Effects of intake of breakfast or caffeine-containing beverages on measurement of circulating chromogranin A in plasma

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Summary

Context: Sampling in the nonfasted state might result in false-high measurements of plasma chromogranin A (CgA), a key biomarker for patients with gastroentero-pancreatic neuroendocrine neoplasms (GEP-NENs).

Objective: To investigate whether intake of a 5-item English breakfast together with tea/coffee (Bfast-T/C) or intake of tea/coffee (T/C) alone relevantly influences postprandial CgA in GEP-NENs and controls.

Methods: In a randomised, controlled, double crossover study, we investigated in >10-hour overnight fasted individuals the effects of Bfast-T/C vs T/C vs the ongoing fasted state on 180-minute postprandial plasma CgA concentrations (28 patients with GEP-NENs, of those 22 on treatment with long-acting somatostatin analogues (SSA); and 11 controls). Ten participants (8 GEP-NEN, 2 controls) were on treatment with proton pump inhibitors (PPI).

Results: Intake of Bfast-T/C but not T/C alone increased CgA in the pooled cohort, reflecting the situation in screening, from 90 minute [area under the curve (AUC)CgA0–180 minute, ongoing fasted 172.6 ± 4.6 vs T/C 173.3 ± 5.2 vs Bfast-T/C 204.2 ± 7.9, P = 0.0002]. Postprandial responses to Bfast-T/C in controls and GEP-NENs were comparable. PPI usage was associated with markedly increased fasted CgA in the pooled cohort (429.3 ± 90.4 vs 91.0 ± 14.7 µg/L; P < 0.0001). AUC CgA 0–180 minute remained higher following Bfast-T/C after exclusion of PPI users (P < 0.05). In GEP-NENs, effects of Bfast-T/C on postprandial CgA raises were more pronounced in patients not treated with SSA.

Conclusions: Intake of Bfast-T/C, but not T/C alone, increased postprandial CgA in both patients with GEP-NENs and controls up to 34%. Fasted CgA measurements should be sought, if possible, and PPIs paused prior to measurement.

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Helen L. Robbins and Megan Symington shared first authorship.
Chromogranin A (CgA) is a 439-amino-acid glycoprotein that is present in the secretory dense-core granules of most neuroendocrine cells.\(^6\) CgA is also frequently secreted from both functioning and non-functioning gastro-entero-pancreatic neuroendocrine neoplasm (GEP-NENs) and is consequently used as a biomarker for both screening and follow-up in GEP-NENs.\(^2\) Although promising novel techniques such as assessment of micro RNAs, circulating tumour cells and gene transcript analyses have been recently proposed as superior to CgA methods,\(^3\) in current routine clinical practice measurement of plasma or serum CgA remains the most widely used biomarker for GEP-NENs.\(^6\) Sensitivity is considered to be reasonable, between 60% and 90%; however, reported specificity of CgA measurements are varying between below 50%\(^5\) and up to 95%,\(^7\) related to the fact that circulating CgA can be raised in various non-GEP-NEN related conditions including certain pituitary endocrine tumours, medullary thyroid carcinoma, pheochromocytoma and paraganglioma, primary hyperparathyroidism and various other endocrine conditions, chronic atrophic gastritis, renal failure, chronic liver disease, inflammatory bowel disease, rheumatoid disorders, cardiac disease,\(^1\) and presence of heterophile antibodies.\(^1\) Further, long-term gastric acid inhibition, especially by proton pump inhibitors (PPI) but possibly also by histamine type-2 receptor antagonists\(^10\) increases serum CgA levels\(^12\) and may reflect the presence and severity of fundic enterochromaffin-like (ECL) cell hyperplasia.\(^13\) Therefore, intake of PPI may give rise to false positive results when screening for a GEP-NEN.\(^13\) Although effects of PPI usage on increasing CgA in people without a diagnosis of a GEP-NEN are well-documented,\(^13\) it is less clear whether it is also associated with increased CgA in patients with GEP-NENs.

Whether food intake affects measurement of CgA is controversial.\(^7\)\(^,\)\(^14\)\(^,\)\(^15\)\(^,\)\(^17\)\(^,\)\(^18\) Early studies in animals indicate that gastric luminal contents can change circulating CgA levels in parallel with plasma gastrin concentrations.\(^19\) However, only few studies have assessed the possible impact of intake of food on circulating CgA levels in humans. Most of these studies were performed in healthy participants,\(^20\)\(^,\)\(^21\) or in patients without a diagnosis of GEP-NEN who were on treatment with PPI.\(^14\) We are aware of only two previous relatively small studies that assessed the effects of a meal test in patients with GEP-NENs.\(^15\)\(^,\)\(^18\) In a study including six patients with gastric NENs and six patients with non-gastric NENs a meal test significantly increased plasma CgA levels in healthy controls and long-term PPI users, but not in the patients with GEP-NENs.\(^15\) However, in another study in seven patients with multiple endocrine neoplasia type 1 who had evidence of a pancreatic NEN, a meal test increased plasma CgA by 20%.\(^18\) Meal induced significant increases in CgA in healthy controls were also observed,\(^18\) although in another larger study in 120 healthy individuals no statistical significant difference between fasting and non-fasting serum CgA measurements was found.\(^20\)

Based on above discrepancies and the paucity of studies that have assessed the effects of food intake on measurement of CgA in patients with GEP-NENs, practice related to the measurement of CgA is not standardised across Centres. Although most Centres dealing with GEP-NETs sample full gut hormone profiles (ie measurements including also serum gastrin and various other, more pancreas specific neuroendocrine markers) in the overnight fasted state, in a recently conducted survey by us amongst ENETS centres of Excellence, we found that in the responding Centres in the UK, only 50% of the Centres by default invite patients after an >10-hour overnight fast for an isolated measurement of CgA. Importantly, although sampling of CgA in the fasted state had been recommended in some of the previous guidelines,\(^17\) in recent guidelines of the leading Neuroendocrine Tumour Societies either no clear recommendation is made how CgA should be sampled,\(^2\)\(^,\)\(^8\) or it is explicitly stated that a fasting specimen for CgA is not required.\(^22\)

The impact of nonfasted sampling of CgA in patients with GEP-NENs remains particularly unclear and it is also unknown whether consumption of caffeine-containing beverages alone affects CgA measurements. We therefore sought to investigate the effects of a 5-item English breakfast together with caffeine-containing beverages on plasma CgA levels, as compared to intake of caffeine-containing beverages alone; or the ongoing fasted state; in a randomised, double-crossover, intervention study in 28 patients with a histologically confirmed diagnosis of GEP-NENs and 11 healthy controls. Given that in our experience it can be difficult to pause PPI treatment for 2 weeks in all patients subjected to a CgA measurement and assuming that effects of PPI treatment may override any possible effects of intake of food or caffeine-containing beverages on CgA measurements, in the present study we chose to also include a subset of patients who were on long-term treatment with PPI.

2  |  METHODS

2.1  |  Ethics approval

This study was approved by the East Midlands Research Ethics Committee (REC; number MW165915/IRAS ID 197653) and the University Hospitals Coventry and Warwickshire (UHCW) Research & Development Department. Written informed consent was obtained from all participants. The study followed the Declaration of Helsinki guidelines for research involving human individuals. Participants were enrolled from February 2017 to June 2017.

2.2  |  Study design

This was a randomised, controlled, double-crossover interventional study in 39 participants, of those 28 patients with GEP-NENs (of those 8 patients on treatment with PPI) and 11 controls without a diagnosis of a GEP-NEN (of those 2 patients on PPI; other known causes of false positive CgA being excluded). After >10 hour overnight fasts, respectively, participants attended the clinical trials unit
in The ARDEN NET Centre for three separate visits, in random order, using a computerised random-number generator. On arrival, intravenous (IV) cannulas were inserted into a forearm vein suitable for repeated blood sampling. At the three respective study days, participants consumed (a) a 5-item English breakfast (available items to choose from: one slice of bacon, one sausage, one fried egg or scrambled egg, fried bread, toast, baked beans, plum tomato, black pudding or one hash brown) together with 250 mL tea or coffee over 30 minutes (Bfast-T/C), or (b) 250 mL tea or coffee over 30 minutes, without additional food intake (T/C) or (c) remained fasted throughout the 180 minutes observation period. Use of milk and/or sugar to be consumed together with caffeine-containing beverages was not restricted. None of the participants did use sweeteners. On each arrival at the respective study days, history was taken about fasted state, the exact timing of the most recent injection of long-acting somatostatin analogues (SSA) (if applicable), medication with drugs if appropriate, and recent events or illnesses. Assessment of anthropometrics was performed by trained staff. Adherence to a strict overnight fast was checked verbally before proceeding at the respective study days and confirmed by all participants at all study days, respectively.

Routine blood markers including urea and electrolytes, full blood count, liver function tests and estimated renal function were drawn (0–30 minutes). Following the intake of Bfast-T/C, T/C alone, or the ongoing fasted state (Figure 1), series blood samples for the measurement of CgA were drawn at baseline (0 minute) and every 30 minutes over 180 minutes. IV cannulas were flushed with 10 mL 0.9% normal saline after obtaining each venous sample. For each blood draw, an initial 10 mL aliquot was discarded to account for saline flush and 4 mL venous blood was drawn into EDTA/Aprotinin tubes thereafter. All visits were supervised by at least one medical professional.

### 2.3 Recruitment and inclusion/exclusion criteria

Patient with GEP-NENs were recruited from the GEP-NEN database of currently n = 480 patients, and the GEP-NEN Out-patient Clinics of the ARDEN NET Centre, ENETS Centre of Excellence. Inclusion criteria were: adults (>18 years) with a histologically confirmed diagnosis of GEP-NEN; ability to adequately understand English and to provide written informed consent; ability to safely fast overnight and to commit to three hospital visits. Exclusion criteria were: no histologically confirmed diagnosis of a GEP-NEN, inability to provide informed consent, inability to adequately read/write or speak English; any patient who was unwell on the day of their routine clinical appointment; pregnancy and inability to safely fast overnight. Two additional patients with GEP-NENs were recruited but withdrew early, related to difficulties in sitting peripheral cannulas suitable for series blood draws.

The control group was recruited via advertisements in the hospital intranet and posters in Out-patient departments. Inclusion criteria for the control group were: adults (>18 years) with no known diagnosis of GEP-NENs; ability to provide written informed consent and speak/read/write English adequately; ability to commit to three hospital visits; and ability to safely fast overnight. Exclusion criteria were: confirmed diagnosis of GEP-NEN, inability to provide written informed consent or read/write speak English; pregnancy; any participant who was unwell on the day of their routine clinical appointment; and inability to fast overnight.

### 2.4 Participant characteristics

Patients with GEP-NEN and Controls were comparable by age, gender distribution, BMI, estimated renal function (expressed as eGFR), PPI usage and presence or absence of cardiovascular disease (all \( P > 0.11 \), Table 1). Most of the participants (n = 7/10) who were on treatment with PPI were using Omeprazole 20 mg or 40 mg OD. Three of the participants were on treatment with Lansoprazole 30 mg OD. Figure 2 shows key characteristics of the GEP-NEN cohort. Overall, 64.3% of the GEP-NEN patients had midgut NENs and 25% had pancreatic NENs. Most (82.1%) patients had stage IV disease, 39.3% of patients had functioning symptoms and 78.6% of the patients were on treatment with SSA, with an even distribution of timing of the intervention at the beginning (within 7 days) or the end (within 7 days) of the usually 4-weekly administration interval of

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**FIGURE 1** Study design
TABLE 1 Participant characteristics

|                  | GEP-NEN | Controls | p. value |
|------------------|---------|----------|----------|
| n                | 28      | 11       |          |
| Age (years)      | 67.6 ± 1.5 | 61.7 ± 4.4 | 0.11    |
| Gender (male/female) | 15/13   | 6/5      | 0.96     |
| BMI (kg/m²)      | 29.5 ± 1.8 | 26.8 ± 0.6 | 0.36    |
| eGFR (mL min⁻¹ 1.73 m⁻²) | 68.0 ± 4.7 | 80.0 ± 5.8 | 0.21    |
| PPI use (n; %)   | 8; 2    |          | 0.25     |
| Baseline CgA (ng/mL)—all, including PPI users | 208.8 ± 37.5 | 92.1 ± 28.2 | 0.063   |
| Baseline CgA (ng/mL)—excluding PPI users | 118.4 ± 20.4  | 30.2 ± 1.8  | 0.005   |
| (n = 20)         | (n = 9)  |          |          |
| Baseline CgA (ng/mL)—PPI users only | 444.6 ± 112.0 | 370.7 ± 94.3 | 0.75    |
| Surgical resection of NEN | 20    | n/a      |          |
| Residual disease | 24      | n/a      |          |
| Cardiovascular disease (not NEN related) | 4     | 2        | 0.74     |

Use of PPI: significant effect on baseline CgA concentrations; both in NENs (one-way ANOVA, \( P = 0.030 \)) and in controls (\( P < 0.0001 \)). CgA (ng/mL), upper limit of normal 100 ng/mL.

SSAs (Figure 2). The timing of visits for GEP-NEN patients on treatment with SSA was scheduled carefully such that each visit coincided with a similar point in their cycle of SSA injections.

2.5 | Assays/measurements

Sampling of a fasting venous blood was performed utilising the VACUETTE system (VACUTAINER®, 4 mL EDTA/Aprotinin 250 KIU tubes). Each blood sample was immediately centrifuged at 3000 RPM for 10 minutes. The plasma fraction was extracted and immediately transferred to a −80°C freezer for storage until measurement. Plasma CgA concentrations were quantified using an Enzyme Linked Immunosorbent Assay [Labor Diagnostika Nord (LDN) Chromogranin] according to the manufacturer’s protocol. The upper limit of normal CgA concentrations is given as 100 µg/L; limit of detection 5 µg/L; mean intra-assay variability 9%; mean interassay variability 5.25% for plasma samples. All samples were analysed within an 8 weeks window, in random order. All CgA levels were measured in duplicates, with the mean value used for analyses. Haemolytic or lipemic samples were excluded from further analyses, considering that these could cause inaccurate results, according to the manufacturer’s instructions.

2.6 | Statistical analyses

Data are presented as mean ± standard error (SE). Normal distribution was checked using Kolmogorov-Smirnov tests. Changes in plasma CgA following intake of Bfast-T/C, T/C only or in the ongoing fasted state over 180 minutes are presented after correction for the baseline (0 minute). One-way ANOVA was used to compare baseline and longitudinal CgA concentrations. Bonferroni corrections were applied for subgroup analyses. When there were no significant differences between groups after Bonferroni corrections, presentation of uncorrected values has been highlighted, as appropriate. Time courses of CgA were additionally compared by calculating the total area under the curve (AUC), using the trapezoidal method; and by presenting the respective individual concentration maximum (Cmax) and the individual time of maximal concentration (Tmax). Repeated measures ANOVA (generalised linear model) with treatment and time as within-subject factors and Huynh-Feldt epsilon correction was used to determine significant main effects and interactions. When there were significant interactions or a main effect of time, mean contrasts according to Bonferroni inequalities were used to analyse significance at specific time points. Additional presentation of uncorrected analyses has been highlighted, where appropriate.

To investigate factors that influence fasting CgA concentrations, a linear regression model was used with combined fasting CgA levels as the dependent variable and GEP-NEN vs Control status, gender, age on day of consent, BMI, eGFR and use of PPI as the independent variables. Breusch-Pagan test and auxiliary regressions were used to investigate for heteroscedasticity and significant relationships between fitted predicted values and squared residuals. A \( P \) value <0.05 was considered statistically significant. Data analyses were performed using IBM SPSS Statistics V.24 (Chicago, IL).

3 | RESULTS

3.1 | Fasting CgA concentrations

Fasting plasma CgA concentrations are presented in Table 1. Results refer to sampling of plasma CgA in the >10 hours overnight fasted state, with all measurements before the respective intervention (ongoing fasted vs T/C vs Bfast-T/C) being pooled. In patients with GEP-NENs, an additional measurement of CgA was available from the clinical database, using a different, in-house radioimmunoassay (RIA) as provided by Hammersmith Hospital, London. The closest routine measurement of CgA prior to the conduction of this study was used. Using the RIA and under less thoroughly standardised, routine clinical conditions, mean CgA in the patients with GEP-NENs was 156.0 ± 58.2 pmol/L (upper limit of normal given as 61 pmol/L).

Treatment with PPI was associated with markedly raised fasting CgA concentrations in the entire cohort (429.3 ± 90.4 vs 91.0 ± 14.7 µg/L; \( P < 0.0001 \)). This effect of PPIs on raised fasting CgA levels remained marked and statistically significant also when analysing Controls and patients with GEP-NENs separately: in Control participants, baseline pooled fasted CgA were 370.7 ± 94.3 vs 30.2 ± 1.8 µg/L in Control participants on and off PPI, respectively (\( P < 0.0001 \)); likewise, in patients with GEP-NET, baseline pooled fasted CgA levels were 444.6 ± 112.0 vs 118.4 ± 20.4 µg/L in participants with GEP-NET on and off PPI, respectively (\( P < 0.0001 \)). Excessively high CgA in Controls who were on treatment with PPI increased the mean CgA levels in the
Control cohort well within “pathologically” raised levels, so that the fasted baseline difference in CgA between GEP-NENs and Controls lost significance level ($P = 0.063$, Table 1). There was no significant difference in baseline CgA concentrations when comparing users of Omeprazole vs users of Lansoprazole ($P = 0.23$). After exclusion of PPI users, the difference in fasting CgA between GEP-NEN and Control participants was statistically significant ($P = 0.005$, Table 1).

### 3.2 Predictors of fasting CgA concentrations

A linear regression model including age, gender, BMI, status GEP-NEN vs control and PPI usage explained 36% of the variation in fasted CgA (adjusted $R^2$). At baseline, significant predictors of plasma CgA concentrations were PPI use ($P = 0.015$) and eGFR ($P = 0.003$). With PPI users included, there was no significant effect of age, gender, BMI or GEP-NEN vs control status (all $P > 0.13$).
FIGURE 3  Changes in plasma CgA concentrations relative to baseline (0 min) in the entire cohort, following ongoing fasted state (white circles), intake of tea or coffee only (black circles), or intake of 5-item English breakfast together with tea or coffee (black squares). Panels show: (A) entire cohort (n = 11 Controls and n = 28 patients with GEP-NEN combined), resembling the situation in screening; (C) Controls only; (E) Patients with GEP-NENs only. Panels (B), (D) and (F) show respective changes of plasma CgA expressed in total area under the curve (AUC). Bonferroni correction for multiple testing was applied. Panel D: Significant difference fasting vs breakfast (P = 0.022) was lost after adjustment for multiple testing. Data are presented as means ± SE. CgA, chromogranin A.
FIGURE 4  Changes in plasma CgA concentrations relative to the baseline (0 min), after exclusion of all participants who were taking proton pump inhibitors (PPI), following ongoing fasted state (white circles), intake of tea or coffee only (black circles), or intake of 5-item English breakfast together with tea or coffee (black squares). Panels show (A) entire cohort (n = 9 Controls and n = 20 patients with GEP-NEN combined), resembling the situation in screening after withdrawal of PPI; (C) Controls only; (E) Patients with GEP-NENs only. Panels (B), (D) and (F) show respective changes of plasma CgA expressed in total area under the curve (AUC). Bonferroni correction for multiple testing was applied. After exclusion of patients who were on PPI, in Controls plasma CgA was significantly higher 120 and 150 min following the intake of Bfast, as compared with the ongoing fasted state (P = 0.026 and P = 0.047, respectively, but significance level was lost after Bonferroni correction
3.3 | Postprandial changes of circulating CgA in the entire cohort, resembling the situation in screening

The respective changes in plasma CgA over 180 min following the ongoing fasted state, intake of T/C or intake of Bfast-T/C are shown in Figure 3. Results are presented after correction for the baseline (0 minute). In the entire cohort (GEP-NEN and Controls combined), repeated measures ANOVA showed a significant effect of the intervention \((P = 0.006)\) and a significant effect of time \((P = 0.034)\), but no significant intervention \(\times\) time interaction \((P = 0.44)\). In the full model, between 0 and 60 minutes, relative changes of plasma CgA vs baseline were comparable between the ongoing fasted, T/C and Bfast-T/C groups \((P > 0.12)\), respectively. However, there was significant difference between groups after 90 minutes \((P = 0.019)\), 120 minutes \((P = 0.020)\) and 150 minutes \((P = 0.024)\). No significant difference between groups was observed after 180 minutes \((P = 0.085)\). Differences in specific time points derived from post hoc analyses are shown in Figure 3. After Bonferroni correction, significant differences were observed only when comparing the ongoing fasted vs Bfast-T/C groups, but not when comparing ongoing fasted vs T/C (Figure 3).

The per cent increase in maximal CgA concentrations (Cmax) was significantly higher after Bfast-T/C \((67\% \pm 20\%)\), as compared with both the ongoing fasted state \((27\% \pm 6\%)\) and intake of T/C alone \((28\% \pm 5\%)\) \((P < 0.05\) in uncorrected model). There was no difference in per cent increase CgA between ongoing fasted state and T/C \((P = 0.93)\). Time of maximal CgA concentration was significantly different between Bfast-T/C and T/C only \((P = 0.032\) in the uncorrected model).

3.4 | Subgroup analyses

3.4.1 | Ongoing fasted state

In the ongoing fasted state over 180 minutes following a >10 hours overnight fast, circulating CgA concentrations remained unchanged \((all \ P > 0.09\) when comparing single time points) (Figure 3A), arguing against a relevant circadian fluctuation of plasma CgA at least until midday.

3.4.2 | Intake of T/C or Bfast-T/C vs ongoing fasted state

Both in the entire cohort and when analysing patients with GEP-NEN isolated, responses in plasma CgA concentrations after ingestion of T/C closely resembled the ongoing fasted state (Figure 3A), with no significant difference between the intervention groups \((all \ P > 0.49\) in the entire cohort and all \(P > 0.40\) in patients with GEP-NEN, respectively \((uncorrected analyses)\). Relative to baseline changes in AUC-CgA180 min \(\text{in the entire cohort were comparable between ongoing fasted and T/C (P = 0.90)}\), but significantly higher after intake of Bfast-T/C, both when compared to the ongoing fasted state and to T/C \((both \ P < 0.01, \text{Figure 3B})\). When analysing Controls separately (Figure 3C), a mild trend to raised CgA was seen after intake of T/C as compared to the ongoing fasted state, but significance level was not reached \((all \ P > 0.27\) in uncorrected analyses). A significant difference \((P = 0.022)\) in AUC-CgA180 min in ongoing fasted vs Bfast-T/C controls was lost after adjustment for multiple testing (Figure 3D).

3.5 | Postprandial changes of circulating CgA after exclusion of proton pump inhibitor (PPI) users

Changes in plasma CgA concentrations relative to the baseline (0 minute) after exclusion of the 10 participants who were taking PPI are presented in Figure 4. When excluding PPI users, in the pooled cohort \(\text{patients with GEP-NENs and Controls combined, resembling the situation in screening)}\), AUC-CgA180 min was significantly higher after the intake of Bfast-T/C, both compared with the ongoing fasted state and with intake of T/C only \((P < 0.05, \text{respectively, after Bonferroni correction})\) (Figure 4A and B). However, significance levels for individual time points were lost after Bonferroni correction, both in the pooled cohort \((Figure 4A)\) and when analysing Controls separately \((Figure 4C and D)\). No significant influences of the intervention were observed when analysing patients with GEP-NENs who were not on PPI separately \((all \ P > 0.05\) in uncorrected analyses) \((Figure 4E and F)\).

3.6 | Influence of treatment of patients with GEP-NENs with long-acting somatostatin analogues (SSA) on postprandial changes of circulating CgA

Changes in plasma CgA concentrations relative to the baseline (0 minute) in patients with GEP-NEN dependent on treatment with SSA are shown in Figure 5. In patients with GEP-NENs who were neither on treatment with SSA nor with PPI, intake of Bfast-T/C significantly increased postprandial plasma CgA concentrations from 60 minutes \((Figure 5 A)\). The effect of intake of Bfast-T/C was

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**FIGURE 5** Influence of treatment with long-acting somatostatin analogues (SSA) on changes in plasma CgA concentrations relative to the baseline (0 min) in patients with GEP-NEN, following ongoing fasted state (white circles), intake of tea or coffee only (black circles), or intake of 5-item English breakfast together with tea of coffee (black squares). Panels (A) and (B) show effects of the intervention on circulating CgA levels in GEP-NEN patients who were not on treatment with SSA \((n = 6)\). None of the patients in this cohort who were not on SSA were taking PPI. Panels (C) and (D) show effects of the intervention on circulating CgA levels in patients who were on treatment with SSA \((n = 22)\), including \(n = 8\) patients who were on treatment with long-term PPI. Panels (E) and (F) show effects of the intervention on circulating CgA levels in \(n = 14\) patients who were on treatment with SSA, after exclusion of patients who were on treatment with long-term PPI.
We here demonstrate that intake of a 5-item English breakfast together with caffeine-containing beverages, but not consumption of tea or coffee alone, significantly increased postprandial plasma CgA concentrations in the entire cohort (ie patients with GEP-NENs and controls without a diagnosis of GEP-NENs combined and PPI users included, reflecting the situation in screening). We also confirm previous findings from others that use of PPI is associated with markedly increased fasting CgA concentrations in Controls.\textsuperscript{13–15} given raise to false positive results when screening for a possible GEP-NEN and thereby decreasing sensitivity of CgA measurements in patients who are on treatment with PPI. In addition, we show that use of PPI was also associated with markedly raised fasting CgA concentrations in the majority of patients with GEP-NENs. Higher CgA concentrations in PPI users were sustained during the 180 minutes observation period, with intake of breakfast showing comparable further increases of plasma CgA as observed in non-PPI users.

While advanced and more sensitive methods such as measurement of circulating tumour cells or analysis of multiple neuroendocrine tumour transcripts are promising future clinical tools,\textsuperscript{7} to date these methods are not yet widely available in the routine clinical setting, also related to constraints on available funding. Therefore, CgA remains the key GEP-NEN biomarker in current clinical practice.\textsuperscript{8,22} Our presented findings have direct implications on clinical practice when using CgA as a biomarker. In screening, both intake of breakfast and PPI usage (but not intake of caffeine-containing beverages alone) appeared to increase the risk of misleading high CgA measurements and ideally should be avoided. Further, in follow-up of patients with a known GEP-NEN, despite relevant limitations plasma CgA levels have been proposed to correlate with tumour mass, differentiation and functional status and are thought to have significant prognostic relevance.\textsuperscript{24–27} Therefore, intake of breakfast prior to CgA sampling or adding a PPI to the treatment in patients who are under follow-up for their GEP-NEN and not pausing PPI prior to repeat CgA sampling could falsely indicate tumour progression, thereby triggering unnecessary additional tests and anxiety for the patients.

A reliable 10 hours fasting state can be challenging in some patient groups, including individuals with secondary diabetes following pancreatic resection, and frail, elderly patients. Anecdotal evidence would also suggest that patient compliance with fasting instructions is variable, potentially complicating interpretations of “fasting” results. Further, in Centres with large numbers of patients with GEP-NENs, it is logistically less challenging to invite patients throughout the day for blood tests, rather than restricting the sampling window to the earlier part of the morning for an overnight fasted blood sample. Although intake of breakfast appeared to have only relatively modest effects on raising CgA levels in our study, with observed raises in postprandial CgA concentrations up to 34%, we suggest seeking a repeat CgA measurement in the >10 hours overnight fasted state in case of borderline pathological nonfasted CgA measurements. According to our here presented findings, this could be particularly relevant in screening for a GEP-NEN, or in GEP-NEN patients with assumed fully resected disease who are not on treatment with long-acting SSA. Finally, according to our results, intake of caffeine-containing beverages prior to sampling of CgA does not need to be discouraged.

Strengths of our study include the systematic, controlled investigation of the effects of intake of food and/or caffeine-containing beverages in a well characterised cohort of patients with GEP-NENs. Limitations of our study should be mentioned. Although our here presented double-crossover intervention in 28 patients with GEP-NENs was considerably larger than the two previous studies that had investigated effects of meal intake on circulating CgA,\textsuperscript{15,18} number of patients in the respective subgroups (ie exact GEP-NEN type, functioning vs non-functioning status, treatment modalities including usage of PPI and/or SSA) was small. Only two of the patients in our Control group were on treatment with PPI; however, marked increases in circulating CgA in healthy controls have been well-documented in previous studies by others\textsuperscript{12–15} and were confirmed in our study. Further, reference intervals and individual patient results differ significantly between different CgA assays and cannot be directly compared.\textsuperscript{2} Therefore, if other assays are used, our here demonstrated findings may not necessarily apply and ideally patients should undergo additional assessments, which may include a one-off measurement of CgA both in the fasted and in the nonfasted state.

In conclusion, our findings suggest that intake of caffeine-containing beverages prior to blood sampling has no impact on CgA measurements and therefore does not need to be discouraged. Intake of a 5-item English breakfast moderately raised plasma CgA levels in all participant groups but was more pronounced in Controls and in patients with GEP-NENs who were not on treatment with long-acting SSA. Therefore, use of nonfasted plasma CgA measurements may result in false positive findings especially in screening and in patients with GEP-NENs with assumed resected disease and should be discouraged; or a repeat CgA measurement could be sought in case of borderline pathological results in nonfasted individuals.

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