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RESEARCH

Cafestol and Kahweol Acutely Reduce Glucose Levels In Subjects With Type 2 Diabetes

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■ Abstract

Objectives: Coffee consumption is inversely associated with development of Type 2 Diabetes (T2D). The bioactive coffee substance cafestol has shown promising preventive effects towards T2D in cell and animal studies. The acute and combined effects of the main diterpenes in coffee, cafestol and kahweol, on glucose metabolism have not previously been investigated in subjects with T2D. **Methods:** In this randomized, double-blinded crossover study, 15 subjects with T2D ingested a cafestol/kahweol mixture or placebo along with a 75-g glucose load during two oral glucose tolerance tests (OGTT). **Results:** Results showed that the cafestol/kahweol mixture compared to placebo increased gastric inhibitory peptide (GIP) after 15-minutes by 19 pmol/l (p=0.055), and reduced glucose by 1.5 mmol/l after 60-minutes (p = 0.007). The area under the curve (AUC) for glucose after 1.5 hours appeared 4% lower after cafestol/kahweol capsule ingestion compared to placebo (p = 0.11), while the 3-hour AUC for GIP tended to be increased by 12% (p=0.16). **Conclusions:** This study is the first to demonstrate that a mixture of cafestol and kahweol acutely increases GIP levels and reduces blood glucose levels during an oral glucose tolerance test in subjects with T2D. Our findings suggest that cafestol and/or kahweol are potential contributors to the inverse association between coffee consumption and development of T2D. This indicates, that a cafestol/kahweol mixture may be used in the prevention of T2D in the future.

Keywords: Cafestol, Kahweol, Coffee, Glucose, Type 2 Diabetes, Human, Randomized, Crossover, Placebo-controlled.

1. Introduction

According to the International Diabetes Federation, 537 million adults were living with diabetes in 2021 and the number is projected to increase to 783 million adults by 2045 [1]. Most patients live in low- or middle-income countries and lack access to the costly and new pharmaceutical glucose lowering innovations that have emerged over the last decade. Treating and managing the disease is challenging, and inadequate glycemic control can result in serious complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy [2]. Apart from effective and affordable treatment, disease prevention is critical in combating the steadily increasing prevalence and its implications. Diet and lifestyle interventions can prevent or delay the development of type 2 diabetes (T2D) [3]. Coffee consumption has been associated with a reduced risk of developing T2D; consumption of 3-4 cups of coffee daily is associated with a 30% lower relative risk of developing T2D compared to consumption of only one cup or no coffee at all [4]. Furthermore, decaffeinated coffee shows the same positive associations as coffee containing caffeine [4, 5].

The question arises what are the potential antidiabetic substances in coffee? The composition of bioactive substances in coffee depends on various factors such as the species of coffee beans, the country of origin, the soil mineral composition, altitude, coffee bean harvest, processing and roasting methods, and brewing technique. Most human trials investigating the potential benefits of coffee have utilized freeze-dried instant coffee with minimal amounts of the bioactive compounds diterpenes cafestol and kahweol [6-8]. Till now no definitive causal explanation of the preventive effects of coffee on T2D has been established and the underlying mechanisms of action of coffee remain unclear. The disparity in results between interventional and observational studies on coffee consumption and T2D may be attributed to various factors including inadequate treatment duration, low dosages, or the absence of suggested glucose-lowering bioactive substances such as cafestol and kahweol. Cafestol and kahweol are lipophilic diterpenes that naturally occur in coffee beans. While both compounds share structural similarities, kahweol is distinguished by an additional double bond and a comparatively lower melting point. They are mainly present in unfiltered coffee brews such as French Press, Greek, Turkish or Scandinavian boiled coffee. We have previously demonstrated that cafestol has an acute stimulatory effect on insulin secretion from rat insulinoma

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INS-1E cells and increases glucose uptake in human skeletal muscle cells *in vitro* [9]. Subsequently, we found that a 10-week intervention with pure cafestol in KKAy-mice, a T2D animal model, leads to a reduction in blood glucose levels and a glucose-dependent increase in insulin secretion from islets of Langerhans [10]. Kahweol, another coffee diterpene, has been found to reduce lipid accumulation and suppress adipogenesis-related genes in a 3T3-L1 adipocyte model through activation of adenosine 5'-monophosphate-activated protein kinase (AMPK) [11]. Furthermore, kahweol increases glucose uptake in a 3T3-L1 cell line and decrease blood glucose levels in C57BL/6 mice [11].

There have been relatively few studies on the effects of cafestol or kahweol in humans. Most human studies involving these compounds have utilized coffee oils or unfiltered coffee brews, rather than pure cafestol or kahweol [12-15]. The primary focus of these studies has been the impact of cafestol and kahweol on serum lipids [16]. Recently, we conducted a study on the effects of oral administration of a mixture of cafestol (86%) and kahweol (14%) on glucose metabolism in humans [17]. A combination of cafestol and kahweol, purified from spent coffee grounds, was used as there was no pure cafestol commercially available. The study aimed to compare the effects of placebo and different dosages of cafestol on glucose, insulin, and incretin hormone responses in non-diabetic, healthy adults with increased waist circumference and an assumed elevated risk of developing T2D [17]. Although this study did not show convincing effects on glucose, insulin, or incretin hormones in the total number of participants, a subgroup with impaired glucose tolerance and/or impaired fasting glucose showed glucose-lowering effects and increased gastric inhibitory peptide (GIP) levels during an oral glucose tolerance test (OGTT) after consuming 12 mg cafestol and 2 mg kahweol. Physiologically, GIP is released from intestinal K-cells as a response to glucose and fat intake. GIP acts directly on GIP receptors expressed by pancreatic beta cells and stimulates insulin secretion [18]. It is noteworthy that this effect of GIP is lower in subjects with T2D than in healthy subjects [19]. We aimed to examine the acute effects of a mixture of cafestol and kahweol in subjects with T2D. The present study tested a 50%/50% cafestol/kahweol mixture reflecting the composition of diterpenes in coffee. Consequently, we looked at the acute effects of a mixture of cafestol and kahweol on glucose metabolism in subjects with T2D using a double-blinded, placebo-controlled crossover design with two 75 g OGTTs being administered one week apart. We hypothesize that the intake of the mixture of cafestol and kahweol will increase GIP-secretion and cause a reduction in the glycemic response during an OGTT compared to placebo, and that the mixture of cafestol and kahweol concomitantly will improve the insulin secretory capacity during the OGTT.

2. MATERIALS AND METHODS

2.1. Study Design

The trial was an acute, randomized, double-blind placebo-controlled crossover study in participants with T2D. The subjects participated in two study days with OGTTs and approximately one week-washout between the two visits. The participants were not allowed to drink coffee, tea, or alcohol for two days prior to the visits, nor were they allowed to conduct strenuous exercise. Glucose lowering drugs or other T2D-related medication were paused on the day before- and on both study days. Participants in treatment with weekly Glucagon-like peptide-1 receptor agonist injections conducted study days 5-6 days after their last injection. After an overnight fast, participants arrived at the research facility at 7AM, had a peripheral venous catheter placed retrogradely into the median cubital vein in the antecubital fossa, and had blood samples drawn at time points -15, 0, 15, 30, 60, 90, 120 and 180 minutes. At time point 0 minutes, the participants ingested either a cafestol/kahweol- or placebo capsule, and immediately hereafter drank a premade solution of 75 g glucose dissolved in 225 ml water. The next study day, the participants underwent the same experiment, but this time with the opposite capsule (intervention/placebo).

2.2. Participants

Participants were recruited from responders to posts at Aarhus University Hospital social media platforms and Danish participant-recruitment webpages. The inclusion criteria were age between 18 and 80 years, T2D-diagnosis by Danish guidelines and HbA1c > 48 mmol/l unless successfully treated with glucose lowering drugs and/or diet/exercise intervention. Exclusion criteria were insulin treatment, pregnancy, planned pregnancy, breastfeeding or significant comorbidity disabling the participant from completing visits at the research site. The study was conducted at Steno Diabetes Center Aarhus at Aarhus University Hospital, Denmark.

2.3. Interventions

Intervention capsules were produced by Kaffe Bueno Aps, Søborg, Denmark, who purified spent coffee grounds into a mixture of 50% cafestol and 50% kahweol. The mixture was dissolved in sunflower oil and sealed in starch capsules. The intervention capsules were filled with 24 mg cafestol and kahweol-rich compound (12 mg cafestol and 12 mg kahweol). The placebo capsules contained sunflower oil only.

2.4. Materials

The blood samples were collected into tubes coated with dipotassium ethylenediaminetetraacetic acid (K2EDTA) from Becton Dickinson, New Jersey, U.S.A. The tubes were placed on ice until centrifuged for 10 minutes at 4˚C with a relative centrifugal force of 2985. After centrifugation, the supernatant was transferred to tubes and frozen at -80˚C. All samples were batch-analyzed at the end of the study. Glucose was analyzed using Roche Diagnostics COBAS C 111 analyzer (Roche Diagnostics International AG, Rotkreuz, Switzerland). Insulin and GIP was analyzed using Mercodia Insulin ELISA plates (Mercodia, Uppsala, Sweden), and PerkinElmer Victor 3 1420 Multilabel counter (PerkinElmer Massachusetts, U.S.A.).

2.5. Outcomes

Primary outcome was change in area under the curve (AUC) for glucose during the OGTT. Secondary outcomes

were change in AUC for insulin and GIP during the OGTT.

2.6. Randomization and Blinding

The participants were randomized to have cafestol/kahweol capsule on the first study day and placebo capsule on the second study day or vice versa through virtual dice rolls by a trusted third party. The dice result corresponded to color-coded capsule containers, from which independent laboratory technicians dispensed capsules for each participant on each study day, ensuring that investigator, laboratory technicians and study participants were all blinded from knowing which capsule was administered at the study day. The investigator was unblinded after blood sample analysis and initial data analysis was complete.

2.7. Statistical Methods

All statistical analyses were performed in R using R-Studio (RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, Massachusetts, U.S.A. URL http://www.rstudio.com/.)

Glucose, insulin and GIP levels were compared at each time point after both treatments (cafestol/kahweol and placebo) using two-way repeated measures ANOVA and pairwise t-tests between treatments. Area under the curve (AUC) denoted total AUC and all AUC values were calculated individually for each participant on both study days separately using the auc()-function from the *MESS* package, with minutes 0-90 (1.5-hour) and 0-180 (3-hour) as x and glucose and insulin levels as y. The cafestol/kahweol AUCs and placebo AUCs were compared using paired t-tests. Normal distribution was assessed using the Shapiro-Wilks test ($p<0.05$ considered not normally distributed) for both glucose and insulin blood samples and calculated AUCs. Normal distribution is to be presumed unless stated otherwise. In the case of a skewed distribution, data were also compared using Wilcoxon signed rank exact tests and after log-transformation. Log transformation ensured normally distributed data but did not substantially alter the ANOVAs or the pairwise t-tests. Data points were considered extreme outliers if they fell 3 interquartile ranges below the lower quartile (Q1) or 3 interquartile ranges above the upper quartile (Q3). In case of extreme outliers, data were re-analyzed with the outliers excluded. The ANOVAs or the pairwise t-tests results were not altered substantially by this, and therefore all data are displayed without exclusions. Prior to the study, a power calculation was performed. To detect a significant 10% reduction in AUC for glucose (within subject SD ±9% of anticipated mean) with a power of 80% and a two-sided 5% significance level, we determined that a sample size of 15 participants would be necessary. A total of 16 participants were included to accommodate one potential dropout. The effect of baseline characteristics on AUCs was evaluated using a linear regression model.

3. RESULTS

3.1. Participants and Recruitment

Twenty-five persons contacted us after having seen the study advertised or heard about it from friends, family, or a colleague. Nineteen potential participants met all inclusion criteria and no exclusion criteria. After having read informational material and had sufficient time to decide, 16 participants agreed to be enrolled in the study and signed informed consent forms. All 16 participants participated in both study days. After initial data analysis, it was obvious that one participant had not been fasting at their first study day, due to -15 and 0-minute GIP levels severalfold above normal fasting GIP ranges before capsule and glucose load [20]. This participant was excluded from the study.

3.2. Baseline Data

All remaining 15 participants with T2D received treatment with metformin. Furthermore, 10 participants also received other antidiabetic medications. Additional baseline data is provided in Table 1.

Table 1: Baseline Data.

	Male (N=8)	**Female (N=7)**	**Total (N=15)**
Age (years)			
Mean	65	64	65
Range	42 - 77	55 - 73	42 - 77
SD	12	6	9
Weight (kg)			
Mean	82	75	79
Range	52 - 105	63 - 86	52 - 105
SD	17	7	14
Height (cm)			
Mean	177	164	171
Range	146 - 188	155 - 172	146 - 188
SD	14	6	12
Body Mass Index - (kg/m²)			
Mean	25.9	28.0	26.9
Range	21.7 - 31.0	24.0 - 32.0	21.7 - 32.0
SD	3.2	3.2	3.3
Waist Circumference (cm)			
Mean	94	96	95
Range	82 - 110	81 - 116	81 - 116
SD	11	12	11
HbA1c (mmol/mol)			
Mean	51.1	46.3	48.9
Range	40.0 - 56.0	39.0 - 58.0	39.0 - 58.0
SD	5.9	6.8	6.6
HOMA-IR			
Mean	3.7	4.3	4.0
Range	0.9 – 5.9	1.8 - 8.0	0.9 – 8.0
SD	1.8	2.4	2.1
Hours of Workout Pr. Week			
0	3 (38%)	2 (29%)	5 (33%)
0-2 hours	1 (12%)	1 (14%)	2 (13%)
2-4 hours	2 (25%)	3 (43%)	5 (33%)
4-8 hours	2 (25%)	1 (14%)	3 (20%)
Metformin Treatment (per day)			
500 mg			1 (7%)
1000 mg			8 (53%)
2000 mg			6 (40%)
Additional Diabetes Treatment			
Semaglutide			4 (27%)
Empagliflozin			1 (7%)
Dapagliflozin			1 (7%)
Empagliflozin + Semaglutide			2 (13%)
Empagliflozin + Sitagliptin			2 (13%)

HOMA-IR (Homeostatic Model Assessment for Insulin Resistance)

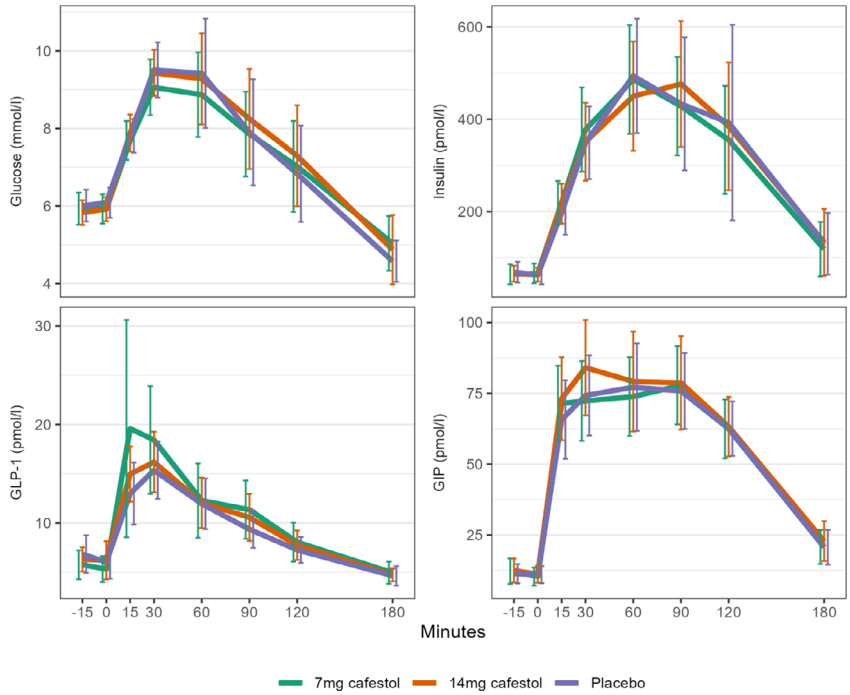

Figure 1: Mean Curves for the Impact of Ingestion of 7 mg Cafestol, 14 mg Cafestol or Placebo at Time Point 0 Minutes on Glucose, Insulin, GLP-1 and GIP Plasma Concentrations During an Oral 75 g Glucose Tolerance Test in 15 Non-diabetic Subjects with Abdominal Obesity. Error Bars Represent 95% CI.

3.3. Outcomes

All glucose AUCs were normally distributed except for the 1.5-hour AUC on the cafestol/kahweol day. The 1.5-hour AUC for glucose was 4% lower on the day when participants consumed cafestol/kahweol compared to the placebo day (-48 mmol/L x 90 min; 95% CI -108-13 mmol/L x 90 min, paired t-test p=0.11, Wilcoxon signed rank exact test p=0.14, paired t-test on log-transformed data p=0.08). The 3-hour AUC for GIP was increased by 12% on cafestol/kahweol day compared to placebo (1,078 pmol/l x 180 min, CI -463 – 2,620 pmol/l x 180 min, paired t-test p=0.16). Insulin levels and AUCs were not normally distributed, and there were no significant differences in 1.5-hour AUCs and 3-hour AUCs for insulin during OGTTs between the cafestol/kahweol day and the placebo day. Remaining AUC results are provided in Table 2.

Table 2: 1.5- and 3-hour Area Under the Curves for Glucose and Insulin.

	Mean	SD	Δ-mean	95% CI	T-test p-value	Wilcoxon p-value
Glucose						
1.5-hour AUC (mmol / l x 90 min)						
Placebo	1,288	243				
Cafestol/Kahweol	1,240	261	-48	-108 - 13	0.11	0.14
3-hour AUC (mmol / l x 180 min)						
Placebo	2,463	584				
Cafestol/Kahweol	2,407	572	-55	-191 - 80	0.40	
Insulin						
1.5-hour AUC (pmol / l x 90 min)						
Placebo	21,466	16,777				
Cafestol/Kahweol	22,820	19,121	1354	-3,364 - 6,082	0.54	0.36
3-hour AUC (pmol / l x 180 min)						
Placebo	60,681	61,232				
Cafestol/Kahweol	56,968	53,749	-3713	-13,939 - 6,514	0.45	0.93
GIP						
1.5-hour AUC (pmol / l x 90 min)						
Placebo	5,507	2,376				
Cafestol/Kahweol	6,086	3,016	580	-451 - 1,610	0.25	
3-hour AUC (pmol / l x 180 min)						
Placebo	9,284	4,037				
Cafestol/Kahweol	10,363	4,920	1,078	-463 - 2,620	0.16	

Mean 1.5- and 3-hour area under the curves (AUC) for glucose and insulin during oral glucose tolerance tests (OGTT) on placebo-day and cafestol/kahweol day. Standard deviation (SD), mean difference between placebo day and cafestol days (Δ-mean), 95% confidence interval for mean difference (95%CI), p-value from paired t-test with placebo, p-value from Wilcoxon signed rank exact test with placebo.

At the 60-minute time point, the mean glucose level was reduced by 1.5 mmol/L on the cafestol/kahweol day compared to the placebo day (95% CI -2.4 - -0.5 mmol/L, paired t-test p=0.007, Wilcoxon signed rank exact test p=0.006). Glucose levels were not normally distributed on either the placebo day or the cafestol/kahweol day at this time point. At the 15-minute time point, the mean GIP level was increased by 19.4 pmol/l on the cafestol/kahweol day compared to placebo day (95% CI -0.5 – 39.3 pmol/l, paired t-test p=0.055). There was no effect of the cafestol/kahweol capsule on glucose, insulin or GIP levels at any other time point.

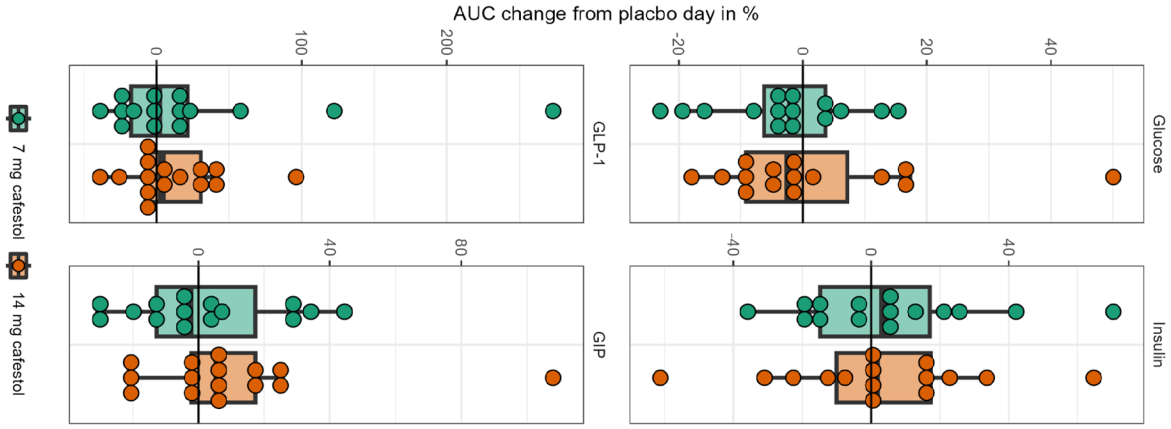

Figure 2: Changes in each Participant's 1.5-hour Area Under the Curve from Placebo (0%) to 7 mg Cafestol and 14 mg Cafestol Intervention, Respectively. Boxes Represent Inter-quartile Ranges. Dots Represent each Participant (N=15).

3.4. Ancillary Analysis

The effects of cafestol/kahweol vs placebo combined with baseline variables on 1.5-hour AUC and 3-hour AUC for glucose, insulin and GIP were evaluated using a linear regression model. The variables analyzed were HbA1c (mmol/mol), BMI (kg/m2), age (years), sex (female/male), workout (0, 0-2, 2-4, 4-8 hours pr. week), metformin dose (500+1000mg daily vs 2000mg daily) and daily habitual coffee cup intake. The 1.5-hour and 3-hour AUC for glucose was increased with higher HbA1c ($\beta = 16$, $p = 0.017$ and $\beta = 49$, $p < 0.001$, respectively). The 1.5-hour AUC for glucose was reduced with higher daily habitual intake of coffee cups ($\beta = -64$, $p < 0.001$). The 1.5-hour AUC for insulin was increased with higher BMI ($\beta = 2{,}483$, $p = 0.009$) and higher HOMA-IR ($\beta = 1{,}035$, $p < 0.0001$) and reduced with higher HbA1c ($\beta = -1{,}191$ $p = 0.012$) and more weekly workout (0-2 hours $\beta = -24{,}467$, $p = 0.009$, 2-4 hours $\beta = -13{,}920$, $p = 0.049$ and 4-8 hours $\beta = -23{,}011$, $p = 0.005$). Similarly, the 3-hour AUC for insulin was also increased with higher BMI and higher HOMA-IR and reduced with higher HbA1c and in workout groups. The 3-hour AUC for GIP was increased in subjects in treatment with 2,000 mg metformin ($\beta = 4{,}119$, $p = 0.005$) and reduced with higher HbA1c ($\beta = -333$, $p = 0.004$). No baseline variable modified the effect of the intervention capsule vs. placebo significantly.

3.5. Harms

No participants experienced any form of discomfort during or after study days.

4. DISCUSSION

To our knowledge, this is the first study on the acute effects on glucose metabolism of a mixture of cafestol and kahweol in humans with T2D. In the present study, we found that intake of cafestol/kahweol-capsule with a glucose load acutely increases the 15-minute GIP level by 19 pmol/l, and subsequently reduces the 60-minute plasma glucose by 1.5 mmol/l, compared to placebo. We also found that the 1.5-hour AUC for glucose appeared to be 4% lower, and the 3-hour AUC for GIP appeared to be 12% increased, after ingestion of the cafestol/kahweol capsule compared to the placebo capsule. These findings are coherent with our previous findings [17], where we found that an impaired glucose tolerance- and/or impaired fasting glucose subgroup of participants with increased waist circumference tended to have a lower 1.5-hour AUC for glucose and higher AUC for GIP during an OGTT with 12 mg cafestol and only 2 mg kahweol ingested with the glucose load. Most participants were in adequate antidiabetic treatment and displayed satisfying glycemic control, which may explain why there were only discrete and statistically insignificant improvements in glucose AUC.

We judge the 1.5 mmol/l plasma glucose level reduction at time point 60 minutes after the glucose load compared to placebo as of clinical relevance. The present study is the first study to show time-specific glucose lowering and GIP increasing effects of the mixture of cafestol and kahweol in humans. Our previous acute study on cafestol/kahweol in healthy, non-diabetic subjects with abdominal obesity found a tendency towards lower 60-minute glucose levels after 86%/14% cafestol/kahweol ingestion in a subgroup of participants with impaired fasting glucose and/or impaired glucose tolerance, however, not reaching statistical significance. The difference in results, specifically the clear 60-minute glucose lowering and 15-minute GIP increase in response to cafestol/kahweol in the present study, but not in our previous study, may be explained by two factors.

Firstly, the subjects with T2D in the present study had a greater potential for glycemic improvement compared to normoglycemic subjects. Secondly, there may be a beneficial synergistic effect of the combination of kahweol and cafestol compared to cafestol at the dosage of 6 mg or 12 mg and only a negligible kahweol dosage (1 or 2 mg). Thus, the combined larger total diterpene dosage used in this study (12 mg cafestol + 12 mg kahweol) may possess a potentially more effective glucose lowering impact.

The ingestion of the cafestol/kahweol mixture compared to placebo shows interesting differences the AUC for glucose; the 1.5-hour glucose AUC appears to be 4% lower after the mixture, whereas the 3-hour glucose AUC only appears 2% lower (Figure 2). Figure 1 suggests that the differences between the placebo and cafestol/kahweol curves are most prominent in the 30- to 90-minute interval, while they appear to be indistinguishable in the final 1.5 hours of the OGTT. This may reflect the bioavailability of the cafestol/kahweol capsules, i.e., suggesting that their absorption may take about 30 minutes and have an effect of approximately one hour. The GIP levels, however, suggest that the cafestol/kahweol mixture has an impact as early as 15 minutes after OGTT start and intake of diterpene mixture. Incretin hormones such as GIP and glucagon-like peptide 1 (GLP-1) are secreted within minutes of nutrient ingestion [18] and our results could indicate that the cafestol/kahweol mixture enhances the GIP response. GIP is known to directly increase the insulin secretion from beta cells [18]. The enhanced GIP secretion may contribute to the lower glucose levels in response to the cafestol/kahweol ingestion. Additional studies are needed to explore the mechanisms of action along with measures of cafestol/kahweol uptake and circulating levels after ingestion. Unfortunately, we did not have access to analytical methods allowing measurements of cafestol or kahweol levels in blood samples. Based on a previous study in subjects with ileostomy, it is presumed that approximately 70% of the cafestol is absorbed in the small intestines [21].

Although the 1.5-hour insulin AUC after cafestol/kahweol mixture ingestion appeared to be higher and the 3-hour insulin AUC lower than placebo, the statistical uncertainties were too great to draw definitive conclusions. The uncertainties could be due to large variance in the OGTT, small sample size, low dosage, or insufficient time for cafestol/kahweol absorption before the glucose load. We cannot exclude that the cafestol/kahweol mixture does not have acute effects on insulin secretion. However, if the insulin curve after kahweol/cafestol intake actually is higher in the first half of the OGTT and lower in the second, this may reflect a more immediate response but with lower amplitude (Figure 1). We have previously shown *in vitro* that cafestol acutely increases insulin stimulated glucose uptake in human skeletal muscle cells [9].

In our auxiliary analysis examining the effects of baseline characteristics on AUCs, several interesting correlations emerged. Higher HbA1c levels were positively correlated with a higher glucose AUC, which may reflect a more advanced disease status. High coffee consumption was associated with a smaller glucose AUC indicating a favorable effect of coffee on the glucose tolerance. This corroborates the epidemiological data showing an inverse association between coffee intake and risk of developing T2D [4, 5]. Higher BMI and HOMA-IR were associated with a higher insulin AUC, probably reflecting increased insulin resistance. In contrast, higher HbA1c was associated with lower insulin AUC possibly due to an insufficient insulin secretory capacity. Participants who exercised had a lower insulin AUC than participants who did not exercise at all. Higher HbA1c was inversely associated with GIP-levels, possibly reflecting that participants with higher incretin response have better glycemic control, although causal conclusions cannot be drawn from the findings in this study design. A 2,000 mg metformin dosage was associated with higher GIP levels than 1000 mg and 500 mg dosages combined, which is interesting as metformin is known to increase incretin hormone GLP-1 levels, but not GIP levels [22].

To ensure participant safety and to mimic the intake of diterpenes from regular to high unfiltered coffee consumption, we adopted a conservative approach by administering a very small dosage of cafestol and kahweol. This dosage is equivalent to consuming 3-4 cups of unfiltered coffee (such as boiled, Turkish, Greek, or French press). For instance, a 150 ml cup of French press coffee typically contains 3.5 mg cafestol and 4.4 mg kahweol [23]. Our current study investigated the effects of a cafestol dosage of 12 mg, which is consistent with the high-cafestol dosage used in the comparable study we previously conducted in non-diabetic subjects with increased waist circumference. However, we increased the kahweol dosage to 12 mg, which is six times greater than the highest dosage (2 mg) used in our previous study, to reflect the composition of diterpenes more accurately in unfiltered coffee. A limitation of this study is that we cannot differentiate if the beneficial effects on glucose tolerance and increased GIP secretion stems from cafestol, kahweol or the combination of the two. Although cafestol and kahweol share similar molecular structures, our *in vitro* study surprisingly did not reveal any effects of oxokahweol on beta cell insulin secretion [9]. Nevertheless, future studies examining the potential health benefits of coffee should consider the total diterpene dosage and composition, while also acknowledging the potential positive effects of other compounds present in coffee. Presently, pure diterpene substances are not commercially available. The cafestol and kahweol used in this study were purified from spent coffee grounds, which are typically discarded but can be recycled in various ways. While the purification and production costs are currently high on a small scale, we anticipate that they will not pose a significant challenge if these substances prove to be promising therapeutic options.

In conclusion, the present study shows that a mixture of cafestol/kahweol can increase GIP levels 15 minutes after and reduce blood glucose levels 60 minutes after a glucose load. The study also shows that the cafestol/kahweol mixture appears to reduce 1.5-hour AUC for glucose and increase

3-hour AUC for GIP during an OGTT in subjects with T2D. These results and our previous studies, showing lowered glucose and increased GIP levels after cafestol/kahweol ingestion, raise the question if increased GIP secretion is the causal explanation for the lowered glucose levels. Further studies are needed to clarify the potential mechanism of action, to determine how much cafestol and kahweol independently contribute to the glucose lowering effects in subjects with T2D, and if there is a beneficial additive effect of the combination. Importantly, additional studies are needed to clarify the longer-term metabolic effects of cafestol and/or kahweol interventions.

4.1. Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

4.2. Acknowledgment

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4.4. Notes

The study is registered at ClinicalTrials.gov with identifier NCT04908904 and is approved by The Central Denmark Region Committees on Health Research Ethics, Viborg, Denmark with approval Number: 1-10-72-307-20.

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