Universidade Federal do Rio Grande Do Sul (UFRGS)

Programa de Pós-Graduação em Medicina: Endocrinologia

Hospital de Clínicas de Porto Alegre (HCPA)

Omentina-1 e diabetes mellitus pós-transplante: Polimorfismo rs2274907 no gene *ITLN1* e níveis plasmáticos de omentina-1 em pacientes transplantados renais.

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Porto Alegre, novembro de 2019

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, da Universidade Federal do Rio Grande do Sul (UFRGS) como requisito parcial para obtenção do título de Doutora em Endocrinologia.

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O formato desta tese de doutorado segue o modelo recomendado pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, da Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de uma breve introdução sobre o tema, seguido dos dois manuscritos originais, finalizando com as considerações finais e perspectivas futuras.

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SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

Akt, proteína kinase B

COX, ciclooxigenase

DCV, doença cardiovascular

DM2, diabetes mellitus tipo 2

DMPT, diabetes mellitus pós-transplante

DRC, doença renal crônica

HDL, *high density lipoprotein*

HOMA-IR, *homeostasis model assessment estimated insulin resistance*

IMC, índice de massa corporal

JNK, c-Jun N-terminal kinase

SNP, *single nucleotide polymorphism*

TNF, fator de necrose tumoral

RESUMO

O diabetes mellitus pós-transplante (DMPT) é uma complicação metabólica comum nos pacientes após o transplante renal. Possui fatores de risco heterogêneos, sendo alguns deles específicos do período pós-transplante relacionados ao uso de imunossupressores, e outros similares aos observados no diabetes mellitus tipo 2 (DM2), como síndrome metabólica, obesidade e idade avançada. O caráter inflamatório desta complicação metabólica vem sendo descrito e associado a uma maior ocorrência de morbimortalidade nesta população. O tecido adiposo desempenha papel fundamental na fisiopatogênese da inflamação sistêmica subclínica, secretando proteínas com atividades inflamatórias e anti-inflamatórias. Omentina-1 é uma adipocina anti-inflamatória associada a parâmetros metabólicos, incluindo resistência periférica à ação da insulina e obesidade. Neste sentido desenvolvemos este estudo, com o objetivo de: 1) avaliar a associação dos níveis plasmáticos de omentina-1 com DMPT e; 2) avaliar a associação de um polimorfismo no gene da omentina-1 com DMPT. No nosso primeiro estudo, alocamos pacientes provenientes de uma coorte de pacientes transplantados renais da região sul do Brasil, para um estudo de caso-controle (54 casos e 53 controles). Os pacientes foram selecionados pelas variáveis idade, sexo, tempo de transplante, índice de massa corporal (IMC) e taxa de filtração glomerular. Como principal resultado, observamos que os níveis plasmáticos de omentina-1 foram significativamente reduzidos no grupo DMPT (3.83 [1.67– 6.52] ng/mL) comparado ao grupo controle (5.62 [2.70 – 9.47] ng/mL, p = 0.036). Avaliando o valor incremental de omentina-1 para predição de risco de DMPT, verificamos que para cada 1ng/mL de aumento nos níveis plasmáticos de omentina-1, as chances de desenvolver DMPT reduziram em 8% (OR=0.92 [0.854 – 0.997], p=0.041).

No segundo estudo, avaliamos a associação do polimorfismo rs2274907 no gene da omentina-1 em pacientes com DMPT e sem DMPT, num desenho de caso-controle aninhado a uma coorte retrospectiva (105 casos e 211 controles). Verificamos que o alelo A foi associado com risco para DMPT nos modelos de herança recessivo e aditivo (ambos p < 0,0001) na população estudada. Após ajuste para idade, etnia, tipo de doador e IMC pré-transplante, o genótipo A/A permaneceu associado ao risco nestes mesmos modelos (recessivo: OR = 3.711, 95% CI 1.659 – 8.302; aditivo: OR = 4.799, 95% CI 1.896 – 12.143; p=0,001).

Este projeto é o primeiro, de nosso conhecimento, a avaliar a associação da omentina-1 com DMPT. Nossos resultados apontam para um significativo papel protetor da omentina-1 no risco de desenvolvimento de DMPT. Mais estudos são necessários para confirmar esse achado. Contudo, acreditamos que nossos resultados possam contribuir para um melhor entendimento da fisiopatologia do DMPT bem como instigar a realização de estudos objetivando estratégias terapêuticas que possam prevenir ou reduzir a ocorrência desta complicação.

ABSTRACT

Posttransplant diabetes mellitus (PTDM) is a common metabolic complication after kidney transplantation. The risk factors are heterogeneous and related to both the transplant process itself, such as the immunosuppressive drugs use and to other factors commonly found in type 2 diabetes mellitus (T2DM) patients, such as metabolic syndrome, obesity, and age.

An inflammatory profile has been associated with PTDM and may lead to increase morbimortality in this population. The adipose tissue has a significant role in chronic low-grade inflammation by secreting inflammatory and anti-inflammatory proteins. Omentin-1 is an anti-inflammatory adipokine associated with metabolic factors, including insulin resistance and obesity.

Therefore, this study aimed to 1) evaluate the association of plasma omentin-1 levels with PTDM and, 2) evaluate the association of rs2274907 polymorphism in the omentin-1 gene with PTDM. For the first aim, we performed a case-control study (54 cases and 53 controls) of a cohort of kidney transplant recipients from southern Brazil. Patients were matched by age, gender, time after transplantation, body mass index (BMI), and glomerular filtration rate. As a main result, we observed that the plasma omentin-1 levels were significantly reduced in the PTDM group (3.83 [1.67– 6.52] ng/mL) compared to the control group (5.62 [2.70 – 9.47] ng/mL, P = 0.036). Evaluating the incremental value of plasma omentin-1 to predict risk of PTDM, we found that for each increased 1ng/mL the odds to develop PTDM decreased by 8% (OR=0.92 [0.854 – 0.997], P=0.041).

For the second aim, we evaluated the association of the rs2274907 polymorphism in the omentin-1 gene in patients with and without PTDM, in a nested case-control study within a retrospective cohort of kidney transplant recipients (105 cases and 211 controls). We observed that the A allele was associated with risk for PTDM under recessive and additive inheritance models (both P < 0.0001). After adjustment for age, ethnicity, type of donor, and BMI pre-transplant, the A/A genotype remained independently associated with risk for PTDM under recessive model: OR = 3.711, 95% CI 1.659 – 8.302 and under additive model: OR = 4.799, 95% CI 1.896 – 12.143; P=0.001.

This is the first study, of our knowledge, to evaluate the association between omentin-1 and PTDM. Our results showed a significant protector pattern of the omentin-

1 in PTDM risk. Further studies are needed to confirm these findings. However, we believe our results can contribute to a better understanding of the PTDM physiopathology, and may also instigate further studies aiming therapeutic strategies to prevent or reduce the occurrence of this complication.

CAPÍTULO 1

1 INTRODUÇÃO

Diabetes Mellitus Pós-Transplante (DMPT) é definido como o diabetes que ocorre em receptores de órgãos ou tecidos, previamente não diabéticos, após o transplante (1). Seu diagnóstico é realizado conforme os critérios da *American Diabetes Association* (2), citados na tabela 1, devendo ser realizado somente após 45 dias da data do transplante, evitando diagnósticos errôneos associados a hiperglicemia transitória do período pós-transplante recente, que pode ocorrer devido às altas doses de imunossupressores utilizados nesta fase.

Tabela 1. Critérios Diagnósticos de Diabetes Mellitus (2)

Hemoglobina glicada (HbA1C)	Maior ou igual a 6,5% ou
Glicemia de jejum	Maior ou igual a 125 mg/dL ou
Teste oral de tolerância a glicose	Maior ou igual a 200 mg/dL ou
Glicemia randômica	Maior ou igual a 200 mg/dL em pacientes com sintomas clássicos de hiperglicemia

O DMPT ocorre em 15 a 40% dos pacientes que realizam transplante renal durante o primeiro ano após transplante (3, 4) e está associado com redução da sobrevida do enxerto e aumento de mortalidade por complicações cardiovasculares (5). O DMPT compartilha alguns fatores de risco e mecanismos fisiopatológicos com o diabetes mellitus tipo 2 (DM2). No entanto, complicações microvasculares a longo prazo parecem

se comportar de maneira diferente (6). Dentre os fatores de risco conhecidos para o desenvolvimento de DMPT estão: idade avançada, obesidade, etnia, história familiar de DM2, infecções por hepatite C e citomegalovírus e drogas imunossupressoras, entre outros, conforme demostrado na tabela 2 (7-9). Os inibidores de calcineurina e os corticosteróides desempenham um papel importante no desenvolvimento de DMPT, tanto pelos efeitos tóxicos diretos nas células beta, levando à diminuição da secreção de insulina quanto pelo aumento da resistência à insulina nos tecidos periféricos e hepáticos (10-12)

Tabela 2. Fatores de risco para DMPT

Não modificáveis	**Potencialmente modificáveis**	**Modificáveis**
Idade > 45 anos Raça negra Doador falecido Gênero masculino História familiar de DM HLA incompatível	Hiperglicemia de jejum ou intolerância à glicose antes do transplante Infecção por vírus da hepatite C Infecção por citomegalovírus	Drogas imunossupressoras; Obesidade e outros componentes da síndrome metabólica.

Além destes fatores de risco acima citados, alguns outros fatores têm sido estudados com o objetivo de melhor conhecer a fisiopatogênese do DMPT, tendo como base estudos associados com DM2 (13).

O tecido adiposo é um dos maiores órgãos endócrinos do corpo e é responsável pela produção de citocinas chamadas adipocinas. As adipocinas influenciam uma

variedade de processos metabólicos (21-26) e estão associadas a efeitos pró-inflamatórios e anti-inflamatórios. Nos últimos anos, estudos com foco nos efeitos anti-inflamatórios de algumas adipocinas e seu papel na regulação do tecido adiposo tem sido realizados, destacando-se a adiponectina, o antagonista do receptor de interleucina-1 e a omentina-1 (14-16).

1.1 Omentina

A Omentina (intelectin-1: *ITLN1*, OMIM: 609873) tem sido descrita como uma importante adipocina secretada pelo tecido adiposo visceral (17). Também chamada de intelectina, trata-se de uma proteína com 313 aminoácidos de 38-40 kDa, sendo seu RNAm predominantemente expresso na fração do estroma vascular do tecido adiposo visceral humano (18). Há uma isoforma homóloga de omentina-1, o qual compartilha 83% dos aminoácidos e é identificada como omentina-2. Esta última é pouco expressa, de forma que os níveis plasmáticos frequentemente se encontram abaixo do limite de detecção (19).

As duas isoformas da omentina, a omentina-1 e a omentina-2, têm sido associadas ao DM2 (20, 21). Omentina-1 foi identificada a partir do tecido adiposo visceral por Yang *et al* em 2003 (22). É a mais abundante e tem sido relacionada a atividades cardiometabólicas via ação anti-inflamatória (23). Baixos níveis circulantes desta adipocina são associados com vários fatores de risco metabólicos, incluindo hipertensão, aumento da circunferência da cintura, dislipidemia e intolerância à glicose (19, 24, 25).

O mecanismo de ação da omentina-1 relacionado ao aumento da captação de glicose em adipócitos humanos, se dá através do aumento da fosforilação da proteína kinase B (Akt) *in vitro* (**figura 1**) (26) tanto na presença como na ausência de insulina. A

expressão de omentina-1 no tecido adiposo visceral é reduzida na presença de obesidade e resistência à ação da insulina (19, 27, 28).

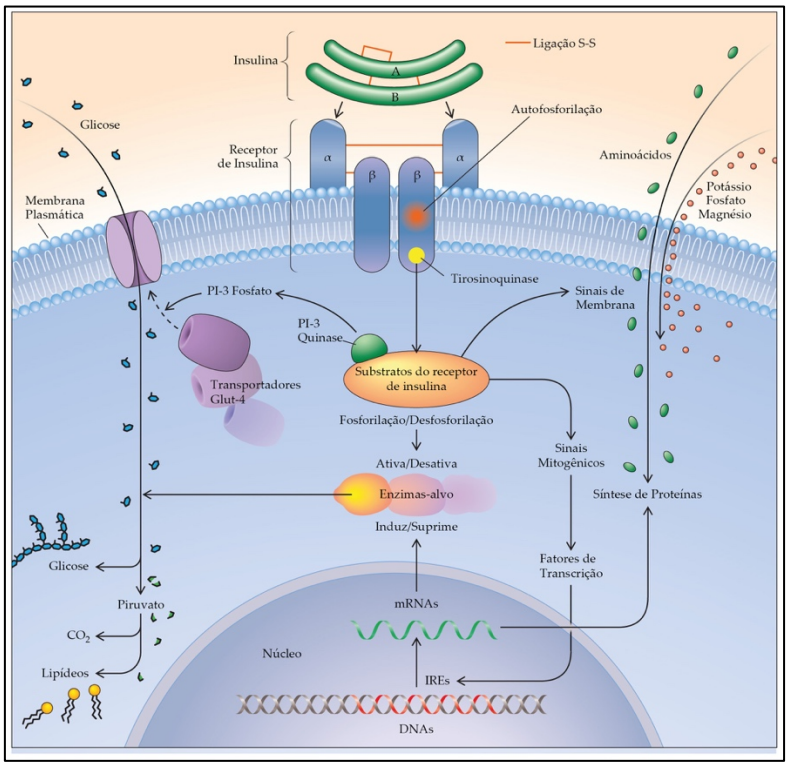

Figura 1 – Insulina atua via receptores do tipo tirosina quinase presentes na membrana celular, estimulando a fosforilação dos substratos do receptor insulínico (IRSs). A fosforilação das proteínas IRSs inicia uma complexa cascata de transdução de sinal, envolvendo a captação da glicose, síntese proteica e sobrevivência celular. A fosforilação da Fosfatidilinositol 3 quinase (PI-3K) e AKT são fundamentais para a translocação do transportador de glicose GLUT4 e consequente entrada da molécula na célula (26).

A associação da omentina-1 com o DM2 já foi descrita em diversos estudos que demonstraram que os níveis circulantes de omentina-1 estavam inversamente associados com a resistência à ação da insulina, avaliado através de *homeostasis model assessment estimated insulin resistance* (HOMA-IR) (19, 28-31). Zhang *et al* encontraram níveis séricos de omentina-1 reduzidos em chineses com DM2, sejam eutróficos sejam com obesidade quando comparados aos controles sem diabetes. Neste estudo, a omentina-1 correlacionou-se negativamente com índice de massa corporal (IMC), HOMA-IR, glicemia em jejum, glicemia pós-prandial, triglicerídemia e positivamente com HDL-c (*high density lipoprotein)* (32). Essa associação inversa entre a omentina-1 e os

marcadores de risco metabólicos pode ser explicada através de propriedades anti-inflamatórias observadas em estudos *in vitro* em células endoteliais e células de músculo liso (**Figura 2**) (33-36). Em aorta de rato, isolada com endotélio preservado, Yamawaki H *et al* demonstraram que o tratamento com omentina-1 levou a vasodilatação através do óxido nítrico derivado do endotélio (48). Já em células endoteliais umbilicais de linhagem humana, o pré-tratamento com omentina-1 inibiu significativamente a fosforilação de c-Jun N-terminal kinase (JNK), bem como a expressão da ciclooxigenase-2 (COX-2) induzida por TNF (34, 37), demostrando um papel anti-inflamatório significativo da omentina-1. Ainda, disfunção endotelial e aterosclerose foram significativamente associadas com baixos níveis séricos de omentina-1 em pacientes com e sem DCV (38, 39).

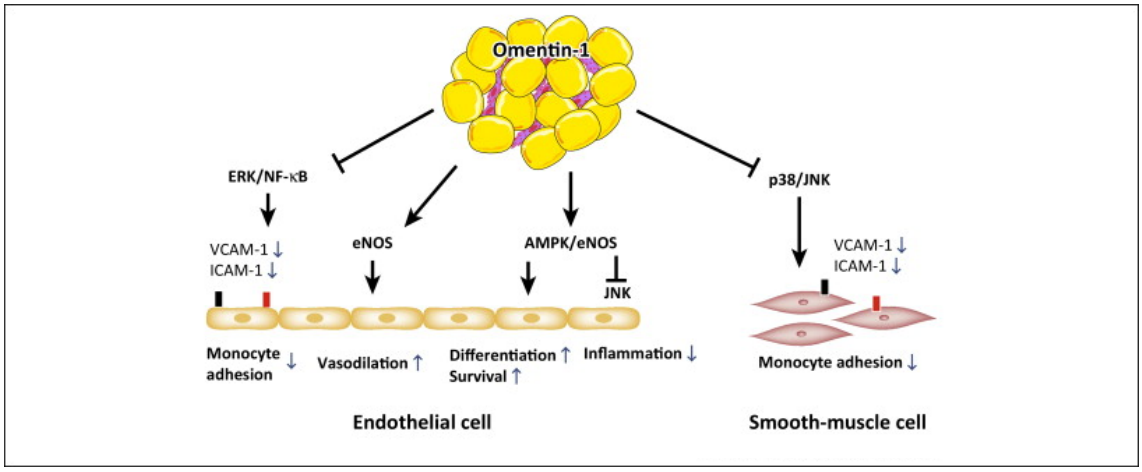

Figura 2 – Ação da omentina-1 nas células endoteliais, demonstrando seu efeito antiaterogênico e anti-inflamatório (36).

A omentina-1 também parece estar envolvida na regulação do apetite. Brunetti *et al* demostraram, em modelo animal, que a administração de omentina-1 diminuiu a expressão de *cocaine-and amphetamine-regulated transcript* (*CART*) e aumentou liberação de norepinefrina no hipotálamo de camundongos (40). Em pacientes com obesidade, a redução de peso levou a um aumento nos níveis circulantes de omentina-1

(41, 42).

1.2 Variantes genéticas da omentina-1

A estrutura do gene *ITLN1* foi determinada por Tsuji *et al* (2001). É composto por 8 exons e está localizado no cromossomo 1, na região 1q21.3-q22 (43, 44). Esta localização cromossomal foi relacionada à susceptibilidade para DM2 em várias populações (20, 44-51). Os níveis séricos de omentina-1 tem sido positivamente correlacionados com a expressão de seu respectivo RNAm (52).

Através de sequenciamento do DNA genômico obtido de indivíduos saudáveis, dois polimorfismos de nucleotídeo único (SNP – *single nucleotide polymorphism*) foram identificados no gene *ITLN1* (44). O primeiro está localizado no éxon 4, sendo que o nucleotídeo +326 (numeração relacionada ao códon de iniciação ATG) é polimórfico. Nele, o códon GTC é substituído por GAC que muda o aminoácido Val para Asp na posição 109 (rs2274907). O segundo polimorfismo identificado (His86His) não altera o aminoácido e está localizado no nucleotídeo 258 (C/T) dentro do éxon-4.

A relação entre a expressão gênica de *ITLN1* e níveis plasmáticos da proteína omentina-1 tem sido estudada em pacientes com doenças cardiovasculares e condições correlatas como obesidade, diabetes e síndrome metabólica (53, 54). O SNP rs2274907 foi associado com doença arterial coronariana (DAC) em homens de uma população Iraniana, onde o alelo A demonstrou ser significativamente mais prevalente nos doentes em relação aos controles (55). Este mesmo polimorfismo foi avaliado em pacientes com doença hepática gordurosa não-alcoólica (56), psoríase e com doença inflamatória intestinal (44, 57). Em relação ao consumo alimentar, Splichal *et al* observaram que o

SNP rs2274907 teve predição para ingestão energética independente de idade e sexo, sendo o genótipo TT associado com menor ingestão e AA com maior ingestão calórica, bem como com ingestão diária de gorduras e proteínas em adultos com obesidade e eutróficos (58).

1.3 Omentina-1 e Doença Renal Crônica

A prevalência de doença renal crônica (DRC) tem aumentado mundialmente e é considerado um importante problema de saúde que acarreta aumento da morbimortalidade da população (59). Inflamação é uma condição clínica altamente prevalente nesta população, conforme demostrado no estudo *Chronic Renal Insufficiency Cohort*, onde 86% dos indivíduos com DRC tinham alguma evidência de inflamação (60).

A relação da omentina-1 com a função renal ainda não está bem estabelecida. O primeiro estudo relacionando os níveis plasmáticos de omentina-1 com DRC data de 2012, onde pacientes em hemodiálise apresentaram níveis mais elevados desta adipocina quando comparados aos controles saudáveis. Este achado foi explicado pela reduzida degradação e excreção da omentina-1, devido a DRC (61). Um mecanismo similar foi discutido por Tekce *et al*, onde pacientes em tratamento conservador, sem terapia de substituição renal, apresentaram omentina-1 sérica significativamente mais alta em estágios mais avançados da DRC quando comparados aos indivíduos em estágios iniciais da doença (62). Por outro lado, os níveis de omentina-1 destes indivíduos foram mais baixos do que os encontrados nos controles saudáveis, corroborando com a hipótese de que a DRC leva a uma redução nos níveis séricos desta adipocina. Kocijancic *et al* concluíram que a redução nos níveis de omentina-1 pode ser um preditor de mortalidade

por doença cardiovascular (DCV) em pacientes em hemodiálise com aterosclerose subclínica (63).

Com a progressão da DRC, torna-se necessária a implementação de uma terapia renal substitutiva, seja através de terapia dialítica ou de transplante renal. A sobrevida geral, risco cardiovascular e qualidade de vida são superiores nos pacientes submetidos a transplante renal em comparação com pacientes em terapia dialítica ou em lista de espera para transplante (64, 65). Entretanto, o transplante renal, apesar de seus reconhecidos benefícios, também pode levar ao desenvolvimento de outras patologias, dentre elas, o DMPT, e que está associado negativamente à desfechos clínicos (obesidade, HAS, dislipidemia) e a desfechos propriamente ligados ao transplante (sobrevida do enxerto, rejeição aguda, infecções) (66).

Levando-se em consideração os conhecimentos mencionados acima sobre o possível papel protetor da omentina-1 em relação a desordens metabólicas, acreditamos ser relevante o estudo da associação da omentina-1 com o DMPT, tanto através da avaliação dos níveis plasmáticos quanto por estudo de polimorfismo no gene *ITLN1* que codifica esta proteína, em uma população de pacientes transplantados, com e sem DMPT.

2 OBJETIVOS

Objetivos primários:

1) Avaliar a associação dos níveis plasmáticos de omentina-1 com a presença de DMPT em pacientes transplantados renais.

2) Avaliar a associação do polimorfismo rs2274907 no gene *ITLN-1* e a presença de DMPT em pacientes transplantados renais.

Objetivos Secundários:

- Analisar a associação entre níveis plasmáticos de omentina-1 e consumo alimentar de energia, macronutrientes e micronutrientes em pacientes transplantados renais.
- Verificar associações entre níveis plasmáticos de omentina-1 com perfil antropométrico, composição corporal e variáveis bioquímicas em pacientes transplantados renais.
- Verificar associações entre os genótipos do polimorfismo rs2274907 no gene *ITLN1* com perfil antropométrico, composição corporal e variáveis bioquímicas em pacientes transplantados renais.

REFERÊNCIAS

1. Sharif A, Hecking M, de Vries AP, Porrini E, Hornum M, Rasoul-Rockenschaub S, et al. Proceedings from an international consensus meeting on posttransplantation diabetes mellitus: recommendations and future directions. Am J Transplant. 2014;14(9):1992-2000.
2. Association AD. 2. Classification and Diagnosis of Diabetes:. Diabetes Care. 2019;42(Suppl 1):S13-S28.
3. Eckhard M, Schindler RA, Renner FC, Schief W, Padberg W, Weimer R, et al. New-onset diabetes mellitus after renal transplantation. Transplant Proc. 2009;41(6):2544-5.
4. Hecking M, Werzowa J, Haidinger M, Hörl WH, Pascual J, Budde K, et al. Novel views on new-onset diabetes after transplantation: development, prevention and treatment. Nephrol Dial Transplant. 2013;28(3):550-66.
5. Jenssen T, Hartmann A. Emerging treatments for post-transplantation diabetes mellitus. Nat Rev Nephrol. 2015;11(8):465-77.
6. Londero TM, Giaretta LS, Farenzena LP, Manfro RC, Canani LH, Lavinsky D, et al. Microvascular Complications of Posttransplant Diabetes Mellitus in Kidney Transplant Recipients: A Longitudinal Study. J Clin Endocrinol Metab. 2019;104(2):557-67.
7. Cascais de Sá D, Santos L, Rodrigues L, Romãozinho C, Guedes Marques M, Macário F, et al. Post-Kidney-Transplant Diabetes: Evaluation of Risk Factors. Transplant Proc. 2019;51(5):1597-600.
8. Lima C, Grden A, Skare T, Jaworski P, Nisihara R. Risk factors for new-onset diabetes mellitus after kidney transplantation (NODAT): a Brazilian single center study. Arch Endocrinol Metab. 2018;62(6):597-601.
9. Kleinsteuber A, Halleck F, Khadzhynov D, Staeck A, Lehner L, Duerr M, et al. Impact of Pre-existing Comorbidities on Long-term Outcomes in Kidney Transplant Recipients. Transplant Proc. 2018;50(10):3232-41.
10. El Essawy B, Kandeel F. Pre, peri and posttransplant diabetes mellitus. Curr Opin Nephrol Hypertens. 2019;28(1):47-57.
11. Anagnostis P, Paschou SA, Spartalis E, Sarno G, De Rosa P, Muscogiuri G. Metabolic complications and kidney transplantation: focus on glycaemia and dyslipidaemia. Curr Vasc Pharmacol. 2019.
12. Shimada H, Uchida J, Nishide S, Kabei K, Kosoku A, Maeda K, et al. Comparison of Glucose Tolerance between Kidney Transplant Recipients and Healthy Controls. J Clin Med. 2019;8(7).
13. Chowdhury TA. Post-transplant diabetes mellitus. Clin Med (Lond). 2019;19(5):392-5.
14. Herder C, Carstensen M, Ouwens DM. Anti-inflammatory cytokines and risk of type 2 diabetes. Diabetes Obes Metab. 2013;15 Suppl 3:39-50.
15. Wittenbecher C, Menzel J, Carstensen-Kirberg M, Biemann R, di Giuseppe R, Fritsche A, et al. Omentin-1, Adiponectin, and the Risk of Developing Type 2 Diabetes. Diabetes Care. 39. United States2016. p. e79-80.
16. Mancuso P. The role of adipokines in chronic inflammation. Immunotargets Ther. 2016;5:47-56.
17. Lee JK, Schnee J, Pang M, Wolfert M, Baum LG, Moremen KW, et al. Human homologs of the Xenopus oocyte cortical granule lectin XL35. Glycobiology. 2001;11(1):65-73.

18. Matsubara T, Mita A, Minami K, Hosooka T, Kitazawa S, Takahashi K, et al. PGRN is a Key Adipokine Mediating High Fat Diet-Induced Insulin Resistance and Obesity through IL-6 in Adipose Tissue. Cell Metabolism. 2012;15(1):38-50.
19. de Souza Batista CM, Yang RZ, Lee MJ, Glynn NM, Yu DZ, Pray J, et al. Omentin plasma levels and gene expression are decreased in obesity. Diabetes. 2007;56(6):1655-61.
20. Xiang K, Wang Y, Zheng T, Jia W, Li J, Chen L, et al. Genome-wide search for type 2 diabetes/impaired glucose homeostasis susceptibility genes in the Chinese: significant linkage to chromosome 6q21-q23 and chromosome 1q21-q24. Diabetes. 2004;53(1):228-34.
21. Fu M, Gong D-w, Damcott C, Sabra M, Yang R, Pollin TI, et al. Systematic Analysis of Omentin 1 and Omentin 2 on 1q23 as Candidate Genes for Type 2 Diabetes in the Old Order Amish. Diabetes. 2004;53.
22. Yang R, Xu A, Pray J, Hu H, Jadhao S, Hansen B, et al. Cloning of Omentin, a New Adipocytokine from Omental Fat Tissue in Humans. Diabetes. 2017;52.
23. Tan YL, Zheng XL, Tang CK. The protective functions of omentin in cardiovascular diseases. Clin Chim Acta. 2015;448:98-106.
24. Shibata R, Ouchi N, Takahashi R, Terakura Y, Ohashi K, Ikeda N, et al. Omentin as a novel biomarker of metabolic risk factors. Diabetol Metab Syndr. 2012;4(1):37.
25. Yoo HJ, Hwang SY, Hong HC, Choi HY, Yang SJ, Seo JA, et al. Association of circulating omentin-1 level with arterial stiffness and carotid plaque in type 2 diabetes. Cardiovasc Diabetol. 2011;10:103.
26. NELSON DL, COX M. Lehninger – Princípios de Bioquímica . 3 ed. São Paulo: Sarvier; 2002.
27. Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, et al. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. Am J Physiol Endocrinol Metab. 2006;290(6):E1253-61.
28. Pan HY, Guo L, Li Q. Changes of serum omentin-1 levels in normal subjects and in patients with impaired glucose regulation and with newly diagnosed and untreated type 2 diabetes. Diabetes Res Clin Pract. 2010;88(1):29-33.
29. Yan P, Liu D, Long M, Ren Y, Pang J, Li R. Changes of serum omentin levels and relationship between omentin and adiponectin concentrations in type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes. 2011;119(4):257-63.
30. Catli G, Anik A, Abaci A, Kume T, Bober E. Low omentin-1 levels are related with clinical and metabolic parameters in obese children. Exp Clin Endocrinol Diabetes. 2013;121(10):595-600.
31. Saremi A, Asghari M, Ghorbani A. Effects of aerobic training on serum omentin-1 and cardiometabolic risk factors in overweight and obese men. J Sports Sci. 2010;28(9):993-8.
32. Zhang Q, Zhu L, Zheng M, Fan C, Li Y, Zhang D, et al. Changes of serum omentin-1 levels in normal subjects, type 2 diabetes and type 2 diabetes with overweight and obesity in Chinese adults. Ann Endocrinol (Paris). 2014;75(3):171-5.
33. Kazama K, Usui T, Okada M, Hara Y, Yamawaki H. Omentin plays an anti-inflammatory role through inhibition of TNF-alpha-induced superoxide production in vascular smooth muscle cells. Eur J Pharmacol. 2012;686(1-3):116-23.
34. Yamawaki H, Kuramoto J, Kameshima S, Usui T, Okada M, Hara Y. Omentin, a novel adipocytokine inhibits TNF-induced vascular inflammation in human endothelial cells. Biochem Biophys Res Commun. 2011;408(2):339-43.

35. Zhong X, Li X, Liu F, Tan H, Shang D. Omentin inhibits TNF-alpha-induced expression of adhesion molecules in endothelial cells via ERK/NF-kappaB pathway. Biochem Biophys Res Commun. 2012;425(2):401-6.
36. Ohashi K, Shibata R, Murohara T, Ouchi N. Role of anti-inflammatory adipokines in obesity-related diseases. Trends Endocrinol Metab. 2014;25(7):348-55.
37. Yamawaki H, Tsubaki N, Mukohda M, Okada M, Hara Y. Omentin, a novel adipokine, induces vasodilation in rat isolated blood vessels. Biochem Biophys Res Commun. 2010;393(4):668-72.
38. Zhong X, Zhang HY, Tan H, Zhou Y, Liu FL, Chen FQ, et al. Association of serum omentin-1 levels with coronary artery disease. Acta Pharmacol Sin. 2011;32(7):873-8.
39. Shibata R, Takahashi R, Kataoka Y, Ohashi K, Ikeda N, Kihara S, et al. Association of a fat-derived plasma protein omentin with carotid artery intima-media thickness in apparently healthy men. Hypertens Res. 2011;34(12):1309-12.
40. Brunetti L, Orlando G, Ferrante C, Recinella L, Leone S, Chiavaroli A, et al. Orexigenic effects of omentin-1 related to decreased CART and CRH gene expression and increased norepinephrine synthesis and release in the hypothalamus. Peptides. 2013;44:66-74.
41. Moreno-Navarrete JM, Catalán V, Ortega F, Gómez-Ambrosi J, Ricart W, Frühbeck G, et al. Circulating omentin concentration increases after weight loss. Nutr Metab (Lond). 2010;7:27.
42. Shaker M, Mashhadani ZI, Mehdi AA. Effect of Treatment with Metformin on Omentin-1, Ghrelin and other Biochemical, Clinical Features in PCOS Patients. Oman Med J. 2010;25(4):289-93.
43. Tsuji S, Uehori J, Matsumoto M, Suzuki Y, Matsuhisa A, Toyoshima K, et al. Human intelectin is a novel soluble lectin that recognizes galactofuranose in carbohydrate chains of bacterial cell wall. J Biol Chem. 2001;276(26):23456-63.
44. Schaffler A, Zeitoun M, Wobser H, Buechler C, Aslanidis C, Herfarth H. Frequency and significance of the novel single nucleotide missense polymorphism Val109Asp in the human gene encoding omentin in Caucasian patients with type 2 diabetes mellitus or chronic inflammatory bowel diseases. Cardiovasc Diabetol. 2007;6:3.
45. Hsueh WC, St Jean PL, Mitchell BD, Pollin TI, Knowler WC, Ehm MG, et al. Genome-wide and fine-mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish: evidence for a new diabetes locus on chromosome 14q11 and confirmation of a locus on chromosome 1q21-q24. Diabetes. 2003;52(2):550-7.
46. Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, et al. An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. Am J Hum Genet. 1998;63(4):1130-8.
47. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ. A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. Diabetes. 1999;48(5):1175-82.
48. Vionnet N, Hani EH, Dupont S, Gallina S, Francke S, Dotte S, et al. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am J Hum Genet. 2000;67(6):1470-80.
49. Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, Sampson M, et al. A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): analysis of 573 pedigrees provides independent

replication of a susceptibility locus on chromosome 1q. Am J Hum Genet. 2001;69(3):553-69.
50. Meigs JB, Panhuysen CI, Myers RH, Wilson PW, Cupples LA. A genome-wide scan for loci linked to plasma levels of glucose and HbA(1c) in a community-based sample of Caucasian pedigrees: The Framingham Offspring Study. Diabetes. 2002;51(3):833-40.
51. Langefeld CD, Wagenknecht LE, Rotter JI, Williams AH, Hokanson JE, Saad MF, et al. Linkage of the metabolic syndrome to 1q23-q31 in Hispanic families: the Insulin Resistance Atherosclerosis Study Family Study. Diabetes. 2004;53(4):1170-4.
52. Cai RC, Wei L, DI JZ, Yu HY, Bao YQ, Jia WP. [Expression of omentin in adipose tissues in obese and type 2 diabetic patients]. Zhonghua Yi Xue Za Zhi. 2009;89(6):381-4.
53. Tan BK, Adya R, Randeva HS. Omentin: a novel link between inflammation, diabesity, and cardiovascular disease. Trends Cardiovasc Med. 2010;20(5):143-8.
54. Zhou JY, Chan L, Zhou SW. Omentin: linking metabolic syndrome and cardiovascular disease. Curr Vasc Pharmacol. 2014;12(1):136-43.
55. Jamshidi J, Ghanbari M, Asnaashari A, Jafari N, Valizadeh GA. Omentin Val109Asp polymorphism and risk of coronary artery disease. Asian Cardiovasc Thorac Ann. 2017;25(3):199-203.
56. Kohan L, Safarpur M, Abdollahi H. Omentin-1 rs2274907 and resistin rs1862513 polymorphisms influence genetic susceptibility to nonalcoholic fatty liver disease. Mol Biol Res Commun. 2016;5(1):11-7.
57. Zhang C, Zhu KJ, Liu JL, Xu GX, Liu W, Jiang FX, et al. Omentin-1 plasma levels and omentin-1 expression are decreased in psoriatic lesions of psoriasis patients. Arch Dermatol Res. 2015;307(5):455-9.
58. Splichal Z, Bienertova-Vasku J, Novak J, Zlamal F, Tomandl J, Tomandlova M, et al. The common polymorphism Val109Asp in the omentin gene is associated with daily energy intake in the Central-European population. Nutr Neurosci. 2015;18(1):41-8.
59. Wen CP, Matsushita K, Coresh J, Iseki K, Islam M, Katz R, et al. Relative risks of chronic kidney disease for mortality and end-stage renal disease across races are similar. Kidney Int. 2014;86(4):819-27.
60. Gupta J, Mitra N, Kanetsky PA, Devaney J, Wing MR, Reilly M, et al. Association between albuminuria, kidney function, and inflammatory biomarker profile in CKD in CRIC. Clin J Am Soc Nephrol. 2012;7(12):1938-46.
61. Alcelik A, Tosun M, Ozlu MF, Eroglu M, Aktas G, Kemahli E, et al. Serum levels of omentin in end-stage renal disease patients. Kidney Blood Press Res. 2012;35(6):511-6.
62. Tekce H, Tekce BK, Aktas G, Alcelik A, Sengul E. Serum omentin-1 levels in diabetic and nondiabetic patients with chronic kidney disease. Exp Clin Endocrinol Diabetes. 2014;122(8):451-6.
63. Kocijancic M, Cubranic Z, Vujicic B, Racki S, Dvornik S, Zaputovic L. Soluble intracellular adhesion molecule-1 and omentin-1 as potential biomarkers of subclinical atherosclerosis in hemodialysis patients. Int Urol Nephrol. 2016;48(7):1145-54.
64. Lippe Nvd, Waldum-Grevbo B, Reisæter AV, Os I. Is HRQOL in dialysis associated with patient survival or graft function after kidney transplantation? BMC Nephrology. 2016;17(1):94.
65. Ali A, Macphee I, Kaski JC, Banerjee D. Cardiac and vascular changes with kidney transplantation. Indian J Nephrol. 2016;26(1):1-9.

66. Yates CJ, Fourlanos S, Hjelmesaeth J, Colman PG, Cohney SJ. New-onset diabetes after kidney transplantation-changes and challenges. Am J Transplant. 2012;12(4):820-8.

CAPÍTULO 2

ARTIGO ORIGINAL 1

“Low plasma omentin-1 levels are associated with posttransplant diabetes mellitus in kidney transplant recipients.”

Low plasma omentin-1 levels are associated with posttransplant diabetes mellitus in kidney transplant recipients.

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The authors declare no conflict of interest.

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ABBREVIATIONS

BMI, body mass index

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration

CKD, chronic kidney disease

CMV, cytomegalovirus

DM, diabetes mellitus

eGFR, estimated glomerular filtration rate

HbA1C, glycated hemoglobin

HCV, hepatitis C virus

HOMA-ß, homeostatic model assessment of beta cell function

HOMA-IR, homeostatic model assessment of insulin resistance

ITLN1, omentin-1 gene

LAP, lipid accumulation product

MAC, mid-arm circumference

MAMC, mid-arm muscle circumference

PTDM, posttransplantation diabetes mellitus

T2DM, type 2 diabetes mellitus

TG, triglycerides

TyG, triglycerides/glucose index

VAI, visceral adiposity index

WHR, waist-hip ratio

ω6: ω3, omega 6: omega 3 ratio

ABSTRACT

BACKGROUND: Posttransplant diabetes mellitus (PTDM) is a common complication after kidney transplantation. Plasma levels of omentin-1 are decreased in obesity, insulin-resistant states and type 2 diabetes mellitus. The aim of this study was to evaluate the association between omentin-1 plasma levels and PTDM in kidney transplant recipients.

METHODS: Plasma levels of omentin-1 were measured in PTDM (n=54) and non-PTDM patients (n=53) in a nested case-control study within a cohort of kidney transplant recipients from southern Brazil. Patients were evaluated through clinical examination, anthropometric measurements and dietary records. Fasting blood glucose, insulin, lipid profile and other biochemical data were also measured. A logistic regression was used to evaluate the association between plasma omentin-1 levels and PTDM.

RESULTS: Plasma omentin-1 levels were significantly reduced in the PTDM group (3.83 [1.67– 6.52] ng/mL) compared to the control group (5.62 [2.70 – 9.47] ng/mL, P = 0.036). The odds ratio (OR) and 95% confidence interval (CI) comparing the highest vs. lowest strata was 0.36 (0.16 - 0.79; P=0.018), considering the cut-off point of 4.84 ng/mL. Additionally, the incremental value of omentin-1 in PTDM risk prediction also was assessed. For each 1ng/mL increased in plasma omentin-1 levels, the odds to develop PTDM reduced by 8% (OR=0.92 [0.854 – 0.997], P=0.041). Women showed a higher concentration of plasma omentin-1 levels than men. The patients who presented higher waist circumference had decreased omentin-1 levels. Furthermore, a significant negative correlation was observed between plasma omentin-1 levels and the omega 6: omega 3 ingestion (ρ= -0.854; P<0.0001).

CONCLUSIONS: Our findings support an association between low plasma omentin-1 levels and risk for PTDM in renal transplant recipients. Further prospective studies are needed to confirm and elucidate the functional significance of this association.

INTRODUCTION

Renal transplantation is the most effective renal replacement therapy for patients with end stage renal disease. It is cost effective and is associated with a better patient quality of life and survival compared to dialysis therapy. However, transplant-related complications and metabolic complications are not uncommon after kidney transplantation. Among the most frequent metabolic dysfunctions are obesity, dyslipidemia and post-transplantation diabetes mellitus (PTDM), which contribute to reduced graft and patient survival [1-4].

PTDM is a well-established complication that occurs mainly during the first year after transplantation with the incidence ranging from 10 to 40% [5-9]. PTDM is associated with increased morbidity and mortality from cardiovascular complications in transplant patients [10,11]. Several risk factors for PTDM have been identified, such as older age, obesity, ethnicity, hepatitis C and cytomegalovirus infections, and immunosuppressive medications [12-14]. Calcineurin inhibitors and corticosteroids play a significant role in the development of PTDM, both by direct toxic effects on beta-cell leading to impaired insulin secretion and by increasing insulin resistance on peripheral and hepatic tissues [15-17].

In addition to the known pathophysiology of PTDM associated with the aforementioned risk factors, some other potential factors have been speculated to be involved in the development of PTDM. Adipose tissue is one of the largest endocrine organs in the body and is responsible for producing proteins called adipokines. Adipokines are known to influence a variety of physiological and pathophysiological metabolic processes [18-23]. Among these adipokines are the acute-phase reactants, cytokines, chemokines, damage-associated molecular pattern molecules, as well as pro-inflammatory and anti-inflammatory factors [24,25]. In the last years, studies focusing on

the anti-inflammatory effects of some adipokines and its role in the regulation of adipose tissue have been conducted [20,26-28].

Omentin (also known as intelectin, intestinal lactoferrin receptor, endothelian lectin HL-1, galactofuranose-binding lectin), is an anti-inflammatory adipokine with a molecular weight of 34 kDa. It is composed of 313 amino acids and is encoded by a gene present in chromosomal region 1q22–q23, which has been related to type 2 diabetes mellitus (T2DM) [29-32]. Omentin is expressed in two isoforms: the omentin-1, which is the major circulating form in human plasma, and the omentin-2, which is released into the intestinal lumen and has not been detected in plasma. Omentin-1 has been positively associated with adiponectin, which is an anti-inflammatory adipokine with anti-atherosclerotic and insulin-sensitizing proprieties [33,34]. Both of them are also associations with anthropometric and metabolic features [34]. Studies in humans have found circulating plasma levels and visceral adipose gene expression of omentin-1 to be negatively correlated with body mass index (BMI), fasting insulin, and measures of homeostatic model assessment of insulin resistance (HOMA-IR) [30,32,35]. Furthermore, hyperinsulinemic induction in healthy subjects significantly reduced plasma omentin-1 levels [36]. This adipokine is decreased in T2DM subjects [37,38] and is associated with risk for developing diabetes [39]. Also, plasma omentin-1 levels are independently associated with endothelial function in subgroups of patients with T2DM at elevated cardiovascular risk [40].

In chronic kidney disease (CKD), some studies also indicate an association of omentin-1 levels with diabetes mellitus (DM) [41,42]. However, in kidney transplant patients, no study, of our knowledge, has evaluated the relation of omentin-1 levels with PTDM. Taking into account the metabolic effects related to omentin-1 and its association

with T2DM, we sought to investigate the association of omentin-1 plasma levels with PTDM and related metabolic parameters in kidney transplant patients.

MATERIALS AND METHODS

Study population

This is a nested case-control study within a cohort of 504 kidney transplant recipients from a tertiary hospital in southern Brazil, from 2000 to 2014. We matched cases (PTDM) and controls (non-PTDM) by age, gender, estimated glomerular filtration rate (eGFR) and BMI to evaluate the association between plasma omentin-1 levels and PTDM. A total of 142 patients fulfilled the matching criteria and were further selected according to eligibility. PTDM diagnostic criteria were applied only after 45 days after transplantation and followed the American Diabetes Association standards. Exclusion criteria included DM prior to transplantation, malignancy, bowel inflammatory disease, acute inflammation or acute infection disease.

Clinical and laboratory data

Plasma omentin-1 levels were determined by a commercially available ELISA kit (E-EL-H2028, Elabscience) according to protocol. The intra and inter-assay coefficients of variation were 3.5% and 10.5%, respectively. The sensitivity, defined as the mean ± 3 SD of the 0 standard, was calculated to be 0.15 pmol/ml. For each patient, the plasma levels were measured twice, and the results were averaged. Fasting glucose, glycated hemoglobin (HbA1C), fasting insulin, serum and urine creatinine, serum urea, triglycerides (TG), total cholesterol, and high-density lipoprotein (HDL) were also determined. All blood samples were drawn after overnight fasting. The low-density lipoprotein (LDL)-cholesterol concentration was estimated using the Friedewald formula

[43]. Renal function was evaluated by eGFR, through CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). The presence of cytomegalovirus (CMV) and hepatitis C virus (HCV) were assessed by medical records. Information about smoking, alcohol consumption and physical activities were self-reported at the interview. Food intake data were obtained from patients by a trained nutrition professional through the completion of a 24-hours food record. In order to establish the absolute and percentage of daily energy intake from carbohydrates, fat, protein and micronutrients, as well as the intake of omega 6: omega 3 ratio (ω6: ω3), were performed the nutritional analysis on Dietbox® software (version 2.0). The study protocol was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre (17-0327, project number) and all methods were performed in accordance with the relevant guidelines and regulations. Informed consent was provided with completion of the interview.

Anthropometric and biochemical indexes

Measurements of height, body weight, body circumference and skinfold thickness were performed in all patients. Mid-arm circumference (MAC), waist and hip circumferences were measured using a flexible non-elastic measuring tape. MAC was measured around the upper arm, at the midpoint between the acromion and the olecranon. Waist circumference (WC) was measured at the midpoint between the lower border of the rib cage and the iliac crest. Hip circumference was measured as the maximum circumference at the level of the buttocks. All skinfold thicknesses were measured according to the International Standards for Anthropometric Assessment [44].

BMI was measured on a continuous scale and groups were classified according to the World Health Organization. Mid-arm muscle circumference (MAMC) was calculated using the standard formula: MAMC = MAC– (3.14 x triceps skinfold thickness).

The HOMA-IR index was calculated as the product of basal glucose and insulin levels divided by 22.5, while the HOMA of β-cell function (HOMA-B) index, computed as the product of 20 and basal insulin levels divided by the value of basal glucose concentrations minus 3.5 [45].

The triglycerides/glucose index (TyG) index was calculated as log [TG×fasting glucose/2]. A cut off was placed at the TyG index value of 4.49, according to Salazar *et al* [46]. Visceral adiposity index (VAI) was calculated using gender-specific formulas: men [WC/39.68+(1.88×BMI)] × (TG/1.03) × (1.31/HDL-C); women: [WC/36.58 + (1.89×BMI)] × (TG/0.81) × (1.52/HDL-C) [47].

Lipid accumulation product (LAP) index was calculated as [WC-65]×[TG] in men, and [WC-58]×[TG] in women [48].

Statistical Analysis

Data were analyzed using IBM SPSS® v20 (New York, USA). Continuous data were presented as mean ± standard deviation or median (quartile interval) as appropriate. Prior to analyses, data were log transformed as needed. Comparisons of continuous data between two independent groups were done by student's t-test assuming that the study variable is a normal variate in the population and the variances in the two groups are homogeneous. Mann-Whitney U test was used for non-normally distributed values. χ^2 test were used to assess the relationship between variables in categorical data. The relationships between variables were analyzed by simple bivariate correlation (Pearson's r or Spearman's rho). Binary logistic regression analysis was performed to estimate odds ratios and 95% CI for the association between plasma omentin-1 levels and PTDM. All statistical tests were two tailed. The value $P > 0.05$ was considered to be significant.

RESULTS

From the 142 patients identified by the matching factors, 107 patients were eligible for inclusion, which 54 cases and 53 in controls. Clinical and demographic data are shown in Table 1 and the anthropometric characteristics are shown in Table 2. The groups were similar according to BMI, body circumferences, waist-hip ratio (WHR) and VAI. PTDM patients had significantly higher TyG index, LAP and HOMA-IR and lower HOMA-β compared to the control patients.

Plasma omentin-1 levels were markedly reduced in the PTDM group (3.83 [1.67–6.52] ng/mL) compared to the control group (5.62 [2.70 – 9.47] ng/mL; P = 0.036) (**Figure 1**). The whole sample was stratified into high and low omentin-1 levels. The best cut-off point of plasma omentin-1 was 4.84 ng/mL, evaluated by receiver operating characteristic (ROC) curve analysis. This result was in agreement with the median value (4.89 ng/mL). Higher plasma omentin-1 levels were associated with a lower PTDM risk. The odds ratio (OR) and 95% confidence interval (CI) comparing the highest vs. lowest strata was 0.36 (0.16 - 0.79; P=0.018). We also assessed the incremental value of omentin-1 in PTDM risk prediction. For each 1ng/mL increased in plasma omentin-1 levels, the odds to develop PTDM reduced by 8% (OR=0.92 [0.854 – 0.997], P=0.041).

When analyzing demographic and clinical variables related to plasma omentin-1 levels, we observed that women showed a higher concentration of plasma omentin-1 levels than men (5.73 [3.1 – 9.81] and 3.03 [1.25 – 6.36] ng/mL, P=0.001). Raised waist circumference (WC) was defined as 88 cm for women and 102 cm for men [49]. In our study, the patients who presented higher WC, had decreased omentin-1 levels (3.62 [1.19-5.95] vs. 5.16 [2.70-12.28] ng/mL, P=0.031).

There was no difference in the omentin-1 levels according to the insulin or metformin use. The correlation analysis between omentin-1 serum levels, insulin and

beta-cell function parameters, showed that there is a positive correlation between omentin-1 and Homa-β (ρ=0.201; p=0.044).

In order to analyze the association between lipid profile and omentin-1 levels, we classified total cholesterol (TC) value according to the cut-off point 190mg/dL [50] Patients with an adequate level of cholesterol presented a higher level of omentin-1 (5.56 [2.51-9.33] ng/mL) compared to those with a high level of cholesterol (4.07 [1.99-6.74] ng/mL, P=0.042).

Analyzing the food consumption, a negative correlation between omentin-1 levels and the omega 6: omega 3 ratio (ω6: ω3) ingestion (ρ= -0.832; p<0.0001) was observed (**Figure 2**). This ratio did not differ between PTDM and non-PTDM patients [9 (7 – 12) *vs* 8 (6 – 12); p=0.195]. Total carbohydrate and sodium ingestion also presented a negative correlation with plasma omentin-1 levels (r= -0.221; P=0.025 and ρ= -0.254; P=0.009, respectively).

DISCUSSION

In the present study, we investigated the plasma levels of omentin-1 in kidney transplant recipients. The main finding was a significant association of low plasma omentin-1 levels with risk for PTDM. This is the first study that demonstrated the association between omentin-1 and PTDM.

It has been previously reported that omentin-1, a visceral protein, is inversely correlated with overweight/obesity and insulin resistance [35,51-53]. In our study, low plasma omentin-1 levels were associated with PTDM, regardless of BMI. In a meta-analysis of observational studies, omentin-1 levels were significantly lower in T2DM and in patients with impaired glucose tolerance [38].

The PTDM physiopathology shares similar mechanisms with T2DM, including insulin resistance and impairment of insulin secretion. In this context, our data suggest that omentin-1 levels are correlated with beta-cell function, which was assessed through HOMA-β. Plasma omentin-1 levels also seem to be associated with gender. De Souza Batista *et al* found an increased level of plasma omentin-1 in women compared to men, after adjustment for BMI [35]. This is in line with our data that showed women to have higher concentration of plasma omentin-1 compared to men, independently of the presence of PTDM.

Studies with mouse models point towards atheroprotective properties of omentin-1 [54-56]. Hiramatsu-Ito M *et al*, showed that omentin-1 reduces atherosclerosis development by reducing the inflammatory response of macrophages through the Akt-dependent mechanisms [55]. Yang *et al*. showed that treatment with recombinant omentin-1 enhanced insulin-stimulated glucose uptake in human subcutaneous and omental adipocytes. They also demonstrate that omentin-1 stimulated Akt phosphorylation in both the absence and presence of insulin [32]. It is well known that insulin-stimulated glucose transporter 4 (GLUT4) translocation via activation of Akt signaling is important in maintaining glucose homeostasis [57,58]. Watanabe *et al* suggested that omentin-1 based medicines, including omentin-1 analogues and omentin-1 receptor agonists, could be a new therapeutic strategy for some metabolic diseases including diabetes and obesity [59].

Of interest, we describe for the first time, a negative correlation between plasma omentin-1 levels and ω-6: ω-3 fatty acid ratio intake. The typical Western dietary pattern has an elevated ω-6: ω-3 fatty acid ratio and it is linked with high prevalence of atherosclerosis, obesity, and diabetes through mechanisms of adipogenesis, lipid homeostasis, brain-gut-adipose tissue axis, and systemic inflammation [60,61]. Jacobo-Cejudo MG *et al* described that the supplementation of ω-3 polyunsaturated fatty acid as

a strategy to reduce the ω-6: ω-3 fatty acid ratio, had a beneficial effect on waist circumference, serum glucose, Hb1Ac, leptin, leptin/adiponectin ratio, and lipid profile in T2DM patients [62]. In a longitudinal study, dietary ω-3 intake was inversely associated with the incidence of CKD among American young adults in 25 years of follow-up [63]. However, ω-3 supplementation was not sufficient to preserve kidney function in patients with T2DM [64]. Also, regarding omega-3 fatty acid, studies have shown a positive relationship between the omega-3 fatty acid supplementation and increased levels of adiponectin [65,66].

In respect to chronic kidney disease, results of plasma omentin-1 levels are conflicting. Alcelik *et al* demonstrated that in hemodialysis patients, omentin-1 levels were higher in the subgroup of diabetic patients when compared to non-diabetic CKD patients. The authors discussed that the possible reason for the increased levels of omentin-1 might be related to impaired renal clearance of the protein and not to a protective effect on HD patients [42]. In this context, Tekce *et al* suggested that DM and inflammation are associated with lower omentin-1 levels in the CKD population. They showed that omentin-1 were significantly lower in diabetic CKD patients subgroup compared to the control group and to non-diabetic CKD patients. When stratified into stages of the CKD, stage 4 CKD patients had a higher omentin-1 levels compared to patients in the earlier stages and in the control group, and these results could be justified by lack of degradation and excretion of the omentin-1 secondary to renal dysfunction [41]. Furthermore, Kocijancik *et al* showed that serum omentin-1 levels were associated with mortality risk in diabetic hemodialysis patients [67]. In our study, plasma omentin-1 levels did not differ among the CKD stages. This may have been due to a small number of patients in stages 4 and 5.

The main strength of this study is the matched case-control design. When individuals were selected into a specific range of factors, we reduced the possibility of bias to the association between omentin-1 and PTDM. Although we attempted to appropriately control for confounding through this statistical approach, there is always a possibility of residual confounding by other serum molecules and genetic factors. Regarding the limitations of our study, the relatively small sample size and to the fact of being a retrospective study can be cited.

In summary, this is the first study to evaluate the association between plasma omentin-1 levels with PTDM. Our findings support an association between low plasma omentin-1 levels and risk for PTDM in renal transplant recipients. Given the pioneering character of our findings, further prospective studies are needed to confirm and elucidate the functional significance of this association. New insight into the PTDM arises from our data and even treatment strategies based on omentin-1 analogs and omentin-1 receptor agonists may gain place with further studies on this field.

REFERENCES

1. Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ. Diabetes mellitus after kidney transplantation in the United States. *Am J Transplant.* 2003;3(2):178-185.
2. Cosio FG, Pesavento TE, Kim S, Osei K, Henry M, Ferguson RM. Patient survival after renal transplantation: IV. Impact of post-transplant diabetes. *Kidney Int.* 2002;62(4):1440-1446.
3. Israni AK, Snyder JJ, Skeans MA, et al. Predicting coronary heart disease after kidney transplantation: Patient Outcomes in Renal Transplantation (PORT) Study. *Am J Transplant.* 2010;10(2):338-353.
4. Phillips S, Heuberger R. Metabolic disorders following kidney transplantation. *J Ren Nutr.* 2012;22(5):451-460.e451.
5. Eckhard M, Schindler RA, Renner FC, et al. New-onset diabetes mellitus after renal transplantation. *Transplant Proc.* 2009;41(6):2544-2545.
6. Hecking M, Werzowa J, Haidinger M, et al. Novel views on new-onset diabetes after transplantation: development, prevention and treatment. *Nephrol Dial Transplant.* 2013;28(3):550-566.
7. Chowdhury TA. Post-transplant diabetes mellitus. *Clin Med (Lond).* 2019;19(5):392-395.
8. Londero TM, Giaretta LS, Farenzena LP, et al. Microvascular Complications of Posttransplant Diabetes Mellitus in Kidney Transplant Recipients: A Longitudinal Study. *J Clin Endocrinol Metab.* 2019;104(2):557-567.
9. Paek JH, Kang SS, Park WY, et al. Incidence of Post-transplantation Diabetes Mellitus Within 1 Year After Kidney Transplantation and Related Factors in Korean Cohort Study. *Transplant Proc.* 2019;51(8):2714-2717.
10. Jenssen T, Hartmann A. Emerging treatments for post-transplantation diabetes mellitus. *Nat Rev Nephrol.* 2015;11(8):465-477.
11. Borda B, Lázár G, Kormányos Á, et al. New-onset Diabetes Mellitus Following Successful Kidney Transplantation Facilitates Aortic Stiffening. *Transplant Proc.* 2019;51(4):1239-1243.
12. Cascais de Sá D, Santos L, Rodrigues L, et al. Post-Kidney-Transplant Diabetes: Evaluation of Risk Factors. *Transplant Proc.* 2019;51(5):1597-1600.
13. Lima C, Grden A, Skare T, Jaworski P, Nisihara R. Risk factors for new-onset diabetes mellitus after kidney transplantation (NODAT): a Brazilian single center study. *Arch Endocrinol Metab.* 2018;62(6):597-601.
14. Kleinsteuber A, Halleck F, Khadzhynov D, et al. Impact of Pre-existing Comorbidities on Long-term Outcomes in Kidney Transplant Recipients. *Transplant Proc.* 2018;50(10):3232-3241.
15. El Essawy B, Kandeel F. Pre, peri and posttransplant diabetes mellitus. *Curr Opin Nephrol Hypertens.* 2019;28(1):47-57.
16. Anagnostis P, Paschou SA, Spartalis E, Sarno G, De Rosa P, Muscogiuri G. Metabolic complications and kidney transplantation: focus on glycaemia and dyslipidaemia. *Curr Vasc Pharmacol.* 2019.
17. Shimada H, Uchida J, Nishide S, et al. Comparison of Glucose Tolerance between Kidney Transplant Recipients and Healthy Controls. *J Clin Med.* 2019;8(7).
18. Mancuso P. The role of adipokines in chronic inflammation. *Immunotargets Ther.* 2016;5:47-56.
19. Lehr S, Hartwig S, Sell H. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. *Proteomics Clin Appl.* 2012;6(1-2):91-101.

20. Liang W, Ye DD. The potential of adipokines as biomarkers and therapeutic agents for vascular complications in type 2 diabetes mellitus. *Cytokine Growth Factor Rev.* 2019;48:32-39.
21. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol.* 2010;314(1):1-16.
22. Reddy P, Lent-Schochet D, Ramakrishnan N, McLaughlin M, Jialal I. Metabolic syndrome is an inflammatory disorder: A conspiracy between adipose tissue and phagocytes. *Clin Chim Acta.* 2019;496:35-44.
23. Singh M, Benencia F. Inflammatory processes in obesity: focus on endothelial dysfunction and the role of adipokines as inflammatory mediators. *Int Rev Immunol.* 2019;38(4):157-171.
24. Kim WK, Bae KH, Lee SC, Oh KJ. The Latest Insights into Adipokines in Diabetes. *J Clin Med.* 2019;8(11).
25. Martínez-García M, Moncayo S, Insenser M, Álvarez-Blasco F, Luque-Ramírez M, Escobar-Morreale HF. Metabolic Cytokines at Fasting and During Macronutrient Challenges: Influence of Obesity, Female Androgen Excess and Sex. *Nutrients.* 2019;11(11).
26. Gateva A, Assyov Y, Tsakova A, Kamenov Z. Classical (adiponectin, leptin, resistin) and new (chemerin, vaspin, omentin) adipocytokines in patients with prediabetes. *Horm Mol Biol Clin Investig.* 2018;34(1).
27. Romanowski M, Dziedziejko V, Maciejewska-Karlowska A, et al. Adiponectin and leptin gene polymorphisms in patients with post-transplant diabetes mellitus. *Pharmacogenomics.* 2015;16(11):1243-1251.
28. Tan BK, Adya R, Randeva HS. Omentin: a novel link between inflammation, diabesity, and cardiovascular disease. *Trends Cardiovasc Med.* 2010;20(5):143-148.
29. Shibata R, Ouchi N, Takahashi R, et al. Omentin as a novel biomarker of metabolic risk factors. *Diabetol Metab Syndr.* 2012;4(1):37.
30. Tan YL, Zheng XL, Tang CK. The protective functions of omentin in cardiovascular diseases. *Clin Chim Acta.* 2015;448:98-106.
31. Yamawaki H, Tsubaki N, Mukohda M, Okada M, Hara Y. Omentin, a novel adipokine, induces vasodilation in rat isolated blood vessels. *Biochem Biophys Res Commun.* 2010;393(4):668-672.
32. Yang RZ, Lee MJ, Hu H, et al. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. *Am J Physiol Endocrinol Metab.* 2006;290(6):E1253-1261.
33. Yan P, Liu D, Long M, Ren Y, Pang J, Li R. Changes of serum omentin levels and relationship between omentin and adiponectin concentrations in type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes.* 2011;119(4):257-263.
34. Herder C, Kannenberg JM, Niersmann C, et al. Independent and opposite associations of serum levels of omentin-1 and adiponectin with increases of glycaemia and incident type 2 diabetes in an older population: KORA F4/FF4 study. *Eur J Endocrinol.* 2017;177(4):277-286.
35. de Souza Batista CM, Yang RZ, Lee MJ, et al. Omentin plasma levels and gene expression are decreased in obesity. *Diabetes.* 2007;56(6):1655-1661.
36. Tan BK, Adya R, Farhatullah S, et al. Omentin-1, a novel adipokine, is decreased in overweight insulin-resistant women with polycystic ovary syndrome: ex vivo and in vivo regulation of omentin-1 by insulin and glucose. *Diabetes.* 2008;57(4):801-808.

37. Rathwa N, Patel R, Pramanik Palit S, et al. Circulatory Omentin-1 levels but not genetic variants influence the pathophysiology of Type 2 diabetes. *Cytokine.* 2019;119:144-151.
38. As Habi A, Sadeghi M, Arab A, Hajianfar H. The association between omentin and diabetes: a systematic review and meta-analysis of observational studies. *Diabetes Metab Syndr Obes.* 2019;12:1277-1286.
39. Wittenbecher C, Menzel J, Carstensen-Kirberg M, et al. Omentin-1, Adiponectin, and the Risk of Developing Type 2 Diabetes. In: *Diabetes Care.* Vol 39. United States2016:e79-80.
40. Hayashi M, Morioka T, Hatamori M, et al. Plasma omentin levels are associated with vascular endothelial function in patients with type 2 diabetes at elevated cardiovascular risk. *Diabetes Res Clin Pract.* 2019;148:160-168.
41. Tekce H, Tekce BK, Aktas G, Alcelik A, Sengul E. Serum omentin-1 levels in diabetic and nondiabetic patients with chronic kidney disease. *Exp Clin Endocrinol Diabetes.* 2014;122(8):451-456.
42. Alcelik A, Tosun M, Ozlu MF, et al. Serum levels of omentin in end-stage renal disease patients. *Kidney Blood Press Res.* 2012;35(6):511-516.
43. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18(6):499-502.
44. Stewart A, Marfell-Jones M, Olds T, De Ridder J. *International Standards for Anthropometric Assessment.* Vol 1372011.
45. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28(7):412-419.
46. Salazar J, Bermúdez V, Olivar LC, et al. Insulin resistance indices and coronary risk in adults from Maracaibo city, Venezuela: A cross sectional study. *F1000Res.* 2018;7:44.
47. Amato MC, Giordano C, Galia M, et al. Visceral Adiposity Index: a reliable indicator of visceral fat function associated with cardiometabolic risk. *Diabetes Care.* 2010;33(4):920-922.
48. Kahn HS, Valdez R. Metabolic risks identified by the combination of enlarged waist and elevated triacylglycerol concentration. *Am J Clin Nutr.* 2003;78(5):928-934.
49. Nishida C, Ko GT, Kumanyika S. Body fat distribution and noncommunicable diseases in populations: overview of the 2008 WHO Expert Consultation on Waist Circumference and Waist-Hip Ratio. *Eur J Clin Nutr.* 2010;64(1):2-5.
50. Arnett DK, Blumenthal RS, Albert MA, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation.* 2019;140(11):e563-e595.
51. Pan HY, Guo L, Li Q. Changes of serum omentin-1 levels in normal subjects and in patients with impaired glucose regulation and with newly diagnosed and untreated type 2 diabetes. *Diabetes Res Clin Pract.* 2010;88(1):29-33.
52. Cai RC, Wei L, DI JZ, Yu HY, Bao YQ, Jia WP. [Expression of omentin in adipose tissues in obese and type 2 diabetic patients]. *Zhonghua Yi Xue Za Zhi.* 2009;89(6):381-384.
53. Moreno-Navarrete JM, Catalán V, Ortega F, et al. Circulating omentin concentration increases after weight loss. *Nutr Metab (Lond).* 2010;7:27.

54. Kataoka Y, Shibata R, Ohashi K, et al. Omentin prevents myocardial ischemic injury through AMP-activated protein kinase- and Akt-dependent mechanisms. *J Am Coll Cardiol.* 2014;63(24):2722-2733.
55. Hiramatsu-Ito M, Shibata R, Ohashi K, et al. Omentin attenuates atherosclerotic lesion formation in apolipoprotein E-deficient mice. *Cardiovasc Res.* 2016;110(1):107-117.
56. Watanabe K, Watanabe R, Konii H, et al. Counteractive effects of omentin-1 against atherogenesis†. *Cardiovasc Res.* 2016;110(1):118-128.
57. Watson RT, Kanzaki M, Pessin JE. Regulated membrane trafficking of the insulin-responsive glucose transporter 4 in adipocytes. *Endocr Rev.* 2004;25(2):177-204.
58. Kioumourtzoglou D, Gould GW, Bryant NJ. Proximity Ligation Assay to Study the GLUT4 Membrane Trafficking Machinery. *Methods Mol Biol.* 2018;1713:217-227.
59. Watanabe T, Watanabe-Kominato K, Takahashi Y, Kojima M, Watanabe R. Adipose Tissue-Derived Omentin-1 Function and Regulation. *Compr Physiol.* 2017;7(3):765-781.
60. Schwinkendorf DR, Tsatsos NG, Gosnell BA, Mashek DG. Effects of central administration of distinct fatty acids on hypothalamic neuropeptide expression and energy metabolism. *Int J Obes (Lond).* 2011;35(3):336-344.
61. Simopoulos AP. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients.* 2016;8(3):128.
62. Jacobo-Cejudo MG, Valdés-Ramos R, Guadarrama-López AL, Pardo-Morales RV, Martínez-Carrillo BE, Harbige LS. Effect of n-3 Polyunsaturated Fatty Acid Supplementation on Metabolic and Inflammatory Biomarkers in Type 2 Diabetes Mellitus Patients. *Nutrients.* 2017;9(6).
63. Kim YS, Xun P, Iribarren C, et al. Intake of fish and long-chain omega-3 polyunsaturated fatty acids and incidence of metabolic syndrome among American young adults: a 25-year follow-up study. *Eur J Nutr.* 2016;55(4):1707-1716.
64. de Boer IH, Zelnick LR, Ruzinski J, et al. Effect of Vitamin D and Omega-3 Fatty Acid Supplementation on Kidney Function in Patients With Type 2 Diabetes: A Randomized Clinical Trial. *JAMA.* 2019.
65. Gray B, Steyn F, Davies PS, Vitetta L. Omega-3 fatty acids: a review of the effects on adiponectin and leptin and potential implications for obesity management. *Eur J Clin Nutr.* 2013;67(12):1234-1242.
66. DeClercq V, d'Eon B, McLeod RS. Fatty acids increase adiponectin secretion through both classical and exosome pathways. *Biochim Biophys Acta.* 2015;1851(9):1123-1133.
67. Kocijancic M, Vujicic B, Racki S, Cubranic Z, Zaputovic L, Dvornik S. Serum omentin-1 levels as a possible risk factor of mortality in patients with diabetes on haemodialysis. *Diabetes Res Clin Pract.* 2015;110(1):44-50.

Table 1. Clinical and laboratory characteristics of posttransplant diabetes mellitus patients and non-diabetic kidney recipients

Characteristics	Controls (n= 53)	PTDM patients (n= 54)	P*
Age (years)	60 (52–65)	59 (54–64)	0.772
Gender (% male)	27 (50.9)	27 (50)	0.999
Ethnicity (% nonwhite)	3 (5.66)	3 (5.55)	0.999
Hypertension (%)	33 (62)	38 (70)	0.409
Smoking current (%)	6 (11.3)	4 (7.4)	0.526
Alcohol consumption (%)	8 (15)	2 (3.7)	0.052
CMV (%)	10 (18.9)	9 (16.7)	0.419
HCV (%)	5 (9.4)	12 (22.2)	0.103
Sedentarism	43 (81.1)	42 (77.8)	0.812
Fasting insulin	8.8 (6.5 – 12.6)	9.5 (6.9 – 15.2)	0.326
HOMA-IR	2.2 (1.4 – 3.5)	3.2 (1.7 – 5.6)	0.002
HOMA-β	106.8 (80.1 – 162.7)	56.8 (32 – 93.8)	<0.0001

Data are shown by median and interquartile interval or percentages. CMV (cytomegalovirus); HCV (hepatite C virus); HOMA IR (homeostatic model assessment of insulin resistance); HOMA-β (homeostatic model assessment of β-cell function). *P values are according to $\chi 2$ test, U-Mann-Whitney or t-test as appropriate.

Table 2. Anthropometric characteristics of PTDM patients and non-diabetic kidney recipients

Characteristics	**Controls (n= 53)**	**PTDM patients (n= 54)**	**P***
Weight (kg)	74.4 ± 10.7	73.3 ± 9.9	0.578
BMI (kg/m2)	27.7 (24.3–30.0)	27.0 (24.8–30.9)	0.665
Waist C (cm)	92.5 ± 10.1	93.8 ± 10.1	0.533
Hip C (cm)	100.3 ± 7.09	100.23 ± 7.23	0.958
Waist-hip Ratio	0.92 ± 0.09	0.93 ± 0.10	0.467
Neck C (cm)	37.08 ± 3.17	37.60 ± 3.96	0.456
MAMC (%)	24.99 ± 10.08	25.45 ± 10.08	0.466
TyG index	3.87 ± 0.20	4.13 ± 0.35	<0.0001
VAI index	2.51 ± 0.29	2.59 ±0.33	0.215
LAP index	56.7 (34.1 – 82.8)	71.1 (40.5 – 110.5)	0.047

Data are shown as mean and standard deviation, or median and interquartile interval, or percentages. BMI (body mass index); C (circumference); MAMC (Mid-arm muscle circumference); VAI (visceral adiposity index); LAP (lipid accumulation product). *P values are according to $\chi 2$ test, U-Mann-Whitney or t-test as appropriate.

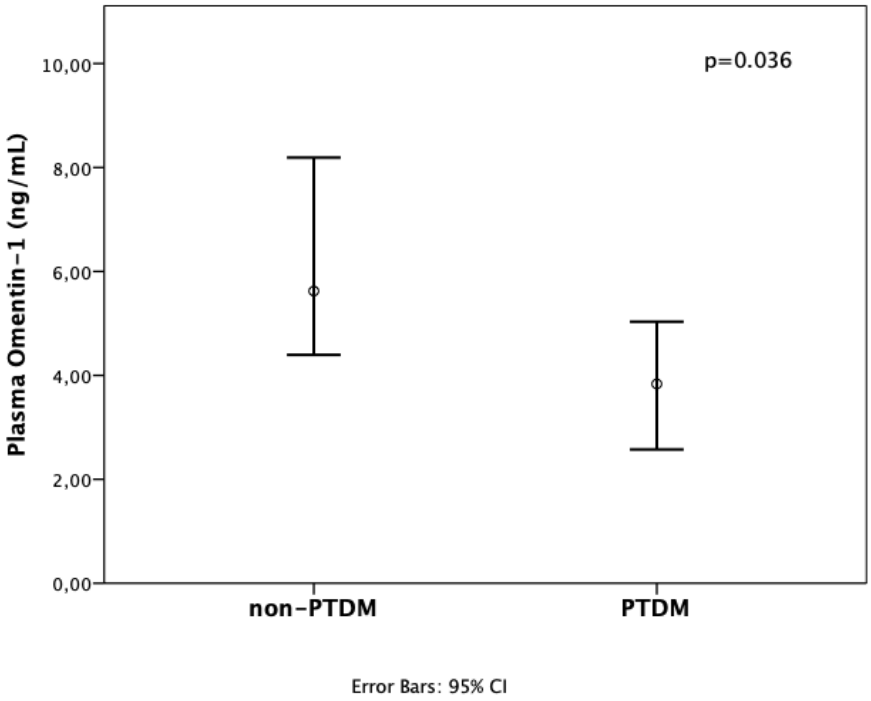

Figure 1. Plasma omentin-1 levels in non-PTDM (controls) and PTDM (cases) patients. Results are expressed as median ± SE in comparison with control patients.

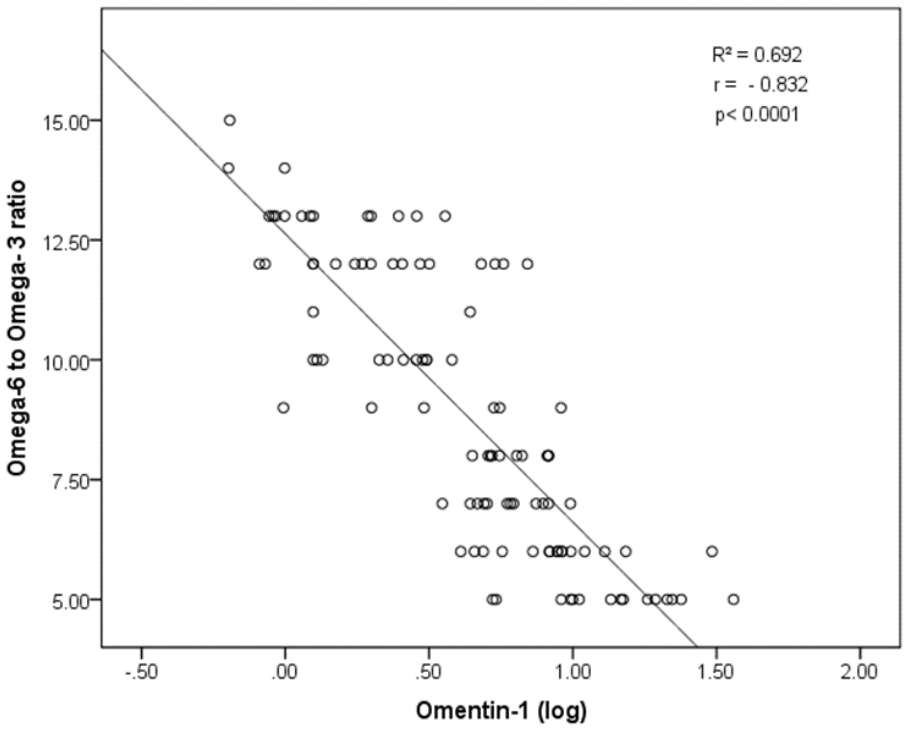

Figure 2. Correlation between plasma omentin-1 levels and omega 6 to omega 3 ratio ingestion. Plasma omentin-1 is shown by log transformation.

ARTIGO ORIGINAL 2

"The *ITLN1* rs2274907 polymorphism is associated with posttransplantation diabetes mellitus in kidney transplant recipients."

Manuscrito original

The *ITLN1* rs2274907 polymorphism is associated with posttransplantation diabetes mellitus in kidney transplant recipients.

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KEYWORDS: Kidney transplantation; post transplantation diabetes mellitus; *ITLN1* gene, omentin, single nucleotide polymorphism.

ABBREVIATIONS

Akt, Protein kinase B

BMI, body mass index

CMV, cytomegalovirus

COX-2, cyclooxygenase-2

CVD, cardiovascular disease

ERK, Extracellular signal-regulated kinases

HCV, hepatitis C virus

HWE, Hardy–Weinberg equilibrium

IFNγ, interferon gamma

IL-6, interleukin 6

ITLN1, omentin-1 gene

NF-κB, factor nuclear κB

PTDM, posttransplantation diabetes mellitus

SNP, single nucleotide polymorphism

T2DM, type 2 diabetes mellitus

ABSTRACT

BACKGROUND:

Posttransplant diabetes mellitus (PTDM) is a common complication after renal transplantation. Previous studies have demonstrated that single nucleotide polymorphism (SNP) rs2274907 in the *ITLN1* gene is associated with metabolic disorders. We aimed to determine the association between rs2274907 SNP and PTDM in kidney transplant recipients.

MATERIALS AND METHODS:

A total of 105 PTDM patients and 211 controls were enrolled in this nested case-control study within a retrospective cohort of kidney transplant recipients. *ITLN1* rs2274907 SNP was evaluated by real time polymerase chain reaction restriction fragment length (RT-PCR). Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the association between rs2274907 polymorphism and PTDM.

RESULTS:

Genotype frequencies were different between PTDM and non-PTDM groups ($P < 0.001$). The A allele was associated with risk for PTDM under recessive and additive inheritance models (both $P < 0.0001$). After adjustment for age, ethnicity, type of donor, and pre-transplant BMI, the A/A genotype remained independently associated with risk for PTDM under recessive model: OR = 3.711, 95% CI 1.659 – 8.302; and additive model: OR = 4.799, 95% CI 1.896 – 12.143.

CONCLUSION:

Our findings suggested a significant association between *ITLN1* rs2274907 SNP and susceptibility to PTDM in renal transplant recipients from a southern Brazilian population. The results of the present study should be confirmed with further studies in larger and geographically distinct populations.

INTRODUCTION

Kidney transplantation is the most cost-effective therapy for end-stage renal disease, leading patients to a better quality of life and higher survival rates than dialytic therapies. Several factors, however, may undermine the outcomes of kidney transplantation, such as acute and chronic rejection, infections and metabolic disorders [1,2]. One of the high incident metabolic complications after transplantation is post-transplantation diabetes mellitus (PTDM), which may influence both short and long-term outcomes of kidney transplant recipients [3]. PTDM shares some risk factors and clinical presentation with type 2 diabetes mellitus (T2DM), however, long term chronic complications appear to behave in a different manner [4]. The cumulative incidence of PTDM ranges from 4% to 25% among renal graft recipients [5-7] and, although many risk factors are known, including aging, ethnicity, obesity, and immunosuppressive drugs [6], the whole picture of this disorder still needs to be clarified.

Adipose tissue, besides its established characteristics of energy storage, heat and mechanical insulation and regulation of thermogenesis, has been recently recognized as an active endocrine organ, responsible for releasing a large number of bioactive cytokines called adipokines [8,9]. These adipokines are involved in a wide range of functions in physiological and pathological processes such as inflammation, autoimmunity, glucose and lipid metabolism, insulin resistance, diabetes, among others [10,11]. The imbalanced production between pro-inflammatory and anti-inflammatory adipokines appears to play a role in the development of some diseases [12-16].

Some adipokines secreted from the adipose tissue are involved in T2DM and insulin resistance [10,17]. One of them, mainly expressed in the human omental adipose tissue is the Omentin-1 (also known as interlectin-1). Omentin-1 is an anti-inflammatory

adipokine that has 313 amino acids codified by the *ITLN1* gene, which is located on the long arm of chromosome 1 (1q22-23) [18]. Two homologous isoforms, omentin-1 and omentin-2, have been described, being the omentin-1 the major omentin present in the human blood. Omentin-1 increases the insulin signal transduction through activation of the protein kinase B (Akt/protein kinase B) [19,20]. Consequently, omentin-1 is linked with regulation of insulin sensitivity by paracrine and endocrine factors, enhancing insulin sensitivity and glucose metabolism on the local level of omental adipose tissue [21].

Polymorphisms in the omentin-1 gene (*ITLN1*), including the rs2274907, have been associated with many disorders, such as insulin resistance, diabetic foot, coronary artery disease, nonalcoholic fatty liver disease, increased daily energy intake, and higher body mass index (BMI) in T2DM subjects in different population [22-27]. The rs2274907 is a single nucleotide polymorphism (SNP) in exon 4 of *ITLN1* where a T > A substitution at the position +326 leads to an amino acid change (Val109Asp) [28]. To date, no study evaluated the association between this SNP and PTDM. Therefore, we aimed to investigate the association between rs2274907 SNP and PTDM in kidney transplant recipients from southern Brazil.

MATERIALS AND METHODS

Study subjects

This nested case-control study was undertaken within a cohort of kidney transplant recipients from Hospital de Clínicas de Porto Alegre, Rio Grande do Sul, southern Brazil. It was designed in accordance with STROBE and STREGA guidelines for reporting of genetic association studies [29,30]. Three-hundred and sixteen transplant recipients were followed-up for at least one year after transplantation. Of them, 105 patients developed PTDM (cases) and 211 patients did not (controls). PTDM was diagnosed according to

American Diabetes Association criteria [31]. Exclusion criteria were age below 18 years old, pre-transplantation diabetes mellitus, and multiorgan transplantation.

Demographic, laboratory and clinical data were collected retrospectively from electronic records and included: donor type (living or deceased), recipient age at transplantation, gender, underlying kidney disease, family history of diabetes, cytomegalovirus (CMV) and hepatitis C virus (HCV) infections, lipid profile, time in dialysis, retransplantation, immunosuppressive drugs, BMI before transplantation, and time of PTDM diagnosis. The ethnic group was defined based on self-classification. All laboratory data were collected at baseline and after transplantation according to the institutional protocol for renal transplant follow-up. The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre and all subjects received adequate information about this study and gave informed consent.

Genotyping of the *ITLN1* rs2274907 polymorphism

Peripheral blood samples were collected from all patients for DNA extraction and genotyping of the *ITLN1* rs2274907 SNP. DNA was extracted using a standardized salting-out procedure. Genotyping was performed by allele discrimination-real time PCR technique using specific Human Custom TaqMan Genotyping Assay 40× (Thermo Fisher Scientific Inc., Waltham, MA, USA). Real-Time PCR reactions were performed in 96-well plates, in a total of 5 µl volume using 2 ng of genomic DNA, TaqMan Genotyping Master Mix 1x (Thermo Fisher Scientific) and Custom TaqMan Genotyping Assay 1x. Then, plates were placed in a real-time PCR thermal cycler (7500 Fast Real-Time PCR System; Thermo Fisher Scientific) and heated for 10 min at 95 °C, followed by 50 cycles of 95 °C for 15 s and 60 °C for 90 s.

Statistical analyses

Allele frequencies were determined by gene counting, and deviations from the Hardy–Weinberg equilibrium (HWE) were verified using χ^2 test. Allele and genotype frequencies were compared between groups of patients using χ^2 tests. Additionally, we also compared genotypes grouped in different inheritance models (additive, recessive and dominant) between case and control groups [32,33]. Clinical and laboratory characteristics were compared between groups using unpaired Student's *t* test or χ^2, as appropriate. Normality of continuous variables was assessed using Shapiro Wilk and Kolmogorov-Smirnov tests. Variables with normal distributions are shown as mean ± SD or absolute numbers (percentages) while variables with skewed distributions that were normalized after log-transformation are shown as median (25^{th}–75^{th} percentile values). The magnitude of the associations with PTDM were estimated using odds ratios (ORs) with 95% confidence intervals (95% CI). Statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA). The significance level was set at $P < 0.05$.

RESULTS

Sample description

Baseline clinical and demographics characteristics of the recipients with and without PTDM are shown in Table 1. The mean follow-up duration was 50.6 months and the median time for the diagnostic of PTDM was 89 days. Seventy-one percent of the recipients developed PTDM within six months after transplantation, and 75% within the first-year post-transplantation. PTDM recipients were older than controls (54.6 ± 10.1 vs. 45.4 ± 12.1 years, $P < 0.0001$), and had higher BMI before transplantation (25.8 ± 4.8 vs. 24.0 ± 3.9 kg/m^2, $p = 0.001$). White and nonwhite frequencies were not significantly

different between PTDM recipients and controls (28.6% vs. 20%, P = 0.080). Regarding donor type, there was more PTDM patients who received deceased donor kidneys compared to non-PTDM (74.1% vs. 60.0%, P = 0.009). Pre-transplant fasting plasma glucose levels were higher in patients who developed PTDM (94 [84 – 107] vs. 89 [82 – 97] mg/dL, P = 0.016). The induction therapy was not different between groups as well as the prevalence of acute rejection. The other clinical variables were similar between cases and controls.

Genotype and allele distributions

Genotype and allele frequencies of the *ITLN1* rs2274907 SNP between PTDM and control groups are summarized in Table 2. Genotype frequencies were differently distributed between PTDM and non-PTDM groups (P < 0.001), and all genotypes were in agreement with those predicted by the HWE (P > 0.05). The A allele frequency was 43% in PTDM patients and 30% in controls (P = 0.001). Accordingly, the A allele was associated with risk for PTDM under recessive and additive inheritance models (both P < 0.0001). After adjustment for age, ethnicity, type of donor, and BMI pre-transplantation, the A/A genotype remained independently associated with risk for PTDM under these models (recessive: OR = 3.711, 95% CI 1.659 – 8.302; additive: OR = 4.799, 95% CI 1.896 – 12.143; Table 2).

Additionally, clinical and laboratory characteristics were compared between the different genotypes of *ITLN1* rs2274907 SNP in the PTDM group and are shown in Table 3. Age, gender, ethnicity, type of donor, BMI pre-transplantation, and waist circumference did not differ significantly between genotypes. It is worth mentioning that none of these variables attained statistical significance irrespective of whether recessive

(T/T - T/A *vs.* A/A), dominant (T/T *vs.* T/A – A/A) or additive (T/T *vs.* A/A) models of inheritance were assumed for the A allele (data not shown).

DISCUSSION

This is the first study to evaluate the association of rs2274907 SNP of *ITLN1* and PTDM in a cohort of renal transplant recipients. Our present results demonstrated that the A/A genotype of rs2274907 SNP was independently associated with increased risk for PTDM in a Southern Brazilian population.

Many studies have investigated polymorphisms associated with risk for T2DM in different populations [34,35]. Since PTDM shares similar pathogenic mechanisms with T2DM, including insulin resistance and impairment of insulin secretion, a number of T2DM candidate genes have also been associated with risk for PTDM [35,36]. The gene that codifies omentin-1 (*ITLN1*) is located in the 1q22-q23 chromosomal region, which has been linked to T2DM in Pima Indians, Amish and European populations [19,37-39], suggesting that *ITLN1* could be a candidate gene for T2DM susceptibility in humans.

However, studies evaluating the association between the rs2274907 SNP and T2DM are conflicting. Previous studies were not able to find any association between this polymorphism and T2DM in Indians and Caucasians [23,27,28]. On the other hand, Khoshi *et al* found an association between A allele and T2DM in Iranian population [24]. Accordingly, Mrozikiewicz-Rakowska *et al* demonstrated that A allele of rs2274907 SNP was more frequent in patients with diabetic foot in both additive and recessive models [22]. In our study, regardless of the traditional risk factors associated with PTDM, our data showed that A/A genotype of the rs2274907 SNP was strongly associated with PTDM after adjustment for type of donor, ethnicity, and BMI, and fasting glycemia in the pre-

transplant time, suggesting this polymorphism might be an independent risk factor for PTDM susceptibility.

Other metabolic disorders have also been associated with *ITLN1* rs2274907 SNP. Splichal *et al* showed that the rs2274907 allelic variant affects the food ingestion volume in patients from central Europe, suggesting orexigenic/anorexigenic effects if this polymorphism. A/A allele carriers were characterized by a higher energy supply (8764 ± 2467 J/d) compared to T/T allele carriers (7977 ± 2780 J/d) [26]. In this context, in a prepubertal healthy children population, there was correlation between fat percentage and BMI with A/T-A/A carriers [40], corroborating the hypothesis that A/A genotype could lead to the higher food consumption and consequently higher BMI.

Furthermore, studies have investigated the relationship of rs2274907 polymorphism with other diseases. Yorük *et al*. suggested that omentin-1 may serve as a biomarker for cardiovascular disease (CVD). This study found a higher frequency of the A/A genotype in Turkish patients with CVD [41], although no statistical difference was demonstrated. The authors assumed the small sample size as a limitation for the results. In Saudi Arabians, the genotype A/T was associated with CVD [23] and in Iranians, the allele was more prevalent among men with CVD compared to healthy men [42]. Nazar *et al*. indicated that the A/T genotype of the *ITLN1* rs2274907 polymorphism was more frequent in Pakistani patients with CVD [43]. The reasons for these discordant findings are not evident, however, we can speculate that ethnicity may play a relevant role in these genetic effects.

The role of the adipokines in the subclinical inflammation and physiopathology of insulin resistance is largely described [44,45]. Several proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukin 6 (IL-6), and interferon gamma (IFNγ), are related with the impairment of insulin sensitive glucose transporters and insulin signaling

in T2DM [11,46]. Omentin is an anti-inflammatory adipokine described to enhance insulin-stimulated glucose transport and Akt phosphorylation in human adipocytes [19]. This adipokine also reduces vascular cell adhesion protein-1 expression on the surface of monocytes and decreases intercellular adhesion molecule-1 expression (via suppression of extracellular ERK/NF-κB), which results in reduced adhesion of monocytes to endothelial cells [47]. Moreover, this protein inhibits TNF-induced endothelial cell cyclooxygenase-2 (COX-2) expression and induces endothelial nitric oxide synthase [48]. Considering the low-grade inflammation status established after kidney transplantation, it is reasonable to suggest that adipokines may be involved in the insulin resistance mechanism leading to the development of PTDM.

As abovementioned, there is a good scientific background to justify our research. Omental adiposity precedes the development of insulin resistance and T2DM and it is one of the major components of the metabolic syndrome [49,50]. Since adipokines derived from omental adipose tissue are clearly involved in insulin resistance, dyslipidemia, and coronary artery disease [21], it is reasonable to suggest that omentin-1 might play an important role in the pathogenesis of diabetes and associated metabolic disorders, including PTDM.

In conclusion, our findings suggested a significant association between *ITLN1* rs2274907 polymorphism and PTDM susceptibility in renal transplant recipients in a southern Brazilian population. The results of the present study should be confirmed with further studies in larger and geographically distinct populations.

REFERENCES

1. Chen YX, Li R, Gu L, Xu KY, Liu YZ, Zhang RW. Risk factors and etiology of repeat infection in kidney transplant recipients. *Medicine (Baltimore).* 2019;98(38):e17312.
2. Ali A, Macphee I, Kaski JC, Banerjee D. Cardiac and vascular changes with kidney transplantation. *Indian J Nephrol.* 2016;26(1):1-9.
3. Tomkins M, Tudor RM, Cronin K, et al. Risk factors and long-term consequences of new-onset diabetes after renal transplantation. *Ir J Med Sci.* 2019.
4. Londero TM, Giaretta LS, Farenzena LP, et al. Microvascular Complications of Posttransplant Diabetes Mellitus in Kidney Transplant Recipients: A Longitudinal Study. *J Clin Endocrinol Metab.* 2019;104(2):557-567.
5. Chowdhury TA. Post-transplant diabetes mellitus. *Clin Med (Lond).* 2019;19(5):392-395.
6. Conte C, Secchi A. Post-transplantation diabetes in kidney transplant recipients: an update on management and prevention. *Acta Diabetol.* 2018;55(8):763-779.
7. Hecking M, Werzowa J, Haidinger M, et al. Novel views on new-onset diabetes after transplantation: development, prevention and treatment. *Nephrol Dial Transplant.* 2013;28(3):550-566.
8. Lehr S, Hartwig S, Sell H. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. *Proteomics Clin Appl.* 2012;6(1-2):91-101.
9. Iannone F, Lapadula G. Obesity and inflammation--targets for OA therapy. *Curr Drug Targets.* 2010;11(5):586-598.
10. Kusminski CM, Bickel PE, Scherer PE. Targeting adipose tissue in the treatment of obesity-associated diabetes. *Nat Rev Drug Discov.* 2016;15(9):639-660.
11. Mancuso P. The role of adipokines in chronic inflammation. *Immunotargets Ther.* 2016;5:47-56.
12. Kahn CR, Wang G, Lee KY. Altered adipose tissue and adipocyte function in the pathogenesis of metabolic syndrome. *J Clin Invest.* 2019;129(10):3990-4000.
13. Lackey DE, Burk DH, Ali MR, et al. Contributions of adipose tissue architectural and tensile properties toward defining healthy and unhealthy obesity. *Am J Physiol Endocrinol Metab.* 2014;306(3):E233-246.
14. Hausmann J, Waechtershaeuser A, Behnken I, et al. The role of adipokines in the improvement of diabetic and cardiovascular risk factors within a 52-week weight-loss programme for obesity. *Obes Res Clin Pract.* 2019.
15. Singh M, Benencia F. Inflammatory processes in obesity: focus on endothelial dysfunction and the role of adipokines as inflammatory mediators. *Int Rev Immunol.* 2019;38(4):157-171.
16. Reddy P, Lent-Schochet D, Ramakrishnan N, McLaughlin M, Jialal I. Metabolic syndrome is an inflammatory disorder: A conspiracy between adipose tissue and phagocytes. *Clin Chim Acta.* 2019;496:35-44.
17. Liang W, Ye DD. The potential of adipokines as biomarkers and therapeutic agents for vascular complications in type 2 diabetes mellitus. *Cytokine Growth Factor Rev.* 2019;48:32-39.
18. Schäffler A, Neumeier M, Herfarth H, Fürst A, Schölmerich J, Büchler C. Genomic structure of human omentin, a new adipocytokine expressed in omental adipose tissue. *Biochim Biophys Acta.* 2005;1732(1-3):96-102.

19. Yang RZ, Lee MJ, Hu H, et al. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. *Am J Physiol Endocrinol Metab.* 2006;290(6):E1253-1261.
20. Lee JK, Schnee J, Pang M, et al. Human homologs of the Xenopus oocyte cortical granule lectin XL35. *Glycobiology.* 2001;11(1):65-73.
21. Watanabe T, Watanabe-Kominato K, Takahashi Y, Kojima M, Watanabe R. Adipose Tissue-Derived Omentin-1 Function and Regulation. *Compr Physiol.* 2017;7(3):765-781.
22. Mrozikiewicz-Rakowska B, Sobczyk-Kopciol A, Szymanski K, et al. Role of the rs2274907 allelic variant of the ITLN1 gene in patients with diabetic foot. *Pol Arch Intern Med.* 2017;127(5):319-327.
23. Jha CK, Mir R, Elfaki I, et al. Evaluation of the Association of Omentin 1 rs2274907 A>T and rs2274908 G>A Gene Polymorphisms with Coronary Artery Disease in Indian Population: A Case Control Study. *J Pers Med.* 2019;9(2).
24. Khoshi A, Bajestani MK, Shakeri H, Goodarzi G, Azizi F. Association of Omentin rs2274907 and FTO rs9939609 gene polymorphisms with insulin resistance in Iranian individuals with newly diagnosed type 2 diabetes. *Lipids Health Dis.* 2019;18(1):142.
25. Kohan L, Safarpur M, Abdollahi H. Omentin-1 rs2274907 and resistin rs1862513 polymorphisms influence genetic susceptibility to nonalcoholic fatty liver disease. *Mol Biol Res Commun.* 2016;5(1):11-17.
26. Splichal Z, Bienertova-Vasku J, Novak J, et al. The common polymorphism Val109Asp in the omentin gene is associated with daily energy intake in the Central-European population. *Nutr Neurosci.* 2015;18(1):41-48.
27. Rathwa N, Patel R, Pramanik Palit S, et al. Circulatory Omentin-1 levels but not genetic variants influence the pathophysiology of Type 2 diabetes. *Cytokine.* 2019;119:144-151.
28. Schaffler A, Zeitoun M, Wobser H, Buechler C, Aslanidis C, Herfarth H. Frequency and significance of the novel single nucleotide missense polymorphism Val109Asp in the human gene encoding omentin in Caucasian patients with type 2 diabetes mellitus or chronic inflammatory bowel diseases. *Cardiovasc Diabetol.* 2007;6:3.
29. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. *Int J Surg.* 2014;12(12):1495-1499.
30. Little J, Higgins JP, Ioannidis JP, et al. STrengthening the REporting of Genetic Association studies (STREGA)--an extension of the STROBE statement. *Eur J Clin Invest.* 2009;39(4):247-266.
31. Association AD. 2. Classification and Diagnosis of Diabetes:. *Diabetes Care.* 2019;42(Suppl 1):S13-S28.
32. Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J. The choice of a genetic model in the meta-analysis of molecular association studies. *Int J Epidemiol.* 2005;34(6):1319-1328.
33. Zintzaras E, Lau J. Synthesis of genetic association studies for pertinent gene-disease associations requires appropriate methodological and statistical approaches. *J Clin Epidemiol.* 2008;61(7):634-645.
34. Goodarzi MO, Palmer ND, Cui J, et al. Classification of Type 2 Diabetes Genetic Variants and a Novel Genetic Risk Score Association with Insulin Clearance. *J Clin Endocrinol Metab.* 2019.

35. Abuhendi N, Qush A, Naji F, et al. Genetic polymorphisms associated with type 2 diabetes in the Arab world: A systematic review and meta-analysis. *Diabetes Res Clin Pract.* 2019;151:198-208.
36. Chakkera HA, Hanson RL, Raza SM, et al. Pilot study: association of traditional and genetic risk factors and new-onset diabetes mellitus following kidney transplantation. *Transplant Proc.* 2009;41(10):4172-4177.
37. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ. A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes.* 1999;48(5):1175-1182.
38. Hanson RL, Ehm MG, Pettitt DJ, et al. An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet.* 1998;63(4):1130-1138.
39. Vionnet N, Hani EH, Dupont S, et al. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *Am J Hum Genet.* 2000;67(6):1470-1480.
40. Gajewska J, Kurylowicz A, Mierzejewska E, et al. Are Omentin Rs2274907 and Vaspin Rs2236242 Gene Polymorphisms Related to Body Composition, Lipid Profile and Other Adipokines in Prepubertal Healthy Children? *Endocr Res.* 2019:1-8.
41. Yoruk U, Yaykasli KO, Ozhan H, et al. Association of omentin Val109Asp polymorphism with coronary artery disease. *Anadolu Kardiyol Derg.* 2014;14(6):511-514.
42. Jamshidi J, Ghanbari M, Asnaashari A, Jafari N, Valizadeh GA. Omentin Val109Asp polymorphism and risk of coronary artery disease. *Asian Cardiovasc Thorac Ann.* 2017;25(3):199-203.
43. Nazar S, Zehra S, Azhar A. Association of single Nucleotide Missence Polymorphism Val109Asp of Omentin-1 gene and coronary artery disease in Pakistani population: Multicenter study. *Pak J Med Sci.* 2017;33(5):1128-1133.
44. Gateva A, Assyov Y, Tsakova A, Kamenov Z. Classical (adiponectin, leptin, resistin) and new (chemerin, vaspin, omentin) adipocytokines in patients with prediabetes. *Horm Mol Biol Clin Investig.* 2018;34(1).
45. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol.* 2010;314(1):1-16.
46. Akour A, Kasabri V, Boulatova N, et al. Levels of metabolic markers in drug-naive prediabetic and type 2 diabetic patients. *Acta Diabetol.* 2017;54(2):163-170.
47. Zhong X, Li X, Liu F, Tan H, Shang D. Omentin inhibits TNF-alpha-induced expression of adhesion molecules in endothelial cells via ERK/NF-kappaB pathway. *Biochem Biophys Res Commun.* 2012;425(2):401-406.
48. Yamawaki H, Kuramoto J, Kameshima S, Usui T, Okada M, Hara Y. Omentin, a novel adipocytokine inhibits TNF-induced vascular inflammation in human endothelial cells. *Biochem Biophys Res Commun.* 2011;408(2):339-343.
49. Klein M, Varga I. Microenvironment of Immune Cells Within the Visceral Adipose Tissue Sensu Lato vs. Epicardial Adipose Tissue: What Do We Know? *Inflammation.* 2018;41(4):1142-1156.
50. García-Ruiz I, Solís-Muñoz P, Fernández-Moreira D, Grau M, Muñoz-Yagüe MT, Solís-Herruzo JA. Omentectomy Prevents Metabolic Syndrome By

Reducing Appetite and Body Weight In A Diet-Induced Obesity Rat Model. *Sci Rep.* 2018;8(1):1540.

Table 1. Clinical and laboratory characteristics of PTDM and non-PTDM patients.

Characteristics	Non PTDM (n = 211)	PTDM (n = 105)	P*
Age (y)	45 ± 12	55 ± 10	<0.0001
Gender, n (% male)	126 (59.7)	63 (60)	0.999
Ethnicity, n (% black)	42 (20)	30 (28.6)	0.08
Pretransplantation BMI (kg/m^2)	24.0 ± 3.9	25.8 ± 4.8	0.001
Hypertension, n (%)	189 (89.6)	94 (89.5)	0.999
Donor type (deceased), n (%)	138 (60)	86 (74.1)	0.009
Time in dialysis, (mo)	31 (16 – 60)	40 (18 – 68)	0.111
HCV infection, n (%)	38 (18)	24 (22.8)	0.366
CMV infection, n (%)	47 (22.3)	20 (19)	0.560
Pretransplantation fasting glycemia (mg/dL)	90 (83 – 97.8)	96 (85 – 108)	0.047
Fasting glycemia (mg/dL)	89 (82 – 97)	94 (84 – 107)	0.016

Data are shown by mean ± standard deviation, median (25th – 75th percentile values) or %. HCV: Hepatitis C virus; CMV: cytomegalovirus; BMI: body mass index. *P values were computed using Student's ***t*** tests, U-Mann Whitney or Chi-square tests, as appropriate.

Table 2. Genotype and allele frequencies of the *ITLN1* rs2274907 polymorphism in patients with PTDM (cases) and without PTDM (controls).

	Non-PTDM patients	**PTDM patients**	***P-value**	**Adjusted OR (95% CI) /†P**
	n = 211	n = 105		
Genotype				
TT	98 (46.5)	36 (34.3)	< 0.001	1
T/A	99 (46.9)	47 (44.8)		1.297 (0.736 – 2.287)/ 0.369
A/A	14 (6.6)	22 (20.9)		4.273 (1.800 – 10.145) / 0.001
Allele				
T	0.70	0.57	0.001	
A	0.30	0.43		
Recessive model				
T/T + T/A	197 (93.4)	83 (79.0)	< 0.0001	1
A/A	14 (6.6)	22 (21.0)		3.711 (1.659 – 8.302)/0.001
Additive model				
T/T	98 (87.5)	36 (62.1)	< 0.0001	1
A/A	14 (12.5)	22 (37.9)		4.799 (1.896 – 12.143)/ 0.001
Dominant model				
T/T	98 (46.4)	36 (34.3)	0.052	1
T/A + A/A	113 (53.6)	69 (65.7)		1.657 (0.970 – 2.830)/ 0.065

Data are shown as number (%) or proportion. DKD: diabetic kidney disease; T1DM: type 1 diabetes mellitus; UAE: urinary albumin excretion. *P-values were calculated using Chi-square tests. † P-values and OR (95% CI) obtained using logistic regression analyses adjusting for BMI pre-transplantation, age, ethnicity and donor type.

Table 3. Clinical and laboratory characteristics among the genotypes of *ITLN1* rs2274907 in PTDM patients

	Genotypes			
	T/T (n = 36)	**T/A (n = 47)**	**A/A (n = 22)**	**P***
Age (y)	57 (46 – 63)	56 (49 – 60)	56 (51 – 61)	0.473
Gender, n (% male)	24 (66)	25 (53)	14 (63)	0.428
Ethnicity, n (% black)	11 (30)	13 (27)	6 (27)	0.948
Pretransplantation BMI (kg/m^2)	26.3 ± 4.5	25.3 ± 5.4	26.2 ± 3.8	0.585
HCV infection, n (%)	25 (19.1)	31 (21.7)	6 (17.1)	0.780
CMV infection, n (%)	30 (23.3)	30 (21)	7 (19.4)	0.846
Time in dialysis, (mo)	35 (16 – 60)	34.5 (18 – 66)	33 (12 – 65)	0.511

Data are shown by mean ± standard deviation, median (25^{th} – 75^{th} percentile values) or %. BMI: body mass index; HCV, hepatitis C virus; CMV: cytomegalovirus. *P values were computed using ANOVA one Way, Kruskal-Wallis or Chi-square tests, as appropriate.

CAPÍTULO 3

CONSIDERAÇÕES FINAIS

Avaliamos, de forma inédita, a associação entre os níveis plasmáticos da omentina-1 e a presença de DMPT bem como a associação entre o polimorfismo rs2274907 no gene *ITLN1* e DMPT em pacientes transplantados renais.

Demostramos que níveis plasmáticos elevados de omentina-1 parecem exercer um efeito protetor no risco para desenvolver DMPT, independente da presença de fatores de risco conhecidos e observados nesta população.

Também verificamos, através de um estudo de caso-controle aninhado a uma coorte retrospectiva, que pacientes portadores do polimorfismo rs2274907 no gene da omentina-1 apresentaram elevado risco para ocorrência de DMPT.

Portanto, os achados resultantes desta tese apontam para uma significativa associação entre a omentina-1 e DMPT. Estudos em outras populações e com maior número de pacientes são necessários para confirmar estes achados; contudo, acreditamos que estes resultados possam contribuir para um melhor entendimento da fisiopatologia do DMPT e para o embasamento de novos estudos focados em estratégias terapêuticas preventivas para o DMPT.

Temos como perspectivas futuras avaliar a relação da omentina-1 como marcador para complicações crônicas associadas ao diabetes mellitus. Para tanto, iniciamos uma revisão sistemática que encontra-se em fase de análise dos resultados, registrada na plataforma PROSPERO sob o número CRD42019140495.