We suppose that constitutive DPP4 activity might be directly associated not only to fat mass and markers of insulin resistance, but also to lean mass. Our

aim was to investigate if constitutive DPP4 activity correlates to body composition parameters, measures of adiposity, and insulin resistance in subjects with excessive adiposity and different degrees of glucose tolerance. Additionally, we evaluated the relationships between DPP4 activity, adipokines, and gut peptides during post-prandial period.

METHODS

Subjects

Subjects with excessive adiposity (n=52) were recruited after clinical and laboratorial assessments. The study protocol was approved by the local Ethics Committee of the University Hospital Pedro Ernesto (CAAE: 24360513.1.0000.5282) and all participants provided their written informed consent.

Participants were subjected to a screening phase before being eligible for the study, which comprised individual clinical history, physical examination, and weight and height measurements. Fasting plasma glucose (FPG), total cholesterol, high-density lipoprotein, and triglycerides, with a calculated low-density lipoprotein by Friedewald equation, were assessed after 8-h overnight fast. A 2-h plasma glucose (PG) after a 75-g oral glucose tolerance test (OGTT) was also performed in all volunteers without previous diagnosis of diabetes mellitus.

The inclusion criteria were: men and women aged between 18 and 50 years, BMI $\geq$25.0 kg/m² and the presence of different degrees of glucose tolerance comprising three groups according to the American Diabetes Association (ADA) criteria [9]: (a) normoglycemia/normotolerance (NGT group): FPG <100 mg/dL and 2-h PG in the 75-g OGTT <140 mg/dL; (b) prediabetes (impaired fasting glucose and/or impaired glucose intolerance; Pre-DM group): FPG 100 mg/dL to 125 mg/dL and/or 2-h PG in the 75-g OGTT 140 mg/dL to 199 mg/dL; and (c)

diabetes mellitus (DM group): FPG ≥126 mg/dL and/or 2-h PG in the 75-g OGTT ≥200 mg/dL or a pre-existing diagnosis, exclusively in use of insulin and/or metformin.

Exclusion criteria were: type 1 diabetes mellitus; the use of any antidiabetic drug, except metformin and insulin (metformin was discontinued for 14 days prior to the exams due to its interference on DPP4 activity [10] and insulin doses were adjusted to avoid hyperglycemia); BMI <25.0 kg/m²; uncontrolled chronic diseases, such as arterial hypertension; smoking; severe alcoholism; moderate to severe chronic kidney disease, heart failure, chronic lung disease, and chronic liver disease; fasting serum triglycerides >400 mg/dL; bariatric surgery; acute disease at the time of sampling, defined as the presence of moderate to severe malaise, with or without fever.

Study design

This is a cross-sectional study. On the day of the exams, after 8-h overnight fast, baseline assessments of body composition and anthropometry (weight, height, waist circumference [WC] and hip circumference [HC]) were performed as described below. Then, the participants were subjected to three venous blood collections for laboratory measurements (detailed below): baseline (fasting state) and 30 and 60 min after a standardized meal intake (247 kcal, 64.5% carbohydrates, 19.5% protein, 16.0% fat) ingested during up to 5 min.

Body composition and measures of adiposity

We assessed body composition by bioelectrical impedance analysis (BIA, Biodynamic 450 Body Composition Analyzer, BioDynamics®, USA). This method estimated absolute and relative values of fat and lean masses from each participant, who were instructed to avoid

exercise and alcohol consumption at least 48 hours prior to the test. BIA was performed in subjects with an empty bladder and wearing light clothing and bare feet.

WC and HC were measured as centimeters at the midway level between the lowest rib margin and the iliac crest and at the widest circumference around the buttocks over the greater trochanters, respectively. The average of two measures of WC and HC was considered for analysis and waist-to-hip ratio (WHR) was calculated. Height and weight were measured to the nearest 0.5 cm and 0.1 kg using a digital scale with stadiometer (Personal Line 180, Filizola®, Brazil). BMI and body adiposity index (BAI) were calculated. BAI is an index of body adiposity that directly reflects the percentage of body fat, as follows: BAI = [HC as centimeters/(height as meter × the square root of height as meter)] – 18 [11].

Laboratory analysis and markers of insulin resistance

An intravenous catheter was inserted in the subject's right antecubital vein and kept in place during the exam for venous blood collections. EDTA tubes containing blood samples were centrifuged immediately after collections at 1.300 g for 15 min at 4°C and plasma samples were clarified by filtration (Millex filter with polyethersulfone membrane, 33 mm, Merck Millipore®, Tullagreen, Ireland), aliquoted and stored at -80°C.

All laboratory measurements were performed in duplicate, as follows: (a) baseline (fasting state): plasma DPP4 activity (colorimetry; sensibility: 0.1 μM/mL/min; intra-assay coefficient of variation [IACV]: <3%); PG (glucose oxidase); GLP-1 (chemioluminescent ELISA; sensibility: 0.14 pM; IACV: <10%; inter-assay coefficient of variation [IECV]: <15%); sex hormone-binding globulin (SHBG, radioimmune assay); insulin (sensibility: 15 pmol/L), C-peptide (sensibility: 9.5 pg/mL), glucagon (sensibility: 13 pg/mL), leptin (sensibility: 41 pg/mL), and GIP (sensibility: 0.16 pg/mL) (Luminex; Milliplex®, HMHMAG-34K; all IACV

and IECV were <10% and <20%, respectively); adiponectin (Luminex; Milliplex®, HADK1MAG-61K; sensibility: 11 pg/mL; IACV: <4%; IECV: <10%); resistin (ELISA; R&D Systems®; DRSN00; sensibility: 0.055 ng/mL; IACV: <6%; IECV: <10%); (b) at 30 and 60 min after meal intake, additional samples for plasma DPP4 activity, PG, insulin, C-peptide, glucagon, GLP-1, GIP, SHBG, leptin, adiponectin, and resistin were collected, prepared and stored.

FPG and insulin were used to calculate the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) as follows: HOMA-IR = insulin (μUI/mL) x FPG (mmol/mL)/22.5 [12] and QUICKI = 1/[log(insulin, μUI/mL) + log(FPG, mg/dL)] [13]. Since low SHBG level is a recognized marker of insulin resistance [14], we used SHBG, fasting insulin, and HOMA-IR to evaluate insulin resistance and QUICKI as a surrogate marker of insulin sensitivity.

Statistical analysis

We used GraphPad Prism® 5 (GraphPad Software Inc., San Diego, CA, USA) and STATISTICA® 7.0 (StatSoft Inc., Tulsa, OK, USA) for statistical analysis. Gaussian distribution was checked and parametric and non-parametric variables data are expressed as mean±SD and median [1st-3rd quartiles], respectively. We used one-way analysis of variance (ANOVA) to compare groups. In the pooled analysis, linear correlations between DPP4 activity and the other variables was conducted. In a step further, multiple regression analysis was also performed to test whether some body composition–anthropometric–laboratorial variables could influence DPP4 activity. Values of $P<0.05$ were considered statistically significant.

RESULTS

Fifty-two subjects with excessive adiposity, aged 38.9±8.36 years and BMI 29.09±2.55 kg/m², were included and almost half (48.0%) were women.At baseline, we noticed significant differences in gender, BMI, BAI, absolute fat mass, FPG, and GIP among NGT, Pre-DM, and DM groups (Table 1). Fasting insulin and HOMA-IR were higher and QUICKI was lower in Pre-DM compared to NGT group (DM group were not included in this analysis since some of these patients were on use of exogenous insulin). Of note, there was no difference in DPP4 activity among groups. We calculated the AUC of DPP4 activity, insulin, C-peptide, glucagon, GLP-1, GIP, leptin, adiponectin and resistin during meal test and investigated possible differences that could be expressed only after this stimulus. We found differences among groups only for PG_{AUC} (6311.59±859.54 vs. 7170±731.77 vs. 10879.5±4421.83 for NGT, Pre-DM and DM groups, respectively; $P<0.001$) and GIP_{AUC} (5513 [4411.75–7242.75] vs. 7321 [5450–8977] vs. 7671 [6033.75–9989.25] for the same groups, respectively; $P<0.05$). No other difference was observed in this analysis.

Table 1. Clinical and biochemical characteristics of subjects at baseline.

	All groups (n = 52)	NGT group (n = 22)	Pre-DM group (n = 20)	DM group (n = 10)	P-value
Age (years)	38.9 ± 8.36	38 ± 8.21	39.65 ± 9.18	39.4 ± 7.56	NS
Female [n, (%)]	25 (48%)	8 (36.3%)	11 (55%)	6 (60%)	<0.01
BMI (kg/m²)	29.09 ± 2.55	27.52 ± 1.63	30.74 ± 2.74‡	29.23 ± 1.63	<0.001
BAI	29.93 ± 4.84	27.95 ± 4.52	31.79 ± 4.93*	30.56 ± 4.03	<0.05
WC (cm)	94.85 ± 9.32	90.95 ± 8.93	77.29 ± 7.7	98.55 ± 10.77	NS
HC (cm)	104.9 ± 5.8	102.8 ± 4.62	107.7 ± 6.57	103.7 ± 4.51	NS
WHR	0.9 ± 0.08	0.88 ± 0.08	0.9 ± 0.07	0.95 ± 0.09	NS
Total cell mass (kg)	28.8 ± 6.15	29.14 ± 5.92	28.98 ± 6.31	27.7 ± 6.82	NS
Total cell mass (%)	33.65 [31.28–35.98]	34.95 [33.48–37.75]	32.75 [30.55–35.3]	32.6 [30.58–36.73]	NS
Fat mass (kg)	25.81 ± 4.9	23.25 ± 3.69	28.73 ± 4.83†	25.63 ± 4.47	<0.01
Fat mass (%)	31.22 ± 5.7	29.05 ± 5.87	33.39 ± 5.27	31.66 ± 4.82	NS
Lean mass (kg)	57.68 ± 11.04	58.07 ± 11.14	58.22 ± 11.74	55.74 ± 10.26	NS
Lean mass (%)	68.78 ± 5.7	70.95 ± 5.87	66.62 ± 5.27	68.34 ± 4.82	NS
HOMA-IR	1.99 ± 1.66[a]	1.24 ± 0.82	2.82 ± 1.96	—	<0.01
QUICKI	0.35 [0.33–0.38][a]	0.33 [0.31–0.35]	0.3 [0.29–0.36]	—	<0.01
SHBG (nmol/L)	31.63 [22.47–49.42]	37.05 [22.68–51.37]	28.96 [22.51–46.77]	28.28 [17.75–60.57]	NS
THR	2.16 [1.78–3.52]	2.16 [1.72–3.66]	2.62 [1.49–3.55]	2 [1.82–3.38]	NS
DPP4 activity (µM/mL/min)	13.46 ± 3.41	13.79 ± 2.69	13.28 ± 3.64	13.1 ± 4.56	NS
FPG (mg/dL)	106.3 ± 38.73	87.45 ± 8.4	104.7 ± 9.7‡	151 ± 71.01‡	<0.001
Insulin (pmol/L)	62.86 ± 48.31[a]	40.87 ± 27.98	74.74 ± 49.35	—	<0.05
C-peptide (pg/mL)	691.3 ± 330.7[a]	629.6 ± 327.3	759.2 ± 329.2	—	NS
Glucagon (pg/mL)	13.28 [15.31–51.01]	9.76 [5.08–26.76]	24.5 [10.42–29.7]	16.42 [5.62–31.99]	NS
GLP-1 (pM)	0.73 [0.11–1.28]	0.66 [0.11–1.49]	0.81 [0.37–1.3]	0.61 [0.11–1.06]	NS
GIP (pg/mL)	23.63 [15.31–51.01]	19.45 [12.22–25.94]	27.88 [18.64–48.81]	49.3 [31.15–67.44]*	<0.05
Leptin (pg/mL)	6278 [3766–11650]	4231 [2496–9251]	7697 [4790–12735]	6117 [4284–13365]	NS
Adiponectin (pg/mL)	12.67 [8.25–17.17]	11.92 [8.76–16.2]	13.55 [6.01–21.36]	11.76 [7.14–23.99]	NS
Resistin (ng/mL)	6.34 [5.23–8]	6.74 [5.28–13.73]	6.32 [5.19–10.64]	6.3 [4.63–10.44]	NS
Total cholesterol (mg/dL)	200.5 [170–234.3]	200.5 [175.3–236]	201.5 [170.3–231]	194.5 [145.5–253]	NS
HDL (mg/dL)	49.5 [41–59]	48 [40.75–58.25]	53 [40–60]	49.5 [40.5–63]	NS
LDL (mg/dL)	126.5 [96.25–153.5]	127.5 [108.5–153.8]	119.5 [96–155.8]	129 [79–160.3]	NS
TG (mg/dL)	133.1 ± 60.12	131.1 ± 62.08	138.3 ± 65.6	127.4 ± 47.93	NS
Screening phase					
FPG (mg/dL)	99.5 [92.25–113]	92 [85.5–96.25]	106.5 [100.8–112.3] ‡	169 [152–267.8]‡§	<0.001
2-h PG in the 75-g OGTT (mg/dL)	127.3 ± 39.11	102.5 ± 21.1	138.8 ± 28.42†	206.8 ± 29.2‡[b]	<0.001
Total cholesterol (mg/dL)	198.3 ± 50.81	194.7 ± 40.52	210.5 ± 58.53	181.9 ± 53.89	NS
HDL (mg/dL)	56.04 ± 16	48.27 ± 11.68	62.25 ± 16.22*	60.7 ± 17.88	<0.01
LDL (mg/dL)	115.4 ± 41.62	121.8 ± 39.06	117.6 ± 47.34	97 ± 32.17	NS
TG (mg/dL)	119.5 [83–170.8]	116.5 [83–155.8]	141.5 [82.25–199.8]	106 [82.25–162.5]	NS

Data are presented as mean ± SD, median [1st–3rd quartiles], or n (%). *P<0.05, †P<0.01, and ‡P<0.001 in comparison to NGT group. §P<0.05 in comparison to Pre-DM group. [a]Pooled data of NGT and Pre-DM groups. [b]Only 04 subjects performed OGTT in the DM group. NGT: normoglycemia; Pre-DM: prediabetes; DM: diabetes mellitus; BMI: body mass index; BAI: body adiposity index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; HOMA-IR: homeostasis model assessment to quantify insulin resistance; QUICKI: quantitative insulin sensitivity check index; SHBG: sex hormone-binding globulin; THR: triglycerides-to-high-density lipoprotein ratio; DPP4: dipeptidyl peptidase 4; FPG: fasting plasma glucose; GLP-1: glucagon-like peptide-1; GIP: glucose-dependent insulinotropic polypeptide; HDL: high-density lipoprotein; LDL:low-density lipoprotein; TG: triglycerides; PG: plasma glucose; OGTT: oral glucose tolerance test.

To test our hypothesis, we correlated DPP4 activity with anthropometry, body composition variables, and insulin resistance markers in the pooled sample. Table 2 presents the observed correlations. Of interest, we observed some associations between baseline DPP4 activity and anthropometric measures and derived indexes, as follows: direct correlations to weight, WC,

and WHR and an inverse association to BAI. Considering body composition variables, baseline DPP4 activity was correlated directly to absolute and percent values of total cell mass and lean mass and inversely to percentage of fat mass. No correlation between baseline DPP4 activity and absolute fat mass was found. Additonally, there were significant relationships between baseline DPP4 activity and all insulin resistance markers herein tested, with the exception for the triglycerides-to-high-density lipoprotein ratio. As noted, we found direct correlations to insulin and HOMA-IR and inverse correlations to SHBG and QUICKI. Some of these correlations are shown on Fig. 1.

Table 2. Linear correlations between DPP4 activity and variables of the pooled group at baseline (n=52).

	r-value	P-value
Age	-0.17	NS
Weight	0.56	<0.001
BMI	0.22	NS
BAI	-0.48	<0.001
WC	0.34	<0.05
HC	-0.03	NS
WHR	0.35	<0.01
Total cell mass (kg)	0.58	<0.001
Total cell mass (%)	0.47	<0.001
Fat mass (kg)	0.11	NS
Fat mass (%)	-0.33	<0.05
Lean mass (kg)	0.57	<0.001
Lean mass (%)	0.33	<0.05
HOMA-IR[a]	0.29	<0.05
QUICKI[a]	-0.29	<0.05
SHBG	-0.56	<0.001
THR	0.25	NS
FPG	-0.01	NS
Insulin[a]	0.32	<0.05
C-peptide[a]	0.25	NS
Glucagon	0.18	NS
GLP-1	0.008	NS
GIP	0.06	NS
Leptin	-0.31	<0.05
Adiponectin	-0.26	NS
Resistin	-0.32	<0.05
Total cholesterol	0.31	<0.05
HDL	-0.1	NS
LDL	0.36	<0.01
TG	0.22	NS

[a]All six insulinized subjects from DM group were excluded from analysis. DM: diabetes mellitus; DPP4: dipeptidyl peptidase 4; BMI: body mass index; BAI: body adiposity index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; HOMA-IR: homeostasis model assessment to quantify insulin resistance; QUICKI: quantitative insulin sensitivity check index; SHBG: sex hormone-binding globulin; THR: triglycerides-to-high-density lipoprotein ratio; FPG: fasting plasma glucose; GLP-1: glucagon-like peptide-1; GIP: glucose-dependent insulinotropic polypeptide; HDL: high-density lipoprotein; LDL:low-density lipoprotein; TG: triglycerides.

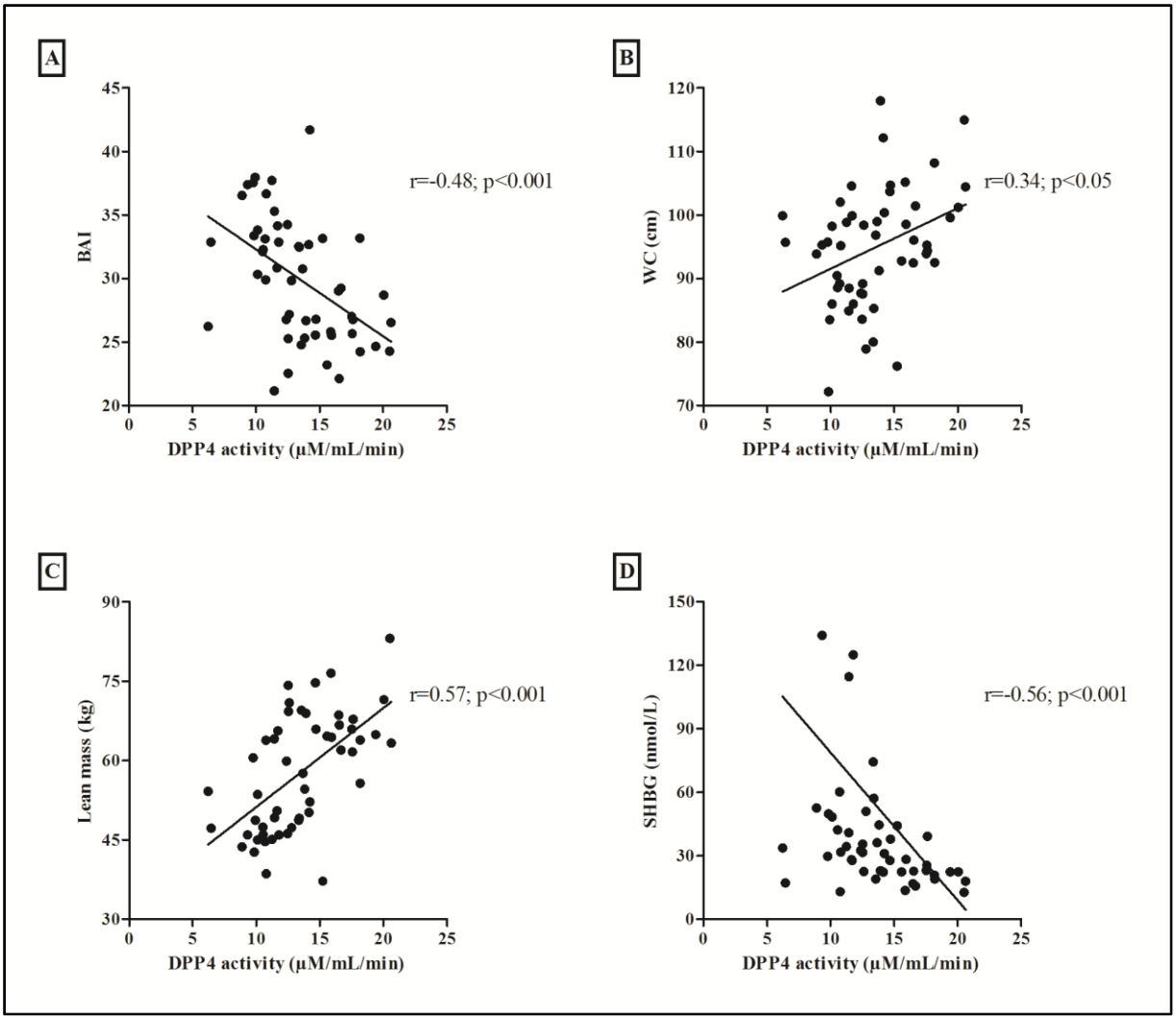

Fig. 1 – Scatter plots demonstrating bivariate correlations between DPP4 activity and other variables in the pooled group. DPP4 activity correlated to BAI (A), WC (B), absolute lean mass (C), and SHBG (D) (n=52). On Fig. D, four data points are outside the Y-axis limits. DPP4: dipeptidyl peptidase 4; BAI: body adiposity index; WC: waist circumference; SHBG: sex hormone-binding globulin.

Since body composition parameters, measures of adiposity, and SHBG levels are all influenced by gender, we performed sex-specific analysis and observed that some baseline DPP4 activity correlations previously found, remained for men but not for women, as follows: WHR (r=0.44, P<0.05), insulin levels (r=0.40, P<0.05), and SHBG (r=-0.40, P<0.05). By including data from post-prandial period of all non-insulinized subjects (n=46), we also observed positive linear correlations of DPP4 activity$_{AUC}$ with insulin$_{AUC}$ (r=0.36, P<0.05)

and a trend toward a correlation to C-peptide$_{AUC}$ (P=0.05) (Table 3), which suggests DPP4 activity is associated with insulin resistance also after meal challenge.

Table 3. Linear correlations between DPP4 activity$_{AUC}$ and the AUC of biochemical variables of the pooled group (n=52).

	r-value	P-value
PG	-0.06	NS
Insulin[a]	0.35	<0.05
C-peptide[a]	0.28	NS
Glucagon	0.22	NS
GLP-1	-0.13	NS
GIP	0.23	NS
Leptin	-0.31	<0.05
Adiponectin	-0.27	NS
Resistin	-0.32	<0.05

[a]All six insulinized subjects from DM group were excluded from analysis (n=46). DM: diabetes mellitus; DPP4: dipeptidyl peptidase 4; PG: plasma glucose; GLP-1: glucagon-like peptide-1; GIP: glucose-dependent insulinotropic polypeptide.

Other interesting associations were observed between DPP4 activity and biochemical variables. At baseline, DPP4 activity was associated to total cholesterol (r=0.31, P<0.05) and LDL (r=0.36, P<0.01). Baseline DPP4 activity was also associated to leptin (r=-0.31, P<0.05) and resistin (r=-0.32, P<0.05) and there was a trend toward a correlation to adiponectin (r=-0.26, P=0.05) (Table 2). This same pattern was observed for DPP4 activity$_{AUC}$, once a correlation to leptin$_{AUC}$ (r=-0.31, P<0.05) and resistin$_{AUC}$ (r=-0.32, P<0.05) and a trend toward an association with adiponectin$_{AUC}$ (r=-0.27, P=0.05) were also evidenced (Table 3). In a step further, we tested whether some body composition–anthropometric–laboratorial variables could, together, influence the DPP4 activity (Table 4). Our results on multiple regression analysis showed that BAI, WHR, percent lean mass, HOMA-IR, and leptin influenced DPP4 activity and explained approximately 26% of the variance on it. BAI and HOMA-IR exerted the greater effect on DPP4 activity, reassuring the existence of a role derived from body composition–anthropometric–laboratorial variables herein tested on plasma DPP4 activity in subjects with excessive adiposity and different stages of glucose tolerance.

Table 4. Multiple regression analysis of the pooled group.

Model	β	P-value	Adjusted R^2	P-value
			0.265	<0.001
BAI	**-0.473**	**0.0002**		
HOMA-IR	**0.291**	**0.019**		

DPP4 activity was considered as dependent variable and BAI, WHR, percent lean mass, HOMA-IR, and leptinas independent variables in the model. Mean substitution strategy was adopted to HOMA-IR values of six insulinized subjects. DPP4: dipeptidyl peptidase 4; BAI: body adiposity index; WHR: waist-to-hip ratio; HOMA-IR: homeostasis model assessment to quantify insulin resistance.

DISCUSSION

Beyond DPP4 enzyme role on pathophysiology of type 2 diabetes, this protein is also an adipo-myokine [5,6] and, therefore, it is supposed to be related to lean and fat masses. However, as far as we know, there are very few studies [15,16] assessing the relationship between DPP4 activity and anthropometric/body composition measures and all of them evaluated healthy people. In the present study, interesting original findings were observed regarding DPP4 activity associations with some anthropometric, body composition, and laboratorial variables in subjects with excessive adiposity and different stages of glucose tolerance. At first, baseline DPP4 activity was not surprisingly direct associated to weight and total cell mass, a finding that could be expected, since DPP4 is an adipo-myokine [5,6] and body weight is largely composed of fat and lean masses [17]. Furthermore, DPP4 activity was associated with all insulin resistance markers herein tested, as follows: directly associated to fasting insulin and HOMA-IR and inversely to SHBG and QUICKI. By testing it in a sex-specific fashion, the observed results were not kept unchanged, except for fasting insulin and SHBG in men. Possibly, the former finding may have occurred because of low statistical power, since insulinized subjects were excluded from almost all of these subanalysis. We should emphasize that DPP4 $activity_{AUC}$ was also correlated to $insulin_{AUC}$. Altogether, these results indicate a direct association between DPP4 activity and insulin resistance, which is

consistent with two studies that found positive associations between DPP4 activity and HOMA-IR in Chinese populations without diabetes [18,19]. Additionally, we also noted that baseline DPP4 activity was inversely correlated to baseline resistin, and this same pattern was reproduced on the observed correlation between DPP4 activity$_{AUC}$ and resistin$_{AUC}$. As its own name expresses, resistin appears to have a role in insulin resistance, but some evidence on this issue are paradoxical and hard to explain [20,21]. For example, factors known to be related to insulin resistance, such as hyperinsulinemia and TNF-α, counter regulate expression of resistin mRNA and protein in cultured adipocytes [21]. Considering our findings that DPP4 activity and insulin levels were positively associated, the latter might be a confounder on the negative correlations between baseline and AUC of DPP4 activity and resistin, respectively, although a causal relationship cannot be excluded.

DPP4 activity was positively associated to WC and WHR, both known as measures of central adiposity and visceral adipose tissue (VAT) as well, but it was inversely correlated to: (a) the percentage of fat mass assessed by BIA; (b) the BAI, an index of general body adiposity [11]; and (c) leptin, an adipokine mainly released by subcutaneous adipose tissue (SAT) [22]. Moreover, there were some trends toward inverse correlations between DPP4 activity and adiponectin (P=0.05), an adipokine with insulin sensitizer properties that is inversely related to general obesity and central fat distribution [23], and also between DPP4 activity$_{AUC}$ and adiponectin$_{AUC}$ (P=0.05) during post-prandial period. Altogether, these findings suggest that VAT may be a determinant of higher DPP4 activity and SAT may have a negative impact on it, and this hypothesis can be partly supported by some bench studies [5,7], by a clinical study evaluating DPP4 levels and body composition in patients with familial partial lipodystrophy type 2 (an inherited disease characterized by lack of SAT and fat deposition in VAT and ectopic sites) [24], and also by the knowledge that SAT is a determinant of metabolic health [25]. Curiously, a meta-analysis of ten randomized controlled trials evaluating the impact of

DPP4 inhibitors on serum adiponectin of patients with type 2 diabetes evidenced significantly elevated adiponectin levels after the pharmacological inhibition of DPP4 [26].

Neidert and co-workers [15] evaluated the correlations between DPP4 activity and body composition in healthy lean subjects and yielded relevant information. Using multiple regression analysis, these authors provided the first evidence that DPP4 activity is positively related to absolute lean mass assessed by dual energy X-ray absorptiometry and we added the same observations, but originally, in a different population. Additionally, DPP4 activity was negatively correlated to absolute fat mass and gynoid fat and no significant relationship between the former and BMI was found [15]. Although we did not find an association between DPP4 activity and BMI either, these results differ from those of three studies involving people without diabetes [16,18,19], in which mild but significant positive correlations were found. Furthermore, Neidert and co-workers found that DPP4 activity was not associated to absolute android fat mass and VAT [15], which contrasts with: (a) our findings of positive associations between DPP4 activity and measures of central obesity; (b) the results of other studies evidencing higher DPP4 expression and release by VAT than by SAT [7]; and (c) positive correlations of DPP4 levels with visceral adipocyte surface [5] and android fat [24]. The authors justify this discrepancy by the low amounts of VAT found in their subjects, since most of them fall into the eutrophic range (mean BMI of approximately 24 kg/m^2) [15].

As pioneered by the above-mentioned authors [15] in healthy normal-weight subjects and now evidenced by us in a different population, the association of DPP4 activity with lean mass reinforces the characterization of DPP4 as a myokine [6]. Curiously, since lean mass is inversely related to insulin resistance [27], our findings that DPP4 activity is direct associated to lean mass and also to measures of central obesity and insulin resistance might seem paradoxical. However, it seems to be the case for many adipo-myokines [8], such as

interleukin-6 (IL-6). This biomarker is released by human skeletal muscle, and it seems to have beneficial effects on insulin-stimulated glucose disposal and fatty acid oxidation after acute stimulation, which support a role for this myokine on muscle metabolism during contraction [8]. On the other hand, IL-6 is overexpressed in human fat cells from insulin-resistant subjects and the chronic elevation of this adipokine may induce insulin resistance [8]. Taken together, these findings suggest the adipo-myokine IL-6 have different influences depending on its source, time of action, and target tissue [8], and herein we hypothesize a parallel mode of action of the adipo-myokine DPP4 tested.

Finally, we performed a multiple regression analysis to test whether some body composition–anthropometric–laboratorial variables could influence the plasma DPP4 activity and evidenced that BAI, WHR, percent lean mass, HOMA-IR, and leptin influenced it, with BAI and HOMA-IR exerting the greater effect on it.

Our study has strengths and limitations. Absolute majority of studies assessed DPP4 levels instead of its activity and, as far as we know, it is the first study evaluating DPP4 activity and its relation to measures of adiposity, body composition, and insulin resistance in subjects with excessive adiposity and different stages of glucose tolerance. Moreover, we assessed adipokines and other biochemical variables to better understand our possible results of our main analysis. Limitations of the study are associated with its cross-sectional design, implying it is explicitly correlational and cannot directly demonstrate causality, and with the small sample size, limiting the power of our subgroup analysis. Therefore, we highlight the results cannot be generalized since the study sample is limited and involved only subjects with excessive adiposity.

CONCLUSIONS

Constitutive DPP4 activity is directly associated to muscle mass, measures of central adiposity, and insulin resistance, but it is inversely related to indexes of general body adiposity, which is consistent with the hypothesis that VAT is a determinant of higher DPP4 activity and SAT may have a negative impact on it. Additionally, DPP4 activity seems to be influenced by some body composition–anthropometric–laboratorial variables, suggesting this adipo-myokine activity could be influenced by body composition aspects of subjects with excessive adiposity and different stages of glucose tolerance.

LIST OF ABBREVIATIONS

ADA: American Diabetes Association; ANOVA: analysis of variance; AUC: area under the curve; BAI: body adiposity index; BIA: bioelectrical impedance analysis; BMI: body mass index; CD26: cluster of differentiation 26; DM: diabetes mellitus; DPP4: dipeptidyl peptidase 4; EDTA: ethylenediamine tetraacetic acid; ELISA: enzyme-linked immunosorbent assay; FPG: fasting plasma glucose; GIP: glucose-dependent insulinotropic polypeptide; GLP-1: glucagon-like peptide-1; HC: hip circumference; HOMA-IR: homeostasis model assessment of insulin resistance; IACV: intra-assay coefficient of variation; IECV: inter-assay coefficient of variation; IL-6: interleukin-6; IR: insulin resistance; mRNA: messenger ribonucleic acid; NGT: normal glucose tolerance; OGTT: oral glucose tolerance test; PG: plasma glucose; Pre-DM: prediabetes; QUICKI: quantitative insulin sensitivity check index; SAT: subcutaneous adipose tissue; SHBG: sex hormone-binding globulin; TNF-α: tumor necrosis factor α; VAT: visceral adipose tissue; WC: waist circumference; WHR: waist-to-hip ratio.

DECLARATIONS

Ethics approval and consent to participate

The protocol was approved by the Ethics Committee of the University Hospital Pedro Ernesto (CAAE: 24360513.1.0000.5282) and carried out in accordance with the principles of the Declaration of Helsinki. All subjects gave written informed consent.

Consent for publication

If the manuscript is accepted, we approve it for publication in Diabetology & Metabolic Syndrome.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

W.S.S.J. researched and analysed data and wrote the manuscript. M.G.C.S. and J.F.N.N. researched data. E.B. reviewed the manuscript. L.G.K.A. designed the study, analysed data, contributed to the discussion, and reviewed/edited the manuscript.

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ANEXO –Aprovação do Comitê de Ética do HUPE-UERJ

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Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Atividade da dipeptidil peptidase 4 (DPP4) e suas associações com disfunção endotelial, marcadores inflamatórios, peptídeos intestinais, índices de resistência insulínica e medidas de adiposidade em indivíduos com diferentes graus de tolerância à glicose.

Pesquisador: Luiz Guilherme Kraemer de Aguiar

Área Temática:

Versão: 2

CAAE: 24360513.1.0000.5282

Instituição Proponente: Laboratório de pesquisas clinicas e experimentais em biologia vascular

Patrocinador Principal: Hospital Universitário Pedro Ernesto

DADOS DO PARECER

Número do Parecer: 940.029

Data da Relatoria: 14/01/2015

Apresentação do Projeto:

Este projeto é de uma tese de doutorado. A aterosclerose é a principal causa de mortalidade em diabéticos e está intimamente relacionada à disfunção endotelial e à inflamação. Esses fatores estão presentes desde fases muito iniciais do comprometimento do metabolismo glicídico, determinando um aumento de risco já nos indivíduos com pré-diabetes. Nos últimos anos, dentre as novas drogas para o tratamento do diabetes, os chamados inibidores da dipeptidil peptidase 4 (DPP4) têm sido alvo de atenção especial.A DPP4 é uma enzima ubíqua, cuja principal fonte é o endotélio. Considerando-se: 1) a participação da DPP4 na fisiopatologia do diabetes, através da inativação das incretinas; 2) a sua capacidade de regular fisiologicamente algumas citocinas, quimiocinas e neuropeptídeos possivelmente associados à inflamação e à função vascular; e 3) as evidências crescentes de que o uso de inibidores da DPP4 está associado à diminuição de marcadores inflamatórios, pode-se especular que um aumento na atividade da DPP4 representaria um elo entre o comprometimento do metabolismo glicídico, a disfunção endotelial e a inflamação.

Objetivo da Pesquisa:

Objetivo Primário:

Estudar as associações entre a atividade de DPP4 e a presença de disfunção endotelial e de

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marcadores inflamatórios em indivíduos com diferentes graus de tolerância à glicose (euglicêmicos/normotolerantes, pré-diabéticos e diabéticos).

Objetivo Secundário:

1) Verificar se a atividade da DPP4 se correlaciona com índices de resistência insulínica. 2) Avaliar a relação entre a atividade da DPP4 e as concentrações séricas de peptídeos intestinais e de adipocinas em pacientes com diferentes graus de tolerância à glicose. 3) Estudar as correlações entre atividade da DPP4 e medidas de adiposidade geral e de distribuição de gordura. 4) Realizar a análise espectral da variabilidade da frequência cardíaca e correlacionar os seus componentes com a atividade da DPP4, os resultados dos testes de reatividade microvascular, marcadores inflamatórios e índices de resistência insulínica entre os grupos de euglicêmicos/normotolerantes, pré-diabéticos, diabéticos e indivíduos submetidos a procedimento bariátrico. 5) Determinar as associações entre a atividade da DPP4 e a presença de disfunção endotelial e de marcadores inflamatórios em indivíduos submetidos a procedimento bariátrico. 6) Comparar a função endotelial, a atividade da DPP4, os marcadores inflamatórios e a variabilidade da frequência cardíaca e da pressão arterial entre indivíduos submetidos e não submetidos à cirurgia bariátrica.

Avaliação dos Riscos e Benefícios:

Poderá haver suspensão de antidiabéticos orais no grupo de diabéticos tipo 2 insulinizados. Mas, os autores justificam dizendo que pretendem ajustar as doses de insulina para que não haja hiperglicemia. Não há benefícios diretos para os participantes.

Comentários e Considerações sobre a Pesquisa:

O projeto da pesquisa está bem apresentado e contém informações essenciais para o seu entendimento. A pesquisa está bem fundamentada e seus resultados podem gerar subsídios para que se abram novas linhas de pesquisa, buscando compreender mais profundamente as associações relatadas e encontrar novas possibilidades de tratamento.

Considerações sobre os Termos de apresentação obrigatória:

Os termos de apresentação obrigatória estão considerando todos os itens da RES.466/12 e estão adequados e sem problemas éticos.

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadequações:

Ante o exposto, a COEP deliberou pela aprovação do projeto, visto que não há implicações éticas.

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Continuação do Parecer: 940.029

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Considerações Finais a critério do CEP:
Faz-se necessário apresentar Relatório Anual - previsto para janeiro de 2016. A COEP deverá ser informada de fatos relevantes que alterem o curso normal do estudo, devendo o pesquisador apresentar justificativa, caso o projeto venha a ser interrompido e/ou os resultados não sejam publicados.

RIO DE JANEIRO, 28 de Janeiro de 2015

Assinado por:
Patricia Fernandes Campos de Moraes
(Coordenador)

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