# Decaffeinated coffee improves insulin sensitivity in healthy men

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## Abstract

Epidemiological studies have found coffee consumption is associated with a lower risk for type 2 diabetes mellitus, but the underlying mechanisms remain unclear. Thus, the aim of this randomised, cross-over single-blind study was to investigate the effects of regular coffee, regular coffee with sugar and decaffeinated coffee consumption on glucose metabolism and incretin hormones. Seventeen healthy men participated in five trials each, during which they consumed coffee (decaffeinated, regular (containing caffeine) or regular with sugar) or water (with or without sugar). After 1 h of each intervention, they received an oral glucose tolerance test with one intravenous dose of [1-$^{13}$C]glucose. The Oral Dose Intravenous Label Experiment was applied and glucose and insulin levels were interpreted using a stable isotope two-compartment minimal model. A mixed-model procedure (PROC MIXED), with subject as random effect and time as repeated measure, was used to compare the effects of the beverages on glucose metabolism and incretin parameters (glucose-dependent insulinotropic peptide (GIP)) and glucagon-like peptide-1 (GLP-1)). Insulin sensitivity was higher with decaffeinated coffee than with water ($P < 0.05$). Regular coffee with sugar did not significantly affect glucose, insulin, C-peptide and incretin hormones, compared with water with sugar. Glucose, insulin, C-peptide, GLP-1 and GIP levels were not statistically different after regular and decaffeinated coffee compared with water. Our findings demonstrated that the consumption of decaffeinated coffee improves insulin sensitivity without changing incretin hormones levels. There was no short-term adverse effect on glucose homoeostasis, after an oral glucose challenge, attributable to the consumption of regular coffee with sugar.

**Keywords: Glucose: Stable isotopes: Insulin sensitivity assessment: Intestinal cytokines**

Coffee is one of the most popular beverages worldwide, and the investigation of the association between coffee consumption and incidence of various diseases has important public health implications. A growing body of evidence from epidemiological studies suggests that coffee consumption is beneficial and inversely associated with the risk of developing chronic diseases[1–3]. Recently, a number of prospective studies reported a negative association between high coffee consumption (>3 cups/d) and risk of type 2 diabetes mellitus (T2DM)[4–6]. In one study, participants who drank three to four cups of coffee per day had an approximately 25 % lower risk of developing T2DM than those who did not drink coffee, or drank less than two cups per day[5]. Moreover, a recent meta-analysis reported that every two-cup increase in regular or decaffeinated coffee intake per day correlated with, respectively, 12 and 11 % decreases in T2DM incidence[6]. Coffee is a mixture of many components, including vitamins, minerals and bioactive compounds (caffeine, chlorogenic acids (CGA),

trigonelline and diterpenes, among others), which may have different effects on glucose metabolism[7–9]. Thus, the protective effects of coffee on T2DM may involve different mechanisms such as those affecting intestinal glucose absorption, glucose metabolism, and antioxidant and inflammatory activities[10].

Few studies have addressed the mechanisms underlying the effects of coffee on T2DM risk[1,11]. Their results indicated that coffee (regular or decaffeinated) decreases the postprandial glycaemic response compared with caffeine alone or water, with a neutral or reduced effect in serum insulin levels and improved insulin sensitivity (Si) index[1,11]. This suggests that the effects of coffee on glucose homoeostasis may depend on or are balanced by its non-caffeine constituents, such as CGA or trigonelline[11,12]. In Brazil and other countries, regular coffee is mainly consumed with sugar, but the effect of this coffee preparation is rarely examined and its impact on glycaemic response is not well described[13]. Accordingly, the aim of this

study was to investigate the effects of consuming regular (containing caffeine) and decaffeinated coffee, and study the effect of regular coffee with sugar on glucose metabolism parameters and incretin hormones in healthy men. We thus used a stable isotope minimal model protocol with oral glucose dose – Oral Dose Intravenous Label Experiment (ODILE). The ODILE has been previously validated against the euglycaemic hyperinsulinaemic clamp[14], providing rigorous estimates of glucose metabolism under realistic physiological conditions. Therefore, this study tested two hypotheses: (a) whether regular and decaffeinated coffee improves Si and increases incretin levels, and (b) whether adding sugar to regular coffee imposes a high response of glycaemia and insulinaemia.

## Methods

### Participants

Study subjects were recruited through public advertisements in the University of Brasilia, Brazil from February 2013 to June 2014. Subjects were all male, aged 18–40 years, with BMI between 18·5 and 25·0 kg/m², habitual breakfast consumers ($\geq 418$ kJ ($\geq 100$ kcal) ingested within 2 h of waking up $\geq 4$ d/week) and habitual coffee consumers ($\geq 100$ ml at least five times/week). They had limited body weight fluctuation in the past 3 months (<5 kg), no self-reported sleep disorders, and were not taking medications. Subjects were excluded if they suffered from diabetes mellitus, cardiovascular diseases, hypothyroidism, anaemia and clotting disorders, or had a history of fainting and/or convulsions, smoking or blood donation in the last 3 months, or a total consumption of more than 4·5 cups of coffee per d[15].

Literature containing the information we needed to carry out a sample size calculation[14,16] was scarce. Information about Si and the expected effect size that we wanted to detect was derived from our earlier research[14]. We used G*Power v 3.1.9.2 (http://www.gpower.hhu.de/en.html) to calculate the sample size that would result in power of at least 80 % to detect a treatment difference of 0·01 litre/pmol per h with 95 % confidence, where the standard deviation of the difference ($S_D$) between treatment means is 0·0126. The $S_D$ is computed as the square root of the sum of the between person variance equal to 0·004litre/pmol per h minus twice the covariance between treatment means, to account for the fact that the comparisons were carried out within individual. We assumed a correlation of 0·8 for measurements obtained on the same person. With these input parameters, G*Power estimated an effect size of 0·8 and a sample size equal to fifteen persons.

Initially, seventy-eight men responded to the announcement, twenty-six of whom completed the first screening visit. Of those, twenty were selected to complete the second screening visit and were eligible for the study. In all, seventeen subjects met all eligibility criteria and completed the study protocol (Fig. 1). Dropouts were offset by over-recruiting and extra subjects were assigned to experimental sessions according to the William square sequence.

### Study design

This randomised cross-over single-blind clinical trial required subjects to complete five experimental sessions, in which they
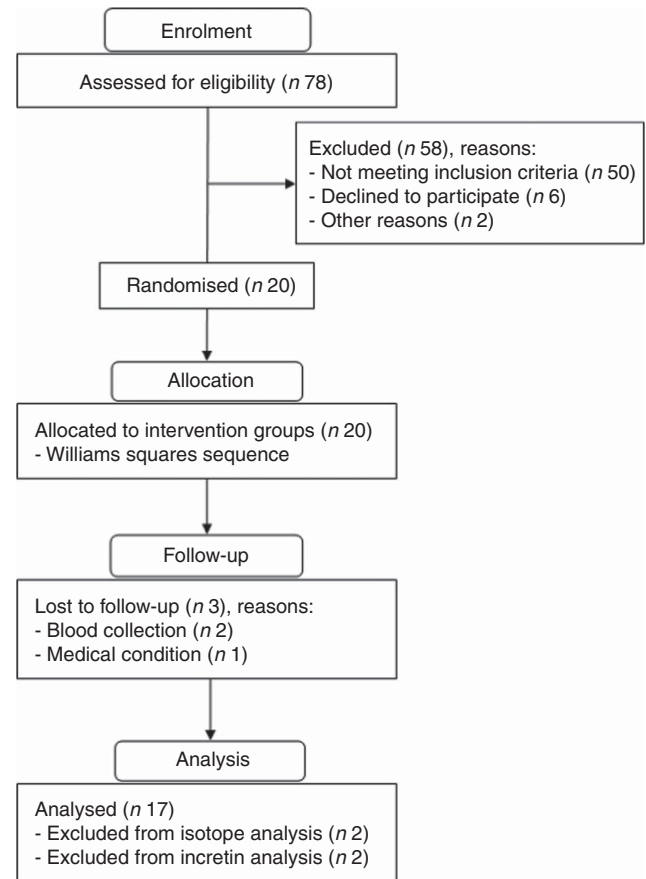


**Fig. 1.** Flow chart for screening and enrolment of study participants.

consumed either regular coffee, regular coffee with sugar, decaffeinated coffee or two controls – water with sugar or water alone, followed by an ODILE test 1 h after consumption[17]. The principal researchers were blinded to all experimental treatments. Subjects were randomised into one of ten treatment sequences, constructed using a Williams square design. The Williams design ensures that every sequence of treatment follows every other treatment once, balancing any treatment effect at the subsequent experimental session. The first sequence was randomly assigned to the first subject and subsequent sequences assigned to each subject enrolled in the study. The sequence was restarted with the eleventh subject and followed sequentially. The treatment sequences constructed with the Williams design were given as[18]:

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 the regular coffee with sugar, B the regular coffee, C the decaffeinated coffee, D the water with sugar and E the water. A single researcher (T. H. M. C.) was responsible for assigning each participant to the respective treatment sequence and supervising beverage preparation.

Subjects were instructed to refrain from consuming coffee, caffeine and alcohol, and also from practicing any non-habitual physical activity for 24 h before the sessions. In addition,

participants were instructed to consume a low-carbohydrate meal (about 38–40 % of energy in the form of carbohydrates) in the evening before the experimental sessions.

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

## Experimental protocol

Subjects fasted for 12 h overnight prior to arriving between 07.00 and 09.00 hours on each experimental day and capillary glucose, height, body weight, waist circumference, body composition, blood pressure and complete blood count were measured. Before the first trial, subjects also answered questionnaires based on the recruitment criteria and coffee intake. In each experimental session, body weight, capillary fasting glucose, the number of hours slept in the previous night, coffee and caffeine intake and the time and composition of the dinner were recorded. Blood glucose was measured with a glucometer (Accu-check Performa; Roche Diagnostics) to confirm that subjects were fasting (glucose <5·5 mmol/l).

On each experimental day, a cannula was inserted into the antecubital veins of each forearm, one for [1-$^{13}$C]glucose administration and the other for blood collection. After a 10-min resting period, basal blood samples were drawn at −10 and at 0 min – just before test beverage intake. The subjects then consumed the test beverage assigned according to the sequence in the Williams square design. Regular and decaffeinated roasted and ground coffee (brewed by drip filtering) provided caffeine to the participants at 1·4–2·0 and 0·24–0·33 (mg/kg body weight), respectively (online Electronic Supplementary Material – coffee brand selection and preparation). Blood samples were taken 30 and 60 min after beverage intake. Immediately after the 60-min sample, 75 g of glucose (Gluc UP 75; Newprov) dissolved in 300 ml of water was orally administered (oral glucose load). In all, twenty-three blood samples were taken at 75, 90, 105, 107, 109, 111, 113, 115, 117, 119, 121, 125, 130, 135, 140, 145, 150, 160, 175, 195, 225, 255 and 285 min after test beverage intake. Immediately after the 105-min sample (45 min after the oral glucose load), a 250-mg intravenous bolus of pyrogen-free [1-$^{13}$C]glucose (Cambridge Isotope Laboratories) was administered through the contralateral arm.

The cannula was then removed to prevent blood from being drawn at the site of isotope infusion. After each blood collection, the sample tubes were kept at room temperature to enable clotting for 30 min, centrifuged, and serum samples stored at −80°C until analysis. The experimental design is presented in Fig. 2. The washout period between study sessions increased after the third session to ensure hematopoietic recovery.

During the experimental sessions, subjects rested comfortably on a bed. Subjects were allowed to read, listen to music, watch TV, use a notebook or other portable electronic devices, and walk around the laboratory to use the toilet, but not allowed to eat or drink (except water).

## Clinical assessments

Body weight was measured to the nearest 0·1 kg on a 150 kg capacity electronic platform scale (Plenna) and height measured to the nearest 0·1 cm on a stadiometer (Alturaexata) mounted to its own platform. BMI (kg/m$^2$) was computed as the ratio of weight:squared height and classified according to the World Health Organization categories[19]. Waist circumference was measured midway between the lowest rib and the iliac crest to the nearest 0·1 cm and classified according to criteria outlined in the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults[20]. Body fat percentage was measured by tetrapolar bioelectrical impedance using a Quantum II body composition analyzer (RJL Systems) according to the protocol described by Lukaski et al.[21]. Blood pressure was measured by a trained professional[22] by auscultation with a calibrated aneroid sphygmomanometer.

## Biochemical measurements

At each blood draw (twenty-seven samples), 4 ml of blood were collected into a serum collection anticoagulant-free vacutainer for glucose, insulin, and [1-$^{13}$C]glucose analysis. C-peptide concentration was measured at 0, 30, 60, 90, 105, 121, 135, 150, 195 and 285 min, in ten blood samples.

In addition, 1 ml of blood was collected into cooled siliconised tubes containing EDTA and 10 µl of dipeptidyl peptidase-4 inhibitor at 0, 30, 60, 90, 121, 150, 175, 225, 255 and 285 min (ten blood samples) for determination of glucagon-like
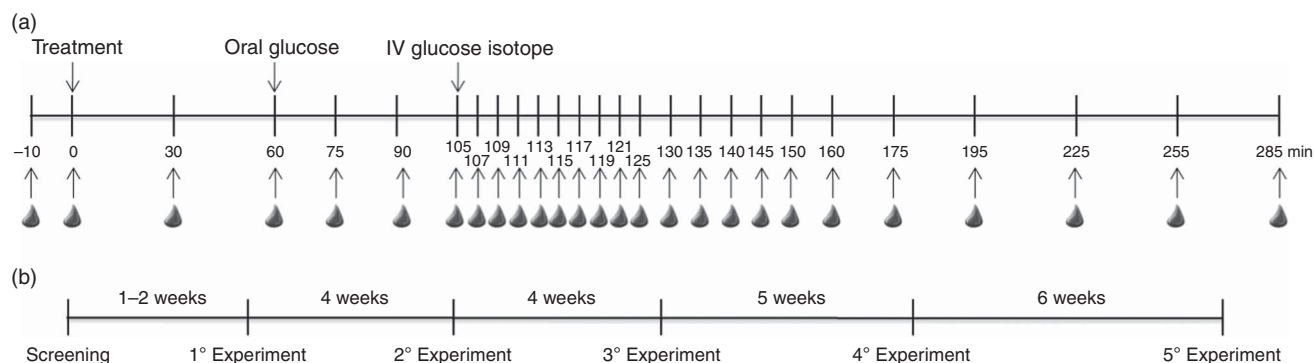


**Fig. 2.** Schematic representation of the experimental design (a) and washout periods between experimental sessions (b). IV, intravenous.

peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) concentrations.

## Analytical determinations

Glucose, insulin and C-peptide concentrations were measured by glucose oxidase, electrochemiluminescence and ELISA methods, respectively. Sensitivity of glucose oxidase was 0·12 mmol/l (within-run CV of 0·41 %), insulin immunoassay was 1·39 pmol/l (within-run CV of 1·9 %), and C-peptide ELISA was 0·01665 nmol/l (within-run CV of 3·1 %). The [1-$^{13}$C] isotopic composition of glucose was determined by GC-combustion-isotope ratio MS (GC/C/IRMS) on a 20–22 isotope ratio mass spectrometer (Sercon Limited) with an Orchid combustion/pyrolysis module (Elemental Microanalysis Ltd) and Agilent 7890B GC gas chromatograph (Agilent Technologies) with a combustion furnace operating at 860°C (within-run CV of 0·8 % and between-run CV of 2·0 %). The ratio of the 44 and 45 Th isotopologues of the generated $CO_2$ was determined using the method of Bluck & Coward[23] and these were converted[24,25] to tracer/tracee ratios using a derived value for pure tracer of 10121 G for the glucose α-D-glucofuranose cyclic 1, 2 : 3, 5-bis (methyl boronate)-6-trifluoroacetate derivative used in this study[26]. The data obtained from GC/C/IRMS analysis were interpreted by the two-compartment minimal model as implemented by Bluck et al.[17]. The indices of glucose metabolism parameters obtained from the ODILE test were Si and glucose effectiveness (Sg). GLP-1 and GIP concentrations were measured by ELISA; detection sensitivity was 2 pM for GLP-1 (within-run CV of 7·4 %) and 0·9545 pmol/l for GIP (within-run CV of 6·5 %).

## Food intake assessment

The dietary record of the evening meal prior to testing was checked with each participant to ensure accuracy and completeness. Food portions were converted into nutrients and energy intake, glycaemic index, and glycaemic load were analysed using the Nutrition Data System for Research software version 2015 (University of Minnesota) with the inclusion of typical Brazilian foods and standardised recipes.

## Statistical analysis

Levene's test and the Shapiro–Wilk tests were performed to determine the homogeneity of variance and normality respectively. The data passed both the homogeneity and normality tests. One-way ANOVA was used to detect differences in baseline characteristics and food intake and, when appropriate, the Tukey's test was used for *post hoc* comparisons. Repeated-measures analysis using the mixed models procedure (PROC MIXED), which takes into account the covariance structure of the data[27], was implemented on SAS® version 9.4 (SAS Institute Inc.). The protocol defined in PROC MIXED used the REML (restricted maximum likelihood) option with subjects as random effect and time as repeated measure with an autoregressive heterogenous (ARh) covariance matrix option and time × treatment interaction for each hormone and glucose measurement. The ARh covariance matrix was used because the time

period between treatment sessions varied in the protocol (Fig. 2). The model was constructed considering baseline values and the Williams design. Fat intake in the evening meal prior to testing was included in the model for GLP-1 concentration. For the analysis of Sg and Si, the model was constructed assuming compound symmetry for the covariance structure and considering the Williams square sequence. The compound symmetry matrix was used because each Sg and Si is a single integrated value for each subject on each treatment. The least square means (LS means) statement of SAS PROC MIXED was used and compared among treatments and also treatments at each time point using the Student's *t* test at a significance level of 0·05. Baseline values are reported as means and ± standard deviations, whereas glucose metabolism parameters (glucose, insulin, C-peptide, GLP-1 and GIP) and ODILE values (Si and Sg) from the repeated measures analysis are reported as LS means with their standard errors. We used a planned comparison against the control conditions, water or water with sugar, to reduce the risk of type I error due to multiple comparisons and to respond to the hypotheses raised. A B-Y method (Benjamin–Yekutieli method)[28] was applied to the *P* value for comparisons between water, regular and decaffeinated coffee treatments, with a critical value of $P < 0.02727$.

In addition, we performed the same analysis of the least square means (SEM) for the Matsuda index[29]. The Matsuda index was determined using glucose and insulin data taken at 60, 90, 121, 150 and 175 min, comprising the period from before (60 min) to approximately 2 h after the oral glucose load (175 min).

## Results

### Participant characteristics

A total of seventeen participants completed the study protocol and their baseline characteristics are summarised in Table 1. All participants met the fasting blood glucose requirement (4·10–5·44 mmol/l) before each experimental session.

There were no significant differences in body weight ($P = 0.99$), capillary glucose ($P = 0.97$), or number of hours slept ($P = 0.66$) at the beginning of each experimental session. In addition, there were no significant differences in food intake (energy, macronutrients, fibre and caffeine consumption, glycaemic index, or glycaemic load; $P > 0.08$) in the evening meal before each experimental session, except for fat intake, which was significantly higher in the water group ($P < 0.05$). Mean fat intake (g) for the groups were: coffee with sugar 32·5 (SD 17·8) and water with sugar 23·5 (SD 12·9); regular coffee 30·1 (SD 19·3); decaffeinated coffee 32·0 (SD 20·7); and water 50·5 (SD 18·7).

### Insulin sensitivity and glucose effectiveness

Decaffeinated coffee induced a 97·5 % increase in Si compared with water treatment ($P < 0.05$), whereas no significant difference in Si was detected for regular coffee compared with water. In addition, there was a tendency for higher Sg values in the regular coffee with or without sugar compared with either control condition ($P = 0.07$). The indices of glucose homoeostasis derived from the ODILE test are presented in Table 2.

**Table 1.** Baseline characteristics of study participants (n 17)
(Mean values and standard deviations)

| Variables | Mean | SD | Range |
|---|---|---|---|
| Age (years) | 26 | 4·4 | 20–35 |
| Body weight (kg) | 72·2 | 5·7 | 61·1–80·9 |
| BMI (kg/m$^2$) | 23·4 | 2·0 | 20·0–27·7 |
| Waist circumference (cm) | 84·1 | 4·4 | 77·0–92·0 |
| Body fat (%) | 18·6 | 5·6 | 8·8–25·2 |
| Systolic blood pressure (mmHg) | 115 | 7·3 | 100–128 |
| Diastolic blood pressure (mmHg) | 74 | 6·1 | 60–84 |
| Fasting blood glucose (mmol/l) | 4·9 | 0·4 | 4·1–5·4 |
| Coffee intake (ml/d) | 247 | 121·8 | 100–500 |
| Time of coffee intake (years) | 8 | 5·1 | 2–20 |

**Table 2.** Glucose homoeostasis indices derived from the Oral Dose Intravenous Label Experiment test
(Least square means with their standard errors; n 17)

| | Si (litres/pmol per h) | | Sg (/h) | |
|---|---|---|---|---|
| Treatment | Mean | SEM | Mean | SEM |
| Coffee with sugar | 0·01897 | 0·003802 | 1·2931 | 0·1496 |
| Water with sugar | 0·01458 | 0·003617 | 0·8733 | 0·1442 |
| Regular coffee | 0·01900[a,b] | 0·003617 | 1·2571 | 0·1396 |
| Decaffeinated coffee | 0·02457[a] | 0·003595 | 1·1152 | 0·1384 |
| Water | 0·01244[b] | 0·003700 | 0·8059 | 0·1495 |
| P* | 0·04 | | 0·07 | |

Si, insulin sensitivity; Sg, glucose effectiveness.
[a,b] Mean values within a column with unlike superscript letters are significantly different (P < 0·05) according to a planned comparison of the groups for the presence or absence of sugar. There is no significant difference between beverages with sugar for Si.
*P value refers to mixed-model procedure (PROC MIXED) with subject as random effect and time as repeated measure to compare the effects of the beverages.

Matsuda indices were 3·79 (SD 1·60) for coffee with sugar, and 5·07 (SD 1·51) for water with sugar; 9·72 (SD 1·65) for regular coffee, 11·58 (SD 1·54) for decaffeinated coffee and 9·98 (SD 1·56) for water, with significant differences between treatments (P < 0·001). However, for the planned comparison groups there were no differences between regular coffee with sugar and water with sugar. In addition, there were no differences between regular coffee, decaffeinated coffee and water.

### Glucose metabolism parameters

Changes of least square mean concentration over time are presented in Fig. 3. As expected, post-absorptive glucose, insulin and C-peptide levels increased in the two treatments containing sugar, both at 30 and 60 min after beverage intake. Following the oral glucose load, insulin and C-peptide peaks at 90 min (30 min after the oral glucose load). Regular coffee with sugar primed insulin, C-peptide and GIP response compared with water with sugar (Fig. 3). At 90 min, 30 min after the oral glucose challenge, regular coffee increased glucose level when compared with water (Fig. 4). C-peptide was significantly higher for regular coffee compared with decaffeinated coffee at 135 min. Insulin increased after regular coffee but did not reach statistical significance. GLP-1 was higher compared with decaffeinated coffee 30 min after the beverage intake (Fig. 4). Table 3 shows the overall results of the mixed-model analysis with metabolic parameters for each treatment reported as LS means with their standard errors. No significant differences in glucose (P = 0·72), insulin (P = 0·49) and GLP-1 (P = 0·43) levels were detected between the treatments over the 285-min trials. C-peptide and GIP levels showed a significant difference between treatments attributed to the presence of sugar. Observing the planned comparison groups, no statistical differences were detected. For example, in the comparison between decaffeinated and regular coffee for C-peptide, the calculated P value was 0·0292 but after B-Y method correction it failed to reach significance (Table 3).

### Discussion

On the basis of epidemiological studies associating coffee drinking with reduced risk of T2DM, we hypothesised that coffee without sugar (regular or decaffeinated) would improve glucose metabolism in response to a glycaemic load, but our findings only partially support this hypothesis. This clinical trial showed that moderate decaffeinated coffee consumption (2·5 cups or 300 ml) improved Si in healthy young adult men (20–35 years) following an oral glucose challenge, compared with water intake. However, there were no significant effects of regular coffee consumption on glucose, insulin, GLP-1 responses and Si and Sg indexes after the oral challenge. The lack of changes in glucose metabolism parameters after regular coffee consumption may result from the bioactive compounds in coffee, such as CGA and trigonelin, counteracting the effect of caffeine, as the former were previously shown to favourably affect glucose homoeostasis[30,31].

Moreover, the addition of 30 g of sugar to regular coffee consumed 1 h before a glucose meal-equivalent had no additional effect on blood levels of glucose, and insulin, and Si over the 285-min response. However, the addition of sugar increased the early insulin secretion response (1 h post-absorption period) and induced a rapid increase in plasma insulin concentration, with a non-significant decrease in the glycaemic response 30–75 min after the oral glucose load follow-up. Louie et al.[13] reported that adding 10 g of sugar to coffee reduced the postprandial glycaemic response by approximately 40 %, compared with coffee without sugar[13]. In contrast to this, our data do not indicate a favourable effect of coffee with sugar when compared with coffee without sugar. However, the reduction of blood glucose increases after an oral glucose overload following coffee with sugar when compared with sugar alone could suggest a favourable effect on postprandial glucose homoeostasis. Regular coffee with sugar primed the secretion of the incretins GLP-1 and GIP. GLP-1 presented a significant increase 30 min after coffee with sugar intake and then GIP at 90 and 120 min after the oral glucose challenge. Blood glucose was significantly decreased at 30 min after coffee consumption compared with water with sugar. These results linked the incretins, GLP-1 and GIP with glucose reduction in the presence of sweetened coffee. In addition, recent identification of norharman β-carboline in coffee, with α-glucosidase inhibiting activity, is another potential explanation for the favourable effects of coffee on glucose homoeostasis, given that this enzyme reduces carbohydrate absorption leading to reduced
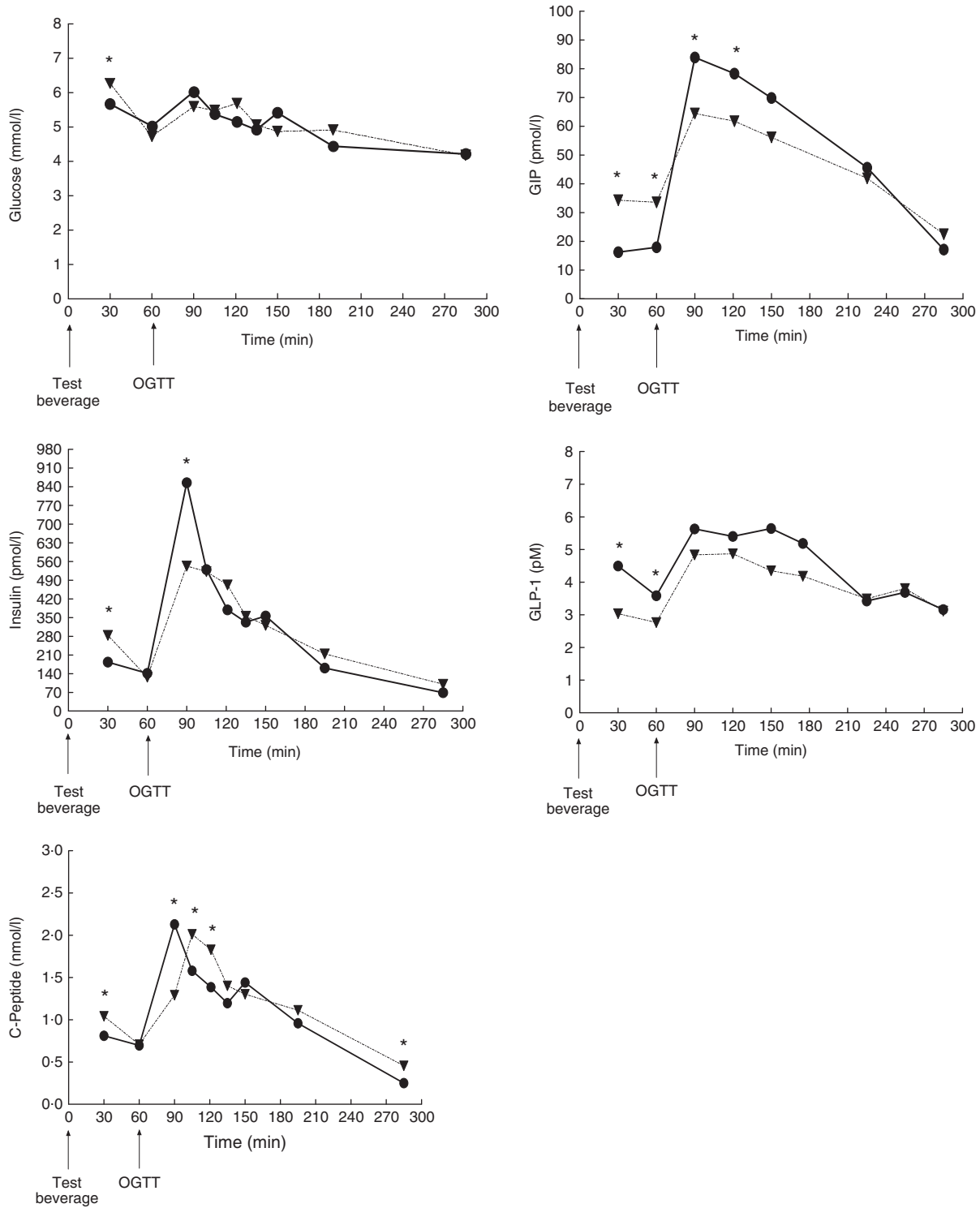
**Fig. 3.** Response during the Oral Dose Intravenous Label Experiment for glucose, insulin, C-peptide, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) after consumption of regular coffee with sugar (—●—) and water with sugar (--▼--). * Mean values were significantly different for planned comparisons with *t* test between the two groups. Time of consumption of the test beverage and the oral glucose tolerance test (OGTT) are indicated in graphs.

postprandial glucose levels[32]. It should be emphasised that the consumption of the sweetened beverages (regular coffee with sugar or water with sugar) promoted the expected increase in glycaemia and that coffee reduced the initial rise in the response by priming the physiological response.

Studies examining the effect of coffee on glucose metabolism have reported controversial results. Whereas some studies, like ours, found similar glucose and insulin responses 2–3 h after consumption of either regular coffee or water[33–39], others showed increased 2-h glucose and insulin AUC for regular
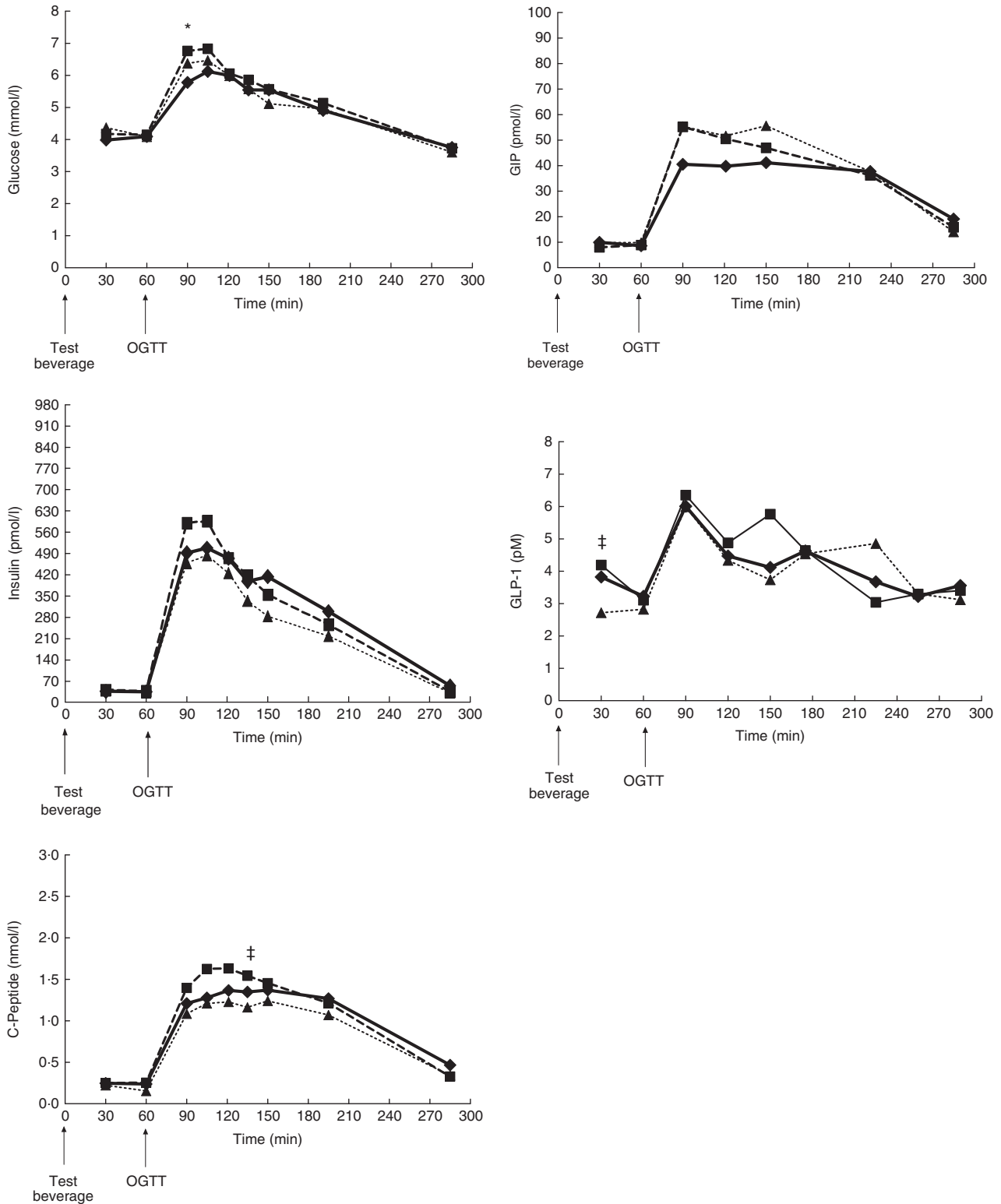
Fig. 4. Response during the Oral Dose Intravenous Label Experiment for glucose, insulin, C-peptide, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) after consumption of regular coffee (━■━), decaffeinated coffee (---▲---) and water (━◆━). Data were analysed using Proc Mixed procedure and data are presented as least square means with omitted standard error for clarity. Mean values compared with Student $t$ test. Significant differences: * regular coffee $v$. water; ‡ regular coffee $v$. decaffeinated coffee. A critical value correction for the multiple comparison was applied with B-Y method ($P < 0.02727$). Time of consumption of the test beverage and the oral glucose tolerance test (OGTT) are indicated in graphs.

**Table 3.** Least square means of metabolic parameters in each treatment group
(Mean values with their standard errors; *n* 17)

| Treatments | Glycaemia (mmol/l) | | Insulin (pmol/l) | | C-peptide (nmol/l) | | GLP-1 (pM) | | GIP (pmol/l) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Coffee with sugar | 5·14 | 0·15 | 334·18 | 33·11 | 1·16 | 0·08 | 4·47 | 0·27 | 47·00 | 2·80 |
| Water with sugar | 5·20 | 0·15 | 326·53 | 33·09 | 1·24 | 0·08 | 3·83 | 0·27 | 44·92 | 3·00 |
| Regular coffee | 5·36 | 0·15 | 311·54 | 34·2 | 1·08 | 0·07 | 4·30 | 0·28 | 31·63 | 2·98 |
| Decaffeinated coffee | 5·17 | 0·15 | 256·13 | 33·15 | 0·86 | 0·07 | 3·93 | 0·28 | 33·42 | 2·88 |
| Water | 5·08 | 0·15 | 300·99 | 33·17 | 0·98 | 0·07 | 4·08 | 0·29 | 28·10 | 3·02 |
| *P** | 0·72 | | 0·49 | | 0·002 | | 0·43 | | <0·0001 | |

GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic peptide.
**P* value refers to mixed-model procedure (PROC MIXED) with subject as random effect and time as repeated measure to compare the effects of the beverages. No significant difference was detected for planned comparison between the beverages containing sugar. Also no significant difference was detected for comparisons between the beverages without sugar.

coffee compared with either water[40] or decaffeinated coffee[13,40,41]. Moreover, two studies reported a lower glucose AUC for decaffeinated coffee compared with regular coffee[42] or water[43]. Regarding the effect of coffee on Si indexes, the scenario is similar: Greenberg *et al.*[42] found a higher Belfiore index (Si) for decaffeinated coffee, Moisey *et al.*[36] showed a reduction in the Matsuda index for regular coffee and Buscemi *et al.*[44] observed a reduction in insulin resistance (homoeostasis model-estimated insulin resistance index (HOMA-IR)) for regular coffee. In addition, other studies showed no effect of coffee consumption on the Matsuda index[40,41,43], or failed to find any consistent short-term (2–3 h) effects of coffee on Si indices[45,46]. Ingestion of the sweetened beverages, resulted in the reduction of Matsuda index values, but no significant difference was observed for other treatments. It is acknowledged that the use of the Matsuda index is limited under study protocols involving a beverage/meal stimulus before the OGTT[36,40,41,43], and is only suitable to compare treatments in this setting. Therefore, the Matsuda index provided limited information to assess Si under the proposed protocol. Thus, our finding of improved Si adds an important contribution to the field, as it relies on the strengths of the ODILE test, specifically the determination of an Si index under physiological conditions in a cross-over randomised trial. The ODILE test was validated against the golden standard assessment of Si, the euglycaemic hyperinsulinaemic clamp[14]. However, although insulin dependent glucose disposal data from the ODILE and the clamp are highly correlated, it is noteworthy that those two tests do not measure the same metabolic parameter, possibly because, unlike the clamp, the ODILE test is not carried out under steady conditions. Therefore, it is expected that the partitioning of the glucose load between the various metabolic routes might be different[14]. In fact, conflicting results have been reported for the effects of coffee consumption on glucose metabolism using different indices of Si/resistance assessment (Matsuda and HOMA-IR)[11]. Our study presents a robust analysis under physiological conditions of the short-term effects of regular and decaffeinated coffee consumption on glucose metabolism.

The accurate determination of Si/resistance is essential in understanding the pathophysiology of T2DM. Currently, there are two widely accepted methodologies that give accurate measures of Si: the euglycaemic hyperinsulinaemic clamp, and the frequently sampled intravenous glucose tolerance test

(FSIVGTT)[47]. Although widely employed, the FSIVGTT has some limitations, which are overcome by the incorporation of a small bolus dose of labelled glucose near the period of maximum hyperglycaemia after the oral glucose load. The ODILE protocol has been proposed by Bluck *et al.* and has been shown to be effective, as it enables the separation of glucose disposal measurements from endogenous glucose production, and increases the accuracy of the estimates of the physiological parameters[14,17]. Accordingly, this study offers a novel approach to understanding the effects of coffee consumption on glucose homoeostasis. To our knowledge, this is the first randomised clinical trial to have used the ODILE test as the experimental model for glucose and insulin assessment.

Our finding of improved Si after consumption of decaffeinated coffee is consistent with epidemiological data showing reduced T2DM incidence in regular consumers of decaffeinated coffee[4–6]. Moreover, the tendency for higher Sg values in the regular coffee treatments requires further investigation under normal physiological conditions due to the role of Sg in the regulation of glucose tolerance by the glucose-induced stimulation of glucose uptake and suppression of its own production[48]. However, even though our study was not powered to detect differences in Sg it shows a moderate trend.

### Strengths and limitations

This study has some strengths and limitations. The cross-over randomised design and the use of the ODILE test interpreted by the two-compartment minimal model are the main strengths. Similarly, the sample size was defined according to the power calculations performed for the Si parameter, and the male-only sample eliminated any potential sex-specific effects. The PROC MIXED analysis is consistent with the protocol (time follow-up) and the study design (William square). In addition, the coffee dose used was moderate and calculated to correspond to a commonly consumed dose. However, it should be highlighted that there are some challenges related to studying coffee consumption because there is no standardised definition for the volume of coffee that makes up one cup, the caffeine content of different coffees can vary, and the method of preparation influences the amount of caffeine and non-caffeine constituents. Our protocol measured the effect of one coffee treatment followed by a glucose tolerance test based on the

45–60 min peak caffeine plasma levels after a coffee beverage intake[49]. Other polyphenolic compounds that might undergo extensive metabolism and modification may not have been detected in our follow-up. It should also be noted that we selected habitual coffee consumers in order to minimise possible adverse effects of acute coffee consumption in less frequent consumers. In addition, subjects were asked to abstain from coffee in the 24 h preceding each intervention so that recent coffee consumption would not affect the effects of the tests beverages on glucose homoeostasis. However, we did not assess adherence to coffee abstinence by tools other than the interview. In addition, we cannot rule out that preference for sweetened coffee over non-sweetened coffee might have affected our findings, given that sweetening coffee is a common habit among Brazilians and that alimentary chemosensation has been shown to be linked with glucose homoeostasis[50]. Moreover, although the sample size of seventeen subjects was calculated to be sufficient to provide power, we acknowledge that the number of subjects included in the present study is smaller when compared with other coffee trials. Also, our findings cannot be generalised to other population groups, such as people with diabetes, women or non-habitual coffee consumers.

In conclusion, our results indicate that, in the short-term, consumption of decaffeinated coffee improves Si compared with water, and that the consumption of coffee with sugar has no adverse effects on glucose metabolism in healthy men. Nevertheless, further research, including long-term interventional studies, is needed to fully elucidate the mechanisms underlying the effects of coffee on glucose homoeostasis and the reduced risk for T2DM.

## Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S000711451800034X

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