Glucagon-like peptide-1 and beta cell glucose sensitivity - a glucose ramp study in mice

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**ABSTRACT**

The incretin glucagon-like peptide-1 (GLP-1) is a gut hormone but also locally produced in pancreatic islets. We evaluated effects of GLP-1 on the insulin response to a gradual increase in glucose in mice within physiological levels. We initially developed a glucose ramp technique in mice. Glucose levels were slowly increased by 0.2 mmol/l/min for 40 min under control conditions, during intravenous infusion of GLP-1 and in GLP-1 receptor knockout mice. In control mice, glucose levels increased from 8.5 ± 0.3 to 16.1 ± 0.3 mmol/l over the 40 min, i.e., by 0.22 ± 0.01 mmol/l/min. This resulted in a slow increase in insulin levels by 96 ± 38 pmol/l from the baseline of 319 ± 53 pmol/l. GLP-1 at 0.5 nmol/kg as bolus plus 0.3 nmol/kg/min over 40 min progressively increased this insulin response by 100-fold, to 9.5 ± 0.2 nmol/l (P < 0.001). Higher doses of GLP-1 enhanced the insulin response similarly (1.0 or 3.0 nmol/kg bolus followed by 0.4 or 1.2 nmol/kg/min), whereas a lower dose (0.3 nmol/kg bolus plus 0.15 nmol/kg/min) had no significant effect compared to controls. Moreover, there was no significant difference in insulin responses between controls and GLP-1 receptor knockout mice. Since the increase in glucose levels were standardized, there was no significant difference in glucose levels between the experimental groups. We conclude that the glucose ramp technique is a tool for studies on insulin responses to slow changes in circulating glucose levels in mice. We also conclude that GLP-1 is extraordinarily potent in enhancing the insulin response to a slow increase in glucose levels.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is an incretin hormone which is produced in and released from intestinal L-cells in response to meal ingestion and which stimulates insulin secretion and inhibits glucagon secretion [1–3]. GLP-1 also reduces food intake and body weight and today GLP-1-based therapies are of importance for treatment of type 2 diabetes and obesity worldwide [4,5]. In such therapy, GLP-1 receptor agonists are also beneficial for cardiovascular health [6].

Besides the expression in intestinal L-cells, GLP-1 is also expressed in pancreatic islets where it is produced in alpha-cells [7–10]. This locally produced GLP-1 is released from islet cells [9] and stimulates insulin secretion [10,11]. Also the GLP-1 inactivating enzyme dipeptidyl peptidase-4 (DPP-4) is expressed locally in islets [12] and, therefore, a local islet GLP-1 mechanism may be involved in the regulation of islet function.

A most important characteristic of the stimulation of insulin secretion by GLP-1 is the glucose dependency, as was first demonstrated in humans by Kreymann et al. in 1987, when they showed that GLP-1 stimulates insulin secretion more efficiently at high glucose levels than at fasting glucose levels [13]. The glucose dependency of the beta cell action of GLP-1 has in subsequent studies been demonstrated by comparing effects during stepped glucose clamp [14], during graded glucose infusions [15], and after modeling of beta cell secretion after an intravenous glucose administration [16]. Studies in vitro have also demonstrated the glucose dependency of the beta cell stimulation by GLP-1 during incubation of islets at low versus high glucose [17] and during a graded glucose perfusion of the isolated pancreas [18]. Therefore, the glucose dependency of beta cell stimulation by GLP-1 is well established. This characteristic is a success factor for the anti-hyperglycemic action of GLP-1-based therapy since it reduces the risk for hypoglycemia [19]. However, the glucose dependency of beta cell action may be an important function also of the local action of GLP-1, since changes in GLP-1 activation would enable beta cells to respond more rapidly and adjust more efficiently to dynamic changes in glucose levels.

Most studies have examined the glucose dependency of GLP-1 on top of a rapid and large change in glucose, such as after intravenous glucose
injection, by incubating islets in high glucose levels, or during a constant glucose level, such as in the glucose clamp. However, these models do not reflect the physiological changes in glucose, which are more slow. Hence, the influence of GLP-1 during more physiological changes in glucose is not established. In fact, the process of dynamic regulation of islets function may be most important for the minute-to-minute function of islets to secrete insulin to which GLP-1 may importantly contribute.

The aim of this study was to evaluate the effect of GLP-1 to insulin responses to a slow dynamic change in glucose in mice. To accomplish this aim, we initially developed a glucose ramp technique in anesthetized mice. Glucose levels were slowly, progressively and linearly increased by 0.2 mmol/l/min under standardized conditions for 40 min reaching a total increase by 8 mmol/l during 40 min. The insulin response to this change in glucose was analyzed and the results in control mice were compared with results during intravenous infusion of GLP-1 at four different doses. Furthermore, we also evaluated the insulin response to glucose ramp in anesthetized GLP-1 receptor knockout mice, which is a model exploring the physiology of GLP-1 [20]. For comparison, we also explored the effect of GLP-1 infusion on the insulin responses during a euglycemic clamp, when glucose levels were clamped at fasting level.

The idea of a glucose ramp emanates from the 1970s when a glucose ramp technique was described in humans [21]. This technique has, however, been rarely used, in contrast to static clamp conditions or rapid glucose ramp to the fasting level.

2. Materials and methods

2.1. Animals

The studies were performed in wildtype C57BL/6 J mice and in GLP-1 receptor knockout mice from our colony, which has been established as previously described [23]. All experiments were undertaken in female mice of 4–6 months of age with a body weight range of 18.6–26.5 g. A total of 58 animals were used in the study (51 wildtype animals and 7 GLP-1 receptor knockout mice). The animals were maintained in a temperature-controlled room (22 °C) on a 12:12 h light-dark cycle (light on at 7:00 AM). Mice were fed a standard pellet diet (total 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 7:30 AM and the actual experiments started at 12:30, i.e., during the light cycle. We used female mice only, to avoid the stress of single housing, which is used in male mice, and to be in line with our previous studies in GLP-1 receptor knockout mice [23,24]. We used the mice randomly during the estrous cycle. The study was approved by the Lund/Malmö Animal Ethics Committee (Approval No. 5.8.18-06417/2020) and performed according to Good Laboratory Practice.

2.2. Experiments

The experimental design is illustrated in Fig. 1. After a 5-h fast, mice were anesthetized with the combination of Fluafent (i.e., a mixture of fluanisone and fentanyl citrate) and midazolam, as previously described [25]. 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 (Sigma-Aldrich, St Louis, MO; 0.315 mg/mL); 150 μl of this solution were given intraperitoneally to each mouse (0.024 mg fentanyl citrate and 0.75 mg fluanisone/mouse). Midazolam (0.250 mg/mouse; Roche, Basel, Switzerland) was also given (150 μl/mouse). After induction of anesthesia, catheters were surgically inserted into the carotid artery and the jugular vein. Glucose was infused from time 0 to time 40 at a variable rate to increase circulating glucose from baseline (8.3 ± 0.3 mmol/l in control mice) aiming at an increase by 0.2 mmol/l/min. Samples were taken every 5 min to adjust the glucose infusion rate to achieve this increase. In experimental groups with GLP-1 infusion, GLP-1 was injected as a bolus at time 0 followed by a constant infusion during 40 min (exact doses shown in Table 1). Samples for determination of insulin were taken every 10 min.

![Fig. 1. Outline of the experiments. Mice were anesthetized at minute -20, thereafter catheters were inserted into the carotid artery and the jugular vein. Glucose was infused from time 0 to time 40 at a variable rate to increase circulating glucose from baseline (8.3 ± 0.3 mmol/l in control mice) aiming at an increase by 0.2 mmol/l/min. Samples were taken every 5 min to adjust the glucose infusion rate to achieve this increase. In experimental groups with GLP-1 infusion, GLP-1 was injected as a bolus at time 0 followed by a constant infusion during 40 min (exact doses shown in Table 1). Samples for determination of insulin were taken every 10 min.](image-url)

Table 1

| Experiment                              | Bolus GLP-1 injection at time t = 0 (mmol/kg) | GLP-1 infusion dose during min 0–40 (mmol/kg/min) | Number of animals |
|-----------------------------------------|---------------------------------------------|-------------------------------------------------|------------------|
| Glucose ramp                            | 0.3                                         | 0.15                                            | 6                |
| Glucose ramp                            | 0.5                                         | 0.3                                             | 6                |
| Glucose ramp                            | 1.0                                         | 0.4                                             | 6                |
| Glucose ramp                            | 3.0                                         | 1.2                                             | 9                |
| Euglycemic clamp                        | 0.5                                         | 0.3                                             | 7                |
maximum 85 μl, which is ≈3% of blood volume. Samples were stored at –20 °C until analysis for insulin.

2.3. Assays

Glucose was detected with the glucose oxidase method using Accu Chek Aviva (Hoffman-La Roche, Basel, Switzerland). Insulin was determined by ELISA (Mercodia, Uppsala, Sweden). The intra-assay coefficient of variation (CV) of the method is 4% at both low and high levels, and the interassay CV is 5% at both low and high levels. The lower limit of quantification of the assay is 6 pmol/l.

2.4. Data analysis and statistics

Data are presented as means ± SEM. Linear regression was calculated for the increase in insulin levels versus the increase in glucose levels. Differences between experimental groups in the glucose ramp were determined using a two-way analysis of variance (ANOVA) followed by a Sidak’s multiple comparisons test. Analyses were carried out using SPSS, v. 27.

3. Results

3.1. The glucose ramp technique

The glucose ramp was developed in wildtype mice (Fig. 2; n = 10). Mean fasting glucose was 8.5 ± 0.3 mmol/l and the target for the glucose ramp model was to continuously and progressively increase glucose levels by 0.2 mmol/l per minute for 40 min to reach a 40 min value of ≈16 mmol/l. This was achieved by an intravenous infusion of glucose at a variable rate with analyses of glucose levels every five minutes. Fig. 2A shows the glucose levels that were achieved during the glucose ramp. It is seen that there was, as expected, a progressive increase in glucose levels until the final measurement at minute 40 of 16.1 ± 0.3 mmol/l. This was equivalent to an increase by 7.6 ± 0.5 mmol/l, i.e., 0.19 ± 0.01 mmol/l per min. The steady state increase in glucose by the target of 0.2 mmol/l per min was reached after 10 min, after which the change in glucose was 0.22 ± 0.01 mmol/l/min. The total amount of glucose infused was 77.9 ± 7.5 μmol and the mean increase in glucose infusion during the second phase (min 10–40) was 1.95 ± 0.19 μmol/min. The total volume of the infusion was 70.1 ± 6.8 μl.

Baseline insulin levels were 319 ± 53 pmol/l. Fig. 2C shows the increase in plasma insulin levels by time during the glucose ramp. It is seen that there was a gradual increase in plasma insulin levels during the initial 30 min, whereas at 40 min there was no further increase. The 40 min increase in insulin levels was 96 ± 38 pmol/l (Table 2). Fig. 2D shows the relation between the increase in insulin levels versus the glucose levels. The regression of the slope of increase in insulin versus increase in glucose levels was 12.9 ± 4.9 pmol/mmol (P = 0.039).

3.2. Comparison between glucose ramp and glucose clamp

The results of the glucose ramp was compared with previously obtained results with a glucose clamp targeting 16 mmol/l during a static hyperglycemic clamp [22]. Fig. 3 shows the differences in glucose levels and glucose infusion rates with the two techniques. In the hyperglycemic clamp, glucose levels reached the target of 16 mmol/l already after 5 min, whereafter the glucose levels were stable, whereas during the glucose ramp there was the gradual increase in glucose to reach the same target (Fig. 3A). The glucose levels reached after 40 min were similar in the two tests. Fig. 3B shows that the glucose infusion rate was rapidly increased in the hyperglycemic clamp to rapidly reach the target of 16 mmol/l, whereas there was a slower increase in glucose infusion to reach the various steps in the glucose ramp. The resulting insulin levels were strikingly different, in that during the hyperglycemic clamp, a clear first phase of insulin secretion was obtained, whereas the second phase, during min 20–40 min, did not differ between the two techniques (Fig. 3C).

3.3. GLP-1 infusion during the glucose ramp

To explore the impact of GLP-1 on the insulin response during the

Fig. 2. A Plasma glucose levels by time, B glucose infusion rate by time (constant during each 5 min), C increase in insulin levels (delta insulin) by time, and D increase in insulin levels in relation to glucose levels during the glucose ramp technique in control mice (n = 10). Means ± SEM are shown.
Table 2
Body weight, fasting and 40 min glucose levels, rate of increase in glucose levels, total volume infused, fasting and 40 min insulin levels, the 40 min increase in insulin levels, and the slope between 40 min changes (Δ) in insulin versus 40 min changes (Δ) glucose in control mice, GLP-1 receptor knockout (GLP-1R KO) mice and during infusion of GLP-1 at four different dose levels in normal (wildtype) mice (dose explanation in Table 1) during the glucose ramp and in control mice and after infusion of GLP-1 at one of the doses during euglycemia.

| Mouse genotype | Controls Wildtype | GLP-1R KO Wildtype | GLP-1 Wildtype | GLP-1 Wildtype | GLP-1 Wildtype | GLP-1 Wildtype | Controls Wildtype |
|----------------|------------------|--------------------|--------------|--------------|--------------|--------------|----------------|
| GLP-1 dose     | NA               | NA                | 0.3          | 0.5          | 1.0          | 3.0          | 0.5           |
| n              | 10               | 7                 | 6            | 6            | 9            | 9            | 7             |
| Body weight (g) | 23.0 ± 0.4       | 20.9 ± 0.4        | 21.2 ± 0.6   | 20.7 ± 0.5   | 22.2 ± 0.7   | 22.0 ± 0.7   | 20.8 ± 0.6    |
| Fasting glucose (mmol/l) | 8.5 ± 0.3 | 10.0 ± 3.0        | 8.7 ± 0.3    | 8.7 ± 0.1    | 7.9 ± 0.3    | 8.3 ± 0.2    | 8.3 ± 0.2     |
| 40 min glucose (mmol/l) | 16.1 ± 0.3 | 16.5 ± 0.5        | 16.1 ± 0.7   | 15.7 ± 0.6   | 15.3 ± 0.6   | 15.9 ± 0.4   | 19.0 ± 0.7    |
| Rate of increase in glucose min 10–40 (mmol/l/min) | 0.22 ± 0.01 | 0.20 ± 0.01       | 0.23 ± 0.01  | 0.19 ± 0.03  | 0.20 ± 0.04  | 0.19 ± 0.02  | NA            |
| Infusion volume (μl) | 70.1 ± 6.8 | 48.7 ± 0.7        | 93.6 ± 7.8   | 125.7 ± 12.1 | 168.6 ± 15.6 | 160.0 ± 9.8  | 25.9 ± 6.8    |
| Fasting insulin (pmol/l) | 319 ± 53 | 293 ± 36          | 294 ± 37     | 328 ± 35     | 285 ± 33     | 288 ± 38     | 341 ± 26      |
| 40 min increase in insulin (pmol/l) | 96 ± 38 | 146 ± 6           | 502 ± 186    | 9478 ± 2442  | 12,124 ± 1033 | 1902 ± 834   | 1038 ± 52     |
| Slope of regression between Δ insulin versus Δ glucose between 0 and 40 min (pmol/mmol) | 12.9 ± 4.9 | 18.3 ± 13.0       | 153 ± 101    | 1393 ± 332   | 1639 ± 333   | 1512 ± 284   | NA            |

NA not applicable.

a \( P = 0.002 \) versus controls.
b \( P = 0.001 \) versus controls.
c \( P < 0.001 \) versus controls.
d \( P = 0.020 \) versus controls in the glucose ramp.

Fig. 3. A Plasma glucose levels, B glucose infusion rate (constant during each 5 min; dot is placed in the middle of the 5 min period), and C increase in insulin levels (delta insulin) during the glucose ramp technique in control mice (n = 10) compared to results from a previous study using the hyperglycemic clamp technique in control mice (n = 12) [22]. Means ± SEM are shown.

glucose ramp, GLP-1 was infused at four different dose levels, and the glucose ramp was undertaken in GLP-1 receptor knockout mice. Fig. 4 and Table 2 show the results in GLP-1 receptor knockout mice and the GLP-1 infusions versus control mice in the glucose ramp. Baseline glucose levels were the same in the groups, except a significantly higher fasting glucose in GLP-1 receptor knockout mice (Table 2). Fig. 4A shows that the glucose levels were similarly increased in all groups to reach the target increase of 0.2 mmol/l/min to the 40 min value of 16 mmol/l. The rate of change in glucose levels by time was ≈0.2 mmol/l/minute, as targeted, with no significant difference between the groups (Table 2). Fig. 4B shows the glucose infusion rate, Fig. 4C the total amount of infused glucose during the 40 min period and Table 2 shows...
the total volume infused. Statistics showed that the total amount of infused glucose was not significantly different from controls and GLP-1 receptor knockout mice or during infusion of GLP-1 at the 0.3-dose. However, the amount of infused glucose was significantly higher during infusion of GLP-1 at the three highest doses (0.5-dose, 1.0-dose and 3.0-dose; for dosing details see Table 1) than in controls (all P < 0.001), with no significant difference between the three highest GLP-1 doses. Table 2 shows that rate of increase in glucose infusion per minute in all groups, showing similarly no significant difference from controls in GLP-1 receptor knockout mice or in the 0.3-dose of GLP-1 infusion, and markedly higher following the GLP-1 doses of 0.5, 1.0 and 3.0 than in controls (all P < 0.001), but with no significant difference between these three high GLP-1 infusion doses.

Fig. 4D shows the insulin levels by time and Fig. 4E shows the increase in insulin levels in relation to glucose levels. Table 2 shows the increase in insulin levels after 40 min and the slope of change in insulin levels versus change in glucose levels. Statistics were performed with these different measures of the insulin responses in the glucose ramp and these statistics showed similar results for all different measures: the insulin responses were not significantly different between control mice, GLP-1 receptor knockout mice and the 0.3-dose of GLP-1 infusion. However, during infusion of GLP-1 at the three highest doses, insulin levels rose markedly higher than in controls (>100-fold enhancement; P < 0.001 for all three doses), but with no significant difference between the three infusion doses.

3.4. GLP-1 infusion during euglycemic clamp

One series of experiments was performed to examine the influence of GLP-1 when glucose levels were not allowed to change from baseline for a period of 40 min (euglycemic clamp). Fig. 5A shows glucose levels during the euglycemic clamp in controls and during infusion of GLP-1 (the 0.5-dose) and as comparison, the results in the glucose ramp is also illustrated. It is seen that in the euglycemic clamp, glucose levels were fairly stable during the 40 min study period. Fig. 5B and C show the glucose infusion rates. It is seen that during the euglycemic clamp, very little glucose had to be infused. The amount of infused glucose was, however, higher during GLP-1 infusion than in controls (P = 0.028). Fig. 5D and E (different scales on y-axis) and Table 2 show the insulin responses. It is seen that insulin levels were stable during the euglycemic clamp, reaching lower levels than during the glucose ramp (P = 0.020). During infusion of GLP-1 in the euglycemic clamp, the 40 min level increased without, however, reaching significance versus in controls. Interestingly, the insulin response to GLP-1 infusion during euglycemic clamp was also not significantly different from the insulin response to the glucose ramp in controls.

4. Discussion

An important physiological role of the pancreatic islets is to adjust insulin secretion to the ambient circulating glucose levels. We have previously shown in model experiments in mice that during rapid administration of glucose, as after the rapid intravenous injection or
during a hyperglycemic clamp [22,28], a clear and prompt insulin secretion is evident. This secretion occurs very rapidly and is divided in two phases, with the first rapid phase lasting \( \approx 10 \) min and the second phase lasting for at least 30 min [29]. However, rapid glucose changes, as in an intravenous glucose tolerance test, or sustained constant glucose levels, as in the hyperglycemic clamp, occur very rarely physiologically, if ever. Instead, in normal physiology, changes in glucose levels are more slow and dynamic. To study such patterns, we developed the glucose ramp technique. Glucose was infused at an increasing pace to achieve a progressive linear increase in circulating glucose by 0.2 mmol/l/min. During the 40 min study period, glucose levels were thereby slowly increased from \( \approx 8 \) mmol/l to \( \approx 16 \) mmol/l. This was associated with an increase in insulin levels. The increase was, however, slow and there was no clear peak. This may be surprising considering that the amount of glucose infused was quite large. A conclusion is, therefore, that there indeed is a different pattern of insulin release when glucose levels are gradually increased compared to a sudden increase following a bolus injection. In fact, the pattern of insulin levels after the gradual increase in glucose levels during the glucose ramp is similar to a second phase of insulin secretion after intravenous glucose injection. A gradual increase in glucose will therefore bypass the first phase, as defined from intravenous glucose injections. This difference between the slow increase in insulin levels after gradual increase in glucose compared to the prompt and rapid release of insulin after the intravenous injection of glucose is also evident when we compared our present results from those of a previous study of a hyperglycemic glucose clamp to similar levels as in our glucose ramp but with a sudden increase [22]. It should be emphasized that the absolute increase in circulating glucose was similar between glucose ramp and glucose clamp; the difference was the pattern of change in glucose. Mechanistically, this difference is most likely related to sudden and rapid increase in glucose after intravenous glucose injection which may have elicited the release of a rapid releasable pool of insulin in the beta cells [30]. In physiology, this may occur after ingestion of large amount of carbohydrates. The glucose ramp may therefore be a tool to study slow dynamic change in glucose, bypassing the rapid release of insulin that is seen during rapid and sustained increase in glucose levels.

Besides the development of the glucose ramp technique, a main aim of this study was to examine the insulin response to GLP-1 during the slow increase in glucose as is achieved by the glucose ramp. We found that the insulin response in the glucose clamp was markedly exaggerated by GLP-1. This shows that GLP-1 receptor activation enhances glucose-stimulated insulin secretion during a slow dynamic change in glucose. This confirms a previous study where we showed that the second phase of insulin secretion is sensitive to GLP-1 [29]. Our results showed, however, that the marked increase in insulin levels during the GLP-1 infusion (during the glucose ramp) is mainly dependent on GLP-1; less than 1% would be regarded as independent on GLP-1 (see numbers in Table 2). Therefore, a robust increase in insulin levels during a continuous and slow rise in glucose levels requires presence of GLP-1. The dose response study of varying the GLP-1 infusion dose showed that the effect of GLP-1 was evident by the 0.5-dose and higher doses, whereas there
was no clear effect of the 0.3-dose. Furthermore, the maximal effect was seen at the 1.0 dose, since there was no further increase in insulin by the 3.0-dose. This suggests a narrow window between no effect and maximal effect of GLP-1 in enhancing glucose-stimulated insulin secretion. Hence, when the threshold of a GLP-1 effect is reached, an almost maximal effect is seen. This was also evident from results of the amount of glucose that had to be infused to maintain the target increase in circulating glucose by 0.2 mmol/l, which was comparable to the controls by the 0.3-dose, whereas already at the 0.5-dose, a maximal effect was seen. These results are different from the dose-response relationship when giving GLP-1 at different dose levels during an intravenous glucose tolerance test, in which a dose-related progressive increase in first phase insulin secretion is evident by increasing the GLP-1 dose [31]. This would suggest different dose-response characteristics for GLP-1 in relation to first versus second phase of insulin secretion.

The intravenous infusion of GLP-1 is an unphysiological model to study physiology of gut GLP-1, since the normally circulating GLP-1 is the result of secretion of the hormone from the gut after meal ingestion and therefore its plasma levels increase in relation to meal ingestion. It has been shown that this islet GLP-1 is secreted [9] and, therefore, locally high concentrations of GLP-1 most likely exist in close vicinity to the beta cells. Hence, the suggested function of GLP-1 based on the results in this study that GLP-1 and circulating glucose exert synergistic actions to enhance insulin secretion, is more likely a reflection of function of GLP-1 as an islet hormone than as an incretin hormone. As such, therefore, GLP-1 seems to be of importance for the physiological adjustment of the insulin response to slow and dynamic changes in glucose.

In GLP-1 receptor knockout mice, the amount of infused glucose to reach the glucose target was not significantly different from in controls, and, similarly, the insulin response to the increase in glucose levels were similar in controls and GLP-1 receptor knockout mice. This suggests that GLP-1 receptors are not required for the insulin response to slow dynamic changes in glucose. This is similar as in our previous study on the effect after intravenous glucose injection, in which the insulin response was not different between controls and GLP-1 receptor knockout mice [24].

In this study, we also compared the effect of GLP-1 during a euglycemic clamp when glucose levels were not allowed to change from baseline. We found that there was a small numerical increase in insulin levels during infusion of GLP-1 (the 0.5-dose) during euglycemia, although this increase did not reach significance versus the controls. In fact, insulin levels were similar during infusion of GLP-1 at euglycemia and in controls during the glucose ramp. In contrast, the increase in insulin was considerably higher during GLP-1 infusion in the glucose ramp. This shows that there is a clear and potent synergy between glucose and GLP-1 infusion in regard to insulin response, and, therefore, that the GLP-1 receptor activation is a potent enhancing factor for beta cells to respond to changes in glucose.

It was expected that GLP-1 would enhance the insulin response to the raise of glucose during the glucose ramp. However, such a marked increase in insulin that was observed (GLP-1 infusion elicited a more than 100-fold enhancement of the insulin response) was not expected. In fact, when GLP-1 was injected together with glucose in a rapid intravenous glucose test, the insulin response to glucose was augmented only by ≈7-fold [31]. Therefore, a novel conclusion from this work is that GLP-1 seems of particular potency when enhancing the beta-cell response to small changes in glucose.

A strength of this study is the development of the novel model with standardized predefined slow changes in glucose levels within the physiological range. A limitation is that the glucose ramp technique is an advanced technique requiring experience with in vivo-models for execution. Another limitation is the limited blood volume than can be drawn during the experiment, which, for example, did not allow to have also C-peptide to be measured in these experiments. A limitation is also that we performed the studies in anesthetized animals, although we used the same anesthesia in all animals and the established neuroleptic anesthesia we used has been shown to be a preferred anesthesia in experimental animal studies in inflammatory, hemodynamic and metabolic research [32,33]. It is also a limitation that we administered GLP-1 systemically and not locally in the pancreas, and therefore we can not exclude that extrapancreatic effects of GLP-1, through its effects on liver, adipose tissue and skeletal muscles, may have contributed to the observed effects. Thus, GLP-1 is known to have effects in the liver [34], possibly mediated by GLP-1 receptors located in the portal system [35,36], skeletal muscles [37,38] and adipose tissue [39]. Therefore, it would be of interest to reproduce these studies in a beta-cell specific GLP-1 receptor knockout model.

Based on the results of this study, we conclude that our novel glucose ramp model is a tool for examining the insulin response to slow dynamic and physiological changes in glucose levels in mice. We also conclude that GLP-1 very potently enhances the insulin response to the slow and dynamic changes in glucose yielding a 100-fold augmentation of the insulin response to glucose under these conditions. Our results therefore suggest that GLP-1 is involved in the local regulation of the beta cell response to slow changes in glucose.

Author contribution

BA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing - original draft, review and editing.

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Declaration of Competing Interest

The authors report no declarations of interest.

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