CAIO EDUARDO GONÇALVES REIS

CAFÉ E METABOLISMO DA GLICOSE:
ENSAIO CLÍNICO CRUZADO RANDOMIZADO COM ISÓTOPOS ESTÁVEIS

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UNIVERSIDADE DE BRASÍLIA
FACULDADE DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

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Orientadora: Profa. Dra. Teresa Helena Macedo da Costa

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RESUMO

Introdução: Dados epidemiológicos mostram uma associação inversa do consumo de café com o risco de diabetes tipo 2. No entanto, os resultados dos estudos em longo prazo (semanas) mostram que o café cafeinado pode melhorar o metabolismo da glicose reduzindo a curva glicêmica e aumentando a resposta insulinêmica, ao passo que nos estudos em curto prazo (horas) o café cafeinado pode aumentar a área abaixo da curva da resposta glicêmica. Já os mecanismos por trás desses efeitos benéficos ainda não foram completamente elucidados. Desta forma, esta pesquisa tem por objetivo investigar o efeito agudo do consumo de café sobre a taxa de captação de glicose e sensibilidade à insulina utilizando uma metodologia com isótopo estável após um teste oral de tolerância à glicose.

Métodos: Quinze homens saudáveis foram submetidos a um ensaio clinico randomizado cruzado duplo cego com cinco grupos experimentais: café descafeinado, café cafeínado (com e sem açúcar) e controles - água (com e sem açúcar); seguido 1 hora após pelo teste oral de tolerância à glicose (75 g de carboidrato disponível) com marcação isotópica intravenosa da glicose ([1]-^{13}C-glicose) analisada pelo índice dos modelos mínimos (225 minutos). Foi aplicado one-way ANOVA com ajuste de Bonferroni para comparar os efeitos das bebidas testes nos parâmetros do metabolismo da glicose.

Resultados: O café descafeinado resultou em maior sensibilidade à insulina em comparação com o café cafeinado e água. Já o café cafeinado apresentou uma maior taxa de captação de glicose em comparação com o café descafeinado e água. No entanto, na análise global (0-225 min) não houve diferenças significativas entre os grupos nos índices da taxa de captação de glicose e sensibilidade insulina.

Conclusão: Os resultados do atual estudo mostram que o consumo de café cafeinado e descafeinado, com ou sem açúcar, não exerce efeitos agudos significativos sobre o metabolismo da glicose. Já os resultados dos estudos em longo prazo (semanas) indicam que a redução do risco de diabetes tipo 2 deve ocorrer devido ao consumo crônico de café como os estudos epidemiológicos veêm mostrando.

Palavras-chave: Café, Diabetes Melittus tipo 2, Glicose, Insulina.

ABSTRACT

Background: Epidemiological data show an inverse association of coffee consumption with risk of type 2 diabetes. However, the results of long-term studies (weeks) showed that caffeinated coffee may improve the glycemic metabolism by reducing the glucose curve and increasing insulin response, while for short-term studies (hours) caffeinated coffee may increase the area under the curve for glucose response. In addition, the mechanisms behind these beneficial effects have not been fully elucidated. Thus, this research aims to investigate the acute effects of coffee on glucose effectiveness and insulin sensitivity using the stable isotope minimal model protocol with oral glucose administration.

Methods: Fifteen healthy men underwent a randomized crossover double-blinding clinical trial with five experimental groups. They consumed decaffeinated coffee, caffeinated coffee (with and without sugar), and controls – water (with and without sugar) followed 1 hour later by an oral glucose tolerance test (75 g of available carbohydrate) with intravenous labeled dosing ([1]-^{13}C-glucose) interpreted by the two-compartment minimal model (225 minutes). One-way ANOVA with Bonferroni adjustment was used to compare the effects of the tested beverages on glucose metabolism parameters.

Results: Decaffeinated coffee resulted in higher insulin sensitivity compared with caffeinated coffee and water, and the caffeinated coffee showed higher glucose effectiveness compared with decaffeinated coffee and water. However, in the overall analysis (0 – 225 min) there were no significant differences in glucose effectiveness and insulin sensitivity among the groups.

Conclusion: The findings of the experimental study demonstrate that the consumption of caffeinated and decaffeinated coffee with or without sugar has no acute effects on glucose metabolism in healthy men. The results obtained from the long-term trials reviewed may indicate that a reduction in the risk of type 2 diabetes should occur due to chronic coffee consumption, as the epidemiology studies have shown.

Key words: Coffee, Type 2 Diabetes Melittus, Glucose, Insulin.

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LISTA DE ABREVIATURAS E SIGLAS

AAC: Área abaixo da curva;

ACT: Água corporal total;

ANOVA: Análise de variância;

AUC: *Area under the curve*;

BMI: *Body mass index*;

CC: *Caffeinated coffee*;

CGA: Chlorogenic acids;

CQA: Ácidos cafeoilquínicos;

CS: *Caffeinated coffee with sugar*;

CV: *Coefficient of variation*;

DC: *Decaffeinated coffee*;

DM2: Diabetes Mellitus tipo 2;

IDF: Federação Internacional de Diabetes;

GC/C/IRMS*: Gas Chromatography/ Combustion/ Isotope Ratio Mass Spectrometry*;

GLP-1: *Glucagon-like peptide-1*;

HOMA-IR: *Homeostasis model assessment insulin resistance index*;

IGT: *Impaired glucose tolerance*;

IL: *Interleukin*;

IMC: Índice de massa corporal;

ISI: *Insulin sensitivity index*;

IVGTT: *Intravenous glucose tolerance test*;

ODILE: *Oral dose intravenous label experimente*;

OGTT: *Oral glucose tolerance test*;

PRISMA: *Preferred reporting items for systematic reviews and meta-analyses*;

RCT: *Randomized controlled trial*;

SD: *Standard deviation*;

SEM: *Standard error of the mean*;

Sg: Taxa de captação de glicose;

Si: sensibilidade à insulina;

T2DM: *Type 2 Diabetes Mellitus*;

W: *Water* ;

WS: *Water with sugar*.

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CAPÍTULO 1

1.1 Introdução

O café é um dos produtos mais comercializados mundialmente, sendo o Brasil seu maior produtor com mais de 25% da produção, e o segundo maior consumidor, com cerca de 12,5%[1] com ingestão média diária estimada em 163 mL[2]. Pesquisas clínicas importantes foram realizadas na última década, mostrando que o café possui propriedades funcionais e nutricionais benéficas para a saúde humana[3-5]. Todavia, apesar da representatividade que o Brasil possui em termos de produção e consumo de café, são poucos os trabalhos realizados que avaliaram os efeitos da sua ingestão na saúde da população brasileira.

Verifica-se grande variedade de compostos biologicamente ativos no café, como a cafeína, os ácidos clorogênicos, o ácido cafeico e demais compostos fenólicos que resultam em uma gama de efeitos positivos para a saúde, tais como: respostas psicoativas (melhora do estado de alerta, modulação do estado de humor), alterações neurológicas (menor risco de doenças de Parkinson e Alzheimer), efeitos nas funções hepáticas e desordens metabólicas, com destaque para a redução de risco de diabetes mellitus tipo 2 (DM2)[3-6].

A DM2 se caracteriza pela redução da sensibilidade à insulina nos tecidos periféricos acompanhada pela diminuição da sua produção pancraética[7] e sua prevalência vem crescendo de forma epidêmica a nível mundial. Dados da Federação Internacional de Diabetes (IDF) estimam prevalência de 382 milhões de pessoas acometidas em 2013 com prespectiva de aumento para 592 milhoes até 2035[8]. Em nível nacional, o Ministério da Saúde (Brasil, 2006) estima uma prevalência de 11% para pessoas com idade superior a 40 anos[8] e dados da IDF publicados em 2013 mostram prevalência de 11,9% para indivíduos de 20 a 79 anos com estimativa de 19,2% em 2035[8].

Diante disso, existe crescente interesse mundial sobre os efeitos do consumo de café na saúde humana. Trabalhos epidemiológicos recentes confirmam resultados já publicados de associação negativa entre o consumo de café e o desenvolvimento da DM2[10-15]. No contexto do Brasil, destaca-se o estudo da Machado et al. (2011) que ao entrevistar indivíduos residentes do Distrito Federal obtiveram associação do consumo moderado de café (100 – 400 mL por dia) como

fator protetor em relação a DM2[16]. Este é o primeiro trabalho a mostrar associação negativa entre o consumo de café e DM2 na população brasileira.

A esse menor risco observado, atribui-se à presença de algumas substâncias no café, como os ácidos clorogênicos, que parecem estar relacionados com a redução da absorção de glicose, via aumento dos hormônios intestinais, como o *glucagon-like peptide-1* (GLP-1), que parece aumentar a produção e secreção de insulina[17]. Não existem ainda dados contundentes que mostrem o efeito do café na sensibilidade à insulina[18], bem como do papel do café descafeinado nos mecanismos biológicos que permeiam essa ação[19].

Em relação a adição de açúcar, hábito tradicional da população brasileira, o estudo da Louie et al. (2008) verificaram que a adição de 10 gramas de açúcar de mesa (sacarose) levou à redução da glicemia pós-prandial em um período de 2 horas[20]. Essa observação foi inédita e paradoxal, pois o consumo de açúcar por si está associado com aumento da glicemia pós-prandial. Desta forma, uma avaliação precisa do efeito do café adoçado no metabolismo da glicose é necessária.

Diante disso, este projeto visa avaliar de forma integrada a estimulação oral (consumo) da glicose e a marcação isotópica intravenosa que favorece a mensuração das respostas bioquímicas e metabólicas da glicemia frente à ingestão prévia de café. A utilização da metodologia com isótopo estável da glicose ([1]-^{13}C-glicose) aplicado no teste de estimulação oral e intravenosa de tolerância à glicose (*Oral Dose Intravenous Label Experiment* – ODILE) (Bluck et al., 2006)[21] permite a separação da glicose que aparece na circulação sanguínea daquela que é removida do sangue e aumenta a precisão da estimativa de utilização glicêmica e de sensibilidade à insulina. Desta forma pretende-se contribuir para o esclarecimento do efeito do consumo de café no controle da glicemia.

1.2 Objetivos

1.2.1 Objetivo Geral

- Revisar sistematicamente a literatura em busca de estudos clínicos randomizados sobre café e metabolismo da glicose;
- Investigar o efeito do consumo de café sobre o metabolismo da glicose utilizando o teste oral de tolerância à glicose com um marcador isotópico intravenoso.

1.2.2 Objetivos Específicos

- Analisar os efeitos do café no metabolismo da glicose dos resultados encontrados nos estudos captados pela revisão.
- Dosar a concentração plasmática de glicose e insulina durante o teste do ODILE;
- Determinar o índice de sensibilidade à insulina (Si) e a taxa de captação de glicose (Sg) em resposta a ingestão das bebidas testes;
- Comparar o efeito dos tratamentos sobre as variáveis Si e Sg.

1.3 Referências

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CAPÍTULO 2

Artigo de Revisão

Coffee consumption and glucose metabolism: a systematic review of clinical trials

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2.1 ABSTRACT

Objective: Epidemiological data shows an inverse association of coffee consumption with risk of type 2 diabetes. However, the clinical effects of coffee consumption on the glucose metabolism biomarkers remain controversial. Thus, this paper reviews clinical trials that evaluated the effects of coffee consumption on glucose metabolism.
Research Design and Methods: We identified studies published until December 2014 by searching electronic databases and reference lists. We included randomized clinical trials which the intervention group received caffeinated and/or decaffeinated coffee and the control group received water or placebo treatments and measured biomarkers of glucose metabolism. The Jadad Score was applied to evaluate the quality of the studies whereas studies that scored ≥ 3 points were considered for the analyses.
Results: Seven clinical trials (total of 237 subjects) were analyzed involving adult healthy, overweight and diabetic subjects. The studies were divided in short-term (1 to 3h) and long-term (2 to 16 weeks) duration. The results for short-term studies showed that caffeinated coffee consumption may increase the area under the curve for glucose response, while for long-term studies caffeinated coffee may improve the glycemic metabolism by reducing the glucose curve and increasing insulin response.
Conclusion: The findings suggest that caffeinated coffee may impairs glucose metabolism in short-term but in the long-term the studies indicate reduction of T2DM risk. More clinical trials with comparable methodology are needed to unravel this paradox.

Keywords: Coffee, Glucose, Diabetes Mellitus Type 2, Insulin.

2.2 INTRODUCTION

Coffee is one of the most frequently consumed beverages worldwide and has gained special attention regarding its beneficial effects on several chronic diseases, specially type 2 diabetes mellitus (T2DM) [1]. Coffee is a complex beverage composed of several substances, such as caffeine, phenolic compounds, including chlorogenic acids (CGA), and nutrients (minerals and vitamins). Collectively, coffee exerts functional and beneficial effects on human health [2]. Epidemiological evidence links moderate coffee consumption with a reduced risk of developing T2DM. This association has been shown in several studies with different populations showing a consistent dose response effect [3-7].

Drinking 3 to 4 cups of coffee per day is associated with an approximately 25% lower risk of developing T2DM compared to consuming none or less than 2 cups per day [3,6]. In addition, participants who increased their coffee consumption (+1 cup/day) had a decrease of 11% on T2DM risk in the subsequent 4 years compared with those who made no changes in its consumption. Coincidentally, people who decreased their coffee intake by more than 1 cup/day had a 17% higher risk for T2DM in these subsequent 4 years [5]. Despite the acute intake of caffeine impairing insulin sensitivity [8], the relative risk of T2DM for the highest level of coffee intake (> 6 cups/day) was 0.71 (0.67-0.76) for caffeinated coffee and 0.79 (0.69-0.91) for decaffeinated coffee intake [7]. It seems that both caffeinated and decaffeinated coffee intake can decrease T2DM risk. Dose-response analysis suggested that incidence of T2DM decreased by 12 % [0.88 (0.86-0.90)] for every 2 cups/day of caffeinated coffee intake and 11 % [0.89 (0.82-0.98)] for every 2 cups/day of decaffeinated coffee intake [7].

Several studies have linked the effects of coffee consumption on biomarkers of glucose metabolism [blood glucose and insulin concentrations, homeostasis model assessment insulin resistance index (HOMA-IR), and insulin sensitivity index] that could explain its beneficial epidemiological findings [9-11]. Thus, the aim of this review study is to analyse the clinical trials that evaluated the effects of coffee consumption on biomarkers of glucose metabolism.

2.3 METHODS

2.3.1 Literature Searching

An evaluation of clinical trials reporting the effects of coffee consumption on glucose metabolism was undertaken in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement [12]. After a comprehensive literature search we identified trials published in English until January 2015 on PubMed and Web of Science database using a combination of the following key words: coffee AND (glucose OR insulin OR type 2 diabetes OR glucose metabolism). Constraints were used for advanced search: adults (19 years or more), human, clinical trial, and search fields: title / abstract. The total hits obtained from searching the databases were screened by reading the article 'title' and 'abstracts'. Studies that did not match the aims of the review were excluded. The selected trials had the methods section analysed and those that did not satisfy the established criteria were excluded. We also scrutinized references within identified papers as well as articles that had come to our attention through other means.

2.3.2 Study selection

Clinical trials were included if they met the following criteria: (i) sample who had not been previously diagnosed with type 1 Diabetes Mellitus; (ii) consumption of coffee (caffeinated and/or decaffeinated) versus a control (placebo or water); (iii) evaluated the effects of coffee consumption on biomarkers of glucose metabolism (i.e. blood glucose and / or insulin concentrations and / or insulin resistance and / or insulin sensitivity). These inclusion criteria were assessed by reading the respective study protocol. When necessary, additional information data were requested from the study's corresponding author. Studies were discarded if they were deemed irrelevant to the review's objectives, duplicate publications, reported an inappropriate protocol or population type, and did not report defined outcomes.

2.3.3 Study quality - Jadad Score

Quality of the studies was judged by two independent reviewers (C.E.G.R. and T.H.M.C.) based on the Jadad Score (1996) [13] that includes randomization, generation of random order, double-blinding, discrimination of the blinding method, and reporting of withdrawals. For each trial a score was attributed to each addressed item, with a possible score of 0 – 5 (5 being the highest level of quality). Studies with a score ≥ 3 reflect 'good' reporting quality, whereas score of < 3 indicates a poor quality study, impacted by a lower relative validity. The analysis was performed

separately by the researchers and the results were compared to obtain a final conclusion. Those articles evaluated with discrepant scores were analysed again for a final score. Studies that scored < 3 points were not considered for further evaluation.

2.3.4 Summary measures and analysis

Data summary of selected studies included country and year of publication, characteristics of the subjects (age, sex, health status and co-morbidities at baseline), study design and duration, sample size, treatment, coffee dosage, study outcomes, and whether or not statistical significance was reported. The descriptive data were summarized in Tables 1 and 2. The studies are sorted as short (follow-up of 1 to 3h) and long-term effects (follow-up of 2 to 16 weeks). Risk of bias was included within the narrative synthesis and was based on Jadad score (1996) [13]. The units of glucose and insulin were standardized according the International System of Units: mmol/L for glucose and pmol/L for insulin. Data are presented as mean ± SEM values. Standard error of the mean (SEM) was used to standardize the study data variability. For studies where standard deviation (SD) was reported, the SEM was calculated dividing the SD by the square root of the sample size of the corresponding arm of the trial.

2.4 RESULTS

2.4.1 Literature search

The literature search identified (n = 740) the trials distributed in PubMed and Web of Science. No additional articles were identified by manually searching the reference lists. After removing not suitable (n = 700), duplicates (n = 14) and that did not match the aims (n = 5) the total number of articles screened were 21 trials [14-34]. After Jadad evaluation 7 trials were included in the analysis [14-17;31-33]. Search strategy is summarized in **Figure 1**.

2.4.2 Data quality

Table 1 ranks all the clinical trials by the Jadad score. Of 21 studies reviewed, 5% (n = 1) high quality (score = 5), 28% (n = 6) good quality (score = 3-4), 19% (n = 4) low quality (score = 1-2), and 48% (n = 10) were classified as poor quality (score = 0). Overall, the Jadad score showed a methodological weakness (score < 3) for 14

trials that were not included in the review analyzes. Seven studies showed a score ≥ 3: 4 short-term [14-17] and 3 long-term follow-up [31-33].

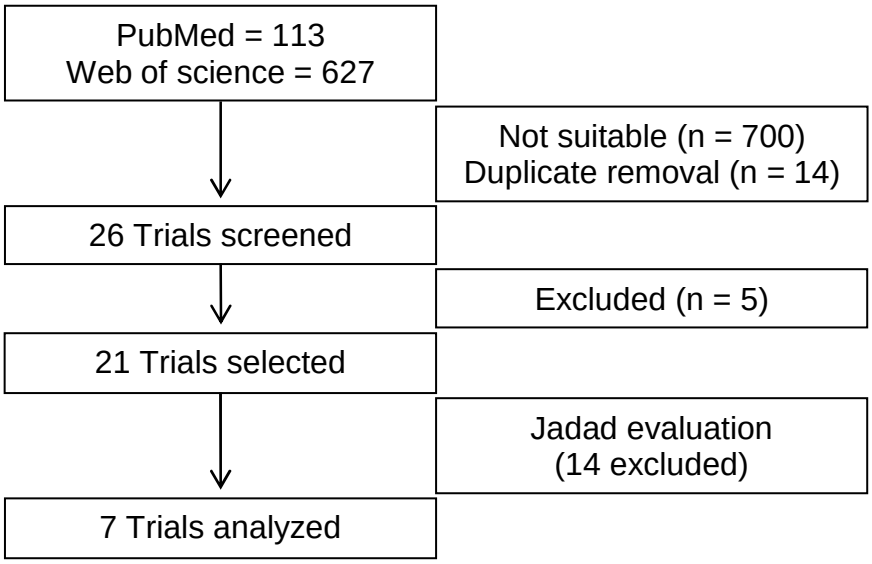

Figure 1. Study selection process that identified clinical trials evaluating the effects of coffee consumption on biomarkers of glucose metabolism.

2.4.3 Characteristics of the studies

Table 2 summarizes relevant data of the short (n = 4) and long-term (n = 3) studies. Overall, the trials were from various countries / populations: three were from Netherlands and one from each of the following countries: Canada, Greece, the United States of America, Japan, and New Zealand. Three studies enrolled exclusively males while four enrolled both males and females combined. Five studies evaluated healthy subjects [mean body mass index (BMI) ranging from 21.3 to 30.0 kg/m²]; one study evaluated individuals with T2DM, and one that enrolled overweight individuals diagnosed with impaired glucose tolerance (IGT). The mean age and BMI of the short-term trials were respectively 44.5 (SD 11.1) years and 26.7 (SD 3.8) kg/m^2; for the long-term trials mean age and BMI were 44.2 (SD 11.0) years and 26.8 (SD 2.7) kg/m^2, respectively. The coffee and caffeine dose in the short-term ranged from 200 to 633 mL and 180 to 526 mg, respectively, whereas in the long-term trials the coffee dose ranged from 500 to 1000 mL and the caffeine dose ranged from 345 mg to 1100 mg. Regarding the coffee preparation, the method used were regular paper-filtered, instant and espresso coffee. The trapezoidal method was used for the calculation of the area under the curve (AUC) of glucose and insulin response curve in all studies.

Table 1 - Jadad score value of clinical trials that evaluated the effects of coffee consumption on biomarkers of glucose metabolism

Author, year	Jadad Score	Randomization[1]	Randomization method reported[2]	Double-blind[1]	Blinding method reported[2]	Dropouts described[1]
Short-term						
Moisey et al., 2008 [14]	4	1	1	1	1	0
van Dijk et al., 2009 [15]	4	1	1	1	1	0
Krebs et al., 2012 [16]	4	1	1	1	1	0
Gavriel et al., 2013 [17]	3	1	1	0	N.A.	1
Thom et al., 2007 [18]	2	1	-1	1	1	0
Moisey et al., 2010 [19]	2	1	-1	1	1	0
Gavrieli et al., 2011 [20]	2	1	1	0	N.A.	0
Hatonen et al., 2012 [21]	1	1	-1	0	N.A.	1

Johnston et al., 2003 [22]	0	1	-1	0	N.A.	0
Battram et al., 2006 [23]	0	1	-1	1	-1	0
Louie et al., 2008 [24]	0	1	-1	0	N.A.	0
Aldughpassi et al., 2009 [25]	0	1	-1	0	N.A.	0
Greenberg et al., 2010 [26]	0	1	-1	0	N.A.	0
Buscemi et al., 2010 [27]	0	1	-1	1	-1	0
Beaudoin et al., 2011 [28]	0	1	-1	0	N.A.	0
Al-Mssallem et al., 2013 [29]	0	1	- 1	0	N.A.	0
Alkaabi et al., 2013 [30]	0	0	N.A.	0	N.A.	0
Long-term						
Wedick et al., 2011	5	1	1	1	1	1

[31]						
vanDam et al., 2004 [32]	3	1	1	0	N.A.	1
Ohnaka et al., 2012 [33]	3	1	1	0	N.A.	1
Kempf et al., 2010 [34]	0	0	N.A.	0	N.A.	0

[1]: Yes = + 1 point; Not = 0 point;

[2]: Yes = + 1 point; Not = - 1 point;

N.A.: Not applied.

Table 2 - Description of the studies of short and long-term coffee consumption effects on glucose metabolism

Authors, year	**Country**[1]	**Sample size**[2]	**Health condition**[3]	**Design**[4]	**Follow-up time**[5]	**Coffee dose**	**Coffee preparation**	**Caffeine dose**
Short-term								
Moisey et al., 2008 [14]	Canada	10 males	Healthy, lean	Crossover RCT	1h + 2h meal test[6]	633mL[7]	Filtered	393mg
van Dijk et al., 2009 [15]	Netherlands	15 males	Healthy, Overweight	Crossover RCT	30 min + 2h OGTT	270mL	Instant	N.A.
Krebs et al., 2012 [16]	New Zealand	18 (9M/9F)	T2DM	Single-blind crossover RCT	1h + 2h OGTT = 3h	200mL	Espresso	180mg
Gavriel et al., 2013 [17]	Greece	16 (8M/8F) 17 (9M/8F)	Healthy, lean Overweight	Crossover RCT	3h meal test[8]	200mL	Instant	383mg 526mg
Long-term								
Wedick et al. 2011 [31]	U.S.A.	45 (16M/29F)	Healthy, Overweight	Double-blind RCT	8wk	885mL	Instant	345mg
van Dam et al. 2004 (Study 1) [32]	Netherlands	26 (10M/16F)	Healthy, lean	Crossover RCT	4wk	1000mL	Filtered	1100mg

van Dam et al. 2004 (Study 2) [32]	Netherlands	45 (20M/25 F)	Healthy, lean	Crossover RCT	2wk	900mL	Filtered	820mg
Ohnaka et al. 2012 [33]	Japan	45 males	Overweight IGT	RCT	16wk	5 cups[9]	Instant	250mg

[1] U.S.A.: United States of America.

[2] M: Males; F: Females.

[3] T2DM: Type 2 diabetes mellitus; IGT: Impaired glucose tolerance.

[4] RCT: Randomized controlled trial.

[5] h: hours; OGTT: 75g oral glucose tolerance test; wk: week;

1h + 2h: Carbohydrate consumption 1 hour before (time -1h) the beverage test (time 0), follow by 2h of evaluation;

30min + 2h: Carbohydrate consumption 30 minutes before (time -30min) the beverage test (time 0), follow by 2h of evaluation;

1h + 2h OGTT = 3h: Carbohydrate consumption 1 hour before (time -1h) the beverage test (time 0), follow by 2h of evaluation, data analysis of 3h of intervention.

3h: Carbohydrate consumption with beverage test (time 0), follow by 3h of evaluation;

[6]: 75g of available carbohydrate.

[7]: Mean coffee volume was calculated using the data shown in the paper (dose of caffeine / kg body weight, average weight of the subjects and coffee caffeine content).

[8]: 22g of available carbohydrate.

[9:] Coffee dose is not clear in the text, but seems to be of 500 ml.

N.A.: Not applied.

2.4.4 Metabolic response

2.4.4.1 Short-term trials

The results for short-term trials have shown that caffeinated coffee consumption may impair the glycemic response compared to others tested beverages (decaffeinated coffee and water) [14,16,17]. There are no significant differences for insulin concentration and AUC, and insulin sensitivity index. In addition, no consistent results were found for decaffeinated coffee consumption on glucose metabolism biomarkers. **Table 3** shows the results of glucose and insulin AUC of the trials.

2.4.4.2 Lean and healthy subjects

Moisey et al. (2008) showed an improvement on glucose metabolism after consumption of decaffeinated compared with caffeinated coffee. They studied 10 healthy men in a crossover randomized clinical trial (RCT); caffeinated and decaffeinated coffee was taken 1h after a high glycemic index meal (75g of available carbohydrate). Compared with caffeinated coffee, decaffeinated coffee resulted in 145.6% and 28.5% lower 2h AUC for glucose ($p < 0.001$) and insulin ($p = 0.16$) respectively. In addition, the Matsuda index had a significantly improvement of 40% ($p < 0.05$) after decaffeinated compared with caffeinated coffee [14]. Gavrieli et al. (2013) reported no significant effects of caffeinated coffee on glucose and insulin concentrations, and AUCs compared with control (water). In this crossover RCT 16 healthy males and females were evaluated for coffee intake after 3h post-prandial response [17] (**Table 3**).

2.4.4.3 Overweight subjects

Gavrieli et al. (2013) showed that overweight / obese males and females ($n = 17$) had significantly elevated glucose 3h AUC after 3 mg (8.7%) and 6 mg (13.3%) of caffeinated coffee intake ($p = 0.03$ and $p = 0.02$, respectively) compared with control intervention (water). In addition 6 mg caffeinated coffee results in lower insulin concentrations at 15 and 30 minutes and elevated glucose concentrations at 60 and 90 minutes compared to water ($p < 0.05$) [17]. However, no significant difference was found for insulin AUC between the treatments. On the other hand, van Dijk et al. (2009) conducted a crossover RCT to analyse the effects of decaffeinated coffee on glucose and insulin concentrations during a 2h OGTT in 15 healthy overweight men.

Decaffeinated coffee did not significantly change glucose or insulin concentrations at any time points or AUC values compared with control [15] (**Table 3**).

2.4.4.4 T2DM subjects

Krebs et al. (2012) studied the effects of espresso caffeinated and decaffeinated coffee compared to water consumed 1h before the 2h OGTT on glucose tolerance and insulin sensitivity in 18 participants with T2DM in a crossover RCT. Glucose 3h AUC was higher after caffeinated coffee than water ($p = 0.03$) and marginally when compared with decaffeinated coffee ($p = 0.055$). There were no differences in insulin AUC ($p = 0.87$) or Matsuda index ($p = 0.47$) following beverage consumption [16]. Black espresso coffee in people with T2DM results in a marginally greater excursion of glucose during a following OGTT compared with water or decaffeinated coffee. This effect does not appear to be mediated by changes in insulin sensitivity (**Table 3**).

2.4.4.5 Long-term trials

Ohnaka et al. (2012) in a 16-week randomized clinical trial (n = 45) with 5 cups per day of caffeinated and decaffeinated coffee or no coffee showed that caffeinated coffee decreases the 2h glucose AUC compared with the baseline values and also is statistically significantly differed from the changes observed in the no coffee group ($p < 0.05$). In addition, 2h insulin AUC was 21.5% higher for caffeinated coffee compared with control, but non-significative. No significant differences were found for decaffeinated coffee and no coffee groups on glucose and insulin response, and insulin sensitivity index (composite ISI and HOMA-IR) [33]. Van Dam et al., (2004) conducted 2 clinical trials to evaluate the effects of caffeinated coffee and caffeine on glucose metabolism. The first study was a 4-week crossover trial that compared the effects of 1L caffeinated coffee consumption with coffee abstinence in 26 volunteers. After 4 weeks, fasting insulin concentrations were higher after the caffeinated coffee than no coffee group ($p = 0.002$) and no appreciable effect was observed to glucose response ($p = 0.94$). However, the second study had no significant effects on fasting glucose and insulin concentrations ($p = 0.42$ and $p = 0.15$, respectively) [32]. Similar to this results, Wedick et al. (2011) (n = 45) have shown that after the consumption of 5 cups per day of caffeinated and decaffeinated coffee, or no coffee for 8 weeks no

significant differences were found for glucose tolerance, insulin sensitivity, and insulin secretion between the groups [31] (**Table 3**).

Despite the limited data of long-term trials, the results shown that caffeinated coffee consumption may improve the glycemic metabolism by the reduction on glucose and increase on insulin response [32,33]. In addition, no impairment on glucose metabolism was found opposing the results suggested by short-term trials.

Table 3 - Short and long-term of coffee intake clinical trials on glucose and insulin AUC in lean healthy, overweight and T2DM subjects*

Authors, year	**GLUCOSE§**						**INSULIN†**						
	Coffee		**Control**		**Decaf**		**Coffee**		**Control**		**Decaf**		**ISI‡**
	AUC	**SEM**	**AUC**	**SEM**	**AUC**	**SEM**	**AUC**	**SEM**	**AUC**	**SEM**	**AUC**	**SEM**	
Short-term													
Lean healthy													
Moisey et al., 2008 [14]	253[a]	40	---	---	103[b]	39	42727	11155	---	---	33241	14541	DC↑[1]
Gavrieli et al., 2013 [17]	8.98	0.43	8.20	0.30	---	---	283.35	23.61	278.49	30.55	---	---	---
Overweight													
van Dijk et al., 2009 [15]	---	---	962	134	958	134	---	---	54727	21658	52324	21658	---
Gavrieli et al., 2013 [17][§]	9.41[a]	0.30	8.35[b]	0.24	---	---	332.66	28.47	324.33	25.00	---	---	---
T2DM													
Krebs et al., 2012 [16]	2547[a]	120	2443[b]	101	2455[ab]	118	66769	10528	65866	9299	68943	9695	†[1]
Long-term‡													
Wedick et al., 2011 [31]	13.0	#	13.8	#	14.3	#	618	#	697.5	#	771	#	†[1,2]
Ohnaka et al., 2012 [33]	16.9[a]	#	21.1[b]	#	20.3[ab]	#	1114	#	917	#	633	#	†[1,2]

*: Data extracted from the originals papers.

§: Means in the same row without a common superscript letter differ significantly $p < 0.05$.

†: No statistically significant difference was found between study groups.

ǂ: van Dam et al. (2004) [32] was removed from the table because they did not performed the AUC calculation.

---: Non applicable.

[#]: Raw data not provided.

[ǂ]: ISI: Insulin sensitivity index.

[1]: Matsuda index.

[2]: HOMA-IR: Homeostasis model assessment of insulin resistance.

2.5 DISCUSSION

This is a systematic review of clinical trials that evaluated the effects of coffee consumption on glucose metabolism summarizing data from seven studies involving a total of 237 subjects. Although there is heterogeneity among the studies, the results seem to show impairment on glucose response for caffeinated coffee consumption in short-term and an improvement on glucose metabolism (glucose and insulin response) in long-term duration. Moreover, no significant change was observed for insulin sensitivity. These results seem to show that the benefits of coffee consumption occur in the long-term as has been shown in the reduction of T2DM risk in epidemiological studies.

Contradicting the results of the trials with caffeinated coffee consumption on glucose metabolism, the epidemiological studies have consistently indicated that regular consumption of coffee (caffeinated or not) is associated with lower risk of T2DM [5-7]. The protective effect of habitual coffee consumption is seen with both caffeinated and decaffeinated coffee with one study suggesting the effect may be greater for decaffeinated coffee [35]. Nonetheless, these results need to be interpreted with caution because the high correlation between coffee and caffeine consumption which makes it difficult to separate their effects [6].

There are some debate about the role of caffeine effects in the development of insulin resistance and T2DM. Short-term administration of caffeine impairs the insulin resistance and glucose tolerance through the antagonism of the A1 and A2 subtypes of the adenosine receptor relating to glucose uptake in skeletal muscles. In addition caffeine has a synergistic interaction with adrenalin and noradrenalin, the main neurotransmitters of the sympathetic nervous system [36]. These negative effects of caffeine on acute glucose metabolism are consistent with the short-term trials results and contradictory to the epidemiological findings that suggest a reduction of T2DM risk with habitual coffee consumption.

The results obtained from the long-term trials may indicate that the reduction of the T2DM risk should occur due to the chronic coffee consumption as the epidemiology studies have shown. This scenario is attributed to reflect what we denominate as the ‘coffee paradox’, where consistently epidemiologic observations are not confirmed by clinical trials. The possible explanation for this ‘coffee paradox’ is that in the short-term trials the hyperglycemic effects of caffeine are dominant over the possible beneficial effects of coffee. Regarding the long-term studies there are

more time (weeks) for the bioactive compounds of coffee exert their antioxidant and anti-inflammatory effects that can lead to an improvement on glucose metabolism.

The epidemiological results suggest that components of coffee other than caffeine are responsible for the beneficial effect [6]. Also there is indication that habitual consumers develop a tolerance to the effects caused by caffeine in insulin sensitivity and glucose tolerance [37]. Additionally, the protective properties of coffee consumption on T2DM involve a multiple mechanisms that include not only antioxidant but also anti-inflammatory effects, factors that play a crucial role on blood glucose control. The coffee benefits can be related to the bioactive compounds content (as CGA, trigonelline, lignans, quinides, cafestol and kahweol) that has anti-inflammatory properties [36,38].

The CGA are the main phenolic components in coffee. Coffee beverage is the richest dietary source of CGA and this phenolic compound has been shown to reduce blood glucose concentrations in animal experiments [36,39]. Unfortunately the fate and mode of action of CGA in the human body after ingestion is more complex than originally anticipated because very little of the absorbed molecules retain the structure of the parent CGA present in the drink [36]. So, the health effects of polyphenols have been shown to go beyond simple antioxidant activity, because polyphenols exert modulatory effects in cells through selective action on cell-signaling pathways involved in pathogenesis of chronic diseases [40]. On the other hand, in the intestine CGA implicated in reducing glucose absorption by competitively inhibiting glucose-6-phosphate translocase and reducing sodium-dependent glucose transport in the brush border membrane [41]. In addition, previous studies have been shown that trigonelline reduces blood glucose concentrations in human [15,42,43]. This result can be explained by the regulation of key enzymes of glucose metabolism as glucokinase, glucose-6-phosphatase [44-46], a-glucosidase, and DPP- 4 activity [43.47].

Several studies conducted on humans and animals have demonstrated that regular coffee consumption may significantly reduce the concentrations of pro-inflammatory biomarkers such as interleukin (IL)-1 b, IL-6, tumor necrosis factor a, C-reactive protein, monocyte chemotactic protein 1, vascular cell adhesion molecule 1, C-peptides, endothelial-leukocyte adhesion molecule 1, and IL-18, 8-isoprostane, and and fetuin-A; and significantly increased the concentrations of anti-inflammatory biomarkers such as adiponectin, IL-4, and IL-10 [31,34,38] that can contribute to a

reduced T2DM risk [48]. In this regard coffee has been shown to be associated with a protection against various types of chemical stresses through the stimulation of enzymes involved in cellular antioxidant defenses resulting in increased endogenous defense mechanisms against electrophilic but also oxidative insults [49]. Therefore, the reduction of T2DM risk found in epidemiological studies may be explained by the long-term effects (years) of the bioactive compounds that reduce the chronic inflammation system favouring an improvement on glucose metabolism.

The evaluation according to the Jaded criteria showed limitations of the studies´ methodology. Although almost all the studies specified randomization, most of them fail to report the method of randomization. Double blinding was also not present in half of the published trials. An explanation is the difficulty in effectively blind the participants and researchers due to the characteristics and aroma of coffee itself, but double blinding is observed in trials using caffeinated and decaffeinated coffee arms [16,19,31]. Description of drop-out was not reported in the short-term follow-up trials except for Hatonen et al. (2012) [21] and Gavriel et al. (2013) [17]. Consequently there is limited information of possible side or harmful effects on the participants. In fact no side or harmful effect of coffee was reported by the subjects participating in all trials which majority were healthy male subjects. The results are then limited to this group of people and cannot be extrapolated to the general population including vulnerable groups, including T2DM subjects.

The advantage of this review relies on the demonstration that short-term follow-up studies are limited to effects on fasting glucose and insulin concentrations which are insufficient markers to effectively explain the effect of coffee on reducing the risk of T2DM. These changes in the antioxidant status in vivo are unlikely to be a direct consequence of the antioxidant activity of coffee, and are more likely to be the result of coffee compounds being able to augment endogenous antioxidant defenses [50]. Additionally, it is evident that long follow-up randomized trials are still insufficient and should be the way forward for new research on the subject. Verifying the direct effect of coffee consumption on biomarkers of glucose and insulin metabolism is not satisfactory model to investigate the mechanism. Data on the effects of coffee intake on glucose metabolism from randomized trials lasting more than 24 hours are sparse and limited to effects on fasting glucose and insulin concentrations. Nevertheless, until the relationship between long-term coffee consumption and T2DM is better

understood and any mechanism involved identified, it is premature to make claims about coffee preventing T2DM.

In conclusion caffeinated coffee may show non-favourable effects on short-term and an improvement on glucose metabolism on long-term trials. Therefore the 'coffee paradox' remains unresolved, further long-term randomized clinical trials are needed to investigate how the substances contained in coffee can overcome caffeine effects and explain the protective effects of coffee consumption on T2DM.

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CAPÍTULO 3

3.1 Métodos

O presente estudo foi realizado no Laboratório de Bioquímica da Nutrição do Núcleo de Nutrição, contando com o apoio de demais Laboratórios da Faculdade de Saúde da Universidade de Brasília e do *Human Nutrition Research*, *Medical Research Council* (Cambridge, Inglaterra).

3.1.1 Amostragem

Considerando a importância do efeito do café no metabolismo da glicose, foi selecionado como variável principal do estudo para o cálculo do tamanho amostral o índice de sensibilidade à insulina. O número amostral de 12 indivíduos foi determinado a fim de permitir a detecção de uma diferença entre as médias do índice de sensibilidade à insulina (Si) de 1,754 comparando-se os grupos com nível de significância de 5% e poder de 90%.

O recrutamento foi realizado nos meios de comunicação da cidade (jornal, rádio e TV) e através da distribuição de cartazes e panfletos de divulgação da pesquisa na Universidade de Brasília. Foram selecionados voluntários do sexo masculino, com idade de 18 a 40 anos, apresentando índice de massa corporal (IMC) entre 18,5 - 24,9 kg/m^2.

Os critérios de inclusão foram:

- Não apresentar flutuação de peso ≥ 5 kg nos últimos 3 meses;
- Não utilizar medicamentos que alterem o metabolismo, o apetite e o sono;
- Não apresentar intolerância aos alimentos fornecidos durante o estudo;
- Não apresentar distúrbios do sono;
- Não ter doado sangue nos últimos 3 meses e não planejar doar nos próximos 3 meses;
- Apresentar consumo regular de desjejum (≥ 100 kcal ingeridas no máximo 2 horas após acordar em mais de 4 dias por semana);
- Ser consumidor regular e moderado de café (≥ 100 mL pelo menos 5 vezes por semana);
- Se comprometer a consumir todos os alimentos do estudo no tempo estipulado;

- Estar de acordo com o Termo de Consentimento Livre e Esclarecido.

Os critérios de exclusão incluíram:

- Diagnóstico de Diabetes Mellitus tipo 2, dislipidemia e hipertensão arterial;
- Problemas cardíacos;
- Hipotireoidismo não tratado;
- Evento prévio de desmaio súbito e convulsão;
- Anemia;
- Etilistas e tabagistas;
-Doação recente (últimos três meses) de sangue em hemocentros;
- Consumo superior a 500mg de cafeína/dia (5 xícaras de café, chá e refrigerantes a base de cola).

Depois de preenchidos os critérios de inclusão e exclusão pelo contato inicial (telefone ou e-mail), foi agendada uma entrevista presencial onde foi aplicado o questionário de triagem (**Apêndice 1**) a fim de confirmar os dados relativos aos critérios de inclusão e exclusão e coletar informações sócio demográficas, sendo também explicado o delineamento experimental do estudo. A seguir, se o voluntário estivesse apto, foi agendado o dia para realização das avaliações iniciais e das etapas experimentais, sendo transmitidas as orientações para a realização das mesmas. Os voluntários que ingressaram no estudo foram orientados a manter constante o nível de atividade física e os hábitos de vida durante a pesquisa.

3.1.2 Delineamento Experimental

Trata-se de um ensaio clínico randomizado, cruzado e duplo cego. O desenho experimental e o tamanho amostral foram baseados na distribuição randomizada dos sujeitos nos cinco tratamentos sequenciais. Os cinco tratamentos foram determinados pelo delineamento de quadrado de Williams, onde os sujeitos foram alocados de modo que cada um dos tratamentos seguiu o próximo tratamento uma vez, alocando cada combinação como a sequência de experimentos a ser seguida para cada voluntário. O primeiro sujeito foi iniciado aleatorizado na sequência de tratamentos, seguindo a sequência proposta no delineamento descrito abaixo mantendo o equilíbrio entre as sequências [1]:

A B E C D ; B C A D E ; C D B E A ; D E C A B; E A D B C ;

D C E B A ; E D A C B ; A E B D C ; B A C E D ; C B D A E ;
Onde A: café com açúcar; B: café sem açúcar; C: café descafeinado sem açúcar;
D: água com açúcar; E: água sem açúcar.

3.1.3 Avaliações iniciais

Antes do início das coletas experimentais, os voluntários se apresentaram ao Laboratório de Bioquímica da Nutrição (Núcleo de Nutrição, Universidade de Brasília) entre 7 - 9h da manhã, em jejum de 12h para a realização das seguintes avaliações: hemograma completo, glicemia capilar de jejum, anamnese alimentar (recordatório 24 horas), avaliação clínica (antropometria, composição corporal e pressão arterial), avaliação do nível de atividade física e do gasto energético (**Apêndice 1**).

3.1.4 Avaliação Clínica: antropometria, composição corporal e pressão arterial

O peso dos voluntários foi determinado na avaliação inicial e ao início de cada dia de intervenção do estudo. Para isso foi utilizada balança eletrônica digital do tipo plataforma, com capacidade para 150 kg e precisão de 100g (Plenna, São Paulo, Brasil). A estatura foi aferida durante a avaliação inicial utilizando um antropômetro vertical milimetrado, com extensão de 2,15 m e escala de 0,1 cm (Alturaexata, Belo Horizonte, Brasil). Para a determinação do peso e da estatura, os voluntários foram posicionados em pé, em posição firme, com os braços relaxados, cabeça no plano horizontal e com roupas leves, aferidos segundo as recomendações de Jelliffe *et al.* (1968) [2]. Foi determinado o valor do IMC e classificado segundo os pontos de corte preconizados pela Organização Mundial de Saúde [3]. O perímetro da cintura foi aferido com o uso de uma fita métrica flexível e inelástica, com extensão de 2 metros e graduada em milímetros, determinado no ponto médio entre a última costela e a crista ilíaca e classificado segundo os critérios do *National Cholesterol Education Program* [4].

A composição corporal dos voluntários foi avaliada utilizando o método da bioimpedância elétrica vertical tetrapolar (RJL – 101 modelo Quantum II, EUA) [5]. Para tal, os voluntários seguiram as seguintes orientações: jejum absoluto de 12h,

não consumir café e bebidas cafeinadas por 24h e álcool por 48h antes da avaliação, não praticar atividade física nas últimas 12h antes, na noite anterior ao teste não consumir refeição rica em carboidratos, não beber água nas horas precedentes ao teste e ter realizado micção no máximo 30 minutos antes do teste (**Apêndice 2**). Os voluntários permaneceram em posição supina horizontal sobre uma superfície não condutora, em ambiente calmo e tranquilo, com braços e pernas abduzidos a 45 graus a partir do corpo, sem meias e objetos metálicos. Um eletrodo emissor foi posicionado próximo à articulação metacarpo-falangea da superfície dorsal da mão direita e outro distal do arco transverso da superfície superior do pé direito. Um eletrodo detector foi posicionado entre as proeminências distais do rádio e da ulna do punho direito e outro entre os maléolos medial e lateral do tornozelo direito. A determinação da água corporal total (ACT) foi feita pelas equações descritas por Sun et al. (2003) [6]. A massa livre de gordura foi determinada pela multiplicação da ACT pela constante de hidratação de 0,74 [7] e a massa gorda foi determinada por diferença da massa corporal total.

Para aferição da pressão arterial dos voluntários foi utilizado o método indireto, com a técnica auscultatória, utilizando um esfigmomanômetro com coluna de mercúrio calibrado. A aferição foi realizada durante a avaliação inicial e nos dias de coleta, por um profissional treinado, segundo as recomendações e classificações da VI Diretrizes Brasileiras de Hipertensão (2010) [8].

3.1.5 Avaliação da Ingestão Alimentar

A fim de se avaliar a ingestão alimentar antes de cada dia de experimento, os voluntários preencheram um registro alimentar referente à última refeição contendo informações sobre horário, alimentos, forma de preparo e quantidades. Cada registro alimentar foi conferido pelo pesquisador na presença do voluntário, a fim de assegurar sua exatidão. As porções de alimentos foram convertidas em gramas e posteriormente a ingestão de energia, macronutrientes, fibras, índice e carga glicêmica foram analisadas utilizando o programa *Nutrition Data System for Research*, versão 2013 (University of Minnesota, USA). Para isso foi incluído alimentos típicos brasileiros e receitas regionais.

3.1.6 Análise do café

A fim de se obter a composição do café a ser utilizado na pesquisa, amostras do café Melitta® tradicional (cafeinado) e descafeinado foram enviadas para análise no Laboratório de Bioquímica Nutricional e de Alimentos, do Departamento de Química da Universidade Federal do Rio de Janeiro coordenado pela Profa. Dra. Adriana Farah. Foram obtidos os teores de ácidos cafeoilquínicos (CQA) e cafeína por meio da cromatografia líquida de alta eficiência. O café cafeinado obteve 856,3 mg / 100g de CQA e 1530,1 mg / 100g de cafeína e café descafeinado obteve 1234,6 mg / 100g de CQA e 340,2 mg de cafeína.

3.1.7 Preparo das Bebidas Testes

Para o preparo do café, foi comprado o café torrado e moído em pó do tipo Arábica (Coffea arabica L. Arbusto). O preparo ocorreu da seguinte forma: Colocar 300 mL de água mineral em jarra pirex. Ferver a água por 2,5 minutos em micro-ondas em potencia alta. Pesar 30 gramas de pó de café torrado e moído no coador de papel filtro espalhando uniformemente e encaixando-o no filtro. Imediatamente antes da fervura (90ºC), despejar a água sobre o pó umedecendo-o uniformemente. Comece molhando o pó de café da extremidade para o centro do coador. Em seguida, despeje a água lentamente em fio bem no centro do filtro sem misturar com a colher. Não exceder o tempo de 5 minutos para despejar a água. Servir imediatamente após o preparo. Para o preparo do café adoçado, pesar 30g de açúcar e despejar no copo, percolar o café no copo sobre o açúcar, homogeneizar bem o açúcar e servir em seguida. Para o preparo do café descafeinado, foi utilizado café do tipo Arábica (Coffea arabica L. Arbusto) com modo de preparo igual ao café comum (cafeinado) e servido imediatamente após o preparo. Para os controles (água com e sem açúcar), foi mensurado o volume de água (300 mL), adoçado ou não, bem homogeneizado quando adoçado e fornecido à temperatura ambiente. O peso do copo antes e depois da ingestão das bebidas foi mesurado e essa diferença forneceu a quantidade de bebida consumida em gramas. As bebidas foram fornecidas em copos idênticos, opaco, com tampa utilizando um canudo preto para a ingestão da bebida. Foi utilizado um copo para as bebidas com café (cafeinado com e sem açúcar e descafeinado) e outro para água (com e sem açúcar) a fim de não

permitir que o sabor residual do café atrapalhasse o cegamento do estudo. Os pesquisadores foram cegos para os tratamentos e os voluntários para os tipos de café ofertado (descafeinado ou não). As bebidas foram preparadas e ingeridas no laboratório, local no qual os voluntários permaneceram durante todo o período de intervenção.

3.1.8 Avaliação Metabólica: Oral Dose Intravenous Label Experiment (ODILE)

Nos dias das etapas experimentais, os voluntários compareceram ao laboratório entre 7 - 9h da manhã, em jejum de 12h para participar de uma das cinco etapas do estudo: Café com açúcar, Café sem açúcar, Café descafeinado sem açúcar e dois controles (água com e sem açúcar). Eles foram instruídos a não ingerir bebida alcoólica, não realizar exercícios físicos não habituais e extenuantes por 24h antecedentes ao teste e a ingerir uma refeição normoglicídica na noite anterior.

Ao se apresentarem ao laboratório para participação em cada etapa, foram mensurados o peso corporal e a glicemia capilar de jejum (Accu-Check Performa, Roche Diagnostics, Alemanhã), registrado o número de horas de sono na noite anterior, o horário da última refeição, o tipo e quantidade de alimentos ingeridos (**Apêndice 3**).

O procedimento de coleta de sangue foi realizado por uma técnica de enfermagem supervisionada pelas médicas endocrinologistas Prof[a]. Dra. Adriana Lofrano Porto, Prof[a]. Dra. Angélica Amato ou Cicilia Rocha. Em cada dia de intervenção do estudo, foi posicionado em cada braço um cateter do tipo *scalp* na veia antecubital para a realização das coletas de sangue. A fim de manter o acesso venoso, após cada coleta foi injetado 1 mL de soro fisiológico estéril para lavar a via de acesso e evitar seu entupimento. No momento da próxima coleta, foram aspirados 2 mL de sangue (sangue + soro fisiológico) para descarte e então foi realizada a coleta de sangue definitiva. Durante a realização do experimento foram coletadas 20 amostras de 5 mL e 7 amostras de 4 mL de sangue, as quais foram distribuídas em tubos siliconizados BD® tipo Vacutainer® soro e do tipo Vacutainer® com K_3EDTA plasma.

Em cada etapa do estudo, os voluntários consumiram uma das bebidas teste no período de 10 minutos. Foram realizadas coletas de sangue aos -10 minutos e

imediatamente antes da ingestão da bebida teste e nos tempos 30 e 60 minutos após. O teste de estimulação oral ocorreu uma hora após a ingestão da bebida teste. Nesse momento foi fornecida uma solução de 75 g glicose dissolvidos em 300 mL de água potável (Gluc UP 75, NewProv, Brasil), e posteriormente foram coletadas amostras de sangue nos seguintes tempos: 15, 30, 45, 47, 49, 51, 53, 55, 57, 59, 61, 65, 70, 75, 80, 85, 90, 100, 115, 135, 165, 195 e 225 minutos. Nos tempos 47, 51, 55, 59, 61, 70, 85 foram coletadas amostras de 4 mL e nos demais tempos 5 mL de sangue. Aos 45 minutos, foi infundida 5 mL de solução salina contendo 250mg de [1]-^{13}C-glicose (Promochem, Inglaterra) na veia do antebraço contral-lateral a coleta de sangue. A duração total de cada visita foi de aproximadamente 6 horas, com um período de separação (*wash-out*) entre os tratamentos de acordo com a figura 2. A Figura 1 mostra o delineamento experimental do estudo.

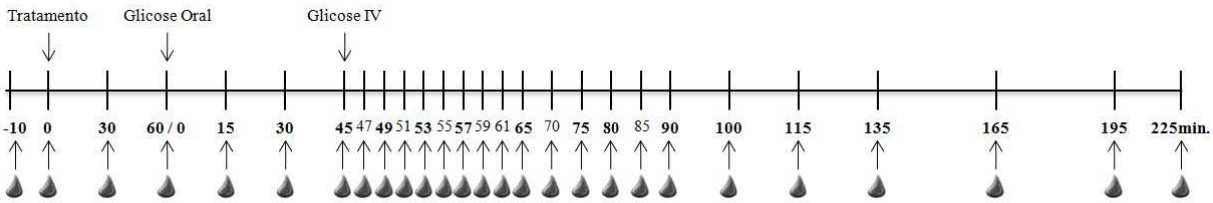

Figura 1. Delineamento experimental do estudo.
Tratamento: Ingestão da bebida teste.
: Coleta sanguínea.

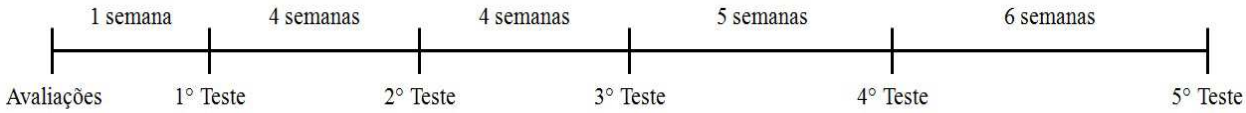

Figura 2. Esquema de periodicidade das coletas de dados do estudo.

Durante o experimento não foi permitido aos voluntários comer ou beber (exceto água) e assistir ou conversar sobre temas que possam influenciar os parâmetros avaliados (que gerem excitação, comidas e alimentos, ou sobre o tipo de bebida recebida de modo a manter o caráter monocego do experimento aos investigadores que aplicam o tratamento). Foi permitido ler, ouvir música, assistir televisão, usar computador e utilizar o banheiro. Os voluntários foram monitorados todo tempo por membros da equipe da pesquisa.

3.1.9 Análises Bioquímicas

A determinação do hemograma completo foi feita pelo laboratório de análises clínicas do Hospital Universitário da Brasília. As análises de glicose e insulina foram realizadas por meio de convênio estabelecido com o Laboratório Sabin de Análises Clínicas (15 sujeitos, 5 tratamentos e 27 pontos coleta, total de 2025 dosagens). Todo material coletado foi centrifugado (4.000 rpm por 15 minutos) e aliquotado em microtubos do tipo *eppendorf* de 1,5 mL, congelado e armazenado a -80°C no Laboratório de Bioquímica da Nutrição para posterior análise. As determinações dos isótopos da glicose ([1]-^{13}C-glicose) foram realizadas utilizando a cromatografia gasosa com espectrometria de massas de razão isotópica (*Gas Chromatography/ Combustion/ Isotope Ratio Mass Spectrometry* - GC/C/IRMS) no *Human Nutrition Research*, *Medical Research Council* (Cambridge, Inglaterra) [9-11]. A modelagem do ODILE foi realizada pelo índice de modelos mínimos [12,13] das curvas de tolerância (glicose, insulina e isótopo da glicose) obtendo-se como resposta o índice de sensibilidade à insulina (Si) e a taxa de captação de glicose (Sg).

3.1.10 Cálculo da área sob a curva

As áreas abaixo da curva (AAC) da resposta glicêmica e insulinêmica foram calculadas considerando as diferenças entre os valores obtidos com o tempo 0, sendo utilizado apenas os valores maiores do que o nível basal. Para isso foi aplicado o método trapezoidal (FAO, 1998) [14], utilizando o programa *GraphPad Prism*, versão 6 (GraphPad Software, Inc., USA). Foram determinadas as AAC para os intervalos: 0 – 60 minutos (período pós-absortivo), 0/60 – 135 minutos (2h teste oral de tolerância a glicose), 0/60 – 225 minutos (4h oral de tolerância a glicose), e 0 – 225 minutos.

3.1.11 Análise Estatística

Foi aplicado o teste Kolmogorov-Smirnov para verificar a normalidade, sendo utilizados os testes paramétricos e não-paramétricos de acordo com os resultados obtidos. Foi utilizada a análise de variância (ANOVA) com post hoc de Tukey ou

Kruskal-Wallis para comparar as médias das variáveis coletadas em cada dia de experimento (horas de sono da noite anterior, glicemia capilar de jejum e peso), análise alimentar da última refeição (macronutrientes, fibras, índice glicêmico e carga glicêmica) e as respostas da AAC. Para analisar os efeitos das bebidas testes nos parâmetros do metabolismo da glicose (Si e Sg) foi aplicado a ANOVA com post hoc de Bonferroni. Os dados coletados foram tabulados no programa Excel versão 2007 (Microsoft, EUA) e analisado no *IBM SPSS Statistics*, versão 21 (IBM Corporation, EUA), adotando como critério de significância estatística $p<0,05$. Os resultados da caracterização da amostra estão apresentados como média ± desvio padrão e os resultados bioquímicos como média ± erro padrão.

3.1.12 Procedimentos em caso de emergência

Quando o voluntário relatasse ou a equipe de pesquisadores percebesse algum mal-estar do sujeito durante a realização do experimento foi estabelecido um procedimento de conduta. Inicialmente era determinada a glicemia capilar utilizando um glicosímetro digital (Accu-Check Performa, Roche Diagnostics, Alemanha) a fim de verificar o nível glicêmico. Em situações de hipoglicemia foram realizados os "Procedimentos Padronizados em casos de Emergência" (**Apêndice 4**). Quatro voluntários apresentaram síncope em um experimento, onde foi determinada a glicemia capilar e aferida a pressão arterial sendo observado o quadro de normalidade e rápida recuperação. O experimento foi suspenso e remarcado.

3.1.13 Aspectos éticos

O protocolo do presente estudo foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Ciências da Saúde da Universidade de Brasília, registro do projeto 005/2012 (**Anexo 1**). Todos os voluntários foram esclarecidos quanto aos objetivos da pesquisa e assinaram o termo de consentimento livre e esclarecido (**Apêndice 5**) antes do início do estudo.

3.2 Referências

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CAPÍTULO 4

Artigo Original

Coffee consumption has no acute effects on glucose metabolism in healthy men: a randomized crossover clinical trial

Short title: Coffee and glucose metabolism

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This trial was registered at clinicaltrials.gov as NCT02099929.

4.1 ABSTRACT

Background: Multiple epidemiologic studies have consistently reported association between increased coffee consumption and a lowered risk of Type 2 Diabetes Mellitus. However, the mechanisms behind this finding have not been fully elucidated.

Objective: We investigate the effect of coffee (caffeinated and decaffeinated) on glucose effectiveness and insulin sensitivity using the stable isotope minimal model protocol with oral glucose administration in healthy men.

Design: Fifteen healthy men underwent 5 arms randomized crossover single-blinding (researchers) clinical trial. They consumed decaffeinated coffee, caffeinated coffee (with and without sugar), and controls – water (with and without sugar) followed 1 hour by an oral glucose tolerance test (75 g of available carbohydrate) with intravenous labeled dosing interpreted by the two compartment minimal model (225 minutes). One-way ANOVA with Bonferroni adjustment were used to compare the effects of the tested beverages on glucose metabolism parameters.

Results: Decaffeinated coffee resulted in higher insulin sensitivity compared with caffeinated coffee and water, and the caffeinated coffee showed higher glucose effectiveness compared with decaffeinated coffee and water. However, these differences were not significant. In overall analyze (0 – 225 min) there were no significant differences on glucose effectiveness, insulin sensitivity, and glucose and insulin area under the curve between the groups.

Conclusions: The findings of this study demonstrate that the consumption of caffeinated and decaffeinated coffee with or without sugar has no acute effects on glucose metabolism in healthy men.

Keywords: Coffee, Glucose, Diabetes Mellitus Type 2, Insulin.

4.2 INTRODUCTION

Coffee is one of the most popular beverages in the world, thus, investigate its association with several diseases is important to public health. A growing body of evidence from recent epidemiological studies suggests that coffee consumption is beneficial and inversely associated with risk for some diseases (1–3). Coffee is a mixture of many components such as vitamins, minerals, and bioactive compounds as caffeine, chlorogenic acids (CGA), trigonelline, and diterpenes that may have different effects on glucose metabolism (3–6).

A number of recent prospective studies have reported a negative association between high coffee consumption (> 3 cups/day) and risk of type 2 diabetes mellitus (T2DM) (7–9). Drinking 3 to 4 cups of coffee per day is associated with an approximately 25% lower risk of developing T2DM compared to consuming none or less than 2 cups per day (8). According to a recent meta-analysis, the incidence of T2DM decreased by 12% for every two cups of caffeinated coffee per day increased, and by 11% for every two cups of per day increased in decaffeinated coffee consumption (9). Coffee may have different mechanism factors involving in the pathogenesis of T2DM, such as glucose metabolism, intestinal glucose absorption, and antioxidant and inflammatory activity (4).

Experimental studies to analyze the mechanisms related to this epidemiological observation have not been widely performed. Reis et al. (2015) in a systematic review study analysed the effects of coffee consumption on biomarkers of glucose metabolism summarizing data from seven randomized clinical trials (n = 237 participants). The results of the short-term studies (1 to 3h) showed that the caffeinated coffee consumption may impair the postprandial glycemic response compared to decaffeinated coffee and water. In addition, there were no significant differences for insulin and insulin sensitivity index; and no consistent results were found for decaffeinated coffee (10). Thus, these findings have shown some doubt on the beneficial effects of caffeinated coffee on glucose metabolism and suggest that non-caffeine constituents of coffee may have important effects on glucose homeostasis. In addition, these results contrast to the epidemiological evidences showing that habitual coffee consumption is protective against development of T2DM. This coffee paradox justified further research to explore the mechanisms behind this beneficial association.

Therefore, the aim of this study was to investigate the effects of coffee (caffeinated and decaffeinated) consumption on glucose effectiveness (Sg) and insulin sensitivity (Si) using the stable isotope minimal model protocol with oral glucose dose (Oral Dose Intravenous Label Experiment – ODILE) in healthy men.

4.3 SUBJECTS AND METHODS

4.3.1 Participants

Study participants were recruited through public advertisements. Eligibility criteria included: age 18 – 40 years, body mass index (BMI) between 18.5 – 25.0 kg/m^2, not taking any medications that affect the metabolism, regular breakfast consumer (≥ 100 kcal ingested within 2 hours of waking on ≥ 4 days/week), limited body weight fluctuation (< 5 kg in the past 3 months), no self-reported sleep disorders, and habitual coffee consumer (≥ 100 mL at least 5 times/week). Exclusion criteria included: any chronic and cardiovascular disease, hypothyroidism, sudden fainting and convulsions, anemia and coagulations disorders, non-smokers, donated blood in the last three months, consumption of more than 500 mg of caffeine/day.

A sample size of 12 subjects would provide a detection of a difference of mean Si of 1.754 comparing between the groups at a 5% significance level and a 90% power. This assumes a within subject coefficient of variation of 0.393 estimated from internal data (11). Dropouts were handled by over-recruiting and extra subjects were allocated to the treatment sequence. A total of 78 people made the initial contact by email or telephone, 26 of these completed the first screening visit (interview), of which 18 completed the second screening visit (clinical assessments) and were eligible for the study. At final, 15 participants met all screening criteria and completed the full study protocol (**Figure 1**).

4.3.2 Study design

This randomized crossover single-blinding (researchers) clinical trial required subjects to complete 5 experimental sessions where caffeinated coffee (CC), caffeinated coffee with sugar (CS), decaffeinated coffee (DC), and 2 controls –water with sugar (WS), and water (W) were consumed followed 1 hour by Oral Dosing Intravenous Labeled Experiment (ODILE) test (12). The principal researchers were blinded to all of the experimental treatments.

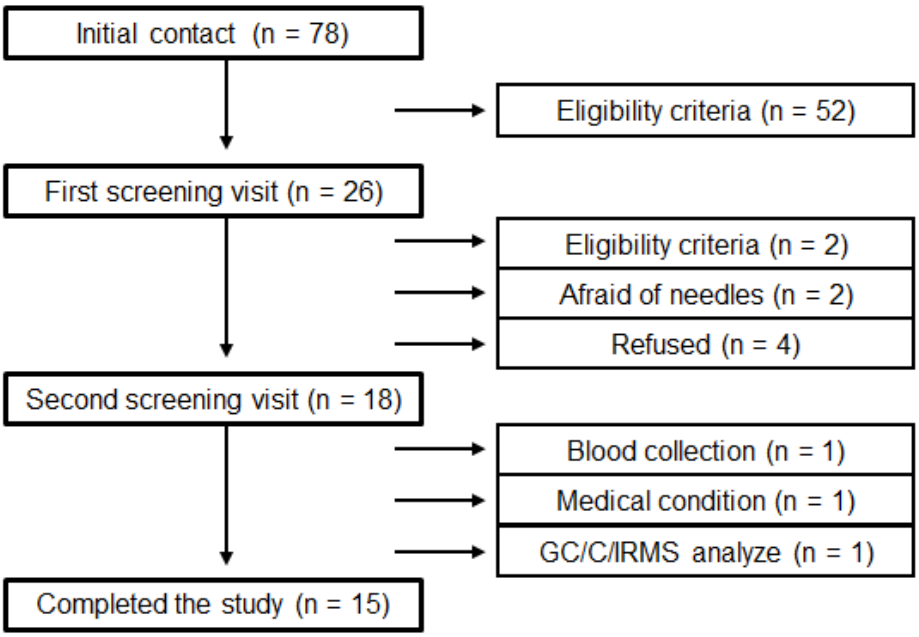

Figure 1. Study flow diagram.

Subjects were randomized to 1 of 5 sequences of treatments. The 5 sequences constitute a Williams squares design and has the property that every treatment follows every other treatment once. As this is a Latin square treatments are balanced with respect to period effects. Subjects will be allocated randomly to the following sequences of treatment maintaining balance between the sequences (13):

A B E C D; B C A D E; C D B E A; D E C A B; E A D B C;

D C E B A; E D A C B; A E B D C; B A C E D; C B D A E;

where A is caffeinated coffee with sugar, B is caffeinated coffee, C is decaffeinated coffee, D is water with sugar, and E is water.

Participants were instructed not to consume coffee, caffeine, alcohol or conduct any non-habitual physical activity 24 hours before the sessions, and to consume a low carbohydrate meal the night before experimental sessions. This meal was intended to not contain high quantity of carbohydrate foods (i.e. breads, rice, pasta, potatoes, cassava, pizza, etc.). The washout period and hematopoiesis recovery is presented in **Figure 2**.

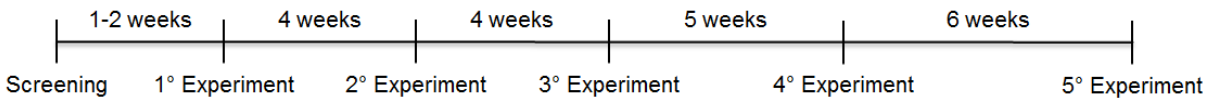

Figure 2. Flowchart of washout period.

4.3.3 Preexperimental protocol

For screening, participants arrived in the laboratory between 0700h and 0900h after a 12 hours overnight fluid and feed-deprivation for measurement of height, body weight, waist circumference, body composition, complete blood count, capillary fasting glucose and blood pressure. Subjects were also asked to answer questionnaires regarding the recruitment criteria and food intake. At each experimental session, body weight, capillary fasting glucose, the number of hours of sleep the night before, and the time and composition of the last meal were assessed. Blood glucose were measured using a glucometer to ensure the subjects were feed-deprived (glucose < 5.5 mmol/L).

4.3.4 Experimental protocol

At the each experimental day, the subjects were admitted to the unit between 0700 and 0900 following 12 hours overnight fast and submitted to the ODILE test. A cannula was inserted into the antecubital vein in each forearm, one specifically for the administration of [1]-13C-glucose, and the other for blood sampling. After 10 minutes of rest period, basal blood samples were taken at -10 and 0 minutes to establish fasting plasma glucose and insulin concentrations. Then the subjects drank one of each tested beverage according the sequence of the Williams squares design and blood samples were taken at times 30 and 60 minutes after. At this time a 75 g of glucose solution dissolved in 300 ml of water (Gluc UP 75, NewProv, Brazil) was provided, and later blood samples were taken at the following times: 15, 30, 45, 47, 49, 51, 53, 55, 57, 59, 61, 65, 70, 75, 80, 85, 90, 100, 115, 135, 165, 195 e 225 minutes. Immediately after the 45 minute sample, an intravenous bolus of 250 mg of pyrogen-free [1]-13C-glucose (Cambridge Isotope Laboratories, USA) was administered in the contralateral arm and the cannula removed for the volunteer comfort and to avoid collection from that arm. Care was taken to discard gloves and apparatus related to the isotopic solution (**Figure 3**).

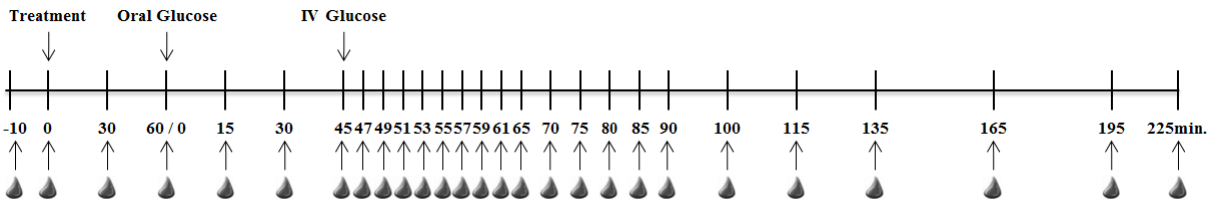

Figure 3. Study desing.

: blood sample.

Participants were not allowed to eat or drink anything (except water) during the study sessions. They were also not allowed to watch any television show or talk about anything related to food, or anything that could affect the assessed parameters. They were allowed to read, listen to music, watch TV, use the computer, and walk inside the laboratory.

The protocol was approved by the Human Research Ethics Committee of the University of Brasília, Brazil (nº 050/2012). All volunteers were informed about the protocol of the study and provided written informed consent.

4.3.5 Coffee preparation

The method for prepare the paper drip-filtered coffee (*Coffea arabica L.*) (Brazil) was always performed in the same fashion (30 g of ground coffee and 300 ml of water) with or without sugar (10 g). The coffee was served immediately after the brewing in a dark closed top mug and drank through a black straw. Water (300 ml) with or without sugar (10 g) were served at room temperature.

The caffeine amount of the coffee used in the study was analyzed by high performance liquid chromatography at the Laboratory of Nutritional Biochemistry and Food Chemistry of the Federal University of Rio de Janeiro, Brazil. The caffeinated and decaffeinated coffee provided to the participants had 459 mg and 102 mg of caffeine, respectively.

4.3.6 Clinical Assessments

Body weight was assessed using an electronic platform scale (Plenna, São Paulo, Brazil), with a capacity for 150 kg and precision of 100 g. Height was measured using a stadiometer (Alturaexata, Brazil) fixed to its proper platform with precision of 0.1 cm. BMI was computed based on weight (kg) and height (m^2) (kg/m^2), and classified according to the parameters of the World Health Organization (14). Waist circumference was measured midway between the lowest rib and the iliac crest with a precision of 0.1 cm, and classified according to the parameters of the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (15). Body fat percentage was measured by tetrapolar electrical bioimpedance (RJL – 101 model Quantum II, USA) according to the protocol of Lukaski et al. (1986) (16). Blood pressure was assessed by auscultation with a calibrated aneroid

sphygmomanometer. The measurement was performed 3 times during the initial evaluation by a trained professional according to recommendations by Brazilian Society of Cardiology (2010) (17).

4.3.7 Biochemical measurements

Five milliliters of blood were collected in a red top vacutainer at each draw. After clotting and centrifugation, insulin, glucose, and [1]-13C-glucose concentrations were measured. Insulin and glucose concentration were measured by electrochemiluminescence and the glucose oxidase method, respectively. Sensitivity of the insulin immunoassay was 1.39 pmol/L (within-run CV of 1.9%) and glucose oxidase sensitivity was 0.12 mmol/L (within-run CV of 0.41%). C isotopic composition of the glucose was determined by Gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) on a Sercon 20-22 Isotope Ratio Mass Spectrometer with Orchid combustion/pyrolysis module and Agilent 7890B GC with a combustion furnace operating at 860°C. The ratio of the 44 and 45Th isotopologues of the generated CO2 was determined using the method of Bluck and Coward (2004) (18), and these were converted (19,20) to tracer/tracee ratios using a derived value for pure tracer of 10121G for the glucose derivative used in this work. The data obtained from GC/C/IRMS analyzes were interpreted by the two-compartment minimal model as implemented by Bluck et al. (2006) (12) and validity with euglycaemic clamp (21).

4.3.8 Food intake assessment

Each dinner dietary record was reviewed in the presence of the volunteer in order to ensure its accuracy and completeness. Food portions were converted into grams and the subsequent meal energy intake, macronutrients, fiber consumption, glycemic index and load were analyzed using the Nutrition Data System for Research software (version 2013) (University of Minnesota, USA) with the inclusion of typical Brazilian foods and standardized recipes.

4.3.9 Area under the curve (AUC) calculation

The incremental AUC for glucose and insulin were calculated excluding the values below the baseline values using the GraphPad Prism software, version 6 (GraphPad Software, Inc., USA). Data analyses were conducted considering the

following periods of time: 0 – 60 min (defined as 1h post-absorption period), 60/0 – 115 min (defined as ~2h OGTT response), 60/0 – 225 min (defined as ~4h OGTT response), and 0 – 225 min (defined as the whole study response).

4.3.10 Statistical analyses

Levene test was performed to assess the homogeneity of variance and the Shapiro-Wilk test to determine the normality distribution of the data. One-way ANOVA test were used to examine differences on baseline characteristics, food intake and AUC values, and, when appropriate, Tukey's test was used to post hoc comparisons. In addition, one-way ANOVA with Bonferroni adjustment were used to compare the effects of the tested beverages on Si and Sg parameters. All statistical analyses were performed using the IBM SPSS Statistics software, version 21 (IBM Corporation, USA) with $p < 0.05$ of criterion for statistical significance. Participants data are presented as mean and standard deviation (SD) and results from ODILE test as mean and standard error of the mean (SEM).

4.4 RESULTS

4.4.1 Participants characteristics

Fifteen participants completed the study protocol and their baseline characteristics are summarized in **Table 1**. All participants were habitual coffee consumers (1 – 3 cups/day) and met the fasting blood glucose requirement (4.10 – 5.44 mmol/L) before each experimental session.

4.4.2 Baseline characteristics

There were no differences in body weight ($p = 0.99$), capillary glucose ($p = 0.97$), and number of hours of sleep ($p = 0.66$) at the beginning of each experimental session. Furthermore, no differences were found for food intake (energy, macronutrients and fiber consumption, glycemic index and glycemic load) ($p > 0.08$) at the last meal before each experimental session.

Table 1 - Baseline participants characteristics (n = 15)

Variables	Mean	SD	Range
Age (y)	27	4.4	20 – 35
Body weight (kg)	71.4	5.5	61.1 – 80.9
Body mass index (kg/m²)	23.4	2.1	20.0 – 24.7
Waist circumference (cm)	83.8	4.6	77.0 – 92.0
Body fat (%)	18.4	5.6	8.8 – 24.5
Systolic blood pressure (mmHg)	115.6	6.5	108 – 128
Diastolic blood pressure (mmHg)	74.1	5.1	68 – 84
Fasting blood glucose (mmol/L)	4.9	0.4	4.1 – 5.4
Coffee intake (ml/day)	226.7	108.3	100 – 450
Time of coffee intake (years)	7.5	5.2	2 – 20

4.4.3 Glucose and Insulin response

As expected, the beverages with sugar (CS and WS) increased the post-absorptive glucose and insulin AUC (0 – 60 min) compared with no-sugar groups (CC, DC, W) ($p < 0.001$). In addition, CS and WS showed lower glucose AUC at 60/0 – 135 min and 60/0 – 225 min period compared with CC, DC, W groups ($p < 0.001$). However, in overall analyze (0 – 225 min) there were no significant differences on glucose and insulin AUC between the groups (**Figure 4**). Figure 5 show the glycemic and insulinemic response over the time.

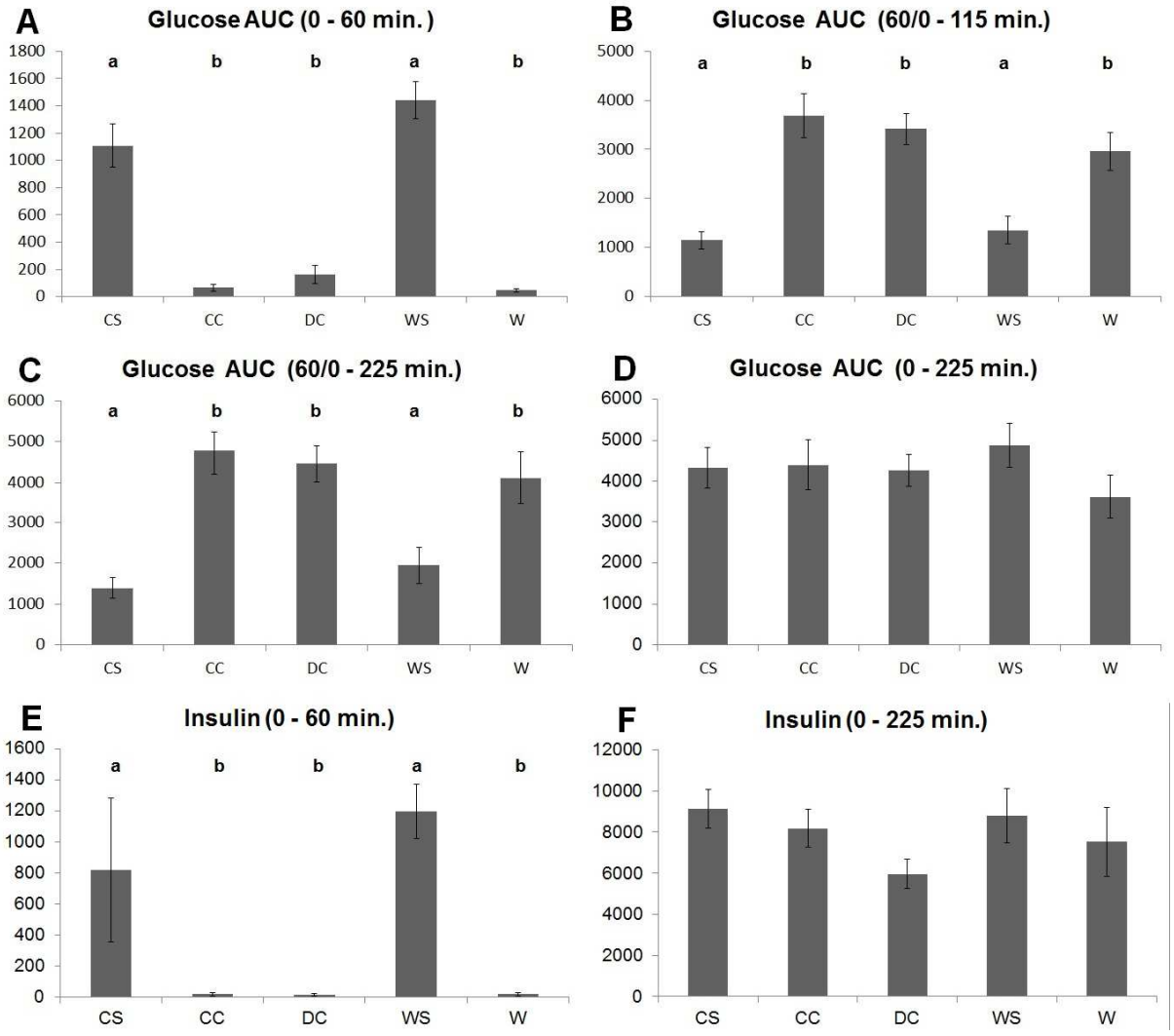

Figure 4. Serum glucose and insulin AUC response. (**A**) Glucose 0 – 60 min AUC (1h post-absorption period), (**B**) Glucose 60/0 – 115 min AUC (~2h OGTT response), (**C**) Glucose 60/0 – 225 min AUC (~4h OGTT response), (**D**) Glucose 0 – 225 min AUC (whole study response), (**E**) Insulin 0 – 60 min AUC (1h post-absorption period), and (**F**) Insulin 0 – 225 min AUC (whole study response). Caffeinated coffee with sugar (**CS**), Caffeinated coffee (**CC**), Decaffeinated coffee (**DC**), Water with sugar (**WS**), and Water (**W**). One-way ANOVA with Tukey's post hoc test were applied to compare the groups. Different letters above the bars indicate statistically significant differences ($p < 0.001$). Data are presented as means with SEM represented by vertical bars ($n = 15$).

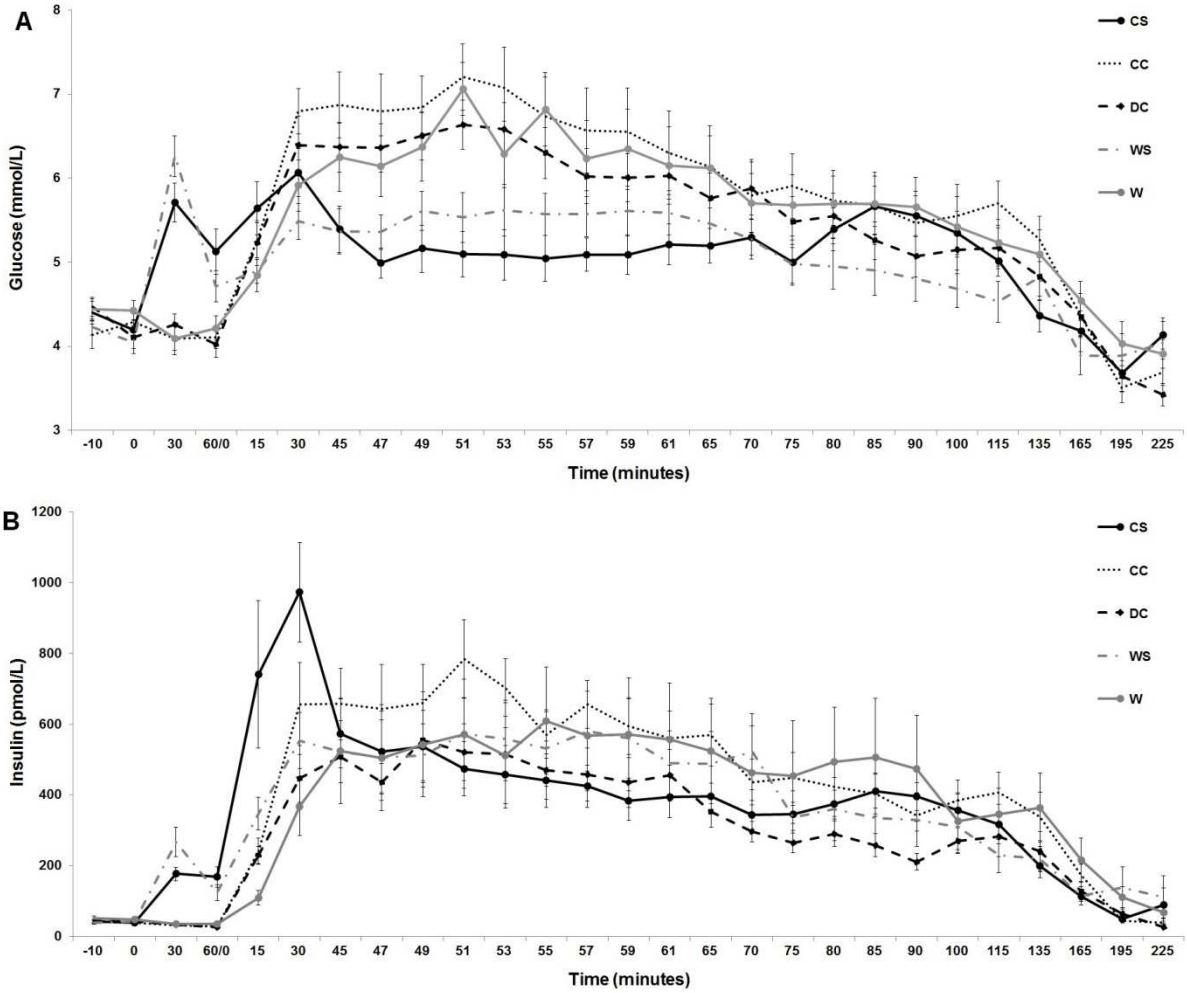

Figure 5. Serum glucose (**A**) and insulin (**B**) concentrations. Caffeinated coffee with sugar (**CS**), Caffeinated coffee (**CC**), Decaffeinated coffee (**DC**), Water with sugar (**WS**), and Water (**W**). All data were interpreted by the two compartment minimal model and analyzed by the one-way ANOVA with Bonferroni adjustment. Data are presented as means with SEM represented by vertical bars (n = 15).

4.4.4 Insulin Sensitivity (Si) and Glucose effectiveness (Sg)

Decaffeinated coffee resulted in 29.2% and 85.5% higher Si compared with caffeinated coffee and water, respectively. In addition, caffeinated coffee showed 15.5% and 60.7% higher Sg compared with decaffeinated coffee and water, respectively. However, there are no significant differences between groups for Si and Sg when caffeinated (with or without sugar) and decaffeinated coffee were consumed 1 hour before the ODILE test (**Table 2**).

Table 2 - Parameters of glucose metabolism derived from ODILE test[1]

	Si ($dm^3.hr^{-1}.pMol^{-1})^2$	**SEM**	**Sg (hr−1)**	**SEM**
Caffeinated coffee with Sugar	1.82	0.27	1.30	0.16
Caffeinated coffee	1.88	0.33	1.27	0.15
Decaffeinated coffee	2.43	0.38	1.10	0.16
Water with sugar	1.43	0.27	0.85	0.09
Water	1.31	0.19	0.79	0.08
P value	0.10		0.17	

[1] All values are present as mean ± SEM.

[2] Expressed as 10^{-2}.

4.5 DISCUSSION

This study is among the first attempts to understand the effects of coffee consumption on glucose metabolism using the ODILE test. The ODILE methodology has some strengths compared to others tests because it is longer and has more times of blood collection, and due the validation against the euglycemic hyperinsulinemic clamp (21). We hypothesized that coffee consumption would reduce postprandial glycemia and insulinemia but the current findings do not supported this theory. This randomized cross-over clinical trial showed that the moderate coffee consumption (2 cups: 300 mL) has no acute effects (5h) on insulin sensitivity and glucose effectiveness in healthy young adult men. Furthermore, the addition of 30g of sugar (2.5 tablespoons) has no additional influence on post-prandial glucose and insulin AUC response in overall analysis. The lack of change on insulin sensitivity may have been mediated by the antagonist effects of the caffeine and bioactive compounds present in coffee on glucose metabolism.

The common habit of sweetening coffee to mask the bitterness had no effects on glucose metabolism over 4 hours when was consumed 1h before a “glucose meal” (75g OGTT). However, 2.5 teaspoons of sugar (30g) acts as a priming in the first phase response (1h post-absorption period) increasing the insulin concentration that reduced the glycemic response at the second phase over 2 and 4 h follow-up ($p < 0.001$). Similar result was found by Loiue et al. (2008) with the addition of 10 g of sugar (sucrose) to the coffee reduced the postprandial glycemic response by ~40% compared with coffee without sugar ($p < 0.001$) (22). However, in the present study

the overall analysis (0 – 225 min) shows no significant effects of coffee consumption on glucose and insulin AUC response.

Previous clinical studies have indicated significant acute increase on postprandial glucose and/or insulin concentrations after caffeinated coffee consumption in different population. In healthy lean subjects, Beaudoin et al. (2011) showed that 2h glucose AUC for caffeinated coffee was elevated by 25% and 13% compared with the water and decaffeinated coffee, respectively ($p < 0.05$) (23); Moisey et al., (2008) observed 147% and 29% higher 2h glucose and insulin AUC for caffeinated coffee compared with decaffeinated coffee ($p < 0.001$ and 0.1, respectively) (24); and Louie et al., (2008) showed increased 2h glucose AUC after caffeinated coffee compared with decaffeinated coffee ($p = 0.02$) (22). Controversially, several trials (25-31) corroborate with the present findings that showed no significant differences for glucose and insulin AUC between the treatments (coffee versus control) in overall analysis (2 or 3h). However, Greenberg et al., (2010) showed significantly lower 3h glucose AUC for decaffeinated coffee than for caffeine (32); and Battram et al., (2006) found 50% lower 2h glucose AUC for decaffeinated coffee compared with water ($p < 0.05$) (33). These acute effects of coffee consumption on glucose metabolism biomarkers are controversial due to post-prandial hyperglycemic and lowering whole-body insulin sensitivity effects of caffeine (34) and the improvement on insulin sensitivity by the coffee bioactive compounds (mainly the chlorogenic acids) (35).

The ODILE test provides rigorous estimates of glucose metabolism parameters (Si and Sg) with the power of tracer methodology under truly physiologically realistic conditions (21). Some trials presented mixed results regarding the effects of coffee consumption on insulin sensitivity (HOMA-IR, Matsuda, and Belfiore indexes). They have some weaknesses compared with the present study due the validation of ODILE against the clamp technique (21). Moisey et al., (2010) showed improvement on insulin sensitivity (Matsuda index) for caffeinated coffee compared with both decaffeinated coffee and water ($p < 0.001$) (28). However, others studies showed no effects of coffee consumption on Matsuda index (23,24,33). In addition, Buscemi et al. (2010) observed reduction on insulin resistance (Homeostatic Model Assessment - HOMA-IR) ($p = 0.02$) for caffeinated coffee compared with water (36); and Greenberg et al., (2010) showed higher insulin sensitivity (Belfiore index) for decaffeinated coffee compared with caffeine (32).

These results are mixed and confirm the findings of our study that there are no consistent significant effects of caffeinated and decaffeinated coffee (with or without sugar) consumption on insulin sensitivity. In this context, the present results are the most effective at the moment. The beneficial effects of coffee did not seem to act in the short-term (hours) on glucose metabolism parameters mainly on insulin sensitivity indices as shown by Reis et al. (2015) (10) which gives a controversial response in the literature. This observation is in contrast to the epidemiological literature which demonstrates reduced T2DM prevalence rates in habitual consumers of coffee (7-9).

The effective measurement of insulin resistance is important in understanding the etiology of T2DM. Currently, there are two accredited methodologies that are considered to give accurate measures of insulin sensitivity: the euglycaemic hyperinsulinaemic clamp and the frequently sampled intravenous glucose tolerance test (FSIVGTT) (37). The FSIVGTT is widely employed, but there are some limitations such as only first phase insulin secretion can be reliably determined and the endogenous insulin response is too small and transitory to be a satisfactory stimulus for peripheral glucose uptake. These objections could be overcome with an oral administration of the glucose load. The drawback of the oral load is that the rate of appearance of glucose in the blood is unknown, and separation of the kinetics of appearance and disappearance is not possible without additional information. The power of this methodology can be enhanced by the addition of stable isotopically labelled tracer to the intravenous glucose bolus. This allows the separation of glucose disposal from endogenous glucose production and also increases the precision of the estimates of the physiological parameters measured. The incorporation of a small bolus dose of labeled glucose near to the maximum hyperglycemia has been proposed by Bluck and colleagues can overcome this problem (12,21). The ODILE test provides rigorous estimates of the parameters of glucose disposal and production under normal physiological conditions using the two-compartment minimal model, is of great interest to both clinicians and epidemiologists interested in the pathology of T2DM. In addition, the protocol was validated against the euglycemic hyperinsulinemic clamp (21).

The current study has some strengths and limitations. The crossover randomized design and the ODILE test interpreted by the two compartment minimal model are some of the study strengths. Furthermore, the sample size was selected according to the power calculations performed for insulin sensitivity parameter, only

men were included in order to eliminate potential sex-specific effects, and the coffee dose was moderate to correspond to a commonly consumed dose. On the other hand, the results cannot be generalized to other population groups, i.e. to diabetics people or non-coffee consumers.

The clinical relevance of the present findings is that there is no need to avoid coffee as the drink choice for healthy people. In conclusion, the findings of this study demonstrate that the consumption of either a caffeinated and decaffeinated coffee with or without sugar has no deleterious or improvement effects on glucose metabolism in healthy men in a short-term period then that expected with the intervene challenge. However further researches, including long-term interventional studies, are needed to fully elucidate the mechanisms behind the coffee effects on reduced risk for T2DM.

4.6 ACKNOWLEDGMENTS

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The authors' responsibilities were as follows: CEGR recruited participants, performed the research, contributed to the laboratory and data analysis, and wrote the manuscript; SW contributed to the laboratory and data analysis (modelling), and revised the manuscript; LJCB contributed to the study design and data analysis (modelling); THMC contributed to the study design and coordination, data analysis, and wrote the manuscript; all authors approved the final manuscript and had access to the full data set. The authors declare no conflict of interest.

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CAPÍTULO 5

5.1 Considerações Finais

A metodologia ODILE apresenta alguns pontos fortes em comparação a outros métodos que avaliam o metabolismo da glicose, pois é uma avaliação mais longa (5 horas) e com mais pontos de coleta de sangue (27 pontos), utiliza a marcação isotópica intravenosa da glicose ([1]-^{13}C-glicose) sendo analisada pelo índice dos modelos mínimos que aumenta a precisão da estimativa de sensibilidade à insulina (Si) e da taxa de captação da glicose (Sg), além de ser uma técnica validada contra o clamp hiperinsulinêmico euglicêmico. Entretanto, nosso experimento utilizando o referido método não mostrou efeitos significativos do consumo de café (cafeinado com ou sem açúcar e descafeinado) nos parâmetros de metabolismo da glicose: Si ($p = 0,13$) e Sg ($p = 0,17$). Já os resultados apresentados pelo artigo de revisão mostram que em curto prazo (horas) o consumo de café cafeinado pode aumentar a área abaixo da curva (AAC) da resposta glicêmica, enquanto que os estudos em longo prazo (semanas) apresentam uma melhora no metabolismo da glicose reduzindo a AAC da glicose e aumentando a resposta insulinêmica. Esses dados sugerem que o café cafeinado pode prejudicar o metabolismo da glicose de forma aguda, mas no longo prazo pode favorecer a redução do risco da diabetes mellitus tipo 2 (DM2). Esses resultados não corroboram completamente os resultados encontrados em estudos epidemiológicos que mostram redução de risco da DM2 decorrente do consumo crônico (anos) de café. O entendimento desse panorama mostra que os efeitos benéficos do consumo de café sobre a DM2 ocorrem devido ao seu consumo crônico e não são demonstrados efeitos consistente em curto (horas) e longo prazo (semanas) sobre o metabolismo da glicose. A tabela 1 compila os principais resultados do consumo de café cafeinado ao longo do tempo (horas, semanas, anos) (tabela 1).

Tabela 1 - Efeito do consumo de café no metabolismo da glicose ao longo do tempo

ODILE	Horas	Semanas	Anos
Sem efeito: Si e Sg	↑ AAC glicose	↓ AAC glicose ↑ AAC insulina	↓ Risco para DM2 (efeito dose resposta)

↓: Diminuição; ↑: Aumento; AAC: área abaixo da curva; Si: Sensibilidade à insulina; Sg: Taxa de captação de glicose; DM2: Diabetes Mellitus tipo 2.

5.2 Limitações do estudo

Inicialmente o estudo previa a utilização da metodologia duplamente marcada, que além da marcação intravenosa com o isótopo da glicose ([1]-^{13}C-glicose) inclui a marcação da glicose oral ([6,6]-$^{2}H_2$-glicose). A inclusão desse segundo marcador isotópico traria uma vantagem adicional, pois seria possível discriminar a contribuição da glicose endógena produzida pelo fígado daquela absorvida pelo intestino aumentando ainda mais a precisão da metodologia ODILE. Além disso, a não realização do grupo 'café descafeinado com açúcar' não permitiu avaliar o efeito do açúcar juntamente com o café sem cafeína o que inviabilizou a comparação par a par dos grupos analisados. Essas limitações ocorreram devido a cortes orçamentários do estudo.

5.3 Conclusões gerais

O consumo de café cafeinado (com e sem açúcar) e descafeinado não exercem efeitos significativos no metabolismo da glicose em curto prazo (5 horas). Já em longo prazo (semanas) é possível observar, dos resultados obtidos dos estudos realizados, uma possível melhora no metabolismo da glicose que pode corroborar com os resultados dos estudos epidemiológicos que mostram o benefício na redução da DM2 decorrente do consumo crônico de café (anos). Esses resultados demonstram a importância de mais estudo clínicos bem delineados e conduzidos que venham a avaliar o efeito do consumo de café em longo prazo no metabolismo da glicose na busca de explicações dos mecanismos por trás da redução desse risco.

5.2 **Apêndices**

5.2.1 Apêndice 1

Universidade de Brasília
Faculdade de Ciências da Saúde
Laboratório de Bioquímica da Nutrição

Café e Metabolismo da Glicose:
ensaio clínico cruzado randomizado com isótopos estáveis

Coordenadora: Profª. Dra. Teresa Helena M. da Costa

Pesquisadores: Prof. Dr. José Dórea, Profª. Dra. Adriana Porto, Profª. Dra. Angélica Amato e Dr. Leslie Bluck

Pós-graduandos: Caio Eduardo Gonçalves Reis (Doutorando) e Cicilia Luíza Rocha dos Santos (Mestrando)

Colaborador: Werte de Sousa Chaves e Leandro Faleiros Garcia

QUESTIONÁRIO DE TRIAGEM

I) Dados pessoais: Data: _____/_____/_____

Nome:_____ **Código:**_____

Endereço: _____

Tel. Residencial: (__)_____Trabalho: (__)_____ Celular: (__)_____

Data de nascimento: _____/_____/_____ Idade: _____ Estado Civil: _____

Escolaridade: _____ Profissão: _____ Ocupação: _____

E-mail: _____

II) História médica

História patológica pregressa:_____

Problemas Gastrointestinais: Sim ( ) Não ( ) Qual(s)? _____

História Familiar de doença metabólica:

Pai: ( ) Obesidade ( ) Diabetes Mellitus ( ) Hipertensão ( ) Dislipidemia

Mãe: ( ) Obesidade ( )Diabetes Mellitus ( )Hipertensão ( ) Dislipidemia

Irmãos: ( ) Obesidade ( ) Diabetes Mellitus ( ) Hipertensão ( ) Dislipidemia
Avós: ( ) Obesidade ( ) Diabetes Mellitus ( ) Hipertensão ( ) Dislipidemia

III) Outras informações

Você tem hábito de tomar café? Sim ( ) Não ( ) Solúvel ( ) Infusão ( )
Há quanto tempo? _____
Açúcar? Sim ( ) Não ( ) Quanto? _____Adoçante? Sim ( ) Não ( ) Quanto? ____
Qual quantidade por dia? _____
Como é o preparo? _____
Consome outras bebidas cafeinadas, como coca-cola ou similares, guaraná em pó, bebidas energéticas, medicamentos com cafeína? Sim ( ) Não ( ) Qual(s)?
_____
Você faz uso de algum remédio ou suplemento alimentar (vitaminas, minerais, proteínas...)? Sim ( ) Não ( ) Qual(s)? _____
Você tem alguma alergia a remédios, alimentos ou outras substâncias?
Sim ( ) Não ( ) Qual(s)? _____
Sintomas:_____
_____
Você fuma ou usa outro tipo de fumo?
Sim ( ) Não ( ) Qual(s)? _____
Quantos cigarros (ou outro tipo de fumo) por dia? _____
Você consome bebida alcoólica? Sim ( ) Não ( ) Qual(s)?
_____
Quantidade por semana: _____
Você pratica exercícios físicos regulares? Sim ( ) Não ( ) Qual(s)?
_____
Tempo por dia: _____
Dias por semana: _____
Você tem distúrbio do sono ou faz uso de medicação para dormir?
Sim ( ) Não ( ) Qual (s)? _____
Você doou sangue nos últimos nos últimos três meses ou planeja doar nos próximos três meses? Sim ( ) Não ( ) Planeja Doar ( )
Seu peso variou em mais de 5 kg nos últimos 3 meses? Sim ( ) Não ( )
Faz dieta ou participa de algum programa de controle de peso? Sim ( ) Não ( )

IV) Avaliação antropométrica, composição corporal, PA e glicemia capilar.

	1	2	3	Média
Peso (kg)				
Estatura (m)			---	
IMC				
Perímetro da Cintura				
Resistência / Reactância	/		/	
Gordura Corporal (%)				
Pressão Sistólica				
Pressão Diastólica				
Glicemia de jejum				

Nome:_____

Código:_____

Data: ____/_____/_____ **Dia da semana**: _____

Recordatório 24h

	ALIMENTOS	QUANTIDADE
Refeição: **Hora:** **Local:**		
Refeição: **Hora:** **Local:**		
Refeição: **Hora:**		

Local:		
Refeição: **Hora:** **Local:**		
Refeição: **Hora:** **Local:**		
Refeição: **Hora:** **Local:**		

Dia fora do padrão alimentar: Não ( ) Sim ( ). Se sim como?

_____

5.2.2 Apêndice 2

Universidade de Brasília
Faculdade de Ciências da Saúde
Laboratório de Bioquímica da Nutrição

Café e Metabolismo da Glicose:

ensaio clínico cruzado randomizado com isótopos estáveis

Coordenadora: Profª. Dra. Teresa Helena Macedo da Costa

Pesquisador Responsável: Caio Eduardo Gonçalves Reis (Doutorando)

ORIENTAÇÕES PARA REALIZAÇÃO DAS AVALIAÇÕES

- Realizar jejum absoluto de 12 horas (não pode água);
- Não beber água nas horas anteriores ao exame;
- Não praticar atividade física nas últimas 12 horas que antecedem o teste;
- Na noite anterior ao teste não consumir refeição rica em carboidratos;
- Urinar 30 minutos antes do teste;
- Não consumir álcool nas 48h anteriores ao teste;
- Não consumir café nas 24h anterior ao teste;

Contato: Caio Eduardo G. Reis

Telefone: (61) 3307.2193 / 8184.6185

5.2.3 Apêndice 3

Universidade de Brasília
Faculdade de Ciências da Saúde
Laboratório de Bioquímica da Nutrição

Café e Metabolismo da Glicose: ensaio clínico cruzado randomizado com isótopos estáveis

Coordenadora: Profª. Dra. Teresa Helena M. da Costa

Pesquisadores: Prof. Dr. José Dórea, Profª. Dra. Adriana Porto, Profª. Dra. Angélica Amato e Dr. Leslie Bluck

Pós-graduandos: Caio Eduardo Gonçalves Reis (Doutorando) e Cicilia Luíza Rocha dos Santos (Mestrando)

Colaborador: Werte de Sousa Chaves e Leandro Faleiros Garcia

FICHA DE ACOMPANHAMENTO DIÁRIO - EXPERIMENTO

Voluntário: _____ **Código:**_____

Data: ____/_____/_____ Hora: _____ Tratamento: _____

Peso: _____ Glicemia: _____ Horas de sono: _____

a) Coleta de Sangue para Hematócrito e Hemoglobina: ( ) SIM ( ) NÃO

b) Medicação em uso ( ) SIM ( ) NÃO Qual: _____

c) Intercorrências ( ) SIM ( ) NÃO Qual: _____

d) Ingeriu bebida alcoólica (24h) ( ) SIM ( ) NÃO Qual/Quant.: _____

e) Praticou atividade física (24h) ( ) SIM ( ) NÃO Qual/Quant.: _____

f) Ingeriu Café / bebida cafeinada (24h) ( ) SIM ( ) NÃO Qual/Quant.: _____

g) Horário da última refeição? _____

h) Recordatório da última refeição?

_____

_____

_____

_____

5.2.4 Apêndice 4

Universidade de Brasília
Faculdade de Ciências da Saúde
Núcleo de Nutrição
Laboratório de Bioquímica da Nutrição

TESTE INTRAVENOSO DE TOLERÂNCIA À GLICOSE (IVGTT)
PROCEDIMENTOS PADRONIZADOS EM CASOS DE EMERGÊNCIA

- **Procedimentos em Situações de Hipoglicemia**

Glicose (mg/dL)	Procedimento
70 – 55	Medir novamente em 5 minutos
54 – 45	Medir novamente em 5 minutos e Comunicar Assistência Médica
44 – 35	Assistência Médica Assintomático: Observar e Medir novamente em 3 minutos Sintomático: Infundir 20 mL de Glicose (50%) e Medir em 3 minutos Repetir a dose de Glicose se os sintomas continuarem ou a glicemia se manter < 45 mg/dL até a recuperação do paciente
Sintomas de Hipoglicemia	Medir novamente a glicemia e Comunicar Assistência Médica

Desobstrução da cânula de acesso venoso

Havendo dificuldade para coleta de sangue durante o IVGTT, o pesquisador deverá:

- Infundir generosamente solução salina;
- Infundir 0,5 mL de Solução Heparinizada;
- Retirar a Solução Heparinizada antes da próxima coleta.

Perda da Consciência

- A perda de consciência pode ocorrer durante o Teste Intravenoso De Tolerância à Glicose. Os primeiros socorros devem ser imediato, com o contato de uma ambulância se necessário.
- A causa mais comum de perda de consciência é o desmaio simples (episódio vasovagal) e a hipoglicemia. O desmaio é mais comum no momento da realização do acesso venoso, mas deve ser considerado durante todo o teste.
- Todo voluntário que estiver sendo submetido ao IVGTT e tiver perda de consciência deve ter a glicemia determinada através de glicosímetro e mantida em níveis de segurança.
- A perda de consciência pode ocorrer também devido a queda da pressão arterial. Isso pode acontecer devido ao grande retirada de sangue levando a redução do volume sanguíneo ou devido ao um episódio vasovagal. Se a pressão arterial continuar baixa deverá ser discutido com um médico a necessidade de infusão de solução salina.
- Há outras várias condições clínicas que podem levar a perda de consciência incluindo arritmia e isquemia cardíaca, comprometimento respiratório, eventos neurológicos. Essas possibilidade deverão ter a assistência médica.

TELEFONES DE EMERGÊNCIA

Serviço de Atendimento Médico de Urgência: 192

Corpo de Bombeiros: 193

Esse documento deve ser seguido pelos pesquisadores que estiverem realizando o Teste Intravenoso De Tolerância à Glicose no voluntário.

5.2.5 Apêndice 5

Universidade de Brasília
Faculdade de Ciências da Saúde
Núcleo de Nutrição
Laboratório de Bioquímica da Nutrição

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Eu, _____,
estou sendo convidado a participar de uma pesquisa do Núcleo de Nutrição da Universidade de Brasília que visa estudar os efeitos do café no risco de desenvolvimento da diabetes mellitus tipo 2. Serão realizados cinco testes no Laboratório de Bioquímica da Nutrição no tal você deverá comparecer pela manhã em jejum de 12 horas em cada dia de teste. Os testes ocorrerão em dias previamente agendados, com intervalo de quatro a seis semanas entre eles.

Em cada dia teste você deverá consumir 300 mL de uma das seguintes bebidas: Café cafeinado com açúcar, Café cafeinado sem açúcar, Café descafeinado sem açúcar e água com e sem açúcar. Uma hora após o consumo da bebida teste você deverá consumir 300 mL de água potável contendo 75g de glicose dissolvidos. Uma hora e 45 minutos após isso, será aplicada na veia do seu antebraço 250mg glicose dissolvida em soro. Uma agulha será inserida na veia do seu outro braço para que 20 amostras de 5 mL e 7 amostras de 4 mL de sangue sejam coletadas durante 6 horas de avaliação, totalizando 128 mL de sangue por dia de visita. Desta forma serão coletados no total 640 mL de sangue ao longo de 5 meses de duração do experimento. Nessas amostras serão medidas as quantidades de glicose e insulina a fim de determinar os efeitos do café nos níveis de glicose no seu sangue.

Todos os procedimentos serão realizados minimizando os riscos a sua saúde, entretanto tonturas, vertigens e queda da pressão arterial podem ocorrer. Para se evitar esses riscos, o procedimento será realizado pela Médica Dra. Adriana Lofrano (CRM/DF: 8.093) que assegurará todos os cuidados necessários a sua saúde. Em caso de necessidades você terá assistência médica no Hospital Universitário de Brasília para situações relacionadas aos procedimentos realizados na pesquisa.

Você receberá todos os seus resultados impressos e por meio eletrônico ou correio, sendo explicados os seus significados e dados os aconselhamentos necessários. Seus dados têm caráter confidencial e serão posteriormente apresentados no meio científico da área de saúde, mantendo em sigilo sua identificação. Você receberá uma ajuda de custo (transporte) no valor de 6,00 (seis) reais e um almoço no Restaurante Universitário da Universidade de Brasília após a participação em cada dia de teste. Além disso, você não terá nenhum tipo de vantagem econômica ou material por participar da pesquisa, além de poder abandonar a pesquisa em qualquer etapa de seu desenvolvimento.

Em caso de dúvida, você pode entrar em contato com o Comitê de Ética em Pesquisa da Universidade de Brasília pelo telefone 3107-1947, pelo e-mail: cepfs@unb.br ou pessoalmente na sala AT 145/34 da Faculdade de Ciências da Saúde da Universidade de Brasília. O Comitê de ética é um órgão da Universidade de Brasília que tem a finalidade de defender os interesses dos sujeitos da pesquisa em sua integridade e dignidade, contribuindo para o desenvolvimento da pesquisa dentro de padrões éticos vigentes.

Esse termo foi elaborado em duas vias e deverá ser assinado por você (Sujeito de pesquisa) e o Pesquisador Principal, sendo uma cópia retida com o pesquisador e a outra imediatamente entregue ao sujeito de pesquisa.

A pesquisa é coordenadora pela Profª. Dra. Teresa Helena Macedo da Costa, com a colaboração dos pesquisadores: Doutorando Caio Eduardo Gonçalves Reis, Profº. Dr. José Garrofe Dórea, Profª. Dra. Adriana Lofrano Alves Porto, Dr. Leslie John Charles Bluck e o Técnico de Laboratório Werte de Souza Chaves.

Tendo qualquer dúvida sobre o estudo, em qualquer momento, entre em contato pelos seguintes telefones e e-mail:

- Profª. Dra. Teresa Helena Macedo da Costa: 3307-2193
- Caio Eduardo Gonçalves Reis: 8184-6185 / 3307-2193

E-mail: pesquisacafeunb@gmail.com / caioedureis@gmail.com

Portanto, eu _____,
estando totalmente ciente sobre a Pesquisa “Café e metabolismo da glicose: ensaio clínico cruzado randomizado com isótopos estáveis” autorizo minha inclusão.

Brasília, _____ de _____ de 201___.

_____

Sujeito de Pesquisa

_____

Pesquisador Principal

5.3 Anexo

5.3.1 Anexo 1

Universidade de Brasília
Faculdade de Ciências da Saúde
Comitê de Ética em Pesquisa – CEP/FS

PROCESSO DE ANÁLISE DE PROJETO DE PESQUISA

Registro do Projeto no CEP: **005/12**

Título do Projeto: "Café e metabolismo da glicose: ensaio clínico cruzado randomizado com isótopos estáveis."
Pesquisadora Responsável: Caio Eduardo Reis
Data de Entrada: 29/02/2012

Com base na Resolução 196/96, do CNS/MS, que regulamenta a ética em pesquisa com seres humanos, o Comitê de Ética em Pesquisa com Seres Humanos da Faculdade de Ciências da Saúde da Universidade de Brasília, após análise dos aspectos éticos e do contexto técnico-científico, resolveu **APROVAR** o projeto **005/12** com o título: "Café e metabolismo da glicose: ensaio clínico cruzado randomizado com isótopos estáveis." analisado na 2ª reunião ordinária realizada no dia 21 de março de 2012.

O pesquisador responsável fica, desde já, notificado da obrigatoriedade da apresentação de um relatório semestral e relatório final sucinto e objetivo sobre o desenvolvimento do Projeto, no prazo de 1 (um) ano a contar da presente data (item VII.13 da Resolução 196/96).

Brasília, 29 de maio de 2012.

Prof. Nelson Monsores
Coordenador do CEP-FS/UnB