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ORIGINAL ARTICLE

Glucose-dependent insulinotropic polypeptide secretion after oral macronutrient ingestion: The human literature revisited and a systematic study in model experiments in mice

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Fat, Glucose, Glucose-dependent insulinotropic polypeptide, Humans, Macronutrients, Mice, Protein

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ABSTRACT

Aims/Introduction: The incretin hormone glucose-dependent insulinotropic polypeptide (GIP) is secreted after meal ingestion. This study explored the relative influence of classes of macronutrients on GIP secretion.

Materials and Methods: The human literature was revisited by identifying articles from PubMed using key words GIP, macronutrients, carbohydrates, fat, protein, healthy subjects. In model experiments in anesthetized mice, glucose (25–125 mg), protein (15–120 mg), fat emulsion (6–100 mg) or saline was given orally with determination of GIP levels.

Results: The literature survey identified 15 studies in which glucose, protein or fat was administered to healthy subjects. All three classes of macronutrients stimulated GIP secretion with a 30–45 min peak after glucose and protein, and a more prolonged release after fat. Limitations in study designs preclude firm conclusions on the relative potency of the macronutrients. In mice, glucose was more potent to stimulate GIP secretion than fat and protein, with no significant difference between protein and fat. By co-administration of the macronutrients at moderate caloric combinations, a synergistic stimulation of GIP secretion was observed. In contrast, when raising the glucose challenge together with protein and fat, no synergy, but an additive effect, was evident.

Conclusions: Glucose, protein and fat all stimulate GIP secretion in humans and mice. In mice, glucose is more potent than fat and protein, and there is also a synergy between the macronutrients on GIP secretion at moderate caloric doses. Further studies are warranted in humans to explore the relative potency of macronutrients.

INTRODUCTION

Glucose-dependent insulinotropic peptide (GIP) was isolated from the gut in 1969 and shown to inhibit gastric acid secretion[1–3]. It was subsequently shown that the main effect of the hormone is its glucose-dependent stimulation of insulin secretion[4–6]. Further studies have shown that GIP is produced in the K cells, which are mainly located in the upper portion of the gut[7]; that GIP is released after meal ingestion and oral glucose in humans[8–14], and enhances insulin secretion at physiological levels[15]; and that a GIP antagonist reduces insulin secretion after oral glucose in humans[16]. These data collectively suggest that GIP is an important incretin hormone, that is, a hormone that is released after oral glucose or meal ingestion and that stimulates insulin secretion.

A characteristic of an incretin hormone is that it is released after meal ingestion. For GIP, we have shown that GIP levels are markedly increased during the first 30 min after meal ingestion in healthy individuals, whereafter they remain persistently elevated for 3 h[11–14]. We have, furthermore, also shown that GIP secretion is dependent on the size of the meal[13]. We have

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Table 1 | Studies where individual classes of macronutrients have been ingested in healthy, non-diabetic, non-obese individuals

Challenge	No. participants	Fold increase of GIP levels	Peak time (min)	Reference
Glucose				
Glucose 25 g	12	9.2	30	22
Glucose 50 g	10	3.2	30	17
	10	3.7	30	23
Glucose 75 g	25	2.3	30	24
	18	2.5	30	25
	45	3.1	30	27
	163	7.1	30	28
Glucose 83 g	18	18.4	30	19
Glucose 100 g	?	4.4	45	26
	8	3.0	45	8
Glucose 2.2 g/kg	12	6	30	12
Protein				
Grilled turkey (375 kcal)	8	No effect	No peak	8
Whey protein 18 g	12	7.1	30	22
Whey protein 30 g	18	6.5	30	19
Milk/egg protein (Promax®) 2 g/kg	12	9.5	30	18
Beef extract (Bovril®) 45 g	6	No effect	No peak	26
Fat				
Corn oil 67 g	10	4.9	60	30
	10	2	120	17
Corn oil 70 g	6	5.1	180	26
Oleic acid 0.88 g/kg	12	2.9	120	18
Long-chain triglycerides (110 kcal)	18	8.2	120	19
Double cream 84 mL (375 kcal)	8	3.5	90	8
Soybean oil (Intralipid®) 0.9 g/kg	12	2.4	120	29
Double cream 200 mL 96 g	5	8.5	120	31

also shown that GIP secretion does not seem to be dependent on the time during the day when the meal is ingested[11], and is independent on the rapidity of meal ingestion[14].

Studies on individual classes of macronutrients have shown that carbohydrates, fat and protein all stimulate GIP secretion[8,12,17,18]. Conclusions on the relative potencies of the individual classes of macronutrients for GIP secretion are, however, limited, because there are only a few studies that have examined more than one class of macronutrients[8,18], and that in most studies only one dose of each macronutrient was tested. In addition, although one study suggested a synergy between different macronutrients when they were given together[19], this has not been studied systematically. To more systematically explore the relative potency of different classes of macronutrients for GIP secretion and their potential synergy, we have in the present study, first, revisited the human literature to explore studies in which individual classes of macronutrients have been ingested and, second, carried out model experiments in mice.

MATERIALS AND METHODS

Human studies revisited

Studies in which GIP levels were determined after ingestion of carbohydrates, fat or protein in healthy individuals were included in the literature survey. They were identified from review articles on GIP[3,10], and from PubMed using key words GIP, macronutrients, carbohydrates, fat, protein and healthy subjects. Key aspects of each article were identified and reported in the result section and in Table 1.

Animal studies

Experiments were undertaken in female C57BL/6J mice (Taconic, Skensved, Denmark; 4–6 months-of-age). Animals were maintained in a temperature-controlled room (22°C) on a 12:12 h light–dark cycle (light on at 07.00 hours). Mice were fed a standard pellet diet (energy 14.1 MJ/kg with 14% from fat, 60% from carbohydrate and 26% from protein; SAFE, Augy, France) and tap water ad libitum. During experimental days, food was removed from the cages at 07.30 hours or at 10.30 hours, and the experiments started at 09.30 hours or at 12.30 hours; that is, during the light cycle. We used female mice only to avoid the stress of single housing, which is used for male mice. We used the mice randomly during the estrous cycle.

Experimental design

Mice were fasted for 2 h[20]. After the 2-h fast, mice were anesthetized with the combination of Fluafent (i.e., a mixture of fluanisone and fentanyl citrate) and midazolam, as previously

described[21]. In short, 10 mg fluanisone (Key Organics, Camelford, Cornwall, UK) was dissolved in 1 mL sterile water at 70°C for 60 min. This solution was mixed with 1 mL of fentanyl citrate (0.315 mg/mL; Sigma-Aldrich, St. Louis, MO, USA); 100 µL of this solution were given intraperitoneally to each mouse (0.016 mg fentanyl citrate and 0.5 mg fluanisone/mouse). Midazolam (0.167 mg/mouse; Roche, Basel, Switzerland) was also given (100 µL/mouse). Mice were then given glucose (25–125 mg per mouse, dissolved in saline; Sigma-Aldrich), protein mixture (whey protein, 15–120 mg/mouse; dissolved in saline; SELF Omninutrition, Skogås, Sweden) or fat emulsion (Calogen^{R} Neutral, which is based on vegetable fat (from rapeseed and sunflower oil) with 50% long-chain triglycerides (10.6% saturated, 61% monounsaturated and 28.6% polyunsaturated) and 50% water (6–100 mg per mouse; Nutricia Nordica AB, Solna, Sweden) or saline alone or macronutrients mixed together in the stomach through a gastric tube (outer diameter 1.2 mm). Whole blood was sampled in heparinized pipettes from the intraorbital retrobulbar sinus plexus (40 µL) at 0, 15, 30 and 60 min. Plasma was separated by centrifugation and stored at −20°C until analysis for GIP and insulin.

Analyses

Glucose in whole blood was detected with the glucose oxidase method using AccuChek Aviva (Hoffman-La Roche, Basel, Switzerland). Insulin was determined by enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). The intra-assay coefficient of variation of the method was 4% at both low and high levels, and the interassay coefficient of variation was 5% at both low and high levels. The lower limit of quantification of the assay was 6 pmoL/L. Total GIP levels were determined with a mouse GIP enzyme-linked immunosorbent assay kit (Crystal Chem, Elk Grove Village, IL, USA). The intra- and interassay coefficient of variation was <10% at low and high levels, and the lower limit of quantification was 2.5 pmoL/L. The assay showed no cross-reactivity with glucagon, glucagon-like peptide (GLP)-1 or GLP-2.

Statistical analysis

Data are presented as the mean ± standard error of the mean. Suprabasal (incremental) areas under the curves (AUCs) were calculated with the trapezoid rule using GIP levels throughout the 60-min study period. Differences between experimental groups were determined using a two-way analysis of variance (ANOVA) followed by a Sidak's multiple comparisons test using SPSS (version 27; IBM Corp., Armonk, NY, USA). Statistical significance was defined as $P < 0.05$.

RESULTS

Human literature survey

Glucose ingestion

In 11 human studies, glucose was administered orally.[8,12,17,19,22–28] All these studies reported an increase in GIP levels after oral glucose with the peak seen at 30 or 45 min. In most studies, peak levels were 2–4-fold higher than baseline levels, although a few studies reported a higher fold increase. There was no association between glucose dose and fold increase when comparing the different studies (Table 1).

Protein ingestion

Five studies reported GIP levels after ingestion of a protein load.[8,18,19,22,26] Three of them reported an increase in GIP levels with a 6-9-fold increase of the peak levels at 30 min over baseline.[18,19,22] In contrast, in two of the studies, no increase in GIP levels were observed.[8,26]

Fat ingestion

In eight studies, humans were challenged with various types of fat, and GIP levels increased in all of them.[8,17–19,26,29–31] Peak levels were in most studies reported at 2 h after fat ingestion. Fold increase in peak GIP levels over baseline levels varied between two and 8.5, with no association with amount of fat ingested.

Model experiments in mice

Administration of macronutrients alone

Glucose, whey protein and fat emulsion all increased GIP levels (Figures 1 and 2; Table 2). Glucose was most efficient and increased GIP levels to >300 pmoL/L after the highest dose of 125 mg. Peak GIP levels after glucose were seen at 15–30 min, whereafter GIP levels gradually returned to baseline. The threshold dose of glucose to increase GIP levels was between 25 and 50 mg, as after 25 mg glucose, there was no significant increase in GIP levels compared with controls at any time point, whereas after 50 mg glucose, there was a significant increase in GIP levels at 15 min ($P = 0.003$) and 30 min ($P = 0.030$). After 100 mg and 125 mg glucose, GIP levels increased at all time points compared with controls ($P < 0.001$ at all time points). Insulin levels increased by glucose after 100 and 125 mg glucose, with significantly increased GIP levels after 100 mg glucose at 15 min ($P = 0.004$) and 30 min ($P < 0.001$), and after 125 mg glucose also at 60 min ($P = 0.018$). Glucose levels were significantly higher after all four doses of glucose than in controls at 15, 30 and 60 min ($P < 0.001$), except at 60 min after the low dose of 25 mg glucose. AUC_{GIP} was significantly higher after 50 mg ($P = 0.011$), 100 mg ($P < 0.001$) and 125 mg glucose ($P < 0.001$) than in controls, but not after 25 mg. $AUC_{insulin}$ was significantly increased after 100 mg and 125 mg glucose (both $P < 0.001$), and also close to significantly increased after 50 mg glucose ($P = 0.058$). $AUC_{glucose}$ was significantly increased after all four doses of glucose (all $P < 0.001$). Protein administration increased GIP levels after 60 mg and 100 mg, but not after 15 mg. After 60 mg protein, GIP levels were significantly higher than in controls at 15 min ($P < 0.001$) and 30 min ($P = 0.012$), with a trend to be increased also at 60 min

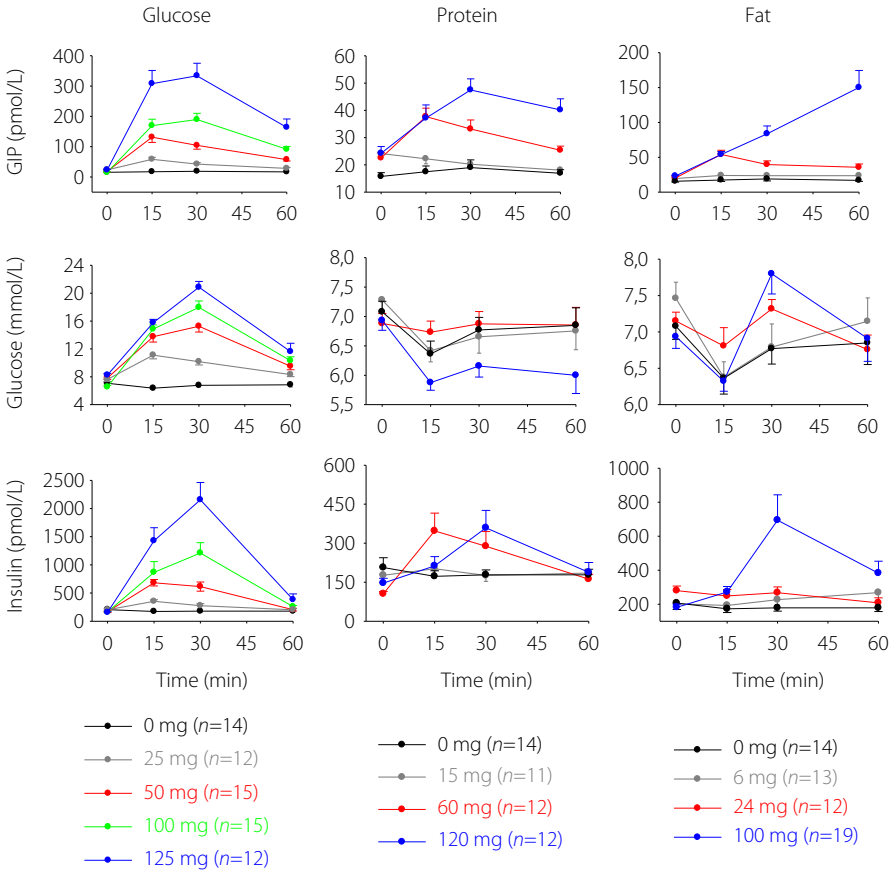


Figure 1 | Glucose-dependent insulinotropic polypeptide, glucose and insulin levels before and after oral administration of glucose (0–125 mg per mouse), whey protein (0–120 mg per mouse) or fat emulsion (0–100 mg per mouse) in normal C57BL/6J mice. Means ± standard error of the mean are shown. Observe that the *y*-axes for the respective panels have been adjusted for the actual levels and are therefore different for the different loads.

($P = 0.089$). After 120 mg protein, GIP levels were significantly higher than in controls at 15, 30 and 60 min (all $P < 0.001$). Insulin levels were not increased after 15 mg protein. In contrast, insulin levels were higher than in controls after 60 mg protein at 15 min ($P = 0.030$) and after 120 mg protein at 30 min ($P = 0.035$). Glucose levels were not significantly affected by protein at any dose. AUC_{GIP} was higher than in controls after 120 mg protein ($P < 0.001$), but not after the lower doses, whereas $AUC_{insulin}$ was higher than in controls after both 60 mg protein ($P = 0.011$) and 120 mg protein ($P = 0.021$). Finally, after 6 mg fat, there was no increase in GIP levels. After 24 mg fat, there was a transient increase in GIP levels with significantly higher levels than in controls at 15 min ($P < 0.001$), but not after 30 or 60 min. After 100 mg fat, GIP levels continuously and gradually increased, with no peak observed within the studied time period of 60 min with significantly higher levels than in controls at 15, 60 and 60 min (all $P < 0.001$). Insulin levels were significantly higher after 100 mg fat compared with controls at 30 min ($P = 0.002$) and 60 min ($P = 0.046$), but not different from controls at any time point after 6 or 24 mg. Glucose levels were not different from controls after any of the fat doses. AUC_{GIP} and $AUC_{insulin}$ were significantly higher after 100 mg fat than in controls (both $P < 0.001$), whereas there was no significant difference between controls and fat after 6 or 24 mg in AUC_{GIP} or $AUC_{insulin}$. $AUC_{glucose}$ did not differ from controls after any of the fat doses.

Figure 2 shows the dose–response relationships between doses of glucose, protein and fat, and AUC_{GIP}, $AUC_{insulin}$ and $AUC_{glucose}$. When statistically analyzing effects of the highest doses, glucose was more potent in increasing AUC_{GIP} than protein and fat (both $P < 0.001$), whereas there was no significant difference between fat and protein ($P = 0.139$). Furthermore, glucose was more potent in increasing $AUC_{insulin}$ and $AUC_{glucose}$ than both protein and fat (both $P < 0.001$), whereas there was no difference between protein and fat administration in regard to $AUC_{insulin}$ and $AUC_{glucose}$.

Co-administration of glucose, protein and fat

To study the potential synergistic effect on GIP levels after co-administering glucose, protein and fat, we used the model of meal tolerance test (MTT; 32). Five different combinations were

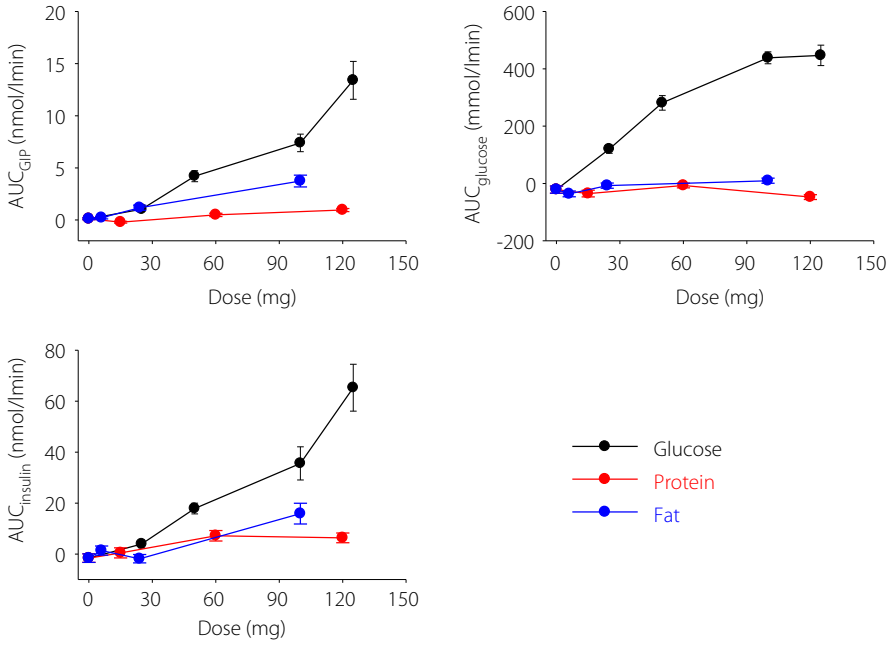


Figure 2 | Suprabasal area under the curve for glucose-dependent insulinotropic polypeptide (AUC_{GIP}), $AUC_{glucose}$ and $AUC_{insulin}$ after oral administration of glucose (0–125 mg per mouse), whey protein (0–120 mg per mouse) or fat emulsion (0–100 mg per mouse) in C57BL/6J mice. Means ± standard error of the mean are shown. There were 11–15 animals in each individual group (see Figure 1 for details).

used. First, we administered a mixed meal with 314 calories (50 mg glucose, 15 mg protein and 6 mg fat) (Figure 3; Table 3), which was selected to be in the same range as carried out in the previous study on GLP-1 secretion[32]. It was found that after this challenge, GIP levels were enhanced similarly as after glucose alone; that is, the addition of protein and fat did not affect the response to glucose alone. Protein and fat addition, however, reduced the glucose and insulin responses compared with glucose alone. To further study the effects of the combination of the nutrients, we increased the dose of protein to 60 mg and fat to 24 mg, and examined how glucose addition from zero to 100 mg would affect the responses. It was found that at 456 and 556 calories (i.e., with glucose 0 or glucose 25 mg added to protein 60 mg and fat 24 mg), there was a synergistic action of the combinations on GIP levels, as these were enhanced significantly more than by adding up the response of each of the nutrients alone (Figure 3; Table 3). At the higher doses (656 and 856 calories, i.e., adding 50 or 100 mg glucose to protein 60 mg and fat 24 mg), the GIP responses were not significantly different from the sum of responses for each of the nutrients given alone; that is, an additive effect. Insulin responses were additive at 456, 556 and 656 calories, whereas a synergy was observed at 856 calories, showing that protein and fat addition enhanced the insulin response to glucose at these higher doses (Figure 4; Table 3). Furthermore, glucose levels were reduced after MTT at 556, 656 and 856 calories compared with the sum of the individual nutrients given alone; that is, adding the nutrients together reduced the expected increase in glucose levels. Table 3 shows the observed AUC_{GIP}, $AUC_{glucose}$ and $AUC_{insulin}$ after each of the MTT tests compared with the sum or responses of the individual macronutrients when given alone. Similarly, Figure 5 summarizes the GIP data for all five MTT challenges. A slight significant synergy was observed at the dose of 456 and 556 calories, whereas when glucose levels were raised to 50 and 100 mg, there was no additional synergy by protein and fat.

DISCUSSION

Glucose-dependent insulinotropic polypeptide is an important incretin hormone in humans, which is evident by a study showing that infusion of a GIP receptor antagonist reduces insulin secretion after combined oral glucose and mixed meal challenge by 30%[16]. Another experimental study in humans also showed that GIP is equally important as GLP-1 as an incretin hormone[33]. To act as an incretin hormone, GIP is secreted into the circulation from the gut K cells after meal ingestion, with a peak level seen after approximately 30 min[3,10–14]. The postprandial levels are, furthermore, sufficient to stimulate insulin secretion[15].

Previous studies have presented characteristics of GIP secretion in humans. It has thus been shown that the size of the meal matters[13]. Thus, ingestion of a meal with 743 kcal elicited a ≈25% higher GIP response than a 511 kcal meal with exactly the same macronutrient composition, and a 1,046 kcal meal

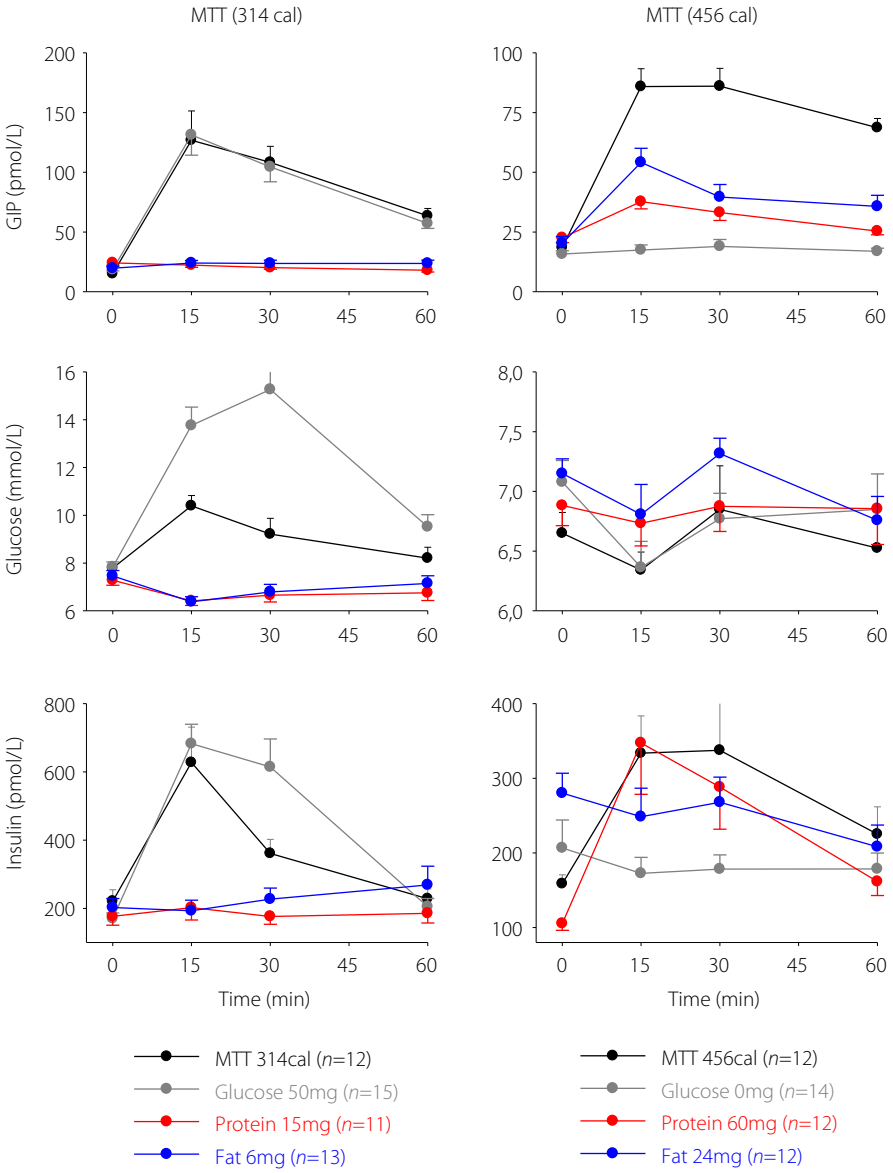


Figure 3 | Glucose-dependent insulinotropic polypeptide (GIP), glucose and insulin levels before and after oral administration of a mixture of glucose, whey protein and fat emulsion or each of glucose, whey protein and fat emulsion alone in C57BL/6J mice. The caloric content of the mixed meal tolerance test (MTT) was 314 and 456 calories, respectively. Bottom of figure shows the composition of each text and number (*n*) of animals in each group. Observe that the *y*-axes for the respective panels have been adjusted for the actual levels and are therefore different for the different loads.

enhanced the GIP response ≈20% further[13]. In contrast, the time of the day does not seem to be of importance for GIP secretion, as the same meal elicited the same GIP response whether ingested at 08.00 hours versus at 17.00 hours[11]. Similarly, rapid or slow ingestion of a meal does not seem to affect GIP secretion, as the same meal ingested over 5 versus over 12 min elicited the same GIP response[14].

The present study revisited the human literature on studies with ingestion of single classes of macronutrients with measurement of GIP levels. The survey clearly showed that there is a consensus between studies that glucose, protein and fat are classes of macronutrients that by themselves stimulate GIP secretion in humans (Table 1). One intention of carrying out this revisit was to explore whether it would be possible to conclude that one or the other of the macronutrients is more potent. Such a conclusion has previously been suggested based on early findings that protein ingestion seemed weaker to stimulate GIP secretion than glucose or fat[3,8,10,26]. However, when

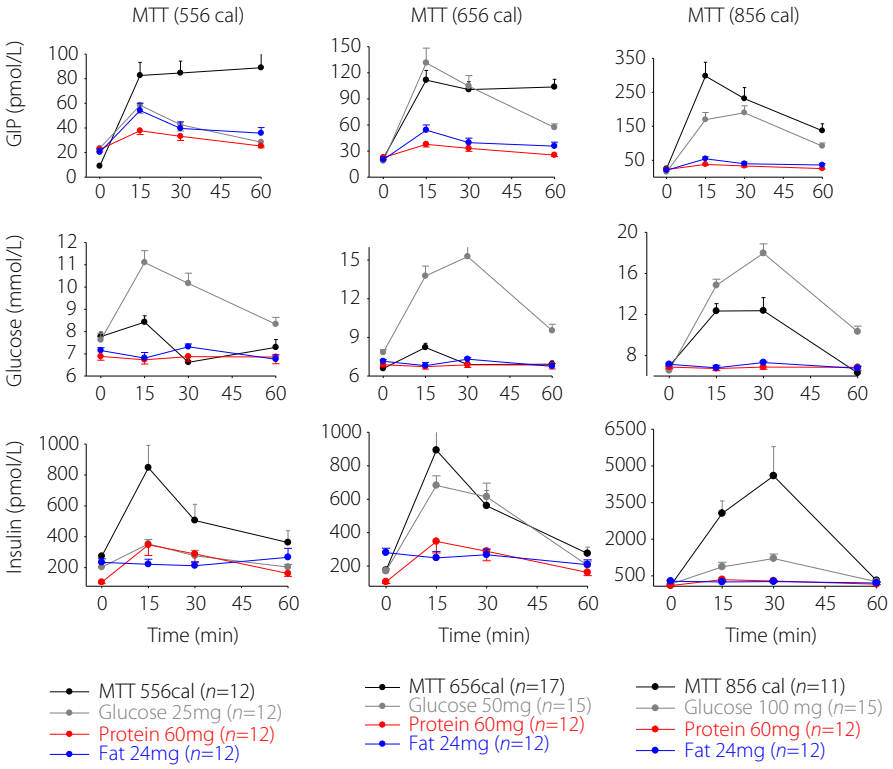
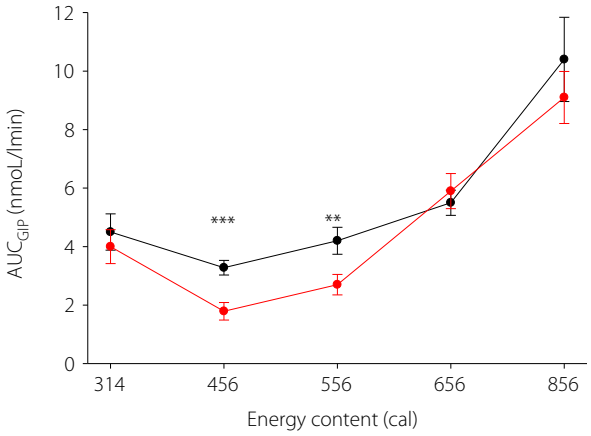


Figure 4 | Glucose-dependent insulinotropic polypeptide (GIP), glucose and insulin levels before and after oral administration of a mixture of glucose, whey protein and fat emulsion or each of glucose, whey protein and fat emulsion alone in C57BL/6J mice. The caloric content of the mixed meal tolerance test (MTT) was 556, 656 and 856 calories, respectively. Bottom of figure shows the composition of each text and number (*n*) of animals in each group. Observe that the *y*-axes for the respective panels have been adjusted for the actual levels and are therefore different for the different loads.

going through all relevant articles, it was obvious that it is not possible to draw such a conclusion. First, there are only very few studies in which more than one class of macronutrient is given[8,18,22] and, second, in most studies, only one single dose of a given macronutrient is given. Third, for protein and fat, different types are given. All this makes it hard to compare the different studies. In fact, not even by analyzing a potential dose–response relationship of the effect of glucose on GIP secretion is possible. Therefore, the conclusion from the human literature is that glucose, protein and fat all have the capacity to stimulate GIP secretion.

Another consistent finding in the human literature is that the peak of GIP levels after macronutrient challenge differ between the macronutrients. Thus, after glucose and protein ingestion, GIP levels peak in most studies at 30 or 45 min; that is, within the same time frame as after a mixed meal[8,12,17–19,22–28]. In contrast, fat administration is associated with a more prolonged and gradual increase in GIP levels, with a peak after approximately 2–3 h[8,17–19,26,29–31]. Although the mechanism of this different time pattern needs to be established, the marked inhibition of gastric emptying, which is evident after fat ingestion, does probably contribute[34,35].

To study the relative potency of different classes of macronutrients more systematically, we turned to model experiments in mice, where we orally administered glucose, whey protein and a lipid emulsion with 50% long-chain triglycerides, and measured GIP levels. We administered glucose, protein and fat emulsion over a large dose range to enable conclusions of their relative potency. We found that glucose, protein and fat all increased GIP levels. Glucose administration increased GIP levels in a glucose-dependent manner with the threshold between 25 and 50 mg, and the strongest effect was seen by the highest dose of 125 mg. Also, protein administration increased GIP levels in a dose-dependent manner, with no increase after 15 mg, whereas after 60 mg, there was significantly increased GIP levels at 15 and 30 min. At the highest dose of 120 mg, GIP levels increased at all time points, also resulting in a significantly increased AUC_{GIP}. Fat administration did not increase GIP levels at 6 mg, whereas at 24 mg there was a transient increase in GIP levels, and after 100 mg, there was a large and continuous increase in GIP levels. Peak GIP levels after glucose and protein were in general seen at 15 or 30 min, whereas after the high dose of fat emulsion, a prolonged and continuous increase in GIP levels was seen with no



Glucose (mg)	50	0	25	50	100
Protein (mg)	15	60	60	60	60
Fat (mg)	6	24	24	24	24
n	12	12	12	17	11

Figure 5 | Suprabasal area under the curve for glucose-dependent insulinotropic polypeptide (AUC$_{GIP}$) after oral administration of a mixture of glucose, whey protein and fat emulsion in C57BL/6J mice compared with the sum of administration of individual macronutrients. The actually observed AUC$_{GIP}$ after each of the mixed meal tolerance test (MTT) challenges are reported in black, whereas the sum of AUC$_{GIP}$ after each of the individual macronutrients when given alone are shown in red. The *y*-axis shows caloric content of each mixture. The composition of each mixture is shown in at the bottom of the figure. Means ± standard error of the mean are shown. There were 11–15 animals in each individual group when the macronutrients were given alone (see Figure 1 for details). Asterisks indicate the probability level of random significance between the groups, $^{**}P < 0.01$, $^{***}P < 0.001$.

observed peak within the 60-min study period. Hence, the different time patterns between glucose and fat, as were identified in human studies, were also evident in mice. An explanation is probably the inhibition of gastric emptying by fat[34,35], which results in different kinetics between glucose, protein and fat, as evident from plasma levels of glucose, amino acids and fat after administration. Thus, glucose levels are rapidly increased after administration, with a peak seen at 15 or 30 min (Figure 1). Also, amino acids are rapidly increased after protein administration, with peak levels at 30–60 min in humans[36], whereas fat levels are more slowly increased after fat administration, as we observed in a previous human study, where it took 180 min for triglyceride levels to be maximal after oral fat administration[29].

Our main finding of the present study was, however, that there indeed is a difference in the potency to stimulate GIP secretion between the classes of nutrients, as glucose was more potent than protein and fat. The earlier conclusion that protein would be less potent to stimulate GIP secretion than glucose[3,8,10,26] seems therefore to be valid, although, as in several human studies[18,19,22], there is a clear effect of protein ingestion to increase GIP levels. There was no significant difference in increasing GIP levels by the highest doses of protein versus fat, which would suggest that these nutrients stimulate GIP secretion by similar potency in mice. However, it should be emphasized that the continuous increase in GIP levels after 100 mg fat mg underestimates the true potency of fat administration by the design of the study with no data point after 60 min.

In our model experiments in mice, we also explored whether macronutrients when given together would have any synergistic action on GIP levels; that is, if their combined action on GIP levels would be significantly higher than expected from adding the responses of the individual macronutrients together or whether there is an additive response of the individual macronutrients. We previously carried out a similar study for GLP-1 secretion, and found that there is a clear synergism by the macronutrients to stimulate GLP-1 secretion in mice, as the GLP-1 response to macronutrients given together exceeded that of the sum of each individual macronutrient[32]. We found that

Table 2 | Suprabasal (incremental) areas under the 0–60 min curves for glucose-dependent insulinotropic polypeptide, glucose and insulin levels after oral administration of glucose, whey protein or fat emulsion in normal C57BL/6J mice

Challenge	*n*	AUC$_{GIP}$ (nmol/L min)	AUC$_{glucose}$ (mmol/L min)	AUC$_{insulin}$ (nmol/L min)
Saline	14	0.12 ± 0.09	−21 ± 13	−1.6 ± 1.7
Glucose 25 mg	12	1.0 ± 0.2	120 ± 14***	4.0 ± 1.5
Glucose 50 mg	15	4.2 ± 0.5*	281 ± 25***	17.9 ± 2.1
Glucose 100 mg	15	7.4 ± 0.9***	439 ± 21***	35.6 ± 6.5***
Glucose 125 mg	12	13.4 ± 1.8***	447 ± 35***	65.3 ± 9.2***
Protein 15 mg	11	−0.2 ± 0.1	−35 ± 11	0.5 ± 2.0
Protein 60 mg	12	0.5 ± 0.2	−7 ± 8	7.2 ± 2.1*
Protein 120 mg	12	1.0 ± 0.1***	−47 ± 8	6.4 ± 2.1*
Fat 6 mg	13	0.2 ± 0.1	−36 ± 10	1.4 ± 1.8
Fat 24 mg	12	1.2 ± 0.2	−7 ± 9	−1.8 ± 1.7
Fat 100 mg	19	3.7 ± 0.6***	10 ± 9	15.9 ± 4.0***

Means ± standard error of the mean are shown. Asterisks show the probability level of random difference compared with the saline control; $^{*}P < 0.05$; $^{***}P < 0.001$. AUC, area under the curve.

Table 3 | Suprabasal (incremental) areas under the 0–60 min curves for glucose-dependent insulinotropic polypeptide, glucose and insulin levels after the mixed meal tolerance test in five different combinations of glucose, protein and fat in normal C57BL/6J mice in comparison with the sum of areas under the curve after each of the individual macronutrients

MTT test (calories)	Composition (mg per mouse)	*n*	AUC_{GIP} (nmol/L min)	$AUC_{glucose}$ (mmol/L min)	$AUC_{insulin}$ (nmol/L min)
314 calories	G50 P15 F6	12	4.5 ± 0.6	77 ± 16**	9.4 ± 1.8*
Sum of individual tests			4.2 ± 0.6	210 ± 35	19.8 ± 4.3
456 calories	G0 P60 F24	12	3.2 ± 0.2***	−2 ± 13	7.6 ± 2.7
Sum of individual tests			1.7 ± 0.3	−14 ± 21	5.4 ± 2.3
556 calories	G25 P60 F24	12	4.0 ± 0.5**	−24 ± 11**	14.0 ± 3.3
Sum of individual tests			2.7 ± 0.4	106 ± 35	9.4 ± 2.9
656 calories	G50 P60 F24	17	4.4 ± 0.4	36 ± 11***	21.0 ± 4.0
Sum of individual tests			5.9 ± 0.6	267 ± 45	23.3 ± 5.4
856 calories	G100 P60 F24	11	10.4 ± 1.4	191 ± 26***	148 ± 33**
Sum of individual tests			9.1 ± 0.9	425 ± 56	70.7 ± 18

Means ± standard error of the mean are shown. Asterisks indicate probability level of random difference were the saline control; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. AUC, area under the curve; F, fat; G, glucose, GIP, glucose-dependent insulinotropic polypeptide; MTT, mixed meal tolerance test; P, protein.

there is also a trend for a synergy between the macronutrients for GIP secretion, as after 456 and 556 calorie ingestion of macronutrients together, the GIP response was significantly higher than by adding the response of each individual macronutrient together.

However, when increasing the caloric content by adding more glucose, it seems as if the effect of glucose dominates, such that there is no further augmentation by the protein and fat ingestion. This is most likely explained by the potent action of glucose alone, such that when GIP secretion is markedly stimulated by the high doses of glucose, the lower actions of protein and fat will not cause a synergistic enhancement, but only an additive. There is also a possibility that synergistic and saturated additive effects of different macronutrients are mediated by direct effects on the GIP secreting K cells, based on previous data on mechanistic aspects of stimulation of these cells[37,38]. However, to what extent the synergistic action of the macronutrients at lower glucose levels is explained by direct effects on the GIP producing K cells and whether such effects are saturated at high-glucose doses remains to be studied.

In contrast, there is a clear effect on insulin levels (significantly enhanced) and glucose levels (being markedly reduced) by adding protein and fat to the glucose challenge. This shows that a mixed meal has a glucose-lowering effect compared with glucose alone, which also has been shown in humans[19]. Our current data suggest, however, that this synergy of a mixed meal on glucose and insulin levels is not mediated by GIP.

A strength of our human literature survey was that we collected many studies with macronutrient ingestion, which allows a more general conclusion than is possible from each of the studies. Furthermore, a strength of the mouse study was that we used many different doses, both when giving macronutrients alone and together. A limitation of the present study was that we carried out the intervention part in mice only, which limits the potential conclusions for the human condition. Another potential limitation was that residual food content in the gut at the time of the experiments would have affected GIP secretion. However, it has been shown that gastrointestinal transit time in mice is such that 80% has passed through after 60 min[39], which suggests that this effect is probably minor in the present experiments in which the mice were fasted for 2 h.

In conclusion, we have identified several human studies where single classes of macronutrients have been given with determination of GIP levels, and we conclude that glucose, protein and fat all stimulate GIP secretion. Furthermore, there is no clear difference between the macronutrients in potency to stimulate GIP secretion, whereas there is a clear difference in time pattern, with peak levels seen at approximately 30–45 min after glucose and protein, whereas a prolonged action with peak levels after 2–3 h is seen after fat ingestion. Furthermore, also in model experiments in mice, we showed that glucose, protein and fat administration stimulate GIP secretion. In mice, glucose is more potent than protein and fat, with no significant difference between protein and fat. Finally, in mixed macronutrient ingestions, there is a synergy between the macronutrients on GIP secretion at moderate caloric doses, whereas at higher caloric doses by raising the glucose content, such synergy is lost and the effect is additive. We also suggest that further studies are warranted in humans to explore the relative potency of macronutrients, and whether synergistic effects might allow nutritional advice to optimize the secretion of this incretin hormone.

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DISCLOSURE

The author declares no conflict of interest.
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Informed consent: N/A.
Registry and the registration no. of the study/trial: N/A.
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DEDICATION

This work is dedicated to Dr Susumo Seino, the great researcher and inspirer, who sadly passed away in 2021.

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