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Metabolism of glucagon-like peptide-2 in pigs: Role of dipeptidyl peptidase IV

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Abstract

Little is known about the metabolism of the intestinotropic factor glucagon-like peptide-2 (GLP-2); except that it is a substrate for dipeptidyl peptidase IV (DPP-IV) and that it appears to be eliminated by the kidneys. We, therefore, investigated GLP-2 metabolism in six multicatheterized pigs receiving intravenous GLP-2 infusions (2 pmol/kg/min) before and after administration of valine-pyrrolidide (300 μmol/kg; a well characterized DPP-IV inhibitor). Plasma samples were analyzed by radioimmunoassays allowing determination of intact, biologically active GLP-2 and the DPP-IV metabolite GLP-2 (3–33). During infusion of GLP-2 alone, 30.9±1.7% of the infused peptide was degraded to GLP-2 (3–33). After valine-pyrrolidide, there was no significant formation of the metabolite. Significant extraction of intact GLP-2 was observed across the kidneys, the extremities (represented by a leg), and the splanchnic bed, resulting in a metabolic clearance rate (MCR) of 6.80±0.47 ml/kg/min and a plasma half-life of 6.8±0.8 min. Hepatic extraction was not detected. Valine-pyrrolidide addition did not affect extraction ratios significantly, but decreased ($p=0.003$) MCR to 4.18±0.27 ml/kg/min and increased ($p=0.052$) plasma half-life to 9.9±0.8 min. The metabolite was eliminated with a half-life of 22.1±2.6 min and a clearance of 2.07±0.11 ml/kg/min. In conclusion, intact GLP-2 is eliminated in the peripheral tissues, the splanchnic bed and the kidneys, but not in the liver, by mechanisms unrelated to DPP-IV. However, DPP-IV is involved in the overall GLP-2 metabolism and seems to be the sole enzyme responsible for N-terminal degradation of GLP-2.

Keywords: Valine-pyrrolidide; GLP-2; MCR; DPP-IV

1. Introduction

The intestinotropic factor, glucagon-like peptide-2 (GLP-2) has attracted considerable attention because of its ability to stimulate intestinal growth in rodents [1,2] and to improve both intestinal absorption and nutritional status in short-bowel patients [3]. Therefore, GLP-2 has been suggested for the treatment of intestinal insufficiency [4].

GLP-2 originates from tissue-specific posttranslational processing of the glucagon precursor, proglucagon, in the intestinal L-cells [5]. This processing also leads to the formation of one of the major incretin hormones, glucagon-like peptide-1 (GLP-1). Because of the great interest in GLP-1 as a potential therapeutic agent for type 2 diabetes [6], more is now known about GLP-1 metabolism [7,8]. GLP-2 has approximately 40% sequence homology with GLP-1, including the two N-terminal amino acids. GLP-2 metabolism might, therefore, resemble that of GLP-1. However, little is actually known about this.

It was suggested by Mentlein et al. in 1993, that the enzyme dipeptidyl peptidase IV (DPP-IV) could be capable of cleaving GLP-2, in agreement with its N-terminal similarity to GLP-1 [9]. Subsequently, it has been shown in a number of *in vivo* studies [10–14] that intact GLP-2 (1–33), like GLP-1 [8], is a substrate for DPP-IV degradation leading to the formation of the partial agonist GLP-2 (3–33) as demonstrated in studies involving high-performance liquid chromatography and sequencing

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; DPP-IV, dipeptidyl peptidase IV; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; IR, immunoreactivity; MCR, metabolic clearance rate; RIA, radioimmunoassay; $T_{½}$, half-life.

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[10,14]. Studies have shown that full length (intact) GLP-2 (1–33) is required for receptor activation [15], and that the DPP-IV cleavage product, GLP-2 (3–33), may even act as a GLP-2 receptor antagonist [16].

The few studies focusing on GLP-2 clearance suggested that the kidneys play a role [17,18], as is the case for GLP-1 [7]. However, it was suggested by Tavares et al. [18] that other organs and mechanisms may also be involved. Again, because of the resemblance to GLP-1, it was suggested that the liver and lungs may contribute to GLP-2 clearance.

The present study was undertaken to elucidate in detail the contribution of the different organs to GLP-2 metabolism, as well as the involvement of DPP-IV, using a previously described porcine model [7,8,19]. A processing independent and an N-terminal-specific assay for GLP-2 were employed, allowing determination of the intact, biologically active molecule as well as the metabolite generated by DPP-IV degradation, GLP-2 (3–33) [10].

2. Materials and methods

This study conformed to the Danish legislation governing animal experimentation, and was carried out after permission was granted from the National Superintendence for Experimental Animals.

2.1. Anesthetized pigs

Danish LYD-pigs (32–37 kg, mean 35.1±0.7 kg; $n=6$) were used. Food was withdrawn 24 h before surgery, but the animals were allowed free access to drinking water. After premedication with midazolam (0.5 mg/kg, Dormicum; Roche, Basal, Switzerland) and ketamine (10 mg/kg, Ketaminol; Veterinarie AG, Zurich, Switzerland), the animals were anesthetized with intravenous administration of alpha-chloralose (66 mg/kg; Merck, Darmstadt, Germany) and ventilated with intermittent positive pressure using N_2O/O_2. Catheters were placed in the right carotid artery for sampling of arterial blood and in an ear vein for GLP-2 and valine-pyrrolidide administration. In addition, non-obstructing catheters were placed in the pulmonary artery and in the renal, hepatic, femoral and portal veins as previously described [7]. After surgical preparation, the animals were heparinized and left undisturbed for 30 min. Anesthesia was maintained with the administration of additional chloralose as necessary.

2.2. Experimental procedure

The animals received two intravenous infusions of GLP-2, one before and one after valine-pyrrolidide administration. Synthetic human GLP-2 (Polypeptide, Wolfenbüttel, Germany), dissolved in 0.9% NaCl containing 1% human serum albumin (Behringwerke, Marburg, Germany), was given as a bolus intravenous injection of 0.75 nmol followed by 40 min infusion at a rate of approximately 2 pmol/kg/min using a syringe pump, commencing at time 0. Arterial blood samples (4 ml) were collected at − 10, 0, 10, 20, 30, 35, and 40 min from the start of infusion. After 40 min, the GLP-2 infusion was stopped, and further blood samples were collected at 5, 10, 15, 20, 30, 45, 60, and 90 min from infusion stop. For determination of arteriovenous GLP-2 concentration differences, simultaneous blood samples (2 ml) were collected from the pulmonary artery and the renal, hepatic, femoral and portal veins at minutes 30 and 35 from start of infusion. At 95 min after the cessation of the first GLP-2 infusion, valine-pyrrolidide (300 μmol/kg, dissolved in 0.9% NaCl; a gift from Drs. R. D. Carr and L. Christensen, Novo Nordisk, Bagsværd, Denmark) was given as a bolus intravenous injection over 2 min. We have previously shown that this dose lowers the activity of DPP-IV in pigs to undetectable levels for several hours [19]. Blood samples were collected at 15 and 30 min after valine-pyrrolidide administration and, thereafter, the second GLP-2 infusion was started and the protocol for blood sampling repeated. The total volume of blood collected over the entire procedure amounted to 164 ml, which for a 40 kg pig, is 4% of the total blood volume. This blood loss has previously been shown not to affect blood pressure and heart rate [7]. Both heart rate and blood pressure were monitored continuously and remained stable throughout the experiment (mean heart rate: 96±2 beats/min; mean arterial pressure: 108±4 mm Hg).

Blood samples were collected into chilled tubes containing EDTA (7.4 mmol/l, final concentration), aprotinin (500 KIE/ml blood; Novo Nordisk, Bagsværd, Denmark) and valine-pyrrolidide (0.01 mmol/l, final concentration). Samples were kept on ice until centrifugation at 4 °C. Plasma were separated and stored at −20 °C awaiting analysis.

2.3. Hormonal analysis

Plasma samples were assayed for GLP-2 contents using two specific radioimmunoassays (RIAs). Total GLP-2 was measured using a "side-viewing" GLP-2 antiserum (Peninsula Laboratories, Cat. No RAS 7167) described in [10,20], which recognizes all peptides containing the GLP-2 sequence. Intact, biologically active GLP-2 was measured using antiserum 92160 (previously described in [10,20,21]), which recognizes the N-terminus of GLP-2 (1–33) and, therefore, only fully processed, intact and biologically active GLP-2. Both assays had detection limits below 5 pmol/l. Before RIA was performed, the plasma samples were extracted with ethanol (75% vol/vol, final concentration), resulting in a recovery of 68% and an intra-assay variation of 5%.

2.4. Calculations and statistical analysis

Concentrations of the metabolite, GLP-2 (3–33) were calculated by subtracting intact GLP-2 (1–33) measured by antiserum 92160 from total GLP-2 measured by the assay based on the Peninsula antiserum. The plasma half-lives ($T_{½}$) for GLP-2 (1–33), GLP-2 (3–33) and total GLP-2 were calculated by $\log_e$-linear regression analysis of peptide concentrations in samples collected after termination of infusion (and after subtraction of endogenous values). Elimination constants, k, were calculated from $k=\ln 2 / T_{½}$. The metabolic clearance rate (MCR) for total GLP-2 was calculated by dividing the actual infusion

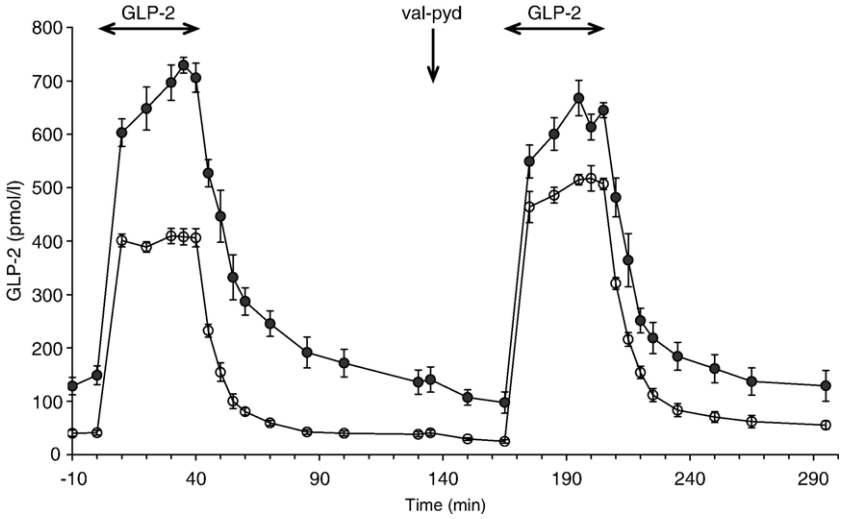

Fig. 1. Plasma GLP-2 immunoreactivity in blood samples from the carotid artery, measured for contents of total GLP-2 (●) and intact GLP-2 (1–33) (○). Animals received a 40 min intravenous GLP-2 infusion (2 pmol/kg/min). Valine-pyrrolidide (val-pyd; 300 μmol/kg) was given 90 min after the end of the first GLP-2 infusion, and after further 30 min, the second GLP-2 was started. Data are means ± SEM; $n = 6$. Arrows indicate the infusions.

rate for each animal with the plateau plasma concentration, after subtraction of basal values. The apparent distribution volume (V_d) for total GLP-2 was calculated from

$$V_d = \text{MCR}_{\text{total GLP-2}} / k_{\text{total GLP-2}}$$

MCR for GLP-2 (1–33) and GLP-2 (3–33) were calculated from V_d for total GLP-2 and the respective k-values, again using $\text{MCR} = V_d * k$. For these calculations it was assumed that the distribution volumes of GLP-2 (1–33) and GLP-2 (3–33) are identical. The incremental area under the curve (AUC) during GLP-2 infusion was calculated using the trapezoidal method, after subtraction of the basal, endogenous GLP-2.

For each animal, the extraction of exogenous GLP-2 across the hind limb (leg), kidney, and portal/splanchnic bed was calculated as a ratio, defined as

$$([\text{GLP-2-IR}]_{\text{carotid artery}} - [\text{GLP-2-IR}]_{\text{vein}}) / [\text{GLP-2-IR}]_{\text{carotid artery}}$$

where $[\text{GLP-2-IR}]_{\text{carotid artery}}$ is the concentration of GLP-2 immunoreactivity (IR) in the carotid artery and $[\text{GLP-2-IR}]_{\text{vein}}$ is the concentration of GLP-2 immunoreactivity in the vein.

For the liver, the hepatic blood supply was calculated on the assumption that 75% originates from the portal vein and 25% is of arterial origin.

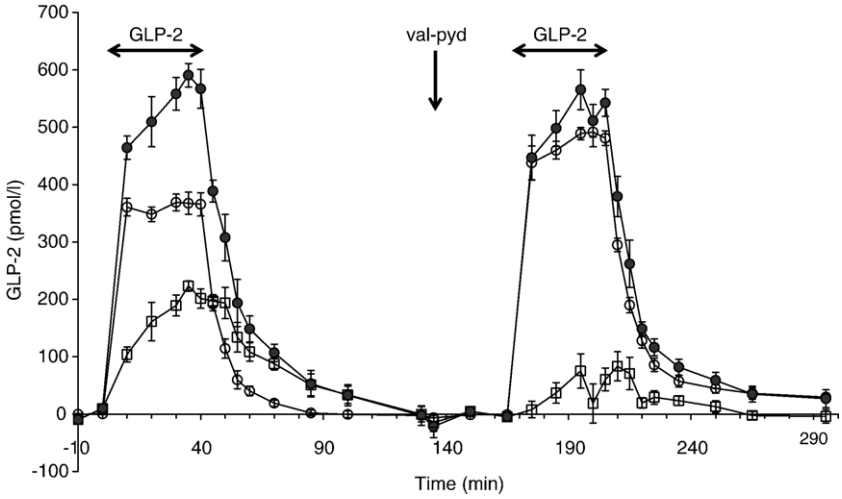

Fig. 2. Concentrations of total GLP-2 (●), intact GLP-2 (1–33) (○) and the metabolite GLP-2 (3–33) (□) after subtraction of basal values. The concentration of GLP-2 (3–33) is obtained by subtracting intact GLP-2 from total GLP-2. Animals received a 40 min intravenous GLP-2 infusion (2 pmol/kg/min). Valine-pyrrolidide (val-pyd; 300 μmol/kg) was given 90 min after the end of the first GLP-2 infusion, and after further 30 min, the second GLP-2 was started. Data are means ± SEM; $n = 6$. Arrows indicate the infusions.

Table 1
Plasma concentrations of exogenous GLP-2 during intravenous infusion

	Total GLP-2 immunoreactivity (pmol/l)		NH_2-terminal immunoreactivity (pmol/l)		GLP-2 (3–33) (pmol/l)
	Before	After	Before	After	Before
Carotid artery	713±23	641±28	409±13	516±11	311±18
Femoral vein	590±18‡	558±24†	326±13‡	405±13‡	263±15
Renal vein	556±22‡	542±27*	305±8‡	400±11‡	252±16*
Portal vein	598±26†	546±33†	307±14†	380±12‡	292±14
Pulmonary artery	647±28†	615±9	357±12*	472±17*	290±23
Hepatic blood supply	627±23	570±23	332±12	414±10	295±13
Hepatic vein	635±28	583±10	324±11	415±12	311±23

Values are expressed as mean±SEM of individual plasma concentrations determined during intravenous GLP-2 infusion before and after valine-pyrrolidide (300 μmol/kg) administration ($n=6$). GLP-2 (3–33) concentrations are calculated as the difference between total and intact GLP-2 (NH_2-terminal immunoreactivity). Hepatic blood supply was calculated on the assumption that 75% originates from the portal vein and 25% from arterial origin. Differences from carotid artery and between hepatic blood supply and hepatic vein: *$p<0.050$; †$p<0.010$; ‡$p<0.001$.

The extraction across the lungs was defined as

$$\left([\text{GLP}{-}2{-}\text{IR}]_{\text{pulmonary artery}} - [\text{GLP}{-}2{-}\text{IR}]_{\text{carotid artery}}\right) / [\text{GLP}{-}2{-}\text{IR}]_{\text{pulmonary artery}}$$

where $[\text{GLP-2-IR}]_{\text{pulmonary artery}}$ is the concentration of GLP-2 immunoreactivity in the pulmonary artery.

Data are expressed as mean±SEM, and all statistical analyses were carried out using Statistica software (Statistica for Windows, Statsoft, Tulsa, OK, USA). Two-way analysis of variance (ANOVA) for repeated measures was used to analyze time–course curves before and after DPP-IV inhibition. The $T_{½}$, AUC, and the extraction ratios and MCR were analyzed using either ANOVA or *t*-test (for paired or non-paired data) as appropriate. Differences resulting in *p*-values less than 0.05 were considered significant.

3. Results

3.1. Effect of valine-pyrrolidide

The concentration profiles for intact GLP-2 (1–33), the metabolite GLP-2 (3–33) and total GLP-2 (GLP-2 (1–33)+GLP-2 (3–33)) during GLP-2 infusion without and with subtraction of basal levels and before and after valine-pyrrolidide administration are shown in Figs. 1 and 2. During infusion of GLP-2 alone, plasma concentrations of intact GLP-2 and were lower (ANOVA, repeated measurement: both $p<0.001$) than concentrations of total GLP-2. After termination of the infusion, basal levels were reached after 90 min for total GLP-2 and the metabolite, and after 35 min for intact GLP-2. Administration of valine-pyrrolidide had no effect on total GLP-2 levels, but intact GLP-2 levels increased significantly (ANOVA, repeated measurement: $p<0.001$), and now did not differ significantly (ANOVA, repeated measurement: $p=0.135$) from total GLP-2 concentrations. Metabolite concentrations decreased (ANOVA, repeated measurement: $p<0.001$) and were not significantly different from zero after valine-pyrrolidide, calculated as the incremental AUC for the metabolite. During infusion of GLP-2 alone, the intact peptide accounted for 69.1±1.7% ($p<0.001$ compared to 100%) of total GLP-2 (calculated as incremental AUCs). After valine-pyrrolidide administration this increased ($p<0.001$) to 93.6±3.1% ($p=0.092$ compared to 100%).

3.2. In vivo half-life

When GLP-2 was infused alone, the plasma $T_{½}$ for intact GLP-2 (6.8±0.8 min) was significantly shorter ($p<0.001$) than

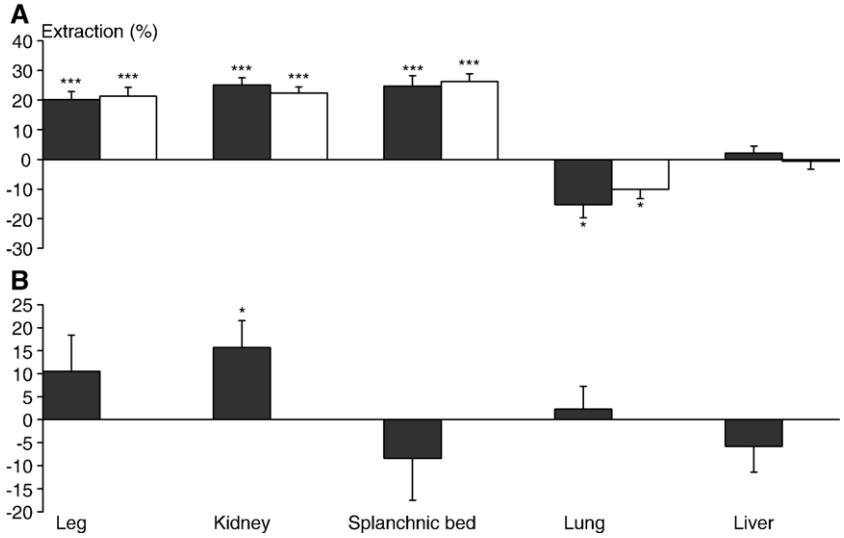

Fig. 3. Regional extractions of intact GLP-2 (1–33) (A) and the metabolite GLP-2 (3–33) (B) during intravenous infusion before (black) and after (white) valine-pyrrolidide administration. Regional extractions of the metabolite were not determined after valine-pyrrolidide addition, due to the total inhibition of GLP-2 degradation. Data are means±SEM of individual extractions calculated across each organ for each animal; $n=6$. *$p<0.050$, **$p<0.010$, ***$p<0.001$; indicating difference from 0. A positive balance indicates a net extraction of GLP-2.

$T_{½}$ for total GLP-2 (12.0±1.0 min). On the other hand, the plasma $T_{½}$ for the metabolite (22.1±2.6 min) was significantly ($p=0.002$) longer compared to $T_{½}$ for total GLP-2. After valine-pyrrolidide administration, the $T_{½}$ for intact GLP-2 was prolonged ($p=0.052$) to 9.9±0.8 min, and did not differ ($p=0.259$) from the $T_{½}$ for total GLP-2 (10.5±0.9 min). Since DPP-IV inhibition resulted in overall metabolite levels that were not significantly different from zero, no attempt was made to calculate the $T_{½}$ for the metabolite after valine-pyrrolidide addition. There was no difference ($p=0.350$) between $T_{½}$ for total GLP-2 before and after valine-pyrrolidide addition.

3.3. Metabolic clearance rate

The MCRs determined for intact GLP-2 (6.80±0.47 ml/kg/min) and the metabolite (2.07±0.11 ml/kg/min) were significantly different (both $p<0.001$) from total GLP-2 (3.73±0.12 ml/kg/min) and from each other ($p<0.001$). After valine-pyrrolidide, the difference between intact GLP-2 and total GLP-2 disappeared (total GLP-2: 3.93±0.21 ml/kg/min; intact GLP-2: 4.17±0.27 ml/kg/min; $p=0.244$ for the difference). No attempts were made to calculate MCR for the metabolite after valine-pyrrolidide. MCR for total GLP-2 was unaffected ($p=0.457$) by valine-pyrrolidide administration, whereas MCR for intact GLP-2 was significantly decreased ($p=0.003$).

3.4. Organ extraction

Plasma concentrations of exogenous GLP-2 during infusion before and after valine-pyrrolidide administration are shown in Table 1, and the regional extractions are shown in Fig. 3. Hepatic extraction was detected neither when GLP-2 was infused alone nor in the presence of valine-pyrrolidide. However, a significant extraction was observed across the hind limb, the kidneys and the splanchnic bed. For the lungs, there was a negative extraction ratio, which was similar after valine-pyrrolidide administration. The extraction ratios for the metabolite were variable and insignificant, except for the kidneys which extracted 15.7±5.9% of the metabolite.

4. Discussion

The main findings of the present study are, firstly, that GLP-2 is significantly degraded by DPP-IV but not nearly to the same extent as GLP-1. Secondly, that GLP-2 is significantly extracted by DPP-IV independent mechanisms in the peripheral tissues in general (here exemplified by extraction across a leg), as well as in the splanchnic bed and the kidneys. Thirdly, that the metabolite formed, GLP-2 (3–33), is eliminated much more slowly and mainly by the kidneys.

The protease DPP-IV cleaves an N-terminal dipeptide from peptides with either proline or alanine as the second amino acid [22], and DPP-IV mediated cleavage of GLP-2 (1–33) therefore results in the formation of the metabolite GLP-2 (3–33), which may function as a partial GLP-2 receptor agonist [16]. Two *in vitro* studies involving incubation of GLP-2 with either rat serum [14] or human plasma [10] have shown that degradation of GLP-2 under these conditions results in only one metabolite, namely GLP-2 (3–33). Although GLP-2 (3–33) appears to function as a partial agonist with some GLP-2-like effects, protection from DPP-IV degradation enhances not only the concentration of intact GLP-2, but also the intestinotropic effect of GLP-2 in both mice and rats [11], supporting that intact GLP-2 is by far the most biologically active form and that DPP-IV mediated degradation has physiological significance. Since intact GLP-2 seems important for GLP-2 receptor activation [11,15], the formation of GLP-2 (3–33) reduces overall GLP-2 activity in two ways: 1) via reducing the concentration of biologically active, intact GLP-2, and 2) via formation of a weak agonist with possible competitive antagonistic characteristics [16]. However, the extent of DPP-IV mediated degradation of GLP-2 contrasts strongly to that of GLP-1, which leads to a similar truncation of the molecule (forming the so-called GLP-1 (9–36)amide [23]). GLP-2 is produced in the same cells as GLP-1 and has the same route of secretion. In addition, it shares about 40% sequence homology with GLP-1, including an identical N-terminal sequence, so one might expect that its susceptibility to DPP-IV degradation would be similar to that of GLP-1. Nevertheless, whereas in pigs, around 70% of infused GLP-2 remains undegraded, only about 20% of intravenously infused GLP-1 survives as the intact peptide [8], resulting in GLP-2 having a more stable pharmacokinetic profile than GLP-1, which has an apparent half-life of between 1 and 2 min, and a plasma clearance which exceeds the cardiac plasma output by a factor of 2 (107 ml/kg/min). Similar findings have been made in humans where GLP-2 appears to be more resistant to DPP-IV degradation than GLP-1. Here, 57% of intravenously infused GLP-2 survived in the intact form [10] as opposed to about 20% of GLP-1 [24], giving rise to a correspondingly slower MCR for intact GLP-2 (6 ml/kg/min in the present study; 7 ml/kg/min in human [10]), compared to intact GLP-1 (66–166 ml/kg/min [25]). The reason for this difference between GLP-2 and GLP-1 is unclear, but it may be that the GLP-2 sequence contains a structural element making the N-terminus less accessible to DPP-IV degradation. In an *in vitro* kinetic study, Lambeir et al. recently reported that the catalytic rate constant for the DPP-IV mediated degradation of GLP-1 and GLP-2 differed by a factor of 10 [26]. Although that study did not address the molecular origin of this difference, the authors concluded that the interactions between the non-conserved residues could be important in determining substrate binding, and therefore selectivity, by influencing transition-state stabilization rather than the formation of the enzyme–substrate complex per se [26]. In the present studies, the addition of the DPP-IV inhibitor, valine-pyrrolidide completely inhibited the N-terminal degradation of GLP-2, indicating that DPP-IV is the only enzyme degrading the N-terminus of GLP-2. Valine-pyrrolidide may also inhibit the cytosolic enzymes DPP-8 and 9, but their actions would not be anticipated to contribute to GLP-2 degradation in the extracellular compartment, including plasma.

Determination of GLP-2 extraction by differences in arteriovenous concentrations across the organs revealed that the kidneys, the splanchnic bed and the peripheral tissues (hind leg) are major sites of GLP-2 extraction. Our findings confirm previous reports, pointing to the involvement of the kidneys in GLP-2 clearance

[17,18] but reveal that, in addition, GLP-2 is significantly removed across the extremities and in the splanchnic bed. Our studies provide no clues to the mechanisms of this extraction, but a receptor mediated uptake could be envisaged for the splanchnic extraction, the GLP-2 receptor being expressed with the highest density in the small intestine [15]. In contrast to the findings for GLP-1 [7], GLP-2 escaped extraction in the liver, although the liver is a site with high DPP-IV activity (reviewed in [22]). The negative arteriovenous concentration difference measured across the lungs (pulmonary artery to systemic artery) is likely to be explained by the admixture of venous blood returning to the heart from the upper part of the body, receiving the blood from the ear veins where the infusion was given, and blood from the lower part of the body. Because of this it is impossible to conclude anything from these studies about the handling for GLP-2 in the lungs.

The lack of change in extraction ratios after DPP-IV inhibition may indicate that, although DPP-IV has an effect on the whole body metabolism of GLP-2, it is not significantly involved in the extraction in the observed organs. Thus, the reduced degradation of intact GLP-2 seen after valine-pyrrolidide administration (evidenced by the prolonged half-life and reduced MCR) likely reflects the effect of the inhibitor in reducing intravascular degradation by DPP-IV in the capillary endothelial and surrounding muscle cells [27] and, to much lesser extent, the soluble DPP-IV in the plasma itself. In agreement with the long half-life and low clearance rate of the metabolite, GLP-2 (3–33), it was only extracted significantly in the kidney, with a clearance compatible with glomerular filtration rates in pigs of similar size (2.6±0.3 ml/kg/min, [28]), and we therefore assume that the metabolite is mainly eliminated by glomerular filtration in pigs. The renal extraction of intact GLP-2, amounting to about 25%, is of similar magnitude and also indicates that glomerular filtration is the main mechanism for renal elimination in pigs. Again this is in striking contrast to GLP-1, for which up to 70% is extracted across the kidneys [7], presumably by a peritubular uptake mechanism.

The finding that the half-lives of total GLP-2 remained unchanged after valine-pyrrolidide administration most likely reflects the fortuitous result of the removal of one contributing component (the metabolite) and the simultaneous prolongation of the half-life of the other component (the intact hormone). It is of interest, that the metabolite was mainly eliminated by the kidneys (cf its long half-life and small clearance). This could indicate that the two N-terminal amino acids represent a structural requirement for the peripheral and splanchnic uptake of GLP-2.

GLP-1 has been found to inhibit the secretion of the L-cells and therefore the concentrations of endogenous GLP-1 decreases during infusion of exogenous GLP-1 [29–31], which may introduce a small error in the calculation of its clearance. GLP-2, on the other hand has been demonstrated not to influence L-cell secretion indicating that it is safe to subtract basal values for the calculations [29]. The assay for total GLP-2 employed here recognizes all peptides containing the C-terminal GLP-2 sequence, and, therefore, both intact and degraded GLP-2 (GLP-2 (1–33) and GLP-2 (3–33), respectively), but also the major proglucagon fragment (a product of pancreatic processing of the proglucagon gene (reviewed in [5]) which contains the GLP-2 sequence. This may explain part of the difference between total GLP-2 and intact GLP-2 concentrations before infusion. However, the concentration of the major proglucagon fragment is clearly small compared to the actual GLP-2 concentrations during GLP-2 infusion. Further, the subtraction of basal concentrations obviously eliminates this source of error.

In summary, our results show that GLP-2 is eliminated in the peripheral tissues, in the splanchnic bed and in the kidneys all with similar extraction ratios. The magnitude of the renal elimination for both GLP-2 and its metabolite is compatible with glomerular filtration being the major responsible mechanism. DPP-IV is involved in GLP-2 metabolism and seems to be the sole enzyme responsible for N-terminal degradation of GLP-2. However, inhibition of DPP-IV does not affect the extraction ratios across any of the investigated organs, indicating that the tissue-specific elimination of GLP-2 is independent of this enzyme. Nevertheless, DPP-IV inhibition does increase the concentrations of intact GLP-2, prolonging its half-life and reducing its clearance, presumably via inhibition of the DPP-IV associated with the capillary endothelium, attesting to the importance of this enzyme in GLP-2 metabolism.

Acknowledgments

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