UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM ALIMENTAÇÃO, NUTRIÇÃO E SAÚDE

MEDIADORES DA RELAÇÃO ENTRE O GENE FTO E A DOENÇA RENAL DO DIABETES MELITO: ANÁLISE DE CAMINHOS

Júlia Marchetti

Orientadora: Profª. Drª. Thais Steemburgo

Co-orientadora: Profª. Drª. Helen Hermana M. Hermsdorff (Universidade Federal de Viçosa, MG)

DISSERTAÇÃO DE MESTRADO

2020

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM ALIMENTAÇÃO, NUTRIÇÃO E SAÚDE

MEDIADORES DA RELAÇÃO ENTRE O GENE FTO E A DOENÇA RENAL DO DIABETES MELITO: ANÁLISE DE CAMINHOS

Júlia Marchetti

Orientadora: Profª. Drª. Thais Steemburgo

Co-orientadora: Profª. Drª. Helen Hermana M. Hermsdorff (Universidade Federal de Viçosa, MG)

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Nutrição, Alimentação e Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de Mestre.

2020

Agradecimentos

Finalizo este ciclo através do apoio de diversas pessoas que me acompanharam e foram fundamentais para a realização de mais essa etapa da minha formação. Desta forma, agradeço a todos que fizeram parte do meu convívio nessa fase: amigos, professores, colegas e funcionários da Universidade.

Agradeço, em especial, aos meus pais Everton e Sonia e ao meu irmão Felippe pelo apoio incondicional em todos os momentos, muitas vezes sendo privados da minha companhia e atenção. Obrigada por desejarem sempre o melhor pra mim, por todo o amor e incentivo e por me ajudarem sempre. Aos meus tios, Ieda, Vera e Joaquim por sempre estarem ao meu lado e torcendo pela minha felicidade e sucesso. A vocês, sou eternamente grata por tudo que sou e consegui conquistar.

Meu agradecimento a Prof.ª Thais Steemburgo, minha orientadora e, sobretudo, uma querida amiga que me acolheu na vida acadêmica e que tanto admiro pessoalmente e profissionalmente. Obrigada por sempre ter acreditado e depositado confiança em mim ao longo desses anos de trabalho que se iniciaram ainda na graduação. Agradeço as Professoras Helen Hermana M. Hermsforff e Leidjaira L. Juvanhol e a Nutricionista Karla P. Balbino, da Universidade Federal de Viçosa (UFV), que compartilharam importantes conhecimentos comigo durante esse processo do mestrado, fazendo com que esse trabalho fosse possível.

Formato da dissertação

Essa dissertação segue o formato proposto pelo Programa de Pós-Graduação em Alimentação, Nutrição e Saúde da Universidade Federal do Rio Grande do Sul:

1. Revisão da literatura sobre o tema

2. Artigo Original

3. Anexos necessários e normas da revista de publicação

`

SUMÁRIO

Lista de abreviaturas

DM	Diabetes Melito
DRC	Doença Renal Crônica
FTO	*Fat Mass and Obesity Associated*
GWAS	*Genome-Wide Association Studies*
IDF	*International Diabetes Federation*
IMC	Índice de Massa Corporal
SNP	*Single Nucleotide Polymorphism*
TFG	Taxa de Filtração Glomerular

Lista de Tabelas e Figuras

Capítulo I

Capítulo II

Resumo

Objetivo: Investigar quais e de que maneira (direta ou indiretamente) variáveis clinicas e metabólicas mediam a associação entre o gene *Fat Mass and Obesity – Associated (FTO)* e a doença renal crônica (DRC) precoce em pacientes com diabetes tipo 2.

Métodos: Estudo transversal conduzido com 236 pacientes com diabetes tipo 2 (53.4% mulheres, com idade média de 60 ± 10 anos). Amostras de DNA foram genotipadas para o polimorfismo rs7204609 (C/T) do gene FTO. Dados clínicos, antropométricos e metabólicos foram coletados. Análise de caminhos foi utilizada para avaliar associações. Os programas SPSS 23.0 e MPlus 5.0 foram utilizados para análises estatísticas.

Resultados: A presença do alelo de risco (C) foi mais frequente entre os pacientes com DRC (21.8% vs. 10.8%; $P = 0.023$). Esse polimorfismo foi positivamente associado com a obesidade central ($P < 0.001$), que por sua vez foi associada com o mau controle glicêmico ($P = 0.004$) e hipertensão arterial ($P < 0.001$). Hipertensão arterial foi associada a alta excreção urinária de albumina ($P = 0.004$) e, conforme esperado, a alta excreção urinária de albumina foi associada a DRC ($P < 0.001$). A análise de caminhos demonstrou uma relação indireta entre o gene FTO e a DRC precoce, mediada pela obesidade central, hipertensão arterial e alta excreção urinária de albumina ($P = 0.045$). Todas as análises foram ajustadas para idade e sexo.

Conclusão: A presença do alelo de risco C contribui para a suscetibilidade genética á DRC em pacientes com diabetes tipo 2, especialmente na presença de obesidade central, hipertensão arterial e elevada albuminúria.

Palavras chave: diabetes tipo 2; *FTO*; análise de caminhos; albuminúria; doença renal crônica.

Abstract

Objective: To investigate which and how (directly or indirectly) clinical and metabolic variables mediate the association between fat mass and the obesity - associated (FTO) gene and early chronic kidney disease (CKD) in patients with type 2 diabetes.

Methods: A cross-sectional study was conducted in a sample of 236 patients with type 2 diabetes (53.4% women, mean age 60 ± 10 years). DNA samples were genotyped for the rs7204609 polymorphism (C/T) in the FTO gene. Clinical, anthropometric, and metabolic data were collected. Path analysis was used to evaluate associations. MPlus software, version 5.0, and SPSS 23.0 software were used to analyze data.

Results: Presence of the risk allele (*C*) was more frequent among patients with CKD (21.8% vs. 10.8%; $P = 0.023$). This polymorphism was positively associated with central obesity ($P < 0.001$), which in turn was associated with poor glycemic control ($P = 0.004$) and hypertension ($P < 0.001$). Hypertension was associated with high urinary albumin excretion (UAE) ($P = 0.004$), and, as expected, high UAE was associated with CKD ($P < 0.001$). Path analysis showed an indirect relationship between the *FTO* gene and early CKD, mediated by central obesity, hypertension, and high UAE ($P = 0.045$). All analyses were adjusted for age and sex.

Conclusion: The *C* allele contributes to genetic susceptibility to CKD in patients with type 2 diabetes, especially through the presence of central obesity, hypertension, and high UAE.

Keywords: type 2 diabetes; *FTO*; path analysis; albuminuria; chronic kidney disease.

Introdução

O Diabetes Melito (DM) é um distúrbio metabólico caracterizado pela presença de hiperglicemia persistente e acomete uma parcela significativa da população mundial (1). A doença, considerada um importante problema de saúde pública, está associada à elevada mortalidade e a inúmeras complicações clínicas – dentre elas a doença renal crônica (DRC) (2).

O DM tipo 2 é a forma mais comum do DM (2). O entendimento da sua patogênese, bem como das complicações decorrentes da doença, no entanto, é difícil para os profissionais da área. Isso se deve ao fato de que tanto fatores ambientais quanto genéticos contribuem para o desenvolvimento dessa comorbidade, tornando-a uma doença de causa multifatorial (3-5). Dentre os fatores genéticos envolvidos, destaca-se o gene *Fat Mass and Obesity Associated* (*FTO*) (6-8).

O gene *FTO*, tradicionalmente associado à obesidade, foi relacionado ao DM tipo 2 em diferentes populações. (6, 8, 9). Esse gene também demonstrou associação com a DRC em estudos realizados com pacientes com DM tipo 2 (10-12). Ainda, o gene FTO foi associado a hipertensão arterial e ao mau controle glicêmico, principais causas para o desenvolvimento da DRC em pacientes com DM tipo 2 (10, 13).

Posto isto e considerando que: 1) a DRC acomete cerca de 20 a 40% dos pacientes com DM tipo 2 (4, 11); 2) o gene FTO está relacionado à obesidade, a hipertensão arterial e ao mau controle glicêmico (6, 7, 9, 13, 14) e, 3) essas condições são os principais fatores de risco para o desenvolvimento da DRC em pacientes com DM tipo 2 (15), é de extrema importância avaliar a influência do gene FTO no desenvolvimento da DRC em pacientes com DM tipo 2. Sendo assim, o objetivo do presente estudo foi avaliar em pacientes com DM tipo 2 a associação do gene FTO, mediada ou não por outras condições clínicas, com a presença da DRC.

CAPÍTULO I

REVISÃO DA LITERATURA

1. Revisão da Literatura

Diabetes Melito e Doença Renal Crônica

O Diabetes Melito (DM) é um distúrbio metabólico caracterizado pela hiperglicemia persistente, decorrente da deficiência na produção e/ou na ação de insulina. A hiperglicemia persistente está associada a inúmeras complicações crônicas, ao aumento de morbidade, redução da qualidade de vida e elevação da taxa de mortalidade (1).

O DM acomete parte significativa da população mundial - aproximadamente 463 milhões de pessoas - e esse número vem aumentando significativamente. Em 2045, estima-se que 629 milhões de pessoas terão a doença. O DM também representa um problema de saúde de grande magnitude no Brasil, o qual ocupou a quinta posição entre os países com maiores taxas da doença em 2019. Dados da *International Diabetes Federation* (IDF) apontam que 16,8 milhões de brasileiros sofrem de DM (16).

O DM tipo 2 é a forma mais comum da doença e ocorre geralmente na fase adulta, estando associado à obesidade em cerca de 80% dos casos. A hiperglicemia sustentada é uma das principais responsáveis pelo desenvolvimento das complicações neuropáticas, macrovasculares e microvasculares, como a doença renal crônica (DRC) (2).

A DRC é uma doença complexa e progressiva definida pela presença persistente de excreção urinária elevada de albumina (albuminúria), baixa taxa de filtração glomerular (TFG) ou outras manifestações de lesão renal (17, 18) Individuos com DRC são, portanto, todos aqueles que possuem algum grau de lesão renal, independentemente da TFG. Os estágios inicias da DRC (estágios 1 e 2), por exemplo, são definidos pela presença de albuminúria com TFG normal ($\geq$60 mL/min/1.73 m^2) (19) – essa classificação permite identificar pacientes ainda em fase inicial da doença (**Figura 1**).

Ainda, a DRC é atualmente um grave problema de saude pública - 1,2 milhão de pessoas morreram dessa comorbidade ao redor do mundo em 2017 (20). A doença apresenta elevada prevalência – aproximadamente 9,0% entre os brasileiros, enorme custo para o sistema de saúde e redução na expectativa e qualidade de vida dos pacientes com a doença (21, 22). Sua origem primária varia, sendo a hipertensão arterial e o DM as causas mais comuns (15).

De acordo com a literatura, 20 a 40% dos pacientes com diagnóstico de DM tipo 2 apresentam algum estágio de DRC, sendo uma das complicações mais prevalentes relacionadas á doença (17, 23). Ademais, sabe-se que o mau controle glicêmico está fortemente associado a um aumento no ritmo de progressão da DRC. Elevados níveis pressóricos, idade, sexo e obesidade também se apresentam como fatores associados ao pior prognóstico da doença (18).

Posto isto, indivíduos com DRC possuem metas terapêuticas, tais como a manutenção da glicemia em valores próximos do normal, valores de hemoglobina glicada < 7% e valores pressóricos controlados (18), pois a microalbuminúria, uma das manifestações precoces de DRC em pacientes com DM, está fortemente associada aos níveis de pressão arterial (24, 25). Recomenda-se também que mantenham um peso corporal saudável, visto que a obesidade está associada ao aumento na incidência de DM e hipertensão arterial (18), além de ser um fator de risco independente para DRC (26, 27).

Diabetes Melito e o gene *Fat Mass and Obesity Associated* (FTO)

Ainda que o DM tipo 2 seja uma comorbidade com alta prevalência, a sua patogênese, bem como das suas complicações, é de difícil compreensão, visto que é uma doença multifatorial que está associada tanto a fatores ambientais quanto genéticos (3-5).

De fato, a importância dos fatores genéticos na patogênese do DM tipo 2 vem sendo amplamente estudada. Os SNPs (*Single Nucleotide Polymorphisms*) são o tipo mais

comum de variações genéticas dispersas dentro ou fora de uma região gênica no genoma humano. Esses polimorfismos são identificados em pelo menos 1% da população, sendo mais da metade dessas variantes mutações não deletérias (28). No caso do DM tipo 2, essas variantes, em geral, não causam diretamente a doença, mas aumentam o risco para a sua patogênese (29). Na **Tabela 1** descrevemos os principais genes associados ao risco de desenvolvimento do DM tipo 2. Dentre eles está o gene *Fat Mass and Obesity Associated* (FTO) (6-8).

O gene FTO, descrito pela primeira vez em 2007, está localizado no cromossomo 16 na posição 12.2 e consiste em nove éxons, ocupando uma área> 400kb. (30, 31). Esse gene é tradicionalmente associado à obesidade em indivíduos sem e com DM (6, 7, 9). Associações positivas entre obesidade e o gene FTO foram observadas em diferentes grupos étnicos, como caucasianos (32), japoneses (33), indianos (34) e chineses (35). Na população brasileira o gene demonstrou estar fortemente associado à obesidade (36). Estudos prévios demostraram que polimorfismos desse gene apresentam relação com maior índice de massa corporal (IMC) e peso corporal (37, 38) e maior cintura (39). Ademais, o gene FTO foi associado a pior controle glicêmico (14) e á hipertensão arterial (13).

O SNP mais frequente do FTO é o rs9939609 A/T, sendo a frequência desse polimorfismo de 42% em pacientes com DM tipo 2. Esses dados foram demonstrados em um estudo de coorte realizado em 38759 pacientes com DM e indivíduos controles, onde o gene do FTO foi fortemente associado com a obesidade. Neste estudo, 16% dos adultos que eram homozigotos para o alelo de risco A pesavam aproximadamente três quilogramas a mais e tiveram um maior risco para obesidade [Risco Relativo (RR) = 1,67] quando comparados com aqueles que não tinham a presença do alelo de risco (40). Ademais, indivíduos com o alelo de risco A também demonstraram ser mais vulneráveis

no controle da glicemia e mais resistentes a ação da insulina do que indivíduos que não apresentavam o alelo de risco (41-43).

Ainda, estudos sugerem que a presença do alelo de risco nos SNP do gene FTO está associada com o maior consumo calórico, com a diminuição de saciedade e com o aumento do apetite dos indivíduos (44). O gene FTO é predominantemente expresso no hipotálamo, área do cérebro que possui importante papel no controle da ingestão calórica (45). Tanto em adultos quanto em crianças sem DM o gene FTO teve associação com maior consumo de energia total e de determinados nutrientes (46, 47). Já em estudo realizado em pacientes com DM tipo 2 o gene foi associado ao maior consumo de gorduras e menor consumo de fibras totais (g/dia) (48).

O gene do FTO e Doença Renal Crônica (DRC)

Tal qual o gene FTO apresentou relação com a obesidade, este também demonstrou ter associação com os principais fatores de risco para o desenvolvimento da DRC – o DM e a hipertensão arterial (10, 13). Ainda, é possível que esse gene também esteja associado a DRC, sendo mediado por esses três distúrbios ou ainda por outros mecanismos biológicos.

Embora a identificação de loci de suscetibilidade a algumas doenças já tenha sido realizada pela introdução de estudos de associação genômica ampla (*Genome-Wide Association Studies* - GWAS), a identificação do loci para a DRC ainda não está completamente elucidada e permanece como um desafio para os pesquisadores da área. O polimorfismo rs7204609 (C/T) está dentre as variantes genéticas do gene FTO associadas ao DM, entretanto, foi pouco investigado.

Estudo realizado em brasileiros com DM tipo 2 demonstrou associações desta variante do gene FTO com componentes da síndrome metabólica e com elevados valores de

excreção urinária de albumina (49), um importante biomarcador precoce para DRC (50). A influência desse SNP na função renal, entretanto, ainda é desconhecida.

Em estudo realizado em japoneses com DM tipo 2, o gene do FTO (SNP rs56094641) apresentou associação positiva com a suscetibilidade a DRC (11). Na população tcheca o polimorfismo rs17817449 do gene do FTO foi associado ao aumento do risco de complicações comuns do DM, em especial a DRC e neuropatia (12). Já em um trabalho realizado em uma população europeia, baseado em dois grandes conjuntos de casos e controles, sugeriu-se uma associação robusta entre o gene FTO (rs17817449) e o risco de DRC (10). Além disso, estudos epidemiológicos (51-56) demonstraram que a suscetibilidade genética contribuiu para o desenvolvimento de DRC tanto em pacientes com DM tipo 1 quanto em indivíduos com DM tipo 2, sugerindo que fatores genéticos estão envolvidos na patogênese da DRC.

Assim sendo, considerando que o gene FTO está associado ao DM, a obesidade e a hipertensão arterial, a maioria das DRC está intimamente ligada a essas três comorbidades e a DRC é um problema mundial de saúde pública que afeta milhões de pessoas, em especial indivíduos com DM, torna-se muito importante a realização de pesquisas para entender a possível associação entre o gene FTO e a DRC em pacientes com DM tipo 2.

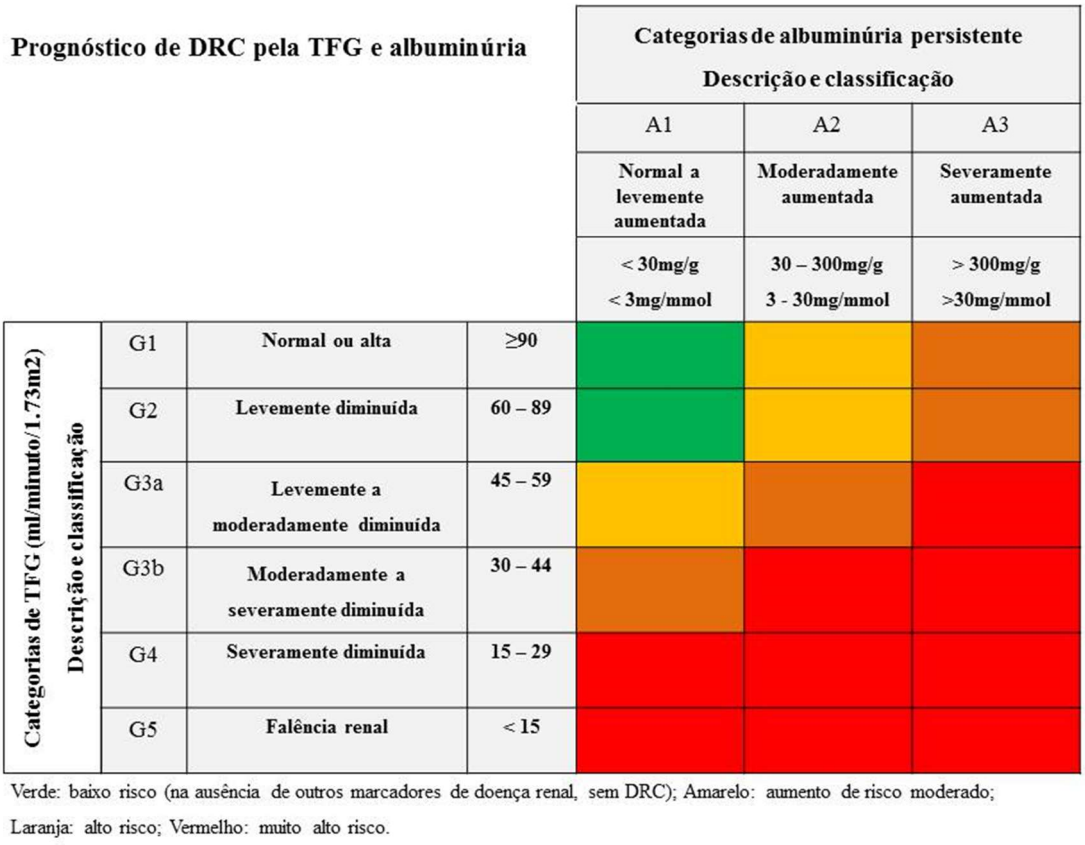
Análise de Caminhos: Método Estatístico Diferencial

A análise de caminhos é um subconjunto da modelagem de equações estruturais que permite a avaliação simultânea das complexas relações entre diferentes variáveis (57, 58). Na análise de caminhos variáveis explicativas podem afetar uma variável desfecho de forma direta ou indireta (59, 60).

Os efeitos diretos representam uma relação direta entre duas variáveis, similar a um coeficiente de regressão. Já os efeitos indiretos expressam um caminho com pelo

menos uma variável intermediária ou mediadora. Através da soma dos efeitos direitos e indiretos temos um efeito total entre duas variáveis de interesse (59, 60).

Conforme demonstrado anteriormente, muitos são os fatores de risco para o desenvolvimento da DRC, em especial em indivíduos com DM tipo 2. Dentre esses fatores está o gene FTO (10-12). O gene FTO apresenta associação com a obesidade, com o mau controle glicêmico e com a hipertensão arterial – fatores de risco para DRC (10, 13). Utilizar esse tipo de modelo de análise estatística permite mensurar simultaneamente as associações entre todas essas variáveis, bem como o papel delas no desenvolvimento da DRC em indivíduos com DM tipo 2.



Adaptado de KDIGO, 2013 (5).

Figura 1. Classificação do prognóstico de DRC de acordo com TFG e albuminúria.

DRC: doença renal crônica; TFG: taxa de filtração glomerular.

Tabela 1. Genes relacionados ao DM tipo 2

Genes					
NOTCH2	PSMD6	VGEFA	CHCHD9	DCD	CMIP
ADAM30	CACNA1D	CDKALI	GAS1	HMGA2	WWOX
SLC44A3	PPARG	C6orf57	CAMK1D	TMEM19	SGSM2
SNX7	SYN2	TP531NP1	CDC123	LGR5	SRR
PROX1	ZPLD1	GCK	VPS26A	TSPAN8	HNF1B
CR2	PLS1	CPVL	K1F11	IGF1	LPIN2
PCNXL2	SLC2A2	JAZF1	HHEK	HNF1A	PAPL
BCL11A	PEX5L	DGKB	ADRA2A	TRIAP1	PEPD
THADA	IGF2BP2	ACHE	TCF7L2	SPRY2	GIPR
GCKR	ST6GAL1	GCC1	TCERG1L	C14orf70	HNF4A
ITGB2	PPP2R2C	PAX4	CRY2	ATP10A	HUNK
RBM43	WFS1	KLF14	MADD	C2CD4A	PCBP3
RND3	MAEA	ZMAT4	KCNJ11	C2CD4B	SEZ6L
ITGB6	ZBED3	KCNU1	GALNTL4	VPS13C	DUSP9
RBMS1	AP3B1	CSMD1	LOC72903	LARP6	
GRB14	CETN3	SLC30AB	KCNQ1	HMG20A	
G6PC2	LOC72901	CDKN2A	ARAP1	ZFAND6	
TMEFF2	PCSK1	CDKN2B	MTNR1B	AP3S2	
IRS1	KCNK16	PTRD	BARX2	PRC1	
ADAMTS9	ZFAND3	GLIS3	TMEM45B	**FTO**	

Adaptado de Berná G. In: Nutrients, 6(11):5338-5369, 2014.

2. Justificativa do estudo

Considerando que: 1) estudos prévios indicam que fatores genéticos exercem papel fundamental na patogênese do DM tipo 2 e de suas complicações, 2) o gene FTO foi associado positivamente á obesidade, á hipertensão arterial e ao mau controle glicêmico e 3) essas três condições são os principais fatores de risco para DRC torna-se relevante um estudo que avalie a possível associação entre o gene FTO e a DRC em pacientes com DM tipo 2.

3. Objetivos

Geral:

Avaliar a associação da variante genética rs7204609 do gene FTO com a presença da DRC em pacientes com DM tipo 2.

Específico:

Avaliar a relação da variante genética rs7204609 do gene FTO, mediada por variáveis clínicas – obesidade central, controle glicêmico, controle pressórico e excreção urinária de albumina -, com a presença DRC-em pacientes com DM tipo 2.

Referências bibliográficas

1. World Health Organization (WHO). Global Report on Diabetes. World Health Organization, Geneva 2016; 1-88.

2. American Diabetes Association (ADA). Standards of Medical Care in Diabetes: Prevention or Delay of Type 2 Diabetes. Diabetes Care 2020; 43:32-36.

3. American Diabetes Association (ADA). Standards of Medical Care in Diabetes: Classification and Diagnosis of Diabetes. Diabetes Care 2020; 43:14-31.

4. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci 2014; 11:1185–1200.

5. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007; 445:881–885.

6. Li H, Kilpeläinen TO, Liu C, et al. Association of genetic variation in FTO with risk of obesity and type 2 diabetes with data from 96,551 East and South Asians. Diabetologia 2012; 55:981–95.

7. Fawwad A, Siddiqui IA, Basit A, et al. Common variant within the FTO gene, rs9939609, obesity and type 2 diabetes in population of Karachi, Pakistan. Diabetes Metab Syndr 2015; 10:43–7.

8. Yang Y, Liu B, Xia W, et al. FTO genotype and type 2 diabetes mellitus: spatial analysis and meta-analysis of 62 case-control studies from different regions. Genes (Basel) 2017; 8(2):70.

9. Zhao X., Yang Y., Sun B.-F., Zhao Y.-L., Yang Y.-G. FTO and Obesity: Mechanisms of Association. Curr Diabetes Rep 2014; 14:486.

10. Hubacek JA, Viklicky O, Dlouha D et al. The FTO gene polymorphism is associated with end-stage renal disease: two large independent case–control studies in a general population. Nephrol Dial Transplant 2012; 27:1030–1035.

11. Taira M, Imamura M, Takahashi A et al. A variant within the FTO confers susceptibility to diabetic nephropathy in Japanese patients with type 2 diabetes. PLoS One 2018; 13(12): e0208654.

12. Hubacek JA, Dlouha D, Klementova M, et al. The FTO variant is associated with chronic complications of diabetes mellitus in Czech population. Gene 2018; 642:220–224.

13. Pausova Z, Syme C, Abrahamowicz M et al. A common variant of the FTO gene is associated with not only increased adiposity but also elevated blood pressure in French Canadians. Circ Cardiovasc Genet 2009; 2: 260–269.

14. Freathy RM, Timpson NJ, Lawlor DA, et al. Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. Diabetes 2008; 57:1419-26.

15. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. Kidney Int 2011; 80:1258–1270.

16. International Diabetes Federation (IDF). IDF DIABETES ATLAS – 9th edition 2019. Acesso em: abril de 2020. Disponível em: http://www.diabetesatlas.org/.

17. Tuttle KR, Bakris GL, Bilous RW, et al. Diabetic kidney disease: a report from an ADA Consensus Conference. Diabetes Care 2014; 37:2864–2883.

18. National Kidney Foundation. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl 2013; 3:1–150.

19. Levey AS, Coresh J, Balk E, et al. National Kidney Foundation. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med.* 2003; 139:137–147.

20. Bikbov B, Purcell CA, Levey AS, et al. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2020; 395:707-733.

21. Barreto SM, Ladeira RM, Duncan BB*, et al.* Chronic kidney disease among adult participants of the ELSA-Brasil cohort: association with race and socioeconomic position. J Epidemiol Community Health 2016; 70:380-389.

22. El Nahas M, Bello AK. Chronic kidney disease: the global challenge. Lancet 2005; 365: 331–340.

23. Afkarian M, Zelnick LR, Hall YN, et al. Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. JAMA 2016; 316:602–610.

24. Adler AI, Stevens RJ, Manley SE, et al. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney Int*. 2003; 63: 225–232.

25. Ritz E, Dikow R. Hypertension and antihypertensive treatment of diabetic nephropathy. *Nat Clin Pract Nephrol* .2006; 2: 562–567.

26. Wahba IM, Mak RH. Obesity and obesity-initiated metabolic syndrome: mechanistic links to chronic kidney disease. *Clin J Am Soc Nephrol.* 2007; 2: 550–562.

27. Wang Y, Chen X, Song Y, et al. Association between obesity and kidney disease: a systematic review and meta-analysis. *Kidney Int.* 2008; 73: 19–33.

28. Mitchell AA, Chakravarti A, Cutler DJ. On the probability that a novel variant is a disease-causing mutation. *Genome Res.* 2005; 15:960–966.

29. Bailey-Wilson JE, Wilson AF. Linkage analysis in the next generation sequencing era. *Hum Hered.* 2011; 72:228–236.

30. Jia G, Fu Y, Zhao X, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat Chem Biol.* 2011;7(12):885–887.

31. Gerken T, Girard CA, Tung Y-CL, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate–dependent nucleic acid demethylase. *Science.* 2007;318(5855):1469–1472.

32. Hunt SC, Stone S, Xin Y, et al. Association of the *FTO* gene with BMI. Obesity 2008; 16: 902-904.

33. Hota K, Nakata Y, Matsuo T, et al. Variations in the *FTO* gene are associated with severe obesity in the Japanese. J Hum Genet 2008; 53: 546-553.

34. Yajnik CS, Janipalli CS, Bhaskar S, et al. FTO gene variants are strongly associated with type 2 diabetes in South Asian Indians. Diabetologia 2009; 52: 247-252.

35. Chang YC, Liu PH, Lee WJ, et al. Common variations in the FTO gene confers risk of obesity and modulates body mass index in the Chinese population. Diabetes 2008; 57: 2245-2252.

36. Fonseca ACP, Marchesini B, Zembrzuski VM, et al. Genetic variants in the fat mass and obesity-associated (FTO) gene confer risk for extreme obesity and modulate adiposity in a Brazilian population. Genetics and Molecular Biology 2020; *43*(1), e20180264.

37. Scuteri A, Sanna S, Chen WM, et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. Plos Genetics 2007; 3:1200-10.

38. Cornes BK, Lind PA, Medland SE, et al. Replication of the association of common rs9939609 variant of FTO with increased BMI in an Australian adult twin population but no evidence for gene by environment (G × E) interaction. Int J Obes 2009; 33:75–9.

39. Loos RJ, Yeo GS. The bigger picture of FTO: the first GWAS-identified obesity gene. Nat Rev Endocrinol 2014; 10(1):51-61.

40. Frayling TM, Tipmson NJ, Weedon MN, et al. A common variant in the *FTO* gene is associated with Body Mass Index and predisposes to childhood and adult obesity. Science 2007; 316: 889-893.

41. Saber-Ayad M, Manzoor S, El Serafi A, et al. The FTO rs9939609 "A" allele is associated with impaired fasting glucose and insulin resistance in Emirati population. Gene 2019; 681:93-8.

42. Shimaoka I, Kamide K, Ohishi M, et al. Association of gene polymorphism of the fat-mass and obesity-associated gene with insulin resistance in Japanese. Hypertens Res 2010;33(3):214–8.

43. Khan SM, El HajjChehadeh S, Abdulrahman M, Osman W, Al Safar H. Establishing a genetic link between FTO and VDR gene polymorphisms and obesity in the Emirati population. BMC Med Genet 2018;19(1).

44. Speakman JR. The "Fat Mass and Obesity Related" (FTO) gene: Mechanisms of Impact on Obesity and Energy Balance. Curr Obes Rep 2015; 4(1):73-91.

45. Gerken T, Girard CA, Tung YL, *et al.* The Obesity-Associated FTO gene encodes a 2-oxoglutarate–dependent nucleic acid demethylase. Science 2007; 318:1469–1472.

46. Sonestedt E, Roos C, Gullberg B, et al. Fat and carbohydrate intake modify the association between genetic variation in the FTO genotype and obesity. Am J Clin Nutr 2009; 90:1418–1425.

47. Wardle J, Llewellyn C, Sanderson S, et al. The FTO gene and measured food intake in children. Int J Obes 2009; 33:42–45.

48. Steemburgo T, Azevedo MJ, Gross JL, et al. The rs9939609 polymorphism in the FTO gene is associated with fat and fiber intakes in patients with type 2 diabetes. J Nutrigenet Nutrigenomics 2013; 6(2): 97-106.

49. Steemburgo T, Azevedo MJ, Gross JL, et al. The rs7204609 Polymorphism in the Fat Massand Obesity-Associated gene is positively associated with central obesity and microalbuminuria in patients with type 2 diabetes from Southern Brazil. J Ren Nutr 2012; 22(2):228-36.

50. Molitch ME, DeFronzo RA, Franz MJ, et al. Nephropathy in diabetes. Diabetes Care 2004; (Suppl 1) S79-83

51. Krolewski AS, Warram JH, Christlieb AR, et al. The changing natural history of nephropathy in type I diabetes. Am J Med 1985; 78:785–794.

52. Quinn M, Angelico MC, Warram JH, et al. Familial factors determine the development of diabetic nephropathy in patients with IDDM. Diabetologia 1996; 39:940 –945.

53. Pettitt DJ, Saad MF, Bennett PH, et al. Familial predisposition to renal disease in two generations of Pima Indians with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1990; 33:438 – 443.

54. Freedman BI, Tuttle AB, Spray BJ. Familial predisposition to nephropathy in African-Americans with non-insulin-dependent diabetes mellitus. Am J Kidney Dis 1995; 25:710 –713.

55. The Diabetes Control and Complications Trial Research: Clustering of longterm complications in families with diabetes in the diabetes control and complications trial. Group. Diabetes 1997; 46: 1829 –1839.

56. Canani LH, Gerchman F, Gross JL. Familial clustering of diabetic nephropathy in Brazilian type 2 diabetic patients. Diabetes 1999; 48:909 –913.

57. Bentler PM, Stein JA. Structural equation models in medical research. Stat Methods *Med Res*. 1992; 1(2):159-81.

58. Gamborg M, Andersen PK, Baker JL, et al. Life course path analysis of birth weight, childhood growth, and adult systolic blood pressure. *Am J Epidemiol*. 2009; 169:1167-78.

59. Kline RB. Principles and practice of structural equation modeling. 2. ed. New York: Guilford Press; 2004.

60. Hair JF, Black WC, Babin BJ, Anderson RE, Tatham RL. Análise multivariada de dados. 6. ed. Porto Alegre: Bookman; 2009.

CAPÍTULO II

ARTIGO ORIGINAL

Esse artigo está sob revisão no

Journal of Renal Nutrition

(Qualis A3, CAPES)

Relationship between *FTO* Genotype and Early Chronic Kidney Disease in Type 2 Diabetes: The Mediating Role of Central Obesity, Hypertension, Glycemic Control and Urinary Albumin Excretion Levels.

Júlia Marchetti, RD[*]

Helen Hermana M. Hermsdorff, PhD[†]

Karla P. Balbino, PhD[†]

Leidjaira L. Juvanhol, PhD[†]

José Alfredo Martinez, PhD[§]

Thais Steemburgo, PhD[*]

[*] Postgraduate Program in Food, Nutrition, and Health, Universidade Federal do Rio Grande do Sul – UFRGS, Ramiro Barcelos, 2400, CEP 90035-003 Porto Alegre, Rio Grande do Sul, Brazil.

[†] Department of Nutrition and Health, Universidade Federal de Viçosa – UFV, Av. PH Rolfs s/n, CEP 36570-900 Viçosa, Minas Gerais, Brazil.

[§] Centro de Nutrición. Universidad de Navarra. Pamplona. IMDEA (CSIC-UAM) Precision Nutrition Program CIBERobn. Instituto Carlos III. Madrid. Spain.

Address correspondence to Thais Steemburgo, PhD.

Ramiro Barcelos Street, 2400, 2º floor.

Porto Alegre, RS 90035-003, Brazil.

Phone: +55 51 33085509

E-mail: tsteemburgo@gmail.com

Word counts for the abstract: 245 words

Word counts for body of the manuscript: 2457 words

Short title: *FTO* gene and early chronic kidney disease

Acknowledgements: The authors are grateful to the special research project "Obesidad, Nutricion y Salud" (LE/97), run by the University of Navarra (Spain), and to Brazil's research funding agency, the ''Conselho Nacional de Desenvolvimento Cientıfico e Tecnológico'' (CNPq), for financial support. HHM Hermsdorff is a CNPq Research Productivity Fellow.

Support: This study was supported by the FIPE – Fundo de Incentivo à Pesquisa do Hospital de Clínicas de Porto Alegre. The study was also financially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico' (CNPq; Brazil). T.S. was a recipient of a CNPq scholarship.

Ethical approval: The Ethics Committee of the Hospital de Clinicas de Porto Alegre approved this project under protocol number 2015.0625.

Conflict of interest: None of the authors declare any conflicts of interest.

Author contributions: J Marchetti, HHM Hermsdorff and T Steemburgo contributed to study concept and design; KP Albino, LL Juvanhol, J. Marchetti and HHM Hermsdorff contributed to the analysis; J Marchetti, JA Martinez and T Steemburgo contributed to data collection. J Marchetti, HHM Hermsdorff, KP Albino, LL Juvanhol, JA Martinez

and T Steemburgo contributed to drafting and revision of the manuscript. All the authors critically revised the manuscript and approved the final version of the manuscript.

Abstract

Objective: To investigate which and how (directly or indirectly) clinical and metabolic variables mediate the association between fat mass and the obesity - associated (FTO) gene and early chronic kidney disease (CKD) in patients with type 2 diabetes.

Methods: A cross-sectional study was conducted in a sample of 236 patients with type 2 diabetes (53.4% women, mean age 60 ± 10 years). DNA samples were genotyped for the rs7204609 polymorphism (C/T) in the FTO gene. Clinical, anthropometric, and metabolic data were collected. Path analysis was used to evaluate associations. MPlus software, version 5.0, and SPSS 23.0 software were used to analyze data.

Results: Presence of the risk allele (*C*) was more frequent among patients with CKD (21.8% vs. 10.8%; $P = 0.023$). This polymorphism was positively associated with central obesity ($P < 0.001$), which in turn was associated with poor glycemic control ($P = 0.004$) and hypertension ($P < 0.001$). Hypertension was associated with high urinary albumin excretion (UAE) ($P = 0.004$), and, as expected, high UAE was associated with CKD ($P < 0.001$). Path analysis showed an indirect relationship between the *FTO* gene and early CKD, mediated by central obesity, hypertension, and high UAE ($P = 0.045$). All analyses were adjusted for age and sex.

Conclusion: The *C* allele contributes to genetic susceptibility to CKD in patients with type 2 diabetes, especially through the presence of central obesity, hypertension, and high UAE.

Keywords: type 2 diabetes; *FTO*; path analysis; albuminuria; chronic kidney disease.

Introduction

Diabetes is a metabolic disorder characterized by sustained hyperglycemia. This is one of the main factors responsible for development and progression of diabetes-associated chronic microvascular damage, which includes chronic kidney disease (CKD).[1] According to the literature, CKD occurs in 20–40% of patients with diabetes and is one of its most prevalent complications.[2-4]

Type 2 diabetes accounts for 90–95% of all diabetes.[1] However, its pathogenesis and complications are difficult to characterize, and both environmental and genetic risk factors play fundamental roles in these processes.[1, 5, 6] The relevance of genetic factors to the development and progression of type 2 diabetes has been widely studied, and one such factor is the fat mass and obesity-associated (*FTO*) gene.[7, 8]

Genome-wide association studies (GWAS) have identified an association between polymorphisms of the *FTO* gene and type 2 diabetes in several populations,[9] as well as associations with CKD.[10-12] Moreover, the *FTO* gene has been associated with central obesity,[13] hypertension,[14] and poor glycemic control.[15] All of these conditions are related to type 2 diabetes[1] and to increased rates of chronic kidney disease,[16,17] suggesting a genetic increase in susceptibility to CKD. In Brazilians with type 2 diabetes, the *rs7204609* polymorphism of the *FTO* gene has been associated with high levels of urinary albumin excretion,[18] an important early clinical marker of CKD.[19] The *rs7204609* polymorphism *(C/T)*, however, has been little investigated, and its influence on renal function is still unknown.

The primary objective of this study is to evaluate whether the *FTO* polymorphism *rs7204609* is associated with presence of CKD in patients with type 2 diabetes and, if such an association exists, whether it is mediated by other conditions. We hypothesized

that this polymorphism would influence CKD either directly or indirectly, i.e. through other clinical and metabolic conditions.

Material and Methods

Patients and Study Design

This cross-sectional study was conducted in a sample of 236 patients with type 2 diabetes (defined as age 30 years or older at onset of diabetes, no history of ketoacidosis or documented ketonuria, and, in insulin users, initiation of insulin therapy at least 5 years after diagnosis).

Consecutive patients were selected on the basis of the following criteria: 24-hour urinary albumin excretion (UAE) < 200 µg/minute, absence of urinary tract infection or other renal diseases, and absence of heart failure (New York Heart Association class IV). Patients with macroalbuminuria (UAE > 200 µg/minute) were not included, because they are usually the subject of specific recommendations. All medications in use were maintained during the study. The Ethics Committee approved the protocol (#2015-0625), and patients gave their written informed consent for participation.

This study was designed and conducted in accordance with the STrengthening the REporting of Genetic Association studies (STREGA) reporting recommendations, an extension of the STROBE Statement.

Anthropometrical, Clinical, and Metabolic Assessment

Body weight and height of patients (not wearing coats or shoes) were obtained using an anthropometric scale, with measurements recorded to the nearest 100 g for weight and to the nearest 0.1 cm for height. Body mass index (BMI) (kg/m^2) was calculated as weight (kilograms) divided by height (meters) squared. Waist circumference was measured midway between the margin of the lowest rib and the iliac

crest, near the umbilicus, using a flexible, non-stretch fiberglass tape. Central obesity was defined by a waist circumference ≥ 94 cm for men and ≥ 80 cm for women.[20]

Blood pressure was measured by the auscultatory method using a standard mercury sphygmomanometer (Korotkoff phases I and V). Two measurements were obtained, to the nearest 2 mmHg, after a 10-minute rest. Hypertension was defined as blood pressure >130/80 mmHg on two occasions or use of antihypertensive drugs.[1, 21]

Blood samples were obtained after a 12-hour fast. Glycated hemoglobin (HbA1C) was determined by ion-exchange high-performance liquid chromatography (Merck-Hitachi L-9100 glycated hemoglobin analyzer, reference range, 4.7% to 6.0%; Merck, Darmstadt, Germany). Urinary albumin was measured by an immunoturbidimetric method (Microalb; Ames-Bayer, Tarrytown, NY). Using urine samples with albumin concentrations of 30 and 100 mg/L, the intra-assay and inter-assay coefficients of variation at our laboratory were both 6%.[22]

The presence of CKD was evaluated as described elsewhere.[1] According to 24-hour UAE results, patients were classified as having normoalbuminuria (UAE < 20 μg/minute) or microalbuminuria (UAE > 20 to 199 μg/minute). Microalbuminuria was always confirmed in a second urine sample.[23] The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[24]

Genotyping for FTO Polymorphism

Peripheral blood samples were collected from all patients for DNA extraction. Detection of the *rs7204609 FTO* SNP was performed by allelic discrimination assay (TaqMan SNP Genotyping Assays, Applied Biosystems, CA) using the ABI PRISM 7000 Real-Time PCR System, and genotypes were read using automated software (SDS1.1, Applied Biosystems, CA). Reactions were run in 10-mL volumes using an

amplification protocol of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, then 60°C for 1.5 minutes.

Statistical Analyses

Statistical analysis was performed in SPSS 23.0 software (SPSS, Chicago, IL). The unpaired Student's *t* test, the Mann–Whitney *U* test, and the chi-square test were used as appropriate. Genotypes were analyzed using risk alleles. In the *rs7204609 (C/T)* polymorphism, the risk allele is the *C* allele. For analysis, all patients with the putative *C* allele were pooled together (*CT* and *CC* genotypes) and compared with patients without the *C* allele (*TT* genotypes). A chi-square test was also used to evaluate Hardy–Weinberg equilibrium and assess the frequency distribution of genotypes. Results were expressed as mean (SD), median (interquartile range), or absolute and relative frequency. *P* values <0.05 were considered statistically significant.

MPlus® software, version 5.0, was used both to analyze the relationship between the presence of the *C* allele and CKD and to investigate, via path analysis, the mediating role of selected variables in relationships involving central obesity, hypertension, glycemic control, and UAE. Path analysis is a subset of structural equation modeling,[25] an extension of regression analysis that allows simultaneous exploration of the complex relationships between multiple variables.[26]

In path analysis, explanatory variables may affect the outcome variable directly or indirectly. Direct effects represent a direct relationship between two variables, i.e., relationships which are not mediated by other variables in the model. They can be interpreted as a regression coefficient. In turn, indirect effects express a sequence of paths with at least one intermediate or mediating variable. They are calculated by multiplying the direct effects between the variables involved in that path. Finally, the total effect is calculated from the sum of direct and indirect effects between two variables.[27, 28] (It bears

stressing that, in this study, the term "effect" is used in the sense of association, not causality.)

Standardized and non-standard coefficients were estimated with their respective *p* values. Odds ratios (ORs) were obtained from exponentiation of non-standard effects. The robust maximum likelihood method, which does not require the assumption of normality in multivariate data, was used to estimate the parameters.[27]

Analyses of the variables associated with CKD included additional adjustments for age and sex. A selection of different measures were used to verify the fit of the model. The root mean square error of approximation (RMSEA) and the standardized root mean square residual (SRMR) are both based on model residuals; values < 0.06 indicate that the theoretical model fits the data.[29-31] The Tucker-Lewis index (TLI) and comparative fit index (CFI) were also estimated, with values > 0.90 indicating good model fit.[27, 29]

Results

A total of 236 patients with type 2 diabetes were enrolled in this study. Most were elderly (mean age 60.1 ± 10.3 years) and female (53.4%), with a median diabetes duration of 11 (6-18) years. The mean BMI was 28.6 ± 4.2 kg/m², and most patients (83.9%) had central obesity. The median UAE was 6.4 (3.5–33.0) μg/min, while the eGFR was 85 (71.2–101.0) mL/min/1.73 m^2. Hypertension were detected in 88.1% of the patients. The genotype frequencies of each *rs7204609* allele were 85.2% for *TT*, 13.1% for *CT*, and 1.7% for *CC*. No deviation from Hardy–Weinberg equilibrium was observed ($\chi 2 = 2.739$; $P = 5.642$).

Table 2 lists the patients' characteristics, stratified by presence of CKD. The proportion of CKD was higher in men and in older patients. There was a higher proportion of the *C* allele presence among patients with CKD than among patients without CKD

(21.8% vs. 10.8%; $P = 0.023$). More aggressive treatment of diabetes was more common in patients without CKD than in patients with CKD. As expected, patients with CKD had higher UAE levels than those without CKD (57.4 [33.6–96.3] vs. 3.5 [3.4–8.6]; $P<0.001$). No significant differences were observed in relation to central obesity, glycemic control, or presence of hypertension.

The path analysis achieved a satisfactory fit according to the RMSEA (0.000), SRMR (0.015), CFI (1.000), and TLI (1.192) indices. **Table 3** shows the OR for CKD. Each 1 mg/min increase in UAE was associated with a 0.8% (CI 95% 0.7-0.9%) increase in CKD odds. The only significant indirect effect was observed in the relationship between the *FTO rs7204609* polymorphism and CKD mediated by central obesity, hypertension and high UAE ($P = 0.045$). Glycemic control did not have a significant direct, indirect, or overall effect on CKD (**Table 3**).

When we analyzed the standardized coefficients for direct effects in path analysis, high UAE was, as expected, positively associated with CKD ($P<0.001$). A positive association was also observed between hypertension and high UAE ($P=0.004$). Central obesity was positively associated with hypertension ($P<0.001$) and with poor glycemic control ($P=0.004$). The presence of the *C* allele of *FTO* was positively associated with central obesity ($p < 0.001$) but did not have a significant direct effect on CKD (**Figure 1**).

Discussion

CKD is one of the most prevalent microvascular complications of diabetes.[1] As expected [2-4], in our sample, 33 % of patients with type 2 diabetes (n = 78) had CKD. Presence of the risk allele (*C*) of the *FTO* gene was more frequent in these participants compared to those without the disease (21.8% vs. 10.8%; $P = 0.023$). These findings suggest a relevant relationship between the *rs7204609* polymorphism and CKD, which, to the best of our knowledge, has not been reported previously. Studies in Czech,

Japanese, and Emirati populations have demonstrated a relationship between CKD and *FTO* variants – *rs17817449, rs56094641* and both *rs1421086* and *rs17817449* polymorphisms, respectively.[10-12]

Studying the factors that trigger CKD caused by diabetes is difficult because of several issues related to the underlying condition.[16, 17] Our path analysis found no significant direct effect of the *FTO* gene on CKD. However, we did detect an indirect effect mediated by central obesity, hypertension, and high UAE, all of which are conditions or biomarkers related to renal function.[16, 17, 32-34] In a Chinese population, components of the metabolic syndrome (including central obesity and hypertension) were associated with high UAE, a well-known predictor of CKD.[35] Although the mechanisms are not fully understood, central obesity in conjunction with hypertension and diabetes could be responsible for increased endothelial permeability and intraglomerular capillary pressure, with consequent development of CKD.[36]

Regarding the effect of genetics, in our sample the *FTO* gene was positively associated with central obesity. This is consistent with previous reports[7, 8, 37] suggesting genetic susceptibility to an obesity phenotype in individuals with type 2 diabetes. Studies have shown that *FTO* polymorphisms are associated with higher food intake and increased hunger/reduced satiety.[38] The role of ethnicity, however, should be considered when interpreting the influence of the *rs7204609* polymorphism on obesity, as the association between *rs7204609* and obesity has been shown to differ across different ethnic groups. In West Africans, for example, this polymorphism was positively associated with obesity, whereas in a Japanese population, the association was negative.[39, 40]

Central obesity is a direct risk factor for deterioration of renal function[32] and influences poor glycemic and blood-pressure control.[41-43] In our sample, central obesity

was associated with poor glycemic control and hypertension, both of which are among the leading risk factors for development and progression of CKD.[16, 17, 34] Furthermore, blood pressure control and glycemic control are part of therapeutic strategies to prevent high UAE in individuals with type 2 diabetes.[44, 45] In our sample, hypertensive patients had higher UAE levels when compared to normotensive individuals. Hypertension can impair vascular endothelial function, leading to an elevated GFR and high UAE.[34, 46] However, the association between glycemic control and high UAE was not confirmed.

As expected, patients with CKD had higher UAE values in our analysis. In 2015, a meta-analysis found that, for every 30% reduction in UAE, the risk of kidney disease decreased by 23.7%.[47] In our study, a one-unit increase in UAE was associated with a 0.8% (CI 95% 0.7-0.9%) increase in the odds of CKD. We should stress that the presence of CKD was significantly more frequent among older and male members of our sample, which is consistent with the literature.[48, 49]

Our findings support the well-established position that lifestyle changes, especially obesity management and blood pressure control, are important factors in diabetes treatment and promote vascular protection in this group of patients. We did not find any studies on this topic that have applied path analysis as a statistical method. Our analysis of the mediating effects of central obesity, glycemic control, hypertension, and high UAE on the relationship between the *FTO* gene and CKD differentiates this study from previous research.

However, the study also has limitations. First, the small sample size prevents generalization of our data. Nonetheless, it is sufficient for the analyses conducted, which demonstrated an interesting association in individuals with type 2 diabetes. This association needs to be confirmed by further replication studies, particularly in other ethnic populations, since this study only enrolled individuals from southern Brazil.

Second, the exclusion of patients with specific medical conditions (such as macroalbuminuria and established renal disease) is also a limiting factor. However, the inclusion of these patients would have biased the results. Third, during clinical follow-up, some patients on the study were receiving antidiabetic therapy. A positive association between diabetes treatment and glycemic control was demonstrated in the analysis. This may at least partly explain why we did not find an association between glycemic control and CKD.

Considering these limitations, the *FTO* gene polymorphism *rs7204609* appeared to contribute to genetic susceptibility to CKD in this sample of patients with type 2 diabetes, in a largely indirect manner, through effects on central obesity, hypertension, and high UAE levels. These research findings may improve our understanding of the underlying mechanisms of CKD caused by diabetes, especially genetic factors.

Practical Application

This study may improve our understanding of the underlying mechanisms of CKD in diabetes, especially as it relates to genetic factors. Furthermore, it provides further support for the long-established position that lifestyle changes, especially control of obesity and blood pressure, are important factors in the treatment of diabetes.

References

1. American Diabetes Association. Standards of Medical Care in Diabetes-2020. *Diabetes Care*. 43 (Suppl. 1): S135–S152.

2. Tuttle KR, Bakris GL, Bilous RW, et al. Diabetic kidney disease: a report from an ADA Consensus Conference. *Diabetes Care*. 2014; 37: 2864–2883.

3. Afkarian M, Zelnick LR, Hall YN, et al. Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. *JAMA*. 2016; 316:602–610.

4. De Boer IH, DCCT/EDIC Research Group. Kidney disease and related findings in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study. *Diabetes Care.* 2014; 37:24–30.

5. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *Int J Med Sci*. 2014; 11:1185–1200.

6. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature*. 2007; 445:881–885.

7. Li H, Kilpeläinen TO, Liu C, et al. Association of genetic variation in FTO with risk of obesity and type 2 diabetes with data from 96,551 East and South Asians. *Diabetologia*. 2012; 55:981–95.

8. Fawwad A, Siddiqui IA, Basit A, et al. Common variant within the FTO gene, rs9939609, obesity and type 2 diabetes in population of Karachi, Pakistan. *Diabetes Metab Syndr.* 2015; 10:43–7.

9. Yang Y, Liu B, Xia W, et al. FTO genotype and type 2 diabetes mellitus: spatial analysis and meta-analysis of 62 case-control studies from different regions. *Genes* (Basel). 2017; 8(2):70.

10. Hubacek JA, Viklicky O, Dlouha D, et al. The FTO gene polymorphism is associated with end-stage renal disease: two large independent case–control studies in a general population. *Nephrol Dial Transplant*. 2012; 27:1030–1035.

11. Taira M, Imamura M, Takahashi A, et al. A variant within the FTO confers susceptibility to diabetic nephropathy in Japanese patients with type 2 diabetes. P*LoS One*. 2018; 13(12): e0208654.

12. Osman WM, Jelinek HF, Tay GK, et al. Genetics of diabetic kidney disease: A follow-up study in the Arab population of the United Arab Emirates. *Mol Genet Genomic Med.* 2019; (12): e985.

13. Loos RJ, Yeo GS. The bigger picture of FTO: the first GWAS-identified obesity gene. *Nat Rev Endocrinol*. 2014; 10(1):51-61.

14. Pausova Z, Syme C, Abrahamowicz M, et al. A common variant of the FTO gene is associated with not only increased adiposity but also elevated blood pressure in French Canadians. *Circ Cardiovasc Genet*. 2009; 2: 260–269.

15. Freathy RM, Timpson NJ, Lawlor DA, et al. Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. *Diabetes*. 2008; 57:1419-26.

16. Kazancioğlu R. Risk factors for chronic kidney disease: an update. *Kidney Int Suppl* (2011). 2013; 3:368-71.

17. National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: evaluation, classification and stratification. *Am J Kidney Dis*. 2002; 39 (Suppl. 1): S1–266.

18. Steemburgo T, Azevedo MJ, Gross JL, et al. The rs7204609 Polymorphism in the Fat Mass and Obesity-Associated gene is positively associated with central obesity and

microalbuminuria in patients with type 2 diabetes from Southern Brazil. *J Ren Nutr*. 2012; 22(2):228-36.

19. Molitch ME, DeFronzo RA, Franz MJ, et al. Nephropathy in diabetes. *Diabetes Care*. 2004; (Suppl 1) S79-83.

20. Alberti KGMM, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome- A Joint Interim Statement of the International Diabetes Federation task force on epidemiology and prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* 2009; 120:1640-1645.

21. Whelton PK, Carey RM, Aronow WS, et al. ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2018;71: e127–248.

22. Camargo JL, Lara GM, Wendland AE, et al. Agreement of different immunoassays for urinary albumin measurement. *Clin Chem*. 2008; 54:925-927.

23. Gross JL, Azevedo MJ, Silveiro SP, et al. Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care*. 2005; 28:164-176.

24. Levey AS, Stevens LA, Schmid CH, et al. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009; 150:604-12.

25. Bentler PM, Stein JA. Structural equation models in medical research. Stat Methods *Med Res*. 1992; 1(2):159-81.

26. Gamborg M, Andersen PK, Baker JL, et al. Life course path analysis of birth weight, childhood growth, and adult systolic blood pressure. *Am J Epidemiol*. 2009; 169:1167-78.

27. Kline RB. Principles and practice of structural equation modeling. 2. ed. New York: Guilford Press; 2004.

28. Hair JF, Black WC, Babin BJ, Anderson RE, Tatham RL. Análise multivariada de dados. 6. ed. Porto Alegre: Bookman; 2009.

29. Hu L, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. *Structural Equation Modeling*. 1999; 6:1-55.

30. Hooper D, Coughlan J, Mullen M. Structural equation modeling: guidelines for determining model fit. Journal of Business Research Methods. 2008; 6:53- 60.

31. Baltar VT, Xun WW, Johansson M, et al. A structural equation modeling approach to explore the role of B vitamins and immune markers in lung cancer risk. *Eur J Epidemiol.* 2013; 28:677-688.

32. Thomas MC, Brownlee M, Suszlak K, et al. Diabetic kidney disease. *Nat Rev Dis Primers.* 2015; 1**:**15018.

33. Wickman C., Kramer H. Obesity and kidney disease: potential mechanisms. *Semin Nephrol.* 2013; 33: 14–22.

34. Marques da Silva P, Carvalho D, Nazare J, et al. Prevalence of microalbuminuria in hypertensive patients with or without type 2 diabetes in a Portuguese primary care setting: The RACE (Microalbumin Screening Survey) study. *Rev Port Cardiol*. 2015; 34(4):237-246.

35. Lee Y-Y, Yang C-K, Weng Y-M, et al. All components of metabolic syndrome are associated with microalbuminuria in a Chinese population. *PLoS One*. 2016; 11(6): e0157303.

36. Bonnet F, Marre M, Halimi JM, et al. Waist circumference and the metabolic syndrome predict the development of elevated albuminuria in non-diabetic subjects: the DESIR Study. *J Hypertens*. 2006; 24:1157–63.

37. Phani NM, Vohra M, Rajesh S, et al. Implications of critical PPARγ2, ADIPOQ and FTO gene polymorphisms in type 2 diabetes and obesity-mediated susceptibility to type 2 diabetes in an Indian population. *Mol Genet Genomics.* 2016; 291:193–204.

38. Speakman JR. The *"Fat Mass and Obesity Related"* (FTO) gene: Mechanisms of Impact on Obesity and Energy Balance. *Curr Obes Rep*. 2015; 4(1):73-91.

39. Adeyemo A, Chen G, Zhou J, et al. FTO genetic variation and association with obesity in West Africans and African Americans. *Diabetes.* 2010; 59:1549-1554.

40. Hotta K, Nakata Y, Matsuo T, et al. Variations in the FTO gene are associated with severe obesity in the Japanese. *J Hum Genet*. 2008; 53:546-553.

41. Silva RC, Silva DA, Bastos JL, et al. Anthropometric measures change and incidence of high blood pressure levels among adults. *J Hypertens*. 2017; 35(1):39-46.

42. Feng RN, Zhao C, Wang C, et al. BMI is strongly associated with hypertension, and waist circumference is strongly associated with type 2 diabetes and dyslipidemia in northern Chinese adults. *J Epidemiol*. 2012; 22:317–323.

43. Fujita M, Sato Y, Nagashima K, Takahashi S, Hata A. Predictive power of a body shape index for development of diabetes, hypertension, and dyslipidemia in Japanese adults: A retrospective cohort study. *PLoS One*. 2015; 10: e0128972.

44. Emdin, CA, Rahimi K, Neal B, et al. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. *JAMA*. 2015; 313(6):603–615.

45. Boussageon R, Bejan-Angoulvant T, Saadatian-Elahi M, et al. Effect of intensive glucose lowering treatment on all-cause mortality, cardiovascular death, and microvascular events in type 2 diabetes: meta-analysis of randomised controlled trials. *BMJ*. 2011; 343: d4169.

46. Thompson JES, Smith W, Ware LJ, et al. Masked hypertension and its associated cardiovascular risk in young individuals: the Africa-PREDICT study. *Hypertens Res*. 2016; 39:158–165.

47. Heerspink HJ, Kröpelin TF, Hoekman J, et al. Drug-induced reduction in albuminuria is associated with subsequent renoprotection: a meta-analysis. *J Am Soc Nephrol*. 2015; 26:2055–2064.

48. Gheith O, Farouk N, Nampoory N, Halim MA, Al-Otaibi T. Diabetic kidney disease: worldwide difference of prevalence and risk factors. *J Nephropharmacol*. 2016; 5(1):49–56.

49. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. *Clin J Am Soc Nephrol*. 2017; 12(12):2032–45.

Table 1. General characteristics of patients with type 2 diabetes

Variable	n (236)
Age (years)	60.1 ± 10.3
Duration of diabetes (years)	11 (6-18)
Sex (female)	126 (53.4%)
Ethnicity (white)	203 (86.4%)
Weight (kg)	75.7 ± 13.7
BMI (kg/m²)	28.6 ± 4.2
Waist circumference (cm)	100.0 ± 10.5
Central obesity*	198 (83.9%)
HbA1C test (%)	7.0 (6.3–8.1)
UAE (μg/minute)	6.4 (3.5 – 33.0)
eGFR (mL/minute per 1.73 m^2)	85 (71.2 – 101.0)
Hypertension (%)	208 (88.1%)
Risk allele of the *rs7204609* polymorphism of the *FTO* gene (C allele)	35 (14.8%)
Diabetes treatment (n, %)	
Diet	12 (5.1%)
Oral antidiabetic agents	140 (59.3%)
Insulin	28 (11.9%)
Insulin + oral antidiabetic agents	56 (23.7%)
ACE inhibitors	131 (55.5%)

BMI: Body mass index; UAE, urinary albumin excretion; eGFR, estimated glomerular filtration rate; *FTO:* Fat Mass and Obesity Associated; ACE: angiotensin-converting enzyme inhibitors. Data are expressed as mean ± standard deviation, median (25th percentile, 75th percentile) or number of patients with the characteristic (%) and compared using t-Student, Mann-Whitney U and chi-square test, respectively.

* Elevated waist circumference: ≥94 cm for men and ≥80 cm for women.

Table 2. Characteristics of patients with type 2 diabetes, by presence of chronic kidney disease.

	Presence of CKD		
	No	**Yes**	
Variables	**(n = 158)**	**(n = 78)**	***P* value**
Sex (Female/Male)	95 (75.4%) / 63 (57.3%)	31 (24.6%) / 47 (42.7%)	0.003**
Age (years)	57.5 ± 10.8	61.3 ± 9.9	0.008**
Risk allele on r*s7204609* SNP of *FTO* gene (C allele)	17 (10.8%)	17 (21.8%)	0.023**
Central obesity*	131 (82.7%)	67 (85.9%)	0.477
HbA1C test (%)	7.4 ± 1.6	7.4 ± 1.5	0.940
Diabetes treatment (%)			
(D/AO/I or I + OA)	5 (41.7%) / 105 (75%)	7 (58.3%) / 35 (25%)	0.009**
	15 (53.6%) / 33 (58.9%)	13 (46.4%) / 23 (41.1%)	
ACE inhibitors	79 (50%)	52 (66.6%)	0.056
Hypertension (yes)	134 (64.4%)	74 (35.6%)	0.072
UAE (µg/minute)	3.5 (3.4-8.6)	57.4 (33.6 – 96.3)	<0.001**
eGFR (mL/minute per 1.73 m^2)	84.5 (71.2 – 98.0)	86.0 (71.5-104.2)	0.454

CKD: chronic kidney disease; SNP single nucleotide polymorphism; *FTO*: Fat Mass and Obesity Associated; D: diet only; OA: oral antidiabetic agents; I: insulin. ACE: angiotensin-converting enzyme inhibitors; UAE, urinary albumin excretion; eGFR, estimated glomerular filtration rate.

Data are expressed as number of patients with the characteristic (%), mean ± SD or median (25th percentile, 75th percentile) and compared using chi-square test, t-Student and Mann-Whitney U, respectively.

* Elevated waist circumference ≥94 cm for men and ≥80 cm for women.

** *P* value has statistical significance ($p < 0.05$).

Table 3. Direct, indirect, and total coefficients for the mediation relations.

Relation	Mediators	Effect	Standardized coefficient	Standard Error (SE)	*P* Value	Odds ratio (OR)	Confidence intervals (CI 95%)
FTO rs7204609 → CKD		**Direct**	0.072	0.044	0.104	1.124	0.981-1.284
	UAE	**Indirect**	0.022	0.036	0.543		
	Glycemic control	**Indirect**	0.003	0.007	0.639		
	Glycemic control → UAE	**Indirect**	-0.001	0.002	0.636		
	Central obesity → Glycemic control → UAE	**Indirect**	0.002	0.003	0.499		
	Hypertension	**Indirect**	-0.003	0.009	0.687		
	Hypertension → UAE	**Indirect**	-0.001	0.002	0.636		
	Central obesity → Hypertension → UAE	**Indirect**	0.005	0.002	**0.045**		
		Total	0.087	0.061	0.153		
Central obesity → CKD	Glycemic control	**Indirect**	-0.027	0.035	0.441		
	Hypertension	**Indirect**	-0.044	0.025	0.084		
	Glycemic control → UAE	**Indirect**	0.009	0.013	0.489		
	Hypertension → UAE	**Indirect**	0.024	0.010	**0.019**		

		Total	-0.038	0.047	0.412		
Glycemic control → CKD	UAE	**Direct**	-0.089	0.093	0.336	0.969	0.912- 1.031
		Indirect	0.029	0.039	0.469		
		Total	-0.060	0.100	0.544		
Hypertension → CKD	UAE	**Direct**	-0.151	0.082	0.066	0.933	0.858-1.013
		Indirect	0.082	0.029	**0.005**		
		Total	-0.069	0.092	0.450		
UAE → CKD		**Direct**	0.584	0.059	**0.000**	1.008	1.007-1.009

CKD: chronic kidney disease; *FTO*: fat mass and obesity-associated; UAE: urinary albumin excretion.

**P* value has statistical significance (*P* <0.05).

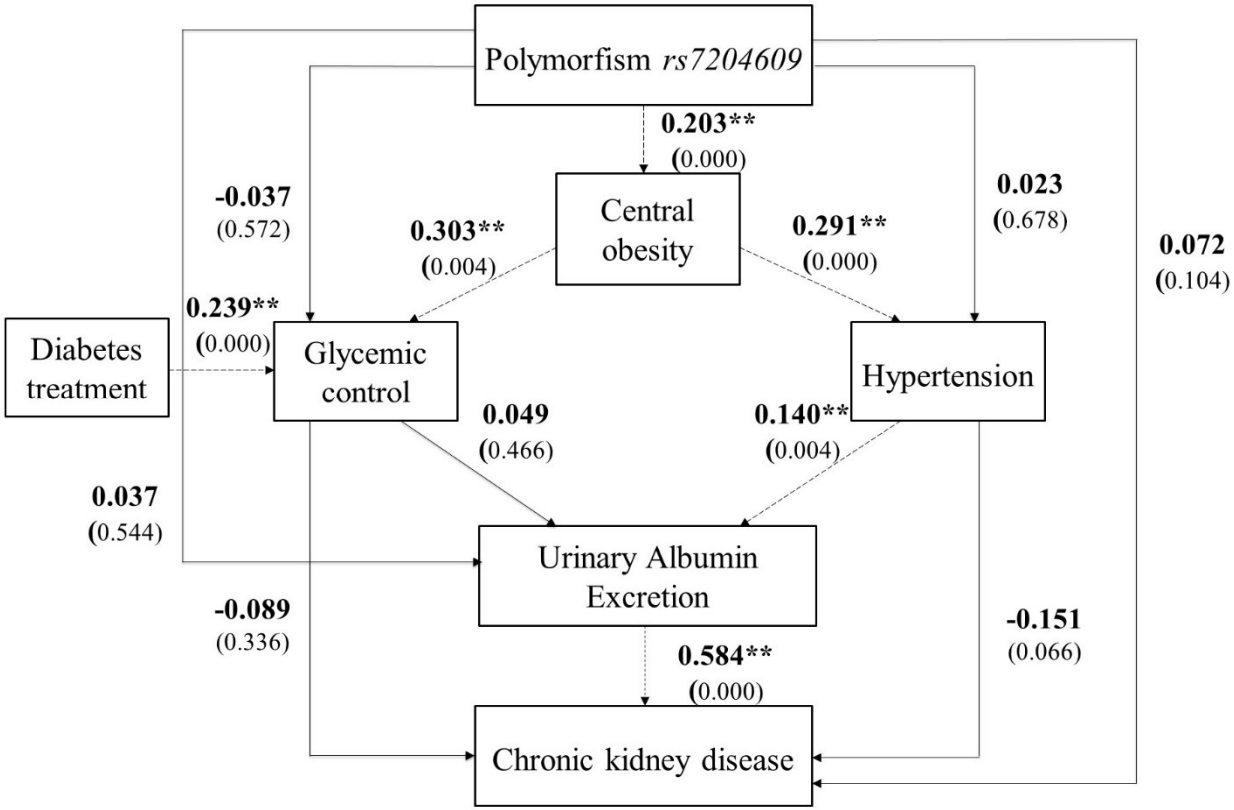


Figure 1. Path analysis for the relationship between the *FTO* gene and chronic kidney disease in patients with type 2 diabetes. In path analysis, all variables were considered continuous, except for the presence of the risk allele of the *rs7204609* polymorphism of the *FTO* gene and for the presence of chronic kidney disease. Associations with chronic kidney disease were further adjusted for age and sex. Standardized coefficients and *P* values were used as parameter estimates in path analysis. Dashed lines indicate paths with statistical significance. **P*<0.05; ***P*<0.01

SUPPLEMENTARY MATERIAL

STrengthening the REporting of Genetic Association studies (STREGA) reporting recommendations, extended from STROBE Statement

Item	Item no	STROBE Guideline	Extension for Genetic Association Studies (STREGA)	Page no
Title and Abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract.		2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found.		2
Introduction				
Background rationale	2	Explain the scientific background and rationale for the investigation being reported.		6
Objectives	3	State specific objectives, including any pre-specified hypotheses	***State if the study is the first report of a genetic association, a replication effort, or both.***	6,7
Methods				
Study design	4	Present key elements of study design early in the paper.		7
Setting	5	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up and data collection.		7
Participants	6	(a) **Cohort study –** Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. **Case–control study –** Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. **Cross-sectional study –** Give the eligibility criteria, and the sources	***Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant.***	7

		and methods of selection of participants. **(b) Cohort study** – For matched studies, give matching criteria and number of exposed and unexposed. **Case–control study –** For matched studies, give matching criteria and the number of controls per case.		
Variables	7	(a) Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	***(b) Clearly define genetic exposures (genetic variants) using a widely – used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).***	7,8
Data sources measurement	8*	(a) For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group.	***(b) Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory /centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.***	8

Bias	9	(a) Describe any efforts to address potential sources of bias.	***(b) For quantitative outcome variables, specify if any investigation of potential bias resulting from pharmacotherapy was undertaken. If relevant, describe the nature and magnitude of the potential bias, and explain what approach was used to deal with this.***	7
Study size	10	Explain how the study size was arrived at.		9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why.	***If applicable, describe how effects of treatment were dealt with.***	No applicable
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding.	***State software version used and options (or settings) chosen.***	9
		(b) Describe any methods used to examine subgroups and interactions.		9
		(c) Explain how missing data were addressed.		
		(d) **Cohort study –** If applicable, explain how loss to follow-up was addressed. **Case–control study –** If applicable, explain how matching of cases and controls was addressed. **Cross-sectional study –** If applicable, describe analytical methods taking account of sampling strategy.		
		(e) Describe any sensitivity analyses.		
			(f) State whether Hardy- Weinberg	9

			equilibrium was considered and, if so, how.	
			(g) Describe any methods used for inferring genotypes or haplotypes.	No applicable
			(h) Describe any methods used to assess or address population stratification.	No applicable
			(i) Describe any methods used to address multiple comparisons or to control risk of false positive findings.	No applicable
			(j) Describe any methods used to address and correct for relatedness among subjects.	No applicable
Results				
Participants	13*	(a) Report the numbers of individuals at each stage of the study – e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up and analysed.	***Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.***	10
		(b) Give reasons for non-participation at each stage.		
		(c) Consider use of a flow diagram.		
Descriptive data	14*	(a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders.	***Consider giving information by genotype.***	10
		(b) Indicate the number of participants with missing data for each variable of interest.		
		(c) **Cohort study –** Summarize follow-up time, e.g. average and total amount.		

Outcome data	15*	**Cohort study –** Report numbers of outcome events or summary measures over time.	***Report outcomes (phenotypes) for each genotype category over time***	11
		Case–control study – Report numbers in each exposure category, or summary measures of exposure.	***Report numbers in each genotype category***	
		Cross-sectional study – Report numbers of outcome events or summary measures.	***Report outcomes (phenotypes) for each genotype category***	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence intervals). Make clear which confounders were adjusted for and why they were included.		11
		(b) Report category boundaries when continuous variables were categorized.		
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.		
			(d) Report results of any adjustments for multiple comparisons.	
Other analyses	17	(a) Report other analyses done – e.g. analyses of subgroups and interactions, and sensitivity analyses.		No applicable
			(b) If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken.	
			(c) If detailed results are available elsewhere, state how they can be accessed.	
Discussion				
Key results	18	Summarize key results with reference to study objectives.		12

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	12-14
Generalizability	21	Discuss the generalizability (external validity) of the study results.	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based.	15

STROBE: STtrengthening the Reporting of Observational Studies in Epidemiology

*Give information separately for cases and controls in case–control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Normas da revista de publicação

GUIDE FOR AUTHORS

INTRODUCTION

The ***Journal of Renal Nutrition*** is the official research publication of the Council on Renal Nutrition of the National Kidney Foundation, Inc. and the International Society of Renal Nutrition and Metabolism. The purpose of JRN is to stimulate interest and research in nutrition pertaining to kidney and urologic diseases, hypertension, dialysis therapies and kidney transplantation in children and adults, as well as to publish information concerning renal nutrition research, practice issues and policies. The goal of JRN is to publish original communications and research that maintain high standards for the profession and that contribute significantly to the overall advancement of the field. The JRN is a refereed publication. Manuscripts are accepted for review with the understanding that the material has not been previously published except in abstract form, and is not concurrently under review for publication elsewhere. Authors submitting a manuscript to JRN must understand that if it is accepted for publication, copyright of the article including the right to reproduce the article in all forms and media, shall be assigned exclusively to the National Kidney Foundation. The Publisher, Elsevier, will not refuse any reasonable request by the author for permission to reproduce any of his or her contributions to the Journal. Information on how to request permission is available on the JRN website (http://www.jrnjournal.org).

Manuscript Categories

The Journal of Renal Nutrition welcomes manuscripts in the following categories: Original Research Articles, Reviews, Clinical/Research Briefs, Practical Aspects

Articles, Case Studies, Patient Education Material, Letters to the Editor, and Supplements. Authors should adhere to the guidelines provided for each type of manuscript.

Original Research: Original research articles are full length reports that cover topics relevant to renal nutrition dietetics or renal nutrition science for both adult and pediatric issues. To be published, the work presented in the manuscript must be original; on occasion, confirmatory studies of timely and important observations will also be acceptable. In addition, other considerations for evaluating the acceptability of a submitted manuscript include its importance, the soundness of the experimental design, the validity of the methods, the appropriateness of the conclusions, and the quality of presentation.

Original Research manuscripts should be organized as follows: title page, support and financial disclosure, abstract, introduction, methods, results, discussion, practical application, acknowledgments, references, tables, and legends and figures. All pages should be numbered consecutively, starting with the title page as page one.

Original Research manuscripts, in general, should range between 2,500 and 4,000 words, but are typically about 3,500 words, not including references. Tables and illustrations range from 2 to 6 and should be limited to those most pertinent to the study without duplicating findings in the text. The editor reserves the right to publish excessively long tables as online-only material. Failure to comply with length restrictions may result in a delay in processing the paper.

1. Introduction: Clearly state the purpose of the research. Summarize the rationale and background for the study or observation; cite only pertinent references. The "Introduction" should be limited to 1.5 typed manuscript pages.

2. Methods: Provide sufficient detail so that the study can be repeated. Describe new methods in detail; report accepted methods briefly with references. Use subheadings as needed for clarity.

Use of Trade Names: Trade names are to be avoided in defining products whenever possible. If naming a product trade name cannot be avoided, the trade names of other like products should also be mentioned, and first use should be accompanied by the superscript symbol ™ or ®, followed in parentheses by the owner's name. If a product trade name is used, it is imperative that the product be described in sufficient detail so that professionally trained readers can understand the nature of the product.

The mention of critical, especially novel, supplies and pieces of equipment ought to be followed, in parenthesis, by name of manufacturer or provider, and on the first mention only, city, state/province, and country (such as Ross Products, Columbus, OH).

Statistics: Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (e.g., CIs, SDs, or SEs), even for differences that were not significant. Report the number of observations. Specify any general use computer programs used, including the version number and the manufacturer's name and location. Include general descriptions of statistical methods in the "Methods" section and specific descriptions in each table and figure legend. Indicate whether variables were transformed for analysis.

Provide details about what hypotheses were tested, what statistical tests were used, and what the outcome and explanatory variables were (where appropriate). Indicate the level of significance used in tests if different from the conventional 2-sided 5% alpha error and whether or what type of adjustment was made for multiple comparisons. When data are summarized in the "Results" section, specify the statistical methods used to analyze them.

3. Results: Present the results in a logical sequence in the text, tables, and figures. Do not duplicate data from tables or figures in the text; emphasize or summarize only important observations. Do not present data from individual subjects except for very compelling reasons.

4. Discussion: This section should not exceed 4 typewritten pages. Emphasize concisely the novel and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material presented in the "Introduction" or "Results." Compare results to those previously reported. Link conclusions with the goals of the study and avoid unqualified statements and conclusions that are not completely supported by the data. Indicate what new information is contributed by the present study.

5. Practical Application: This section is written in terms that the practicing clinician can understand and the potential clinical application of the research presented in the paper. Keep the description short, about 2 to 3 sentences, and in a language that the readers can easily understand. Do not make unreasonable claims that cannot be derived from the work described in the paper. If this section is not included with the manuscript submission, the author acknowledges that an Associate Editor of the Journal will write the practical application of the research findings.

Reviews: Comprehensive, quantitative reviews of specific renal nutrition topics of clinical relevance, traditional or meta-analysis, are usually invited contributions; however, letters of interest are welcomed. Reviews should address topics with an extensive body of literature to provide a critical summary of the current evidence and applications. In some cases, review articles may also address an emerging topic with limited literature to better demonstrate the need for more research, but if the focus of the article is on a clinical practice issue, this might better be presented as a "Practical Aspects" article.

Reviews should include: (1) an unstructured abstract (150 maximum word count) that clearly states the purpose of the review, the methodology employed, brief findings and conclusion; (2) introduction and purpose; (3) body, which develops the subject in logical order using appropriate headings and subheadings; (4) conclusions that specify the needs for further research; (5) a detailed and comprehensive list of references; and (6) relevant tables and/or figures. Maximum word count for a review article is 4,500 words, not including references, tables/figures and title page.

Clinical/Research Briefs: Clinical/research briefs are submitted in an abbreviated manuscript format that presents clinical practice experience, preliminary research findings (basic or clinical), or professional observations in a shortened report form. Length usually should not exceed six doublespaced pages, not including references, tables and figures. Clinical/Research Briefs should be organized as follows: title page, support and financial disclosure, abstract, introduction, methods, results, discussion, practical application, acknowledgments, references, tables, and legends and figures. All pages should be numbered consecutively, starting with the title page as page one.

Practical Aspects: The Journal welcomes manuscripts about a specific renal nutrition topic of clinical relevance for the provider of nutrition or medical care to patients with kidney disease. Contributions to this section are detailed protocols, forms, or other such materials that are successfully utilized for delivery of nutrition care or medical, nursing or psychological care that has a nutrition component.

Material submitted to the Practical Aspects section should include: (1) a title page; (2) an unstructured abstract (150 maximum word count); (3) an introduction and purpose; (4) a body, which develops the subject in logical order using appropriate headings and subheadings; (5) references and, (6) tables and figures, when appropriate.

Case Studies: This detailed scenario should illustrate a patient care situation that benefited from nutrition intervention. Typically, it should consist of a brief clinical and nutrition history, and a detailed nutrition intervention plan with discussion of recommendations focused on practical application. Appropriate laboratory values, anthropometric measurements, and clinical parameters should be provided.

Patient Education*: This section provides renal dietitians with a convenient, easy-to-read, educational handout for patient distribution. Patient education materials are published online only, although they are referenced in the Table of Contents of the print edition. Submissions for Patient Education should be accompanied by an introduction explaining the rationale for the development of the material and, when appropriate, how it should be used. Text should be clear and concise and illustrations should be contained on two pages with expanded type.

Letters to the Editor: Letters should be restricted to scientific commentary about materials published in JRN or to topics of general interest to professionals working in the field of

renal nutrition science and dietetics. Letters must be double-spaced, should include a title page, should have no more than 10 references, and should not exceed 750 words. All letters will be subjected to editorial review and decision before acceptance. The JRN does not accept letters that are unrelated to a specific, recently published article; that contain extensive unpublished data; or that engage in personal slander or invective.

Supplements: The JRN publishes funded supplements after approval and review by the Editorial Office. Initial inquiries and proposals for supplements should be directed to Jerrilynn D. Burrowes, Editor-in-Chief, Journal of Renal Nutrition at jerrilynn.burrowes@liu.edu.

Proceedings Papers: Manuscript submissions from Proceedings must include a 150 word abstract, a main body of about 2,000 words, one figure and no more than 20 references. (If the figure is not possible, then the submission must have one table. In rare circumstances, up to 2 figures may be considered following discussion with the editors. Pure text without a figure should be avoided. *NOTE: Patient Education and Product Updates are accepted for publication solely on the JRN website. They will be listed in the printed table of contents and indexed in MEDLINE. There are no charges for color figures; however, they will be subject to the same copyright laws as the printed edition.

BEFORE YOU BEGIN

Ethics in publishing

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

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Author contributions For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example

Copyright

This journal offers authors a choice in publishing their research: Open access and Subscription.

For subscription articles: Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' and the copyright will be assigned exclusively to the National Kidney Foundation, Inc., including the right to reproduce the article in all forms and media (for more information on this and copyright, see https://www.elsevier.com/copyright). An e-mail will be sent to the corresponding author

confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

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

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

Retained author rights

As an author you (or your employer or institution) retain certain rights. For more information on author rights for:

Subscription articles please see https://www.elsevier.com/journal-authors/author-rights-and-responsibilities.

Open access articles please see https://www.elsevier.com/OAauthoragreement.

Elsevier supports responsible sharing

Find out how you can share your research published in Elsevier journals.

Role of the funding source

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

Open access

Please visit our Open Access page for more information.

Language (usage and editing services)

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

Submission

The Journal of Renal Nutrition utilizes the Elsevier Editorial System (EES), a web-based manuscript submission and peer review system that provides full electronic capabilities for submission, review, and status updates. Manuscripts must be submitted at http://ees.elsevier.com/yjren. Manuscripts that do not comply with these specific guidelines will be returned to the authors for revision prior to being sent out for review or evaluated by the editors.

The submission process of the journal proceeds online only with step-by-step guidance through creating and uploading your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the peer review process, these source files are needed for further processing after acceptance. After submission, the author can log onto the EES to view the status of the manuscript. For problems or questions concerning submission, contact Sheila Gibbons, JRN Managing Editor at managingeditorjrn@gmail.com or visit the Elsevier Support Center.

Please note that the JRN Editorial Office receives hundreds of manuscripts a year. Each new manuscript receives a unique number, and information on the manuscript is recorded in the EES. The editorial staff releases information on manuscripts to authors only. The EES will e-mail a letter to the corresponding author acknowledging receipt of a manuscript, whether new or a resubmission.

All accepted manuscripts are subject to editorial revision and shortening. Authors should avoid redundancy between sections of text and between illustrations and text. Due to page limitations, the Editors may decide that tables, illustrations, appendices, acknowledgments, and other material should be published online and referenced in the print edition.

Peer Review Process

This journal operates a double blind review process, which means the identities of the authors are concealed from the reviewers, and vice versa. All contributions will be initially assessed by an Editor who has expertise in the subject area for suitability for the journal. Papers deemed suitable are then sent to a minimum of two independent expert

reviewers to assess the scientific quality of the paper. The reviewers evaluate each article on the basis of content, originality, scientific accuracy, clarity, and contribution to the field of renal nutrition dietetics and science. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review is available here.

Decisions

The single most important criterion for acceptance is the originality of the work. However, a decision to accept a manuscript is not based solely on the scientific validity of its content. Other factors affecting decisions include the extent and importance of new information in the paper compared with that in other papers being considered, the Journal's need to represent a wide range of topics, and the overall suitability for JRN. Decision letters usually, but not always, convey all factors considered for a particular decision. Occasionally, the comments to the authors may appear to be inconsistent with the editorial decision, which takes into consideration reviewers' comments to the editor, etc.

Revisions and Rejections

Manuscript revisions should be resubmitted within three months of the initial decision; they are carefully re-examined by the editorial team and/or reviewers. However, no guarantee can be made about the final acceptability of the paper. If authors of a rejected manuscript are able to make new advances that go far beyond the original submission, they may consider submitting the manuscript again as a new submission, referring to the original submission in the cover letter.

PREPARATION

Manuscript Format

Manuscripts must adhere to standard layout and length guidelines. Manuscripts must be submitted in Microsoft Word, double-spaced using 12 pt. font (preferably Times New Roman) and unjustified margins. Pages must be numbered starting with the title page. Include a cover letter to the Editor with the submission stating the main aspects of the research findings of the article. Identify the name of the corresponding author. The JRN encourages authors to provide the names, fields of interest, addresses, telephone and fax numbers, and e-mail addresses of 3 to 4 unbiased and qualified potential expert reviewers from outside the authors' institutions.

Do not use the footnote function for references or the comments function. DO NOT UPLOAD MANUSCRIPT TEXT FILES IN PDF FORMAT. Also, manuscripts must not be submitted with track changes.

Use of the present tense is preferable. Refer to patients by number. Actual names or initials should not be used in the text, tables, or illustrations. All clinical laboratory values should be expressed in U.S. conventional units. If necessary, the International System of Units (SI units) can be provided in parentheses immediately after the U.S. convention units. Conversion tables are available at JAMA 1986; 255(17): 2329-2339 or Ann Intern Med 1987;106(1):114-129.

International authors are advised to have their manuscripts reviewed by a scientific colleague who is fluent in English so that the manuscripts will conform to U.S. English usage and grammar.

Title Page

The title page should include: (1) the title of the manuscript; (2) the name and degree(s) of each author(s); (3) the current title(s) and affiliation(s) of the author(s); (4) the corresponding author's name, address, telephone and fax numbers, and email address; (5) word counts for the abstract and the body of the manuscript (excluding, references, tables and figures); (6) a short title (45 characters or fewer, including spaces) to be used as a running head; and (7) acknowledgements if authors wish to express thanks or acknowledge assistance. All individuals who contributed to the writing of the manuscript but who do not qualify as authors must be named in this section. Authors are responsible for informing all listed individuals/parties that they are being mentioned in the manuscript and for obtaining their approval prior to publication.

Support and Financial Disclosure Declaration

The title page should acknowledge research support (from funding agencies or industry) and disclose any potential financial conflicts of interest for each author. Any meaningful affiliation or involvement, either direct or indirect, with any organization or entity with a direct financial interest in the subject matter or materials discussed (e.g., employment, consultancies, grants, honoraria) must be disclosed. Specifics of the disclosure will remain confidential. The Editors may request that authors of a study sponsored by a funder with a proprietary or financial interest in the outcome sign a statement, such as “I had full access to all of the data in this study and I take complete responsibility for the integrity of the data and the accuracy of the data analysis.” If deemed appropriate by the Editor, a general statement regarding disclosure will be included in the Acknowledgment section of the manuscript. The Acknowledgment section must also reveal all sources of support for the work, both financial and material. If no financial conflict of interest is identified, ‘none’ should be written next to the author's name. Note: If the manuscript is

accepted for publication, a summary of the relevant information will be transferred to the “Support” and “Financial Disclosure” sections of the Acknowledgements.

Authorship

All individuals listed as authors must fulfill the following criteria for authorship. Each author must have participated sufficiently in the work to take public responsibility for the content of the paper and must approve of the final version of the manuscript. Authorship should be based on substantive contributions to each of the following: (1) conception and design of the study; (2) generation, collection, assembly, analysis and/or interpretation of data; (3) drafting and/or revision of the manuscript; and (4) approval of the final version of the manuscript. The author who is named as the corresponding author on the manuscript's title page must be the individual to whom all Editorialrelated correspondence is directed.

Abstract

The abstract page should include the title of the manuscript, but no other identifying information. A structured abstract format should be used for "Original Research" and "Clinical/Research Brief " using the following headings: (1) Objective: clear statement of purpose of study; (2) (Design and) Methods: research design including methods and procedures used for the primary outcome and up to one secondary outcome; (3) Results: primary findings of the study; and (4) Conclusion: brief summary of the results directly supported by evidence. Complete sentences should be used throughout the abstract. The abstract limit is 300 words or fewer. A list of approximately five keywords (to be used for indexing) should appear at the end of the abstract.

Abbreviations and Acronyms

Complex terms used frequently in the manuscript may be abbreviated. Abbreviations should be placed in parentheses at first use in the abstract and again at first use in the text. Do not use abbreviations and acronyms in titles.

Formatting of funding sources

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

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

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

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

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

Figures and Tables

Figures

Illustrations include charts, drawings, graphs, and photographs. Charts, drawings and graphs must be computer generated. Each figure should be on a separate page. Number figures consecutively in the order they are to appear in the text (e.g., Figure 1, Figure 2,

Figure 3, etc.). Figures should be uploaded as separate files, not embedded in the manuscript file.

For most types of figures, TIFF or EPS files are the preferred format. The resolution for line art must be at least 1200 dpi; photographs and shaded drawings must have a finished resolution of 300 dpi. The following image file formats are not acceptable because they are low resolution: PowerPoint, JPG, GIF,ONG, PCX, PNG, XBM, and Excel. On a separate page, list the legend for each figure, doublespaced, with abbreviations and symbols identified.

Authors must bear all costs associated with printed color figures. The cost of printing color figures is $600.00, whether for one figure or several. If authors elect not to print figures in color, in most cases, authors will be able to have their color figures produced in black and white for the print version of JRN, but the figures will appear in color online.

Tables

Type each table on a separate page. Do not insert vertical lines in tables. Include a title for each table. Number tables consecutively within the manuscript (Table 1, Table 2, Table 3, etc.). Use standard citation superscript symbols (*, †, ‡, §...). Authors should place footnotes in order, reading from left to right and top to bottom, and should begin a new series of footnotes for each table. Footnotes should not appear in table titles. Tables should be uploaded as separate files, not embedded in the manuscript file.

If any table or figure has been published previously, a copy of the letter of permission from the copyright holder must accompany the manuscript. The original source of the table or figure should be acknowledged in full reference form in the reference section of the manuscript. The figure legend (or table notes) should conclude with: "Reprinted with

permission" followed by the appropriate reference number. Authors are responsible for applying for permission for both print and electronic rights for all borrowed materials, and they are responsible for paying any fees related to the application of these permissions.

References

References must be numbered according to order of appearance in the text using superscript numbers in the text. References should be compiled at the end of the manuscript according to the order of citation in the text and should follow the American Medical Association (AMA) style and format.

Authors using reference-managing software such as EndNote or Reference Manager should select NLM/PubMed output style.

References should be typed, double-spaced starting on a separate page at the end of the manuscript. When listing references, abbreviate names of journals as listed in PubMed. List all authors and/or editors up to 6; if more than 6, list the first 3 followed by "et al." Journal references should include the issue number in parentheses after the volume number. Accuracy of reference information is the author's responsibility. Please indicate if the source is a complete article, abstract, or editorial; give inclusive page numbers for complete articles. All reference information must be complete when the manuscript is submitted.

Data References

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data

repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Examples of References

Journal Article: Six or Fewer Authors

Eyre S, Attman P, Haraldsson B. Positive effects of protein restriction in patients with chronic kidney disease. J Ren Nutr. 2008;18(3):269-280.

Journal Article: More Than Six Authors

Fernandez-Reyes MJ, Sanchez R, Garcýa L, et al. Acute responses of gastrointestinal hormones to both oral and parenteral intradialytic nutrition. Am J Nephrol. 2010;32(3):272-278.

Journal Article in Press

Steiber AL, Kopple JD. Vitamin status and needs for people with stage 3-5 chronic kidney disease. J Ren Nutr. (in press)

Complete Book

Byham-Gray LD, Burrowes JD, Chertow GM, eds. Nutrition in Kidney Disease. Totowa, NJ: Humana Press; 2008.

Chapter in Book

Wilkens KG, Juneja V. Medical nutrition therapy for renal disorders. In: Mahan LK, Escott-Stump S, eds. Krause's Food & Nutrition Therapy. 12th ed. St. Louis, MO: Saunders; 2008:921-958.

Journal Article in a Supplement

Gullett NP, Hebbar G, Ziegler TR. Update on clinical trials of growth factors and anabolic steroids in cachexia and wasting. Am J Clin Nutr. 2010;91(4)(suppl):S1143-S1147.

PubMed Abstract Citation

Szklarek-Kubicka M, Fijalkowska-Morawska J, Zaremba- Drobnik D, Ucinski A, Czekalski S, Nowicki M. Effect of intradialytic intravenous administration of omega-3 fatty acids on nutritional status and inflammatory response in hemodialysis patients: a pilot study [abstract]. J Ren Nutr. 2009;19(6):487-493. http://www.ncbi.nlm.nih.gov/pubmed/19616450. Accessed December 24, 2010. PMID: 19616450.

Editorial

McCarron DA, Drueke TB, Stricker EM. Science trumps politics: urinary sodium data challenge US dietary sodium guideline [editorial]. Am J Clin Nutr. 2010;92(5):1005-1006.

EPub Ahead of Print

Kagoma YK, Weir A, Iansavichus AV, et al. Impact of estimated GFR reporting on patients, clinicians, and health-care systems: a systematic review [published online ahead

of print December 9 2010]. Am J Kidney Dis . 2010. http://www.ncbi.nlm.nih.gov/pubmed/ 21146269. Accessed December 24, 2010.

Data Reference

[dataset] Oguro, M, Imahiro, S, Saito, S, Nakashizuka, T. Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1; 2015. http://dx.doi.org/10.17632/xwj98nb39r.1.

Supplementary Material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. This material should be important to the understanding and interpretation of the paper and should not repeat material in the article. Supplementary material should be original and not previously published. It will under undergo editorial and peer review with the main manuscript. If the manuscript is accepted for publication and if the editors deem the online only material appropriate for publication, it will be posted online at the time of publication of the article as additional material provided by the authors.

Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. Each supplementary item should be cited in the text of the main manuscript (e.g., see Table S1). If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data

This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to Mendeley Data. The datasets will be listed and directly accessible to readers next to your published article online.

For more information, visit the Mendeley Data for journals page.

Data statement

To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

AFTER ACCEPTANCE

Accepted manuscripts are sent to the publisher, Elsevier. Once an article has been copyedited, typeset, and authors have reviewed the proof and submitted corrections, the author-corrected proof will be uploaded to the JRN websites (http://www.jrnjournal.org

and http://www.sciencedirect.com) under "Articles in Press" for pre-print viewing by subscribers. The corrected proof of the article is also delivered to PubMed for indexing. When the article is assigned to a specific issue of JRN, it moves from the Articles in Press section to the table of contents of that issue.

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. Example of a correctly given DOI (in URL format; here an article in the journal Physics Letters): http://dx.doi.org/10.1016/j.physletb.2010.09.059. When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

Proofs

The corresponding author can expect one set of page proofs in PDF format via e-mail approximately two months after acceptance. The PDF proofs can be annotated using Adobe Reader. Instructions on how to annotate PDF files will accompany the proofs. If the author(s) do not wish to use the PDF annotations function, the corrections can be listed (including replies to the Query Form) and returned to Elsevier via email. Please list corrections quoting line number. If, for any reason, this is not possible, the corrections and any other comments can be sent (including replies to the Query Form) on a printout of the proof and returned by fax, scan or e-mail.

Please only check the typesetting, editing, completeness and correctness of the text, tables and illustrations. Significant changes to the article after it has been accepted for publication will only be considered at this stage with permission from the Editor.We will do everything possible to get your article published quickly and accurately. Therefore, it is important to ensure that all corrections are returned to Elsevier in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely the authors' responsibility. Note: With permission from the Editor, Elsevier may proceed with the publication of the article if the proofs are not returned.

Authors are expected to review proofs promptly (within 48 hours of receipt).

Copyediting

Manuscripts are copyedited to make them consistent with the Journal style; if a particular section in the manuscript is not clear or requires additional information, the copy editor will direct questions to the author. These questions, or "author queries," will appear in the margins of the proofs that are sent to the author. Changes made by the copy editors for style, grammar, and readability should not be altered by the author(s) unless a scientific error has been introduced.

Offprints

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

and co-authors may order offprints at any time via Elsevier's Author Services. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.

You can also check the status of your submitted article or find out when your accepted article will be published.