

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

**ASSOCIAÇÃO DAS CONCENTRAÇÕES SÉRICAS DE TESTOSTERONA
COM O CONSUMO DE COLESTEROL E COM A FORÇA MUSCULAR DE
HOMENS**

GEDERSON KARDEC GOMES

UBERLÂNDIA

2022

GEDERSON KARDEC GOMES

**ASSOCIAÇÃO DAS CONCENTRAÇÕES SÉRICAS DE TESTOSTERONA
COM O CONSUMO DE COLESTEROL E COM A FORÇA MUSCULAR DE
HOMENS**

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

Área de concentração: Ciências da Saúde.

Orientador: Prof. Dr. Erick Prado de Oliveira

Coorientadora: Prof^a. Dra. Flávia Moure Simões de Branco

UBERLÂNDIA

2022

Ficha Catalográfica Online do Sistema de Bibliotecas da UFU
com dados informados pelo(a) próprio(a) autor(a).

G633
2022 Gomes, Gederson Kardec, 1979-
ASSOCIAÇÃO DAS CONCENTRAÇÕES SÉRICAS DE TESTOSTERONA
COM O CONSUMO DE COLESTEROL E COM A FORÇA MUSCULAR DE
HOMENS [recurso eletrônico] / Gederson Kardec Gomes. -
2022.

Orientador: Erick Prado de Oliveira.

Coorientadora: Flávia Moure Simões de Branco.

Tese (Doutorado) - Universidade Federal de Uberlândia,
Pós-graduação em Ciências da Saúde.

Modo de acesso: Internet.

Disponível em: <http://doi.org/10.14393/ufu.te.2023.10>

Inclui bibliografia.

1. Ciências médicas. I. Oliveira, Erick Prado de, 1983-
, (Orient.). II. Branco, Flávia Moure Simões de, 1991-
, (Coorient.). III. Universidade Federal de Uberlândia.
Pós-graduação em Ciências da Saúde. IV. Título.

CDU: 61

Bibliotecários responsáveis pela estrutura de acordo com o AACR2:
Gizele Cristine Nunes do Couto - CRB6/2091
Nelson Marcos Ferreira - CRB6/3074



ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Ciências da Saúde				
Defesa de:	Tese de Doutorado Nº 25/PPCSA				
Data:	20.12.2022	Hora de início:	08:00h	Hora de encerramento:	12:00h
Matrícula do Discente:	11913CSD006				
Nome do Discente:	Gederson Kardec Gomes				
Título do Trabalho:	Associação das concentrações séricas de testosterona com o consumo de colesterol e com a força muscular de homens				
Área de concentração:	Ciências da Saúde				
Linha de pesquisa:	2 - DIAGNÓSTICO, TRATAMENTO E PROGNÓSTICO DAS DOENÇAS E AGRAVOS À SAÚDE				
Projeto de Pesquisa de vinculação:	NUTRIÇÃO E EXERCÍCIO FÍSICO APLICADOS À SAÚDE E DESEMPENHO HUMANO				

Reuniu-se em web conferência pela plataforma Zoom, em conformidade com a PORTARIA Nº 36, DE 19 DE MARÇO DE 2020 da COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR - CAPES, pela Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Ciências da Saúde, assim composta: Profs. Drs. Paula Cândido Nahas (UNA), Paulo Ricardo Prado Nunes (UEMG), Geórgia das Graças Pena (UFU), Guilherme Moraes Puga (UFU) e Erick Prado de Oliveira (UFU), orientador do candidato.

Iniciando os trabalhos, o presidente da mesa, Prof. Dr. Erick Prado de Oliveira, apresentou a Comissão Examinadora e o candidato, agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

Aprovado.

Esta defesa faz parte dos requisitos necessários à obtenção do título de Doutor.

O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.



Superior, em 20/12/2022, às 11:55, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Paula Cândido Nahas, Usuário Externo**, em 20/12/2022, às 11:55, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Geórgia das Graças Pena, Professor(a) do Magistério Superior**, em 20/12/2022, às 11:55, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Paulo Ricardo Prado Nunes, Usuário Externo**, em 20/12/2022, às 11:55, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Guilherme Moraes Puga, Professor(a) do Magistério Superior**, em 20/12/2022, às 11:59, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



A autenticidade deste documento pode ser conferida no site

[https://www.sei.ufu.br/sei/controlador_externo.php?](https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0)

[acao=documento_conferir&id_orgao_acesso_externo=0](https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0), informando o código verificador **4135161** e o código CRC **E235D8B2**.

DEDICATÓRIA

Dedico este trabalho primeiramente a Deus, por ter me honrado e me capacitado para chegar até aqui. À minha querida avó, Ana Rosa, com toda sua simplicidade me mostrou que o melhor caminho seria a busca pelo conhecimento e uma formação acadêmica. Aos meus pais, Elizabeth Ramos Gomes e Alancardec Gomes (in memoriam), que, além de me presentear com a vida, souberam de forma simples me mostrar o verdadeiro significado e importância de ser uma pessoa humilde. À minha amada esposa Lilian Roberta, pela compreensão quanto à minha ausência em alguns momentos, pelos conselhos, companheirismo e amor que sempre foi e será dedicado. À minha irmã Geisa Lúcia que, mesmo distante, estava sempre na torcida para que tudo desse certo em minha jornada.

AGRADECIMENTOS

À Deus, por sempre me direcionar e principalmente durante essa caminhada.

Ao meu orientador Prof^o Dr. Erick Prado de Oliveira, pela maestria na condução e orientação para que fosse possível vencer todos os desafios. Obrigado pela disponibilidade, atenção, dedicação e por todos os ensinamentos durante todo esse tempo!

À minha coorientadora Prof^a Dra. Flávia Moure Simões de Branco, pela contribuição e toda a paciência demonstrada durante esse período.

Ao Grupo de pesquisa LaNES, por ter contribuído em vários aspectos durante toda a caminhada.

Ao Me. Heitor Santos, pela parceria durante essa trajetória.

À minha querida sogra Dalva Mendes, por sempre acreditar em mim e por todas as orações depositadas para que eu concluísse com vitória mais essa etapa em minha vida.

À minha amada esposa, Lilian Roberta Gomes, pela paciência e incentivo durante essa etapa.

Ao Programa de Pós-graduação em Ciências da Saúde da Faculdade de Medicina da UFU, pela oportunidade de realização do doutorado.

A todos aqueles que contribuíram de alguma forma para o início e término desse trabalho. Muitíssimo Obrigado!

*“Que diremos, pois, diante dessas coisas?
Se Deus é por nós, quem será contra nós?”
(Romanos 8:31)*

RESUMO

Introdução: A testosterona é um hormônio esteroide androgênico anabólico sintetizado a partir do colesterol, sendo considerado um hormônio importante para a estrutura e função do músculo esquelético. No entanto, pouco se sabe sobre as relações entre a ingestão de colesterol com as concentrações séricas de testosterona e a relação das concentrações séricas de testosterona com a força muscular de homens. **Objetivo:** Esta tese possui dois objetivos principais: 1) avaliar se a ingestão de colesterol e os níveis de colesterol total sérico estão associados com as concentrações séricas de testosterona total em homens; 2) investigar se as concentrações séricas de testosterona total estão associadas com a força de preensão manual em homens. **Metodologia:** Os estudos são transversais e os participantes são homens de 20 a 80 anos participantes do *National Health and Nutrition Examination Survey (NHANES)*. No estudo 1, foi utilizado o biênio de 2013-2014 (n=1996). Foi avaliado a associação entre o consumo de colesterol (por meio de dois recordatórios alimentares de 24 horas) e os níveis sérios de colesterol total com as concentrações séricas de testosterona total. Análise de regressão linear foi realizada para observar se as concentrações séricas de testosterona total estão associadas com a ingestão de colesterol e níveis séricos de colesterol total. No estudo 2, foi utilizado o biênio de 2011-2012 (n=2049), sendo avaliada a associação entre as concentrações séricas de testosterona total e a soma da força de preensão manual (soma da força da mão direita e mão esquerda). Análise de regressão linear foi realizada para observar se os níveis séricos de testosterona total se associam com a soma da força de preensão manual, em homens de 20 a 80 anos e separados por idade, homens jovens (20 a 39 anos); homens de meia-idade (40 a 59 anos) e homens idosos (60 a 80 anos). **Resultados:** No estudo 1, a ingestão de colesterol e os níveis séricos de colesterol total não foram associados com as concentrações séricas de testosterona total nas análises sem ajustes e ajustadas (ajuste para ingestão de energia, gordura total e álcool, tabagismo, idade, atividade física, renda familiar, estado civil, raça, escolaridade, diabetes, hipertensão e índice de massa corporal). No estudo 2, observamos associações positivas ($p<0.001$) entre as concentrações séricas de testosterona total e a soma da força de preensão manual no modelo ajustado (ajuste para ingestão de energia e álcool, tabagismo, idade, atividade física, renda familiar, estado civil, raça, escolaridade, diabetes, hipertensão, artrite e índice de massa corporal). Quando separamos os participantes por idade, encontramos associações positivas entre as concentrações séricas de testosterona total e a soma da força

de preensão manual no modelo ajustado em homens jovens ($p < 0.001$) e meia-idade ($p = 0.036$). **Conclusão:** Concluímos que o consumo de colesterol e os níveis de colesterol sérico não estão associados com as concentrações séricas de testosterona total. As concentrações séricas de testosterona total apresentam associações positivas em homens jovens e de meia-idade, mas não em homens idosos.

Palavras-chave: Andrógenos; Colesterol; Hipogonadismo; Lipídios; Hormônios sexuais; Testosterona; Força de Preensão Manual.

ABSTRACT

Background: Testosterone is an anabolic androgenic steroid hormone synthesized from cholesterol, it is considered an important hormone for the structure and function of skeletal muscle. However, little is known about the relationship between cholesterol intake and serum testosterone concentrations, and the relationship between serum testosterone concentrations and muscle strength in men. **Aim:** 1) to assess whether cholesterol intake and serum total cholesterol levels are associated with serum total testosterone concentrations in men; 2) to investigate whether serum concentrations of total testosterone are associated with handgrip strength in men. **Methods:** The studies are cross-sectional, and the participants are men aged 20 to 80 years from the National Health and Nutrition Examination Survey (NHANES). In study 1, the 2013-2014 biennium (n=1996) was used. We evaluated the association between cholesterol intake (through two 24-hour dietary recalls) and serum total cholesterol levels with serum total testosterone concentrations. Linear regression analysis was performed to see whether serum total testosterone concentrations are associated with cholesterol intake and serum total cholesterol levels. In study 2, the 2011-2012 biennium (n=2049) was used, and the association between serum concentrations of total testosterone and the sum of handgrip strength (sum of right-hand and left-hand strength) was evaluated. Linear regression analysis was performed to observe whether serum levels of total testosterone are associated with the sum of handgrip strength, in men aged 20 to 80 years and separated by age, young man (20 to 39 years); middle-age (40 to 59 years) and older man (60 to 80 years). **Results:** In study 1, cholesterol intake and serum total cholesterol levels were not associated with serum total testosterone concentrations in unadjusted and adjusted analyzes (adjusting for energy intake, total fat and alcohol, smoking, age, activity physical activity, family income, marital status, race, education, diabetes, hypertension and body mass index). In study 2, we observed positive associations ($p < 0.001$) between serum total testosterone concentrations and the sum of handgrip strength in the adjusted model (adjusting for energy and alcohol intake, smoking, age, physical activity, family income, marital status, race, education, diabetes, hypertension, arthritis and body mass index). When we separated the participants by age-range, we found positive associations between serum total testosterone concentrations and the sum of handgrip strength in the adjusted model in individuals between 20 and 39 years old ($p < 0.001$) and between 40 and 59 years old ($p = 0.036$). **Conclusion:** We conclude that cholesterol intake and serum cholesterol

levels are not associated with serum total testosterone concentrations. Serum total testosterone concentrations show positive associations in young and middle-aged men, but not in older men.

Keywords: Androgens; Cholesterol; Hypogonadism; Lipids; Sex hormones; Testosterone; Handgrip Strength.

LISTA DE ILUSTRAÇÕES

ARTIGO 1: CHOLESTEROL INTAKE AND SERUM TOTAL CHOLESTEROL LEVELS ARE NOT ASSOCIATED WITH TOTAL TESTOSTERONE LEVELS IN US MEN: A CROSS-SECTIONAL STUDY FROM NHANES 2013-2014.

Figure 1. Flowchart of the sample selection from NHANES 2013-2014.....46

Figure 2. Linear regression between quintiles of dietary cholesterol and total testosterone levels. NHANES, 2013-2014.....47

Figure 3. Linear regression between quintiles of blood cholesterol and total testosterone levels. NHANES, 2013-2014.....48

ARTIGO 2: GRIP STRENGTH IS ASSOCIATED WITH SERUM TESTOSTERONE LEVELS IN YOUNG AND MIDDLE-AGED MEN, BUT NOT IN OLDER MEN: NHANES 2011-2012.

Figure 1. Flowchart of the sample selection from NHANES 2011 e 2012.....63

LISTA DE TABELAS

ARTIGO 1: CHOLESTEROL INTAKE AND SERUM TOTAL CHOLESTEROL LEVELS ARE NOT ASSOCIATED WITH TOTAL TESTOSTERONE LEVELS IN US MEN: A CROSS-SECTIONAL STUDY FROM NHANES 2013-2014.

Table 1. Sociodemographic, health conditions and habits, physical activity, anthropometric and body composition and dietary intake of adults men by quintiles of dietary cholesterol (NHANES, 2013-2014).....42

Table 2. Linear regression of continuous values of dietary and blood cholesterol with total testosterone levels in men aged 20-80 y. NHANES, 2013-2014.....44

Supplementary Table 1. Linear regression between quintiles of cholesterol intake and serum total cholesterol levels according to total testosterone levels in men aged 20-80 y from the NHANES, 2013-2014.....45

ARTIGO 2: GRIP STRENGTH IS ASSOCIATED WITH SERUM TESTOSTERONE LEVELS IN YOUNG AND MIDDLE-AGED MEN, BUT NOT IN OLDER MEN: NHANES 2011 – 2012.

Table 1. Sociodemographic, health conditions and habits, physical activity, anthropometric and dietary intake of adult's men by tertiles of total testosterone concentration. NHANES, 2011 e 2012.....64

Table 2. Sociodemographic, health conditions and habits, physical activity, anthropometric and dietary intake of adults aged 20-80 years divided by age-range. NHANES, 2011-2012.....65

Table 3. Linear regression between total testosterone concentration (ng.dL) and grip strength in men aged 20-80 years and divided by age-range. NHANES, 2011-2012.....67

LISTA DE ABREVIATURAS E SIGLAS

FUNDAMENTAÇÃO TEÓRICA

1RM	Uma Repetição Máxima
CDC	Centros de Controle e Prevenção de Doenças
dL	Decilitro
EUA	Estados Unidos da América
FPM	Força De Prensão Manual
GnRH	Hormônio Liberador de Gonadotrofina
HMG-CoA	3-hidroxi-3-metil-CoA reductase
LH	Hormônio Luteinizante
mg	Miligrama
NCHS	Centro Nacional de Estatísticas de Saúde
ng	Nanograma por decilitro
NHANES	<i>National Health and Nutrition Examination Survey</i>
T	Testosterona
TT	Testosterona total

ARTIGOS

ALA	<i>Alpha Linolenic Acid</i>
BMI	<i>Body Mass Index</i>
CI	<i>Confidence Interval</i>
CNPQ	<i>The Brazilian National Council for Scientific and Technological Development</i>
DHA	<i>Docosahexaenoic Acid</i>
dL	<i>Deciliter</i>
EPA	<i>Eicosapentaenoic Acid</i>
FNDDS	<i>Nutrient Database for Dietary Studies</i>
g	<i>Gram</i>
HGS	<i>Handgrip Strength</i>
Kcal	<i>Calorie</i>
kg	<i>kilogram</i>

m ²	<i>Square meter</i>
mcg	<i>microgram</i>
mg	<i>Milligram</i>
NCI	<i>National Cancer Institute</i>
ng	<i>Nanogram</i>
NHANES	<i>National Health and Nutrition Examination Survey</i>
Q	<i>Quintile</i>
T	<i>Testosterone</i>
T.K.K 5401	<i>Takei Digital Dynamometer</i>
TC	<i>Serum total cholesterol</i>
TT	<i>Total Testosterone</i>
US	<i>United States</i>
y	<i>Years</i>

SUMÁRIO

1 INTRODUÇÃO	15
1.1 Considerações iniciais	17
2 FUNDAMENTAÇÃO TEÓRICA.....	18
2.1 Colesterol	18
2.1.1 Definição e biossíntese.....	18
2.1.2 Recomendações e efeitos do consumo de colesterol.....	19
2.2 Testosterona	19
2.2.1 Definição e biossíntese.....	19
2.2.2 Ação fisiológica da testosterona	20
2.3 Colesterol e testosterona.....	21
2.4 Força muscular	22
2.5 Testosterona e força muscular	23
2.6 National Health and Nutrition Examination Survey.....	24
3 OBJETIVOS	26
3.1 Objetivo geral.....	26
3.2 Objetivos Específicos.....	26
4 RESULTADOS	27
Artigo 1.	27
Artigo 2.	49
5 CONCLUSÕES	68
6 PERSPECTIVAS FUTURAS	69
REFERÊNCIAS	70

1 INTRODUÇÃO

O colesterol é biossintetizado por todas as células de mamíferos, está localizado predominantemente nas membranas celulares (LUO *et al.*, 2019) e também é um precursor dos hormônios esteroides (JOHNSON; WOOD, 2001). Dado que a testosterona (T) é um hormônio esteroide derivado do colesterol, uma avaliação crítica dessa relação é de grande interesse. Alguns estudos mostraram que abordagens nutricionais que aumentam a ingestão de colesterol, aumentam os níveis de testosterona total (TT) em homens jovens treinados (treinamento de força) (BAGHERI *et al.*, 2021; WILSON *et al.*, 2020). No entanto, os estudos supracitados possuem tamanho amostral pequeno ($n \leq 30$) e não fornecem evidências para estudos de base populacional com diferentes faixas etárias.

Os níveis séricos de TT em homens são considerados normais quando seus valores se apresentam entre ~215 a 915 ng.dL (TRAVISON *et al.*, 2017). Homens com as concentrações séricas de TT abaixo desses valores, são considerados hipogonadais. Nesse sentido, o hipogonadismo está associado com efeitos deletérios para a saúde em geral, como osteopenia e sarcopenia (BORST; YARROW, 2015), obesidade (KELLY; JONES, 2015), diabetes *mellitus* tipo 2, doenças cardiovasculares, síndrome metabólica (ZAROTSKY *et al.*, 2014) e maiores taxas de mortalidade por todas as causas (MURALEEDHARAN *et al.*, 2013). A sarcopenia é definida como a perda da força, qualidade e massa muscular (WIEDMER *et al.*, 2021). Estudos têm mostrado que essa condição está associada a limitações funcionais, sendo fator de risco para quedas, fragilidades, incapacidade e até mesmo a morte (ANAND; GAJRA, 2018). Nesse sentido, a diminuição das concentrações dos níveis séricos de TT é vista como uma das razões para a sarcopenia (PRIEGO *et al.*, 2021; SHIN; JEON; KIM, 2018). Atualmente, o nível da força muscular é considerado o principal fator para o diagnóstico da sarcopenia. Assim, para mensurar os níveis de força muscular, um dos testes sugerido é a força de preensão manual (FPM) (CRUZ-JENTOFT *et al.*, 2019). Portanto, a FPM é considerada um preditor de doenças crônicas e mortalidade precoce e é utilizada como um marcador do estado nutricional, atividades de vida diária e incapacidade subsequente (LEONG *et al.*, 2015; PETERSON *et al.*, 2016).

Isso posto, elucidar fatores que podem modular as concentrações séricas de TT, como estratégias nutricionais, pode ser importante para novas condutas clínicas. Nesse sentido, são necessários estudos que avaliem a relação entre a ingestão de colesterol e as concentrações séricas de TT em uma amostra representativa. Além disso, até onde

sabemos, não existem estudos epidemiológicos que investigaram associações entre as concentrações séricas de TT com a força muscular de homens em diferentes faixas etárias.

Portanto, os objetivos do presente estudo foram investigar a relação das concentrações séricas de TT com o consumo de colesterol e com os níveis séricos de colesterol total, e, investigar se existem associações entre as concentrações de TT com a FPM em homens com diferentes faixas etárias. Nossa hipótese é que as concentrações séricas de TT não se associarão com a ingestão de colesterol e com os níveis séricos de colesterol total; no entanto, as concentrações séricas de TT serão associadas com FPM em todas as faixas etárias.

1.1 Considerações iniciais

Esta tese foi produzida de acordo com as normas instituídas pelo Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Medicina da Universidade Federal de Uberlândia. A formatação segue o modelo alternativo de apresentação, sendo que os resultados do trabalho devem ser apresentados em formato de 02 (dois) artigos científicos.

Inicialmente, a tese foi organizada nos seguintes tópicos: **Fundamentação Teórica**, composta por uma revisão da literatura sobre as temáticas retratada na tese; **Objetivos**, onde são apresentados os propósitos do estudo; **Resultados**, que apresentam os dois artigos científicos desenvolvidos; **Conclusão**, que apresenta a síntese dos principais resultados do trabalho; e, **Perspectivas futuras**, onde são apresentadas possibilidades para novos estudos.

O primeiro artigo, intitulado **“Cholesterol intake and serum total cholesterol levels are not associated with total testosterone levels in US men: A cross-sectional study from NHANES 2013-2014”**, teve como objetivo investigar se as concentrações séricas de TT se associam com a ingestão de colesterol e como os níveis séricos de colesterol de homens entre 20 e 80 anos participantes do NHANES. Este artigo foi submetido à revista *Hormones International Journal of Endocrinology and Metabolism* (*Impact Factor* = 3.419) em 20 de novembro de 2022.

O segundo artigo, intitulado **“Grip strength is associated with serum testosterone levels in young and middle-aged men, but not in older men: NHANES 2011 – 2012”** teve como objetivo investigar se as concentrações séricas de TT se associam com a FPM de homens em diferentes faixas etárias participantes do NHANES e pretendemos submetê-lo à revista *Scientific Reports* (*Impact Factor* = 5.516).

2 FUNDAMENTAÇÃO TEÓRICA

2.1 Colesterol

2.1.1 Definição e biossíntese

O colesterol é um tipo de lipídio encontrado nas células do organismo humano, sendo que aproximadamente 25% dos lipídios de membranas em determinadas células nervosas são constituídos pelo colesterol. Ele modula a fluidez das membranas celulares e constitui o precursor dos hormônios esteroides (testosterona, estrogênio, aldosterona), dos ácidos biliares e da vitamina D (DIETSCHY; WILSON, 1970). Aproximadamente 75% do colesterol do organismo é de origem endógena (biossíntese) sintetizada principalmente no fígado, ao passo que aproximadamente 25% são de origem exógena (dieta) (MILES, 1989), podendo ser encontrado em alimentos de origem animal, como carnes, ovos, laticínios, aves e frutos do mar (DESALVO; OLSON; CASAVALE, 2016). O colesterol é extremamente importante para as funções fisiológicas do organismo. Visto que todas as células sintetizam colesterol a partir da Acetil-Coenzima A (precursora dos átomos de carbono do colesterol), consequentemente, não é necessário a ingestão de colesterol via dieta (LECERF; DE LORGERIL, 2011).

A biossíntese do colesterol é complexa e sua regulação se dá pela concentração intracelular de colesterol (KANE; HARDMAN; PAULUS, 1980). Nesse sentido, o colesterol é sintetizado a partir da Acetil-Coenzima A, ao passo que as enzimas que catalisam sua síntese se encontram no citosol e no retículo endoplasmático. A biossíntese de colesterol pode ser dividida em três estágios. Primeiro, no citoplasma ocorre a síntetização do isopentenil pirofosfato, unidade de isopreno ativada, constituinte da construção básica do colesterol. Segundo, no retículo endoplasmático, ocorre a condensação de seis moléculas de isopentenil pirofosfato para formar o equaleno. Terceiro, ciclização do esqualeno, e o produto tetracíclico é por fim convertido em colesterol no retículo endoplasmático (BERG; TYMOCZKO; STRYER, 2014).

A regulação da biossíntese do colesterol é regulada por diversos mecanismos logo no início da sua síntese, operado pela 3-hidroxi-3-metil-CoA reductase (HMG-CoA). Sendo assim, em uma alimentação rica em colesterol ocorre um *feedback* negativo na síntese endógena. Em contrapartida, uma redução substancial na ingestão de colesterol por via dietética pode aumentar a síntese endógena (HARPER, 1993).

2.1.2 Recomendações e efeitos do consumo de colesterol

Foi recomendado pelas Diretrizes Dietéticas para americanos de 2010, não ultrapassar o consumo de colesterol dietético de 300 mg/dia (U.S., 2010). No entanto, as novas Diretrizes Dietéticas para americanos de 2015-2020 não incluíram essas recomendações, mas destacam a importância de consumir baixas quantidades de colesterol na dieta para a construção de padrões alimentares saudáveis (DESALVO; OLSON; CASAVALLE, 2016). Atualmente, muitos estudos investigaram a relação entre a ingestão de colesterol e doenças cardiovasculares (BERGER *et al.*, 2015; HARMAN; LEEDS; GRIFFIN, 2008; ISO *et al.*, 2001). Uma revisão sistemática e meta-análise conduzida por Berger *et al.* (2015), mostrou que a alta ingestão de colesterol dietético aumenta os níveis de colesterol no sangue, ao passo que esse aumento é um importante fator de risco para doenças cardiovasculares. Em contraste, outros estudos relataram uma diminuição ou nenhuma alteração do risco com maior ingestão de colesterol (HARMAN; LEEDS; GRIFFIN, 2008; ISO *et al.*, 2001). Nesse sentido, podemos observar uma inconsistência entre os estudos, deixando claro que mais estudos são necessários para esclarecer melhor a associação entre o consumo de colesterol e doenças cardiovasculares.

2.2 Testosterona

2.2.1 Definição e biossíntese

A T é um hormônio androgênico anabólico envolvida no desenvolvimento fetal e nas características sexuais adultas (primárias e secundárias), na manutenção da função reprodutora, no comportamento sexual e na espermatogênese (BRINKMANN, 2011; MORALES, 2013). Ademais, a T está envolvida na síntese proteica e no crescimento muscular e ósseo (KICMAN, 2008). Em homens, 95% da T secretada é sintetizada pelas células de Leydig nos testículos e 5% pelas glândulas supra-renais (CONWAY *et al.*, 2000) e possui uma vida média de aproximadamente 12 minutos (VANDERSCHUEREN *et al.*, 2014), nesse sentido, é necessário que seja secretada continuamente. Na mulher, a T é produzida pelas glândulas supra-renais e nos ovários, no entanto, a mulher produz cerca de 10 a 20 vezes menos T do que o homem (VINGREN *et al.*, 2010).

A biossíntese da T integra uma cascata de reações, que são iniciadas pelo transporte de colesterol para a mitocôndria integrada por várias reações enzimáticas (MILLER, 1988). Em sequência, o colesterol se transforma em progesterona, a qual será convertida em androstenediona e logo, é convertida em T (WETTSTEIN, 1961). No

homem, o mecanismo da produção da T tem início no hipotálamo, o qual regula a produção do Hormônio Liberador de Gonadotrofina (GnRH), logo, a hipófise estimula a produção do Hormônio Luteinizante (LH), ativando diretamente a produção de T no interstício do testículo maduro (HSUEH; DAFAU; CATT, 1976). A regulação da produção da T testicular se dá pela secreção do GnRH no hipotálamo, o qual é capaz de controlar a secreção do LH e a própria testosterona exerce o *feedback* negativo inibindo a secreção de GnRH (VELDHUIS; CARLSON; JOHNSON, 1987).

A TT é considerada a soma das concentrações de T ligada a proteínas séricas (globulina ligadora de hormônios sexuais e albumina) e a T ausente de ligações proteicas, enquanto a T que não possui ligação proteica é considerada a testosterona livre (MATSUMOTO; BREMNER, 2004). A dosagem da TT é o método mais utilizado para investigar declínios hormonais em homens (BHASIN *et al.*, 2010; MORLEY *et al.*, 2000).

2.2.2 Ação fisiológica da testosterona

Além da T ser um hormônio fundamental para a estrutura muscular, é importante também para a função muscular (KVORNING *et al.*, 2006). Nesse sentido, manter os níveis de TT dentro dos valores considerados normais pode ser importante para aspectos gerais de saúde (ZAROTSKY *et al.*, 2014b). De acordo com Travison *et al.* (2017), níveis de TT entre ~220 e 915 ng·dL em homens são considerados normais, por conseguinte, valores abaixo de ~220 ng·dL são considerados hipogonadais.

As concentrações séricas de TT declinam progressivamente de acordo com a idade (HUHTANIEMI, 2014). Alguns estudos tem mostrado que a baixa concentração sérica de TT está associada ao aumento da quantidade de gordura corporal (TRABERT *et al.*, 2012), diminuição da massa muscular esquelética e desempenho físico (BAUMGARTNER *et al.*, 1999; YUKI *et al.*, 2013), obesidade, diabetes *mellitus* tipo 2, doenças cardiovasculares, síndrome metabólica, saúde geral ruim e maiores taxas de mortalidade por todas as causas (KELLY; JONES, 2015; MURALEEDHARAN *et al.*, 2013; ZAROTSKY *et al.*, 2014b). Em contrapartida, um estudo clássico conduzido por Bhasin *et al.* (1996) mostrou que a TT em níveis supra fisiológicos (>2.000 ng·dL) foi capaz de aumentar o tamanho e a força muscular independentemente do treinamento de força, neste estudo homens saudáveis de 19 a 40 anos receberam injeções semanais de 600mg de enantato de T durante 10 semanas.

O excesso de gordura corporal pode elevar a secreção de adipocitocinas e citocinas inflamatórias podendo afetar a produção de T nos testículos e levar a condições de hipogonadismo em homens (DANDONA; ALJADA; BANDYOPADHYAY, 2004; GROSSMANN, 2011). Rastrelli et al. (2015), observaram que fatores metabólicos e de estilo de vida relacionados à obesidade podem levar ao desenvolvimento de hipogonadismo, podendo ser revertida com perda de massa corporal. Em concordância com esse estudo, Grossmann e Matsumoto (2017) observaram aumentos significativos nas concentrações de TT em homens com sobrepeso ou obesos após diminuição do peso corporal. É importante ressaltar que o exercício físico associado a planejamento dietético, é visto como uma estratégia para perda de gordura corporal e aumento da TT (FLECK; KRAEMER, 2017).

2.3 Colesterol e testosterona

Já está bem estabelecido que o colesterol é de origem endógena, o qual é sintetizado principalmente pelo fígado, enquanto o colesterol de origem exógena é aquele que consumimos via alimentação (MILES, 1989). Nesse sentido, sabemos que o colesterol pode ser encontrado em vários alimentos como, carnes, ovos, laticínios, aves e frutos do mar. Por conseguinte, mudanças na ingestão desses alimentos podem promover mudanças nos níveis de T (HÄMÄLÄINEN *et al.*, 1984). Algumas dietas ricas em gorduras, como, dietas cetogênicas e dietas utilizando ingestão de ovos de galinhas inteiros, têm sido utilizadas como estratégias nutricionais para aumentar a ingestão de colesterol e supostamente aumentar os níveis séricos de TT em homens. Um estudo recente conduzido por Bagheri et al. (2021) investigou o efeito de 12 semanas de treinamento de força associado à ingestão de colesterol na dieta por meio de ovos de galinhas inteiros sobre os níveis séricos de TT. Esses autores compararam a ingestão de 3 ovos de galinhas inteiros (672 mg de colesterol dos ovos, sendo ~ 842 mg/dia da dieta inteira) com a ingestão de 6 claras de ovos (0 mg de colesterol das claras de ovos, sendo ~ 285 mg/dia da dieta inteira). O estudo mostrou que o grupo que ingeriu mais colesterol (842 mg/dia) aumentou as concentrações séricas de TT em 2,4 ng/ml, enquanto o grupo que ingeriu menos colesterol (285 mg/dia) houve aumento de 0,7 ng/dia nas concentrações séricas de TT. Todavia, esse aumento não foi importante para promover mudanças significativas na massa muscular. Outro estudo conduzido por Wilson et al. (2020) demonstrou que uma intervenção dietética cetogênica foi capaz de aumentar os níveis séricos de TT. Além disso, uma revisão sistemática e meta-análise, investigou o

impacto de dietas com baixa quantidade de gordura *versus* alta quantidade de gordura na produção de testosterona, os autores mostraram que dietas com baixas quantidades de gordura parecem diminuir os níveis séricos de TT (WHITTAKER; WU, 2021; WILSON *et al.*, 2020). No entanto, é importante ressaltar que os estudos supracitados não avaliaram a ingestão de colesterol isoladamente, o que impossibilita concluir que os níveis de TT aumentaram devido a uma maior ingestão de quantidade de gordura total, colesterol ou ambos. Nesse sentido, observamos que as evidências quanto aos efeitos da ingestão de colesterol sobre as concentrações séricas de TT são limitadas. Ademais, até o momento, nenhum estudo avaliou essas associações em uma amostra representativa.

2.4 Força muscular

A força do tecido muscular esquelética máxima é definida como a quantidade máxima de força realizada por um músculo ou grupo muscular em um padrão de movimento e velocidade específico (KNUTTGEN; KRAEMER, 1987). A capacidade de realizar diferentes atividades da vida diária é determinada, em parte, pela capacidade em desenvolver força muscular (BRILL *et al.*, 2000). Já é bem estabelecido que os níveis de força muscular estão relacionados com a idade (PÉREZ-ZEPEDA *et al.*, 2017), sendo consensual que um maior decréscimo da força muscular ocorre a partir dos 60 anos (DOHERTY; VANDERVOORT; BROWN, 1993; KEMMLER *et al.*, 2018). Este decréscimo contribui substancialmente com a diminuição da capacidade física, resultando em aumento do risco de fragilidade e quedas (WIEDMER *et al.*, 2021).

A diminuição da força muscular durante o envelhecimento pode ser devido à degeneração neuromuscular, o que contribui para a redução da capacidade do músculo esquelético de gerar força (ENOKA *et al.*, 2003; LARSSON; GRIMBY; KARLSSON, 1979). Essa perda é resultado de mudanças na capacidade do tecido muscular de produzir força devido à diminuição na tensão específica de fibras musculares individuais e aumento relativo na expressão das características das fibras musculares de contração lenta (LARSSON; LI; FRONTERA, 1997). A diminuição da força muscular pode ser também resultado de mudanças nas ativações neuromusculares, como diminuição do impulso neural para os músculos agonistas, coordenação potencialmente perturbada entre agonista e sinergista e aumento da co-ativação agonista-antagonista (KLEIN; RICE; MARSH, 2001; MACALUSO *et al.*, 2002). Alterações nos mecanismo musculares (sinapse de motoneurônio) também estão envolvidas na diminuição da força muscular, devido a degeneração do cerebelo e outros centros espinhais, essas alterações resultam em

diminuições da excitabilidade e morte de neurônios motores localizados na medula espinhal, assim, levando a atrofia muscular (BARRY; CARSON, 2004). Nesse sentido, muitas pesquisas têm se concentrado na identificação de estratégias para amenizar esses quadros.

É possível medir a força máxima de forma dinâmica (deslocamento de uma resistência com mudanças no comprimento muscular), como o teste de uma repetição máxima (1RM); e estática (não ocorre movimento muscular em determinadas articulações), como o teste de FPM (RIEBE *et al.*, 2018). O teste de FPM é considerado importante, ao passo que, dentre as atividades físicas diárias, muitas delas exigem o uso dos músculos flexores do antebraço e das falanges (dedos) das mãos, os quais estão envolvidos na FPM (ONG *et al.*, 2017). Além disso, a FPM é um teste válido para diagnosticar a sarcopenia, também é considerada um preditor de doenças crônicas e mortalidade precoce em homens (CRUZ-JENTOFT *et al.*, 2019; LEONG *et al.*, 2015; PETERSON *et al.*, 2016).

2.5 Testosterona e força muscular

A força e a qualidade muscular são consideradas os principais critérios para o diagnóstico da sarcopenia, os quais estão correlacionadas com alta morbidade e mortalidade devido ao aumento do risco de fragilidade e queda (WIEDMER *et al.*, 2021).

Os mecanismos determinantes da ação da T no aumento da força muscular continuam incertos, no entanto, alguns estudos têm especulado alguns fatores importantes. O “*Position stand on androgen and human growth hormone use*” (HOFFMAN *et al.*, 2009), mostrou que a T induz processos anabólicos e anticatabólicos, interage com receptores neuronais resultando no aumento de neurotransmissores liberados. Além disso, atua na regeneração de nervos aumentando as dimensões dos dendritos, ao passo que esses fenômenos são importantes para a produção da força muscular (DESCHENES *et al.*, 1993). Nesse sentido, podemos hipoteticamente dizer que os níveis séricos de TT podem ser importantes para o aumento ou manutenção da força muscular.

A idade pode afetar negativamente os níveis séricos de TT, levando a uma diminuição da miogênese muscular e consequentemente uma redução gradual da massa muscular, a qual está associada a uma diminuição concomitante da força muscular (PRIEGO *et al.*, 2021). Alguns estudos mostraram que o declínio da força muscular de

membros inferiores e superiores é dependente da idade (FRONTERA *et al.*, 2000), sendo mais rápido após os 60 anos (KEMMLER *et al.*, 2018; NAHHAS *et al.*, 2010).

Um estudo observacional mostrou que a diminuição das concentrações de TT, força muscular e desempenho físico ocorrem de acordo com a idade (SAAD *et al.*, 2017). Em contraste, outro estudo mostrou que níveis mais baixos de TT não estão associados aos primeiros três anos de diminuição da força muscular em idosos (ROMIJN *et al.*, 2015). Outro estudo mostrou associações positivas entre as concentrações de TT e FPM, mas é preciso ressaltar que nesse estudo foi incluído uma amostra de 7.064 indivíduos, a qual estão inseridos em todas as análises homens juntamente com mulheres. Isto pode ser considerado um fator de confusão importante nesse estudo (CHIU; SHIH; CHEN, 2020). A princípio, está bem elucidado que as mulheres possuem quantidade inferior nas concentrações de TT (cerca de 10 vezes menos) quando comparadas com homens de idade semelhante (VINGREN *et al.*, 2010). Nesta lógica, essa diferença pode ser um grande limitador nas conclusões apresentadas no estudo supracitado (CHIU; SHIH; CHEN, 2020).

2.6 National Health and Nutrition Examination Survey

A população investigada nesse estudo foi extraída do banco de dados *National Health and Nutrition Examination Survey (NHANES)*, contendo informações de histórico médico, medidas físicas, avaliação bioquímica, exame físico e ingestão alimentar de grupos populacionais nos Estados Unidos da América (EUA). O NHANES é realizado pelo Departamento de Saúde e Serviços Humanos dos EUA, em que se examina uma amostra nacionalmente representativa de cerca de 5.000 pessoas a cada ano. Essas pessoas estão localizadas em municípios de todo o país, 15 dos quais são visitados a cada ano. O NHANES é um programa importante do Centro Nacional de Estatísticas de Saúde (NCHS), o qual, faz parte dos Centros de Controle e Prevenção de Doenças (CDC), sendo responsável por produzir estatísticas vitais e de saúde a nível mundial. Neste programa contém entrevistas e exames físicos dos participantes, buscando avaliar a saúde e o estado nutricional de uma amostra representativa da população não institucionalizada dos EUA, além disso, o programa conta com um projeto de amostragem probalística complexa, estratificada em três estágios. O estágio 1 consiste na seleção das unidades primárias de amostragem por probabilidade proporcional. No estágio 2 as unidades primárias de amostragem são divididas em segmentos, por exemplo, bairros, os quais são selecionados por probabilidade. O estágio 3 consiste em sorteios dos domicílios. Já no

estágio 4 a seleção probabilística dos indivíduos dentro da casa previamente selecionada (CDC, 2012). O programa NHANES foi conduzido como uma série de pesquisas com foco em diferentes grupos populacionais ou tópicos de saúde, tendo início na década de 1960. Em 1999, a pesquisa tornou-se um programa contínuo, buscando atender às necessidades emergentes. Os exames compreendem em medições médicas, odontológicas e fisiológicas, bem como exames laboratoriais administrados por pessoal médico altamente treinado. Os resultados são usados para determinar a prevalência de doenças e fatores de risco, as informações têm como objetivo avaliar o estado nutricional e sua associação com a promoção da saúde e prevenção de doenças e são usados em estudos epidemiológicos e pesquisas em ciências da saúde, que ajudam a desenvolver políticas de saúde pública sólidas, para direcionar e projetar programas e serviços de saúde e expandir o conhecimento em saúde mundial (CDC, 2012).

O NHANES realiza entrevistas de saúde em centros móveis em vários locais do país e nas casas dos próprios participantes. Um sistema avançado de computadores auxilia a coleta e processa todos os dados, praticamente eliminando a necessidade de formulários em papel e operações manuais. As informações do NHANES são disponibilizadas através vários documentos, os quais são disponíveis em publicações no próprio site do programa e em artigos em revistas científicas (CDC, 2012). O NHANES foi projetado para facilitar a realização das pesquisas e incentivar a participação da população, sendo que as entrevistas de saúde são realizadas nas casas dos participantes e as avaliações de saúde são realizadas em centros móveis, os quais viajam para todos os locais do país.

O peso amostral do NHANES foi criado com o objetivo de fazer estimativas representativas da população civil não institucionalizada dos EUA, considerando o complexo desenho da pesquisa (incluindo sobreamostragem), a não resposta da pesquisa e o ajuste pós-estratificação para corresponder às contagens totais da população. Quando uma amostra é ponderada no NHANES, ela é representativa da população residente civil não institucionalizada dos EUA. O peso da amostra é criado em três etapas: primeiro, o peso base é calculado, o que explica as probabilidades desiguais de seleção, uma vez que alguns grupos demográficos foram superamostrados; em segundo, os ajustes são feitos para não resposta; e por último, ajustes de pós-estratificação são feitos para corresponder às estimativas da população (CHEN *et al.*, 2018).

Nesta tese, foram utilizados os biênios 2011-2012 e 2013-2014, os quais foram analisados separadamente.

3 OBJETIVOS

3.1 Objetivo geral

Esta tese teve dois objetivos principais: 1. Avaliar a associação das concentrações séricas da testosterona total com o consumo de colesterol e com os níveis séricos de colesterol total em homens entre 20 e 80 anos participantes do *National Health and Nutrition Examination Survey* (NHANES) 2013 e 2014 (Artigo 1); 2. Avaliar a associação das concentrações séricas de testosterona total com a força muscular em homens jovens, de meia idade e idosos participantes do *National Health and Nutrition Examination Survey* (NHANES) 2011 e 2012 (Artigo 2).

3.2 Objetivos Específicos

- Avaliar as características demográficas, condições de saúde e comportamento dos indivíduos avaliados;
- Avaliar a força, parâmetros bioquímicos e dietéticos dos participantes dos estudos.

4 RESULTADOS

Artigo 1.

Artigo intitulado “Cholesterol intake and serum total cholesterol levels are not associated with total testosterone levels in US men: A cross-sectional study from NHANES 2013-2014”, submetido à revista *Hormones International Journal of Endocrinology and Metabolism* (Impact Factor = 3.419) em 20 de novembro de 2022.

Original Article

Cholesterol intake and serum total cholesterol levels are not associated with total testosterone levels in US men: A cross-sectional study from NHANES 2013-2014

Gederson K. Gomes¹, Flávia M.S. de Branco¹, Heitor O. Santos¹, Jaqueline L. Pereira³, Fábio L. Orsatti⁴, Erick P. de Oliveira^{1*}

¹ Laboratory of Nutrition, Exercise and Health (LaNES), School of Medicine, Federal University of Uberlandia (UFU), Uberlandia, Minas Gerais, Brazil.

² School of Medicine, Federal University of Uberlandia (UFU), Uberlandia, Minas Gerais, Brazil.

³ Department of Nutrition, School of Public Health, University of São Paulo (USP), São Paulo - SP, Brazil.

* Corresponding author. Federal University of Uberlandia, School of Medicine, Av. Para, 1.720 Bloco 2U - Sala 20, Campus Umuarama, Umuarama, 38400902, Uberlândia, MG, Brazil. Fax: +55 34 32328620. e-mail address: erick_po@yahoo.com.br, erickdeoliveira@ufu.br

ABSTRACT

Background: Testosterone (T) is an anabolic hormone crucial to the structure and function of skeletal muscle. Testosterone is synthesized from cholesterol, but little is known about the relationship of cholesterol intake and serum cholesterol with T levels.

Aim: To investigate whether cholesterol intake and serum total cholesterol (TC) levels are associated with serum total testosterone (TT) levels in men.

Methods: A cross-sectional study enrolling 1996 men aged 20 to 80 years from National Health and Nutrition Examination Survey (NHANES) 2013-2014 was carried out. Diet assessment was performed using two 24-hour food recalls; and TT levels were measured by liquid chromatography method coupled with tandem mass spectrometry. Regression analyzes were performed to evaluate whether TT is associated with cholesterol intake and serum TC levels.

Results: Neither cholesterol intake nor serum TC levels were associated with TT levels in unadjusted and adjusted analyses (adjustment for energy, total fat and alcohol intakes, smoking, age, physical activity, family income, marital status, race, educational level, diabetes, hypertension, and body mass index).

Conclusion: Dietary cholesterol intake and TC levels are not associated with TT levels in men from USA.

Keywords: Androgens; Cholesterol; Hypogonadism; Lipids; Sex hormones; Testosterone.

Introduction

Testosterone (T) is an important sex hormone for reproductive factors, acting in the process of male genital development and secondary sexual characteristics [1, 2]. T is also fundamental to skeletal muscle structure and function [3]. In males, T production increases rapidly under stimulation of anterior pituitary gonadotropic hormones at the onset of puberty, but values tend to decline after 50 years and may reduce by 20% to 50% at 80 years [4].

Low levels of total testosterone (TT) are associated with increased body fat and decreased skeletal muscle mass during aging [5, 6], as well as an increased likelihood of having low libido and erectile dysfunction [7]. In addition, recent studies show that low TT levels are associated with underlying comorbidities and potentially higher mortality risk in men [8, 9]. Therefore, efforts should be made to identify strategies to manage TT levels.

Given that T is a cholesterol-derived steroid hormone from Leydig cells and adrenals [10], it is possible to speculate that both cholesterol intake and serum total cholesterol (TC) may be associated with TT levels. However, to date, no study has evaluated these associations in a representative sample. The limited evidence as to whether cholesterol intake has an effect on TT levels has been demonstrated by few randomized clinical trials [11, 12]. These studies showed that the increment of egg intake [11] or ketogenic diet intervention [12], which are nutritional approaches that raise cholesterol intake, increased TT levels in trained (resistance training) young men [11, 12]. However, although randomized clinical trials have an important scientific evidence level, it is not yet possible to conclude whether these increases in TT levels occurred due to an isolated effect of cholesterol intake or because other nutrients that can also have effects on TT levels were also more ingested, such as fats [13-15]. For example, Wilson et al. [12] demonstrated that a ketogenic dietary intervention, which increased total fat intake and (likely) cholesterol intake, increased TT levels. However, since this study did not evaluate cholesterol intake, it is not possible to conclude whether T levels increased by a higher intake of fat, cholesterol, or both. In addition, a recent study showed that increased intake of cholesterol through daily intake of 3 whole eggs (672 mg cholesterol from eggs, but ~842 mg/day from whole diet) for a 12-week increased TT levels. However, considering that this elevated intake of cholesterol is not usually consumed at the population level [16], it is not known whether habitual intake of cholesterol is associated with TT levels. Furthermore, the aforementioned studies [11, 12] have a small

sample size ($n \leq 30$) and do not provide evidence for the untrained (resistance training) population.

Therefore, studies evaluating the association between habitual cholesterol intake and TT levels in a representative sample adjusting for important confounders may contribute to the understanding of this issue at the population level. Thus, the present study aimed to evaluate whether cholesterol intake and TC levels (which are partially predicted by cholesterol intake) are associated with serum TT levels in a representative sample of US men from the National Health and Nutrition Examination Survey (NHANES) 2013-2014.

Methods

Survey and Participants

Data were obtained from the National Health and Nutrition Examination Survey (NHANES) 2013-2014. NHANES is a cross-sectional survey, conducted by the National Center for Health Statistics of Centers for Disease Control and Prevention, based on a multi-stage, complex pooled, stratified probability sampling design to select a representative sample of civilians from non-institutionalized U.S. Initially, participants conducted interviews at home, which were administered by trained staff, then all participants attended to a mobile testing clinic, where trained staff collected anthropometric and biochemical data [17]. A total of 10675 individuals were evaluated in NHANES 2013-2014. For the present study, women, individuals under 20 years old, with missing data for TT levels, who did not have two dietary recalls or who had one dietary recall, and who did not have anthropometric measures were excluded. Thus, 1996 men aged 20 to 80 years were evaluated in the present study (Figure 1). NHANES is a public dataset and all participants provided a written informed consent, consistent with approval from the National Center for Health Statistics Research Ethics Review Board (protocol #2011-17 for NHANES 2013-14).

Anthropometrics

The body weight and height of each participant were obtained according to the Lohman's protocol to calculate the body mass index (BMI) [18].

Total testosterone and serum cholesterol

TT values were selected for men who completed the morning laboratory test to better represent TT levels due to the diurnal variation of these hormones. TT levels were measured by the liquid chromatography method coupled with tandem mass spectrometry [19]. Serum TC levels were analyzed in venous samples collected following a standardized protocol, which were measured using coupled enzymatic reactions [20].

Dietary intake

Food intake was obtained for all individuals through two 24-hour dietary recalls [21], in which the first dietary interview was conducted in person at the mobile testing clinic and the second one was collected by phone 3 to 10 days later, using the Automated Multiple Pass Method [22]. This method consists in an interview in 5 steps which includes quick list, forgotten foods, time and occasion, detail cycle and final probe. Additionally, USDA's Food and Nutrient Database for Dietary Studies (FNDDS) 2013-2014 was used for processing the 2013-2014 intakes. The National Cancer Institute (NCI) method [23] was applied to estimate the usual dietary intake. We evaluated the intake of energy (kcal/day), carbohydrate (g/day), total protein (g/day), protein (g/kg/day), lipids (g/day), total fatty acids (g/day) and their main types (saturated, monounsaturated, and polyunsaturated fatty acids), cholesterol (mg/day), total omega-3 (g/day), acid linoleic (g/day), fiber (g/day), zinc (mg/day), vitamin D and A (mcg/day) and alcohol (g/day).

Covariates of interest

We considered as confounders demographic characteristics such as age (years), race/ ethnicity (non-Hispanic white or other), marital status (single/ divorced/ widowed/ never married or married/ living as married), education level (lower/ higher secondary education and some college or more) and annual household income (0 to \$19,999, from \$20,000 to 54,999, from \$55,000 to 74,999 or above \$75,000). In addition, morbidities and lifestyle conditions included in the present study were hypertension (yes or no), diabetes (pre-diabetes, yes or no), and smoking (yes or no). Physical activity variable was evaluated as “yes” or “no”. If the individual did not perform any type of physical activity, that is, answered no to all 3 types, he was classified as “no”. The intake of energy (kcal/day), total fat (g/day) and alcohol (g/day) were also considered as confounders.

Statistical analyzes

Linear regression was used to compare demographic, health and behavior conditions, anthropometric and biochemical parameters, and food intake according to quintiles of cholesterol intake. Continuous variables were described as mean and standard deviation and categorical variables were described as percentage and confidence interval. Linear regression models were used to estimate coefficients and 95% confidence intervals (95% CI) for TT by quintiles of dietary cholesterol intake or blood cholesterol levels. Linear regression analyses were also performed evaluating continuous values of dietary cholesterol and blood cholesterol levels. Analyzes were performed without (Model 1) and with adjustments for confounders (Model 2). Variables included as adjustments were age, BMI, race/ethnicity, educational level, marital status, annual household income, diabetes, hypertension, physical activity, smoking, energy intake (kcal/day), total fat (g/day), alcohol (g/day). A missing variable was created to annual family income, educational level, and smoking. Stata 14.0 software (StataCorp, College Station, TX, US) was used for statistical analysis and p-value <0.05 was considered as significant. All statistical analyzes were performed considering the weight of the examination sample [17].

Results

Subjects' characteristics

Table 1 shows the characteristics of the participants according to the quintile of cholesterol intake (mg). Men ingesting more cholesterol were predominantly younger, but a smaller proportion of non-Hispanic white consumed more cholesterol. In addition, men with the higher intake of cholesterol had a lower prevalence of hypertension and diabetes. Individuals ingesting more cholesterol had higher body weight and BMI, and also ingested more energy, carbohydrates (g/day), protein (g/day and g/kg/day); total, saturated, monounsaturated, and polyunsaturated fats (g/day), total omega-3 (g/day), linoleic acid (g/day), zinc (mg/day) and vitamin A (mcg/day). For biochemical parameters, no differences were found for TC (mg/dL) and TT (ng/dL) values according to the quintile of cholesterol intake (mg).

Cholesterol intake and total testosterone levels

No associations were observed between the quintile of cholesterol intake and TT in the unadjusted analyses (Supplementary Table 1), as well as when adjusted for confounders

(Figure 2 and Supplementary Table 1). Continuous values of cholesterol intake were also not associated with TT levels after adjustments for confounders (Table 2).

Serum cholesterol and Total Testosterone levels

Quintiles of TC were not associated with TT levels in the unadjusted analyses (Supplementary Table 1), as well as when adjusted for confounders (Figure 3 and Supplementary Table 1). Continuous values of TC levels were also not associated with TT levels after adjustments for confounders (Table 2).

Discussion

The main result of the present study was that cholesterol intake and TC levels were not associated with serum TT levels in men (20 to 80 years old) from a representative US sample. The lack of association was observed even considering an average cholesterol intake (347 mg/day) above than that recommended by some guidelines, such as the Dietary Guidelines for Americans, which recommended not to exceed 300 mg/day of dietary cholesterol intake [24]. However, there is no current limit on cholesterol intake as the 2015–2020 Dietary Guidelines for Americans removed the recommendation to restrict cholesterol intake to up to 300 mg/day [25]. Despite the mean values of cholesterol intake from the overall sample size (347 mg/day) and the highest quintile (474 mg/day) exceeded the previous cholesterol intake threshold by 16 to 58%, respectively, we did not find a positive association with serum TT levels. Our results therefore do not support the hypothesis that a higher cholesterol intake is associated with higher TT levels, although cholesterol is the primary precursor for T synthesis [26]. In addition, we also did not observe association between serum cholesterol (that is partially predicted by dietary cholesterol) and TT levels, which shows that TT does not seem to be associated with cholesterol, independent the form that it was evaluated.

To the best of our knowledge, the present study is the first to evaluate the cross-sectional association between cholesterol intake and TT levels in a representative US sample. This limits direct comparison with studies that have the same design (cross-sectional), but other studies have evaluated a possible effect of cholesterol intake on TT levels. A prospective study observed that reducing cholesterol intake from ~633 mg/day to ~342 mg/day for 6 weeks was able to decrease serum TT from ~655 ng/dL to ~557 ng/dL in healthy middle-aged men (40 to 49 years old) [27]. However, this decrease in

serum TT levels is within the range considered normal for men (~300 to 800 ng/dL) and likely have no clinical relevance [28].

Considering that eggs are one of the main food-sources of dietary cholesterol, hypothetically, the intake of eggs itself could increase TT levels [29]. A recent study evaluating physically active eugonadal young men showed that daily intake of 3 whole eggs (672 mg cholesterol from eggs, ~842 mg/day from whole diet) for a 12-week strength-training program increased post-workout serum TT levels (~240 ng/dL) compared to ~70 ng/dL increase for the control group (6 egg whites; 0 mg cholesterol from eggs, ~285 mg/day from whole diet) [11]. Although this [11] and the present study do not have the same study design, which limits comparisons of the results, it is possible to observe that a high intake of cholesterol per day (~842 mg/day) seems to be necessary to promote an increase in TT levels [11]. In the present study, the lack of association can partially be explained due to a lower intake of cholesterol observed in the last quintile (mean intake of 474 mg/day). Therefore, our study shows that a habitual dietary cholesterol intake is not associated with TT levels, but based in the other studies, it is possible to speculate that greater intake of cholesterol could promote increases on TT levels. However, it is unclear whether these increases in TT levels have important clinical implications [29].

Interventional studies using other dietary models with higher cholesterol intake should be considered as well. The ketogenic diet is the dietary model with the highest cholesterol intake [30]. Overall, 500 to 1000 mg of cholesterol per day is expected in the ketogenic diet, but the common ketogenic diet is a cluster of high intake of cholesterol, total fat, and saturated fatty acids [12, 30], which limits the extrapolation of the isolated effect of cholesterol intake on TT levels. Wilson et al. demonstrated that a ketogenic dietary intervention (~220 g/day of total fat; ~110 g/day of saturated fatty acids) associated with 12-week strength training in trained young men increased TT levels by ~120 ng/dL compared to subjects who underwent a low-fat Western diet (~84g/day of total fat; ~37 g/day of saturated fatty acids) [12]. Conversely, these authors did not show the data for cholesterol intake, although a high intake could be conceivable in virtue of the high intake of total and saturated fatty acids in the ketogenic diet.

Given the relationship between cholesterol intake and total fat, a critical appraisal of total fat consumption and its effects on TT levels is of great interest. A study showed that reducing total fat intake from ~112 to ~40 g/day promoted a decrease in serum TT concentrations from ~438 to ~386 ng/dL in healthy middle-aged men (50 to 63 years old)

[13]. This result is in line with a recent meta-analysis of intervention studies that confirmed the inverse relationship between low-fat diets and TT levels in men [14]. In terms of an epidemiological basis, while our study did not show a relationship between cholesterol intake and TT levels, a recent study ($n = 3.128$) employing NHANES analyses (1999 to 2000, 2003 to 2004, and 2011 to 2012) observed that men ($n = 457$) following a low-fat pattern had lower TT levels than men in general (410.8 ± 8.1 vs. 443.5 ± 7.3 , $p = 0.005$) even when controlled for confounding variables (comorbidities, age, BMI, and activity level) [31]. In the present study, we observed that individuals ingesting more cholesterol also ingested more fat that would be an important confounder. However, we showed that the lack of association between cholesterol intake and TT was independent of total fat intake.

The present study has limitations. First, the study design is cross-sectional and causality cannot be established. Second, we did not focus on patients with hypogonadism and thus we cannot state whether the increment in cholesterol intake is useful for males with low TT levels. As strengths, our study evaluated a representative sample of men from USA. The current population of individuals represents a heterogeneous sample of the real practical scenario and hence depicts clinical applicability. The usual cholesterol intake was modeled through the NCI method based on data from two 24HR, which reduces bias caused by measurement error in estimated associations between usual dietary intakes and health outcomes [23]. All analyses were adjusted for important confounders. We performed the regression analyses according to quintiles of cholesterol and also evaluating the continuous values. This shows that TT levels are not associated with dietary and blood cholesterol independent of the form that the analyses were performed.

Conclusion

Dietary cholesterol intake and TC levels were not associated with TT levels in men from US. Longitudinal research is needed to evaluate whether an isolated increment of cholesterol intake can change the TT levels in men.

Acknowledgements

We thank CAPES for the financial support. EPO would like to thank The Brazilian National Council for Scientific and Technological Development (CNPq, Brazil, 304205/2019-2).

Authorship

GKG participated in interpretation of the data, performed statistical analysis, and wrote the manuscript; FMSB participated in interpretation of the data, performed statistical analysis, and revised the manuscript. HOS wrote the manuscript; JLP performed statistical analysis and revised the manuscript, FLO participated in interpretation of the data and revised the manuscript. EPO participated in the interpretation of the data, statistical analysis, wrote, and revised the manuscript. All authors read and approved the final manuscript.

Data availability: The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate: NHANES is a public dataset and all participants provided a written informed consent, consistent with approval from the National Center for Health Statistics Research Ethics Review Board (protocol #2011-17 for NHANES 2013-14).

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Consent for publication: All authors give full consent for publication of the present article.

Competing interests: The authors declare no competing interests.

References

1. Brinkmann AO: **Molecular mechanisms of androgen action—a historical perspective.** *Androgen Action* 2011:3-24. https://doi.org/10.1007/978-1-61779-243-4_1
2. Morales A: **The long and tortuous history of the discovery of testosterone and its clinical application.** *The journal of sexual medicine* 2013, **10**(4):1178-1183. <https://doi.org/10.1111/jsm.12081>

3. Kvorning T, Andersen M, Brixen K, Madsen K: **Suppression of endogenous testosterone production attenuates the response to strength training: a randomized, placebo-controlled, and blinded intervention study.** *American Journal of Physiology-Endocrinology and Metabolism* 2006, **291**(6):E1325-E1332. <https://doi.org/10.1152/ajpendo.00143.2006>
4. Lokeshwar SD, Patel P, Fantus RJ, Halpern J, Chang C, Kargi AY, Ramasamy R: **Decline in serum testosterone levels among adolescent and young adult men in the USA.** *European urology focus* 2021, **7**(4):886-889. <https://doi.org/10.1016/j.euf.2020.02.006>
5. Trabert B, Graubard BI, Nyante SJ, Rifai N, Bradwin G, Platz EA, McQuillan GM, McGlynn KA: **Relationship of sex steroid hormones with body size and with body composition measured by dual-energy X-ray absorptiometry in US men.** *Cancer Causes & Control* 2012, **23**(12):1881-1891. <https://doi.org/10.1007/s10552-012-0024-9>
6. LeBlanc ES, Wang PY, Lee CG, Barrett-Connor E, Cauley JA, Hoffman AR, Laughlin GA, Marshall LM, Orwoll ES: **Higher testosterone levels are associated with less loss of lean body mass in older men.** *The Journal of Clinical Endocrinology & Metabolism* 2011, **96**(12):3855-3863. <https://doi.org/10.1210/jc.2011-0312>
7. Cohen J, Nassau DE, Patel P, Ramasamy R: **Low testosterone in adolescents & young adults.** *Frontiers in endocrinology* 2020, **10**:916. <https://doi.org/10.3389/fendo.2019.00916>
8. Holmboe SA, Skakkebaek NE, Juul A, Scheike T, Jensen TK, Linneberg A, Thuesen BH, Andersson A-M: **Individual testosterone decline and future mortality risk in men.** *European journal of endocrinology* 2018, **178**(1):121-128. <https://doi.org/10.1530/EJE-17-0280>
9. Yeap BB, Marriott RJ, Antonio L, Chan YX, Raj S, Dwivedi G, Reid CM, Anawalt BD, Bhasin S, Dobs AS: **Serum testosterone is inversely and sex hormone-binding globulin is directly associated with all-cause mortality in men.** *The Journal of Clinical Endocrinology & Metabolism* 2021, **106**(2):e625-e637. <https://doi.org/10.1210/clinem/dgaa743>

10. Johnson LR, Wood RI: **Oral testosterone self-administration in male hamsters.** *Neuroendocrinology* 2001, **73**(4):285-292.
<https://doi.org/10.1159/000054645>
11. Bagheri R, Moghadam BH, Ashtary-Larky D, Forbes SC, Candow DG, Galpin AJ, Eskandari M, Kreider RB, Wong A: **Whole egg vs. egg white ingestion during 12 weeks of resistance training in trained young males: A randomized controlled trial.** *The Journal of Strength & Conditioning Research* 2021, **35**(2):411-419. <https://doi.org/10.1519/JSC.0000000000003922>
12. Wilson JM, Lowery RP, Roberts MD, Sharp MH, Joy JM, Shields KA, Partl JM, Volek JS, D'Agostino DP: **Effects of ketogenic dieting on body composition, strength, power, and hormonal profiles in resistance training men.** *The Journal of Strength & Conditioning Research* 2020, **34**(12):3463-3474.
<https://doi.org/10.1519/JSC.0000000000001935>
13. Wang C, Catlin DH, Starcevic B, Heber D, Ambler C, Berman N, Lucas G, Leung A, Schramm K, Lee PW: **Low-fat high-fiber diet decreased serum and urine androgens in men.** *The Journal of Clinical Endocrinology & Metabolism* 2005, **90**(6):3550-3559. <https://doi.org/10.1210/jc.2004-1530>
14. Whittaker J, Wu K: **Low-fat diets and testosterone in men: Systematic review and meta-analysis of intervention studies.** *The Journal of Steroid Biochemistry and Molecular Biology* 2021, **210**:105878.
<https://doi.org/10.1016/j.jsbmb.2021.105878>
15. Volek JS, Kraemer WJ, Bush JA, Incledon T, Boetes M: **Testosterone and cortisol in relationship to dietary nutrients and resistance exercise.** *Journal of Applied Physiology* 1997. <https://doi.org/10.1152/jappl.1997.82.1.49>
16. Xu Z, McClure ST, Appel LJ: **Dietary cholesterol intake and sources among US adults: results from National Health and Nutrition Examination Surveys (NHANES), 2001–2014.** *Nutrients* 2018, **10**(6):771.
<https://doi.org/10.3390/nu10060771>
17. CDC. **National Health and Nutrition Examination Survey questionnaire (or examination protocol, or laboratory protocol).** Hyattsville, MD: US Department of Health and Human Services, Centers for Disease Control and Prevention. 2009. Available at: http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm 2013.

18. Lohman TG, Roche AF, Martorell R: **Anthropometric standardization reference manual**: Human kinetics books; 1988.
19. Pirkle J: **Laboratory Procedure Manual: Total Testosterone**. *National Center for Environmental Health, Centers for Disease Control and Prevention*
Retrieved from https://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/tst_g_met.pdf.
20. CDC. **NHANES MEC Laboratory Procedures Manual**. *Centers for Disease Control and Prevention (CDC): Atlanta, GA, USA*. Available at: <https://www.cdc.gov/nchs/nhanes/index.htm> 2012.
21. CDC. **NHANES MEC Laboratory Procedures Manual**. *Centers for Disease Control and Prevention (CDC): Atlanta, GA, USA* 2013.
22. Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, Paul DR, Sebastian RS, Kuczynski KJ, Ingwersen LA *et al*: **The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes**. *The American journal of clinical nutrition* 2008, **88**(2):324-332. <https://doi.org/10.1093/ajcn/88.2.324>
23. Tooze JA, Kipnis V, Buckman DW, Carroll RJ, Freedman LS, Guenther PM, Krebs-Smith SM, Subar AF, Dodd KW: **A mixed-effects model approach for estimating the distribution of usual intake of nutrients: the NCI method**. *Statistics in medicine* 2010, **29**(27):2857-2868. <https://doi.org/10.1002/sim.4063>
24. Committee USDGA: **Dietary guidelines for Americans, 2010**: US Department of Health and Human Services, US Department of Agriculture; 2010.
25. Health UDo, Services H: **US Department of Agriculture. 2015–2020 dietary guidelines for Americans. December 2015**. In.; 2019.
26. Eacker SM, Agrawal N, Qian K, Dichek HL, Gong E-Y, Lee K, Braun RE: **Hormonal regulation of testicular steroid and cholesterol homeostasis**. *Molecular Endocrinology* 2008, **22**(3):623-635. <https://doi.org/10.1210/me.2006-0534>
27. Hämmäläinen E, Adlercreutz H, Puska P, Pietinen P: **Diet and serum sex hormones in healthy men**. *Journal of steroid biochemistry* 1984, **20**(1):459-464. [https://doi.org/10.1016/0022-4731\(84\)90254-1](https://doi.org/10.1016/0022-4731(84)90254-1)
28. Morton RW *et al*. Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men.

Journal of applied physiology 2016,**121**(1):129-138.

<https://doi.org/10.1152/japplphysiol.00154.2016>

29. Santos HO, Gomes GK, Schoenfeld BJ, de Oliveira EP: **The Effect of Whole Egg Intake on Muscle Mass: Are the Yolk and Its Nutrients Important?** *International Journal of Sport Nutrition and Exercise Metabolism* 2021, **31**(6):514-521. <https://doi.org/10.1123/ijsnem.2021-0086>
30. Santos HO: **Ketogenic diet and testosterone increase: Is the increased cholesterol intake responsible? To what extent and under what circumstances can there be benefits?** *Hormones (Athens)* 2017, **16**(3):266-270. <https://doi.org/10.1007/BF03401520>
31. Fantus RJ, Halpern JA, Chang C, Keeter MK, Bennett NE, Helfand B, Brannigan RE: **The association between popular diets and serum testosterone among men in the United States.** *The Journal of urology* 2020, **203**(2):398-404. <https://doi.org/10.1097/JU.0000000000000482>

Table 1. Sociodemographic, health conditions and habits, physical activity, anthropometric and body composition and dietary intake of adults men by quintiles of dietary cholesterol (NHANES, 2013-2014).

Variables	Dietary Cholesterol quintiles						<i>p-trend</i>
	Total	Q1	Q2	Q3	Q4	Q5	
Dietary Cholesterol (mg)	346.2 (83.1)	238.1 (33.2)	296.1 (11.4)	333.9 (10.5)	377.9 (16.2)	473.8 (63.9)	<0.001
Age, years	46.9 (16.88)	52.3 (19.08)	47.4 (17.25)	47.0 (16.78)	46.2 (14.57)	42.4 (15.65)	<0.001
Non-Hispanic white, %	66.6 (60.6 – 72.1)	68.8 (62.3 – 74.4)	71.6 (64.0 – 78.2)	67.1 (59.1 – 74.2)	67.5 (60.6 – 73.7)	57.9 (48.6 – 66.6)	0.013
<i>Marital status, %</i>							0.276
Single/divorced/widowed/never married	32.8 (28.7 – 37.1)	31.2 (25.6 – 38.1)	32.3 (23.8 – 42.1)	33.1 (26.6 – 40.7)	28.9 (22.5 – 36.2)	38.4 (30.7 – 46.8)	
Married/ living as married	67.1 (62.8 – 71.2)	68.7 (61.8 – 74.9)	67.6 (57.8 – 76.1)	66.6 (59.2 – 73.3)	71.0 – 63.7 – 77.4)	61.5 – 53.1 – 69.2)	
<i>Annual family income, %</i>							0.767
\$0-19.999	14.2 (10.8 – 18.4)	16.7 (12.6 – 21.8)	16.1 (9.8 – 25.2)	9.4 (6.4 – 13.7)	12.2 (8.1 – 17.9)	17.6 (11.3 – 26.3)	
\$20.000-54.999	33.7 (29.6 – 38.0)	38.9 (28.1 – 50.9)	28.2 (22.9 – 34.31)	41.5 (35.7 – 47.5)	28.0 (22.1 – 34.8)	32.5 (27.3 – 38.1)	
\$55.000-74.999	11.3 (8.8 – 14.3)	9.9 (5.5 – 17.2)	12.5 (6.6 – 22.4)	13.8 (8.1 – 22.7)	9.5 (62.05 – 14.4)	10.2 (6.4 – 15.9)	
Over \$75.000	37.5 (31.5 – 44.0)	31.4 (25.7 – 37.8)	40.2 (32.5 – 48.4)	32.9 (27.3 – 39.0)	47.4 (38.0 – 57.0)	34.1 (24.0 – 45.9)	
Missing	3.1 (2.1 – 46.64)	2.8 (0.9 – 8.1)	2.8 (1.0 – 7.4)	2.1 (0.9 – 4.6)	2.6 (1.4 – 4.8)	5.4 (3.1 – 9.1)	
<i>Educational level, %</i>							0.348
High school graduate or under	35.8 (30.8 – 41.1)	39.5 (30.7 – 49.1)	34.6 (25.4 – 45.3)	38.8 (31.5 – 46.5)	27.5 (20.9 – 35.2)	39.6 (32.3 – 47.4)	
Some college or above	64.1 (58.8 – 69.1)	60.4 (50.8 – 69.2)	65.3 (54.6 – 74.5)	61.1 (53.3 – 68.3)	72.4 (64.7 -79.0)	60.3 (52.5 – 67.6)	
Missing	0.005 (0.004 – 0.05)	-	-	0.002 (0.0003 – 0.02)	-	-	
<i>Health conditions and habits</i>							<0.001
Hypertension, %	34.3 (30.5 – 38.4)	40.1 (32.8 – 47.8)	36.8 (29.8 – 44.4)	34.0 (27.8 – 40.9)	34.9 (27.3 – 43.4)	26.3 (20.7 – 32.8)	
Diabetes, %							0.040
Pre-diabetes	3.1 (2.1 – 4.4)	12.6 (5.5 – 28.5)	6.2 (2.7 – 13.3)	16.0 (7.8 – 32.3)	35.1 (15.3 – 78.2)	27.2 (12.2 – 59.3)	
Yes	9.1 (7.3 – 11.4)	13.2 (9.3 – 18.3)	9.9 (6.4 – 15.0)	8.8 (4.5 – 16.7)	5.8 (3.9 – 8.6)	8.7 (6.0 – 12.5)	
No	87.7 (85.2 – 89.8)	85.5 (80.8 – 89.2)	83.8 (75.3 89.7)	89.5 (81.7 – 94.1)	90.6 (86.0 – 93.7)	88.4 (84.0 – 91.8)	
Smoking %							0.707
Yes	19.0 (16.3 – 22.0)	21.4 (13.2 – 32.9)	18.7 (14.2 – 24.1)	16.4 (13.0 – 20.6)	21.2 (15.9 – 27.6)	17.6 (11.9 – 25.4)	
Missing	0.009 (0.001 – 0.08)	-	0.004 (0.0005 – 0.03)	-	-	-	
<i>Physical activity %</i>							0.148
Yes	55.9 (52.4 – 59.3)	49.5 (43.3 – 55.7)	56.4 (48.8 – 63.6)	53.6 (45.4 – 61.6)	61.4 (51.8 – 70.1)	57.4 (48.9 – 65.5)	
No	44.0 (40.6 – 47.5)	50.4 (44.2 – 56.6)	43.5 (36.3 – 51.1)	46.3 (38.3 – 54.5)	38.5 (29.8 – 48.1)	42.5 (34.4 – 51.0)	
<i>Anthropometrics</i>							
Weight, kg	89.2 (20.2)	86.1 (21.9)	87.7 (19.8)	91.5 (18.5)	89.0 (17.7)	91.2 (23.3)	0.030

Height, m	1.75 (0.7)	1.73 (0.7)	1.76 (0.7)	1.76 (0.6)	1.75 (0.7)	1.76 (0.7)	0.106
Body mass index, kg/m ²	28.8 (6.0)	28.4 (6.8)	28.2 (6.4)	29.4 (5.2)	28.9 (5.0)	29.2 (7.0)	0.046
<i>Dietary intake</i>							
Energy, kcal/day	2399.6 (516.4)	2006.9 (453.4)	2264.4 (399.4)	2360.0 (353.6)	2531.4 (430.2)	2789.1 (605.1)	<0.001
Carbohydrate, g/day	279.8 (69.7)	251.6 (67.3)	269.3 (56.3)	279.9 (54.9)	288.0 (66.4)	306.9 (89.9)	<0.001
Protein, g/day	96.4 (20.3)	76.2 (14.5)	89.9 (13.9)	94.2 (14.2)	103.2 (14.4)	116.1 (21.1)	<0.001
Protein, g/kg	1.13 (0.3)	0.9 (0.2)	1.1 (0.3)	1.1 (0.2)	1.2 (0.3)	1.3 (0.4)	<0.001
Lipids, g/day	92.0 (20.7)	74.5 (17.5)	85.3 (14.7)	89.4 (14.5)	99.1 (16.9)	109.5 (22.3)	<0.001
Saturated fat, g/day	29.8 (7.2)	23.6 (5.4)	27.5 (4.9)	29.1 (5.9)	32.4 (6.1)	35.7 (8.3)	<0.001
Monounsaturated fat, g/day	32.3 (7.3)	26.5 (6.7)	30.1 (5.6)	31.4 (5.3)	34.6 (5.7)	38.3 (7.7)	<0.001
Polyunsaturated fat, g/day	21.1 (4.7)	17.9 (4.5)	19.9 (3.8)	20.3 (3.9)	22.3 (4.2)	24.3 (4.8)	<0.001
Total ω -3, g/day	2.3 (0.4)	1.9 (0.4)	2.1 (0.3)	2.2 (0.4)	2.4 (0.4)	2.6 (0.4)	<0.001
EPA, g/day	0.026 (0.004)	0.023 (0.004)	0.025 (0.004)	0.026 (0.004)	0.026 (0.004)	0.028 (0.005)	<0.001
DHA, g/day	0.078 (0.026)	0.061 (0.018)	0.701 (0.024)	0.076 (0.024)	0.081 (0.023)	0.099 (0.02)	<0.001
ALA, g/day	1.9 (0.4)	1.6 (0.4)	1.8 (0.3)	1.9 (0.3)	2.0 (0.4)	2.2 (0.4)	<0.001
Linoleic acid, g/day	18.5 (4.3)	15.8 (4.1)	16.6 (3.6)	17.9 (3.6)	19.7 (3.8)	21.3 (4.3)	<0.001
Fiber, g/day	18.7 (6.0)	17.8 (7.2)	18.2 (5.4)	18.7 (5.7)	18.8 (5.1)	20.2 (6.7)	0.014
Zinc (mg)/day	12.9 (2.9)	10.8 (2.9)	12.1 (2.2)	12.6 (2.1)	13.6 (2.6)	15.0 (3.3)	<0.001
Vitamin D (D2+D3), mcg/day	25.5 (35.6)	24.4 (36.6)	20.4 (19.4)	34.6 (55.8)	23.8 (28.9)	25.1 (22.5)	0.678
Vitamin A (RAE), mcg/day	693.8 (613.0)	537.2 (681.5)	638.9 (516.8)	596.3 (457.1)	727.3 (505.1)	963.6 (815.9)	<0.001
Alcohol, g/day	12.7 (15.7)	10.6 (1.0)	13.3 (1.5)	12.2 (0.9)	12.4 (1.0)	14.4 (1.4)	0.134
<i>Biochemical parameters</i>							
Total Cholesterol (mg/dL)	187.2 (39.9)	180.5 (44.0)	183.3 (40.7)	191.4 (33.7)	193 (41.0)	185.9 (39.3)	0.055
Total Testosterone (ng.dL)	412.9 (176.9)	400.4 (167)	413.2 (177)	402.7 (160)	427.8 (200.2)	418.3 (166.1)	0.158

Notes: ALA: alpha linolenic acid; AMMI: appendicular skeletal muscle mass index; DHA; docosahexaenoic acid; EPA: eicosapentaenoic acid; LDL: low-density lipoprotein; SHBG, sex hormone binding globulin. Data described as mean (standard deviation) or percentage (confidence interval).

Table 2. Linear regression of continuous values of dietary and blood cholesterol with total testosterone levels in men aged 20-80 y. NHANES, 2013-2014.

	Model 1			Model 2		
	β	95%CI	p-value	β	95%CI	p-value
Dietary cholesterol (mg/day)	0.811	-0.221; 0.184	0.115	0.884	-0.233; 0.200	0.112
Blood cholesterol (mg/dL)	0.115	-0.503; 0.202	0.379	-0.005	-0.297; 0.286	0.968

Note: Model 1: Without adjustment. Model 2: adjusted for energy (kcal/d), total fat (g/d) and alcohol intakes, smoking, age, physical activity, family income, marital status, race, educational level, diabetes, hypertension, and BMI. Values shown as coefficients confidence intervals (95%CI).

Supplementary Table 1. Linear regression between quintiles of cholesterol intake and serum total cholesterol levels according to total testosterone levels in men aged 20-80 y from the NHANES, 2013-2014.

	Model 1						Model 2					
	Q1	Q2	Q3	Q4	Q5	p-trend	Q1	Q2	Q3	Q4	Q5	p-trend
Cholesterol intake	ref	12.7 (-19.6; 45.1)	2.3 (-25.7; 30.2)	27.4 (-3.4; 58.7)	17.9 (-11.1; 46.9)	0.158	ref	9.2 (-16.1; 34.7)	11.1 (-21.1; 43.4)	32.2 (-0.9; 65.3)	21.7 (-5.3; 48.8)	0.104
Serum cholesterol	Ref	46.9 (22.5; 71.2)	9.6 (-27.8; 47.0)	-0.13 (-35.1; 34.8)	10.9 (-36.3; 58.1)	0.602	ref	33.9 (6.6; 61.1)	10.9 (-17.6; 39.6)	17.4 (-19.0; 54.0)	20.5 (-16.9; 58.0)	0.575

Notes: Model 1: Without adjustment. Model 2: adjusted for energy (kcal/d), total fat (g/d) and alcohol intakes, smoking, age, physical activity, family income, marital status, race, educational level, diabetes, hypertension and BMI. Values shown as coefficients and 95% confidence intervals (95%CI).

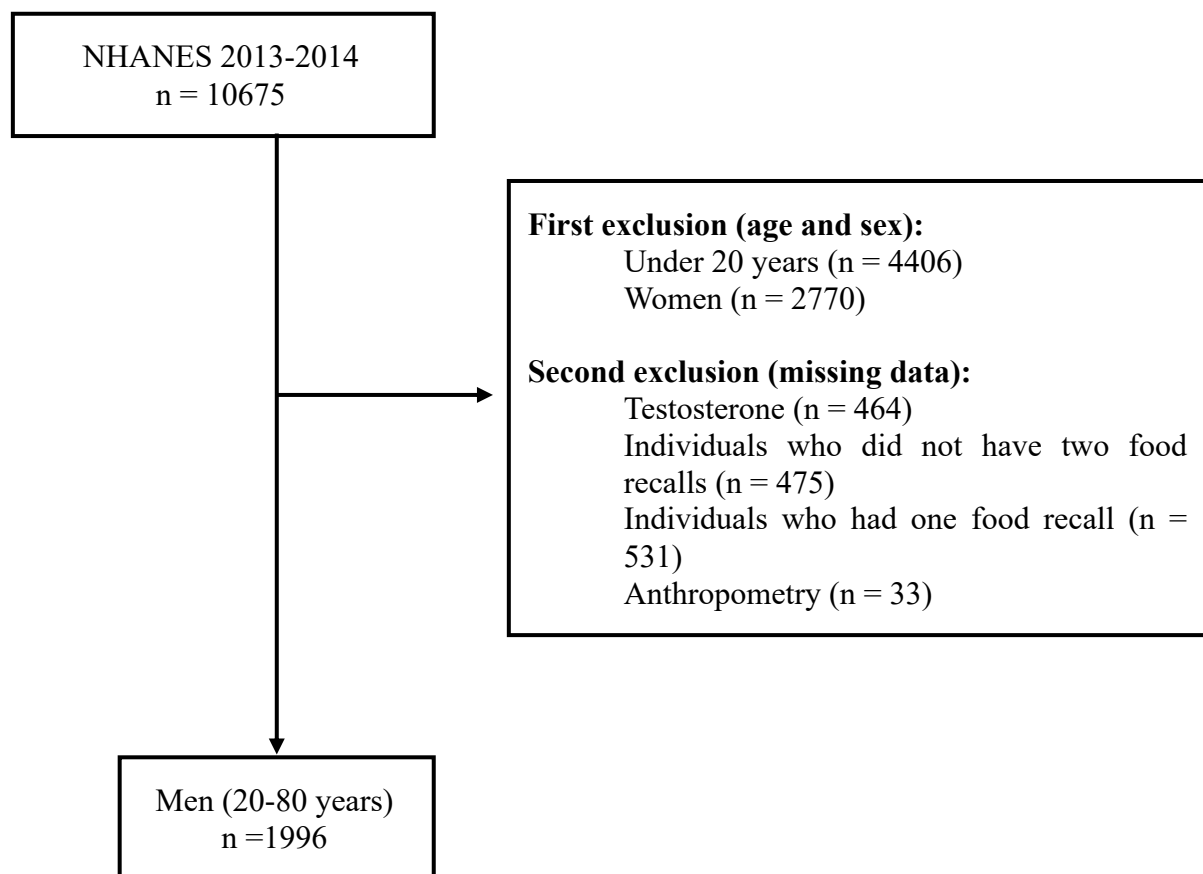


Figure. 1. Flowchart of the sample selection from NHANES 2013-2014.

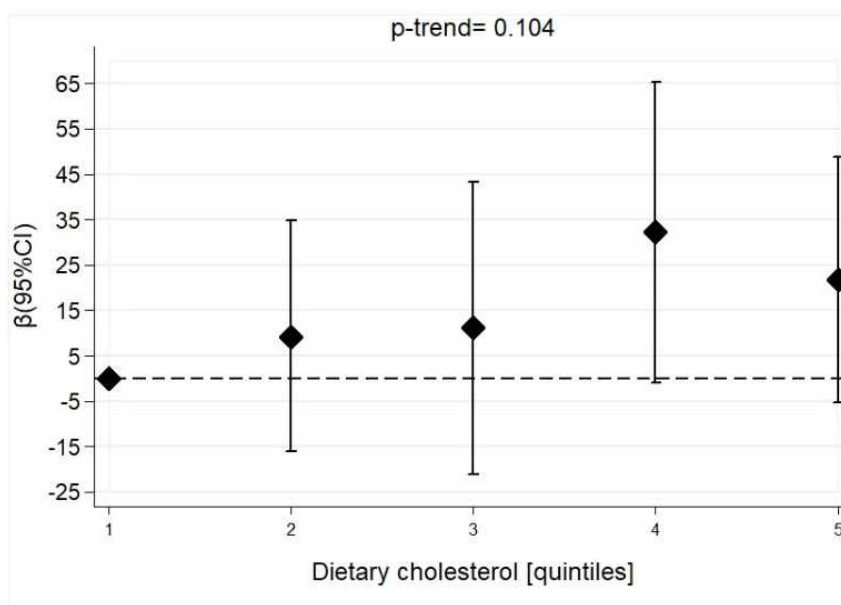


Figure 2. Linear regression between quintiles of dietary cholesterol and total testosterone levels. NHANES, 2013-2014.

Adjusted for adjusted for energy (kcal/d), total fat (g/day) and alcohol intakes, smoking, age, physical activity, family income, marital status, race, educational level, diabetes, hypertension and BMI.

Quintiles of dietary cholesterol (mg): quintile 1: 89.4 – 273.6.; quintile 2: 273.7– 314.6; quintile 3: 314.7– 353.0; quintile 4: 353.1 – 411.1 and quintile 5: 411.4 – 835.2.

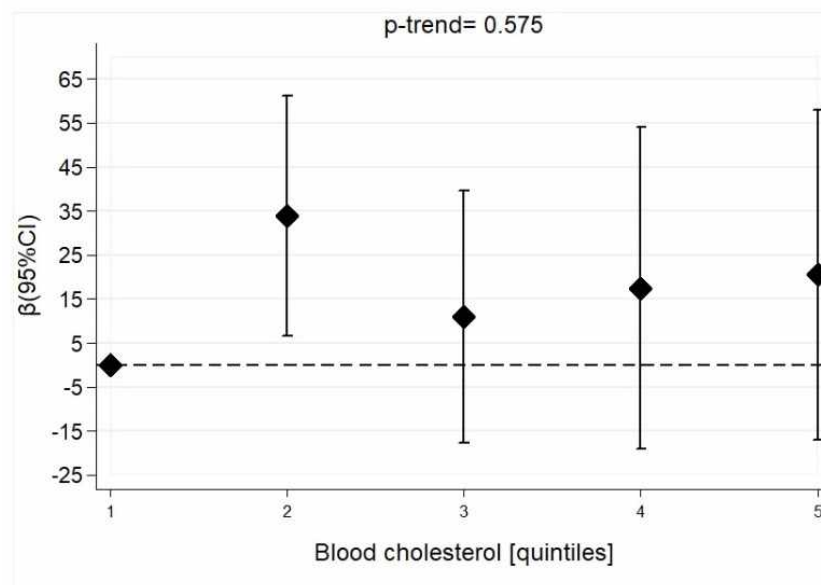


Figure 3. Linear regression between quintiles of blood cholesterol and total testosterone levels. NHANES, 2013-2014.

Adjusted for adjusted for energy (kcal/d), total fat (g/day) and alcohol intakes, smoking, age, physical activity, family income, marital status, race, educational level, diabetes, hypertension and BMI.

Quintiles of blood cholesterol (mg/dL): quintile 1: 82 – 150.; quintile 2: 151– 173; quintile 3: 174 – 194; quintile 4: 195 – 218 and quintile 5: 219 – 362.

Artigo 2.

Artigo intitulado “Grip strength is associated with serum testosterone levels in young and middle-aged men, but not in older men: NHANES 2011 – 2012”, pretendemos submetê-lo à revista *Scientific Reports* (*Impact Factor* = 5.516).

Original Article

Grip strength is associated with serum testosterone levels in young and middle-aged men, but not in older men: NHANES 2011 – 2012.

Gederson K. Gomes¹, Heitor O. Santos¹, Fábio L. Orsatti², Flávia M.S. de Branco¹, Erick P. de Oliveira^{1*}

¹ Laboratory of Nutrition, Exercise, and Health (LaNES), School of Medicine, Federal University of Uberlandia (UFU), Uberlandia, Minas Gerais, Brazil.

² Research Applied Physiology, Nutrition and Exercise Group, Uberaba, Minas Gerais, Brazil.

* Corresponding author. Federal University of Uberlandia, School of Medicine, Av. Para, 1.720 Bloco 2U - Sala 20, Campus Umuarama, Umuarama, 38400902, Uberlândia, MG, Brazil. Fax: +55 34 32328620. e-mail address: erick_po@yahoo.com.br, erickdeoliveira@ufu.br

ABSTRACT

Background: Total testosterone (TT) at normal physiological levels contribute to regulate skeletal muscle mass and muscle strength. However, little is known about the association of TT with muscle strength according to different age-range.

Aim: To investigate the association between TT levels and handgrip strength in men according to age-range (20 to 39, 40 to 59, and 60 to 80 years).

Methods: A cross-sectional study was conducted involving 2049 American men aged between 20 and 80 years, which were separated into age-range groups: 20 to 39 (young men), 40 to 59 (middle-aged men), and 60 to 80 years (older men). TT levels were measured using liquid chromatography and tandem mass spectrometry. Handgrip strength (HGS) was measured using a digital dynamometer following a standardized protocol. The sum of HGS of each hand was used. Linear regression analysis was performed between TT and HGS according to different age-range.

Results: We observed positive associations between TT levels and HGS in total sample (aged 20 to 80 years). When the participants were divided by age-range, a positive association was observed between TT levels and HGS in young ($p < 0.001$) and middle-aged ($p < 0.001$) men. However, TT was not associated with HGS in older men (≥ 60 years).

Conclusion: TT levels are positively associated with HGS in young and middle-aged men, but not in older men.

Keywords: Sex hormone; Testosterone; Grip strength.

Introduction

Testosterone is an anabolic androgenic hormone capable of promoting effects on skeletal muscle tissue by binding to its specific receptor (1). In this sense, concentrations of total testosterone (TT) at normal physiological levels may contribute to the regulation of skeletal muscle mass and muscle strength (2). However, low levels of TT can promote losses in skeletal muscle mass and muscle strength. In this sense, low levels of TT are associated with several comorbidities, such as obesity, type 2 diabetes, cardiovascular disease, metabolic syndrome, poor general health (3, 4) and higher rates mortality (from all causes) (5). Thus, muscle strength, as an example, handgrip strength (HGS) is considered a predictor of chronic diseases and early mortality in men (6, 7).

A recent observational study (8) showed positive associations between TT and HGS levels in young and older men. However, it is necessary to emphasize that in this study the authors did not separate the participants according to sex and age. It is well established that women produce less testosterone (about ten times less) in comparison with men of the same age (9). Also, some studies will show that muscular strength is dependent on the age (10). In this sense, if the authors of the study (8) consider these factors, different results could be found.

Age can negatively affect serum TT levels leading to a decrease in muscle myogenesis and consequently a gradual reduction in muscle mass, which is associated with a concomitant decrease in muscle strength (11). An observational study (12) showed that decreased levels of TT, muscle strength and physical performance occur in older people. Another study found no associations between TT and HGS levels in older man with health and heart failure (13). Furthermore, a cross-sectional study demonstrated a relationship between TT levels with HGS and physical performance of older men (14). An observational study showed that decreased levels of TT, muscle strength, and physical performance occur with aging. Other study did not show associations between TT and HGS levels in patients with heart failure. Similarly, another study showed that TT was not associated with muscle strength after analyzes were adjusted for muscle mass (15).

Therefore, it is possible to observe that the current evidence remains inconclusive (12-14) requiring clarification of the relationship between the levels of TT with muscle strength considering sex and age. Furthermore, the study that evaluated the association between TT and HGS levels did not perform analyzes according to age (8). As we have many different causes of strength loss during aging (16, 17), the association between TT and HGS may differ according to age.

Thus, the present study aimed to assess the relationships between TT levels and HGS in men according to age-range 20 to 39 years (young men), 40 to 59 years (middle-aged men), and 60 to 80 years (older men). Our hypothesis is that TT levels would be positively associated with HGS in men across all age-range.

Methods

Survey and Participants

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional survey conducted by the Centers for Disease Control and Prevention's National Center for Health Statistics, based on a multi-stage probability sampling design to select a representative sample. Of civilians from non-institutionalized US countries. Data were obtained from NHANES 2011-2012; the present study evaluated men aged 20 to 80. Participants completed home interviews, physical exams, biochemical tests, dietary interviews, and other examinations (18). After excluding 1990 women and 4196 individuals younger than 20 years, plus 718 missing data for TT levels, 257 for those who did not have all nutritional information, 2 for anthropometric measurements (body mass index), and 532 missing data of HGS, a total of 2049 men were evaluated and had data on handgrip strength and serum testosterone levels (Figure 1).

Dietary intake

Dietary data were collected by an interviewer using a 24-hour food recall (19). The first interview was in-person at mobile testing centers, and the second was conducted by telephone. The automated multi-step method collected dietary data from (20). This section is divided into five steps containing different information about food consumed during the previous 24-hour period. This procedure allows respondents to remember and report the foods consumed. The estimation of habitual food consumption was applied using the National Cancer Institute (NCI) (21). Dietary variables were energy intake (kcal/day), total protein (g), protein (g/day), lipids (g/day), fiber (g/day), total fatty acids (g/day) and their main types (saturated fat, monounsaturated fat, and polyunsaturated fat), alcohol (g/day) and total omega-3 (g/day).

Measurement of grip strength

The Takei digital dynamometer (T.K.K 5401) was used in all evaluations to measure the manual pressure force. Before each test, calibration was performed, and data

were recorded as part of quality control procedures. The dynamometer handle was adjusted according to the size of each participant's hand; the index finger of each hand should be flexed at 90° (between the proximal and middle phalangeal joints). The participant made a practical attempt (submaximal effort) to ensure an understanding of the proper procedures. The test was performed in a standing position with feet hip-width apart and holding the dynamometer away from the body and aligned with the forearm at thigh height so that it did not touch the body and the arm was fully extended; each participant performed three attempts of each hand, squeezing the device with maximum force, holding for 5 seconds, and separated for 60 seconds (alternate hands). However, if the participant could not stand unaided throughout the test, they could still perform the test. Still, in a sitting position, with the back unsupported and not holding anything, the arm must be at the side of the body. The handgrip strength of each hand was summed to produce the final value. All examiners were trained according to the necessary procedures, the detailed protocol for the handgrip strength exams is provided in the NHANES Muscle Strength/Adherence Test Procedures Manual (22).

Total testosterone

All participants had their blood collected by venipuncture in the morning after an overnight fast. Total testosterone levels were measured in serum using isotope dilution liquid chromatography and tandem mass spectrometry. Detailed descriptions regarding sample collections, laboratory protocols, and information on reliability, validation, and quality control of serum testosterone levels are provided in the NHANES Laboratory Procedures Manual (23).

Covariates of interest

We investigated some potential confounders, which were included in the analysis as adjustments. The body mass index (BMI) was calculated by dividing the weight of individuals by the square of their height (kg/m^2). Demographic characteristics were age (years), race/ ethnicity (non-Hispanic white or other), marital status (single/ divorced/ widowed/ never married or married/ living married), education (lower/ higher secondary education and some college or higher) and annual household income (0 to \$19,999, \$20,000 to \$54,999, \$55,000 to \$74,999, or over \$75,000). The morbidities and lifestyle conditions included in the present study were hypertension (yes or no), diabetes (pre-diabetes, yes or no), arthritis (yes or no), and smoking (yes or no). The physical activity

variable was evaluated as “yes” or “no.” If the individual did not perform any physical activity, that is, answered no to the three types, he was classified as “no.” Energy (kcal/day) and alcohol (g/day) intake were also considered confounding factors. A missing variable was created whenever needed.

Statistical analyzes

The sample was characterized according to age-range and testosterone tertiles. In the characterization analyzes we used linear regression for continuous dependent variables and logistic regression for categorical dependent variables. Linear regression analysis was performed between TT and HGS including all participants and divided by age-range (20-39 years, 40-59 years, and 60-80 years). Continuous variables were described as mean and standard deviation and categorical variables were described as percentage and confidence interval. Linear regression was used to estimate coefficients and 95% confidence intervals (95% CI) for TT and HGS levels. Analyzes were performed without adjustment (Model 1), and with adjustments for confounders (Model 2). Variables included as adjustments were age, BMI, race/ethnicity, education, marital status, annual family income, diabetes, hypertension, arthritis, physical activity, smoking, intake of energy and alcohol (g/day). Stata 14.0 software (StataCorp, College Station, TX, USA) was used in all analysis, and a p -value < 0.05 was considered significant. In all analyzes we include the "SVY" commands for weight information of the examined sample.

Results

Individual's characteristics

The participants' characteristics are described according to the TT tertile in **Table 1**. Men with lower TT concentrations had higher mean ages and were married or living as a married couple. Furthermore, men with higher concentrations of TT had a lower prevalence of diabetes, arthritis, and hypertension, performed more physical activity, had higher body weight and BMI, and ingested more protein (g/kg). Interestingly, individuals with higher TT concentrations consumed more alcohol (g/day) and had a higher prevalence of smokers. **Table 2** presents the characteristics of the participants according to age-range. Older men had lower handgrip strength, had a higher proportion of non-Hispanic whites, and were married or living as a married couple. According to health

conditions, older men had a higher prevalence of hypertension, diabetes, and arthritis and performed less physical activity. In addition, older men were taller, ingested less energy (kcal/day), carbohydrates (g/day), protein (g/day and g/kg), lipids (g/day), saturated, monounsaturated, and polyunsaturated fats (g/day), total Omega-3 (g/day) and ALA (g/day). No differences were found for TT values (ng/dL) for biochemical parameters according to age-range.

Total testosterone levels and levels of grip strength

We did not observe associations between TT concentrations and HGS in model 1 (unadjusted model); however, in model 2, we observed positive associations between TT levels and HGS (Table 3).

Total testosterone levels and levels of grip strength by age

In linear regression analyses between TT concentrations and HGS divided by age, we did not observe associations in the unadjusted model in all age-range (young men middle-aged and older men). However, in the model adjusted for confounders, positive associations were observed for individuals young and middle-aged men, in older men we found no associations (**Table 3**). After additional adjustment for uric acid, we found no significant differences in relation to the results presented in Table 3.

Discussion

The main result of the present study was that serum TT levels were positively associated with HGS for young men and middle-aged, but not for older men. To the best of our knowledge, this is the first study that investigated the association between TT levels and HGS in men dividing the sample by age.

These results corroborate with a recent study by Chiu et al. (2020) which showed associations between TT levels and HGS in men and women with a mean age of 36.2 years (8). Sezer et al. (2018) evaluated 144 men with an average age of 39.8 years demonstrating positive associations between TT and HGS levels (24). Therefore, the result of the present study suggests that TT levels may be important to the muscle strength of young and middle-aged men. The determining mechanisms of testosterone action on muscle strength remain undefined, however the “*Position stand on androgen and human growth hormone use*” (25) discusses some important factors. The study showed that testosterone induces anabolic processes by interacting with neuronal receptors, releasing

greater amounts of neurotransmitters. In addition, T modulates nerve regeneration by increasing the dimensions of the dendrites, and these phenomena are important to produce muscle strength (26).

We did not observe positive associations between TT and HGS levels in older men. These findings corroborate the recent study by Loncar et al. (2017) that investigated 93 men (73 with heart failure and 20 healthy) with a mean age of 67 years, this study did not find associations between serum TT and HGS (13). However, the present study contrasts with other studies (14, 27). The cross-sectional study of Auyeng et al. (2011) investigated 1.489 men (± 72.5 years) and showed a relationship between serum TT levels with HGS and physical performance (14). HSU et al. (2014) accompanied 1.318 men (± 76.3 years) for 2 years. This study showed that men with higher levels of TT have higher levels of HGS, and men with low TT levels were approximately twice as likely to have functional decline (27). It is important to emphasize that the above studies did not use confusing covariables in adjusted models, such as food intake, physical activity level, health conditions and behavior, in addition this study investigated different populations. Thus, the lack of these adjustments may have interfered with the results. Some studies have shown that TT levels progressively decrease with age (28, 29). However, in the present study, older men did not have lower TT levels when compared to young and middle-aged men ($p = 0.07$). In addition, some studies have shown that the decline in muscle strength occurs with age (30, 31) and after 60 years the decline it is faster (32, 33). In the present study, older men had HGS approximately 20% lower than young men, however, TT levels do not differ between them. This can be explained by the fact that during aging there are several causes of loss of muscle strength, such as increased oxidative stress (34), reduced muscle fiber size, thus decreasing contraction capacities (35), increased inflammation (36), loss of skeletal muscle mass (16) and physical inactivity (37).

This study had some limitations. First, cross-sectional studies can define associations, but not causality. Second, NHANES does not include detailed information about muscle strength training to better clarify participants' training level. Finally, we did not analyze hypogonadal participants separately, so we cannot extrapolate our results to this population. However, this study has strengths. We evaluated only male participants aged 20 to 80 years, thus ensuring specific analyzes for this population of a representative US sample. We divided the participants by age-range. The investigated population

represents a heterogeneous sample of the practical scenario, thus portraying the practical applicability. Finally, all analyzes were adjusted for confounders.

Conclusion

We conclude that TT levels are positively associated with the sum of handgrip strength in young and middle-aged men, but not in older men.

References

1. Kadi F. Cellular and molecular mechanisms responsible for the action of testosterone on human skeletal muscle. A basis for illegal performance enhancement. *British journal of pharmacology*. 2008;154(3):522-8. <https://doi.org/10.1038/bjp.2008.118>
2. Oki K, Law TD, Loucks AB, Clark BC. The effects of testosterone and insulin-like growth factor 1 on motor system form and function. *Experimental gerontology*. 2015;64:81-6. <https://doi.org/10.1016/j.exger.2015.02.005>
3. Kelly D, Jones T. Testosterone and obesity. *Obesity Reviews*. 2015;16(7):581-606. <https://doi.org/10.1111/obr.12282>
4. Zarotsky V, Huang MY, Carman W, Morgentaler A, Singhal P, Coffin D, et al. Systematic literature review of the risk factors, comorbidities, and consequences of hypogonadism in men. *Andrology*. 2014;2(6):819-34. <https://doi.org/10.1111/andr.274>
5. Muraleedharan V, Marsh H, Kapoor D, Channer KS, Jones TH. Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. *Eur J Endocrinol*. 2013;169(6):725-33. <https://doi.org/10.1530/EJE-13-0321>
6. Leong DP, Teo KK, Rangarajan S, Lopez-Jaramillo P, Avezum Jr A, Orlandini A, et al. Prognostic value of grip strength: findings from the Prospective Urban Rural Epidemiology (PURE) study. *The Lancet*. 2015;386(9990):266-73. [https://doi.org/10.1016/S0140-6736\(14\)62000-6](https://doi.org/10.1016/S0140-6736(14)62000-6)
7. Peterson MD, Zhang P, Choksi P, Markides KS, Al Snih S. Muscle weakness thresholds for prediction of diabetes in adults. *Sports medicine*. 2016;46(5):619-28. <https://doi.org/10.1007/s40279-015-0463-z>

8. Chiu H-T, Shih M-T, Chen W-L. Examining the association between grip strength and testosterone. *The Aging Male*. 2020;23(5):915-22.
<https://doi.org/10.1080/13685538.2019.1632282>
9. Vingren JL, Kraemer WJ, Ratamess NA, Anderson JM, Volek JS, Maresh CM. Testosterone physiology in resistance exercise and training. *Sports medicine*. 2010;40(12):1037-53. <https://doi.org/10.2165/11536910-000000000-00000>
10. Zammit AR, Robitaille A, Piccinin AM, Muniz-Terrera G, Hofer SM. Associations between aging-related changes in grip strength and cognitive function in older adults: a systematic review. *The Journals of Gerontology: Series A*. 2019;74(4):519-27. <https://doi.org/10.1093/gerona/gly046>
11. Priego T, Martín A, González-Hedström D, Granado M, López-Calderón A. Role of hormones in sarcopenia. *Vitamins and Hormones*. 115: Elsevier; 2021. p. 535-70.
<https://doi.org/10.1016/bs.vh.2020.12.021>
12. Saad F, Roehrig G, von Haehling S, Traish A. Testosterone deficiency and testosterone treatment in older men. *Gerontology*. 2017;63(2):144-56.
<https://doi.org/10.1159/000452499>
13. Loncar G, Bozic B, Neskovic AN, Cvetinovic N, Lainscak M, Prodanovic N, et al. Androgen status in non-diabetic elderly men with heart failure. *The Aging Male*. 2017;20(4):215-24. <https://doi.org/10.1080/13685538.2017.1350155>
14. Auyeung TW, Lee JSW, Kwok T, Leung J, Ohlsson C, Vandenput L, et al. Testosterone but not estradiol level is positively related to muscle strength and physical performance independent of muscle mass: a cross-sectional study in 1489 older men. *European journal of endocrinology*. 2011;164(5):811-7. <https://doi.org/10.1530/EJE-10-0952>
15. Roy TA, Blackman MR, Harman SM, Tobin JD, Schragger M, Metter EJ. Interrelationships of serum testosterone and free testosterone index with FFM and strength in aging men. *American Journal of Physiology-Endocrinology and Metabolism*. 2002;283(2):E284-E94. <https://doi.org/10.1152/ajpendo.00334.2001>
16. Aversa Z, Zhang X, Fielding RA, Lanza I, LeBrasseur NK. The clinical impact and biological mechanisms of skeletal muscle aging. *Bone*. 2019;127:26-36.

<https://doi.org/10.1016/j.bone.2019.05.021>

17. Lexell J, Taylor CC, Sjöström M. What is the cause of the ageing atrophy?: Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15-to 83-year-old men. *Journal of the neurological sciences*. 1988;84(2-3):275-94. [https://doi.org/10.1016/0022-510X\(88\)90132-3](https://doi.org/10.1016/0022-510X(88)90132-3)
18. CDC. National Health and Nutrition Examination Survey questionnaire (or examination protocol, or laboratory protocol). Hyattsville, MD: US Department of Health and Human Services, Centers for Disease Control and Prevention. 2009. Available at: <https://wwwncdcgov/nchs/nhanes/continuousnhanes/questionnaires.aspx?BeginYear=2011>.
19. CDC. National Health and Nutrition Examination Survey (NHANES): MEC in-person dietary interviewers procedures anual. <https://www.cdc.gov/nchs/nhanes/index.htm>.
20. Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, et al. The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes. *The American journal of clinical nutrition*. 2008;88(2):324-32. <https://doi.org/10.1093/ajcn/88.2.324>
21. Tooze JA, Kipnis V, Buckman DW, Carroll RJ, Freedman LS, Guenther PM, et al. A mixed-effects model approach for estimating the distribution of usual intake of nutrients: the NCI method. *Statistics in medicine*. 2010;29(27):2857-68. <https://doi.org/10.1002/sim.4063>
22. CDC. NHANES MEC Laboratory Procedures Manual. Centers for Disease Control and Prevention (CDC): Atlanta, GA, USA. 2012. Available at: <https://wwwcdc.gov/nchs/nhanes/indexhtm>.
23. Pirkle J. Laboratory Procedure Manual: Total Testosterone. National Center for Environmental Health, Centers for Disease Control and Prevention Retrieved from https://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/tst_g_met.pdf.
24. Sezer S, Tural E, Çolak T, Uyanık S, Haberal M. Hand-Grip Strength Is Associated With Serum Testosterone and Albumin Levels in Male Kidney Transplant Recipients. *Experimental and Clinical Transplantation: Official Journal of the Middle*

East Society for Organ Transplantation. 2018;16(Suppl 1):75-9.

25. Hoffman JR, Kraemer WJ, Bhasin S, Storer T, Ratamess NA, Haff GG, et al. Position stand on androgen and human growth hormone use. *The Journal of Strength & Conditioning Research*. 2009;23:S1-S59.

<https://doi.org/10.1519/JSC.0b013e31819df2e6>

26. Deschenes M, Maresh C, Crivello J, Armstrong L, Kraemer W, Covault J. The effects of exercise training of different intensities on neuromuscular junction morphology. *Journal of neurocytology*. 1993;22(8):603-15.

<https://doi.org/10.1007/BF01181487>

27. Hsu B, Cumming RG, Naganathan V, Blyth FM, Le Couteur DG, Seibel MJ, et al. Longitudinal relationships of circulating reproductive hormone with functional disability, muscle mass, and strength in community-dwelling older men: the Concord Health and Ageing in Men project. *The Journal of Clinical Endocrinology & Metabolism*. 2014;99(9):3310-8. <https://doi.org/10.1210/jc.2014-1124>

28. Bayar G, Şirin H, Aydın M, Özağar A, Tanrıverdi O, Kadihasanoğlu M, et al. Low free and bioavailable testosterone levels may predict pathologically-proven high-risk prostate cancer: a prospective, clinical trial. *Turkish journal of urology*.

2017;43(3):289. <https://doi.org/10.5152/tud.2017.35467>

29. Golds G, Houdek D, Arnason T. Male hypogonadism and osteoporosis: the effects, clinical consequences, and treatment of testosterone deficiency in bone health. *International journal of endocrinology*. 2017;2017.

<https://doi.org/10.1155/2017/4602129>

30. Dodds RM, Syddall HE, Cooper R, Benzeval M, Deary IJ, Dennison EM, et al. Grip strength across the life course: normative data from twelve British studies. *PloS one*. 2014;9(12):e113637. <https://doi.org/10.1371/journal.pone.0113637>

31. Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R. Aging of skeletal muscle: a 12-yr longitudinal study. *Journal of applied physiology*. 2000;88(4):1321-6. <https://doi.org/10.1152/jappl.2000.88.4.1321>

32. Kemmler W, Von Stengel S, Schoene D, Kohl M. Changes of maximum leg strength indices during adulthood a cross-sectional study with non-athletic men aged

- 19–91. *Frontiers in Physiology*. 2018;9:1524. <https://doi.org/10.3389/fphys.2018.01524>
33. Nahhas RW, Choh AC, Lee M, Chumlea WMC, Duren DL, Siervogel RM, et al. Bayesian longitudinal plateau model of adult grip strength. *American Journal of Human Biology*. 2010;22(5):648-56. <https://doi.org/10.1002/ajhb.21057>
34. Meng S-J, Yu L-J. Oxidative stress, molecular inflammation and sarcopenia. *International journal of molecular sciences*. 2010;11(4):1509-26. <https://doi.org/10.3390/ijms11041509>
35. Fulle S, Protasi F, Di Tano G, Pietrangelo T, Beltramin A, Boncompagni S, et al. The contribution of reactive oxygen species to sarcopenia and muscle ageing. *Experimental gerontology*. 2004;39(1):17-24. <https://doi.org/10.1016/j.exger.2003.09.012>
36. Peake J, Gatta PD, Cameron-Smith D. Aging and its effects on inflammation in skeletal muscle at rest and following exercise-induced muscle injury. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 2010;298(6):R1485-R95. <https://doi.org/10.1152/ajpregu.00467.2009>
37. Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, et al. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. *Journal of the American Medical Directors Association*. 2011;12(4):249-56. <https://doi.org/10.1016/j.jamda.2011.01.003>

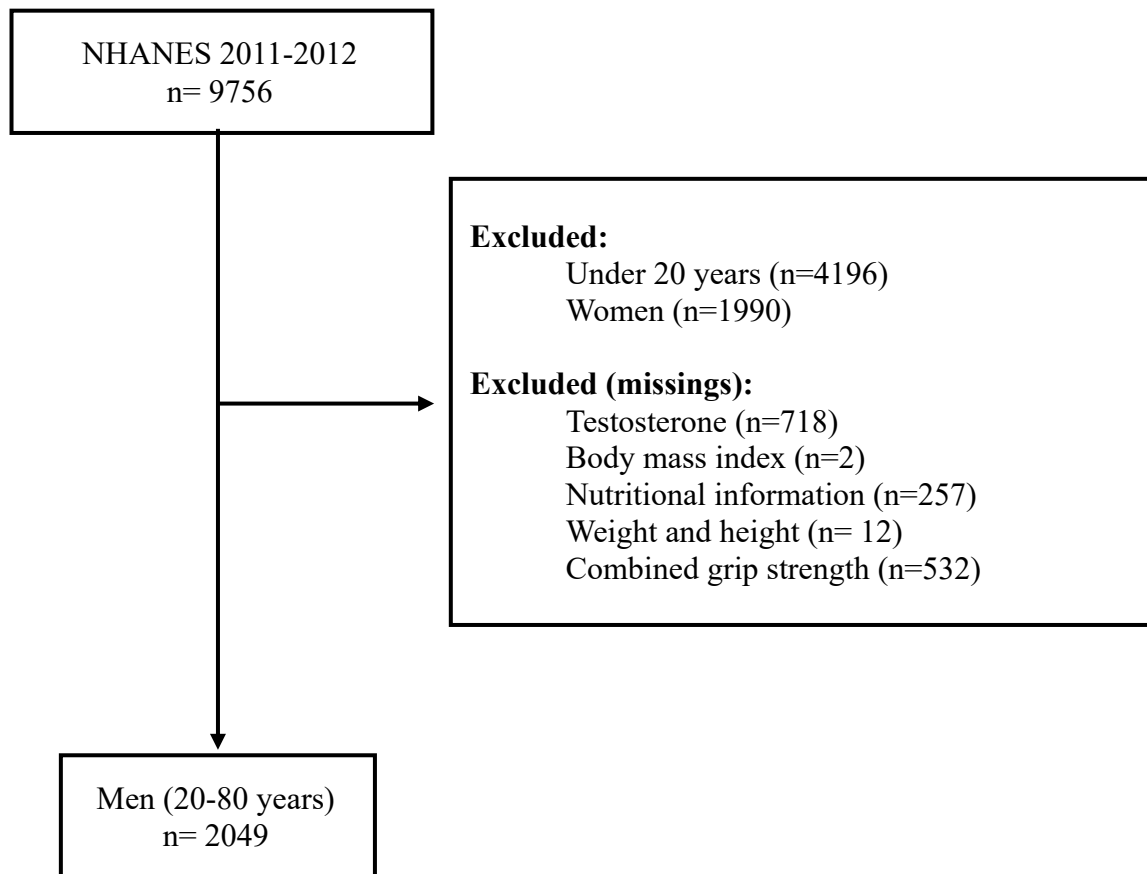


Figure 1. Flowchart of the sample selection from NHANES 2011 e 2012.

Table 1. Sociodemographic, health conditions and habits, physical activity, anthropometric and dietary intake of adult's men by tertiles of total testosterone concentration. NHANES, 2011 e 2012.

Variables	Tertiles of total testosterone				<i>p-trend</i>
	Total 409.8 (182.0)	Tertile 1 228.7 (66.5)	Tertile 2 384.2 (42.8)	Tertile 3 606.8 (141.1)	
Strength					
Combined grip strength (kg)	90.0 (18.2)	88.4 (20.3)	91.1 (17.3)	90.5 (16.8)	0.120
Demographic					
Age, years	46.6 (16.49)	49.2 (16.2)	45.7 (16.11)	45.2(16.7)	<0.001
Non-Hispanic white, %	69.2 (60.9 – 76.4)	70.4 (60.5 – 78.7)	66.7 (58.0 – 74.5)	70.4 (62.3 – 77.30)	0.997
Marital status, %					<0.001
Single/divorced/widowed/never married	35.7 (31.0 – 40.8)	28.9 (23.9 – 34.4)	34.2 (27.1 – 42.1)	43.7 (36.9 – 50.8)	
Married/ living as married	64.2 (59.2 – 69.0)	71.1 (65.5 – 76.0)	65.7 (57.8 – 72.9)	56.2 (49.1 – 63.0)	
Annual family income, %					0.033
\$0-19.999	16.3 (13.3 – 19.9)	13.3 (10.0 – 17.4)	16.4 (12.5 – 21.3)	19.1 (15.0 – 23.9)	
\$20.000-54.999	35.5 (31.3 – 39.6)	33.5 (28.0 – 39.5)	36.9 (29.9 – 44.5)	36.1 (30.7 – 41.9)	
\$55.000-74.999	11.9 (9.6 – 14.5)	12.8 (8.9 – 18.1)	12.6 (9.4 – 16.7)	10.2 (6.4 – 15.7)	
Over \$75.000	33.3 (1.7 – 4.5)	36.6 (29.5 – 44.4)	30.9 (24.0 – 38.9)	32.4 (25.5 – 40.1)	
Missing	2.8 (1.7 – 4.5)	3.5 (1.5 – 7.6)	2.9 (1.4 – 5.5)	2.0 (1.0 – 4.1)	
Educational level, %					0.872
High school graduate or under	37.8 (31.8 – 44.2)	38.5 (31.3 – 46.2)	37.2 (29.5 – 45.6)	37.8 (30.5 – 45.8)	
Some college or above	62.1 (55.7 – 68.1)	61.4 (53.7 – 68.6)	62.7 (54.3 – 70.4)	62.1 (54.1 – 69.4)	
Health conditions and habits					<0.001
Hypertension, %	31.3 (27.4 – 35.4)	37.4 (32.7 – 42.4)	32.0 (26.9 – 37.5)	24.8 (20.0 – 30.3)	
Diabetes, %					0.002
Pre-diabetes	1.7 (1.0 – 2.8)	2.4 (1.3 – 4.4)	1.5 (0.7 – 3.2)	1.1 (0.3 – 3.3)	
Yes	9.3 (7.9 – 11.0)	14.0 (11.4 – 17.1)	8.0 (5.9 – 10.8)	6.2 (3.9 – 9.8)	
No	88.8 (86.9 – 90.5)	83.4 (79.9 – 86.4)	90.3 (87.1 – 92.8)	92.6 (88.4 – 95.3)	
Arthritis, %					0.005
Yes	17.5 (14.9 – 20.4)	22.6 18.4 – 27.5)	14.2 (11.2 – 17.7)	15.9 (11.8 – 21.0)	
No	82.2 (79.2 – 84.8)	77.2 (72.3 – 81.4)	85.5 (81.8 – 88.6)	83.7 (78.3 – 88.0)	
Missing	0.18 (0.12 – 0.02)	0.08 (0.01 – 0.3)	0.10 (0.02 – 0.17)	0.020 (0.006 – 0.12)	
Smoking %					0.002
Yes	23.4 (20.3 – 26.7)	16.0 (12.1 – 20.7)	24.5 (19.5 – 31.0)	29.3 (24.7 – 34.9)	
Physical activity %					0.011
Yes	57.9 (52.2 – 63.5)	51.7 (44.0 – 59.3)	60.6 (55.3 – 65.6)	61.3 (53.7 – 68.4)	
No	42.0 (36.4 – 47.7)	48.2 (40.6 – 55.9)	39.3 (34.3 – 44.6)	38.6 (31.5 – 46.2)	
Anthropometrics					
Weight, kg	89.1 (19.4)	97.6 (22.5)	89.7 (17.5)	80.2 (13.7)	<0.001
Height, m	1.75 (0.7)	1.75 (0.7)	1.76 (0.7)	1.76 (0.7)	0.610
Body mass index, kg/m ²	28.7 (5.7)	31.5 (6.5)	28.8 (5.0)	25.8 (4.0)	<0.001
Dietary intake					
Energy, kcal	2480.0 (873.5)	2453.7 (864.5)	2450.2 (841.0)	2534.0 (908.1)	0.171
Carbohydrate, g	295.9 (115.7)	291.2 (112.4)	292.9 (110.5)	303.4 (122.8)	0.141
Protein, g	96.8 (37.0)	95.8 (36.1)	95.2 (37.3)	98.2 (37.4)	0.524
Protein, g/kg	1.2 (0.4)	1.0 (0.4)	1.1 (0.4)	1.3 (0.5)	<0.001
Lipids, g	93.2 (41.1)	95.1 (43.3)	89.9 (39.2)	94.6 (40.6)	0.869
Alcohol, g/day	14.2 (29.7)	10.7 (22.9)	16.5 (33.0)	15.4 (31.5)	0.039
Saturated fat, g	30.1 (14.9)	30.9 (15.5)	28.6 (13.9)	30.8 (15.1)	0.861
Monounsaturated fat, g	33.6 (15.6)	33.9 (16.2)	32.6 (15.2)	34.3 (15.4)	0.678
Polysaturated fat, g	21.7 (11.2)	22.5 (12.1)	21.2 (10.4)	21.6 (11.0)	0.267
Total ω-3, g	2.0 (0.4)	2.1 (1.1)	1.9 (1.0)	2.0 (1.1)	0.173
EPA, g	0.030 (0.007)	0.031 (0.008)	0.033 (0.007)	0.027 (0.006)	0.581
DHA, g	0.063 (0.006)	0.061 (0.001)	0.069 (0.002)	0.058 (0.001)	0.736
ALA, g	1.9 (1.1)	2.0 (1.1)	1.8 (1.0)	1.9 (1.1)	0.196
Fiber, g	19.8 (10.9)	19.6 (10.5)	19.1 (9.7)	20.6 (12.3)	0.308

Note: DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ALA: alpha linolenic acid.

Data described as mean (standard deviation) or percentage (confidence interval).

$p < 0.05$ are presented in bold type.

Tertiles of Serum testosterone levels: **Tertile 1:** 2.7 – 312.84 ng/dL; **Tertile 2:** 312.88 – 462.68 ng/dL; **Tertile 3:** 462.84 – 2543.99 ng/dL.

Table 2. Sociodemographic, health conditions and habits, physical activity, anthropometric and dietary intake of adults aged 20-80 years divided by age-range. NHANES, 2011-2012.

Variables	Classification by age-range			<i>p-trend</i>
	Young	Middle-aged	Older	
Strength				
Combined grip strength (kg)	97.1 (17.4)	91.4 (14.3)	77.0 (18.1)	<0.001
Demographic				
Age, years	29.1 (5.8)	49.3 (5.2)	68.9 (7.5)	<0.001
Non-Hispanic white, %	58.7 (50.1 – 66.8)	71.5 (62.2 – 79.3)	81.2 (74.4 – 86.6)	<0.001
Marital status, %				<0.001
Single/divorced/widowed/never married	48.3 (42.7 – 54.0)	33.7 (25.7 – 42.8)	19.8 (15.8 – 24.5)	
Married/ living as married	51.6 (45.9 – 57.2)	66.2 (57.1 – 74.2)	80.1 (74.4 – 84.1)	
Annual family income, %				0.052
\$0-19.999	22.4 (15.9 – 30.5)	13.2 (9.4 – 18.3)	12.0 (8.2 – 12.5)	
\$20.000-54.999	36.6 (31.9 – 41.6)	33.8 (28.0 – 40.0)	36.6 (30.9 – 42.7)	
\$55.000-74.999	10.8 (8.1 – 14.1)	9.6 (6.7 – 13.6)	17.1 (11.3 – 25.0)	
Over \$75.000	28.0 (21.7 – 35.2)	39.8 (31.6 – 48.7)	30.7 (23.3 – 39.2)	
Missing	2.0 (1.1 – 3.5)	3.2 (1.5 – 6.9)	3.2 (1.7 – 6.0)	
Educational level, %				0.260
High school graduate or under	34.7 (26.9 – 43.5)	40.3 (34.2 – 46.7)	38.5 (32.8 – 44.6)	
Some college or above	65.2 (56.4 – 73.0)	59.6 (53.2 – 65.7)	61.4 (55.3 – 67.1)	
Health conditions and habits				<0.001
Hypertension, %	11.5 (8.6 – 15.3)	33.5 (26.8 – 40.9)	57.7 (52.6 – 62.7)	
Missing	0.8 (0.2 – 0.3)	0	0.05 (0.008 – 0.03)	
Diabetes, %				<0.001
Pre-diabetes	0.5 (0.1 – 1.6)	0.2 (0.1 – 1.6)	0.2 (0.2 – 0.6)	
Yes	1.5 (0.7 – 3.0)	9.7 (6.5 – 14.3)	20.6 (15.8 – 26.5)	
No	97.8 (96.2 – 98.8)	88.1 (83.8 – 91.4)	76.3 (70.6 – 81.2)	
Missing	0	0	0.04 (0.005 – 0.3)	
Arthritis, %				<0.001
Yes	2.7 (1.4 – 5.3)	18.9 (14.9 – 23.6)	37.7 (32.1 – 43.6)	
No	97.2 (94.6 – 98.5)	80.9 (76.1 – 84.9)	61.6 (55.7 – 67.2)	
Missing	0	0.9 (0.1 – 7.1)	0.6 (0.4 – 0.9)	
Smoking %				0.102
Yes	23.5 (18.4 – 29.4)	27.6 (22.5 – 33.4)	16.4 (13.4 – 19.9)	
Physical activity %				<0.001
Yes	69.1 (63.4 – 74.2)	53.7 (46.0 – 61.2)	47.9 (42.5 – 53.3)	
No	30.8 (25.7 – 36.5)	46.2 (38.7 – 53.9)	52.0 (46.6 – 57.4)	
Anthropometrics				
Weight, kg	87.2 (20.3)	92.1 (17.3)	86.9 (20.5)	0.703
Height, m	1.76 (0.7)	1.76 (0.6)	1.74 (0.8)	<0.001
Body mass index, kg/m ²	27.8 (5.9)	29.6 (5.1)	28.5 (6.1)	0.081
Dietary intake				
Energy, kcal	2.657.6 (995.8)	2522.6 (735.9)	2141.6 (745.3)	<0.001
Carbohydrate, g	321.6 (131.9)	296.2 (95.2)	256.6 (106.2)	<0.001
Protein, g	103.1 (41.7)	98.1 (31.4)	84.9 (31.4)	<0.001
Protein, g/kg	1.2 (0.5)	1.0 (0.3)	1.0 (0.4)	<0.001
Lipids, g	98.4 (45.7)	95.7 (36.1)	81.3 (37.2)	<0.001

Alcohol, g/day	14.6 (29.9)	16.0 (31.0)	10.8 (22.0)	0.080
Saturated fat, g	31.9 (17.0)	31.1 (12.9)	25.7 (12.8)	<0.001
Monounsaturated fat, g	35.1 (16.9)	34.5 (13.7)	29.9 (15.6)	0.001
Polyunsaturated fat, g	23.0 (12.6)	22.1 (9.7)	19.2 (10.2)	<0.001
Total ω -3, g	2.1 (1.2)	2.0 (0.9)	1.8 (1.2)	0.022
EPA, g	0.033 (0.008)	0.028 (0.006)	0.029 (0.007)	0.330
DHA, g	0.06 (0.1)	0.05 (0.09)	0.06 (0.01)	0.704
ALA, g	2.0 (1.1)	1.9 (0.9)	1.8 (1.2)	0.024
Fiber, g	19.9 (11.1)	19.9 (9.0)	19.3 (13.6)	0.602
Biochemical parameters				
Total testosterone (ng/dL)	434.4 (175.9)	388.7 (146.9)	406.6 (243.0)	0.075

Note: DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ALA: alpha linolenic acid.

Data described as mean (standard deviation) or percentage (confidence interval).

p < 0.05 are presented in bold type.

Table 3. Linear regression between total testosterone concentration (ng.dL) and grip strength in men aged 20-80 years and divided by age-range. NHANES, 2011-2012.

		Model 1		Model 2	
		β (95% CI)	p-value	β (95% CI)	p-value
Combined Grip strength	Total	0.049 (-0.009 – 0.107)	0.097	0.981 (0.058 – 0.137)	<0.001*
	Young	0.017 (-0.056 – 0.091)	0.626	0.185 (0.149 – 0.266)	<0.001*
	Middle-aged	0.012 (-0.109 – 0.134)	0.826	0.010 (0.004 – 0.023)	0.041*
	Older	0.060 (-0.026 – 0.147)	0.163	0.018 (-0.060 – 0.097)	0.634

Note: Model 1: Without adjustment. Model 2: adjusted for energy (kcal/d), protein (g) and alcohol intake, smoking, age, physical activity, family income, marital status, race, educational level, diabetes, hypertension, arthritis, and BMI.

Values are shown as coefficients and 95% confidence intervals (95% CI).

5 CONCLUSÕES

Referente as nossas análises transversais, o estudo 1 mostrou que o consumo de colesterol não está associado com os níveis séricos de TT em homens americanos de 20 a 80 anos. No estudo 2, podemos observar associações positivas entre as concentrações séricas de testosterona e a soma da FPM (modelo ajustado por fatores de confusão) em homens jovens e de meia idade, mas não em homens idosos.

Assim, nossos achados sugerem que os níveis séricos de TT em homens de 20 a 80 anos não são dependentes do consumo de colesterol de acordo com os valores encontrados em nosso estudo. Entendemos que a testosterona é um hormônio importante para os níveis da força muscular, no entanto, de acordo com os nossos achados, os níveis de FPM não são determinados pelas concentrações séricas de TT em homens idosos. Hipoteticamente, a força muscular nesses indivíduos sofre interferências de outras variáveis.

6 PERSPECTIVAS FUTURAS

Devido à limitação dos estudos transversais, eles não possibilitam uma relação de causa efeito entre as variáveis investigadas. Nesse sentido, tais resultados necessitam ser confirmados através de desenhos de estudos que permitem evidenciar uma relação causal entre o consumo de colesterol com os níveis séricos de testosterona total e entre os níveis séricos de TT e a FPM em homens jovens, de meia idade e idosos.

REFERÊNCIAS

- ANAND, A.; GAJRA, A. Hand grip dynamometry as prognostic and predictive marker in older patients with cancer. **J Gerontol Geriatr Res**, v. 7, n. 10.4172, p. 2167-7182.1000471. 2018. <https://doi.org/10.4172/2167-7182.1000471>
- BAGHERI, R. *et al.* Whole egg vs. egg white ingestion during 12 weeks of resistance training in trained young males: A randomized controlled trial. **The Journal of Strength & Conditioning Research**, v. 35, n. 2, p. 411-419. 2021. <https://doi.org/10.1519/JSC.0000000000003922>
- BARRY, B. K.; CARSON, R. G. The consequences of resistance training for movement control in older adults. **The Journals of Gerontology Series A: Biological Sciences and Medical Sciences**, v. 59, n. 7, p. M730-M754. 2004. <https://doi.org/10.1093/gerona/59.7.M730>
- BAUMGARTNER, R. N. *et al.* Predictors of skeletal muscle mass in elderly men and women. **Mechanisms of ageing and development**, v. 107, n. 2, p. 123-136. 1999. [https://doi.org/10.1016/S0047-6374\(98\)00130-4](https://doi.org/10.1016/S0047-6374(98)00130-4)
- BERG, J.; TYMOCZKO, J.; STRYER, L. B. **Bioquímica**. 7ª edição. Guanabara Koogan, 2014.
- BERGER, S. *et al.* Dietary cholesterol and cardiovascular disease: a systematic review and meta-analysis. **The American journal of clinical nutrition**, v. 102, n. 2, p. 276-294. 2015. <https://doi.org/10.3945/ajcn.114.100305>
- BHASIN, S. *et al.* Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. **The Journal of Clinical Endocrinology & Metabolism**, v. 95, n. 6, p. 2536-2559. 2010. <https://doi.org/10.1210/jc.2009-2354>
- BHASIN, S. *et al.* The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. **New England Journal of Medicine**, v. 335, n. 1, p. 1-7. 1996. <https://doi.org/10.1056/NEJM199607043350101>

BORST, S. E.; YARROW, J. F. Injection of testosterone may be safer and more effective than transdermal administration for combating loss of muscle and bone in older men. **American Journal of Physiology-Endocrinology and Metabolism**, v. 308, n. 12, p. E1035-E1042. 2015. <https://doi.org/10.1152/ajpendo.00111.2015>

BRILL, P. A. *et al.* Muscular strength and physical function. **Medicine and science in sports and exercise**, v. 32, n. 2, p. 412-416. 2000. <https://doi.org/10.1097/00005768-200002000-00023>

BRINKMANN, A. O. Molecular mechanisms of androgen action—a historical perspective. **Methods Mol Biol.**, v. 776, p. 3-24. 2011. https://doi.org/10.1007/978-1-61779-243-4_1

CHEN, T. C. *et al.* National health and nutrition examination survey: estimation procedures, 2011–2014. *Vital Health Stat*, n. 177, p. 1-26. 2018. <https://doi.org/10.1080/13685538.2019.1632282>

CHIU, H. T.; SHIH, M. T.; CHEN, W. L. Examining the association between grip strength and testosterone. **The Aging Male**, v. 23, n. 5, p. 915-922. 2020.

CDC. CENTERS FOR DISEASE CONTROL AND PREVENTION. National Health and Nutrition Examination Survey questionnaire (or examination protocol, or laboratory protocol). **Hyattsville, MD: US Department of Health and Human Services, Centers for Disease Control and Prevention.** 2012. Acesso em: 08 nov 2022. Disponível em: <https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/questionnaires.aspx?BeginYear=2011>.

CONWAY, A. J. *et al.* Use, misuse and abuse of androgens: The Endocrine Society of Australia consensus guidelines for androgen prescribing. **Medical Journal of Australia**, v. 172, n. 5, p. 220-224. 2000. <https://doi.org/10.5694/j.1326-5377.2000.tb123913.x>

CRUZ-JENTOFT, A. J. *et al.* Sarcopenia: revised European consensus on definition and diagnosis. **Age and ageing**, v. 48, n. 1, p. 16-31. 2019.

<https://doi.org/10.1093/ageing/afz046>

DANDONA, P.; ALJADA, A.; BANDYOPADHYAY, A. Inflammation: the link between insulin resistance, obesity and diabetes. **Trends in immunology**, v. 25, n. 1, p. 4-7. 2004. <https://doi.org/10.1016/j.it.2003.10.013>

DESALVO, K. B.; OLSON, R.; CASAVALLE, K. O. Dietary guidelines for Americans. **JAMA**, v. 315, n. 5, p. 457-458. 2016.

DESCHENES, M. *et al.* The effects of exercise training of different intensities on neuromuscular junction morphology. **Journal of neurocytology**, v. 22, n. 8, p. 603-615. 1993. <https://doi.org/10.1001/jama.2015.18396>

DIETSCHY, J. M.; WILSON, J. D. Regulation of cholesterol metabolism. **New England Journal of Medicine**, v. 282, n. 22, p. 1241-1249. 1970. <https://doi.org/10.1056/NEJM197005282822206>

DOHERTY, T. J.; VANDERVOORT, A. A.; BROWN, W. F. Effects of ageing on the motor unit: a brief review. **Canadian journal of applied physiology**, v. 18, n. 4, p. 331-358. 1993. <https://doi.org/10.1139/h93-029>

ENOKA, R. M. *et al.* Mechanisms that contribute to differences in motor performance between young and old adults. **Journal of Electromyography and Kinesiology**, v. 13, n. 1, p. 1-12. 2003. [https://doi.org/10.1016/S1050-6411\(02\)00084-6](https://doi.org/10.1016/S1050-6411(02)00084-6)

FLECK, S. J.; KRAEMER, W. J. **Fundamentos do treinamento de força muscular**. 4ª Edição. Artmed Editora, 2017.

FRONTERA, W. R. *et al.* Aging of skeletal muscle: a 12-yr longitudinal study. **Journal of applied physiology**, v. 88, n. 4, p. 1321-1326. 2000. <https://doi.org/10.1152/jappl.2000.88.4.1321>

GROSSMANN, M. Low testosterone in men with type 2 diabetes: significance and treatment. **The journal of clinical endocrinology & metabolism**, v. 96, n. 8, p. 2341-2353. 2011. <https://doi.org/10.1210/jc.2011-0118>

GROSSMANN, M.; MATSUMOTO, A. M. A perspective on middle-aged and older men with functional hypogonadism: focus on holistic management. **The Journal of Clinical Endocrinology & Metabolism**, v. 102, n. 3, p. 1067-1075. 2017. <https://doi.org/10.1210/jc.2016-3580>

HÄMÄLÄINEN, E. *et al.* Diet and serum sex hormones in healthy men. **Journal of steroid biochemistry**, v. 20, n. 1, p. 459-464. 1984. [https://doi.org/10.1016/0022-4731\(84\)90254-1](https://doi.org/10.1016/0022-4731(84)90254-1)

HARMAN, N. L.; LEEDS, A. R.; GRIFFIN, B. A. Increased dietary cholesterol does not increase plasma low density lipoprotein when accompanied by an energy-restricted diet and weight loss. **European journal of nutrition**, v. 47, n. 6, p. 287-293. 2008. <https://doi.org/10.1007/s00394-008-0730-y>

HARPER, A. Dietary guidelines challenge animal product consumption. **Feedstuffs**, 1993. [https://doi.org/10.1016/S0006-291X\(76\)80251-3](https://doi.org/10.1016/S0006-291X(76)80251-3)

HOFFMAN, J. R. *et al.* Position stand on androgen and human growth hormone use. **The Journal of Strength & Conditioning Research**, v. 23, p. S1-S59. 2009. <https://doi.org/10.1519/JSC.0b013e31819df2e6>

HSUEH, A.; DAFAU, M.; CATT, K. Regulation of luteinizing hormone receptors in testicular interstitial cells by gonadotropin. **Biochemical and Biophysical Research Communications**, v. 72, n. 3, p. 1145-1152. 1976. [https://doi.org/10.1016/S0006-291X\(76\)80251-3](https://doi.org/10.1016/S0006-291X(76)80251-3)

HUHTANIEMI, I. T. **Andropause—lessons from the European male ageing study**. Elsevier. 128-131. 2014. <https://doi.org/10.1016/j.ando.2014.03.005>

ISO, H. *et al.* Prospective study of fat and protein intake and risk of intraparenchymal hemorrhage in women. **Circulation**, v. 103, n. 6, p. 856-863. 2001.

<https://doi.org/10.1161/01.CIR.103.6.856>

JOHNSON, L. R.; WOOD, R. I. Oral testosterone self-administration in male hamsters. **Neuroendocrinology**, v. 73, n. 4, p. 285-292. 2001. <https://doi.org/10.1159/000054645>

KANE, J. P.; HARDMAN, D. A.; PAULUS, H. E. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. **Proceedings of the National Academy of Sciences**, v. 77, n. 5, p. 2465-2469. 1980.

<https://doi.org/10.1073/pnas.77.5.2465>

KELLY, D.; JONES, T. Testosterone and obesity. **Obesity Reviews**, v. 16, n. 7, p. 581-606. 2015. <https://doi.org/10.1111/obr.12282>

KEMMLER, W. *et al.* Changes of maximum leg strength indices during adulthood a cross-sectional study with non-athletic men aged 19–91. **Frontiers in Physiology**, v. 9, p. 1524. 2018. <https://doi.org/10.3389/fphys.2018.01524>

KICMAN, A. T. Pharmacology of anabolic steroids. **British journal of pharmacology**, v. 154, n. 3, p. 502-521. 2008. <https://doi.org/10.1038/bjp.2008.165>

KLEIN, C.; RICE, C.; MARSH, G. Normalized force, activation, and coactivation in the arm muscles of young and old men. **Journal of Applied Physiology**, v. 91, n. 3, p. 1341-1349. 2001. <https://doi.org/10.1152/jappl.2001.91.3.1341>

KNUTTGEN, H. G.; KRAEMER, W. J. Terminology and measurement. **Journal of applied sport science research**, v. 1, n. 1, p. 1-10. 1987.

<https://doi.org/10.1519/00124278-198702000-00001>

KVORNING, T. *et al.* Suppression of endogenous testosterone production attenuates the response to strength training: a randomized, placebo-controlled, and blinded intervention study. **American Journal of Physiology-Endocrinology and**

Metabolism, v. 291, n. 6, p. E1325-E1332. 2006.

<https://doi.org/10.1152/ajpendo.00143.2006>

LARSSON, L.; GRIMBY, G.; KARLSSON, J. Muscle strength and speed of movement in relation to age and muscle morphology. **Journal of Applied Physiology**, v. 46, n. 3, p. 451-456. 1979. <https://doi.org/10.1152/jappl.1979.46.3.451>

LARSSON, L.; LI, X.; FRONTERA, W. Effects of aging on shortening velocity and myosin isoform composition in single human skeletal muscle cells. **American Journal of Physiology-Cell Physiology**, v. 272, n. 2, p. C638-C649. 1997.

<https://doi.org/10.1152/ajpcell.1997.272.2.C638>

LECERF, J.M.; DE LORGERIL, M. Dietary cholesterol: from physiology to cardiovascular risk. **British Journal of Nutrition**, v. 106, n. 1, p. 6-14. 2011.

<https://doi.org/10.1017/S0007114511000237>

LEONG, D. P. *et al.* Prognostic value of grip strength: findings from the Prospective Urban Rural Epidemiology (PURE) study. **The Lancet**, v. 386, n. 9990, p. 266-273. 2015. [https://doi.org/10.1016/S0140-6736\(14\)62000-6](https://doi.org/10.1016/S0140-6736(14)62000-6)

LUO, J. *et al.* Intracellular cholesterol transport by sterol transfer proteins at membrane contact sites. **Trends in biochemical sciences**, v. 44, n. 3, p. 273-292. 2019.

<https://doi.org/10.1016/j.tibs.2018.10.001>

MACALUSO, A. *et al.* Contractile muscle volume and agonist-antagonist coactivation account for differences in torque between young and older women. **Muscle & nerve**, v. 25, n. 6, p. 858-863. 2002. <https://doi.org/10.1002/mus.10113>

MATSUMOTO, A. M.; BREMNER, W. J. Serum testosterone assays—accuracy matters. **The Journal of Clinical Endocrinology & Metabolism**, v. 89, n. 2, p. 520-524. 2004. <https://doi.org/10.1210/jc.2003-032175>

MILES, R. Eggs important in diet, unfairly criticized as heart disease risk. **Feedstuffs**, p. 26-51. 1989.

- MILLER, W. L. Molecular biology of steroid hormone synthesis. **Endocrine reviews**, v. 9, n. 3, p. 295-318. 1988. <https://doi.org/10.1210/edrv-9-3-295>
- MORALES, A. The long and tortuous history of the discovery of testosterone and its clinical application. **The journal of sexual medicine**, v. 10, n. 4, p. 1178-1183. 2013. <https://doi.org/10.1111/jsm.12081>
- MORLEY, J. E. *et al.* Validation of a screening questionnaire for androgen deficiency in aging males. **Metabolism**, v. 49, n. 9, p. 1239-1242. 2000. <https://doi.org/10.1053/meta.2000.8625>
- MURALEEDHARAN, V. *et al.* Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. **Eur J Endocrinol**, v. 169, n. 6, p. 725-733. 2013. <https://doi.org/10.1530/EJE-13-0321>
- NAHHAS, R. W. *et al.* Bayesian longitudinal plateau model of adult grip strength. **American Journal of Human Biology**, v. 22, n. 5, p. 648-656. 2010. <https://doi.org/10.1002/ajhb.21057>
- ONG, H. L. *et al.* Hand-grip strength among older adults in Singapore: a comparison with international norms and associative factors. **BMC geriatrics**, v. 17, n. 1, p. 1-11. 2017. <https://doi.org/10.1186/s12877-017-0565-6>
- PÉREZ-ZEPEDA, M. U. *et al.* A frailty index from next-of-kin data: a cross-sectional analysis from the Mexican health and aging study. **BioMed research international**, v. 2017. 2017. <https://doi.org/10.1155/2017/6069374>
- PETERSON, M. *et al.* Muscle weakness thresholds for prediction of diabetes in adults. **Sports medicine**, v. 46, n. 5, p. 619-628. 2016. <https://doi.org/10.1007/s40279-015-0463-z>
- PRIEGO, T. *et al.* Role of hormones in sarcopenia. *In: Vitamins and Hormones*: Elsevier. v. 115, p. 535-570. 2021. <https://doi.org/10.1016/bs.vh.2020.12.021>

RASTRELLI, G. *et al.* Development of and recovery from secondary hypogonadism in aging men: prospective results from the EMAS. **The Journal of Clinical Endocrinology & Metabolism**, v. 100, n. 8, p. 3172-3182. 2015.

<https://doi.org/10.1210/jc.2015-1571>

RIEBE, D. *et al.* Diretrizes do ACSM para os testes de esforço e sua prescrição. : Philadelphia: Guanabara Koogan. 2018.

ROMIJN, M. D. *et al.* Mild chronic kidney disease is associated with cognitive function in patients presenting at a memory clinic. **International journal of geriatric psychiatry**, v. 30, n. 7, p. 758-765. 2015. <https://doi.org/10.1002/gps.4226>

SAAD, F. *et al.* Testosterone deficiency and testosterone treatment in older men. **Gerontology**, v. 63, n. 2, p. 144-156. 2017. <https://doi.org/10.1159/000452499>

SHIN, M. J.; JEON, Y. K.; KIM, I. J. Testosterone and sarcopenia. **The world journal of men's health**, v. 36, n. 3, p. 192-198. 2018. <https://doi.org/10.5534/wjmh.180001>

TRABERT, B. *et al.* Relationship of sex steroid hormones with body size and with body composition measured by dual-energy X-ray absorptiometry in US men. **Cancer Causes & Control**, v. 23, n. 12, p. 1881-1891. 2012. <https://doi.org/10.1007/s10552-012-0024-9>

TRAVISON, T. G. *et al.* Harmonized reference ranges for circulating testosterone levels in men of four cohort studies in the United States and Europe. **The Journal of Clinical Endocrinology & Metabolism**, v. 102, n. 4, p. 1161-1173. 2017. <https://doi.org/10.1210/jc.2016-2935>

U.S. Department of Agriculture and U.S. Department of Health and Human Services. **Dietary Guidelines for Americans**, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010.

VANDERSCHUEREN, D. *et al.* Sex steroid actions in male bone. **Endocrine reviews**, v. 35, n. 6, p. 906-960. 2014. <https://doi.org/10.1210/er.2014-1024>

VELDHUIS, J. D.; CARLSON, M. L.; JOHNSON, M. L. The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. **Proceedings of the National Academy of Sciences**, v. 84, n. 21, p. 7686-7690. 1987.

<https://doi.org/10.1073/pnas.84.21.7686>

VINGREN, J. L.; *et al.* Testosterone physiology in resistance exercise and training. **Sports medicine**, v. 40, n. 12, p. 1037-1053. 2010. <https://doi.org/10.2165/11536910-000000000-00000>

WETTSTEIN, A. Biosynthèse des hormones stéroïdes. **Experientia**, v. 17, n. 8, p. 329-344. 1961. <https://doi.org/10.1007/BF02201749>

WHITTAKER, J.; WU, K. Low-fat diets and testosterone in men: Systematic review and meta-analysis of intervention studies. **The Journal of Steroid Biochemistry and Molecular Biology**, v. 210, p. 105878. 2021. <https://doi.org/10.1016/j.jsbmb.2021.105878>

WIEDMER, P. *et al.* Sarcopenia—Molecular mechanisms and open questions. **Ageing research reviews**, v. 65, p. 101200. 2021. <https://doi.org/10.1016/j.arr.2020.101200>

WILSON, J. M. *et al.* Effects of ketogenic dieting on body composition, strength, power, and hormonal profiles in resistance training men. **The Journal of Strength & Conditioning Research**, v. 34, n. 12, p. 3463-3474. 2020. <https://doi.org/10.1519/JSC.0000000000001935>

YUKI, A. *et al.* Relationship between low free testosterone levels and loss of muscle mass. **Scientific reports**, v. 3, n. 1, p. 1-5. 2013. <https://doi.org/10.1038/srep01818>

ZAROTSKY, V. *et al.* Systematic literature review of the epidemiology of nongenetic forms of hypogonadism in adult males. **Journal of Hormones**. 2014a. <https://doi.org/10.1155/2014/190347>

ZAROTSKY, V. *et al.* Systematic literature review of the risk factors, comorbidities, and consequences of hypogonadism in men. **Andrology**, v. 2, n. 6, p. 819-834. 2014b.
<https://doi.org/10.1111/andr.274>