Title: Reproducibility of gastric emptying in overweight and obese males

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Non-standard Abbreviations used: GE, gastric emptying; $t_{lat,}$ latency time; t_{asc}, ascension time; $t_{1/2}$, half time; t_{lag}, lag time; ^{13}C-OBT, ^{13}C-octanoic acid breath test.

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Abstract

Background and aim: To understand whether any change in gastric emptying (GE) is physiologically relevant, it is important to identify its variability. Information regarding the variability of GE in overweight and obese individuals is lacking. The aim of this study was to determine the reproducibility of GE in overweight and obese males.

Methods: Fifteen overweight and obese males [body mass index 30.3 (4.9) kg/m^2] completed two identical GE tests 7 days apart. GE of a standard pancake breakfast was assessed by ^{13}C-octanoic acid breath test. Data are presented as mean (±SD).

Results: There were no significant differences in GE between test days (half time ($t_{1/2}$): 179 (15) and 176 (19 min), p = 0.56; lag time (t_{lag}): 108 (14) and 104 (8) min, p = 0.26). Mean intra-individual coefficient of variation for $t_{1/2}$ was 7.9% and t_{lag} 7.5%. Based on these findings, to detect a treatment effect in a paired design with a power of 80% and $\alpha = 0.05$, minimum mean effect sizes for $t_{1/2}$ would need to be ≥ 14.4 min and t_{lag} ≥ 8.1 min.

Conclusions: These data show that GE is reproducible in overweight and obese males and provide minimum mean effect sizes required to detect a hypothetical treatment effect in this population.

1. Introduction

Measurement of gastric emptying (GE) is essential to understand changes in gastric symptoms and appetite in various pathologic conditions, and in response to treatments. For example, GE could play an important role in the aetiology of obesity through processes of satiety and satiation. However, to understand whether any change in GE is detectable and clinically meaningful, it is important to identify its day-to-day variability. The reproducibility of GE has been studied in various populations, including infants,[1-2] children,[3] critically ill patients,[4] diabetics[5] and healthy lean adult males[6] and females.[7] Surprisingly, despite being implicated in the pathogenesis of obesity[8] and measured in response to numerous interventions[9] information regarding the day-to-day variability of GE in overweight and obese individuals is lacking.

Previous studies have reported a mean intra-individual coefficient of variation for GE half time ranging from 6% in healthy infants[1] to 73% in patients with functional dyspepsia.[15] This illustrates the large variation in the reproducibility of GE that can occur depending on the population studied and highlights the importance of establishing the intra-individual variability in the subject population of interest. Given some evidence that gut peptide[10] and appetite responses may vary according to body composition or body mass index, it should not be assumed that outcomes observed in a group of lean individuals will be identical in overweight and obese individuals[11]. Individual differences in the person studied (e.g. neurological and hormonal differences[12], anatomical differences such as the shape of the stomach[13], diet, physical activity and psychological factors[14]) may all influence the reproducibility of GE. The intra-subject variability in GE might therefore be different in overweight and obese compared to lean individuals.

The test conditions (e.g. the test meal used[5]) and the GE parameters reported[16] may also influence the intra-individual variability. Knowledge of the reproducibility of different phases

of GE and hence GE parameters is important given the kinetic and temporal nature of GE and relation to appetite control.[9] Although half time is generally the focus of GE studies as it is considered the most useful parameter in clinical practice, it does not reflect the complete pattern of GE. Since the ^{13}C-octanoic acid breath test (^{13}C-OBT) was proposed as a safe, reliable and non-radioactive alternative to scintigraphy for measurement of GE,[17] the test has been widely used in a variety of populations including obese individuals.[8] A number of GE parameters have been proposed that reflect the various phases of GE (e.g. Schommartz et al.[18]). However, little information exists on the reproducibility of the different parameters or phases of GE.

Thus, using the ^{13}C-OBT to measure GE, the aims of this study were to (i) determine the reproducibility of GE, (ii) compare the reproducibility of different GE parameters and (iii) calculate minimum effect sizes required to detect a hypothetical treatment effect in GE, in overweight and obese males.

2. Materials and Methods

2.1 Participants and Design

Fifteen overweight and obese men [mean (±SD) body mass index = 30.3 (4.9) kg/m^2, age = 34.9 (10.6) y, percent body fat = 32.1 (8.0) %] participated in the study. Height was measured without shoes to the nearest 0.5cm and weight to the nearest 0.01kg. Body composition was measured using air displacement plethysmography (Bodpod, Concord, CA). All participants had no history of gastrointestinal disease or surgery, significant illness, or were taking any medication known to directly affect gastrointestinal motility or appetite. The medication participants reported taking included albuterol (n = 1), budesonide (n=1), fexofenadine (n = 1), atorvastatin/amlodipine and olmesartan (n = 1). Participants provided written informed consent prior to taking part in the study. The study received ethical approval from the Queensland University of Technology Research Ethics Committee.

Each participant participated in two identical GE test days 7 days apart. Participants were provided with a standardised evening meal to consume at home prior to both test days and were asked to then fast for 12 hours overnight until attending the laboratory the following morning. In addition, participants were instructed to refrain from strenuous exercise and alcohol for 24 hours beforehand and to avoid consumption of naturally ^{13}C-enriched foods (e.g. corn or corn products, pineapple, kiwi fruit, cane sugar and exotic fruits) for at least two days prior to both test days. Test mornings commenced between 6am and 9am, and the time of day for repeat tests was standardised within participants.

2.2 Gastric Emptying

GE parameters were calculated using the ^{13}C-OBT.[17] The egg yolk of a standardized pancake breakfast meal [1676 kJ (400 kcal); 15g (15%) PRO, 17g (37%) Fat, 48g (48%) CHO)] was labelled with 100mg ^{13}C-octanoic acid (Cambridge Isotope Laboratories, Andover, USA).

Participants consumed the meal together with 250ml of water within 10 minutes. Breath samples were collected in 10ml glass Exetainer tubes (Labco, Buckinghamshire, UK) prior to the breakfast, immediately after, and subsequently at 15 minute intervals for 5 hours after breakfast. Participants remained in sedentary activities throughout.

2.2.1 ^{13}C breath test analysis

^{13}C enrichment of breath samples was measured by isotope ratio mass spectrometry (Hydra 20-20) and compared to a reference gas (5% CO_2, 75% N_2, 20% O_2 calibrated with a standard of $^{13}CO_2$). Data were analysed according to Ghoos et al [17] and fitted to the original GE mathematical model by non-linear regression analysis . The r^2 coefficient between the modelled and raw data was calculated and accepted if $r^2>0.9$. The conventional uncorrected time based parameters (t_{lag} and $t_{1/2}$) proposed by Ghoos et al.[17] and the parameters latency time (t_{lat}) and ascension time (t_{asc}) proposed by Schommartz et al. (1998)[18] were calculated. Latency time refers to the the initial delay in the cumulative ^{13}C-excretion curve, thus reflecting the initial emptying phase and ascension time to the time course between the latency phase and the half excretion time, representing a period of high ^{13}C-excretion rates[18].

2.3 Statistical Analysis

Paired t tests were used to compare differences between visits 1 and 2. The difference between results on the two separate days was plotted against the mean of the results for each subject, according to Bland and Altman.[19] Intra-individual variability was expressed as the coefficient of intra-subject variation (CV_{intra}; $CV_{intra} = SD_d/(m\sqrt{2})$ where SDd is the standard deviation of the differences between the repeated tests and *m* is the mean of the repeated tests [4-5, 19]). Based on day to day variability observed in these parameters, minimum effect sizes required to detect a hypothetical treatment effect with 80% power were calculated. Minimum differences that would be detected by a sample of fifteen subjects were also calculated. Statistical analysis was performed using PASW Statistics 18.0 (SPSS Inc., Chicago, IL) and

Graph Pad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). Data are expressed as mean ± standard deviation (SD), unless otherwise stated. Statistical significance was set at $P < 0.05$.

3. Results

Bland Altman plots for GE time based parameters are shown in **Figure 1**. For all GE parameters, no significant difference was found between the two test days (Table 1). The mean difference between test days was small for all parameters. However the 95% limits of agreement were -35.9 to 42.1 min for $t_{1/2}$ (**Figure 1)**. As shown in **Figure 1,** one individual was outside the 95% limits of agreement with a mean difference between test days of 46 min for $t_{1/2}$. This individual reported to adhere to the study protocol, therefore this change may represent the extreme of intra-individual variability. The mean CV_{intra} varied depending on the parameter reported from a minimum of 7.5% (t_{lag}) to a maximum of 11.4% (t_{asc}) (Table 1).

[FIGURE 1]

[TABLE 1]

3.1 Relationships between Variables

Change in t_{lag} from visit 1 to 2 was significantly correlated with change in $t_{1/2}$ ($r = 0.81$, $p < 0.001$, **Figure 2**). Changes in all parameters between visits 1 and 2 were significantly correlated ($p < 0.05$), except for t_{lat}. Change in t_{lat} was significantly correlated with change in t_{lag} ($r = 0.77$, $p = 0.001$). However, there was no significant correlation between change in t_{lat} with change in $t_{1/2}$ ($r = 0.25$, $p = 0.36$) or t_{asc} ($r = -0.011$, $p = 0.97$, Figure 2).

[FIGURE 2]

3.2 Calculation of minimum effect sizes for gastric emptying parameters

Based on the day-to-day variations observed, we calculated that in order to detect a treatment effect, in a paired design with a power of 80% and $\alpha = 0.05$, minimum mean effect sizes for GE $t_{1/2}$ would need to be $\geq$ 14.40min, $t_{lag} \geq$ 8.1 min, $t_{asc} \geq$ 13.9 min and $t_{lat} \geq$ 3.8 min. An estimate of the minimum number of participants needed to detect significant differences in a paired design study assuming $\alpha = 0.05$ and a power of 80% was calculated and used to construct a nomogram (**Figure 3**). To detect a 10% change the minimum number of participants needed for t_{lag} and $t_{1/2}$ would be 10, t_{lat} 25 and t_{asc} 14.

[FIGURE 3]

4. Discussion

Knowledge of the day to day variability of gastric emptying is necessary to assist in both designing studies and interpreting the clinical relevance of any changes if observed. This study provides evidence that GE is reproducible in overweight and obese males. In addition, these data show that the reproducibility and hence the sample size required to detect a significant difference in GE will vary depending on the parameter of interest.

Some evidence indicates that the release of gut peptides and appetite may be altered in obese individuals.[10-11] For example, ghrelin is a significant determinant of GE in lean but not obese individuals.[10] We therefore hypothesized that the day-to-day variability in GE might be different. However, our findings of a mean intra-individual coefficient of variation of ~ 8% for GE half time in healthy overweight and obese males, is comparable to studies in lean individuals using test meals similar in energy content.[5, 20-21] In healthy lean adults, an intra-individual coefficient of variation ranging from 7%[21] to 30%[22] has been reported for half time of solid meals, with the majority of studies indicating an intra-individual variability of between approximately 11-15%.[16, 20-21, 23-25] Although others have reported a higher intra-individual variability in GE of between 20-30% in healthy adults,[17, 22, 26] differences in study methodologies may account for this inconsistency. The low energy content of the test meals used (ranging from 200 to 250 kcal) in the latter studies[17, 22, 26] may contribute to the lower reproducibility.[27] It is also possible that the true variability in GE may go undetected if the test meal does not challenge motility,[28] therefore in the current study we used a test meal (400kcal) which reflects a more typical size of meal. Taken together, our findings indicate that unlike other populations where the variability of GE has been shown to be higher (e.g. diabetics,[5] patients with functional dyspepsia,[15] preterm infants[2] and critically ill patients[4]), the reproducibility of GE in healthy overweight and obese males is similar to that reported in healthy lean adults.

Our findings also indicate that the parameters used to characterise GE differ in their reproducibility. We found that the intra-individual variability was similarly lowest for t_{lag} and $t_{1/2}$ derived from the original ^{13}C-OBT mathematical model[17], than for other GE parameters. This finding is in contrast with others who have shown the lag phase or initial emptying to be less reproducible than subsequent emptying parameters[16, 20]. One explanation may be that GE was measured in these studies by scintigraphy and lag time derived by scintigraphy is known to be difficult to quantify.[28] Chey et al.,[28] examined the reproducibility of GE parameters measured by both ^{13}C breath test and scintigraphy and reported that while for scintigraphy half time was considerably more reproducible, for the breath test lag time was more reproducible. Our findings suggest that in contrast to some scintigraphic studies, lag time derived by breath test is a reproducible GE parameter.

In addition to lag and half times, other parameters attempting to more accurately reflect the biphasic nature of GE have been proposed. By definition the same part of the $^{13}CO_2$ exhalation curve is used for the calculation of both t_{lag} and $t_{1/2}$ and both parameters have been shown to be highly correlated.[18] As a result the different phases of GE (e.g. a delayed initial emptying but accelerated subsequent emptying or vice versa) could be difficult to distinguish. This prompted Schommartz et al.,[18] to propose the parameters latency time and ascension time. Although, little information exists on the reproducibility of these parameters, we found intra-individual coefficient of variations of ~ 11% and 9% for latency and ascension times respectively indicating that both parameters are reproducible in overweight and obese males. Interestingly, we also found that while changes in lag time and half time from visits 1 to 2 were highly correlated, changes in latency and ascension times were not. These findings suggest that these additional parameters may be more sensitive to detecting changes in different phases of GE, and would be useful to determine in addition to the conventional parameters in repeated measures studies.

Information regarding the day-to-day variability of GE is necessary to determine appropriate sample sizes when designing studies. For example, Lartigue et al.,[5] calculated that to detect a 20% change in GE half time in a paired design study, 7 healthy subjects would be required whereas 18 diabetics would be required. We found that in overweight and obese males a 20% change in all parameters (lag, half, latency and ascension times) could be detected with a sample size of 7 participants. These results demonstrate that only a small number of participants are needed to detect clinically relevant changes in GE. As GE studies are often carried out in small numbers e.g. measured pre and post surgical procedure, these findings illustrate the potential efficiency of undertaking smaller studies before larger studies are undertaken.

There are various methodological aspects to this study and GE reproducibility studies in general which deserve further consideration. How best to represent intra-individual variability remains a matter of debate.[29] We have discussed primarily the intra-individual coefficient of variation as it allows comparison across the majority of other studies and hence populations and methods. The parameters used to describe GE measured by ^{13}C-OBT also vary. Some report half and lag times that are corrected to scintigraphy equivalent values.[17, 22, 25] However, the rationale for the correction of values has been questioned as it is possible to obtain negative and physiologically insignificant values.[18] For this reason, we report the original uncorrected lag and half times proposed by Ghoos et al.,[17], similar to others.[1-2, 4, 23-24, 28] It should also be noted that many other parameters in addition to those described in the current study have been proposed for the ^{13}C-OBT. We analysed the reproducibility of four commonly used parameters which aim to characterise the biphasic nature of GE. In addition, the reproducibility of a test meal consisting of a less balanced composition or different energy content may be different. Similarly, our findings apply to healthy overweight and obese males and therefore future studies in conditions such as gastroparesis and in females are warranted.

Lastly, the ^{13}C-OBT does not provide direct imaging of gastric function, and therefore studies measuring GE using other techniques would be useful to confirm the present findings in overweight and obese individuals.

In the current study we did not determine the accuracy of the ^{13}C-OBT against the 'gold standard' scintigraphy. However, scintigraphic measurements may be hampered in obese individuals as defining the gastric areas of interest can be difficult and consequently the acceptance of scintigraphy as the 'gold standard' has been considered by some an arbitrary choice.[25] The optimal method of measuring GE in overweight and obese individuals remains unclear. It is difficult to examine the accuracy of scintigraphy in obese individuals, as it is the 'gold standard'. Other non-invasive non-radioactive methods such as the paracetamol absorption test and ultrasound have only been validated for liquid emptying and ultrasound in particular is considered a suboptimal method for measuring GE in overweight or obese individuals. In contrast, the ^{13}C-OBT measures solid meal emptying, has been validated against scintigraphy, has a day to day variability comparable to scintigraphy,[17] is sensitive enough to detect pharmacological influences on GE[30] and has been successfully used in obese individuals.[8] Our findings further suggest that GE measured by ^{13}C-OBT is reproducible in overweight and obese individuals and therefore the ^{13}C-OBT represents a promising method for measuring changes in GE in this population.

In summary, although studies have measured the reproducibility of GE using different methods and in different populations, to the best of our knowledge, this is the first study to investigate the reproducibility of GE in an overweight and obese population specifically. Our findings demonstrate that (i) the reproducibility of GE is similar to that found in lean adults, (ii) the reproducibility varies depending on the GE parameter reported, and (iii) that relatively small sample sizes are sufficient to detect clinically significant changes in GE in overweight

and obese males. This knowledge is important given the increasing number of GE studies being undertaken in this population.

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Statement of Authorship

The author's responsibilities were as follows – KMH, NMB, GJC and NAK contributed to the design of the study; KMH collected the data, analysed the data and drafted the manuscript; NMB, GJC and NAK contributed to data analysis and critical revision of the manuscript. All authors read and approved the final manuscript.

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Legend for Figures

Figure 1. Bland–Altman plots showing the difference between visits 1 and 2 (y axis) plotted against the mean for the two visits (x axis) for a) t_{lag}, lag time, b) $t_{1/2}$, half time, c) t_{lat}, latency time and d) t_{asc}, ascension time. Solid line indicates mean bias. Dashed lines indicate 95% limits of agreement. n = 15.

Figure 2. Scatter plots of the relation between the change - from visit 1 to visit 2 - in (a) lag time (t_{lag}) and half time ($t_{1/2}$) ($r = 0.81$, $p < 0.001$), and b) latency time (t_{lat}) and ascension time (t_{asc}) ($r = -0.01$, $p = 0.97$). n = 15.

Figure 3. Minimum number of participants needed to detect significant differences in a paired design in a) latency time (t_{lat}), b) ascension time (t_{asc}), c) half time ($t_{1/2}$),and d) lag time (t_{lag}), assuming a power of 80% and $\alpha = 0.05$.