OLIVIA GONÇALVES LEÃO COELHO

EFEITO DO SUCO E DE DERIVADOS DA UVA NA GLICEMIA, NO APETITE E NOS MARCADORES DE GLICAÇÃO, VISANDO O CONTROLE DO EXCESSO DE PESO

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência da Nutrição, para obtenção do título de *Doctor Scientiae*.

Orientadora: Rita de Cássia G. Alfenas

VIÇOSA - MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

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Coelho, Olívia Gonçalves Leão, 1990-
Efeito do suco e de derivados da uva na glicemia, no apetite e nos marcadores de glicação, visando o controle do excesso de peso / Olívia Gonçalves Leão Coelho. - Viçosa, MG, 2021.
110 f. : il. (algumas color.) ; 29 cm.

Orientador: Rita de Cássia Gonçalves Alfenas.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Peso corporal - Regulação. 2. Fenóis. 3. Glicemia. 4. Apetite. 5. Uva. I. Universidade Federal de Viçosa. Departamento de Nutrição e Saúde. Programa de Pós-Graduação em Ciência da Nutrição. II. Título.

CDD 22. ed. 613.25

Bibliotecário(a) responsável: Alice Regina Pinto Pires CRB6 2523

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APROVADA: 22 de julho de 2021.

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Aos meus pais, exemplos de amor e dedicação.

AGRADECIMENTOS

A Deus, pela minha vida, saúde e amparo, por iluminar e guiar meu caminho. Aos meus pais, Alexandre e Ana Angélica, por serem exemplo de resiliência e honestidade. Por acreditarem no meu potencial e me apoiarem em todas as escolhas ao longo da vida.

À minha irmã Iara, por sua presença repleta de bondade, leveza e amizade.

Ao meu marido Fillipe, pelo amor, paciência e incentivo. Sempre presente nos momentos de alegria e angústias e a cada conquista celebrada.

À minha querida orientadora Rita de Cássia Gonçalves Alfenas, faltam palavras para expressar minha gratidão e admiração. Desde o Mestrado, são 8 anos de trabalho, suporte, acolhimento, confiança, amizade e respeito. Exemplo de ser humano e profissional que será sempre minha inspiração.

Ao professor Dr. Richard Mattes, por me receber tão bem durante o estágio sanduíche, e pela orientação de pesquisa no Laboratory of Ingestive and Sensory Studies, na Purdue University (IN, EUA).

Às amigas e companheiras de trabalho do Laboratório de Estudos em Ingestão Alimentar (LEIA), por compartilharem os aprendizados, angústias e alegrias, e por tornarem tudo mais leve e divertido! Em especial, à querida amiga e parceira Priscila, minha “dupla” desde o primeiro dia do doutorado, por nunca largar a minha mão, superando juntas todos os desafios! E aos colegas do Laboratory for Sensory and Ingestive Studies (Purdue University) pelas experiências e aprendizados que enriqueceram o ano que passamos juntos.

Aos meus amigos e familiares, que sempre torceram por mim e me proporcionaram momentos de descontração e alegria. Repouso certo nas horas incertas.

Aos bons amigos que fiz em West Lafayette-USA no período em que lá vivi, pelo suporte, companheirismo e por serem minha família quando eu estava longe de casa.

A todos os participantes dos estudos realizado na Universidade Purdue, pelo comprometimento, trocas e compreensão. Sem eles, não seria possível.

Às professoras Juliana, Júnia, Flávia e ao professor Jorge, por aceitarem o convite para participar da banca examinadora e contribuir para o enriquecimento deste trabalho.

À CAPES pelo apoio financeiro, concedendo não só a bolsa de estudos durante o doutorado, como também a bolsa do doutorado sanduíche, onde fiquei 1 ano na Purdue University, EUA.

Ao Departamento de Nutrição e Saúde da UFV, seus professores e funcionários, pelo prazeroso convívio e aprendizado contínuo, desde 2008, quando ingressei na graduação.

À Universidade Federal de Viçosa, por me proporcionar, com excelência, tamanho crescimento pessoal e profissional…sentirei saudades!!!

“Seja qual for seu sonho, comece. A ousadia contém genialidade, poder e magia.”

(Johann Goethe)

RESUMO

COELHO, Olívia Gonçalves Leão, D.Sc., Universidade Federal de Viçosa, julho de 2021. **Efeito do suco e de derivados da uva na glicemia, no apetite e nos marcadores de glicação, visando o controle do excesso de peso.** Orientadora: Rita de Cássia Gonçalves Alfenas.

O controle da ingestão alimentar pode modular a fisiopatologia do excesso de peso. O suco de uva Concord e outros produtos derivados da uva, ricos em compostos fenólicos, podem aumentar a saciedade e modular a glicemia. No entanto, os efeitos do suco de uva Concord na resposta glicêmica de 24h, sensações de apetite e função cognitiva ainda não foram avaliados. Os efeitos dos derivados da uva na ingestão alimentar, no apetite e nos marcadores de glicação são inconclusivos. Assim, os objetivos dos estudos aqui apresentados foram avaliar os efeitos do consumo do suco e de produtos da uva na resposta glicêmica, no apetite e nos marcadores de glicação visando o controle do excesso de peso. Esta tese é composta de três artigos, sendo dois referentes a estudos de revisão sistemática (artigos 1 e 2) e um estudo clínico (artigo 3). **METODOLOGIA: Artigos 1 e 2 –** Analisou-se criticamente artigos identificados no PubMed, Scopus, The Cochrane Register of Clinical Trial (artigos 1 e 2), e Embase (artigo 1). No artigo 1, os estudos investigaram os efeitos dos compostos fenólicos da uva em marcadores de glicação precoce e avançada e receptores. No artigo 2, selecionaram-se estudos que avaliaram os efeitos dos produtos da uva sobre hormônios intestinais, apetite e ingestão alimentar. **Artigo 3 -** Trata-se de dois ensaios clínicos randomizados, *crossover*, duplo-cegos, em que os participantes consumiram três bebidas: suco integral de uva Concord (CGJ), bebida sem polifenóis e com mesmo sabor do CGJ (LP) e bebida sem polifenóis e com intensidade de sabor reduzida (LPF). As bebidas foram consumidas sozinhas (experimento I) e com alimento (experimento II). Glicemia de 24 h foi medida pelo monitoramento contínuo da glicose. Excreção de polifenóis foi avaliada na urina de 24 h. Apetite e função cognitiva foram avaliados a cada 1h até a hora de dormir, utilizando escalas analógicas visuais. **RESULTADOS: Artigo 1 -** Sete estudos foram selecionados. Polifenóis da uva reduziram frutosamina. Quercetina reduziu metilglioxal. Resveratrol aumentou a expressão do gene do receptor endógeno de produtos de glicação avançada, sem afetar sua concentração sérica. **Artigo 2 -** Seis estudos foram selecionados, sendo avaliados os efeitos do extrato de semente de uva, da uva passa

e do suco de uva. Os produtos da uva modularam hormônios que controlam o apetite, mas a ingestão alimentar não foi afetada. **Artigo 3 -** Quando consumidas com alimento, CGJ e LP reduziram fome, desejo de comer e consumo prospectivo. Quando CGJ foi consumido sozinho, indivíduos considerados maiores excretores de polifenóis tiveram menor resposta glicêmica. **CONCLUSÕES:** Mais estudos clínicos são necessários para compreender o efeito anti-glicativo dos compostos fenólicos da uva. Produtos derivados da uva modularam a secreção de hormônios intestinais capazes de controlar o apetite, sem afetar a ingestão alimentar. Fenólicos naturais e intensidade do sabor do suco de uva moderaram apetite e glicemia de adultos com excesso de peso, sendo esses efeitos modificados pela presença/ausência de alimento na mesma refeição.

Palavras-chave: Compostos fenólicos. Excesso de peso. Glicemia. Saciedade. Uva.

ABSTRACT

COELHO, Olívia Gonçalves Leão, D.Sc., Universidade Federal de Viçosa, July, 2021. **Effect of grape juice and grape products on blood glucose, appetite and glycation markers, on excess body weight control**. Adviser: Rita de Cássia Gonçalves Alfenas.

Food intake can modulate the pathophysiology of excess body weight. Concord grape juice and other grape products, rich in phenolic compounds, can increase satiety and modulate glycemia. However, the Concord grape juice effects on 24-h glycemic response, appetite sensations, and cognitive function have not been evaluated. The effects of grape products on food intake, appetite, and glycation markers are still inconclusive. Thus, the studies presented here aimed to evaluate the effects of grape juice and grape products consumption on glycemic response, appetite, and glycation markers on excess body weight control. This thesis is composed of three articles, from which two are systematic reviews (articles 1 and 2) and one refers to a clinical study (article 3). **METHODOLOGY: Articles 1 and 2** - Studies identified in PubMed, Scopus, The Cochrane Register of Clinical Trial, and Embase (article 1) were critically analyzed. In article 1, the selected studies investigated the effects of grape phenolic compounds on early and advanced glycation markers and receptors. In article 2, we selected studies that evaluated the effects of grape products on intestinal hormones, appetite, and food intake in adults. **Article 3** - These were two randomized, crossover, double-blind clinical trials in which participants consumed three beverages: 100% Concord grape juice (CGJ), a beverage without polyphenols and the same flavor intensity as CGJ (LP), and a beverage without polyphenols and reduced flavor intensity (LPF). The beverages were consumed alone (trial I) and with food (trial II). The 24h glycemia was measured by continuous glucose monitoring and excretion of polyphenols was evaluated through 24h urine collection. Appetite and cognitive function were assessed hourly using visual analog scales during 4h after beverage intake. **RESULTS: Article 1** - Seven studies were selected. Grape polyphenols reduced fructosamine. Quercetin reduced methylglyoxal. Resveratrol increased endogenous receptor gene expression of advanced glycation products, without affecting its serum concentration. **Article 2** - Six studies were selected, which evaluated the effects of grape seed extract, raisins, and grape juice. Grape products modulated hormones that control appetite, without affecting food intake. **Article 3** -

When consumed with food, CGJ and LP reduced hunger, desire to eat, and prospective consumption. When CGJ was consumed alone, higher polyphenol excreters subjects had lower glycemic response. **CONCLUSIONS**: Future clinical trials are necessary to understand better the anti-glycative effect of grape phenolic compounds. Grape products modulated intestinal hormones secretion capable of controlling appetite without affecting food intake. Grape juice natural phenolics and flavor intensity moderate appetite and blood glucose in overweight adults, and these effects are modified by the presence/absence of food at the same meal.

Keywords: Glycemia. Grapes. Excess body weight. Phenolic compounds. Satiety.

LISTA DE ILUSTRAÇÕES

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ARTIGO 1

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LISTA DE SIGLAS E ABREVIATURAS

3-DG	3-deoxyglucosone
μl	Microliter
AGEs	Advanced glycation end products
AgRP	Agouti-related protein
ALT	Alanine transaminase
AST	Aspartate transaminase
$_{t}$AUC	Total area under the curve
BMI	Body mass index
BSA	Body surface area
CART	Cocaine- and amphetamine-regulated transcript
CAT	Catalase
CVD	Cardiovascular disease
CGJ	Concord Grape juice
CGM	Continuous Glucose Monitor
cRAGE	Cleaved isoform of receptor of advanced glycation end product
dAGE	Dietary advanced glycation end product
DBP	Diastolic blood pressure
DCNT	Doença crônica não transmissível
dl	Deciliters
DPP-IV	Dipeptidyl peptidase 4
EGPs	Early glycation end products
ERK	Extracellular signal-regulated kinase
esRAGE	Endogenous secretory receptor of advanced glycation end product
GGT	Gamma-glutamyl transferase
GLO-1	Glyoxalase 1
GLP-1	Glucagon-like peptide-1
GO	Glyoxal
GPE	Grape polyphenol extract
GSE	Grape seed extract
GSH	Reduced glutathione
GSPE	Grape seed proanthocyanidin extract
FBG	Fasting blood glucose
HbA1c	Glycated hemoglobin
HDL-C	High density lipoprotein cholesterol
HED	Human equivalent dose
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
hsCRP	Highly sensitive C-reactive protein
JNK	c-jun N-terminal kinase
kg	Kilograms
Km	Specific constant
LDL-C	Low density lipoprotein cholesterol

LP	Polyphenol-free grape flavored drink with the same flavor essence
LPF	Polyphenol-free grape flavored drink with reduced flavor essence
MDA	Malondialdeyde
mg	Miligram
ml	Militers
MDA	Malondialdeyde
MGO	Methylglyoxal
mRNA	Messenger RNA
MGJ	Muscadine grape juice
NF-κB	Nuclear factor κB
NPY	Neuropeptide Y
Obrb	Leptin longest receptor isoform
PGE	Polyphenolic grape extract
POMC	Pro-opiomelanocortin
PPAR-γ	Peroxisome proliferators-activated receptors
PYY	Peptide YY
RAGE	Receptor of advanced glycation end product
RCT	Randomized clinical trial
SBP	Systolic blood pressure
ROS	Reactive oxygen species
SE	Standard error
SOD	Superoxide dismutase
SGD	Substitute grape-flavored drink
SGOT	Serum glutamate oxaloacetate transaminase
SGPT	Serum glutamate pyruvate transaminase
STAT3	Signal transducer and activator of transcription 3
T2DM	Type 2 diabetes mellitus
TAOS	Total antioxidant status
TBARS	Thiobarbituric acid-reactive substances
TBW	Total body water
TC	Total cholesterol
TG	Triglycerides
TGF-ß1	Transforming growth factor beta 1
VAS	Visual analogue scale

LISTA DE SÍMBOLOS

%	Percentual
<	Less than
>	Bigger than
n	Number
vs	Versus
↑	Increased
↓	Decreased
↔	Unchanged

SUMÁRIO

1. INTRODUÇÃO

Atualmente, a maioria da população mundial vive em países onde o excesso de peso é responsável por uma mortalidade maior que a verificada para o baixo peso (WHO, 2018). No Brasil, cerca de 60% dos adultos possuem excesso de peso, sendo que a prevalência de obesidade aumentou em mais de 100% em 16 anos (IBGE, 2020). Esses dados são preocupantes, pois trata-se de uma doença crônica não transmissível (DCNT) que se configura entre uma das causas centrais de morte do mundo, por aumentar o risco de diabetes mellitus tipo 2 (DM2), hipertensão arterial, doenças cardiovasculares (DCV) e alguns tipos de câncer (WHO, 2018). Dentre as principais causas do excesso de peso, destacam-se a adoção de hábitos alimentares inadequados (IBGE, 2020; WRIGHT et al., 2017), a compulsão alimentar (BOSWELL; KOBER, 2016), o sedentarismo (KOKKINOS, 2012; PUGLISI et al., 2009) e a predisposição genética (GADDE et al., 2018). Sendo assim, é de extrema relevância a adoção de estratégias para a prevenção do excesso de peso, principalmente entre os indivíduos eutróficos, porém com peso corporal no limite superior para o sobrepeso e percentual de gordura ou perímetro da cintura elevados (CHOOI; DING; MAGKOS, 2018).

O consumo alimentar exacerbado e não saudável promove o ganho de peso (WRIGHT et al., 2017) e o aumento da gordura corporal (GEIKER et al., 2018). A hipertrofia do tecido adiposo, decorrente do ganho de peso, gera hipóxia nos adipócitos, ativa a produção de citocinas pró-inflamatórias e a formação de radicais livres, caracterizando o estado de inflamação subclínica e o estresse oxidativo presentes nos indivíduos com excesso de peso (GADDE et al., 2018). Tal condição constitui a base para desordens metabólicas, como a hiperglicemia. A variação da glicemia durante o dia tem implicações à saúde. Sabe-se que a glicemia de jejum não é tão fortemente associada ao risco de DCNT quanto a glicemia pós-prandial (CAVALOT et al., 2006; HANEFELD et al., 1996). Desta maneira, um dos alvos da intervenção dietética para prevenção do diabetes mellitus tipo 2 e suas complicações é o manejo da glicemia pós-prandial durante o dia.

A hiperglicemia permanente, aliada ao estresse oxidativo e à inflamação subclínica (TAVARES et al., 2020), também está associado à formação de produtos de glicação precoce (EGPs) (AHMAD et al., 2013) e avançada (AGEs) (NATARAJAN et al., 2020). Esses produtos são resultantes de reações não-enzimáticas entre moléculas de açúcar e outros compostos, como proteínas, lipídios e ácidos nucléicos (OTT et al.,

2014). EGPs e AGEs podem ser tão prejudiciais quanto os radicais livres, pois inativam a função biológica do composto glicado e seu acúmulo pode desencadear distúrbios metabólicos no indivíduo com excesso de peso (VAN NGUYEN, 2006).

O consumo de alimentos com propriedades anti-obesogênicas é fundamental na prevenção e no tratamento do excesso de peso e de complicações associadas (PARANDOOSH et al., 2019; RIBEIRO et al., 2019). O conteúdo de fitoquímicos das frutas, principalmente seus compostos fenólicos, conferem a esses alimentos a alegação de serem funcionais, devido a seus abundantes benefícios à saúde (DOHADWALA et al., 2010; HYSON, 2015). As uvas roxas possuem uma composição singular desses compostos, sendo uma das frutas mais ricas em fenólicos (BHAGWAT; HAYTOWITZ; HOLDEN, 2013). Os principais fenólicos presentes na uva são os flavonoides (antocianinas e flavonóis), os estilbenos (resveratrol), os ácidos fenólicos (derivados dos ácidos cinâmicos e benzoicos) e uma variedade de taninos (BHAGWAT; HAYTOWITZ; HOLDEN, 2013; RODRÍGUEZ-PÉREZ et al., 2019; STALMACH et al., 2011). Tais compostos são os responsáveis pelos efeitos fisiológicos distintos da uva e dos seus derivados no organismo, como a redução da glicemia de jejum (DOHADWALA et al., 2010), redução da oxidação de LDL (O'BYRNE et al., 2002), supressão do apetite, aumento da saciedade (SERRANO et al., 2016), e melhoria do desempenho cognitivo (TUORILA; CARDELLO, 2002). Evidências de estudos experimentais sugerem que alguns tipos de polifenóis presentes na uva roxa suprimem as atividades das enzimas α-amilase e α-glicosidase (YILMAZER-MUSA et al., 2012), inibem a absorção intestinal de glicose (JOHNSTON et al., 2005) e favorecem a redução da resistência à insulina (BREEN et al., 2008), permitindo um melhor controle glicêmico. No entanto, ainda não foram elucidados os efeitos do consumo destes compostos na glicemia em seres humanos a curto prazo.

Ademais, a regulação da ingestão alimentar é essencial para o tratamento do excesso de peso. Para que essa modulação aconteça, o cérebro depende de informações do organismo (MORTON et al., 2006), como sinais neurais e hormonais (VALASSI; SCACCHI; CAVAGNINI, 2008), e do meio ambiente, como os estímulos hedônicos e sociais (BERTHOUD; MÜNZBERG; MORRISON, 2017; DOUGLAS et al., 2017; WIJNGAARDEN et al., 2015). Estudos *in vitro* (IBARS et al., 2017; SERRANO et al., 2016) e com animais demonstraram que alguns polifenóis da uva roxa modulam a secreção de hormônios anorexígenos (GONZÁLEZ-ABUÍN et al., 2014; SERRANO et al., 2016), contribuindo para supressão da ingestão alimentar, aumento do gasto

energético (SERRANO et al., 2017) e redução do ganho de peso (SERRANO et al., 2016, 2017). Além disso, o suco de uva integral, rico em antocianinas, flavonoides e proantocianidinas (BLUMBERG; VITA; CHEN, 2015), possui o potencial de afetar a saciação, tanto pelo seu conteúdo fenólico, como pelo intenso aroma e sabor, que ativam sinais sacietógenos no organismo (HOLLIS et al., 2009; RAMAEKERS et al., 2014; YIN et al., 2017).

Diante disso, o tratamento do excesso de peso e prevenção da hiperglicemia abrange o controle da ingestão alimentar, a manutenção de concentrações glicêmicas adequadas ao longo do dia, e a redução do processo de glicação no organismo, visando a atenuação do estresse oxidativo e da inflamação subclínica. No entanto, as evidências científicas sobre os benefícios do consumo de produtos derivados da uva e de seus compostos fenólicos em seres humanos são limitadas. Assim, visando a identificação de estratégias nutricionais eficazes, esses efeitos necessitam ser explorados e compreendidos para que tais produtos sejam incorporados ao tratamento do excesso de peso.

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3. OBJETIVOS

3.1. Objetivo geral

Avaliar os efeitos do consumo do suco e de produtos derivados da uva na glicemia, no apetite, e nos marcadores de glicação, visando o controle do excesso de peso.

3.2. Objetivos específicos

- Analisar criticamente os estudos que avaliaram o efeito dos polifenóis da uva sobre os marcadores de glicação precoce e avançada e receptores em adultos com doenças crônicas não transmissíveis;
- Elucidar os possíveis mecanismos envolvidos no efeito anti-glicação dos polifenóis da uva;
- Analisar criticamente os estudos que investigaram os efeitos de produtos da uva na secreção de hormônios gastrointestinais anorexígenos e orexígenos, nas sensações de apetite e ingestão alimentar em adultos;
- Elucidar os mecanismos plausíveis pelos quais os produtos da uva podem regular o apetite e reduzir a ingestão alimentar.

- Avaliar o efeito agudo do suco de uva Concord, consumido sozinho, sobre a glicemia de 24h, sensações de apetite e função cognitiva em adultos com excesso de peso corporal;
- Avaliar o efeito agudo do suco de uva Concord, consumido como parte do café da manhã, sobre a glicemia de 24h, sensações de apetite e função cognitiva em adultos com excesso de peso corporal;
- Avaliar o perfil de excreção urinária dos metabólitos dos polifenóis da uva após o consumo do suco de uva Concord, isolado e como parte de uma refeição;

4. ARTIGO DE REVISÃO 1: Can grape polyphenols affect glycation markers in humans? a systematic review

- Artigo aceito para publicação na revista Critical Reviews in Food Science and Nutrition (fator de impacto: 11,176).

4.1. ABSTRACT

Advanced glycation end-products (AGEs) favor the occurrence of inflammation and oxidative stress, playing an important role in chronic diseases pathogenesis. Grape products polyphenols exert antiglycative and antioxidant effects which may contribute to prevent chronic diseases. However, clinical evidence of grape polyphenols on chronic disease prevention and treatment by glycation markers modulation are limited. Therefore, we aimed to critically analyze studies about that topic to investigate the antiglycative power of dietary grape polyphenol, and to explore the molecular mechanism involved. This systematic review was conducted and reported according to PRISMA guidelines. The following search terms were used: “grape”, “extract”, “grape seed extract”, “grape skin extract”, “polyphenol extract”, “grape polyphenol(s)”, “grape juice”, “resveratrol”, “quercetin”, “catechin”, “epicatechin”, “procyanidin(s)”, and “anthocyanin(s)”. Seven studies were included. Glycated hemoglobin was not affected. The interventions duration may not have been enough to detect changes. Grape polyphenols reduced fructosamine and methylglyoxal (MGO) concentrations, and increased endogenous secretory RAGE (esRAGE) gene expression but did not affect the serum concentration. Resveratrol antiglycative effects are mainly due its ability to trap MGO and downregulate RAGE. In conclusion, grape polyphenols may have a positive impact on early glycation products, AGEs and esRAGE. Future studies are needed to explore how they modulate AGEs and their receptors in chronic diseases.

Keywords: advanced glycation end products, esRAGE, glycated hemoglobin, grape seed extract, chronic disease, phenolic compound

PROSPERO registration: CRD42021241275

4.2. INTRODUCTION

High serum concentrations of advanced glycation end products (AGEs) (Vlassara et al., 2016) leads to a pro-oxidative and pro-inflammatory state, inducing the manifestation of chronic diseases (Natarajan et al., 2020) such as type 2 diabetes mellitus (DM2), cardiovascular diseases, and some types of cancer (Hruby; Hu, 2015; Chooi; Ding; magkos, 2019). Besides favoring inflammation and oxidative stress, high AGEs blood concentrations results in metabolic imbalance (Yubero-Serrano and Pérez-Martínez 2020), causing glucose intolerance, insulin resistance (García-Gómez et al. 2021), and altered lipid profile (Rasool et al. 2019), leading to chronic diseases progression (Yubero-Serrano and Pérez-Martínez 2020).

AGEs are formed throughout non-enzymatic glycation, the Maillard reaction, characterized by the interaction between reducing sugars and free amino groups of proteins, lipids, and nucleic acids (Ott et al. 2014; Mesías et al. 2013). The reaction starts with the formation of a highly unstable Schiff base, which is then transformed into an early glycation product (EGP), such as glycated hemoglobin (HbA1c) and fructosamine (Ahmad et al. 2013). In the advanced stage, these products undergo oxidation reactions, forming the AGEs, which are irreversible compounds with deleterious health effects when associated with their membrane receptor (RAGE) (Van Nguyen 2006). AGEs can also bind to other receptors besides RAGE. One of these receptors is the endogenous secretory receptor for AGEs (esRAGE), which does not cause deleterious effects once it binds to AGEs (Raucci et al., 2008).

Although AGEs are mainly originated from endogenous source, dietary AGEs increase the circulating pool of these substances, increasing their deleterious effects (Koschinsky et al., 1997). Thus, there is an interest in identifying dietary strategies capable of preventing and controlling chronic diseases. Grape products are rich in polyphenols, which have a unique composition (Bhagwat, Haytowitz, and Holden 2013) mainly represented by procyanidins, catechin, quercetin, epicatechin, anthocyanin, and resveratrol (Bhagwat, Haytowitz, and Holden 2013; Stalmach et al. 2011; Rodríguez-Pérez et al. 2019). Polyphenols are known to exert antiglycative and antioxidant effects (Sun et al. 2012, Bo' et al. 2019), besides exerting several biological effects (Luca et al. 2020) such as insulin sensitivity improvement (Costabile et al. 2019), glycemic control enhancement (Pandey and Rizvi 2014), and plasma triglyceride and LDL-cholesterol reduction (Del Bas et al. 2005).

However, clinical evidence of grape polyphenols on chronic disease prevention and treatment by glycation markers modulation are still scarce, indicating the need for robust investigation on the field. Therefore, in this systematic review our question was if grape polyphenols could modulate glycation markers in adults with chronic diseases. Based on the results obtained, we explored the possible molecular mechanism that may be involved in the effects promoted by polyphenols on glycation markers.

4.3. METHODS

4.3.1. Protocol and registration

This systematic review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al. 2009). The protocol was registered in PROSPERO database (registration CRD42021241275).

4.3.2. Literature search

The participants, intervention comparators, outcomes, and study design (PICOS) criteria adopted in this study are shown in Table 1. Two authors (OGLC and PVMR) searched for original articles that investigated the effects of grape polyphenols on serum glycation markers using the following electronic databases: MEDLINE (PubMed, www.pubmed.com), Cochrane (www.cochrane.org), Scopus (www.scopus.com) and EMBASE (www.embase.com). The following search terms were used: (“grape” OR “grapes) AND (“extract” OR “grape seed extract” OR “grape skin extract” OR “polyphenol extract”) AND (“grape polyphenol” OR “grape polyphenols” OR “grape juice”). Additional searches were performed with all individual phenolic compounds reported in grape, using the terms separately: (“resveratrol”), (“quercetin”), (“catechin”), (“epicatechin”), (“procyanidin” OR “procyanidins”), and (“anthocyanin” OR “anthocyanins”).

The search strategy was not restricted by date and language. The last search was done on July 18, 2021. A reverse hand-search was also performed to identify relevant articles cited in all selected studies.

4.3.3. Study Selection

Study selection was performed by three authors (OGLC, PVMR, and RCGA) in three phases: analyses of titles, abstracts, and full texts. All clinical trials that assessed the effects of grape polyphenol intake on glycations markers (early glycation products (EGPs) (fructosamine, glycated hemoglobin), AGEs (methylglyoxal [MGO], glyoxal [GO], 3-deoxyglucosone [3-DG], AGEs receptors (RAGE, and esRAGE)) in adults were included.

Protocols, reviews, letters, case reports, abstracts, and unpublished articles were not included, along with animal, *in vitro,* and epidemiological studies. Besides, red wine (with alcohol), polyphenol mixes extracted from other products (such as cocoa, green tea, berries, etc.), and grape polyphenol mixes combined with other interventions (when only the combined effects are described) or included in a food formulation. By adopting these exclusion criteria, we assured that the studies included reflected the effect solely of grape polyphenols

4.3.4. Data extraction

After reading the selected studies, the authors (OGLC and PVMR) compared the compiled data to guarantee its integrity and reliability. Divergent decisions were discussed with a third author (RCGA) and settled by consensus. For each study included, the following information was extracted: title, author's name, year of publication, study purpose, subjects' characteristics, sample size, intervention, phenolic composition, study duration. Additionally, results regarding serum EGPs, AGEs, and esRAGE concentrations, and RAGE gene expression were extracted.

4.3.5. Assessment of Risk of Bias

The authors assessed the risk of bias using the Cochrane Collaboration method[10]. The studies were analyzed on three levels of bias: high risk, low risk, and unclear (when the information provided was not sufficient to make a clear judgment). The authors considered the following biases: random sequence generation and allocation concealment (selection bias), blinding of participants and staff (performance bias), blinding of outcome assessment (detection bias), selective reporting (notification bias), and incomplete outcome data (attrition bias) (Higgins and Green). Studies were classified as having a low risk of bias when >80% questions were answered as "yes

(low risk), ”moderate risk of bias when 50% to 79% of the questions were answered as “yes (low risk),” and a high risk of bias when <50% questions were answered as “yes (low risk)” (Gomes, Costa, and Alfenas 2017). Different opinions between the authors were settled by consensus.

4.3.6. Data analyses

All studies selected for this systematic review are summarized in Table 2 according to their main characteristics and findings concerning glycation markers and other results. The studies were organized chronologically by year of publication, starting with the first published study. Fructosamine, HbA1c, circulating AGEs (methylglyoxal [MGO], glyoxal [GO], 3-deoxyglucosone [3-DG]), esRAGE, and RAGE gene expression were considered as the primary outcomes. The secondary outcomes were chronic disease markers like cardiometabolic markers (lipid profile, systemic arterial pressure, fasting blood glucose, fasting insulin, and HOMA-IR), highly sensitive C-reactive protein (hsCRP), oxidative stress markers (plasma protein carbonyl, Malondialdeyde [MDA], catalase [CAT], superoxide dismutase [SOD], serum glutamate oxaloacetate transaminase [SGOT], reduced glutathione [GSH], total antioxidant status [TAOS], thiobarbituric acid-reactive substances [TBARS]), anthropometric markers (body mass index [BMI] and waist circumference), renal and hepatic markers (creatinine, aspartate transaminase [AST], alanine transaminase [ALT], gamma-glutamyl transferase [GGT]).

Conducting a statistical meta-analysis was not justified due to the heterogeneity between the included studies. Therefore, in accordance with the Cochrane handbook, we performed a systematic review (Higgins and Green).

4.4. RESULTS

4.4.1. Study selection

We identified 2058 studies after searching the PubMed, SCOPUS, Cochrane, and Embase databases. A total of 441 duplicate studies were removed, resulting in 1617 unique records. Then we excluded 69 review studies, meta-analyses, case reports, and protocols. Next, we also excluded 1497 studies that were considered irrelevant to the topic of interest, and 13 animal or *in vitro* studies. After reading the full

text of the remaining 38 studies, seven studies met all criteria adopted for this systematic review. The study selection flowchart is indicated in Figure 1.

4.4.2. Description of included studies

The seven studies included in this review (Table 2) initially included 304 subjects. In one of the studies, 8 subjects dropped out (Sano et al. 2007) and in another 2 subjects were excluded from the analyses (Van den Eynde et al. 2018), totalizing data from 294 subjects. Sample sizes of the studies ranged from 19 to 61 subjects and intervention duration varied from 4 to 12 weeks. One study did not inform the subjects genders (Van den Eynde et al. 2018), the other six studies included subjects of both genders (female: 45.7%, n = 139; male: 40,5%, n = 123). The mean ± SD age of the subjects from all seven studies was 57 ± 9.1 years. Except for one study (Hokayem et al. 2013), all the others included subjects with normal BMI and excess body weight (Banini et al. 2006; Kar et al. 2009; Sano et al. 2007; Van den Eynde et al. 2018; Seyyedebrahimi et al. 2018; Roggerio et al. 2018). Three (42.9%) studies included subjects with T2DM (Banini et al. 2006; Kar et al. 2009; Seyyedebrahimi et al. 2018).

In the present review, we only included data from subjects who underwent dietary grape polyphenol interventions. Interventions included one study with Muscadine grape juice (Banini et al. 2006), two studies with grape seed extract (GSE) (Sano et al. 2007; Kar et al. 2009), one study with grape polyphenol extract (GPE) (Hokayem et al. 2013). In addition, three studies assessed grape-derived pure compounds, such as epicatechin and quercetin (Van den Eynde et al. 2018), and two studies assessed resveratrol (Roggerio et al. 2018; Seyyedebrahimi et al. 2018). Four studies (57.1%) evaluated the effect of grape polyphenol on EGPs (i.e. HbA1c and fructosamine) (Sano et al. 2007; Hokayem et al. 2013; Kar et al. 2009; Banini et al. 2006), two studies (28.6%) evaluated the effect on AGEs (methylglyoxal, glyoxal, 3-deoxyglucosone) and soluble receptor (esRAGE) (Van den Eynde et al. 2018; Roggerio et al. 2018), and one study (14.3%) assessed HbA1c and RAGE (Seyyedebrahimi et al. 2018). Regarding the geographic distribution, each study was conducted in a different country, United States (Banini et al. 2006), Japan (Sano et al. 2007), United Kingdom (Kar et al. 2009), France (Hokayem et al. 2013), Netherlands (Van den Eynde et al. 2018), Iran (Seyyedebrahimi et al. 2018), and Brazil (Roggerio et al. 2018).

4.4.3. Bias Risk Assessment

The major domains evaluated in the present study were the random generation allocation sequences and the data concerning incomplete results. Two (28.6%) studies were classified as low risk of bias (Van den Eynde et al. 2018; Seyyedebrahimi et al. 2018); four (57.2%) as the moderate risk of bias (Hokayem et al. 2013; Roggerio et al. 2018; Roggerio et al. 2018; Kar et al. 2009), and only one (14.2%) had a high risk of bias (Banini et al. 2006). Three studies were unclear as to how their random allocation sequences were generated (Sano et al. 2007; Kar et al. 2009; Banini et al. 2006). All studies were randomized and reported all outcomes data. However, blinding of treatment allocations was not clearly presented in 2 studies (Roggerio et al. 2018; Banini et al. 2006). Only the study by Seyyedebrahimi and colleagues (Seyyedebrahimi et al. 2018) presented a low risk of bias due to the blinding of the participants or staff and the method used to evaluate the results. In addition, Banini et al. (2006) did not clearly define their selective results report and Kar et al. (2009) had a high risk of bias (Figure 2).

4.4.4. Results of Individual Studies

The consumption of Muscadine juice (150 ml/day) for 4 weeks had neutral effect on HbA1c compared with baseline in subjects with excess body weight with and without T2DM. In subjects with T2DM, that juice reduced HDL-C. Dealcoholized muscadine wine consumed in the same dose (150 ml/day) for 4 weeks reduced fasting insulin in subjects with T2DM, although no effect was observed in the ones without T2DM. Fasting blood glucose and insulin, BMI, waist circumference, systolic and diastolic blood pressure also remained unchanged after juice intake (Banini et al. 2006).

In a 12-week GSE (200mg and 400 mg/day of proanthocyanidin) supplementation study, HbA1c, BMI, total cholesterol, LDL-C, triglycerides, blood pressure, kidney, and hepatic function were not affected in subjects with normal and excess body weight. Contrary to the result obtained in the previously mentioned study (Banini et al. 2006), HDL-C increased in all groups after GSE supplementation (Sano et al. 2007).

The consumption of GPE (2 g/day), for 8 weeks did not alter HbA1c, BMI, waist circumference, blood pressure, total cholesterol, HDL-C, LDL-C, triglycerides, hsCRP,

fasting blood glucose and insulin, TBARS, and hepatic function in subjects with excess body weight (Hokayem et al. 2013). On the other hand, GSE supplementation (600 mg of total phenolics) for 4 weeks reduced fructosamine, glutathione, and hsCRP in subjects with T2DM and excess body weight. Fasting blood glucose, HOMA-IR, HDL-C, and total antioxidant status remained unaltered (Kar et al. 2009).

There was considerable variability in the responses provoked by grape polyphenol interventions on AGEs (i.e. methylglyoxal, glyoxal, 3-deoxyglucosone) concentrations, free and protein-bound AGE, RAGE, and esRAGE. In a 4-week randomized controlled trial (RCT), quercetin (160 mg/day), but not epicatechin (100 mg/day), reduced methylglyoxal while the other AGEs assessed (glyoxal, 3-deoxyglucosone, free and protein-bound AGE) remained unchanged (Van den Eynde et al. 2018).

In another 4-week RCT, 500mg of resveratrol on a daily basis increased esRAGE gene expression although esRAGE concentration remained the same compared with baseline. That supplementation also increased HOMA-IR and total cholesterol, but no changes were observed for HDL-C, LDL-C, triglycerides, fasting blood glucose and insulin, waist circumference, and blood pressure (Roggerio et al. 2018).

Furthermore, a higher dose (800 mg/day) of resveratrol for a longer period (8 weeks) reduced plasma protein carbonyl content but did not affect RAGE and HbA1c concentrations in subjects with T2DM. In that study, there was a reduction in BMI and blood pressure, besides an increase in total antioxidant status and total thiol. Waist circumference, fasting blood glucose and insulin, urea, creatinine, uric acid, triglycerides, total cholesterol, HDL-c, LDL-C, total protein, SGOT, hsCRP, and HOMA-IR remained unchanged (Seyyedebrahimi et al. 2018).

4.5. DISCUSSION

To our knowledge, this is the first systematic review examining the effects of dietary grape polyphenols on glycation markers like EGPs, AGEs and receptors. The small number of studies identified filling the inclusion criteria confirms the fact that this is an emergent topic of research that requires attention of the researchers. There was considerable variability in the polyphenol composition of the products tested in the studies selected for the present review (Table 2). Different compounds will lead to distinct molecular pathways that can beneficially prevent glycation (Li et al. 2014).

The studies included in this review present some heterogeneity regarding the population studied and the glycation marker evaluated. As previously mentioned, each study was conducted in a different country, arising the question if this difference in ethnicity could affect the results obtained. Previous evidence shows that racial and ethnicity differences can affect glycemic control of adults with TD2M (Harris et al. 1999) and type 1 diabetes (Kahkoska et al. 2018), suggesting that geographic factors may affect glycation markers concentrations (Hunt et al. 2020). Although the authors of the studies selected for this review did not indicate the ethnicity and race of the participants, each study was conducted in a different country, allowing a broad representation of the results with respect these demographic characteristics.

According to the results of the studies included in the present review, HbA1c was not affected by grape polyphenols intake, independently of the compound, dose, or intervention duration (Banini et al. 2006; Sano et al. 2007; Hokayem et al. 2013; Seyyedebrahimi et al. 2018). However, HbA1c is an early-stage glycation product formed by the reaction between hemoglobin and glucose but its response to dietary intervention is not rapid. Hemoglobin half-life is approximately 120 days. Thus, an intervention duration of less than three months may not be sufficient to detect any changes in HbA1c (Selvin et al. 2015). Except for one study (Sano et al. 2007), all the others had a duration of less than 12 weeks (i.e. 3 months), which might be the reason for the lack of detected effects on HbA1c. Besides, the validity of the HbA1c measurement can be affected by some conditions (Selvin et al. 2015) that were not evaluated in the studies (Banini et al. 2006; Sano et al. 2007; Hokayem et al. 2013; Seyyedebrahimi et al. 2018), such as occurrence of anemia, altered red blood cell lifespan, kidney disease, liver disease, and abnormal forms of hemoglobin (Selvin et al. 2015). Even though, the 12-week also did not affect HbA1c (Sano et al. 2007), indicating that some other unknown reason might explain the lack of effect.

Based on experimental data, 50 mg of GSE/kg reduced HbA1c concentration in diabetic mice (Hwang et al. 2009). Therefore, we hypothesize that the dose tested (277.5 mg or 555 mg of GSE) in that study (Sano et al. 2007) may not have been enough to reduce HbA1c. Due to the lack of human evidence to compare the results, we must convert the animal dose to a human equivalent dose (HED) (Shin et al. 2015) to compare the dose tested in animal (Hwang et al. 2009) with the one tested in humans (Sano et al. 2007). To convert, we applied the body surface area (BSA) normalization algorithm (Reagan-Shaw, Nihal, and Ahmad 2008), using specific

constants (Shin et al. 2015). Thus, considering the GSE dose tested (50 mg/kg) in mice by Hwang et al. (2009), we have a HED of 4.05 mg/kg of body weight. Based on the mean body weight (63 kg) presented by the subjects of Sano et al. (2007), we get an approximate human dose of 255 mg/day. Therefore, the lower dose tested by Sano et al. (2007) (277 mg of GSE) was a little higher than the dose tested in rats (Hwang et al. 2009). So, the dose tested does not explain the inconsistent results between these two studies. However, while the animals were diabetic (Hwang et al. 2009), the subjects were not (Sano et al. 2007). Therefore, it would be interesting to assess how the consumption of at least 255 mg of GSE/day for more than 12 weeks would affect HbA1c in humans.

Some authors did not assess the outcomes obtained for subjects with excess body weight and normal body weight separately (Banini et al. 2006; Kar et al. 2009; Sano et al. 2007; Van den Eynde et al. 2018; Seyyedebrahimi et al. 2018; Roggerio et al. 2018). Excess body weight is a chronic disease itself, associated with oxidative stress and subclinical inflammation occurrence (Tavares et al. 2020; Gaens et al. 2014), which is a risk factor to increase the production of glycation markers (EGPs and AGEs) (Gaens et al. 2014) and to develop other chronic diseases (Rasool et al. 2019; Tupe et al. 2014). Therefore, the outcome of subjects differing in BMI categories (or health conditions) should not be assessed in the same intervention group since it can interfere with the results.

Instead of assessing HbA1c, we can evaluate fructosamine concentration, which reflects changes in glycemic status over the previous 2 to 4 weeks, which is the turnover of plasma proteins (Selvin et al. 2015). Therefore, fructosamine assessment can reflect the effect of interventions conducted for a shorter period of time. Among the studies selected for this review, only one evaluated fructosamine. In that study, 600 mg of GSE/day for four weeks reduced fructosamine concentration (Kar et al. 2009). Fructosamine is an early glycation product (i.e. glucose bound to circulating serum proteins, mainly albumins but also globulins and other proteins), and its degradation leads to AGEs formation (Ahmed and Thornalley 2003). Unfortunately, in that study (Kar et al. 2009) the phenolic composition of the applied treatment was not informed. According to the literature, GSE is rich in various antioxidants and it is considered among the most powerful plant-derived antioxidant food (Grases et al. 2015).

An *in vitro* assay assessing eight brands of GSE, all tested products effectively inhibited the formation of AGEs in a concentration-dependent manner. Regardless of

different polyphenol compositions, they all contained procyanidin, catechin, and epicatechin (Sun et al. 2012). According to the results obtained in some of the studies included in this systematic review, the consumption of 160mg of quercetin for 4 weeks reduced MGO, but did not affect GO, 3-DG, free and protein-bound AGE (Van den Eynde et al. 2018). MGO and GO are reactive dicarbonyl precursors of AGEs, which have been associated with diabetes-related long-term complications (Li et al. 2014). Due to the reactive carbonyl group, MGO and GO can modify proteins by reacting with aminoacidic residues, and can also exhibit a potential cellular toxicity to DNA. Any reaction that increases MGO or GO concentrations in tissues or plasma can ultimately lead to diabetic complications (Li et al. 2014). MGO is also associated with central nervous system disorders and cardiovascular diseases (Matafome et al. 2017), including cholesterol metabolism dysfunction (Bacchetti et al. 2014). It is important to note that the tested quercetin dose (160mg/day) was higher than the dose of epicatechin (160 mg/day *vs* 100 mg/day), which might explain the different results in MGO concentration, considering the concentration-dependent manner effect verified in previously mentioned *in vitro* study (Van den Eynde et al. 2018).

Quercetin can inhibit AGEs formation through its MGO-trapping capacity. Differently from epicatechin, quercetin molecule has structures to ensure its ability to scavenge MGO and other dicarbonyls (Li et al. 2014). The major active sites for flavonoids bindings are located in the A ring, which has a second hydroxyl group for efficient dicarbonyls trapping. The A ring in quercetin and epicatechin is identical. However, at the C ring, epicatechin lacks a double bond and a ketone group, which may explain why quercetin has a higher MGO-trapping efficacy compared with epicatechin (Shao et al. 2014) and why quercetin, but not epicatechin, reduced MGO in the previously mentioned study (Van den Eynde et al. 2018). Besides, quercetin can indirectly modulate the glyoxalase system. Glyoxalase 1 (*GLO1*) is a key enzyme to convert MGO to d-lactate, reducing AGEs formation (Van den Eynde et al. 2018). Quercetin can activate nuclear factor erythroid 2-related factor-2 (Nrf2) (Karuppagounder et al. 2015), which increases GLO1 gene expression, enhances the glyoxalase pathway, and consequently, reduces MGO concentrations (Xue et al. 2012) (Figure 3).

In two of the selected studies, although resveratrol supplementation did not affect RAGE gene expression (Seyyedebrahimi et al. 2018), it increased esRAGE gene expression (Roggerio et al. 2018). Resveratrol has antioxidant, anti-inflammatory, anti-

proliferative, and antiangiogenic effects, and many signaling pathways are among its molecular targets (Buttari et al. 2013). Based on data from *in vitro* (Shen, Xu, and Sheng 2017; Buttari et al. 2013) and animal (Al-Hussaini and Kilarkaje 2018; Yılmaz et al. 2018) studies, resveratrol can reduce AGEs/RAGE interaction, and consequently reduce glycation reactions. RAGE is a membrane receptor that originates the soluble cleaved isoforms RAGE (cRAGE) and esRAGE. The AGEs-RAGE interaction activates inflammatory and oxidative stress pathways throughout NF-kB signaling (Tavares et al. 2020), which also controls RAGE expression, suggesting the occurrence of a positive inflammatory feedback (Matafome et al. 2017). However, most of the studies that demonstrated these effects were conducted in diabetic rats. In this systematic review, we verified that although RAGE expression was not affected in response to 800 mg of resveratrol/day for 8 weeks (Seyyedebrahimi et al. 2018), there was a reduction in plasma protein carbonyl in subjects with T2DM. In that same study, total antioxidant status (TAOS) and total thiol increased, while BMI and blood pressure decreased after the supplementation (Seyyedebrahimi et al. 2018), representing an improvement in the capacity of plasma factors to counteract oxidative stress (Tupe et al. 2014). Increased concentration of plasma protein glycation products, like protein carbonyl, play a key role in impairing antioxidant status and amplifying erythrocytes oxidative damage in T2DM patients (Tupe et al. 2014). Thus, that antioxidant effect is a possible mechanism that can attenuate AGEs deleterious effects (Hajizadeh-Sharafabad et al. 2019).

On the other hand, in a study involving subjects with normal body weight and overweight, the consumption of 500mg of resveratrol for 30 days led to an increase in esRAGE gene expression but not in esRAGE serum concentration (Roggerio et al. 2018). Despite the increase in gene expression, the cell protein concentration depends on the balance between its production and degradation (Evankovich et al. 2017). Therefore, it would be interesting to assess how the consumption of 500mg/day of resveratrol for a longer period would affect esRAGE concentration. Soluble forms of RAGE (i.e. esRAGE) seem to prevent AGEs/RAGE interaction (Bierhaus et al. 2005), protecting the vascular cells against the activation of the cell-surface receptors and consequently avoiding the AGEs deleterious effects (Yonekura et al. 2003).

Further, the antiglycation effect of resveratrol has been attributed to its ability to trap and degrade MGO, downregulate RAGE, and scavenge ROS. Resveratrol also suppresses cell damage and related diseases in response to AGEs through

mechanisms that mainly target oxidative stress, proinflammatory cytokine production, and immune responses (Hajizadeh-Sharafabad et al. 2019). Like quercetin (Karuppagounder et al. 2015), resveratrol activates Nrf2, which upregulates sestrine2, glyoxalase, and other antioxidant enzymes, that can increase the MGO degradation, reducing AGEs formation (Cheng et al. 2012) (Figure 3).

The AGEs-RAGE interaction is implicated in the pathogenesis of chronic diseases by increasing the production of proinflammatory cytokines, adhesion molecules, and RAGE itself (Tanaka et al. 2000). AGEs-RAGE binding activates NADPH oxidase, protein kinase-C, p21, extracellular signal-regulated kinase (ERK), p38 and c-jun N-terminal kinase (JNK), leading to NF-κB translocation to the nucleus. This pathway culminates in the transcription of inflammatory markers (Matafome et al. 2017). Resveratrol activates PPAR-γ and prevents the inhibitory effects of NF-kB on peroxisome proliferators-activated receptors (PPAR-γ) activity, which results in RAGE downregulation (Zhang et al. 2010). Resveratrol can also suppress RAGE-NFkB signaling pathway, reducing TGF- ß1 mRNA and preventing vasculopathy (Jing et al. 2010) (Figure 3).

Further, resveratrol also increased esRAGE gene expression, although serum concentration remained unchanged (Roggerio et al. 2018). Little is known about the interaction between resveratrol and esRAGE. *In vitro* studies demonstrated that resveratrol can influence esRAGE formation by modulating an alternative splicing in a target-specific and dose-dependent manner (Markus, Marques, and Morris 2011). Hence, the increase in esRAGE expression suggests a role for resveratrol in the control of deleterious effects of the RAGE cascade.

Strengths and limitations

Grapes have a unique and rich polyphenol composition. The studies included in this review tested different polyphenol types allowing a broad investigation about their effects on glycation markers. Besides, the duration of these studies (4 to 12 weeks) was appropriate and sufficient to provide us an overview of the time response of polyphenol consumption on EGPs, AGEs, RAGE, and esRAGE concentrations. Studies from seven countries were included in this review, providing a broad analysis of the results from different races and ethnicities. However, we also had some limitations: (a) some studies evaluated only serum EGPs (Banini et al. 2006; Sano et

al. 2007; Kar et al. 2009; Hokayem et al. 2013) or AGEs (Van den Eynde et al. 2018; Roggerio et al. 2018), instead of evaluating the concentration of both glycation biomarkers, which would allow us to better understand the antiglycative effects of grapes and the mechanisms involved. (b) Two studies (Kar et al. 2009; Banini et al. 2006) did not inform the polyphenol composition tested. (c) All but one study (Hokayem et al. 2013) included subjects with normal BMI and with excess body weight in the same group. This may have masked the results in subjects with overweight and obesity, since they have metabolic alterations that normal weight subjects do not have, and could have responded differently to the dietary intervention. (d) Only one study (Van den Eynde et al. 2018) evaluated AGEs serum concentration; and (e) only one study (Roggerio et al. 2018) evaluated esRAGE, which is an important biomarker in chronic disease prevention.

In conclusion, the results of the studies included in this review indicated that the consumption of grape polyphenols in different doses (200g – 2000g/day) and periods of time (4-12 weeks) has no effect on HbA1c, but the intake of 600mg of GSE for 4 weeks reduced fructosamine. Pure compounds derived from grapes, like quercetin (160 mg/day) and resveratrol (500 mg/day) led to positive effects on AGEs concentration and esRAGe gene expression after 4 weeks of supplementation. The studies published so far, and included in this review after extensive search, show heterogeneity on the types of AGEs evaluated, so their results are not enough to drawn solid conclusions regarding the grape polyphenols effect on AGEs formation. The results of these studies demonstrate that despite the potential role of grape polyphenols on oxidative stress and inflammation control, it is highly recommended that new studies investigate the chronic effect (at least 4 weeks) of these compounds on EGPs, different types of AGEs and their receptor isoforms in subjects with overweight and obesity. Future researches in this matter will provide more precise evidence and mechanistic insights on the antiglycative effect of grape polyphenols on chronic diseases.

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Table 1. PICOS criteria for inclusion of studies

Parameter	Inclusion criterion
Participant	Adults with chronic diseases
Intervention/exposure	Grape polyphenols intake
Comparison	Consumption of placebo or nothing
Outcome	HbA1c, fructosamine, circulating AGEs and receptor isoforms, cardiometabolic, inflammatory, oxidative stress, anthropometric, renal, and hepatic function markers
Study design	Clinical trials

Table 2. Characteristics of the studies in which the effect of the consumption of grape polyphenols on early and advanced glycation end products and secondary outcomes was assessed

Reference (origin)	Sample	Intervention	Duration	Main results
Banini et al., 2006	23 subjects (11M/12F) Age: 53 ± 10.3 y BMI: 28.4 ± 6.7 kg/m^2 19 subjects with T2DM (6M/13F) Age: 58 ± 10 y BMI: 38.1 ± 16.4 kg/m^2	-Muscadine juice (MJ): 150 ml - Control: no treatment - MJ: 150 ml - Dealcohlized muscadine wine (DzW):	4 weeks	↔ HbA1c compared to baseline ↔ FBS, fasting insulin, BMI, SBP, DBP, waist circumference compared to baseline ↔ HbA1c compared to baseline in both groups ↔ FBS, BMI, SBP, DBP, waist circumference in both groups compared to baseline ↓ HDL-C in MJ group compared to baseline ↓ fasting insulin in DzW compared to baseline
Sano et al., 2007	61 subjects (29M/32F) Age: 52.4 ± 16.7 y BMI: 24.2 ± 4.7 kg/m^2	-GSE: 277.5 mg (200mg of proanthocyanidin) -GSE: 555 mg (400 mg of proanthocyanidin) -Control: 0 mg of proanthocyanidin	12 weeks	↔ HbA1c compared to baseline in both test groups ↔ BMI, SBP, DBP, TC, LDL-C, TG, total protein, AST, ALT, GGT, uric acid, creatinine, blood glucose in all groups compared to baseline ↑ HDL-C all groups compared to baseline

Cont. Table 2. Characteristics of the studies in which the effect of the consumption of grape polyphenols on early and advanced glycation end products and secondary outcomes was assessed

Reference	Sample	Intervention	Duration	Main results
Kar et al., 2009	32 subjects with T2DM (16M/16F) Age: 61.8 ± 6.36 y BMI: 30.2 ± 5.9 kg/m^2	- GSE: 600 mg/d - Placebo: 600 mg/d	4 weeks	↓ fructosamine compared to baseline ↓ GSH, hsCRP, TC ↔ FBG, HOMA-IR, HDL-C, TAOS compared to baseline
Hokayem et al., 2013	38 subjects (18M/20F) Age: 49.0 ± 12.0 y BMI: 29.2 ± 4.0 kg/m^2	- GPE: 2g/d (Procyanidin, catechin, epicatechin, anthocyanin, resveratrol) -Placebo: 2g/d	8 weeks	↔ HbA1c compared to baseline ↔ BMI, waist circumference, SBP, DBP, TC, HDL-C, LDL-C, TG, hsCRP, FBG, fasting insulin, TBARS, AST, ALT, GGT compared to baseline
Van den Eynde et al., 2018	37 subjects (gender not informed) Age: 66.4 ± 7.9 y BMI: 26.7 ± 3.3 kg/m^2	- Epicatechin: 100 mg/d - Quercetin: 160 mg/d - Placebo	4 weeks	↓ MGO in quercetin group compared to baseline and placebo ↔ GO, 3-DG, free and protein-bound AGE in all groups group compared to baseline and placebo

Cont. Table 2. Characteristics of the studies in which the effect of the consumption of grape polyphenols on early and advanced glycation end products and secondary outcomes was assessed

Reference (origin)	Sample	Intervention	Duration	Main results
Seyyedebrahim et al., 2018	46 subjects with T2DM (19M/22F, 5 not informed) Age: 56.8 ± 6.2 y BMI: 28.9 ± 4.4 kg/m^2	- Resveratrol: 800 mg/d - Placebo	8 weeks	↔ RAGE gene expression, HbA1c compared to placebo ↑ TAOS, total thiol ↓ BMI, SBP, DBP, plasma protein carbonyl ↔ waist circumference, FBS, urea, creatinine, uric acid, TG, TC, HDL-c, LDL-C, total protein, SGOT, hsCRP, HOMA-IR, fasting insulin compared to placebo
Roggerio et al., 2018	48 subjects (24M/24F) Age: 58.5 ± 3.5 y BMI: 26.7 ± 3.7 kg/m^2	- Resveratrol: 500 mg/d	4 weeks	↑esRAGE gene expression ↔ esRAGE compared to baseline ↑ HOMA-IR, TC ↔ waist circumference, SBP, DBP, HDL-C, LDL-C, TG, FBG, fasting insulin compared to baseline

3-DG: 3-deoxyglucosone; ALT: alanine transaminase; AST: Aspartate transaminase; BMI: body mass index; CAT: catalase; DBP: diastolic blood pressure; FBG: fasting blood glucose; GGT: gamma-glutamyl transferase; GO: glyoxal; GPE: grape polyphenols extract; GSE: grape seed extract; GSH: reduced glutathione; HbA1c: glycated hemoglobin A1c; HDL-C: HDL-cholesterol; HOMA-IR: homeostasis model assessment-insulin resistance; hsCRP: highly sensitive C-reactive protein; LDL-C: LDL-cholesterol; MDA: Malondialdehyde; MGO: methylglyoxal; SBP: systolic blood pressure, SGOT: serum glutamate oxaloacetate transaminase; SGPT: serum glutamate pyruvate transaminase; SOD: superoxide dismutase; TAOS: total antioxidant status; TBARS: thiobarbituric acid-reactive substances; TC: total cholesterol, TG: triglycerides. ↑: increased; ↓: decreased; ↔: unchanged.

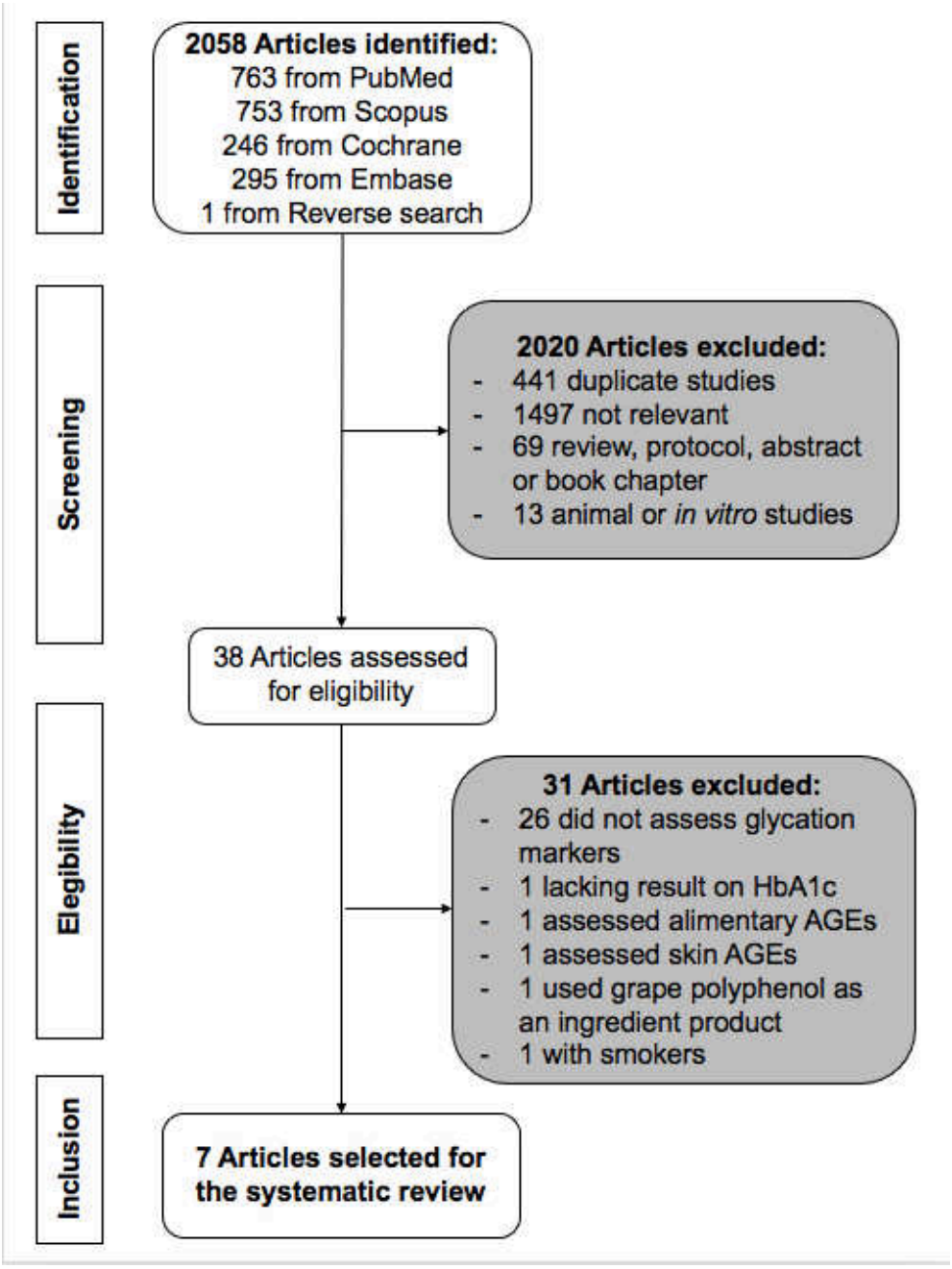

Figure 1. Flowchart of the study selection process.

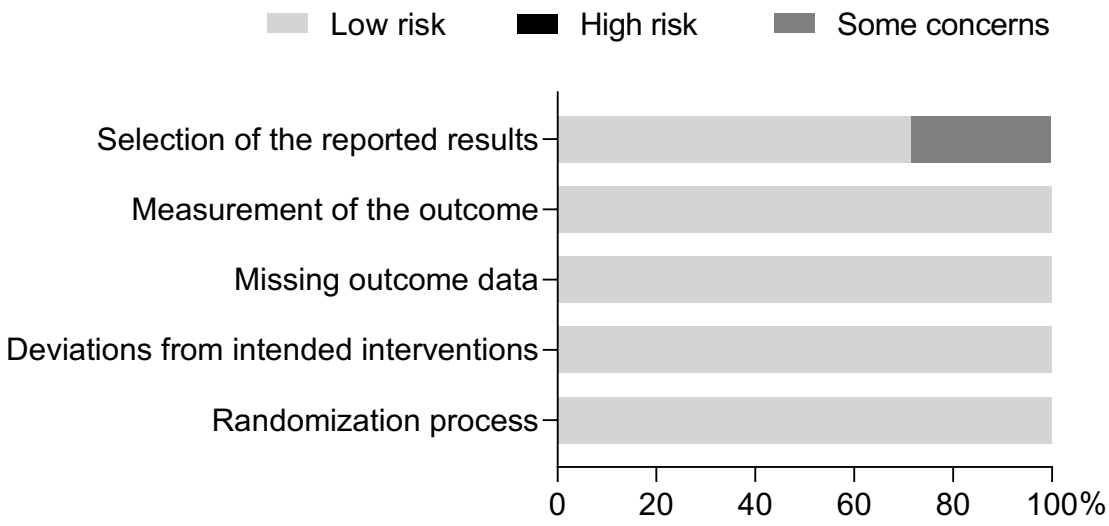

Figure 2. Risk of bias summary: authors' judgments about the five risk of bias domains for the included study. Bias classified as low risk, high risk, and some concerns

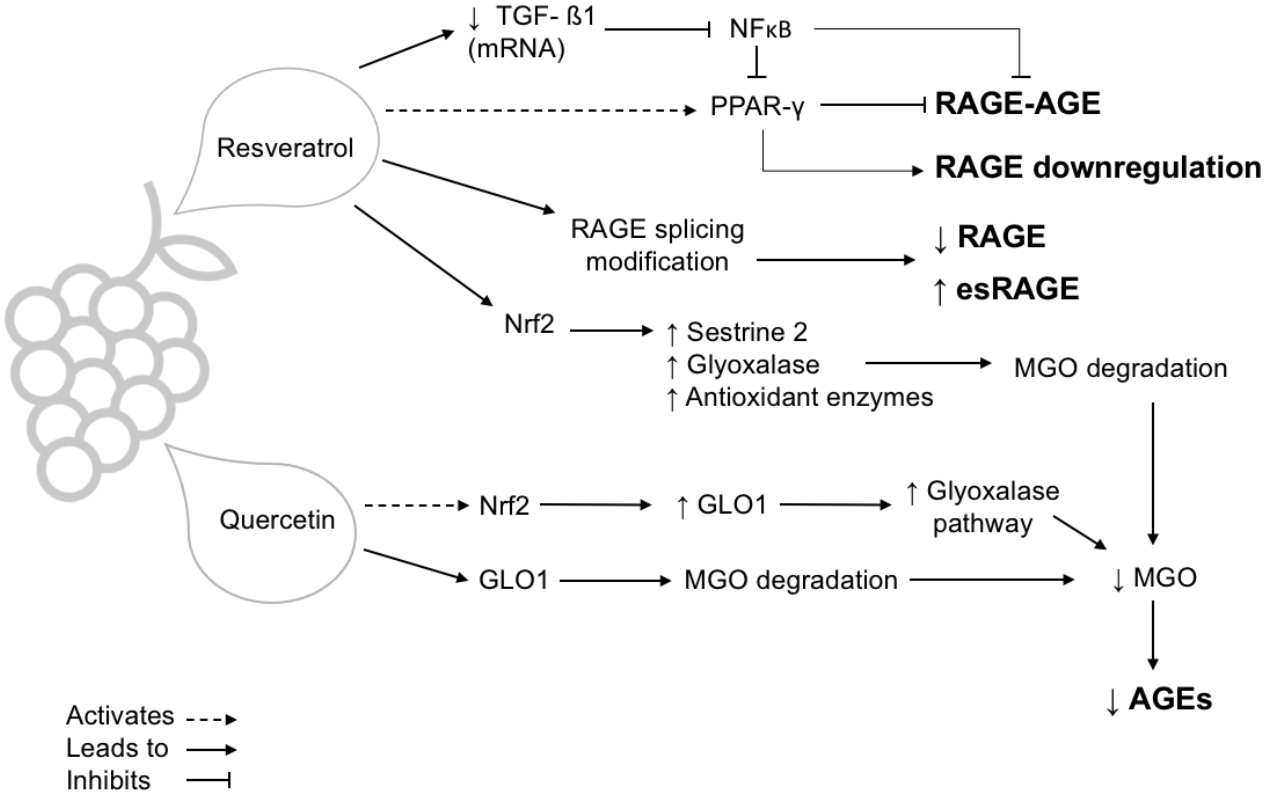

Figure 3. A simplified mechanistic model of resveratrol and quercetin inhibition of RAGE_AGE binding, besides AGEs, RAGE, esRAGE formations. Resveratrol can suppress RAGE-NFkB signaling pathway, reducing TGF- ß1 mRNA, thus inhibiting RAGE-AGE binding. Resveratrol activates PPAR-γ and prevents the inhibitory effects of NF-kB on PPAR-γ activity, resulting in RAGE downregulation. Resveratrol leads to RAGE splicing originating esRAGE, reducing RAGE formation. Quercetin activates Nrf2, which increases GLO1 gene expression, enhances the glyoxalase pathway, reducing MGO concentration, consequently reducing AGEs formation. Nrf2 activation increases antioxidant enzymes Sestrine 2, and glyoxalase contributing to MGO degradation and reducing AGEs formation. ↑: increases; ↓: decreases; AGE: advanced glycation end products; esRAGE: endogenous secretory receptor of AGE; GLO1: glyoxalase 1; MGO: methylglyoxal; NF- κB: nuclear factor κB Nrf2: factor erythroid 2-related factor-2; PPAR-γ: peroxisome proliferators-activated receptors; RAGE: membrane receptor for AGE.

5. ARTIGO DE REVISÃO 2: Can grape products affect appetite and food intake? - A systematic review of randomized clinical trials

- Artigo será submetido à revista Journal of the Academy of Nutrition and Dietetics (fator de impacto 4,151).

5.1. ABSTRACT

The consumption of high satiety food can reduce food intake, controlling obesity and reducing the risk of associated chronic illnesses. Grape products are rich in polyphenols, which have innumerous benefits to human health. Animal studies results indicate that the consumption of grape products can control food intake. However, there is still no clear evidence that the consumption of these products would lead to such effect in humans. Therefore, this study aimed to identify the effects of grapes and grape products on appetitive sensations and food intake. A systematic literature search was conducted using PubMed, Scopus, and The Cochrane Register of Clinical Trials databases to identify randomized clinical trials that assessed the effects of grape products on the previously mentioned outcomes in humans. From a total of 910 studies, six met the criteria adopted for this systematic review and were critically appraised. No study assessed the effects of fresh grapes itself. Grape seed extract associated with energy-restricted diet reduced the orexigenic neuropeptide Y (NPY), body weight, BMI, waist circumference, and waist: hip ratio after 12 weeks in subjects with excess body weight. On the other hand, although the ingestion of one cup of raisins for 6 weeks affected the secretion of hormones that control appetite, it did not affect food intake and BMI in lean and overweight subjects. Overall, the grape products tested in the selected studies did not affect subjective appetitive sensations and food intake in subjects with normal and excess body weight. We encourage long-term controlled clinical trials involving subjects with excess body weight to assess the effect of meals containing high enough doses of polyphenols on satiety and food intake under laboratory conditions. Also, to have a better picture of the effect of the treatments on subjective appetitive sensations, it would be interesting if the concentration of orexigenic and anorexigenic hormones could be evaluated

Keywords: Grape seed extract, proanthocyanin, appetite control, gut hormones.

5.2. INTRODUCTION

Daily consumption of a diversity of fruits and vegetables is recommended to improve health, as well as to reduce the risk of major chronic illnesses.[1] The consumption of high satiety foods avoids excessive food intake and weight gain.[2] Grape seeds, grape skin, and grape juice are rich in polyphenols, which may control food intake. Each grape fraction contains various polyphenols, and the concentrations of these compounds vary depending on the grape's geographic origin and species.[3] The most common compounds in red and purple grapes are anthocyanins, proanthocyanidins, phenolic acids, hydroxycinnamates, resveratrol, and flavanols.[4]

In vitro and animal studies indicate that grape polyphenols can act as satiating agents.[5–7] Acute[5] and chronic[6] consumption of different doses of grape seed extracts (polyphenol-based) can reduce food intake in animals, possibly through mechanisms involving the modulation of neuropeptides,[7] such as neuropeptide Y (NPY), agouti-related protein (AgRP), pro-opiomelanocortin (POMC), and cocaine- and amphetamine-regulated transcript (CART)[8] and the secretion of gastrointestinal hormones, such as glucagon-like peptide-1 (GLP-1).[5] These studies suggest that the ingestion of human doses equivalent to the animal doses may exert beneficial effects in humans.

In addition, grape products, especially grape juice,[9] have a distinct and intense flavor and aroma that can contribute to satiation, reducing food intake.[9–11] However, there is still no consensus about the effect of grape products on appetite and food intake in humans, and the molecular mechanism on such variables is still under investigation.

Therefore, the purpose of the present review was to critically analyze human clinical trials in which the effects of grapes and grape products (seeds, skin, juice, extract, and others) on appetite, and food intake in humans. In order to understand better the results verified, we explored the plausible mechanisms by which grape products may reduce food intake thus preventing weight gain.

5.3. MATERIAL AND METHODS

5.3.1. Registration and Search Strategy

This systematic review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[12] The protocol was registered in PROSPERO (CRD42020200660).

Two authors (OGLC and PVMR) independently searched for articles using the following electronic databases: PubMed (http://www.ncbi.nlm.nih. gov/pubmed/), Scopus (http://www.scopus.com/), and the Cochrane Library (http://www.cochrane.org/) databases to search for randomized clinical trials (RCTs) designed to assess the effects of grapes and/or grape products on appetite and food intake in human adults. Keywords were chosen from the Medical Subject Headings (MeSH) and Descriptors in Health Sciences (DeHS) using the following search strategy: (((grape) OR (grapes) OR (raisin) OR (raisins)) AND (appetite) OR (food intake) OR (satiety response) OR (energy intake) OR (food intake) OR (dietary intake))) using the Cochrane filter for RCT.[13] A reverse hand-search was also performed to identify relevant articles cited in all selected studies. The search strategy was not restricted by date and language. The last search was done on August 5th, 2020.

5.3.2. Study Selection

The population, intervention comparators, outcomes, and study design (PICOS) criteria adopted in this study to formulate eligibility are shown in Table 1. Studies selection criteria adopted were 1) Manuscripts written in English, Spanish or in Portuguese; 2) Human RCTs involving subjects with normal or excess body weight; 3) adopted grapes and/or grape products as intervention; 4) provided the information of baseline and endpoint values for the primary outcomes; 5) did not include subjects that were taking grape phenolic compounds as a multi-component supplement. *In vitro* studies, studies involving animals, smokers, pregnant or lactating women, and interventions in which grape product were used as a food ingredient were excluded. Also, studies testing any type of alcohol containing wine were not included because alcohol may interfere in polyphenol bioavailability[14] and satiety responses.[15] Comments, reviews, protocols, letters, case reports, transversal studies, abstracts,

and unpublished articles were not included.

5.3.3. Data extraction

After reading the selected studies, the authors (OGLC and PVMR) compared the compiled data to guarantee its integrity and reliability. Divergent decisions were settled by consensus. For each study included, the following information was extracted: title, author's name, year of publication, study purpose, subjects' characteristics, sample size, intervention (groups and test foods), test foods phenolic composition, study duration, and results.

5.3.4. Risk of Bias Assessment

The authors assessed the risk of bias using the revised tool for assessing risk of bias in randomized trials (RoB 2).[16] The studies were analyzed on five distinct domains of bias: randomization process; deviations from intended interventions; missing outcome data; measurement of the outcome, and selection of the reported results. For crossover studies, an extra domain was assessed, related to the period and carryover effects. Each domain was judged, with a specific algorithm, in low risk, high risk or some concerns. After that, the authors were able to assess the overall bias (low/high/some concerns).[16] Different opinions between the authors were settled by consensus.

5.3.5. Data analyses

The characteristics presented by the selected studies are summarized in Table 2. The studies were organized according to intervention duration. The primary outcomes were appetite (hormones, appetitive sensations, satiety), and food intake considering the mean difference before and after the intervention or comparing the test group(s) and the control group. The secondary outcomes were macronutrient consumption, meal palatability, glucose response, fat mass, body mass index (BMI).

Conducting a statistical meta-analysis was not justified due to the heterogeneity in terms of the products tested and the outcomes assessed among the included studies. Therefore, in accordance with Cochrane handbook we performed a systematic review.[13]

5.4. RESULTS

5.4.1. Study Selection

The initial search yielded 910 potentially articles. Titles and abstracts were screened. A total of 896 studies were removed, resulting in 14 eligible articles. Then, we excluded three studies that did not assess appetite or food intake, four that did not include adults with excess body weight, and one that was an acute study and did not include a group control. Therefore, six studies met all inclusion criteria adopted for this systematic review (Figure 1).

5.4.2. Description of Included Studies

The six studies included in this review (Table 2) contained data from 244 subjects. The sample sizes of the studies ranged from 20[17] to 76[9] subjects. One study included only men,[17] four studies[14,18–20] included participants of both genders (female: 62,8%, n = 93; male: 37.2%, n = 55), and although both genders were included in one study[9] the authors did not inform the proportion between them. The participants included in the six studies were 37.5 ± 7.2 years old, had an age range of 50-70 years old,[20] and a body mass index (BMI) of 26.8 ± 2.8 kg/m^2. Two studies included healthy subjects with normal body weight or that were overweight,[18,20] two studies included only subjects with overweight,[9,14] one study included subjects with overweight or obesity.[19] One study included only lean subjects.[17] The short-term effect (one day[17] and three days[18] of the treatments were assessed in two studies. The effect of the treatments adopted for a more extended period (4-12 weeks) was assessed in four studies.[9,14,19,20]

Four studies assessed appetitive sensations through the Visual Analogue Scale (VAS),[21] differing somehow in the questions used, although hunger and fullness were present in all four of them. Two studies assessed satiety sensation by measuring appetite hormones.[19,20]

Most studies tested the effects of grape extract in capsules or tablets, of which two were from grape seeds.[18,19] Two studies offered some grape juice, Concord grape juice (CGJ)[9] and Muscadine grape juice (MGJ),[14] and one tested an extract of grape polyphenolics[17]. Raisins were offered in one study.[20]

Regarding the geographic distribution, three studies were conducted in the United States,[9,14,20] one in the Netherlands[18], one in New Zealand,[17] and one in Iran[19] (Table 1).

5.4.3. Bias Risk Assessment

The overall risk of bias of all studies[9,14,17–20] included in this review were “some concerns”. However, in four of the five domains,[16] as randomization process, deviations from intended interventions, missing outcome data, and measurement of the outcome, the six studies [9,14,17–20] were “low risk”. On the other hand, the two crossover studies were “some concerns” for the domain related to the bias arising from period and carryover effect,[17,18] specific for crossover design. The domain about selection of the reported results, shows “some concerns” for most studies,[9,14,18–20] except for one that was “low risk”[17] (Figure 2).

5.4.4. Main results of individual studies

Lean and healthy subjects consumed 500mg or 1500mg of dried polyphenolic grape extract (PGE) capsules or placebo (white bread), followed by a standard high-starch low polyphenol breakfast and a lunch served 3h later in a one-day study. The treatments did not cause any adverse sensory effect or nausea. Subjects remained in the laboratory during the whole testing time. Appetitive sensations, *ad libitum* lunch macronutrient consumption, and energy intake were not affected.[17]

In a 3-day study, the effect of daily consumption of three tablets containing a total of 300mg of grape seeds extract (GSE) or placebo tablets 30 minutes before breakfast, lunch, and dinner was tested in subjects with normal body weight and overweight. Subjects consumed standard breakfast and snacks at home and brought all left-overs to the laboratory. Lunch and dinner were consumed *ad libitum* in the laboratory. Subjective appetitive sensations and food intake did not differ between GSE and placebo groups.[18]

Notwithstanding, in another study with the same dose, subjects with excess body weight were prescribed a ~250 kcal restricted-calorie diet based on the subjects estimated energy requirements and took daily capsules (300mg of GSE) or placebo capsules (containing avicel, gelatin and dicalcium phosphate). between meals (breakfast, lunch, and dinner) for 12 weeks. Subjects from the GSE group had a significant reduction in neuropeptide Y (NPY) compared with the placebo group. GSE also led to a reduction in body weight, BMI, waist circumference, and waist: hip ratio[19]. However, appetite and food intake, based on dietary recall, remained unchanged compared with the placebo group after 12 weeks. However,

Subjects with normal body weight and overweight consumed one cup of raisins daily for six weeks. Raisins consumption increased leptin and ghrelin concentrations compared with baseline, while peptide YY (PYY), glucagon-like peptide-1 (GLP-1), and BMI remained unchanged.[20]

The effect of different types of grape juices were tested in two studies[9,14]. In the most recent one, subjects with overweight consumed either 480ml of CGJ or 480ml of polyphenol-free substitute grape-flavored drink (SGD), matching on energy content, appearance, smell, and taste, or no treatment at all. While the portion of CGJ consumed contained 933mg of total phenols, SGD contained none. Appetitive sensations, food intake, body weight, and BMI were not affected in the CGJ group. However, subjects who had SGD decreased fullness after 12 weeks compared with baseline, suggesting that appetite sensations, food intake, and appetite hormones acted differently on satiety than CGJ. Fasting glucose and insulin were not affected, even though serum glucose and insulin 180 minutes AUC increased in response to CGJ[9]. In the other study that assesed the effect of grape juice, subjects with excess body weight consumed 150ml of Muscadine grape juice (MGJ) for four weeks,[14] a portion equivalent to about one-third of the one offered in the previously mentioned study.[9] Appetitive sensations, satiety response, energy, and macronutrient intake did not differ compared with the control group.[14]

5.5. DISCUSSION

To our knowledge, this is the first systematic review to critically analyze clinical trials that investigated the effects of grape products on appetite and food intake. Even though there is a wide range of grape varieties, all studies included in this review tested the effects of red/purple grapes,[9,14,18–20] except for one, that tested an extract from both red and white grape seeds and skin.[17] Albeit the positive results in gastrointestinal hormones secretion, the grape products tested did not affect subjective appetitive sensations and food intake in healthy subjects.[9,17–19] However, some methodologic aspects may explain the lack of effect verified in these studies.

Appetitive sensations and *ad libitum* lunch intake were not affected in the one-day intervention in healthy lean subjects,[17] suggesting that the acute consumption of that dose of PGE may not be the best strategy to control food intake in these subjects. On the other hand, we do not know how PGE would affect these subjects` food intake for a more extended period. The identification of strategies capable of controlling weight

gain in such subjects could avoid an increase in overweight occurrence. It is worthy to note, however, that according to some authors, appetitive sensations to extraneous stimuli tend to differ between lean individuals and those with BMI above 25.0 kg/m^2.[22,23] Although circulating ghrelin concentrations, a hormone that increases appetite, decreased in lean subjects after mixed meals, the opposite effect was observed in subjects with obesity.[23]

The dose tested (500mg or 1500mg of PGE) in that study[17] may not have been enough to provoke an acute food intake reduction. In a study with Wistar rats, an acute dose of 423mg/kg of body weight of grape seed proanthocyanidin extract (GSPE) reduced food intake by 18% in rats.[5] Due to the lack of human evidence to compare the results, we must consider the result obtained in that study[5] and use the body surface area normalization method[,24] to estimate the human equivalent dose (HED)[17] that may lead to food intake reduction.In that case, based on the mean body weight (about 65kg) of the subjects included in the previously mentioned study,[17] we have an HED of aproximatly 68,6 mg/kg of body weight, resulting in 4459mg of PGE. Therefore, it would be interesting if the lean subjects would have consumed about 1500 mg of PGE three times in the same day. Thereby, something else besides the dose could explain the different results between the animal study[5] and the human study.[17] We presume that the sample size (n=20) may have been insufficient to detect differences between interventions.

The 3-day intervention with 300mg of grape seed extract (GSE) did not alter appetite and food intake in subjects with normal body weight and overweight. However, the dose of PSE tested in that study[18] was lower (about 270mg of proanthocyanidin (PAC)) than the high dose (424mg in 1500 mg of PGE) tested in that previously mentioned acute study.[17] Despite the lack of information about PAC content, in a 12-week study,[19] subjects with overweight and obesity lost weight, which is a positive result for that population, although appetite and food intake were not affected.

Nevertheless, we must mention that the GSE dose tested (300 mg) and the duration (3 days) of that study[18] was probably not enough to achieve higher polyhpenols brain levels,[8] which could, in turn, efficiently stimulate changes in appetitive sensations and food intake. When assessing the neural effects of polyphenols, a critical point is related to these hormones' brain concentrations. However, the precise concentration of these compounds in the brain is not clear and appears to vary according to the type of compound studied. Besides, the administration

of limited concentration of polyphenols can impair their quantification in the brain.[8,25] Moreover, appetite and food intake were not affected in the 12-week interventions,[9,19] suggesting that the dose ingested is more relevant than the intervention duration. Apparently, repeated dosing of polyphenols, similar to what might be identified in a daily diet rich in these compounds, would provoke a different result considering that the longer-term administration of polyphenols may generate higher brain concentrations.[25] Unfortunately, there is still no recommendation on how much polyphenols should be consumed on a daily basis to lead the expected health benefits. However, it is known that an intake higher than 1170mg/day is associated with a lower risk of CDV events,[26,27] and an intake higher than 2632mg/day protects against DM2 related events.[28,29] Besides the dose ingested and the intervention duration, the nutritional status of the subjects may affect the effect of polyphenols on appetite and food intake, since lean subjects are less responsive to stimuli that increase satiety than subjects with excess body weight.[22,23]

So far, regardless of the study duration, no study has documented food intake changes in response to grape product consumption. Measuring energy intake is challenging, especially in subjects with overweight, due to food intake under-reporting,[30] which may result in unpredictable outcomes. When these subjects receive a given treatment at home,[9,18] the reported energy intake is very likely to be underestimated.[30] Assessing food intake requires seriousness and commitment from the subjects, once they cannot share any of the food provided, they must eat these foods as they would typically do,[18] and they must provide veracious information about their consumption. Therefore, assessing the *ad libitum* consumption of a meal served in the laboratory can provide a more reliable outcome, especially in studies involving subjects with overweight.

Only one study tested the effect of raisins.[20] In that study, the consumption of one cup of raisins increased leptin and ghrelin concentrations but it did not affect food intake. The increase in plasma ghrelin associated with leptin is antithetical, given the opposite effects of these two hormones on food intake. However, the increase in circulating leptin may have reduced appetite in individuals consuming raisins, leading to a lower food intake, which in turn would may have increased plasma ghrelin.[20] Interestingly, no study tested the effect of whole fresh grapes on appetite. Grapes and raisins have a high content of fiber and bioactive compounds, which could hold potential benefits on overall health and appetite.[31] Although some polyphenols are lost

in the drying process, raisins can provide various other polyphenols. The consumption of 1 cup of raisins (145g) provided 10g of dietary fiber and approximately 3g of soluble fiber per day. In previous studies, the consumption of 6g of arabinoxylan soluble fiber[32] and of 10.5 g of insoluble wheat fiber[33] also increased postprandial circulating ghrelin as a possibly late and indirect effect of reducing food intake[20] since PYY also increased.[33] The exact signals mediating meal-related ghrelin suppression are not known.[20,23,33]

Two types of grape juices were tested, Muscadine (MGJ) and Concord (CGJ). The portion offered of MGJ was one-third of the CGJ one. The consumed portion of CGJ (480 ml) contained 933mg of total phenols, 191mg of anthocyanins, and 307mg of PACs.[9] The polyphenol content of MGJ was not informed. In obese-induced rats, the consumption of PACs (25mg/kg/day) for three weeks reduced food intake, with no changes in energy expenditure and substrate oxidation.[6] If we once again convert that dose[6] to HED,[17,24] we have similar doses, with the one used in animals[6] slightly higher,which may explain the divergent results. Furthermore, it is worthy of note that in this study[9], the subjects consumed CGJ as a complete food matrix, where interactions between the various nutrients can occur, affecting the bioavailability of the PACs[34,35], thus causing different effects than those observed when the isolated compound is ingested.[6] Although appetite and food intake were not affected in CGJ, subjects who had the placebo decreased fullness after 12 weeks compared with baseline.[9] These results suggest that compared to placebo, CGJ probably increased fullness, although that effect was not detected statistically.

All four studies used VAS to measure appetitive sensations.[9,17–19] Although VAS is a subjective measurement widely used in clinical trials, an alternative and sometimes complementary method to evaluate appetite and food intake control is dosing hormones that regulate those proccesses.[36] However, only two studies[19,20] in this review conducted that type of analysis. One cup of raisins increased leptin and ghrelin, while PYY, GLP-1, and BMI were not affected compared with baseline.[20] Further, although the consumption of 300mg of GSE/day for 12 weeks did not affect appetite and food intake in subjects with excess body weight, it led to a significant reduction in the orexigenic NPY compared with placebo.[19] NPY plays an important role in modulating food intake, its release is enhanced immediately prior to feeding onset and it gradually decreases as food intake continues.[2,37] Nonetheless, food intake, assessed through 3-day dietary recall, was not affected,[19] suggesting that the dietary intake

methodology may have failed in detect the alterations. Some flaws that may occur when this method is used are: incomplete reporting due to the fact that some subjects may not remember consuming specific foods or beverages, inaccurate measuring or portion sizes estimation, and accidentally or purposely failing to record specific items.[38] Thus, GSE[19] and raisins[20] may indeed play a role in controlling food intake by modulating anorexigenic hormones.

Based on the results of *in vitro* and animal studies,[5,39] the long-term consumption of GSE may affect food intake through orexigenic and anorexigenic hormones modulation. Unfortuntely, in the previously mentioned study[19] there was no information about the phenolic composition of the GSE tested. Since different compounds may act through several pathways to modulate appetite and food intake,[7,39] knowing the GSE composition would have enabled us to better explain the results observed in terms of doses and mechanisms involved.

To clarify the effect of grape products on appetite and food intake as a strategy to control obesity in humans, we believe it is interesting to include in future studies only subjects with excess body weight, since lean individuals tend to be less responsive to the applied treatments.[22,23] According to some authors, the acute effect of the consumption of a minimum dose of 69 mg of PAC/kg/day for at least 12 weeks should be tested. Importantly, the PAC content must be offered in the form of a beverage or a meal rather than in capsules. Albeit the fact that beverages are not as satiating as solid meals, their satiating effect is still higher than the observed in response to capsules or tablet administrations, since beverages activate the reward system while capsules do not.[40,41] To have a better picture of the effect of the treatments on subjective appetitive sensations, it would be interesting if the concentration of orexigenic and anorexigenic hormones could be evaluated.[19] *Ad libitum* food intake should be assessed in the laboratory instead of being assessed under free-living conditions.[17] Adopting these approaches associated with the assessment of other variables, such as energy expenditure and substrate oxidation, may clarify the effect of grape products on obesity control and may lead to a better understanding of the possible mechanisms involved. Hereafter, based on the available data about this topic, we will elucidate and discuss the potential pathways by which grape products may modulate appetite and food intake (Figure 3).

Possible mechanisms involved in appetite and food intake modulation

The mechanisms explored bellow are applied to individuals with excess body weight, since normal weight subjects do not have appetite regulation impairment. Grape polyphenol will contribute to recover the normal regulation. Food intake and energy expenditure regulate energy balance through interactions among nutrients and peripheral hormones, including anorexigenic pro-opiomelanocortin (POMC) and orexigenic AgRP, both located in the hypothalamus arcuate nucleus.[42] In POMC and AgRP expressing neurons, leptin interacts with its longest receptor isoform (Obrb), activates STAT3, dimerizes, and translocates from the cytoplasm into the nucleus, where it binds to the POMC and AgRP promoters.[43,44] In that scenario, GSPE increases STAT3 without altering its receptor expression, stimulates POMC expression, and inhibits AgRP, thus recovering leptin signaling in the hypothalamus and reducing food intake[6] (Figure 3). Leptin resistance is a condition mostly present in individuals with obesity.[42] Overall, subjects from the studies included in this review had moderate BMI (mean BMI ranging from 23.1 to 31.8 kg/m^2). That nutritional status might have affected the treatments' response, whereas GSPE was effective in recovering leptin resistance in obese mice.[6]

Another mechanism concerning proanthocyanidin and food intake is related to the modulation of NPY, an important orexigenic hormone, which increased levels or enhanced activity stimulates food intake in an animal model.[8] Resveratrol, an important polyphenol in grapes, has a neuroprotective effect in mice under a high-fat diet (HFD). While HDF promoted NPY neuron production, adding resveratrol to HFD changed new cells' fate to POMC neurons. Because NPY neurons are orexigenic and that POMC ones are anorexigenic, we can hypothesize that cell differentiation induced by resveratrol changes this main feeding center's architecture for satiety response and weight loss.[45]

Strengths and limitations

Our study has many strengths that should be highlighted. All studies included in this review adopted appetite and food intake as primary outcomes. Appetite was assessed using the same method in all studies, allowing us to compare the results even at various time points. Three studies informed the grape products polyphenol composition, supporting a relevant discussion on the molecular mechanism exerted by

proanthocyanidins. Regardless of the lack of information in some studies, the total polyphenol content tested in the studies included in this review ranged from 353mg (>40% proanthocyanidins) to 933mg (33% proanthocyanidins), allowing us to verify the effects of a wide range of doses.

Nevertheless, there are limitations in this study that could contribute with future investigation conception. The studies selected for this review adopted different approaches to assess food intake. Only two studies evaluated appetite hormones concentrations in response to intervention.[19,20] Appetite hormones assessment is considered an objective method to measure appetite control, and it may provide more answers to the researchers in this field. Some studies included subjects with normal weight and excess body weight in the same group[9,18,20], which could have interfered with the intervention effects. Finally, the lack of data regarding the composition of polyphenols showing a positive result on appetite control did not allow us to make further inferences regarding the polyphenols composition and neuromodulators.

5.6. CONCLUSION

The acute consumption (1-3 days) and the long-term (4-12 weeks) consumption of grape products had no effects on subjective appetitive sensations and food intake in men and women with normal body weight and overweight. Besides, the consumption of 300mg of GSE intake for 12 weeks decreased NPY concentration, and one cup of raisins for 6 weeks increased leptin and ghrelin, although these two treatments did not affect food intake. We believe that sufficient amounts (at least 69 mg/kg of body weight) of grapes proanthocyanidin may control appetite and food intake by modulating neuropeptides in the brain and stimulating the secretion of gastrointestinal hormones by the enteroendocrine cells. Hence, the effect of the consumption of grape products providing that amount of proanthocyanidin should be assessed in future long-term studies (at least 12 weeks) involving subjects with excess body weight. The assessment of appetite hormones is necessary to better understand how polyphenols modulate appetite and food intake.

5.7. REFERENCES

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Table 1. PICOS criteria for inclusion of studies

Parameter	Inclusion criterion
Participant	Healthy adults with normal weight or excess body weight
Intervention/exposure	Consumption of grapes or any derived product
Comparison	Consumption of placebo or nothing
Outcome	Appetite hormones, appetitive sensations, food/energy intake, glycemic control markers, anthropometric measures, and intervention acceptance
Study design	Randomized Clinical trials

Table 2. Characteristics of the randomized clinical trials in which the effects of grape products on appetite and food intake measures were assessed.

Reference	**Subject characteristics**	**Intervention arms**	**Phenolic content**	**Duration**	**Main results**
Shin et al., 2015	20 healthy men 26.4 ± 1.7 years old BMI: 23.1 ± 0.7 kg/m^2	- PGE $_{500}$: 500mg of dried PGE - PGE $_{1500}$: 1500mg of dried PGE - Placebo: 3 capsules containing corresponding placebo	PGE $_{500}$: 353mg total polyphenol (>40% of PCAs ~141mg) PGE $_{1500}$: 1059mg (>40% of PCAs ~424mg)	1 day	PGE$_{500}$ and PGE$_{1500}$ x Placebo: ↔ hunger, fullness, desire to eat, thoughts of food, EI, palatability.
Vogels et al., 2004	51 subjects (20M/31F) 48.7 ± 14.3 years old BMI: 25.6 ± 2.6 kg/m^2	- 300mg of GSE - Placebo tablets	>90% of PCAs (~270 mg)	3 days	GSE x Placebo: ↔ hunger, fullness, appetite, satiety, prospective consumption, desire to eat, food intake.
Banini et al 2006	23 subjects (11M/12F) 53 ± 10.3 years old BMI: 28.4 ± 1.4 kg/m^2	- MJ: 150 ml of Muscadine juice - Control: no treatment	Not informed	4 weeks	MJ x Control: ↔ EI, macronutrients intake

Puglisi et al, 2009	34 subjects (17M/17F) 50-70 years old BMI: 24.9 ± 2.3 kg/m^2	- RAISINS: 1 cup of raisins	Not informed	6 weeks	Raisins x baseline: ↑ leptin, ghrelin ↔ PYY, GLP-1, BMI
Parandoosh et al., 2019	40 subjects (7M/33F) 34.2 ± 2.0 years old BMI: 31.8 ± 8.5 kg/m^2	- 300mg of GSE - Placebo tablets	Not informed	12 weeks	GSE X Placebo: ↔ appetite, hunger, fullness, food intake ↓ NPY, BMI, waist circumference, waist:hip ratio compared to placebo
Hollis et al., 2009	76 subjects (gender proportion not informed) 25.33 ± 7.7 years old BMI: 27.1 ± 1.5 kg/m^2	- CGJ: 480 ml of CGJ - SGD: 480 ml of polyphenol-free substitute grape-flavored drink matching on energy content, appearance, odor and taste - NTG: no-treatment control group	933mg of total polyphenol: 191mg of anthocyanins 639mg of PCAs	12 weeks	CGJ x SGC and NTG: ↔ hunger, fullness, appetite, satiety, prospective consumption, desire to eat, EI, palatability, body weight and BMI ↑ Serum glucose and insulin AUC_{180} SGD x NTG:

					↔ fasting glucose and insulin SGD x baseline: ↓ fullness

↔: unchanged; ↓: decreased; ↑: increased. AUC_{180}: Area under the curve after 180 minutes; BMI: body mass index; CGJ: Concord grape juice; EI: energy intake; F: female; GSE: grape seed extract; M: male; MGJ: Muscadine grape juice; NPY: neuropeptide Y; NTG: no-treatment control group; PCAs: proanthocyanins; PGE: polyphenolic grape extract; SGD: substitute grape-flavored drink.

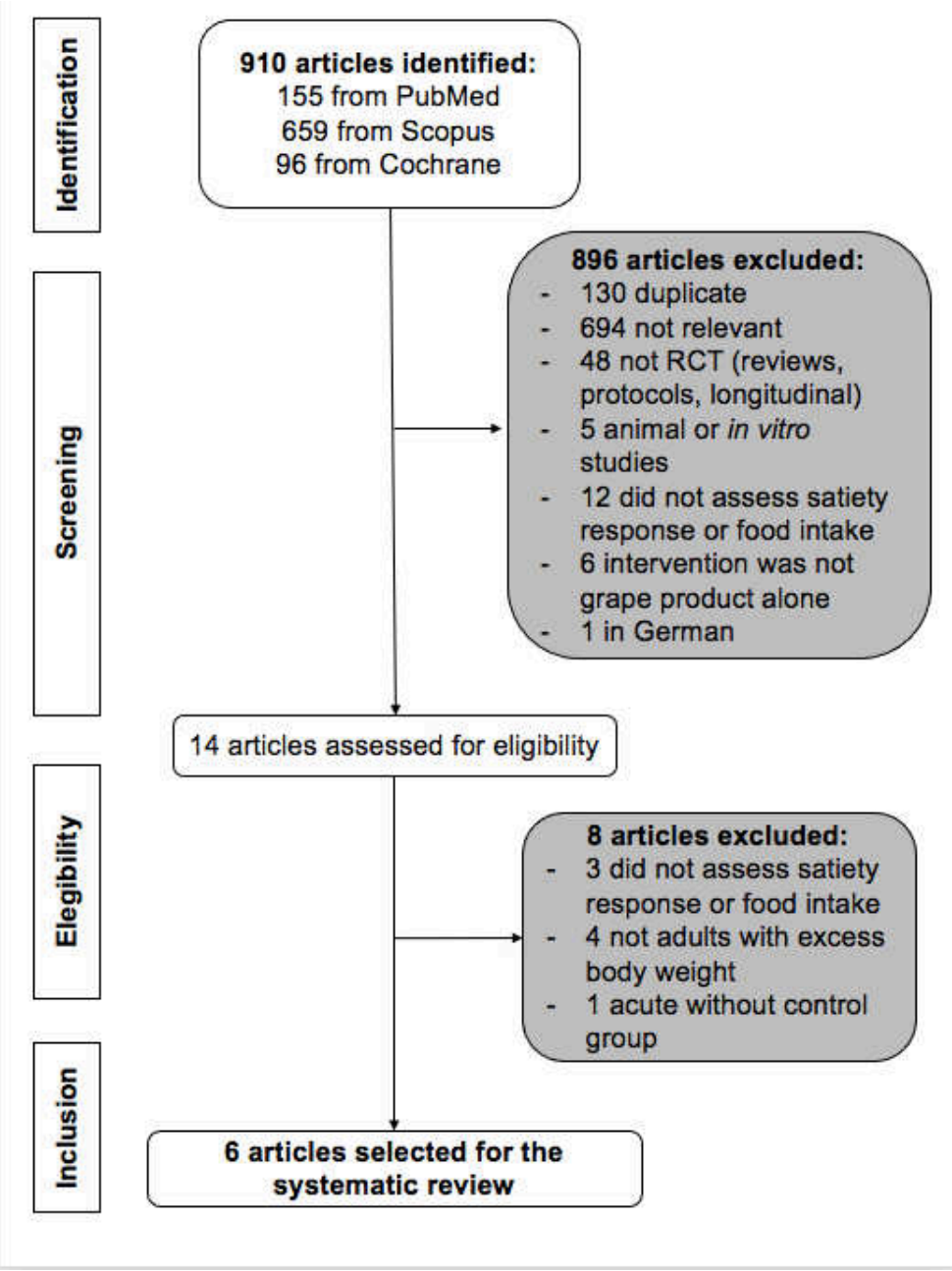


Figure 1. Flowchart of the study selection process.

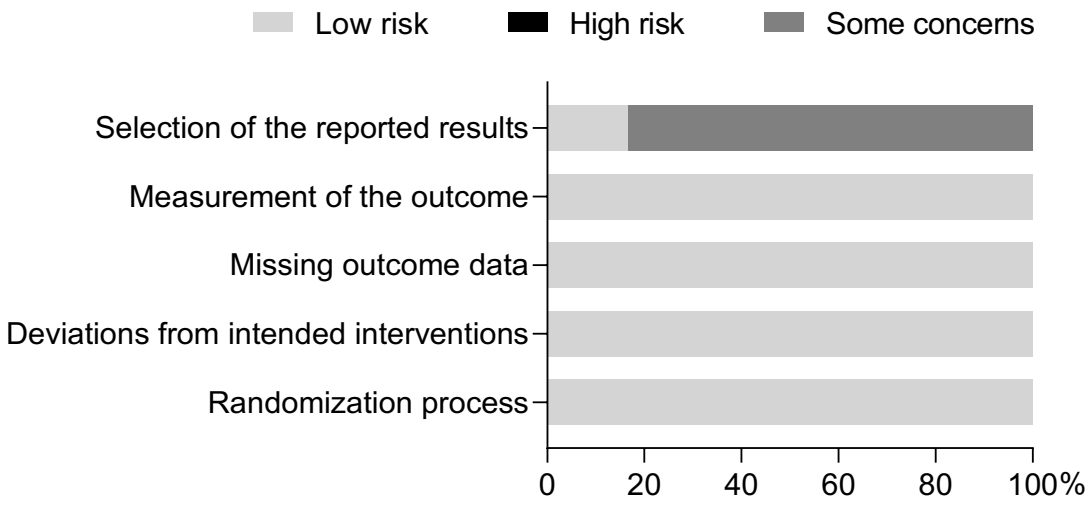


Figure 2. Risk of bias summary: authors' judgments about the five risk of bias domains for the included study. Bias classified as low risk, high risk, and some concerns.

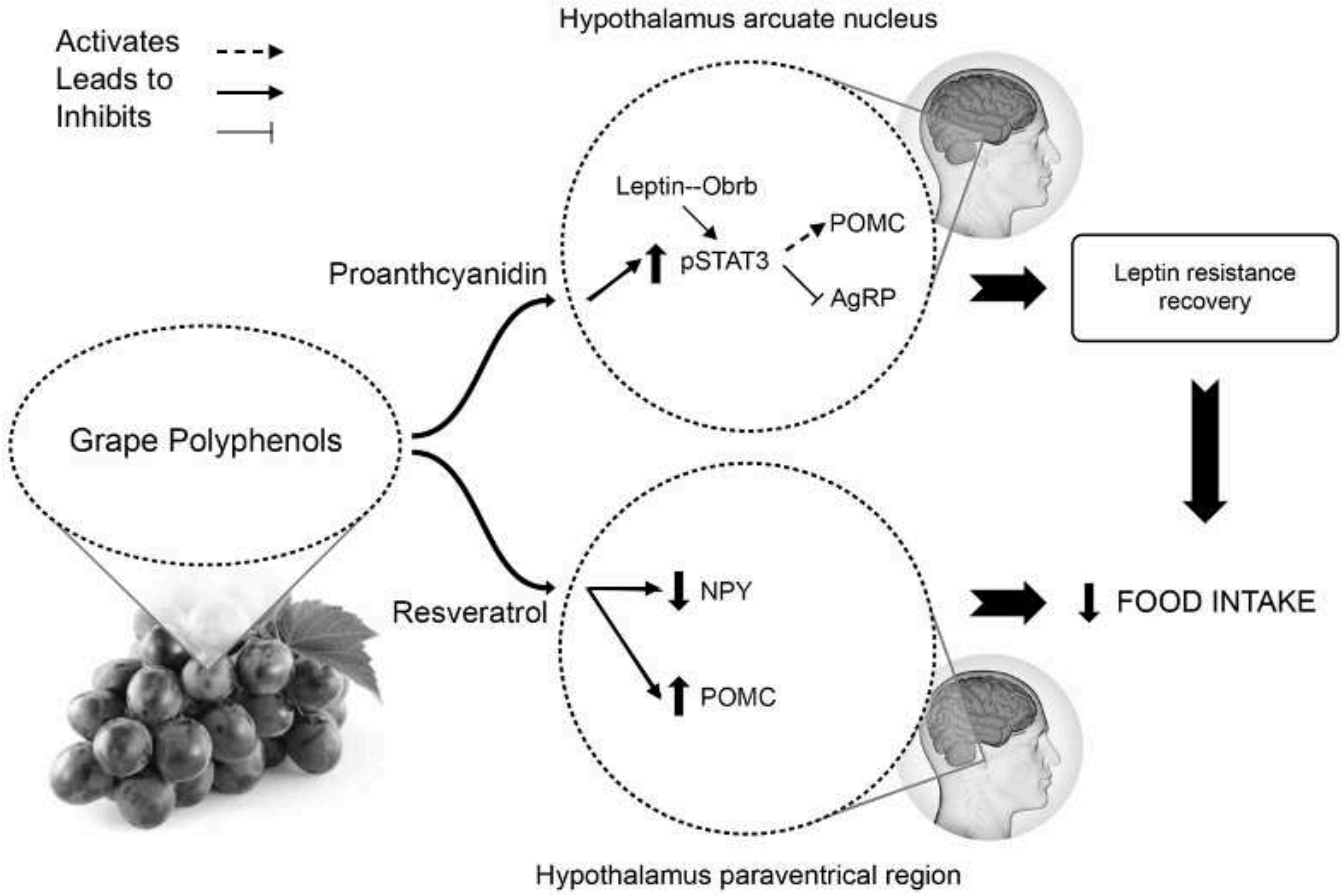


Figure 3. Possible mechanisms by which proanthocyanidins reduces food intake. In the hypothalamus arcuate nucleus, proanthocyanidins increase pSTAT3 concentrations, activate POMC and inhibits AgRP expressions, without affecting Obrb receptor, thus reducing leptin resistance. In the paraventrical region of hypothalamus, resveratrol can reduce NPY expressing neurons and increase POMC expressing neurons. Both actions will contribute to reducing food intake. ↑: Increases; ↓: Decreases; AgRP: Agouti-related protein; NPY: Neuropeptide Y; Obrb: Leptin longest receptor isoform; POMC: Pro-opiomelanocortin; pSTAT3: Signal transducer and activator of transcription 3 phosphorylated.

6. ARTIGO ORIGINAL: Effects of Concord grape juice flavor intensity and phenolic content on glycemia, appetite and cognitive function in adults with excess body weight: a randomized-crossover trial

- Artigo submetido à revista Food & Function (fator de impacto: 4,171)

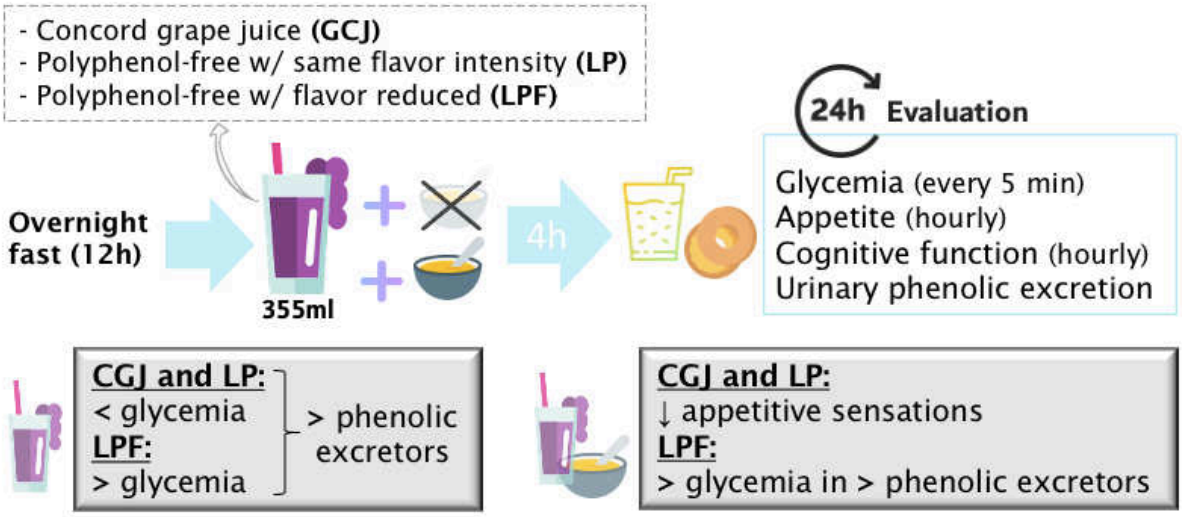
6.1. ABSTRACT

Background & Aims: Concord grape (*Vitis lambrusca*) juice (CGJ) contains a unique combination of phenolic compounds with diverse effects on human health. It also has an intense sensory profile that may modify food choice. Daily consumption of CGJ over 8 weeks reduced fasting blood glucose. However, the impact on 24h-postprandial glucose response from CGJ is still not clear. The purpose of this study was to assess the effect of CGJ flavor intensity and phenolic content on 24h postprandial glucose concentration, appetitive sensations, and cognitive function in adults with excess body weight when consumed alone and with a meal. **Methods:** In a randomized, double-blind, crossover design study, participants consumed 355ml of three types of beverages: 100% CGJ, a polyphenol-free grape flavored drink with the same flavor intensity (LP) or a polyphenol-free grape flavored drink with reduced flavor intensity (LPF) either without (trial I) or with (trial II) a meal. 24-h glucose was measured through continuous glucose monitoring. Phenolic metabolite excretion was assessed in 24-h urine samples. Appetite and cognitive function were assessed hourly through visual analog scales throughout 4 hours after beverage intake. **Results:** Thirty-four adults completed each trial. When consumed with a meal, CGJ and LP reduced hunger, desire to eat, and prospective consumption. The consumption of LPF beverage was associated with higher glucose response. No consistent effects were observed for cognitive outcomes. When consumed alone, CGJ was related to a lower glycemic response by those excreting a higher concentration of phenolic metabolite, but in LPF, glycemia was higher among those excreting higher concentrations of caffeic acid-O-sulfate. **Conclusions:** Both natural phenolics and flavor intensity of CGJ help to moderate appetite and glycemia.

Key words: Grapes, polyphenols, glycemic response, appetite, flavor intensity.

Clinical Trials registered at www.clinicaltrials.gov: NCT03409484 (trial I) and NCT03409497 (trial II).

Graphical abstract



Concord grape juice phenolics and flavor intensity moderate appetite and glycemia in adults with excess body weight, depending on the dietary context in which the juice is consumed.

6.2. INTRODUCTION

The consumption of foods and food supplements with properties that may aid adherence to or the metabolic effects of diets intended to promote negative or neutral energy balance may contribute to the prevention and treatment of obesity and its associated comorbidities, such as diabetes [1,2]. Improved understanding of the functionality of foods is needed as more than 1.9 billion adults have excess body weight [3], and it is projected that 418 million people will have impaired glucose tolerance by 2025 [4]. In the US, an estimated 88 million Americans have pre-diabetes [5]. The current prevalence of Type 2 diabetes is over 8% [6].

Diet is the preferred approach to management of body weight and diabetes [2]. It entails food choices that meet nutrient needs while supplying less energy than needed (to accomplish weight loss) or meeting energy needs (to maintain a healthy body weight). Further benefits may accrue if the properties or bioactive components of

selected foods prevent wide swings in glycemia. This may be accomplished by ingestion of foods that either do not themselves promote wide deviations of glycemia or possess properties that moderate the effects of other ingested high glycemic foods [10,14].

Concord grapes are a native North American purple grape variety cultivated principally in the United States to produce unfermented grape juice products. 100% Concord grape juice (CGJ) contains high concentrations of a unique combination of phenolic compounds including anthocyanins, tartarate esters of hydroxycinnamates, flavonols and flavan-3-ols (including complex proanthocyanidins) [7]. One cup of CGJ (240ml) provides about 30 mg of proanthocyanidins, 64 mg of anthocyanins, 3.3 mg of flavanols, and 8.6 mg of flavonols, equivalent to 105 mg of total flavonoids and 616 mg of total phenolic compounds. Thus, flavonoids from even a modest serving of CGJ can contribute substantially to the total daily intake of polyphenols [8].

Accumulating evidence suggests polyphenols present in purple grapes inhibit α-amylase and α-glucosidase activity [9], reduce fasting blood glucose [10], reduce of LDL oxidation [11], increase platelet-derived nitric oxide production [12], and improve memory function [13]. Additionally, management of glycemia has been linked to the regulation of food intake [14]. Thus, the phenolic content of CGJ, may directly contribute to broader health benefits through moderation of hyperglycemic episodes [10], anti-oxidative and anti-inflammatory potential [15], appetite control [16], and enhanced cognitive performance [17].

The sensory properties of foods are key drivers of food choice as well as energy and nutrient intake. While palatability influences the appeal of foods and beverages, other sensory attributes may more directly modify appetitive sensations. Strong flavor intensity is a property that may augment the sensation of fullness and thereby reduce portion size and total energy intake [18–23]. CGJ has an intense grape aroma and strong sweet taste; properties hypothesized to promote satiation [23–26].

Sensory properties may also modulate cognitive function. Aromas of different qualities [27–29] as well as stimuli leading to altered oro-sensory sensations [30] reportedly enhance or depress mood, cognition, alertness, and vigilance. Similar effects have also been reported for polyphenols [31,32]. The distinct sensory properties and high polyphenol content of CGJ have prompted studies of its impact on these outcomes. Positive effects have been noted in younger [33,34] and older [13,35] adults, though sample sizes have been limited.

Taken together, the literature [7–9,13,18–23,33,34] suggests that the phenolic content and strong flavor intensity of CGJ juice hold potential beneficial effects on appetite, glycemia and cognitive function. However, evidence that CGJ moderates whole day glycemic responses, appetite sensations, and cognitive function in individuals with overweight/obesity is lacking. The assessment of these effects was the primary goal of this study.

Beyond therapeutic interest, consumption of CGJ as a modifier of appetite and glycemia could provide an option to consumers interested in alternatives or compliments to snacking to extend their performance through the day. The post-prandial effects of a meal or snack extend beyond the time between successive eating events. It is expected that inclusion of CGJ with a high glycemic meal will serve to moderate the 24h post-prandial glycemic profile due a reduced rate of carbohydrate digestion and glucose absorption and second meal effects [36]. Thus, an additional aim of this study was to determine the effects of CGJ, either consumed alone (i.e., as a snack) or with a meal (meal modifier), on the 24h-glycemic response, appetite and cognitive function in adults with elevated adiposity.

6.3. MATERIAL AND METHODS

6.3.1. Study Design

The present study consisted of two trials. In trial I, participants consumed test beverages alone, while in trial II the beverages were consumed as part of a defined breakfast. Other than that, all testing was identical. Each trial was an acute randomized, double-blind, crossover study involving three sessions. Randomization was computer generated and were kept in consecutively numbered envelopes opened at the moment of participant enrollment into the study. The lab manager generated the random sequence and one researcher enrolled the participants and assigned them to interventions according to the sequence generated previously. Both researchers and participants remained blinded from the time of randomization until data collection and statistical analyses were completed. The study procedures were in accordance with the ethical standards of Purdue University and the research protocol was reviewed and approved by Purdue University's Institutional Review Board.

During each session, participants consumed, on separate days, one of the three test beverages. Each session lasted 4 days with a 5-day washout period between

them. Study sessions were conducted on the same days of the week. Participants consumed a low phenolics diet at home from day 1 through day 4 of each session. On day 2, a continuous glucose monitoring patch (CGM - Dexcom model G6) was placed onto the abdomen of each participant according to the manufacturer's instructions. Participants were instructed not to take acetaminophen from 24 hours prior to day 2 until the monitor was removed to avoid inaccurate glycemia readings. On day 3, participants reported to the laboratory after a 10-12h fast to consume one of the test beverages (with or without a meal, according to each trial), were released to their activities, and returned 4h later to consume a defined lunch in the laboratory. Participants were instructed not to eat or drink anything (including water) during the 4 hours between breakfast and lunch. Immediately after the first sip of the juice, participants provided flavor ratings on a scale that ranged from 0 to 14. Appetitive and cognitive sensations were recorded hourly during 4 hours. 24-h urine was collected. The CGM was removed on day 4 (Figure 1).

The specific hypotheses tested were:

- CGJ will moderate post-prandial glycemia relative to phenolic-free grape flavored drink (LP) and phenolic-free and low flavor intensity (essence) grape flavored drink (LPF) when consumed alone as the first eating event of the day.
- CGJ will moderate post-prandial glycemia relative to LP and LPF when consumed with a standard meal as the first eating event of the day.
- If flavor intensity (essence) affects appetite, CGJ and LP will reduce appetite compared to LPF.
- If phenolic concentration affects post-prandial glycemia, CGJ will reduce post-prandial glycemia compared to LP and LPF.
- If interactive effects occur between meal constituents or properties and CGJ, differential effects on glycemia, appetite and/or cognition will be observed when the CGJ is ingested alone versus with a meal.

6.3.2. Test beverages and meals

Three different beverages were tested in both trials: Concord grape juice (CGJ), phenolic-free same flavor intensity as CGJ drink (LP), and phenolic-free low flavor intensity drink (LPF). Table 1 shows additional information about beverage characteristics and composition. The beverages were matched on other sensory

properties (e.g., appearance, viscosity). A grape flavor essence was added to LP to match the natural flavor intensity of CGJ. For LPF, the dosage rate of the added grape flavor essence was reduced 20-fold. Beverages were provided by Welch Foods Inc. A portion of 355 ml of each beverage (about 45-50g carbohydrate) was served.

During trial I, participants consumed each of the test beverages alone as the first ingestive event of the day. During trial II, they consumed each test beverage with a bowl of Cream of Wheat® original flavor (28g containing 100kcal, 20g of carbohydrates, 1g of fiber, 0g of fat, and 3g of protein). The cereal was prepared following the manufacturer's instructions. No seasoning, sugar or salt was added. In both trials, participants consumed the same standard lunch within 10 minutes. Participants were asked to eat the entire portion served. It consisted of a white bagel, fat-free mozzarella cheese and fruit-flavored sugar-sweetened beverage (Table 2).

6.3.3. Participants

Potential participants responded to public advertisements and completed a pre-screening questionnaire. Those meeting initial screening criteria had measurements taken of height, body weight, body composition (Tanita Model TBF-410 by Tanita Corporation of America Inc.), capillary finger-stick blood glucose (OneTouch Ultra 2 glucometer (Lifescan, Milpitas, CA), and questionnaires were completed for assessment of physical activity and eating patterns.

The study included adults who had a BMI between 25.0-34.9 kg/m^2 and were 25-60 years of age; had a score $\leq$ 14 in the food intake disinhibition and hunger questionnaire; non-smokers; not post-bariatric surgery patients; regular breakfast consumers (≥100 kcal ingested within 2 hours of waking, at least 4 days a week); willing to eat all test foods; low tea and coffee consumers; and willing to refrain from caffeinated beverages during the 48 hours prior to test days and on the test day. They were also required to have fasting blood glucose below 110 mg/dl; not use any medications, vitamins or other supplements known to affect glycemia, lipid metabolism or appetite; have stable body weight (< 5kg in the 3 months prior to the beginning of the study); were not pregnant or lactating; and did not have any acute or chronic disease.

6.3.4. Outcomes measures

Glycemic response

Blood glucose monitoring started before ingestion of the test beverage and continued for 24h using a commercially available CGM with a wireless receiver (model G6 Platinum, Dexcom, San Diego, CA). Glucose readings were collected every 5 minutes by an accompanying wireless receiver that remained within 20 feet of the sensor. These data were used to compute the postprandial glucose 10, 20, 30, 60, 120, and 180 minutes after beverage intake and after lunch intake, and also the 24h mean, peak and nadir glucose concentrations. Glucose total area under the curve ($_{t}$AUC) was calculated from 0 to 4 hours and from 0 to 24 hours after beverage intake, using the trapezoidal rule.

Appetitive sensations and cognitive function

Appetite (hunger, thirst, fullness, desire to eat, and prospective consumption) [37,38] and cognitive function (alertness, energy, strength, calmness and relaxation) [39] were assessed hourly, starting immediately before test beverage consumption until before lunch intake, using visual analogue scales (VAS). Participants marked scales with bipolar end anchors specific to the trait being evaluated (e.g., hunger, alertness) and the distance from the low anchor to the participant's mark was measured in millimeters. Flavor intensity was recorded on a 14 cm Label Magnitude Scale and responses were coded as the distance in millimeters from the lowest point on the scale to the participant's mark [59]. Samples were rated independently, not relative to each other. Higher appetite scores reflect more intense sensations, but higher cognitive function scores reflect less intense sensations. Qualtrics software was used to capture questionnaire responses and were accessed from each participant's smartphones, tablets, laptops or computers.

Phenolic metabolites excretion

Urine samples were kept refrigerated until analysis. Target phenolic metabolites were extracted from urine samples, aliquoted from the 24h urine collection, with preconditioned 96-well solid-phase extraction cartridges (Strata™-X Polymeric Reversed Phase, Phenomenex, Torrance, CA, USA; microelution 2 mg/well) as previously described [60] with minor modifications. Briefly, the 96 well cartridges were

preconditioned with 200 µl 1% formic acid in methanol followed by 200 µl 1% formic acid in water. 75 µl of urine samples were spiked with ethyl gallate (internal standard for extraction efficiency) and were loaded onto the preconditioned wells. The samples were then placed on a positive pressure nitrogen manifold (Waters Positive Pressure-96 Processor) to facilitate the elution process. Samples were washed twice with acidified water prior to elution with methanol (100 µl, 0.1% formic acid) into a 96-well plate (350 µl Acquity 96-well plate, Waters, Milford, MA, USA).

Phenolic metabolite analysis was conducted using a Waters XEVO TQD (I Class UPLC system equipped with a triple quadrupole detector). The inlet method was optimized for a run time of 6 minutes for 5µl of injection volume on an Acquity BEH C18 column (2.1 um, 1.7 mm id x 50 mm) at 40°. The mobile phase consisted two solvents: 0.1% formic acid in acetonitrile and 2.0% formic acid in water (positive mode) or 0.1% formic acid in water (negative mode) with a gradient system of: 0 min, 100% B; 0.5 min, 94% B; 2 min, 91% B; 3 min, 87% B; 4.5 min, 65% B; 5.2 min, 100% B; 6 min, 100% B. MS conditions were as follows: capillary voltage, 0.5 kV; probe temp, 150°C; source temp, 600°C; desolvation gas flow, 1000 l/hr; cone gas flow, 50 l/hr. MS source parameters for individual compounds were optimized for cone voltage and collision energy by directly infusing individual standards. For glucuronides and sulfate conjugates or other target metabolites where standards were not available, previously reported mass fragmentation patterns with confirmatory secondary transitions were used for their identification.

Creatinine excretion

Creatinine was measured to check the completion of urine collection and analyses were performed according to the manufacturer's protocol (Roche Diagnostics). Creatinine reflects lean body mass, which does not change rapidly. 24h urine samples were stored at -25°C until batch analyses. A sample (0.50 ml) was taken from the mixed total urine volume. No preparation was required. The analyses were conducted on a Roche COBAS 400 Plus analyzer using 10µl of sample plus reagents (30µl) and diluent (107 µl of water).

6.3.5. Statistics

Statistical analyses were carried out with SPSS 22 software for Mac (SPSS, Inc., Chicago, IL, USA) and power was determined with a post hoc power analyses performed with G*Power 3.1 software [40]. The criterion for statistical significance was $p < 0.05$, two-tailed. Analyses were performed within each trial. The number of participants in each trial was calculated [41] based on a confidence interval of 95%, considering a reduction of 10% on mean first 2h postprandial serum glucose concentration [42]. The stated sample size provided a power of 84% with a large size effect size (0.577) in each trial [43].

Descriptive statistics are presented as mean ± standard error of the mean (SEM) unless otherwise indicated. Data normality and homogeneity of variance were assessed by Shapiro-Wilk and Levene tests, respectively. Variables with non-normal distributions were transformed logarithmically and if the distribution remained non normal, a non-parametric test was used for analyses.

One-way repeated measures ANOVA (or non-parametric Friedmann test for non-normal data) was used to compare flavor ratings, glycemic response, $_{t}$AUC, and phenolic metabolite excretion between groups. Beverage effects on appetitive sensations and cognitive function variables from time 0 to 4 hours post prandial were tested by a mixed model, repeated-measures ANOVA.

Additionally, participants were classified as low and high phenolic excreters by dividing groups at the median urinary concentration for each of the selected compounds. Plasma glucose concentrations and appetitive sensations were then compared within each treatment group by Student *t*-test.

6.4. RESULTS

6.4.1. Trials I and II participant baseline characteristics

In trial I, there were 245 participants interested in the study, but only 60 met eligibility criteria, and 39 consented to participate after learning about all procedures. Three participants withdrew because of schedule issues and two participants were withdrawn because of no compliance (Figure 2). In trial II, there were initially 177 participants interested in the study and 41 met eligibility criteria. Among the 38 participants who signed the consent form, four withdrew because of personal issues

(Figure 2). In trial I, 55.9% were male (n=19) while in trial II 67.7% were male (n=23). Baseline characteristics of participants were similar in both trials (Table 3).

In trial I, mean ± SEM 24-hour urinary creatinine excretion was 91.8 ± 6.8 mg/dl, 97.56 ± 8.6 mg/dl, and 97.09 ± 7.9 mg/dl for CGJ, LP, and LPF, respectively. In trial II, creatinine excretion was 112.2 ± 11.0 mg/dl, 103.6 ± 8.5 mg/dl, and 107.9 ± 10.5 mg/dl for CGJ, LP, and LPF, respectively. Creatinine excretion was not significantly different between groups in both trials (p = 0.151; p = 0.590). Flavor intensity ratings were not different (p = 0.386; p = 0.226) between test beverages in both trials (Table 5).

6.4.2. Appetite and cognitive function

When CGJ was consumed with a meal (trial II), there was a significant time*treatment interaction for hunger, desire to eat, prospective consumption, and alertness (Figure 3**)**. CGJ and LP reduced hunger, desire to eat, and prospective consumption 1h and 2h after intake, with baseline concentrations reestablished at 3h. No significant effects were observed with ingestion of the CGJ alone (trial I). We also verified a significant (p=0.038) time*treatment interaction for alertness. Participants consuming LPF were more alert compared to ingestion of CGJ 1h after beverage intake. CGJ promoted greater alertness at 2h than in the fasting state. At 1h postprandial, participants consuming CGJ and LPF were more alert than after LP (Figure 3).

6.4.3. Phenolic excretion

A total of 29 and 26 different phenolic metabolites generated presumably from metabolism of both CGJ and/or other food phenolic and amino acid components were detected in the 24h-urine samples in trial I (Table 4) and II (Table 5), respectively.

In trial I, 4-hydroxybenzaldehyde, 3-hydroxyphenylpropionic acid, 3-hydroxyhippuric acid, caffeic acid-O-sulfate, and Iso/ferulic-3'-O-glucuronide concentrations were significantly higher in urine samples after GCJ compared with LP and LPF (Table 4). High excreters of Iso/ferulic-3'-O-glucuronide had lower 4h $_t$AUC (23.010 ± 682 *vs* 19,596 ± 718; p=0.009), lower 24h $_t$AUC (144,176 ± 3,446 *vs* 124,490 ± 3,485; p=0.003), and lower 24h mean glucose response (100.9 ± 2.4 *vs* 87 ± 2.4; p=0.003) after CGJ consumption (Supplementary Table 1). In addition, participants with higher excretion of 3-hydroxyhippuric acid reported less fullness (41.2 ± 2.7 *vs*

27.5 ± 3.3; *p*=0.003) after CGJ intake alone (Supplementary Table 1). High 3-hydroxyhippuric acid excreters had lower 24h mean glucose in response to LP consumption (103.1 ± 3.2 *vs* 94,5 ± 2.1; *p*= 0.032) (Supplementary Table 1). High caffeic acid-O-sulfate excreters had higher 4h $_t$AUC (20,431 ± 759 *vs* 23,468 ± 876; *p*=0.013), 24h $_t$AUC (128,851 ± 5,130 *vs* 147,614 ± 4,689; *p*=0.011), and 24h mean glucose response after LPF (90.2 ± 3.5 *vs* 103.1 ± 3.2; *p*=0.010) (Supplementary Table 1).

In trial II, seven phenolic metabolites were higher in the CGJ (Table 5). When beverages were consumed with a meal, participants with higher levels of 4-OH-benzaldehyde had lower desire to eat with CGJ (56.6 ± 3.7 *vs* 44.4 ± 3.9; *p*=0.032) and more fullness in LPF (30.1 ± 2.8 *vs* 39.9 ± 3.8; *p*=0.048) (Supplementary Table 2). Participants with higher levels of caffeic acid-O-sulfate showed less thirst (52.1 ± 4.1 *vs* 34.3 ± 4.5 *p*=0.007) and less desire to eat (56.2 ± 4.2 *vs* 44.7 ± 3.6, *p*=0.044) (Supplementary Table 2). When LPF was consumed with a meal, high producers of 3-OH-phenylacetic acid had higher 24h $_t$AUC than low excreters (146309 ± 3669 *vs* 157532 ± 3616; *p*=0.037) (Supplementary Table 2).

6.4.4. Glycemia

Postprandial glucose responses were similar between groups in both trials. In trial II, CGJ nadir glucose was higher than with LPF (Table 6). The three beverages did not promote a significant second meal effect in either trials. In both trials, there was an interaction for postprandial glycemia after beverage intake ($p < 0.001$) and after lunch intake ($p < 0.001$). However, there was no simple main effect of treatment, only of time (Table 7). As expected, postprandial glycemia values at times 20, 30, and 60 minutes after beverage intake were higher than in the fasting state. Also, postprandial glycemia at 30-180 minutes after lunch intake was higher than before (L0) lunch intake. The results were similar for CGJ, LP, and LPF (Table 7).

6.5. DISCUSSION

In the present study, we aimed to assess the effects of natural polyphenols and flavor intensity of CGJ on glycemia, appetite, and cognitive function. Previous studies demonstrated that many phenolic metabolites are detected in plasma and 24h-urine in healthy participants following the acute consumption of 350ml of CGJ [7,44,45]. Among the phenolic metabolites identified in urine samples from this study, 11 were detected

in very low concentrations suggesting they may have come from some diet component other than the test beverages. Five metabolites were significantly higher in CGJ compared with LP and LPF in both trials, permitting assessment of their effects on study outcomes. Urinary creatinine concentrations were similar across treatments indicating comparable efficiency of urinary sample collection. Flavor essence concentration was markedly lower in the LPF beverage compared to the other beverages, but the former was not rated as less intense by the participants. This may be because sweetness and appearance were not altered and this maintained the strong overall flavor profile. Thus, outcomes based on the LPF beverage are based on an absolute reduction in essence, but not to a level that altered perception.

Our hypothesis that CGJ phenolic content would moderate post prandial glycemia was partially confirmed. Post-prandial glycemia did not differ between beverages. However, glycemia was blunted after CGJ ingestion among participants with higher levels of select phenolic metabolites (e.g. Iso/ferulic-3'-O-glucuronide) and glycemia was higher among participants generating higher levels of the metabolite caffeic acid-O-sulfate after consumption of the LPF beverage. These observations suggest that there may be differences in response to CGJ or beverages lacking phenolics or flavor essence based on the ability of the microbiota to metabolize ingested phenolics. This is similar to observations of metabotypes in response to treatment with phenolic rich walnuts and pomegranate where urolithin metabolism was predictive of cardiometabolic benefits in humans [46]. In particular, after CGJ consumption higher excreters of Iso/ferulic-3'-O-glucuronide had lower mean 24h-glucose, 4h and 24h glucose $_{t}$AUC values. Iso/ferulic-3'-O-glucuronide is derived from ferulic acid, a polyphenol with strong antioxidant properties [47]. Ferulic acid has beneficial effects on reducing hyperglycemia [48–50], and it can modulate glucose homeostasis through multiple mechanisms such as inhibition of intestinal α-glucosidase activity [51], activation of glucokinase activity [48,50], improvement in glucose uptake by muscle cells [52], and inhibition of glucose absorption from the intestine [53]. Recently, it was demonstrated that ferulic acid is associated with lower odds of elevated hsCRP [54]. Because similar concentrations of Iso/ferulic-3'-O-glucuronide were observed with and without a meal, this indicates other components of the meal likely contributed to the glycemic responses and may have offset the effects of the additional Iso/ferulic-3'-O-glucuronide.

Participants who consumed CGJ with a meal and were high excreters of 4-OH-benzaldehyde or caffeic acid-O-sulfate phenolic metabolites had a lower desire to eat. The observation that these effects were only noted when the CGJ was consumed with a meal is consistent with a mechanism involving delayed nutrient absorption potentially through effects on gastrointestinal hormones, such as GLP-1 [16], ghrelin [55], and NPY [56]. However, human clinical trials assessing these hormones in response to CGJ are lacking. This result supports our hypothesis regarding the interaction between meal constituents and CGJ leading to appetite modulation, but not for cognition and glycemia.

The sensory properties of foods are key drivers of ingestive behavior. Strong flavor intensity is a property that may modify appetitive sensations and thereby influence intake [18–23]. CGJ has an intense grape aroma and strong sweet taste; properties hypothesized to impact appetite [23–26]. While perceived flavor intensity did not differ between beverages, there was a marked reduction of flavor essence in the LPF beverage. The CGJ and LP beverages had comparable flavor essence and elicited the similar appetitive sensation ratings. No effects were noted with the LPF beverage. These observations are consistent with our hypothesis that flavor intensity affects appetite. It is notable that the strongest effects were observed when the CGJ was consumed with a meal suggesting an interactive effect with eating.

It has been suggested that the polyphenols present in CGJ, can modulate cerebral blood flow, leading to improved glucoregulation and inhibition of monoamine oxidase activity, ultimately contributing to improved brain function [33]. However, no clear pattern of effects of beverage consumption were observed for the cognitive outcomes. The CGJ for the prior study and the present contained 1790 [33] and 1667mg gae/l respectively. The beverage size in this study was 350 ml, compared to 200ml in the prior study [33]. Thus, neither concentration nor absolute amount of polyphenols explains the discrepant findings of these trials. Color and taste may play strong roles in flavor perception [57], which, in turn, may enhance feelings of alertness and energy [57,58]. The similar effects of CGJ and LP on cognitive outcomes in this trial may be due to the similarity of the sweetness, color, viscosity or other sensory properties of the beverages or possibly expectancy effects due to prior experience with CGJ. With the present results, we were not able to confirm two of the 5 hypotheses. The CGJ did not moderate post prandial glycemia relative to LP and LPF when consumed alone or with a meal as the first eating event of the day.

The present study has many strengths, including its robust design: two randomized, double-blind, cross-over, human intervention studies. Besides, their strong statistical power, the defined beverage composition and the use of 24h glucose monitoring to assess treatment effects. There are also limitations. One is the failure to adequately test the flavor intensity hypothesis. While the essence of the beverages was quantitatively modified, it did not lead to a perceptual change in intensity and the subjective sensation may be critical to maximizing the effect. It is also possible that the foods used in the test meals contributed with phenolic compounds themselves thereby diminishing the power to detect beverage effects. Additionally, this trial focused on individuals with high adiposity and did not assess gastrointestinal hormones involved in satiety response. Greater effects on glycemia may be apparent among individuals with impaired glucose tolerance such as those with pre-diabetes and manifest type 2 diabetes. Assessing those hormones could provide more answers on molecular mecanisms concerning appetite regulation.

6.6. CONCLUSION

In summary, this study revealed different effects when the beverages were consumed alone and with a meal. When consumed with a meal, beverages with customarily high flavor intensity (CGJ and LP) reduced hunger, desire to eat, and prospective consumption. Additionally, consumption of polyphenol-free, reduced flavor intensity beverage (LPF) was associated with higher 24h glucose $_{t}$AUC. In contrast, appetitive effects were limited when the beverages were consumed alone. However, CGJ alone was associated with lower glycemic responses by those excreting a higher concentration of Iso/ferulic-3'-O-glucuronide and when no polyphenols were present and flavor intensity was reduced, glycemia was higher among those excreting higher concentrations of Caffeic acid-O-sulfate, suggesting a possible difference in response based on phenolic metabolism by gut microbial communities. No consistent effects were observed for cognitive outcomes under either condition. These findings suggest some potential beneficial effects of CGJ ingestion for moderating chronic disease risk, but these may be based on specific compounds and are modified by the dietary context in which they are consumed.

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Table 1. Characteristics and composition of test beverages by bromatological analysis

Test beverage	Polyphenols	°Brix	Titratable acidity	pH	Glucose	Fructose	Total Sugar
CGJ - I	1667	15.94	0.601	3.23	6.22	7.25	13.5
LP - I	0	14.34	0.499	2.22	6.28	7.38	13.6
LPF - I	0	14.14	0.398	2.33	6.28	7.38	13.7
CGJ - II	1790	16.05	0.568	3.26	6.66	7.55	14.20
LP - II	0	13.94	0.561	2.25	6.74	7.06	13.80
LPF - II	0	13.84	0.467	2.50	6.75	7.12	13.90

CGJ I and II: 100% Concord Grape Juice trial I and trial II; LP - I and II: phenolic-free grape flavored drink with matched flavor essence to CGJ trial I and II. LPF - I and II: phenolic-free and low flavor essence grape flavored drink trial I and II. Polyphenols: mg gallic acid equivalents/L; Titrable acidity: g citric acid/100g; Glucose, Fructose, and total sugar: g/100g.

Table 2. Nutritional composition of the lunch served on test days of both trials

	White bagel	Fat-Free Mozzarella	Fruit Kool-Aid juice	TOTAL
Portion size (g or ml)	131	14	250	-
Energy (kcal)	360	40	52	452
Protein (g)	13.8	9.0	0	22.8
Fat (g)	2.1	0	0	2.1
Carbohydrate (g)	70.0	1.0	13.0	84.0
Fiber (g)	3.0	0	0	3.9

Table 3. Baseline characteristics of study participants according to trials

	Trial I (n=34)	**Trial II (n=34)**
Age (years)	34.0 ± 1.5	31.3 ± 1.2
Gender (Male/Female)	19/15	23/11
Weight (kg)	84.6 ± 2.4	84.0 ± 2.1
BMI (kg/m^2)	28.6 ± 0.5	28.4 ± 0.5
Fat (%)	31.5 ± 1.3	28.3 ± 1.4
Fat mass (kg)	26.6 ± 1.4	23.6 ± 1.2
FFM (kg)	55.0 ± 2.5	60.4 ± 2.1
TBW (%)	42.5 ± 1.4	44.2 ± 1.5
FBS (mg/dl)	95.8 ± 1.3	95.8 ± 1.1

Values are mean ± SEM. Baseline characteristics between trials are not different by independent T-test (p<0.05). FFM: free fat mass; TBW: total body water; FBS: fasting blood sugar.

Table 4. Urinary phenolic compound metabolites (μM) excretion after CGJ, LP, and LPF consumption in trial I.

Phenolic metabolite (μM)	CGJ	LP	LPF
3-hydroxyphenylpropionic acid	**0.93 ± 0.69[a]**	**0.40 ± 0.31[b]**	**0.64 ± 1.08[b]**
3-hydroxy-4-methoxyphenylpropionic	0.20 ± 0.59	0.15 ± 0.40	0.19 ± 0.65
Homovanillic acid	4.93 ± 3.08	4.41 ± 2.25	4.58 ± 2.03
3-hydroxyphenylacetic acid isomer	12.30 ± 21.28	14.78 ± 27.77	17.29 ± 33.70
3-OH-4-methoxyphenyl acetic acid	2.78 ± 1.04	2.87 ± 0.87	2.83 ± 1.22
4-hydroxybenzaldehyde isomer	**0.32 ± 0.27[a]**	**0.10 ± 0.14[b]**	**0.14 ± 0.18[b]**
3-OH-4-methoxybenzoic acid	4.45 ± 4.09	6.05 ± 10.57	6.10 ± 10.58
Hippuric Acid	605.50 ± 300.55	576.20 ± 303.70	606.84 ± 336.81
3-hydroxyhippuric acid	**21.05 ± 11.17[a]**	**10.83 ± 6.86[b]**	**12.92 ± 8.08[b]**
Chlorogenic acid	0.04 ± 0.11	0.011 ± 0.02	0.02 ± 0.04
Ferulic acid	0.09 ± 0.16	0.09 ± 0.21	0.05 ± 0.06
Ferulic acid isomer	0.05 ± 0.12	0.04 ± 0.08	0.06 ± 0.15
Caffeic acid-O-sulfate	**131.94 ± 115.82[a]**	**39.31 ± 51.25[b]**	**52.39 ± 58.71[ab]**
Dihydrocaffeic acid-O-sulfate	36.95 ± 39.87	29.26 ± 37.08	29.76 ± 35.97
Iso/ferulic acid-4'-sulfate	39.91 ± 65.10	32.19 ± 68.64	20.501 ± 24.01
Iso/ferulic-3'-O-glucuronide	**1.00 ± 1.49[a]**	**0.77 ± 1.01[b]**	**0.64 ± 0.42[b]**
(Epi)gallocatechin glucuronide	3.27 ± 1.79	3.55 ± 1.90	3.60 ± 1.97
Methyl (epi)catechin glucuronide	0.83 ± 0.38	0.94 ± 0.57	0.94 ± 0.50
Cyanidin-O-Glucuronide_1	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.02
Cyanidin-O-Glucuronide_2	0.07 ± 0.03	0.08 ± 0.05	0.06 ± 0.03
Cyanidin-O-Glucuronide_3	0.08 ± 0.05	0.09 ± 0.06	0.08 ± 0.05
Peonidin-O-Glucuronide_1	0.09 ± 0.08	0.08 ± 0.10	0.08 ± 0.10
Peonidin-O-Glucuronide_2	0.03 ± 0.03	0.03 ± 0.04	0.03 ± 0.04
Peonidin-O-Glucuronide_3	0.05 ± 0.04	0.05 ± 0.04	0.06 ± 0.05
Delphinidin-O-Glucuronide	0.27 ± 0.13	0.32 ± 0.19	0.25 ± 0.14
Malvidin-O-Glucuronide	0.07 ± 0.04	0.07 ± 0.07	0.08 ± 0.05
Total Cyanidin-O-Glucuronide	0.17 ± 0.08	0.20 ± 0.11	0.17 ± 0.08
Total Peonidin-O-Glucuronide	0.16 ± 0.10	0.16 ± 0.12	0.16 ± 0.14

Values are mean ± SD. Different superscript letters in the same line indicate differences between beverages by ANOVA followed by post hoc Bonferroni ($p < 0.05$). CGJ: 100% Concord grape juice, LP: phenolic-free grape flavored drink with matched flavor intensity to CGJ, LPF: phenolic-free and low flavor intensity grape flavored drink.

Table 5. Urinary phenolic compound metabolites (μM) excretion after test beverages consumption in trial II.

Phenolic metabolite (μM)	CGJ	LP	LPF
4-hydroxybenzaldehyde	**2.17 (1.6-3.02)[a]**	**1.78 (1.38-2.27)[b]**	**1.6 (1.42-2.68)[ab]**
4-hydroxybenzaldehyde	1.42 (1.32-1.54)	1.37 (1.33-1.48)	1.41 (1.33-1.51)
Homovanillic acid	7.68 (4.44-10.11)	5.47 (3.71-8.73)	6.10 (4.53-9.48)
3-hydroxyphenylacetic ac. isomer	**5.55 (3.00-14.83)[a]**	**2.38 (1.90-8.33)[b]**	**2.91 (1.96-11.12)[b]**
3-hydroxy-4-methoxyphenyl	2.00 (1.71-2.90)	1.91 (1.71-2.67)	2.04 (1.78-2.57)
3-hydroxyphenyl propionic	**0.69 (0.39-1.36)[a]**	**0.44 (0.34-0.60)[b]**	**0.43 (0.34-0.64)[b]**
3-hydroxy-4-methoxyphenyl	0.29 (0.27-0.41)	0.28 (0.27-0.31)	0.28 (0.27-0.32)
3-hydroxy-4-methoxy propionic	1.12 (0.55-1.98)	0.78 (0.40-1.35)	0.70 (0.36-2.23)
3-hydroxy-4-methoxy benzoic	2.55 (2.30-2.93)	2.39 (2.19-3.03)	2.42 (2.25-3.03)
Hippuric acid	220.45 (122.98-72.47)	246 (124.87-359.34)	232.01 (135.91-316.50)
3-hydroxy hippuric acid	**11.33 (6.59-19.81)[a]**	**6.78 (3.53-15.74)[b]**	**5.63 (3.32-23.19)[b]**
Dihydrocoumaric acid	**0.66 (0.42-1.46)[a]**	**0.41 (0.35-0.61)[b]**	**0.47 (0.34-0.77)[b]**
Ferulic acid	0.74 (0.00-0.91)	0.73 (0.00-0.88)	0.33 (0.00-0.81)
Caffeic acid-O-sulfate	**58.96 (30.91-178.45)[a]**	**27.23 (12.24-62.48)[b]**	**26.74 (10.94-[illegible])[b]**
Dihydrocaffeic acid-O-sulfate	19.78 (2.98-58.85)	9.96 (2.54-59.6)	7.7 (0.02-59.29)
Ferulic acid-4'-sulfate	66.46 (44.98-198.91)	71.26 (42.00-161.92)	100.56 (35.88-[illegible])
Total Iso/ferulic acid-3'-O-	2.72 (1.88-4.25)	2.48 (1.85-4.13)	2.80 (1.83-3.83)
Epi/gallocatechin glucuronides	1.82 (1.39-2.96)	1.89 (1.35-2.69)	1.71 (1.40-2.62)
Methyl-(epi)catechin-O-	**3.50 (1.74-5.74)[a]**	**0.60 (0.50 - 2.62)[b]**	**0.95 (0.48-2.75)[b]**
Total Epi/catechin	9.38 (7.15-12.87)	9.15 (6.17-14.68)	9.58 (6.93-12.98)
Methyl epi/catechin	0.65 (0.49 - 0.85)	0.60 (0.53 - 0.83)	0.61 (0.49 - 0.79)
Total Cyanidin-3-glucuronide	0.29 (0.18-0.44)	0.22 (0.16-0.44)	0.27 (0.19-0.46)
Total Peonidin-3-Glucuronide	2.16 (1.02-3.30)	1.49 (0.84-2.16)	1.32 (0.93-2.44)
Delphinidin-3-glucuronide	0.63 (0.39-1.09)	0.54 (0.44-1.03)	0.69 (0.43-0.98)
Total Petunidin-3-Glucuronide	0.1 (0.06-0.15)	0.08 (0.06-0.13)	0.08 (0.06-0.10)
Malvidin-3-O-glucuronide	0.19 (0.13-0.35)	0.19 (0.13-0.34)	0.20 (0.15-0.31)

Values are median (P25th-P75th). Differences compared with Friedmann test. Different superscript letters in the same line indicate difference between treatments (p<0.05). CGJ: 100% Concord grape juice, LP: phenolic-free grape flavored drink with matched flavor intensity to CGJ, LPF: phenolic-free and low flavor intensity grape flavored drink.

Table 6. Glycemia after test beverage consumption and flavor ratings assessed in the trials I and II

	Trial I (n=34)			Trial II (n=34)		
	CGJ	**LP**	**LPF**	**CGJ**	**LP**	**LPF**
Baseline glycemia	84.8 ± 2.2	85.3 ± 2.2	82.64 ± 2.7	95.7 ± 2.6	97.5 ± 2.8	94.8 ± 1.9
Glycemia 2h after beverage	85.9 ± 2.8	87.6 ± 2.4	84.5 ± 23.0	104 ± 4.2	101.1 ± 3.2	102.2 ± 3.5
Glycemia 3h after beverage	80.6 ± 2.1	83.6 ± 1.9	80.71 ± 2.3	92.6 ± 2.9	95.7 ± 2.7	93.2 ± 2.7
Glycemia 1h after lunch	113.9 ± 4.1	119.0 ± 4.4	117.8 ± 5.0	121.8 ± 5.4	125.6 ± 3.9	116.7 ± 4.7
Glycemia 24h	97.2 ± 2.2	98.8 ± 2.0	96.6 ± 2.6	112.3 ± 2.4	109.6 ± 1.9	107.1 ± 1.8
24h Peak glycemia	142.7 ± 4.0	145.5 ± 3.7	143.2 ± 4.4	168.5 ± 4.7	163.4 ± 4.4	160.4 ± 19.8
24h Nadir glycemia	66.1 ± 1.9	69.9 ± 1.9	65.3 ± 2.3	80.2 ± 2.0[a]	79.7 ± 2.3	72.1 ± 2.5[b]
4h $_t$AUC	22,106 ± 592	22,288 ± 500	21,949 ± 629	25,589 ± 599	25,706 ± 566	25,222 ± 523
24h $_t$AUC	138,965 ± 3069	141,279 ± 2,899	138,233 ± 3,792	159,689 ± 3,366	156,984 ± 2,837	151,920 ± 2,718
Flavor rating	9.1 ± 0.3	8.6 ± 0.3	8.9 ± 0.3	8.9 ± 0.2	8.4 ± 0.2	8.5 ± 0.2

Values are mean ± SEM. Different superscript letters indicate difference between treatments by ANOVA (p<0.05). Variables unit: mg/dl, except for AUC and flavor rating. $_t$AUC: total area under the curve. CGJ: 100% Concord grape juice, LP: phenolic-free grape flavored drink with matched flavor essence to CGJ, LPF: phenolic-free and low flavor essence grape flavored drink.

Table 7. Mean glucose (mg/dl) at baseline and 10, 20, 30, 60, 120, and 180 minutes after beverage intake (B) and after lunch intake (L) for the 3 groups in trials I and II

	Trial I				**Trial II**			
	CGJ	**LP**	**LPF**	***p***	**CGJ**	**HFI**	**LFI**	***p***
B0	84.8 ± 2.2[a]	85.3 ± 2.2[a]	82.8 ± 2.2[a]		95.5 ± 2.8 [a]	97.5 ± 3.0 [a]	95.9 ± 1.8 [a]	
B10	87.7 ± 2.4	87.6 ± 2.4	87.8 ± 3.4		97.2 ± 3.0	99.4 ± 3.5	97.6 ± 2.1	
B20	96.4 ± 3.2	101.1 ± 3.6 [b]	99.9 ± 4.2 [b]		106.7 ± 3.6	108.4 ± 4.2	108.7 ± 3.2	
B30	108.2 ± 4.1[b]	113.8 ± 1.1 [b]	111.7 ± 4.4 [b]	<0.001	124.3 ± 4.7 [b]	127.3 ± 4.6 [b]	128.4 ± 4.7 [b]	<0.001
B60	108.8 ± 5.1 [b]	103.4 ± 3.9 [b]	105.7 ± 4.2 [b]		128.7 ± 5.2 [b]	129.6 ± 4.5 [b]	126.1 ± 5.1 [b]	
B120	85.9 ± 2.8	87.6 ± 2.4	84.5 ± 2.9		101.6 ± 3.6	101.0 ± 3.44	103.6 ± 3.3	
B180	80.6 ± 2.1	83.6 ± 1.9	80.7 ± 2.3		91.8 ± 3.0	95.5 ± 2.9	93.3 ± 2.8	
L0	83.3 ± 2.1 [a]	82.1 ± 2.0 [a]	83.7 ± 2.7 [a]		93.2 ± 2.8 [a]	95.5 ± 2.9 [a]	93.3 ± 3.1 [a]	
L10	86.7 ± 2.4	85.3 ± 2.7	86.1 ± 3.1		96.0 ± 3.0	98.5 ± 3.25	94.7 ± 3.1	
L20	98.2 ± 3.3	98.4 ± 3.7[b]	96.5 ± 4.0		108.4 ± 3.8	109.6 ± 3.6	106.0 ± 3.6	
L30	112.2 ± 4.0 [b]	112.5 ± 4.2 [b]	110.6 ± 4.3 [b]	<0.001	124.3 ± 4.8 [b]	124.6 ± 3.5 [b]	123.5 ± 4.8 [b]	<0.001
L60	113.9 ± 4.1 [b]	119.0 ± 4.4 [b]	117.8 ± 5.0 [b]		122.6 ± 5.7 [b]	126.2 ± 4.2 [b]	118.5 ± 4.7 [b]	
L120	109.0 ± 4.3 [b]	113.7 ± 3.5 [b]	106.0 ± 4.2 [b]		119.0 ± 3.8 [b]	119.6 ± 3.1 [b]	118.9 ± 2.9 [b]	
L180	102.2 ± 3.8 [b]	108.6 ± 2.6 [b]	102.2 ± 4.5 [b]		109.8 ± 3.8 [b]	111.0 ± 3.5 [b]	108.4 ± 2.9	

Values are mean ± SEM. Interaction time*treatment were assessed using two-way repeated measures ANOVA test ($p < 0.05$). Post hoc Bonferroni was used to correct for multiple comparisons. Different superscript letters indicate simple main effect of time within treatment in comparison with time 0. CGJ: 100% Concord grape juice, LP: phenolic-free grape flavored drink with matched flavor essence to CGJ, LPF: phenolic-free and low flavor essence grape flavored drink.

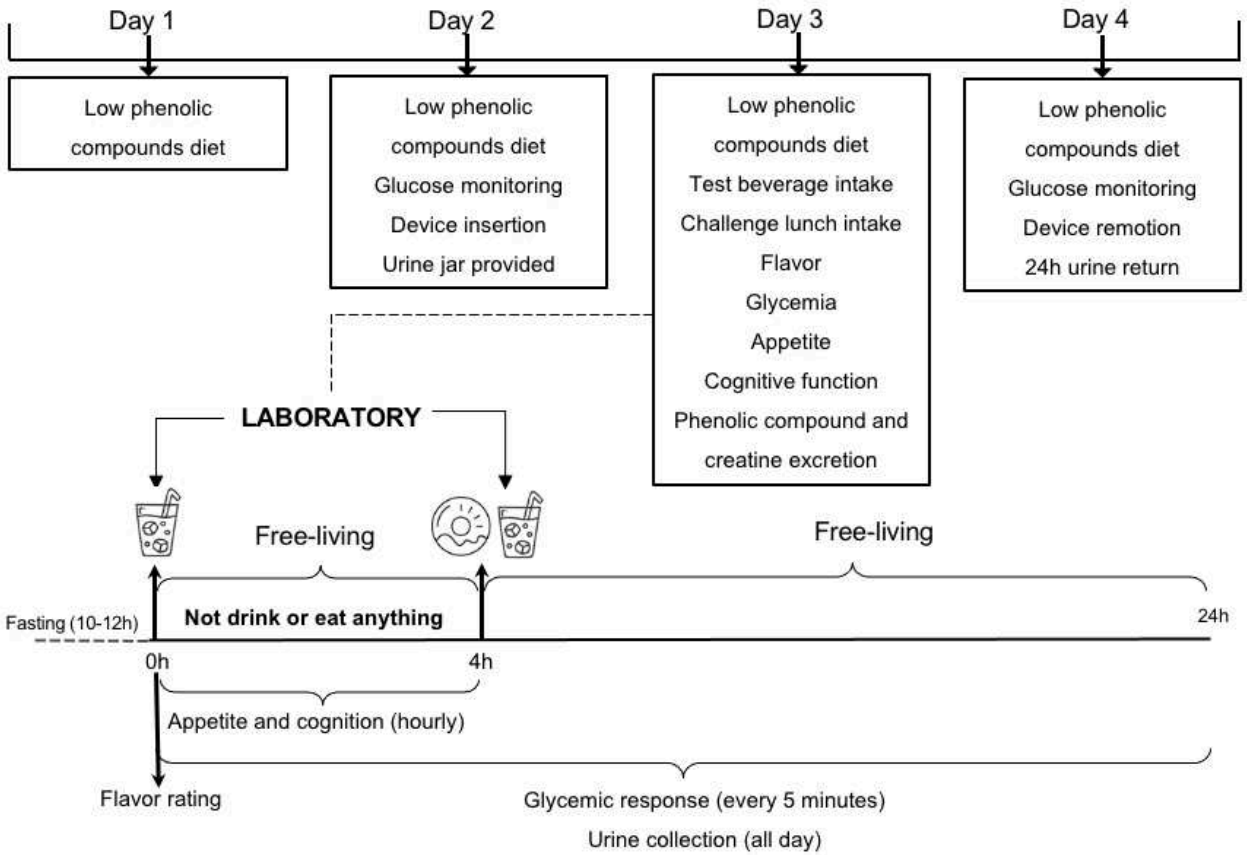

Figure 1. Experimental design. Three types of beverages were consumed on three different days during 4-day sessions separated by a 5-day wash-out period. From day 1 through day 4 of each session, participants consumed a low phenolic compounds diet at home. On day 2, a glucose monitor device was inserted in the lab. On day 3, after 10-12h of hour fasting, participants consumed one of the three test beverages (CGJ: 100% Concord grape juice, LP: phenolic-free grape flavored drink with matched flavor intensity to CGJ or LPF: phenolic-free and low flavor intensity grape flavored drink) in the lab and the urine collection jar was provided. A standard lunch was served in the lab 4 hours later. Participants were instructed not to eat or drink anything between breakfast and lunch. Glycemia was monitored every 5 minutes, appetite and cognitive function were assessed hourly during 4h, and 24h urine was collected throughout day 3. On day 4, the glucose monitoring device was removed, and the urine jar was brought back to the lab.

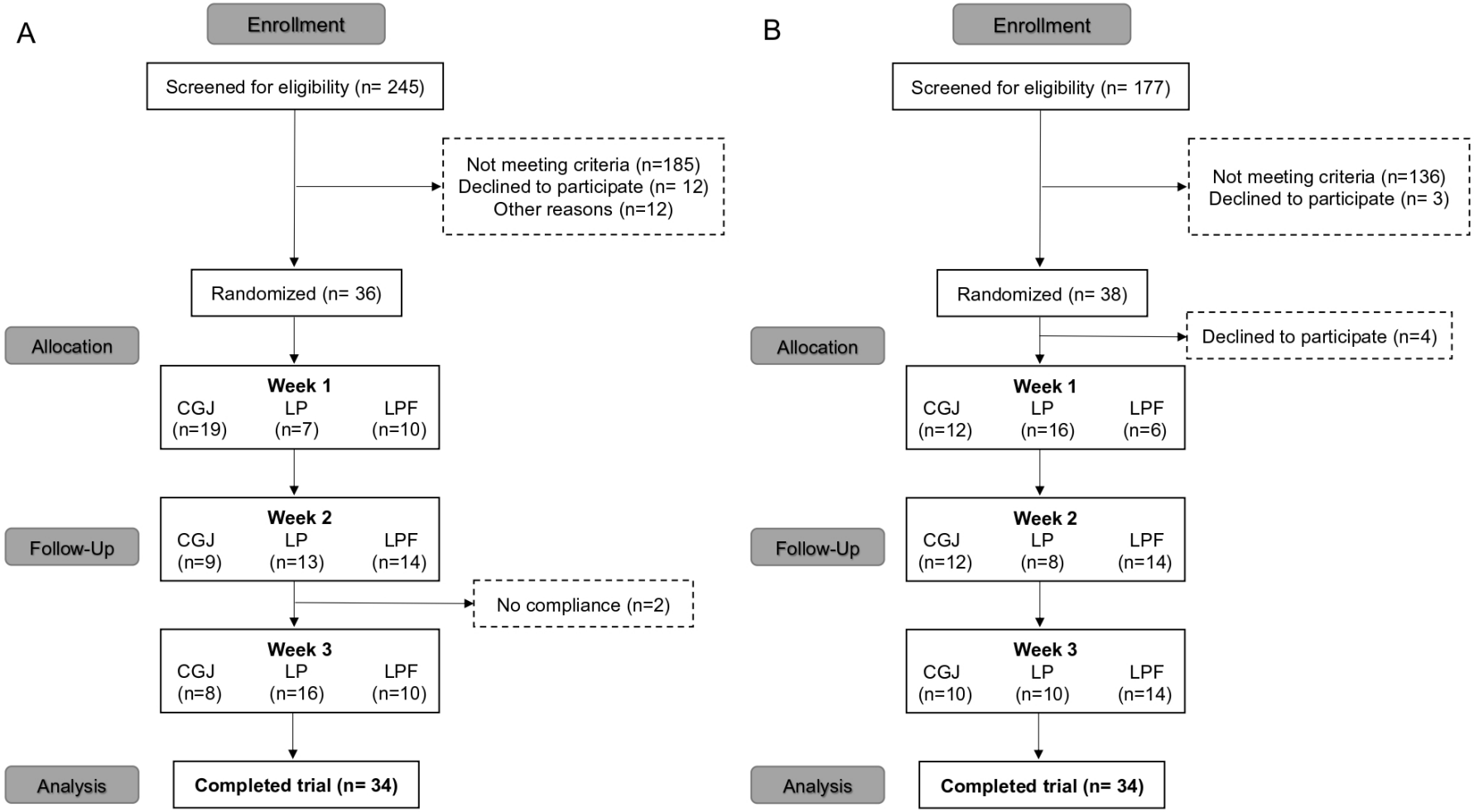

Figure 2. CONSORT Flow Diagram of Participants in trial I (A) and II (B).

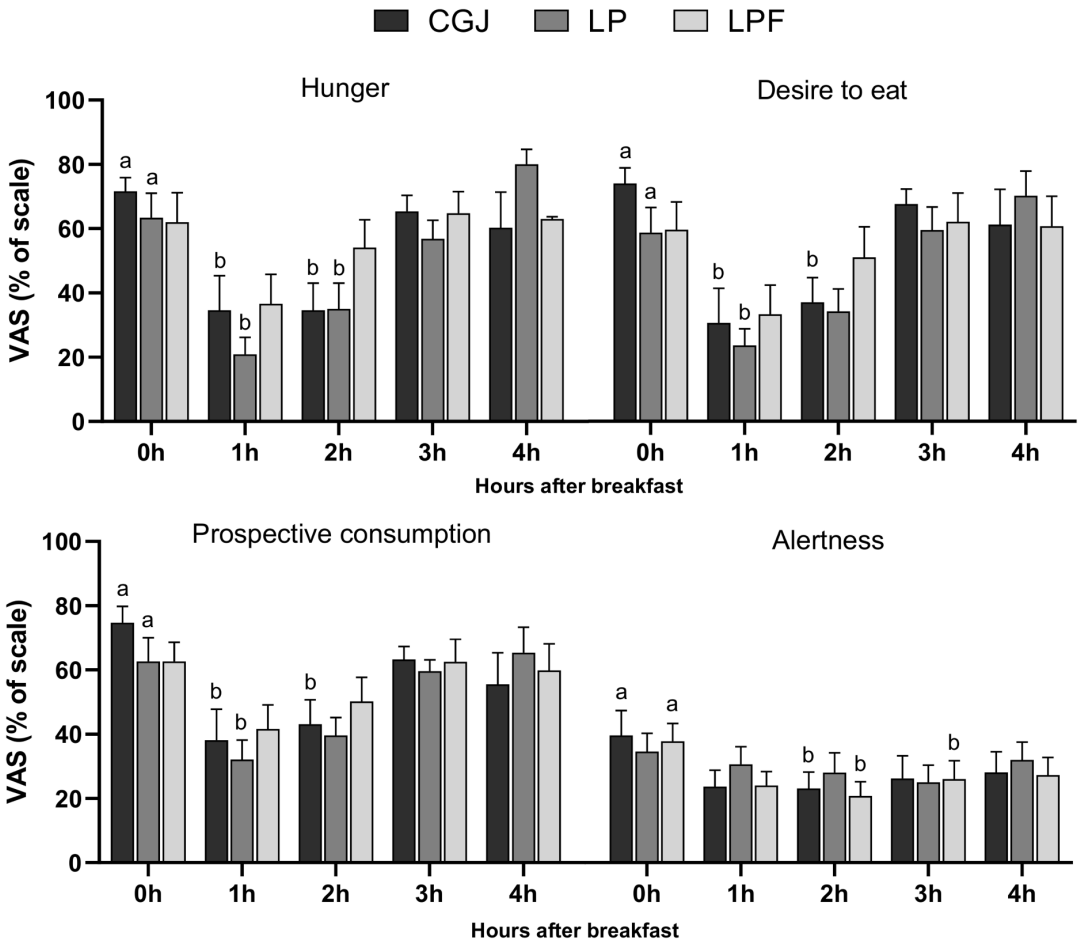

Figure 3. Mean (SEM) hunger, desire to eat, prospective consumption, and alertness ratings up to 4h after beverage consumption with a meal. Time*treatment interactions were assessed by two-way repeated measures ANOVA test (p <0.05) and significant differences are represented by different letters within treatments compared to time 0. Post hoc Bonferroni was used to correct for multiple comparisons. CGJ: 100% Concord grape juice, LP: phenolic-free grape flavored drink with matched flavor essence to CGJ, LPF: phenolic-free and low flavor essence grape flavored drink.

6.8. SUPPLEMENTAR MATERIAL

Table 1s. Glycemic and appetite responses by low and high phenolic excreters after beverages intake alone (trial I)

CGJ	3-hydroxyhippuric acid			Iso/ferulic-3'-O-glucuronide		
	Low	High	*p*	Low	High	*p*
4h glucose $_t$AUC	22,342 ± 818	21,870 ± 877	0.697	23.010 ± 682	19,596 ± 718	0.009
24h glucose $_t$AUC	140,270 ± 3,573	137,660 ± 5,084	0.677	144,176 ± 3,446	124,490 ± 3,485	0.003
24h glucose (mg/dl)	98.3 ± 2.4	96.2 ± 3.7	0.623	100.9 ± 2.4	87 ± 2.4	0.003
Hunger	50.5 ± 4.4	54.7 ± 3.5	0.459	55.5 ± 3.3	44.4 ± 4.6	0.078
Thirst	44 ± 5.6	51.4 ± 3.9	0.287	48.6 ± 4.5	45.2 ± 3.5	0.673
Fullness	41.2 ± 2.7	27.5 ± 3.3	0.003	33.5 ± 3.2	36.9 ± 2.3	0.545
Desire to eat	53.1 ± 4.2	54.4 ± 4.7	0.829	55.9 ± 3.9	47.8 ± 4.2	0.259
Prospective consumption	49.7 ± 4.1	56.7 ± 4.2	0.249	56.1 ± 3.7	45.1 ± 3.8	0.101
LP	**3-hydroxyhippuric acid**			**Iso/ferulic-3'-O-glucuronide**		
	Low	High	*p*	Low	High	*p*
4h glucose $_t$AUC	22,944 ± 538	21,633 ± 830	0.194	22,640 ± 489	21,893 ± 918	0.464
24h glucose $_t$AUC	144,742 ± 4,601	133,189 ± 3,673	0.058	142,493 ± 4358	134,996 ± 4,220	0.228
24h glucose (mg/dl)	103.1 ± 3.2	94,5 ± 2.1	0.032	100.8 ± 3.5	96.5 ± 1.8	0.297
Hunger	51.3 ± 4.3	52.4 ± 4.2	0.860	54.3 ± 4.1	49.1 ± 4.3	0.385
Thirst	39.3 ± 4.3	43.3 ± 5.6	0.573	42.0 ± 4.3	40.5 ± 5.8	0.835
Fullness	28.9 ± 4.7	36.9 ± 4.0	0.205	28.9 ± 4.7	37.5 ± 3.9	0.174
Desire to eat	49.9 ± 4.5	456.5 ± 4.9	0.326	52.8 ± 4.3	53.5 ± 5.3	0.910
Prospective consumption	52.0 ± 4.5	53.8 ± 4.1	0.775	55.4 ± 4.1	50.0 ± 4.4	0.370

Cont. Table 1. Glycemic and appetite responses by low and high phenolic excreters after beverages intake alone (trial I)

LPF	Caffeic acid-O-sulfate			Iso/ferulic-3'-O-glucuronide		
	Low	High	*p*	Low	High	*p*
4h glucose $_{t}$AUC	20,431 ± 759	23,468 ± 876	0.013	22,121 ± 876	21,732 ± 927	0.764
24h glucose $_{t}$AUC	128,851 ± 5,130	147,614 ± 4,689	0.011	139,856 ± 5,302	136,176 ± 5,522	0.637
24h glucose (mg/dl)	90.2 ± 3.5	103.1 ± 3.2	0.010	97.5 ± 3.7	95.5 ± 3.8	0.702
Hunger	58.1 ± 4.3	58.2 ± 4.0	0.980	57.4 ± 3.8	59.1 ± 4.5	0.774
Thirst	46.1 ± 4.3	53.5 ± 5.8	0.314	53.0 ± 4.4	45.7 ± 6.0	0.331
Fullness	37.6 ± 5.0	28.1 ± 2.8	0.107	34.8 ± 4.5	30.5 ± 3.4	0.476
Desire to eat	55.3 ± 4.3	56.7 ± 4.0	0.813	54.4 ± 4.0	58.0 ± 4.2	0.538
Prospective consumption	56.7 ± 4.8	60.6 ± 4.0	0.530	57.2 ± 4.2	60.4 ± 4.7	0.615

Values are mean ± SEM in low and high phenolic excreters classified according to the median value for each

Table 2. Glycemic and appetite responses by low and high phenolic excreters after beverages intake with a meal (trial II)

CGJ	**4-OH-benzaldehyde isomer**			**Caffeic acid-O-sulfate**		
	Low	High	*p*	Low	High	*p*
4h glucose $_t$AUC	26281 ± 985	24897 ± 671	0.254	25943 ± 908	2352.94 ± 800	0.563
24h glucose $_t$AUC	162287 ± 5182	1570.91 ± 4361	0.449	159782 ± 4808	159596 ± 4857	0.979
24h glucose (mg/dl)	115 ± 3.5	109 ± 3.1	0.183	114 ± 3.4	111 ± 3.4	0.523
Hunger	55.1 ± 4.3	45.0 ± 4.1	0.097	55.2 ± 4.7	45.0 ± 3.6	0.094
Thirst	47.7 ± 4.9	38.3 ± 4.5	0.172	52.1 ± 4.1	34.3 ± 4.5	0.007
Fullness	34.1 ± 3.2	36.1 ± 3.1	0.648	35.1 ± 3.3	35.1 ± 3.1	0.994
Desire to eat	56.6 ± 3.7	44.4 ± 3.9	0.032	56.2 ± 4.2	44.7 ± 3.6	0.044
Prospective consumption	57.3 ± 4.0	47.7 ± 4.5	0.123	56.7 ± 4.6	48.3 ± 4.0	0.174
LP	**4-OH-benzaldehyde isomer**			**Caffeic acid-O-sulfate**		
	Low	High	*p*	Low	High	*p*
4h glucose $_t$AUC	25690 ± 981	25722 ± 599	0.978	25806 ± 902	25606 ± 713	0.863
24h glucose $_t$AUC	1562689 ± 4341	157699 ± 3778	0.805	155163 ± 4452	158804 ± 3598	0.529
24h glucose (mg/dl)	109 ± 3.0	110 ± 2.5	0.679	108 ± 3.1	111 ± 2.3	0.450
Hunger	49.0 ± 3.3	51.4 ± 3.9	0.649	46.6 ± 3.6	53.9 ± 3.3	0.147
Thirst	47.2 ± 4.4	42.4 ± 3.2	0.387	45.9 ± 3.9	43.8 ± 3.9	0.706
Fullness	34.4 ± 4.5	38.4 ± 3.8	0.501	35.2 ± 4.2	37.6 ± 4.2	0.689
Desire to eat	47.7 ± 3.9	44.8 ± 3.9	0.601	44.6 ± 3.9	48.1 ± 4.0	0.535
Prospective consumption	50.1 ± 3.5	51.8 ± 3.9	0.746	49.3 ± 3.7	52.6 ± 3.8	0.546

Cont. Table 2. Glycemic and appetite responses by low and high phenolic excreters after beverages intake with a meal (trial II)

LPF	**4-OH-benzaldehyde isomer**			**3-OH-phenylacetic acid isomer**		
	Low	High	*p*	Low	High	*p*
4h glucose $_t$AUC	24980 ± 741	25464 ± 757	0.651	24302 ± 800	26141 ± 619	0.078
24h glucose $_t$AUC	149623 ± 3643	154218 ± 4067	0.406	146309 ± 3669	157532 ± 3616	0.037
24h glucose (mg/dl)	105 ± 2.4	109 ± 2.7	0.198	104 ± 2.8	110 ± 2.2	0.078
Hunger	48.6 ± 3.6	46.6 ± 3.4	0.684	46.2 ± 3.6	49.0 ± 3.4	0.585
Thirst	35.8 ± 4.5	43.8 ± 4.3	0.209	41.5 ± 4.9	38.2 ± 4.0	0.612
Fullness	30.1 ± 2.8	39.9 ± 3.8	0.048	37.3 ± 3.3	32.9 ± 3.8	0.388
Desire to eat	46.9 ± 4.1	47.7 ± 4.2	0.885	43.3 ± 3.6	51.5 ± 4.4	0.160
Prospective consumption	51.0 ± 3.1	46.2 ± 3.0	0.273	47.3 ± 2.9	49.9 ± 3.3	0.564

Values are mean ± SEM in low and high phenolic excreters classified according to the median value for each compound. The variables for each phenolic compound were compared using independent T-test ($p<0.05$). Appetitive sensations were assessed from 0-4h after beverage intake with a meal. CGJ: 100% Concord grape juice, LP: phenolic-free grape flavored drink with matched flavor essence to CGJ, LPF: phenolic-free and low flavor essence grape flavored drink.

7. CONCLUSÕES GERAIS

Os resultados obtidos em nosso estudo de intervenção envolvendo indivíduos adultos com excesso de peso indicaram que:

- Ao serem consumidas com uma refeição, as bebidas com intenso sabor da uva Concord (CGJ e LP) reduziram a fome, o desejo de comer e o consumo prospectivo. A bebida sem polifenol e sabor reduzido de uva (LPF) se relacionou à $_{t}$AUC da glicemia de 24h mais alta.
- O consumo do suco de uva Concord (CGJ) sozinho resultou em menor resposta glicêmica pelos indivíduos que apresentaram maior excreção do metabólito fenólico iso/ferúlico-3'-O-glucuronídeo. Ademais, a bebida LPF resultou em glicemia mais alta nos indivíduos que excretam mais ácido cafeico-O-sulfato.

Esses desfechos sugerem que os fenólicos naturais e o intenso sabor do suco de uva Concord podem ajudar a moderar o apetite e a glicemia. No entanto, esses efeitos podem refletir o metabolismo de compostos fenólicos (Ex: ácido 3-OH-hipúrico, iso/ferúlico-3'-O-glucuronídeo, ácido cafeico-O-sulfato) e são modificados pelo contexto alimentar em que o mesmo é consumido.

Após a análise dos estudos selecionados para as revisões sistemáticas aqui apresentadas, nós concluímos que o consumo:

- De polifenóis da uva em diferentes doses (200 g – 2000 g/dia) e períodos (4-12 semanas) não afetaram a hemoglobina glicada, em indivíduos eutróficos e com excesso de peso corporal.
- De quercetina (160 mg/dia) reduziu a concentração do produto final de glicação avançada (AGE) metilglioxal e de resveratrol (500 mg/dia) aumentou a expressão do gene RAGE secretor endógeno (esRAGE), mas não afetou sua concentração sérica, após 4 semanas de suplementação em indivíduos eutróficos e com excesso de peso corporal.
- Agudo (1-3 dias) e a longo prazo (4-12 semanas) de produtos derivados da uva não afetaram o apetite e a ingestão alimentar em homens e mulheres com peso normal e sobrepeso.
- De GSE (300 mg/dia) associado à dieta restrita em calorias por 12 semanas reduziu o neuropeptídeo Y, peso corporal, IMC, a circunferência da cintura e relação cintura/quadril em indivíduos com excesso de peso. Por outro lado, o consumo desse

extrato (600 mg/dia) por 4 semanas reduziu a frutosamina em indivíduos com excesso de peso corporal.

- De uma xícara (145 g/dia) de uva-passa, durante seis semanas, aumentou a secreção de leptina e grelina, mas não afetou a ingestão alimentar e o IMC em indivíduos com peso normal e excesso de peso.

A ingestão de 160mg de quercetina parece exercer efeito de anti-glicação em indivíduos com excesso de peso. Tais efeitos podem estar relacionados ao poder antioxidante e anti-inflamatório dos polifenóis, os quais participam de vias de sinalização celular que inibem a ligação AGE-RAGE e diminuem a formação de AGEs. Assim, o efeito crônico (consumo por pelo menos 4 semanas) dos polifenóis da uva nos produtos precoces de glicação avançada, nos diferentes tipos de AGEs e nas isoformas do receptor de AGEs deve ser investigado em indivíduos com excesso de peso. Além disso, ensaios clínicos controlados de longo prazo (pelo menos 12 semanas), envolvendo apenas indivíduos com excesso de peso corporal, devem ser conduzidos para avaliar o efeito de doses suficientemente altas de polifenóis da uva (pelo menos 69 mg/kg de peso corporal) no apetite, na ingestão alimentar e na secreção de hormônios que controlam o apetite em condições laboratoriais.