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Synergism by individual macronutrients explains the marked early GLP-1 and islet hormone responses to mixed meal challenge in mice

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Abstract

Apart from glucose, proteins and lipids also stimulate incretin and islet hormone secretion. However, the glucoregulatory effect of macronutrients in combination is poorly understood. We therefore developed an oral mixed meal model in mice to 1) explore the glucagon-like peptide-1 (GLP-1) and islet hormone responses to mixed meal versus isocaloric glucose, and 2) characterize the relative contribution of individual macronutrients to these responses.

Anesthetized C57BL/6J female mice were orally gavaged with 1) a mixed meal (0.285 kcal; glucose, whey protein and peanut oil; 60/20/20 % kcal) vs. an isocaloric glucose load (0.285 kcal), and 2) a mixed meal (0.285 kcal) vs. glucose, whey protein or peanut oil administered individually in their mixed meal caloric quantity, i.e., 0.171, 0.055 and 0.055 kcal, respectively. Plasma was analyzed for glucose, insulin and intact GLP-1 before and during oral challenges.

Plasma glucose was lower after mixed meal vs. after isocaloric glucose ingestion. In spite of this, the peak insulin response (P=0.02), the peak intact GLP-1 levels (P=0.006) and the estimated β-cell function (P=0.005) were higher. Furthermore, the peak insulin (P=0.004) and intact GLP-1 (P=0.006) levels were higher after mixed meal ingestion than the sum of responses to individual macronutrients.

Compared to glucose alone, we conclude that there is a marked early insulin response to mixed meal ingestion, which emanates from a synergistic, rather than an additive, effect of the individual macronutrients in the mixed meal and is in part likely caused by increased levels of GLP-1.

Keywords: macronutrients; insulin; GLP-1, incretin, mixed meal

Abbreviations: GPCR: G-Protein coupled receptors, GLP-1: Glucagon-like peptide 1, sAUC: Suprabasal area under the curve, tAUC: Total area under the curve, QUICKI: Quantitative insulin sensitivity check index, OGIS: Oral glucose insulin sensitivity index

1. Introduction

Glucose tolerance and islet hormone secretion are commonly studied by using a variety of metabolic tests among which the oral glucose tolerance test is one of the most exploited [1]. Such studies have revealed the complexity of glucose regulation, which is elicited by passing glucose through the gastrointestinal tract and the importance of nutrients, neural and hormonal factors for glucose metabolism [2]. Of key importance are the incretin hormones which are released after oral glucose and account for more than 50% of total insulin release after an oral glucose load compared to an isoglycemic intravenous glucose bolus [3, 4]. One key incretin hormone is glucagon-like peptide-1 (GLP-1), which is secreted from intestinal L-cells [5] and potentiates glucose-stimulated insulin release [6] through G_S-protein-coupled rise in intracellular cAMP [7].

Nonetheless, for the study of physiological glucose regulation, intake of pure glucose load is not physiological feeding. Therefore, studies with mixed meal ingestion have been undertaken, and also after mixed meal ingestion there is a marked release of insulin and GLP-1 [8]. Apart from glucose, also proteins and lipids regulate insulin secretion both directly on the islet level and indirectly through the release of incretin hormones, as demonstrated in both rodents [9] and humans [10]. Combined with inhibitors of GLP-1 inactivation, strategies to increase endogenous GLP-1 secretion through dietary interventions is a potential future therapy of type 2 diabetes [11]. This raises the question to what degree the different individual macronutrients in a mixed meal contribute to the insulin and GLP-1 response and whether the individual macronutrients act synergistically. To study this, we have developed an experimental model in mice based on the oral administration of a mixed meal, consisting of glucose, whey protein and peanut oil. With the use of this novel model, the aim of the present study was 1) to explore the GLP-1 and islet hormone responses to a mixed meal versus an isocaloric glucose load, and 2) to characterize the impact of the contribution of the single macronutrients to the GLP-1 and islet responses to mixed meal.

2. Material and methods

2.1. Animals

Female C57BL/6J mice (average 22 g) were obtained from Taconic (Skensved, Denmark). Upon arrival, the mice were fed a standard rodent diet D12450B (Research Diets, New Brunswick, NJ, USA) containing 10% fat, 20% protein and 70% carbohydrates of total energy content. Food and water was provided *ad libitum*. The animals were housed in groups of eight per cage in a temperature-controlled (22°C) room with artificial lighting maintained on a 12h light/12h dark cycle. All experimental procedures were performed in agreement with the Animal Ethics Committee in Lund, Sweden.

2.2. Experimental procedure

The oral tolerance testing was performed during early afternoon after removal of food from cages 5h previously. Fasted mice were anesthetized with an intraperitoneal injection of midazolam (0.4 mg/mouse, Dormicum®, Hoffman-La Roche, Basel, Switzerland) and a combination of fluanisone (0.9 mg/mouse) and fentanyl (0.02 mg/mouse, Hypnorm®, Janssen, Beerse, Belgium). In a first experimental series, at 30 minutes post-anesthesia, the mice were orally challenged (0.5 ml) with either a mixed meal, consisting of a mixture of glucose (45 mg/mouse, Sigma), whey protein (14.9 mg/mouse, SELF Omninutrition) and peanut oil (6.3 mg/mouse, Zeta), with total caloric content of 0.285 kcal with 60/20/20% glucose, protein and lipids, or an isocaloric (0.285 kcal) glucose load (75 mg/mouse). To elucidate the impact of individual macronutrients in the response to mixed meal, a second experimental series was undertaken in which glucose, whey protein or peanut oil was given separately or together in the same quantity as in meal, i.e., 0.171 kcal glucose, 0.057 kcal whey protein and 0.057 kcal peanut oil, all given in a standardized volume of 0.5 ml. All nutrients were administered through a gavage tube (outer diameter 1.2 mm) placed in the stomach. Blood samples were collected from the retrobulbar, intraorbital, capillary plexus before and either 5, 10 and 20 minutes or 15, 30, 60 and 90 minutes after oral gavage for plasma glucose and insulin determination in heparinized tubes. For intact GLP-1 measurements, blood samples were collected before and 5, 10 and 20 minutes after oral challenge into appropriately pre-treated tubes, containing a combination of the protease inhibitor aprotinin (Trasylol; 500 KIE/ml Bayer, Leverkusen, Germany) and the inhibitor of the GLP-1 degrading enzyme dipeptidyl peptidase-4, valine pyrrolidide (0.03 mM, Novartis,

Basel, Switzerland), respectively. After collection, all blood samples were immediately centrifuged (4°C) and plasma was stored (-20°C) for subsequent analysis.

2.3. Plasma analysis

Plasma insulin was analyzed with sandwich immunoassay technique (ELISA) using double monoclonal antibodies against insulin (Mercodia, Uppsala, Sweden). Plasma glucose was measured with the glucose oxidase method using 2,2´-azino-bis(3-ethyl-benzothialozine-6-sulphonate) as an substrate with the absorbance measured at 420 nm on a microtiter plate reader (Fluostar/Polarstar Galaxy; BMG Labtechnologies; Offenburg; Germany). Levels of intact GLP-1 were determined by sandwich immunoassay technique (ELISA) using monoclonal antibodies specific for the intact form of GLP-1 (Meso Scale), whereby electrochemiluminescent labelling enables detection of voltage-mediated chemiluminescence in the SECTOR Imager plate reader (Meso Scale, Gaithersburg, USA).

2.4. Calculations and statistical analysis

Data are reported as mean values ± SEM. Basal insulin sensitivity was determined with the quantitative insulin sensitivity check index (QUICKI) [12], based on fasting plasma glucose and insulin levels, and dynamic insulin sensitivity was estimated with the oral glucose insulin sensitivity index (OGIS) [13]. Suprabasal (sAUC) and total (tAUC) areas under the curve were calculated by the trapezoidal rule for glucose and insulin data during time intervals 0-30 and 0-90 min and for intact GLP-1 during 0-20 min. β-cell function during 30 and 90 min of challenge was calculated with an insulinogenic index, here defined as the ratio between AUC_{INS}/AUC_{GLU}. Statistical significances were assessed by using Student´s t-test.

3. Results

3.1. Mixed meal versus isocaloric glucose: plasma glucose, insulin and GLP-1 responses

To evaluate the GLP-1 and insulin responses to mixed meal versus oral glucose, C57BL/6J mice were orally gavaged with either a mixed meal (0.285 kcal) or an isocaloric glucose load (0.285 kcal). As shown in Figure 1A, plasma glucose levels peak at 15 min after both challenges and thereafter glucose levels

gradually declined throughout the 90 min study period. The peak (15 min) plasma glucose level was markedly lower after mixed meal versus glucose intake (19.8±0.9 versus 27.5±1.8 mM, P=0.001), which is expected considering the different amount of glucose in the two challenges. However, in contrast to plasma glucose levels, the early (15 min) insulin response was significantly higher after mixed meal versus glucose intake (2.8±0.3 versus 1.7±0.3 nM, P=0.02) (Fig. 1B). This early insulin response was followed by a sustained later (30 min) response after oral glucose compared to mixed meal (1.1±0.2 versus 0.6±0.1 nM, P=0.03). After both challenges, insulin levels were back at baseline after 60 min. As for insulin, also intact GLP-1 in plasma was significantly higher in early samples after intake of mixed meal versus glucose. The GLP-1 responses were more rapid than the insulin responses and showed the peak at 5 min (1.2±0.1 pg/ml after mixed meal versus 0.6±0.2 pg/ml after glucose alone, P=0.006), whereas a smaller elevation of intact GLP-1 was observed at the end (20 min) of glucose challenge (0.22±0.07 versus 0.04±0.03 pg/ml, P=0.05) (Fig. 1C). To match the GLP-1 data, plasma insulin data at earlier time points showed a significant increase at both 5 and 10 minute after mixed meal versus isocaloric glucose (5 min: 1.5±0.3 versus 0.7±0.1 nM, P=0.005 and 10 min: 2.3±0.3 versus 1.2±0.1 nM, P=0.006) (insert in Fig. 1B), leading to lowering of plasma glucose levels (20 min: 13.9±0.9 versus 22.4±1.2 mM, P=0.00001) (insert in Fig. 1A).

3.2. Mixed meal versus isocaloric glucose: β-cell function and insulin sensitivity

Table 1 displays basal body weight, indices of basal and dynamic insulin sensitivity as well as insulin secretion and β-cell function data during mixed meal (0.285 kcal) or isocaloric glucose (0.285 kcal) challenges. When observing basal data, there were no difference in body weight or basal insulin sensitivity (QUICKI index) between groups. However, the dynamic OGIS index of glucose clearance was higher after intake of glucose versus mixed meal (241±11 versus 200±11 ml min-1 kg-1, P<0.05), indicating that the isocaloric glucose load improves glucose clearance from the blood. This increased rate of glucose clearance after the pure glucose load was not present during the first 30 min of challenge and thus did not affect the early GLP-1 and insulin responses. The insulinogenic index revealed that early β-cell function was significantly higher after intake of mixed meal compared to glucose (88±11 versus 45±8 nM/mM, P=0.005) whereas no difference was found when considering the whole 0-90 interval. Also, linear regression analysis of the early (30 min) AUC insulin and AUC glucose showed that at each plasma

glucose level the mixed meal challenge elicited a higher ability of the islet β-cells to respond to changes in plasma glucose (Fig. 2).

3.3. Mixed meal versus individual macronutrients: synergistic increase in plasma GLP-1 and insulin levels

We next sought to elucidate whether the exaggerated GLP-1 and insulin responses to mixed meal compared to oral glucose were additive or synergistic in relation to the responses of the individually ingested macronutrients. We therefore orally gavaged the C57BL/6J mice with mixed meal (0.285 kcal) or the individual macronutrients in their respective mixed meal caloric concentrations (glucose 0.171 kcal, protein 0.057 kcal and lipids 0.057 kcal). There were no difference in body weight or basal insulin sensitivity (QUICKI index) between groups (data not shown). After mixed meal, plasma glucose levels peaked at 15 min with no significant difference versus glucose alone (Fig. 3A). Thereafter, glucose levels declined, with a significantly higher elimination after mixed meal compared to glucose load. In contrast, protein or lipid ingestion did not significantly affect plasma glucose levels. All challenges elicited an insulin response; however, the peak (15 min) insulin response to mixed meal was substantially higher than the added responses to the single macronutrients (2.4±0.5 vs. 1.1±0.1 nM, P=0.004) (Fig. 3B). Also, the early sAUC insulin response (0-30 min) after mixed meal ingestion was markedly exaggerated compared to the added responses of the single macronutrients (38.4±9.1 vs. 17.1±2.4 nM x30 min, P=0.004), i.e. glucose (14.5±2.2 nM x30 min), protein (1.8±0.3 nM x30 min) and lipids (0.8±0.3 nM x30 min) (Fig. 4A). Similarly, the peak (5 min) intact GLP-1 response after mixed meal was significantly higher compared to the sum of responses to the single macronutrients (1.2±0.1 versus 0.6±0.2 pg/ml, P=0.006) (Fig. 3C). Also, when adding together the early sAUC GLP-1 response (0-20 min) to the individual macronutrients (glucose 3.1±1.0, protein 0.9±0.4 and lipids 0.2±0.1 pg/ml x 30 min) there was an significantly higher intact GLP-1 levels after mixed meal (9.0±29 vs. 4.2±1.1 pg/ml x 30 min, P=0.02) (Fig. 4B). Thus, by combining glucose with protein and fat there was an potentiating effect on early GLP-1 and insulin secretion that was not obtained by adding together the responses to the individual macronutrients.

4. Discussion

This study describes and explores a novel model, the oral meal tolerance test in C57BL/6J mice, for evaluation of GLP-1 secretion and β-cell function under a more physiological condition than after pure glucose. The first main finding of the current study is that the mixed meal significantly exaggerated early insulin secretion compared to an isocaloric glucose challenge, in spite of lower plasma glucose levels after mixed meal. This is similar as previously inferred from studies in rats [14] and humans [15, 16]. However, an important novelty of the present study compared to the previous observations is that total calories were here completely matched between the mixed meal and glucose challenges, and therefore our investigation controls for the confounding factor of different caloric intake. A consequence of our result is that by replacing part of the glucose in the oral challenge with protein and fat will augment β-cell insulin release and result in increased insulin secretion at lower glucose levels as a sign of increased glucose sensitivity in the ß-cells. A second main finding of this study is that there is a synergistic interaction between macronutrients of the mixed meal to stimulate intestinal GLP-1 and insulin secretion. We suggest that this is the main contributor to the observed synergistic increase in insulin release during the mixed meal challenge. This conclusion was based on the observation that when giving the macronutrients of the mixed meal separately in their meal calorie concentrations, the sum of GLP-1 and insulin responses to the individual macronutrients was less than half of that after intake of mixed meal.

The ability of the body to utilize blood glucose depends on the capacity of the β-cells to respond to changes in blood glucose, and the sensitivity of peripheral tissues to the action of insulin [17]. Since glucose is considered the main regulator of insulin release [18], it may be expected that a pure glucose load would elicit an insulin response that is higher or no less than that of an isocaloric mixed meal (60% glucose). On the contrary, we observed a pronounced elevation of the early insulin secretion after intake of mixed meal versus isocaloric glucose load. Also, at a specific plasma glucose level, intake of the mixed meal leads to an increase in β-cell function in comparison to isocaloric glucose as determined by calculating the insulinogenic index, i.e., relating the increase in insulin levels to the ambient glucose levels. Since non-glucose nutrients have direct effects on β-cells insulin secretion, the glucose-based insulinogenic index may, however, be somewhat misleading to quantify ß-cell function; nevertheless the insulinogenic index is used to illustrate the difference in insulin release between mixed meal and isocaloric glucose load. Glucose-stimulated insulin secretion is known to be biphasic with an early

transient stimulation of insulin release, followed by a second phase of gradually released insulin [19]. It has been demonstrated that first phase insulin secretion is crucial for proper lowering of blood glucose after food ingestion and, interestingly, replacing 40% of the total calories in the pure glucose load with protein and lipids facilitates the early β-cell insulin release. Thus, this observation led us to conclude that nutrient origin seems to be more important for a strong early insulin response than the prevailing plasma glucose levels. Deterioration of this early insulin secretion is one of the earliest sign of type 2 diabetes [20, 21], and thus, it would be beneficial to use the current meal tolerance test methodology to gain more insight into the physiological adaptations that occur during development of glucose intolerance.

Apart from the stimulation of islet insulin release by absorbed nutrients, passing nutrients through the gastrointestinal tract stimulates the release of incretin hormones, such as GLP-1, that also contribute substantially to the insulin response during oral challenge. It has thus been shown that carbohydrate, protein and lipid challenges all stimulate GLP-1 secretion [10, 22]. For this reason we also determined the GLP-1 response to mixed meal challenge and found that the insulin secretion after mixed meal ingestion is accompanied by a marked early increase in intact GLP-1 levels compared to the isocaloric glucose ingestion, again in spite of a lower glucose dosing and plasma level. This increase in intact GLP-1 levels thus may contribute to the difference in insulin secretion that we found between the isocaloric mixed meal and glucose challenge. Also, measuring plasma insulin at early time points confirms the association of increased early GLP-1 and insulin secretion, with improvements in glycemia. The components of the mixed meal; glucose, protein and fat, may raise the plasma level of intact GLP-1 through direct stimulation of L-cell hormone release or possibly through inhibiting the enzymatic cleavage of intact GLP-1 by dipeptidyl peptidase 4 (DDP-4), as previously shown after protein supplementation of glucose load [9]. Due to the lack of reliable assays for intact glucose-dependent insulinotrophic peptide (GIP) levels in mouse plasma, the potential role of GIP, which is the other important incretin hormone, in the synergistic effects of a mixed meal on insulin release cannot be concluded in this study.

Similar to our findings, Zinker *et al* have found that an oral mixed meal (Ensure) was a stronger stimulus of insulin secretion than an oral glucose load in rats, although the amount of Ensure and glucose in this study was not matched for calories [14]. Matching of calories is a foundation for comparison, since the caloric content of a meal has been shown to be an important factor in the release of both incretin

hormones and insulin [23, 24]. Furthermore, pure glucose is compared to other carbohydrates sources in the Ensure liquid meal formula, such as maltodextrine and sucrose, and it is not taken into account that various carbohydrates affect glucose tolerance differentially [25], which is also true for different protein and lipid sources [26-28]. To avoid this bias we have prepared the current mixed meal with glucose, whey protein and peanut oil only and compared the responses to mixed meal with an isocaloric load of pure glucose.

Oral administration of nutrients by gavage in anesthetized mice is a common methodology in animal studies to explore regulation of glucose homeostasis. This technique involves inherent limitations that are highlighted in this section. Oral gavaging during daytime may be considered unphysiological feeding since mice eat continuously throughout the dark period, with 70 % of food intake during night [29]. A gavage with a single meal, leads also to higher peak glycemic excursions that are seen throughout the 24h period in mice. Also, the cephalic phase of metabolic regulation is also lost when delivering the nutrients directly into the stomach. On the other hand, one of the main benefits of oral gavaging is to ensure reproducibility of results in that every mouse receives the same standardized meal during a set time interval. Furthermore, in this study blood sampling is performed via the capillary plexus in the orbital cavity which necessitates the use of anesthesia, which on one hand results in unstressed animals, compared to gavaging and immobilization of conscious mice, but on the other hand may have allowed the anesthesia per se to have some influence. It is important to emphasize, that, as demonstrated by Andrikipoulus *et al*, there are no notable effects between conscious and anesthetized mice on the glycemic responses to an oral glucose challenge [30]. It is also worth pointing out that the rate of entry of nutrients into the small intestine, which is dependent on both volume and caloric load [31], has an effect on glycemia. For example, studies in humans showed that a relatively high intraduodenal glucose load (4 kcal/min) resulted in changes in pylorus-duodenal pressure [32], which is associated with slowing of gastric emptying [33]. Thus, one possible explanation to the difference in insulin secretion after gavage of the mixed meal and isocaloric glucose is that the elevation of plasma glucose after the glucose load will slow down subsequent gastric emptying of glucose and therefore lower and/or delay glucose-stimulated insulin secretion. On the other hand, the mixed meal contains 40% kcal of protein and fat that are also potent inhibitors of gastric emptying [9]. Also, differences in protocol, i.e. the current glucose challenge is

given acutely while Pilichiewicz *et al* have infused glucose into the duodenum during 120 minutes, may explain discrepancies in results. However, this is an unlikely explanation since the peak of insulin occurred at the same time point for both mixed meal and isocaloric glucose load. Nevertheless, the relationship between the rate of entry of nutrients into the intestine and gastric emptying is important to consider and warrants further studies.

In the current study, we show that intake of a mixed meal is superior to an isocaloric glucose load in evoking a strong early insulin release, accompanied by an elevated early plasma level of intact GLP-1. To elucidate the contribution of the individual macronutrients to these responses; glucose, protein and lipids were given seperately in their meal calorie concentration. Then, it was found that there is a 47% increase in intact GLP-1 levels after mixed meal compared to the sum of responses to the individual macronutrients. Hence, when glucose is ingested together with protein and lipids, the early level of intact GLP-1 was greater than the sum of the responses to individual macronutrients, indicative of synergistic, rather than additive effects of the macronutrients. This synergistic increase in early GLP-1 levels thus may explain the difference in insulin secretion that we found between mixed meal and isocaloric glucose. Indeed, there was also an approximate 45% difference in early AUC insulin secretion between the mixed meal and the added responses to the individual macronutrients. The adding of insulin data for the individual macronutrients is, however, somewhat complicated, since the incretin effect is believed to be glucose dependent [34], meaning that without an elevation of blood glucose any increase in plasma GLP-1 levels by protein or lipids will likely not be translated into frank insulin secretion.

The L-cells are open-type endocrine cells in the mucosa of the gastro-intestinal tract and contact with unabsorbed nutrients with their luminal surface is considered the main stimulus of GLP-1 secretion. Similarly to islet β-cells, GLP-1 secreting cell lines respond to glucose stimulation with changes in electrical membrane potential, Ca^{2+} influx and GLP-1 hormone release [35]. Glucose-independent mechanisms of GLP-1 secretion involve various amino acid and peptide transporters [36, 37], whereas fatty acids and lipids have been linked to the activation of various G-protein coupled receptors (GPCR) in the cell membrane [38, 39]. Hence, glucose, protein and lipids are all individually capable of direct stimulation of GLP-1 secretion, however, the mechanism of synergism between the macronutrients remains to be resolved. One possible explanation of synergism may be that protein and lipids potentiate

the stimulatory effect of glucose on the L-cell. There is some evidence that a protein preload will prime the L-cells to response better to a subsequent meal stimulus [40]. In line with this, amino acid transport in GLUTag cells has been shown to induce a small Na^+-dependent inward current, which increases the excitability and firing rate of the cell in response to further stimuli [37]. Furthermore, apart from fatty acid mediated insulin release through activation of various GPCRs, lipid ingestion leads to the secretion of bile acid into the intestine that also has been shown to stimulate GLP-1 release [41]. On the other hand, there is evidence for the majority of L-cells being located in the distal part of the intestine, enabling rapid control of GLP-1 secretion by upper gut neural or hormonal pathways after a meal [42, 43].

In conclusion, with the use of the novel oral meal tolerance test for evaluating islet and incretin hormone secretion in mice, we here show that there is a markedly augmented early insulin secretion after mixed meal compared to an isocaloric glucose challenge, in spite of a lower glycemia, and we suggest that this in part is mediated by the similarly increased levels of intact GLP-1. We also show that the increases in GLP-1 and insulin levels after mixed meal are mediated through synergistic effects of the macronutrients. Thus, replacing part of the total calories in an pure glucose load with protein and fat will lead to a marked increase in early β-cell function. Whether this synergism is due to the direct stimulation of intestinal L-cell GLP-1 release or if other factors, such as inhibiting DPP-4 activity, are involved in the GLP-1 response is to be determined. Hence, compared to glucose alone, combining glucose with protein and lipids as part of a mixed meal offers a physiological tool for exploration of incretin and islet hormone secretion in studies on integrative metabolism and drug development.

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Disclosure statement

All authors have approved the final version of the article. There are no conflicts of interest to disclose for any of the authors.

Author contributions: L Ahlkvist contributed with the design of the study, undertaking of the experiments, analyses and statistics and writing the manuscript. J Vikman contributed with the design of the study and gave comments to the manuscript, G Pacini contributed with calculations and assessment of beta cell function data and gave comments to the manuscript. B Ahrén contributed with design of the study and writing the manuscript.

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Figure legends

Fig. 1. Plasma glucose (A), insulin (B) and intact GLP-1 (C) levels after oral gavage (0-90 min) of mixed meal (0.285 kcal, ●) or isocaloric glucose load (0.285 kcal, ○) in 5h fasted and anesthetized C57BL/6J mice. Early (0-20 min) plasma glucose and insulin levels are inserted into the upper right corner of figure 1A and 1B, respectively. Means ± S.E.M. are shown, n=10-12 mice per group.

Fig. 2. Early β-cell function was determined by linear regression analysis of AUC insulin and glucose in plasma during 30 min of mixed meal (0.285 kcal, ●) versus isocaloric glucose (0.285 kcal, ○) challenge in 5h fasted and anesthetized C57BL/6J mice (n=12 mice per group).

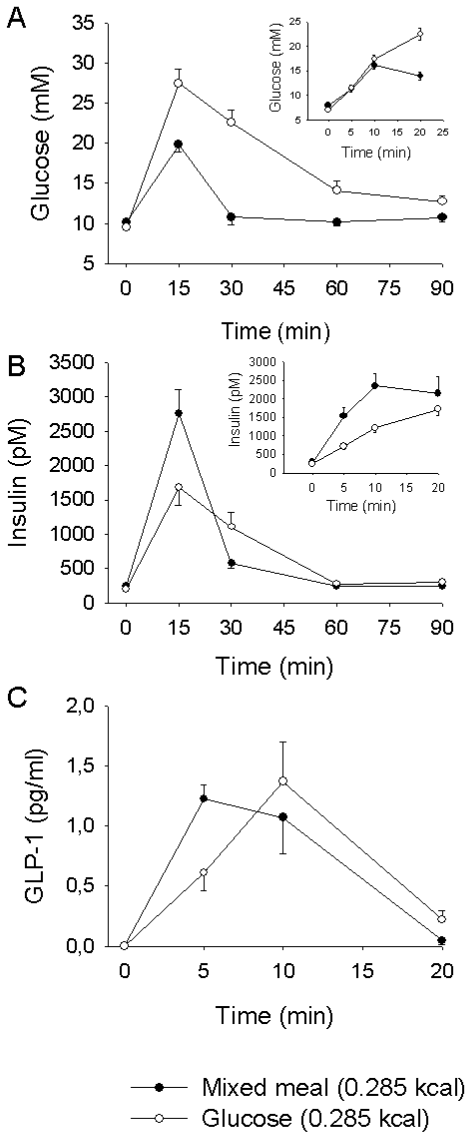
Fig. 3. Plasma glucose (A), insulin (B) and intact GLP-1 (C) levels after oral gavage (0-90 min) of mixed meal (0.285kcal, ●) or individual macronutrients in their mixed meal calorie concentrations; glucose (0.171 kcal, ○), protein (0.057 kcal,▼) and lipids (0.057 kcal, ▽), in 5h fasted and anesthetized C57BL/6J mice. Means ± S.E.M. are shown, n=10-12 mice per group.

Fig. 4. Suprabasal area under the curve (sAUC) of insulin (A, 0-30 min) and intact GLP-1 (B, 0-20 min) after oral gavage of mixed meal (0.285 kcal) versus the sum of individual responses to glucose (0.171 kcal), protein (0.057 kcal) and lipids (0.057 kcal) in 5h fasted and anesthetized C57BL/6J mice. Means ± S.E.M. are shown, n=10-12 mice per group.

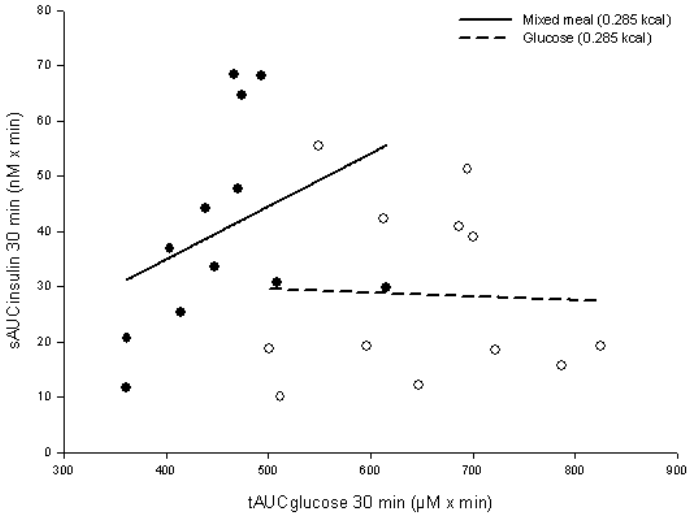
Table 1. *Body weight, insulin sensitivity indexes and β-cell function data in C57BL/6J mice following oral challenge of mixed meal (MTT) versus isocaloric glucose load (OGTT).*

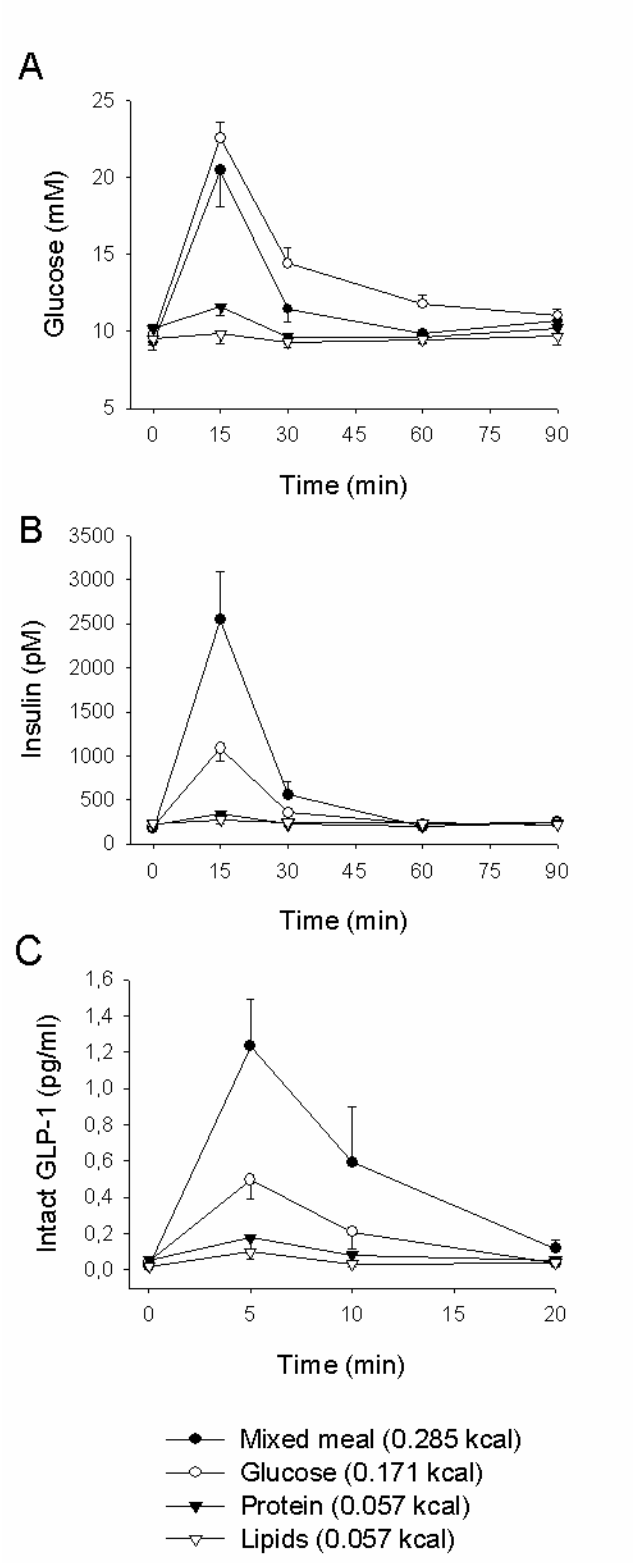
	MTT (0.285 kcal)	OGTT (0.285 kcal)
Body weight (g)	21.6 ± 0.4	22.0 ± 0.3
QUICKI	0.30 ± 0.01	0.31 ± 0.01
OGIS (ml min-1 kg-1)	200 ± 11	241 ± 11 *
$tAUC_{GLU\ 0\text{-}30\ min}$ (mM)	0.45 ± 0.02	0.65 ± 0.03 †
$tAUC_{GLU\ 0\text{-}90\ min}$ (mM)	1.08 ± 0.04	1.61 ± 0.08 †
$sAUC_{INS\ 0\text{-}30\ min}$ (nM)	40.1 ± 5.5	28.5 ± 4.7
$sAUC_{INS\ 0\text{-}90\ min}$ (nM)	45.5 ± 6.0	36.4 ± 6.6
$sAUC_{INS}/tAUC_{GLU\ 0\text{-}30\ min}$	88 ± 11 †	45 ± 8
$sAUC_{INS}/tAUC_{GLU\ 0\text{-}90\ min}$	42 ± 6	29 ± 6

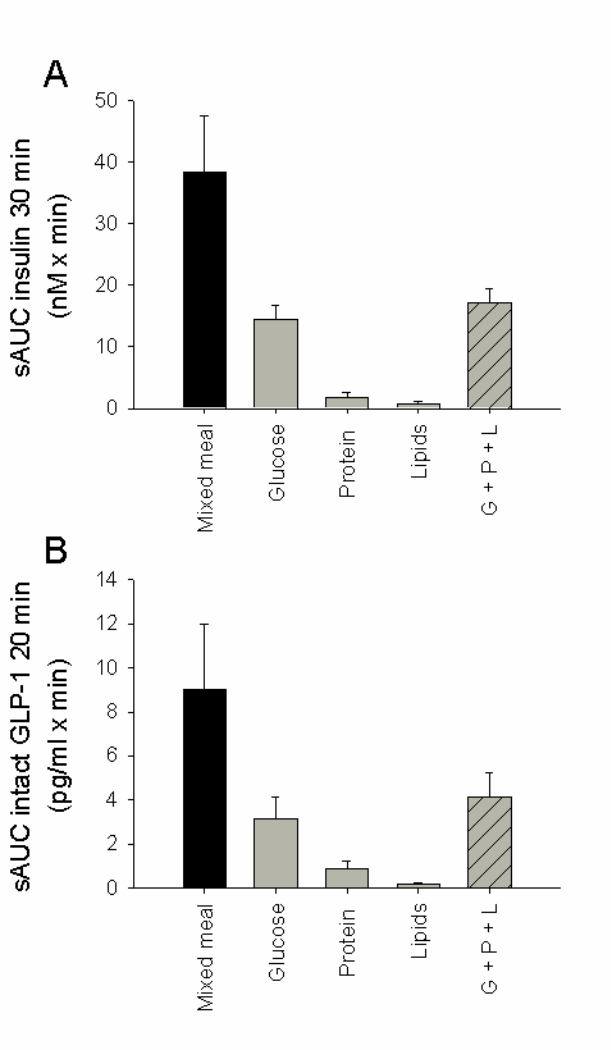
Means ± S.E.M. are shown, n=12 mice per group. QUICKI: quantitative insulin sensitivity check index, OGIS: oral glucose insulin sensitivity index, sAUC and tAUC: suprabasal and total areas under the curve. Beta-cell function is represented by the ratio of sAUC/tAUC. * $P < 0.05$, † $P < 0.01$.


A
Glucose (mM)
Time (min)
B
Insulin (pM)
Time (min)
C
GLP-1 (pg/ml)
Time (min)
Mixed meal (0.285 kcal)
Glucose (0.285 kcal)

Mi
O


Mixed meal (0.285 kcal)
Glucose (0.285 kcal)
sAUCinsulin 30 min (nM x min)
tAUCglucose 30 min (µM x min)
0
10
20
30
40
50
60
70
80
300
400
500
600
700
800
900


A
Glucose (mM)
25
20
15
10
5
0
15
30
45
60
75
90
Time (min)
B
Insulin (pM)
3500
3000
2500
2000
1500
1000
500
0
0
15
30
45
60
75
90
Time (min)
C
Intact GLP-1 (pg/ml)
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0
5
10
15
20
Time (min)
Mixed meal (0.285 kcal)
Glucose (0.171 kcal)
Protein (0.057 kcal)
Lipids (0.057 kcal)


A
sAUC insulin 30 min
(nM x min)
50
40
30
20
10
0
Mixed meal
Glucose
Protein
Lipids
G + P + L
B
sAUC intact GLP-1 20 min
(pg/ml x min)
14
12
10
8
6
4
2
0
Mixed meal
Glucose
Protein
Lipids
G + P + L