

GLP-2 and mesenteric blood flow

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THE THESIS IS BASED ON THE FOLLOWING PUBLICATIONS

1. The effect of Glucagon-Like Peptide-2 on mesenteric blood flow and cardiac parameters in end-jejunoscopy short bowel patients. Bremholm L, Hornum M, Andersen UB, Hartmann B, Holst JJ, Jeppesen PB. Regul Pept. 2011 Jun 7; 168(1-3):32-8. Epub 2011 Mar 21.
2. The effect of glucagon-like peptide-2 on arterial blood flow and cardiac parameters. Bremholm L, Hornum M, Andersen UB, Holst JJ. Regul Pept. 2010 Jan 8; 159(1-3):67-71. Epub .
3. Glucagon-like peptide-2 increases mesenteric blood flow in humans. Bremholm L, Hornum M, Henriksen BM, Larsen S, Holst JJ. Scand J Gastroenterol. 2009; 44(3):314-9.

INTRODUCTION

Research and interest in the family of gut hormones are increasing, and some of the incretin hormones have already had a major clinical impact in the anti diabetic treatment with Glucagon-Like Peptide(GLP)-1(GLP-1 receptor agonist)(1), furthermore GLP-2 was recently commercially introduced for the treatment of short bowel syndrome (SBS) patients. GLP-1 and 2 are derived from the same amino hormone precursors; proglucagon. The mammalian proglucagon gene is expressed in the alpha cells in the pancreas, in the brain and in the gastrointestinal mucosa. In the intestinal mucosa the expression is localized to entero endocrine L-cells(2). The entero endocrine L-cells are primarily localized in the distal small intestines(3) and colon(4). These are epithelial cells derived from multipotent stem cells, which differentiate into all of the epithelial cells. The cells have a turnover time of 3-4 days. Ten different types of hormone producing entero endocrine cells have

been identified(5). The proglucagon gene encodes a 160 amino acid sequence(6;7), which is identical in the pancreas and intestine(2). However posttranslational enzymatic processing of the the proglucagon, in the intestine and in the pancreas produces different products. In the pancreas proglucagon is cleaved by the enzyme prohormone convertase (PC2)(8), forming a molecule named "the major proglucagon fragment" (MPGF)(corresponding to PG 72-160 amide), which remains uncleaved, and glucagon (corresponding PG 33-61 amide) plus a N-terminal fragment (corresponding to PG 1-30 amide) (2;9;10). In the L-cells the peptides GLP 1 and GLP- 2(11) (corresponding to PG 78-107 and PG 126-158 amide), are derived from the MPGF(12), whereas PG 1-69 also called glicentin remains uncleaved (or partially cleaved into PG 1-30) and oxyntomodulin (corresponding to PG 33-69 amide) (2;10).

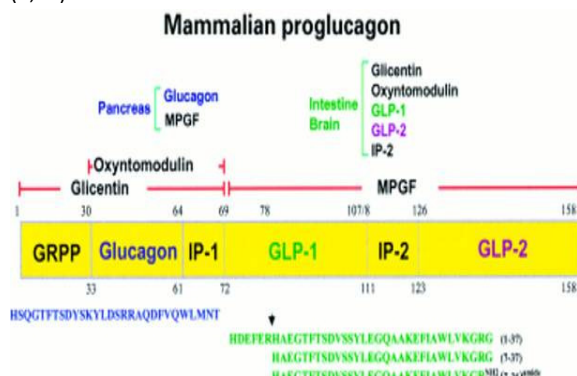


Figure 1. Amino acid sequence of proglucagon, and derived hormones.

The biological effect of the 33 amino acid peptide GLP-2 is mediated by activation of a G-protein coupled 7 transmembrane receptor, expressed mainly in the gut and the brain(13). Thus GLP-2 receptors are expressed in the brainstem, lungs, stomach, small intestine and colon, but not in the heart(14). The receptor sequence is close related to the sequence of the GLP-1 and glucagon receptors (13). The receptor has been reported to localize to subepithelial myofibroblasts(14-16) as well as to enteric neurons and enteroendocrine cells(17;18). Studies have also shown that the receptors colocalizes with nitric oxide (NO) expressing vasoactive intestinal polypeptide-positive enteric neurons, and serotonin in enteroendocrine cells, suggesting that GLP-2 induced increased intestinal blood flow is mediated by vasoactive neurotransmitters. The localization to epithelial endocrine cells (14), has not been supported by subsequent studies.

Elimination of GLP-2 is mainly renal(19), and the T½ of the hormone in humans is approximately 7 minutes(20). Administration of inhibitors of the enzyme; dipeptidyl peptidase 4 (DPP-4) (an incretin enhancer), has been shown to elevate levels of GLP-2, suggesting that the enzyme is responsible for the degradation of the hormone(21), although less extensively than the degradation of the GLP-1 hormone(22;23).

GLP-2 secretion, measured by plasma levels, is increased during enteral feeding both in humans and other mammals(24;25). In bolus and continuously enterally fed piglets, the plasma levels of GLP-2 were increased 5-9 times above baseline, and the maximum increase was reached after 1 hour(25). Xiao et al. showed that enteral feeding in humans increased circulating GLP-2 levels 2-5 times compared to baseline within the first hour of meal ingestion. Interestingly when giving combined meals composed of fat, carbohydrates and proteins, and when giving solely carbohydrates or fat, GLP-2 levels were raised, but when giving solely proteins, GLP-2 levels did not alter. Furthermore, ingestion of protein, carbohydrates and fat increases blood flow in the mesenteric arteries(26;27).

The glucagon like peptides exert different actions, GLP-1 is known regulator of blood glucose, by means of increased insulin secretion, reduced glucagon secretion, delayed gastric emptying, impaired acid secretion and increased satiety. And all ready GLP-1 have as mentioned, therapeutic indications by analogues used in diabetes treatment(1).

Regarding the biological actions of GLP-2, there are several key elements; stimulation of intestinal growth, including increased crypt cell proliferation and increased intestinal blood flow(16;28-31). In 1996 Drucker and co-workers showed that GLP-2 was the likely mediator of the intestinal growth, which could be observed in nude mice bearing transplantable proglucagon-producing subcutaneous tumors. After only 4 days a clear growth of villus height and increase of small bowel weight were observed, and after 6 days the changes were significant. There were no changes in other organs(32). In subsequent studies it has been confirmed that epithelial growth can be induced by administration of GLP-2 to rodents(21). It has further been shown by immunoneutralization that GLP-2 acts as a physiologic growth factor in the small intestine in hyperphagic diabetic rats(33). Subsequently it has been shown that in neonatal piglets total parenteral nutrition(TPN) induces mucosal atrophy, and reduces plasma levels of GLP-2 (34). Furthermore it is shown in rodents on TPN, that mucosal atrophy reverses on supplemented GLP-2 infusion(35). It has been suggested by Dubé et al. that GLP-2 increases intestinal derived Insulin-Like Growth Factor-1(IGF-1) secretion from subepithelial myofibroblast, and that IGF-1 is needed for intestinal growth in response to GLP-2(36). Keratinocyte growth factor expressed in SEMFs, has also been suggested as a mediator of enteric growth(15). In systematic studies of the possible role of gut hormones in meal induced reduction of bone resorption (as measured by serum markers), GLP-2-secretion was found to correlate with this reduction, and it was shown that subcutaneous injections of GLP-2 had a similar effect on bone resorp-

tion(37). Subsequent studies have shown that bed-time sc injection of GLP-2 reduced nocturnal bone resorption in postmenopausal women, and in these studies GLP-2 administration was associated with a small but significant reduction in systolic and diastolic blood pressure(38).

It has been shown in several animal studies that GLP-2 infusion increases intestinal blood flow, and that this increase is confined to the small intestine(28-30). The GLP-2 induced changes in blood flow is rapid, reaching significance already after 10 min.(28). The increased blood flow is probably mainly restricted to the the superior mesenteric artery (SMA). The SMA supplies the duodenum (from the papilla vateri), jejunum, ileum and partial the proximal colon, as will be elaborated later in the thesis.

GLP-2 infusion studies on pigs and humans have shown that the hormone inhibits antral motility and inhibits gastric acid secretion(39;40).

When evaluating the physiological effect of GLP-2, it is natural to consider a possible therapeutical use of GLP-2 or analogues. Such studies have focused on gastro intestinal (GI) disorders, and as mentioned above, an extra intestinal effect as a possible treatment for osteoporosis(37;38;41). As for GI disorders studies on GLP-2 include:

Inflammatory bowel diseases(IBD)(42;43); based on the observation of elevated circulating levels of active GLP-2 in patients with IBD, and on rodent models where GLP-2 induces healing of intestinal damage that was caused experimental.

Chemotherapeutic enteritis(44;45), where rodent models have shown beneficial effect of GLP-2 on intestinal healing, intestinal proliferation, survival, inflammation and bacteremia following chemotherapy.

Experimental induced intestinal ischemia(46;47) in rodents, here GLP-2 significantly increased mucosal mass, based on DNA-measurements.

The above mentioned potential therapeutic effects of GLP-2 are all at an early stage. In the treatment of SBS patients with intestinal failure, GLP-2 had its first commercial breakthrough(48), with the GLP-2 analogue teduglutide, which is a DPP-4 resistant peptide, that has the amino acid alanine in position 2 is substituted by glycine. This markedly increases plasma half-life of the hormone. In a placebo controlled study of SBS patients with intestinal failure(49), teduglutide was shown to reduce amounts of parenteral support. Before this important turning point, the role of GLP-2 in the treatment of SBS patients have been investigated in several studies(49-54), based on its potential influence on the severe complications and consequences of SBS(55) and the fact the SBS patients have almost no endogenous GLP-2, due to the intestinal resections(56). The human trials on SBS patients have shown that GLP-2, improves intestinal wet weight absorption and nutritional status(50;54).

AIM OF THE THESIS STUDIES

The aim of the three studies on which the thesis is based, was to investigate basic physiological effects of the gut hormone GLP-2, in healthy volunteers and in SBS patients with focus on the effects

on mesenteric blood flow, blood flow at other vascular sites and effects on cardiac parameters. These parameters have been evaluated after both meal stimulation and GLP-2 administration. The aim of the first study was to investigate the effects of GLP-2, both subcutaneously (SC) and intravenously (IV), and after meal stimulation, on intestinal blood flow (SMA) and blood pressure in humans.

The aim of the second study was to examine the effect of SC GLP-2 on arterial blood flow in different vascular beds, including SMA, common carotid artery (CCA), celiac artery (CA) and renal artery (RA), and cardiac function.

The aim of the third study was to examine the effect of SC GLP-2 on mesenteric blood flow and cardiac function in end-jejunoscopy SBS patients.

BACKGROUND

GLP-2 and intestinal blood flow

As mentioned above, infusion of GLP-2 increases intestinal blood flow, the blood flow changes may be restricted to the blood supply of the small intestine. Neonatal pigs on total parenteral nutrition respond to GLP-2 with an increase in intestinal blood flow, and the response was demonstrated to be NO dependent, with NO thought to derive from enteric neurons (14;29). Other studies have shown GLP-2R expression in enteric neurons expressing NO synthase. There was also GLP-2R expression in serotonin containing enteroendocrine cells, suggesting that the increased mesenteric blood flow is mediated by secondary neurotransmitters (16). Further studies in piglets demonstrated that GLP-2 infusions increase blood flow in the SMA, but not in the CA(30). These studies all examined the response to a single bolus administration of GLP-2. However recent studies have shown that the increase in blood flow in the small intestine, was reduced when GLP-2 was administered in a longer period(31).

Ultrasound

In order to perform human studies regarding blood flow effects of GLP-2 it is desirable to use a non-invasive and safe method. It is possible to monitor alterations in blood flow velocity by ultrasound scanning (US) combined with the Doppler technique(57). The technique is validated, accurate and reproducible(58;59). Increased intestinal blood flow is a known effect of enteral feeding. Doppler applied to the SMA has previously confirmed, that blood flow in this vessel increases in response to a meal(26). Very few studies have investigated vascular actions of GLP-2, outside the vessels supplying the intestines. Rodent studies using Doppler-US have shown immediate significant changes in blood flow in the SMA, and smaller delayed significant changes in the inferior mesenteric artery (IMA) and CCA. There were no significant changes in the CA and RA(28) under GLP-2 infusion. The same studies showed no significant changes in mean arterial pressure (MAP).

Hemodynamic parameters

Hemodynamic parameters such as stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR), ECG and blood pressure can be measured non invasively. This can be done by automatic beat-to-beat impedance cardiography and beat-to-beat blood pressure (by finger plethysmography) provided by the Task Force Monitor (TFM). The use of thoracic impedance is a rather novel technique, but it has been shown to be highly reproducible and equal to thermo dilution, which is considered "gold standard" for CO determination.(60-62)

Short bowel syndrome

End-jejunoscopy SBS patients have significantly reduced post-prandial GLP-2 production(49). As previous mentioned, studies have shown that exogenous GLP-2 and the DPP-4 degradation resistant analog teduglutide act as growth factors in the small intestine when administered to these patients, and also improve intestinal function(49;52). However, acute increases in stoma nipple size have been observed within minutes after initiating this treatment(49), suggesting that this was due to changes in intestinal blood flow rather than changes in mucosal growth, the induction of which is likely to take hours or days.

No basic vascular and cardiac physiological studies on the effect of GLP-2 or analogues have been made in the SBS intestinal failure population. And very few studies have investigated basic physiological changes in humans when GLP-2 is administered.

GLP-2 measurements

GLP-2 concentrations in plasma can be measured by radioimmunoassay after extraction of plasma with ethanol, employing antiserum and standards of human GLP- and monoiodinated Tyr-12 GLP-1. The antiserum is directed against the N-terminus of GLP-2 and therefore measures only fully processed GLP-2 of intestinal origin(12).

Materials and methods for the studies

Below is described the material and methodological design for the three studies. The three studies have been carried out consecutively, for more elaborated descriptions; the reader is referred to the original manuscripts.

STUDY 1: GLUCAGON-LIKE PEPTIDE 2 INCREASES MESENTERIC BLOOD FLOW IN HUMANS

Procedure and experimental design

10 healthy volunteers were included and participated in a two-day study separated by approximately one week. We first studied the effects of a standardized meal in order to stimulate flow in the superior mesenteric artery while at the same time sampling blood for determination of the plasma concentrations of endogenous GLP-2

The ultrasound examinations were performed with a GE LOGIQ 9 © Ultrasound Unit (General Electric Healthcare, USA). The SMA was examined in the longitudinal direction in the sagittal plane. The sampling cursor was placed within the diameter of the vessel,

2–3 cm distal to its origin, and the angle between the ultrasound beam and the SMA was kept smaller than 60°.

Calculations: The RI can be used to estimate the resistance in a vessel. In this setup, a falling RI was taken to indicate that the flow was increasing. The RI was calculated as

$$RI = 1 - \frac{V_{dia}}{V_{sys}}$$

where V_{dia} is the end diastolic velocity and V_{sys} is the maximal systolic. Each RI value were included in the statistical analysis represents the result of three measurements.

We calculated for each participant the difference between baseline RI and the mean of the values measured in response to the standard meal and GLP-2 injection. This difference thus expresses the integrated RI response to the standard meal/GLP-2 injection.

At day one the subjects ingested a standard liquid meal.

RI in the SMA was recorded, blood pressure (systolic(SYS), diastolic(DIA) and MAP) and pulse rate measured, general wellbeing subjectively evaluated and venous blood samples were obtained at times at times 0, 5, 15, 25, 35, 45, 60, 75 and 90 minutes.

On day two synthetic human GLP-2 were diluted in hydroxyethylen starch solution (Voluven®) and infused IV at rates of 0.5 pmol/kg/min, 1.0 pmol/kg/min and 2.0 pmol/kg/min for 3 x 45 min. between each infusion period, the volunteer rested for approximately 15-20 minutes, in order to achieve baseline levels of RI, equal to the initially measured RI.

RI in the SMA was recorded, blood pressure (SYS, DIA and MAP) and pulse rate measured, general wellbeing subjectively evaluated and venous blood samples were obtained at times at times 0, 5, 15, 25, 35, and 45 minutes.

After a 15-20 min rest, where baseline levels of RI, equal to the initially measured RI was re-established, 450 nmol synthetic GLP-2 was given (SC). RI in the SMA were recorded, blood pressure (SYS, DIA and MAP) and pulse rate measured, general wellbeing subjectively evaluated and venous blood samples were obtained at times at times 0, 5, 15, 25, 35, 45, 60, 75 and 90 minutes.

Height and weight were measured and IV access was established on both days.

GLP-2 concentrations in plasma were measured by radioimmunoassay.

Statistical analysis: Results for IV GLP-2 were analysed for statistical significance by the Friedmann ANOVA test for repeated measurements and the test meal/GLP-2 injection by the Wilcoxon test for paired data.

Blood pressure results were analysed for statistical significance by one sample t-test and Wilcoxon matched pair tests. Results are expressed as medians and ranges or mean \pm SE, and differences resulting in P-values <0.05 were considered statistically significant.

Study 2: THE EFFECT OF GLUCAGON-LIKE PEPTIDE 2 ON ARTERIAL BLOOD FLOW AND CARDIAC PARAMETERS

Procedure and experimental design

10 young healthy volunteers were included. All of the volunteers were given GLP-2 while 5 served as controls, receiving placebo

(isotonic saline). The crossover experiments, with GLP-2 or placebo in randomized order, were carried out over two days, under identical circumstances and in the same facilities. The trial was blinded to the subjects.

Doppler US-scanning: All examinations were performed by the same experienced sonographer. The Doppler-US-equipment was an Acuson® Sequoia 512 (New York, USA). A 2.5-4.0 MHz curvilinear transducer was used for the abdominal examinations, while a linear 12 MHz transducer was used for the carotid examinations. All measurements were performed without moving the subject, who was lying in the same supine position during the whole session.

For each measurement, calculations on the Doppler spectra were made on 5 consecutive heartbeats, using the auto measure software. All Doppler parameters (TAMV, V_{dia} and V_{sys}) were calculated by the equipment, thus avoiding measurement bias.

The SMA and the CA were examined by a longitudinal epigastric view. Measurements were made as close to the ostia as possible, with angle correction as necessary. For each time point, we made three measurements in the SMA, RA, CA, and two in the CCA. In two cases, the CA could not be examined due to bowel gas.

The right renal artery was examined via a transverse epigastric view. Duplicate measurements were made in the proximal down sloping part of the artery.

For the measurements of the diameter of the CCA, we used spatial compounding to enhance the exposition of the trailing edge of the intimal-medial layer. The diameter was measured immediately inferior to the carotid bulb, from the trailing edge of the anterior wall to the leading edge of the posterior wall, on a frozen, zoomed image. For each time point, five diameter measurements were made on each of three images, by a technician blinded to the experiment. For the measurement of the Doppler spectrum of the CCA the beam was angled to less than 60 degrees, and we attempted to use the same angle for all measurements.

Calculations: The RI was calculated as described in study 2.

Changes in the TAMV more directly reflect changes of the flow in a vessel. The software of the equipment calculates this parameter.

Also the two modalities do not reflect identical observations; RI represents resistance change and TAMV change in velocity. There may be dynamic vascular difference in the measured changes in these modalities, as RI depends on vessel diameter change and TAMV on blood flow velocity (63).

We estimated the flow in the CCA as (D is diameter):

$$CCA \text{ flow} = TAMV \times \pi \times (\frac{1}{2}D)^2$$

Cardiovascular measurements: With the TFM HR, BP (by finger plethysmography), CO, SV and TPR (by impedance cardiography) were measured. The continuous beat-to-beat measurement of blood pressure was supplemented with conventional non-invasive oscillometric blood pressure measurement. All data was stored from time -1 minute to 90 minutes.

Analyses: GLP-2 concentrations in plasma were measured by radioimmunoassay.

The examination started after an overnight fast.

On day one 1 milliliter of isotonic saline (placebo solvent) was given SC.

On day two 450 nmol synthetic GLP-2 (volume 1 milliliter) was given SC.

Doppler scanning was performed as described at times 0, 5, 15, 25, 35, 45, 60, 75 and 90 minutes. Venous blood samples were taken at times 0, 30 and 60 minutes.

Cardiac parameters were continuously measured from time -1 minute to 90 minutes. The data material was separated in different log files as stated above.

Statistical analysis: Change in blood flow and cardiac parameters compared to baseline were analyzed for statistical significance by the Friedmann ANOVA test for repeated measurements.

Calculation of changes in blood flow and cardiac parameters compared to placebo was based on the 5 persons who received saline. The changes were analyzed for statistical significance by T-test for paired samples.

Results were expressed as medians and ranges or mean \pm SE, and differences resulting in P-values <0.05 were considered statistically significant.

Study 3: THE EFFECT OF GLUCAGON-LIKE PEPTIDE 2 ON MESENTERIC BLOOD FLOW AND CARDIAC PARAMETERS IN END JEJUNOSTOMY SHORT BOWEL PATIENTS

Procedure and experimental design

The study included 8 end-jejunosomy SBS patients in stable condition, but with less than 200 cm of jejunum remaining. The crossover experiments, with GLP-2 or placebo in randomized order, were carried out over two days, under identical circumstances and in the same facilities. The trial was blinded to both the patient and the sonographer carrying out the investigation. All of the patients were given SC 1600 μ g synthetic GLP-2 (volume 2 ml) and 2 ml of placebo (isotonic saline), respectively, on separate study days.

Doppler US-scanning: In regards to blood flow in SMA and CA, the procedure was as described in study 2.

Calculations: As described in study 2.

Cardiovascular measurements: As described in study 2.

Analyses: for practical purposes as described in study 2.

Statistical analysis: As described in study 2.

RESULTS

In the following paragraphs the results for the three studies are presented in short form, each of the three studies and there after presented with more detailed results. The complete results can be studied in the original papers in the appendix.

Blood flow

In all three studies blood flow changes in the SMA after GLP-2 administration, were similar regarding changes over time and degree of change. Blood flow changes were similar to changes

seen after a standard meal. Only RI changes were registered in all three studies, but the TAMV changes in study 2 and 3 had similar characteristics.

Cardiovascular parameters

In all three studies no significant changes in blood pressure were registered in relation to GLP-2 administration. In study two and three, where cardiac parameters also were registered by impedance cardiography, increases in CO and SV was seen.

Plasma GLP-2

There was as expected measured supra physiological GLP-2 plasma levels after SC and IV administration.

Study 1 results: Glucagon-like peptide-2 increases mesenteric blood flow in humans

Subjects:

Baseline characteristics are presented in Table 1. No serious side effects to administration of GLP-2 or ingestion of the standard meal were observed.

Table 1: demographics of test subject

	Age (years)	weight (kilograms)	height (centimetre)	BMI (kg/m ²)
Mean	26,2	63, 9	171.8	21.6
Range	24-29	48-69	165-178	17.6-23.4

Abbreviation: BMI = body mass index

Blood flow

The standard meal and the SC GLP-2 injection (450 nmol) resulted in similar, significant decreases in RI, ($P<0.005$), representing a proportional rise in mesenteric artery blood flow. The maximum change in mesenteric blood flow after SC GLP-2 averaged 15.6 % (range 5.0-28.1). The maximum change in mesenteric blood flow in response to a standard meal was 13.9 % (range 8.8-20.0) (Figure 2).

The maximum changes in blood flow were observed 25 minutes after ingestion of the standard meal and 35 minutes after injection of 450 nmol SC GLP-2. At 5 min after the application of the stimulus, the blood flow changes amounted to increases of 6.1 % and 8.2 % for the standard meal and SC GLP-2, respectively.

The changes over time in RI in response to a standard meal were similar to those observed after 450 nmol SC GLP-2 (Figure 1) and AUC for the two curves also did not differ.

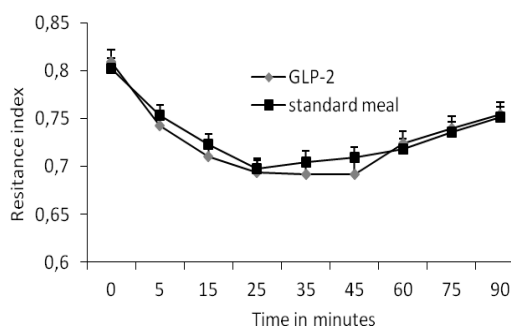


Figure 2. Resistance index expressed as mean \pm SE over time when giving a standard meal, ($P < 0.02$) and subcutaneous injection of GLP-2, ($P < 0.005$).

After IV infusion of GLP-2 the following mean changes in RI were observed: 0.5 pmol/kg/min: 2.7 % (range 0-6.3 %), 1.0 pmol/kg/min: 6.7 % (range 0.4-15.9 %), 2.0 pmol/kg/min: 15.3 % (range 9.6-22.7 %). The change in blood flow in relation to the increasing IV dose was significant, ($P < 0.00802$). The dose-response curve for GLP-2 seemed to fit an exponential function (Figure 3).

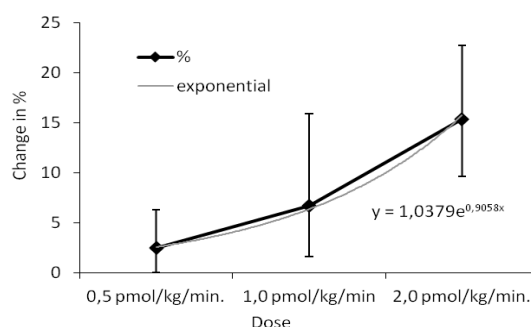


Figure 3. Change in % of resistance index under increasing intra-venous GLP-2 infusion, expressed as mean \pm range.

Cardiovascular parameters

Blood pressure was measured non-invasively. The standard meal resulted in a significant decrease in blood pressure, SYS, MAP (Figure 4 a and b) and DIA. GLP-2 administration did not result in consistent changes in blood pressure values, neither at IV or SC administration, but a rather large variation was noted. DIA showed a significant decrease during SC injection, but there was no clear relationship between DIA, MAP and SYS values upon SC injection.

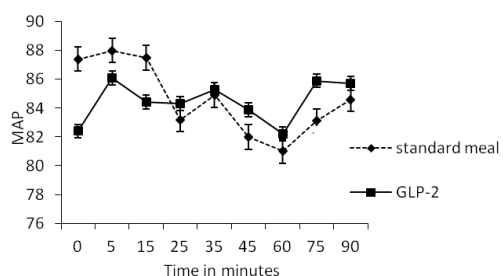


Figure 4 a. Change in mean arterial pressure over time when giving a standard meal and subcutaneous administration of GLP-2, expressed as mean \pm SE. ($P < 0.01$) for standard meal.

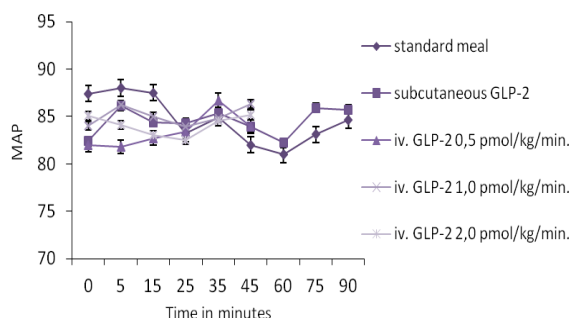


Figure 4 b. Change in mean arterial pressure over time when giving a standard meal, subcutaneous administration and intra-venous infusion of GLP-2, expressed as mean \pm SE.

Plasma GLP-2

Plasma GLP-2 concentrations are shown in figure 5 and 6, which indicate that the low IV infusion rate resulted in physiological elevations of the plasma GLP-2 concentration. Plasma GLP-2 concentrations at higher doses resulted in supra-physiological levels.

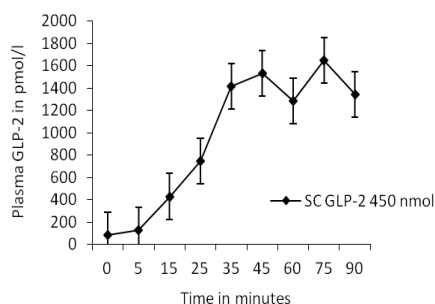


Figure 5. Change in plasma GLP-2 over 90 minutes after subcutaneous injection of GLP-2. Plasma GLP-2 in pmol/l is expressed as mean \pm SE. Plasma levels were elevated to an expected level(50).

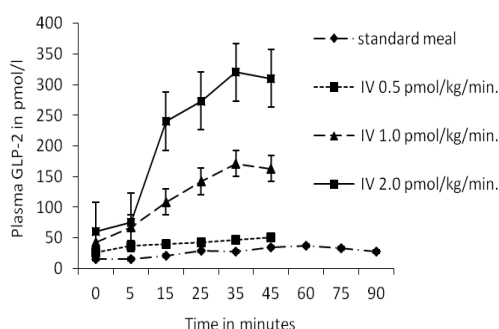


Figure 6. Change in plasma GLP-2 over 45/90 minutes after IV GLP-2 and standard meal. Plasma GLP-2 in pmol/l is expressed as mean \pm SE. The low IV infusion rate resulted in physiological elevations of the plasma GLP-2 concentration.

Study 2 results: The effect of glucagon-like peptide-2 on arterial blood flow and cardiac parameters

Subjects:

Baseline characteristics are presented in table 2. No serious side effects to injection of GLP-2 were observed. One volunteer experienced a short episode of stomach pain and a minor fall in blood pressure, but completely recovered within 20 minutes. The episode occurred 50 minutes after GLP-2 injection. The episode was likely to be a vaso vagal response.

Table 2: Demographic data

	Age	weight	height	BMI
	(years)	(kilograms)	(centimetre)	(kg/m ²)
Mean	24.8	75.95	175.4	24.6
±SD	1.6	13.5	8.0	3.5

Abbreviation: BMI = body mass index

Blood flow in the different vascular beds

GLP-2 significantly increased SMA blood flow, both compared to baseline and placebo. SMA flow remained unchanged in the placebo group. No significant changes in velocities and resistance indexes in the CA or RA, or blood flow in the CCA were observed, neither in the GLP-2 group nor in the placebo group. The results are illustrated in figure 7. The increase in velocity and decline in resistance elicited in the SMA was 269.4 % and 27 % (P<0.01), respectively.

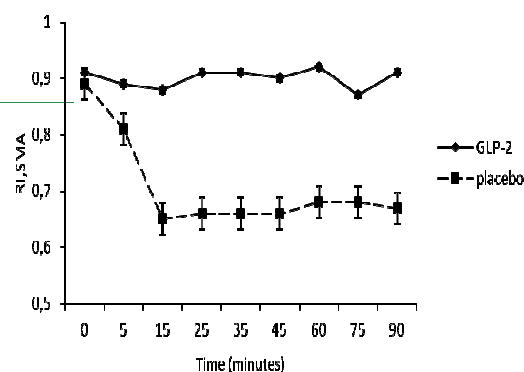
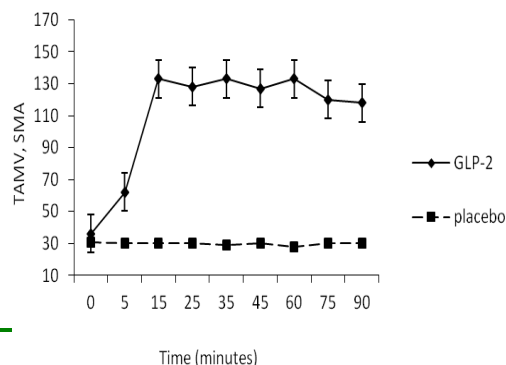
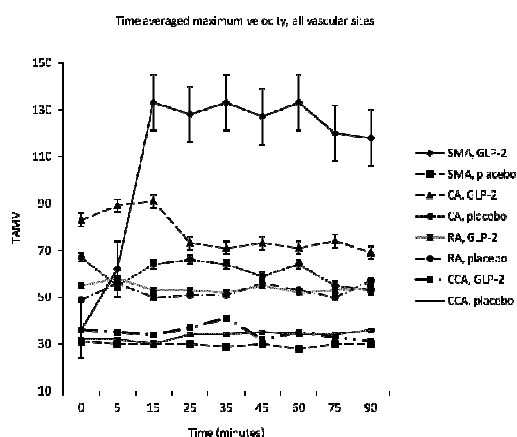


Figure 7 a,b and c. In figure 7 a is shown change in Time Averaged Maximal Velocity (TAMV), comparing subcutaneous injection of 450 nmol GLP-2 with placebo (isotonic saline) expressed as mean \pm SE. GLP-2 significantly increases velocity in the superior mesenteric artery compared both to baseline and placebo, but not in the other examined vascular sites. In figure 7 b and c is shown the SMA blood flow change expressed as TAMV and resistance index.

Cardiovascular parameters

CO, SV and HR increased from baseline in the GLP-2 group (15.3, 4.81 and 8.2 % (P<0.001, P<0.01 and P<0.01), respectively), but compared to the placebo group, there were no significant differences. The CO and HR changes are illustrated in figure 8 and 9. Total peripheral resistance was significantly reduced from baseline. All cardiac parameters are presented in table 3 a and b. The maximal change of blood flow and cardiac parameters was reached after an average of 15 minutes.

There were no significant changes in blood pressure values within or between the GLP-2 and the placebo group during the experiments, even though diastolic and mean arterial pressure tended to be higher in the placebo group during the first 15 minutes. Blood pressure changes is graphically illustrated in figure 10 a, b and c.

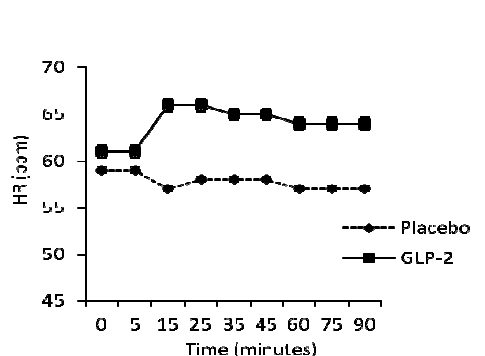


Figure 8. Change in heart rate (HR), comparing subcutaneous injection of GLP-2 with placebo (isotonic saline) expressed as mean \pm SE. GLP-2 significantly increases HR compared to baseline.

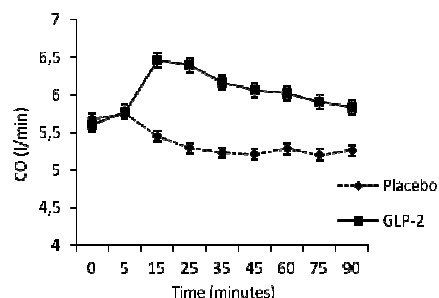


Figure 9. Change in cardiac output (CO), comparing subcutaneous injection of GLP-2 with placebo (isotonic saline) expressed as mean \pm SE. GLP-2 significantly increases CO compared to baseline.

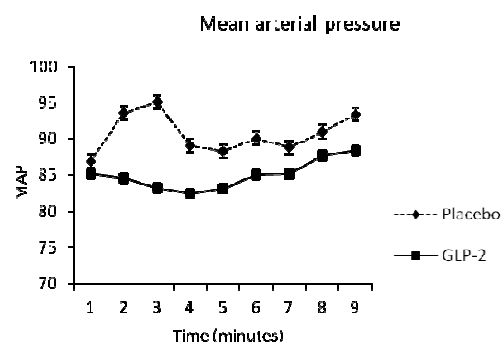
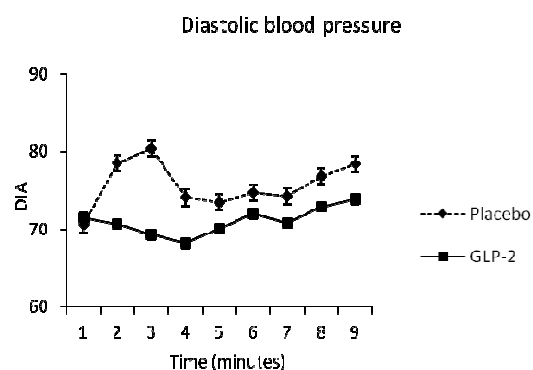
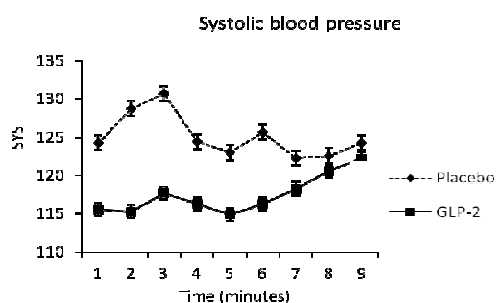


Figure 10 a, b and c. Change in blood pressure values comparing subcutaneous injection of GLP-2 with placebo (isotonic saline) expressed as mean \pm SE. There were no significant changes.

Table 3 a: GLP-2, mean values (\pm SD)

Time	HR	\pm SD	SYS	\pm SD	DIA	\pm SD	MAP	\pm SD	SV	\pm SD	CO	\pm SD
Minutes	(bpm)		(mmHg)		(mmHg)		(mmHg)		(ml)		(l/min)	
0	61	7.75	115.5	9.19	71.5	6.64	85.2	6.78	89.2	17.29	5.61	0.87
5	61	7.68	115.3	7.17	70.7	5.59	84.6	5.52	90.1	17.62	5.81	0.87
15	66	8.19	117.7	8.84	69.3	5.0	83.3	5.51	93.5	18.97	6.47	0.86
25	66	8.62	116.3	10.1	68.2	6.75	82.5	6.96	93	19.91	6.4	0.79
35	65	9.2	114.9	8.29	70.1	8.46	83.1	7.54	90.2	19.60	6.02	0.79
45	65	9.19	116.3	10.7	72	9.94	85	9.22	89.1	19.06	6.17	0.79
60	64	7.2	118.3	9.24	70.8	12.9	85.2	10.3	89.1	19.38	5.83	0.81
75	64	7.18	120.6	11.0	72.9	8.0	87.7	9.4	87.8	19.0	5.82	0.82
90	64	7.76	122.3	12.8	73.9	10.9	88.4	12.2	87.4	18.8	5.74	0.75

Table 3 b: Placebo, mean values (\pm SD)

Time	HR	\pm SD	SYS	\pm SD	DIA	\pm SD	MAP	\pm SD	SV	\pm SD	CO	\pm SD
Minutes	(bpm)		(mmHg)		(mmHg)		(mmHg)		(ml)		(l/min)	
0	59	4.51	124.3	5.6	70.6	6.68	86.9	4.71	101.7	11.22	5.68	0.69
5	59	4.94	128.8	6.72	78.5	4.71	93.6	4.25	101	7.96	5.54	0.58
15	57	3.19	130.7	10.6	80.4	7.15	95.1	8.08	99.1	8.47	5.47	0.29
25	58	2.31	124.4	6.38	74.1	8.57	89.1	8.64	96	9.76	5.27	0.25
35	58	2.68	123	6.84	73.5	4.67	88.3	5.07	94.2	8.49	5.22	0.24
45	58	1.80	125.7	11.6	74.7	2.73	90.1	3.7	93.5	7.97	5.27	0.18
60	57	9.08	122.3	3.93	74.3	6.05	88.8	4.27	94.9	3.79	5.05	0.97
75	57	8.58	122.6	7.74	76.8	6.95	91	7.19	94.4	4.15	5.11	0.92
90	57	8.97	124.3	6.7	78.4	11.03	93.4	9.74	93.7	4.07	5.1	0.93

Abbreviations: HR; heart rate, SYS; systolic blood pressure, DIA; diastolic blood pressure, MAP; mean arterial blood pressure, SV; stroke volume, CO; cardiac output, TPR; total peripheral resistance.

Plasma GLP-2 concentrations

There was no difference in plasma GLP-2 concentrations before GLP-2 or placebo (saline) injection. Placebo did not elicit any change in GLP-2 concentrations. SC GLP-2 induced a marked increase from an average of 20.3 pmol/l to 1725 pmol/l GLP-2. This is illustrated in figure 11.

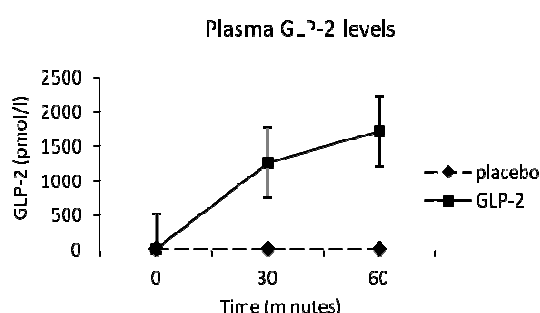


Figure 11. Change in plasma GLP-2 over 60 minutes after subcutaneous injection of GLP-2, plasma GLP-2 in pmol/l is expressed as mean \pm SE. Plasma levels were elevated to an expected level.

Study 3 results: The effect of Glucagon-Like Peptide-2 on mesenteric blood flow and cardiac parameters in end jejunostomy short bowel patients

Patients

Eight end-jejunostomy SBS patients were included (5 women and 3 men). Mean time from last performed intestinal resection was 9.5 ± 9.4 years (range 2-31 years). The etiology of SBS was Crohn's disease in 5 cases, ulcerative colitis with surgical complications in 2 and ischemic infarction in 1. Seven patients received either home parenteral nutrition (n=5) or fluids (n=2), whereas the last patient maintained weight by compensatory hyperphagia. In order to reduce stomal output, seven of the patients received anti-secretory agents (proton pump inhibitors) and 5 received anti-diarrhoeal medications (codeine mixture (n=3), loperamide (n=1) and tincture of opium (n=1), none of the Crohn's disease

patients received any anti-inflammatory drugs, and were all in clinical remission.

Table 4. Demographic data:

	Age	Weight	Height	BMI
	(years)	(kilograms)	(centimetres)	(kg/m ²)
mean	53.6	66.89	168.6	23.19
\pm SD	13.15	15.59	10.04	3.27

Abbreviation: BMI = body mass index

No adverse events were observed in the trial due to administration of placebo or GLP-2.

Blood flow in the different vascular beds

SC administered GLP-2 significantly increased SMA blood flow compared both to baseline and placebo (figures 11 and 12). The changes were measured both as RI and TAMW. Compared to baseline, GLP-2 SC elicited a 19.4 % (from 0.82 ± 0.09 to 0.66 ± 0.08) decrease ($P < 0.01$) in RI and a 97.6 % (from 49.34 ± 42.33 to 97.52 ± 49.3) increase in TAMV in the SMA ($P < 0.01$). The changes are expressed as average change over time, the individual change in each patient measured from baseline time 0 min to the individual maximal change is shown in table 5. The maximum changes in RI and TAMV for the whole patient group were 22.6 ± 11.5 % and 171 ± 129 %, respectively (the individual maximum changes were reached at different times, illustrated in table 1). SC administered placebo elicited no significant changes in blood flow compared to baseline, measured both as RI and TAMV in the CA (figures 11 and 12).

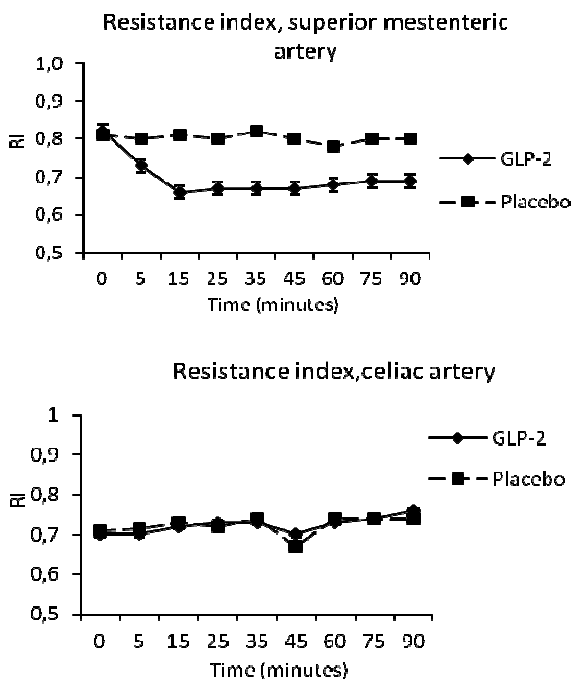


Figure 11 a and b. Resistance index (RI); superior mesenteric artery (SMA), GLP-2 compared to placebo ($P < 0.05$). RI; SMA, GLP-2 compared to baseline ($P < 0.01$). Shown are means \pm SE. RI; celiac artery (CA), GLP-2 compared to placebo (NS). RI; CA, GLP-2 compared to baseline (NS). Shown are means \pm SE.

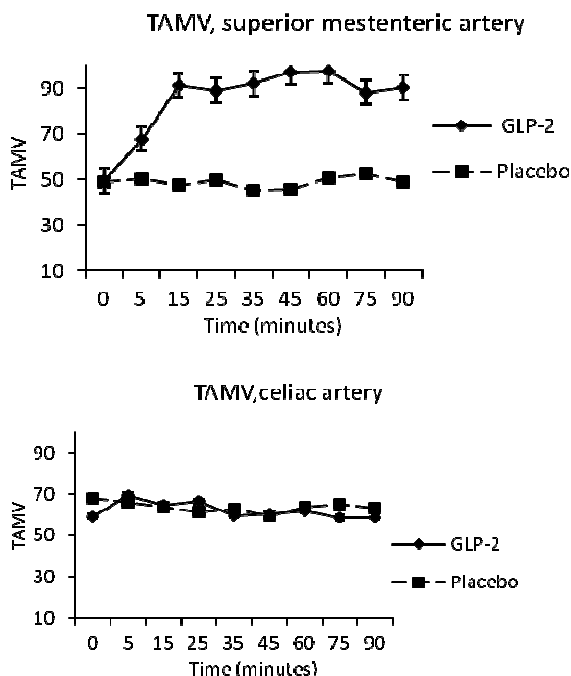


Figure 12 a and b. 12 a; time averaged maximal velocity (TAMV); superior mesenteric artery (SMA), GLP-2 compared to placebo ($P < 0.05$). TAMV; SMA, GLP-2 compared to baseline ($P < 0.01$). Shown are means \pm SE. 12 b; TAMV; celiac artery (CA), GLP-2 compared to placebo (NS). TAMV; CA, GLP-2 compared to baseline (NS). Shown are means \pm SE.

2 of the 8 SBS patients had less than 50 cm small intestine remaining (both approximately 30 cm jejunum). In these patients, the changes in RI and TAMV of the SMA induced by SC GLP-2 were markedly lower than in the other patients in the trial. The two patients elicited a 6.8 % (8.9 % and 4.7 %) maximum decrease in RI compared to a 25.7 ± 8 % maximum decrease in the group of 6 patients with more than 100 cm small intestine, and a 35.7 % (62.2 % and 1.5 %) maximum increase in TAMV compared to 125.8 ± 112 % was seen in the group of 6 patients with more than 100 cm small intestine.

When analyzing the whole group, there was a significant correlation between change in blood flow measured as RI and length of remaining intestine, and a measurable but not significant correlation between change in blood flow measured as TAMV and length of remaining intestine. The correlation was calculated as Pearson Product Moment Correlation, RI ($y = 0.14x + 4.3$, $R = 0.83$, $P < 0.01$) and TAMV ($y = 1.21x + 21.3$, $r = 0.63$, $P < 0.09$) (figure 13).

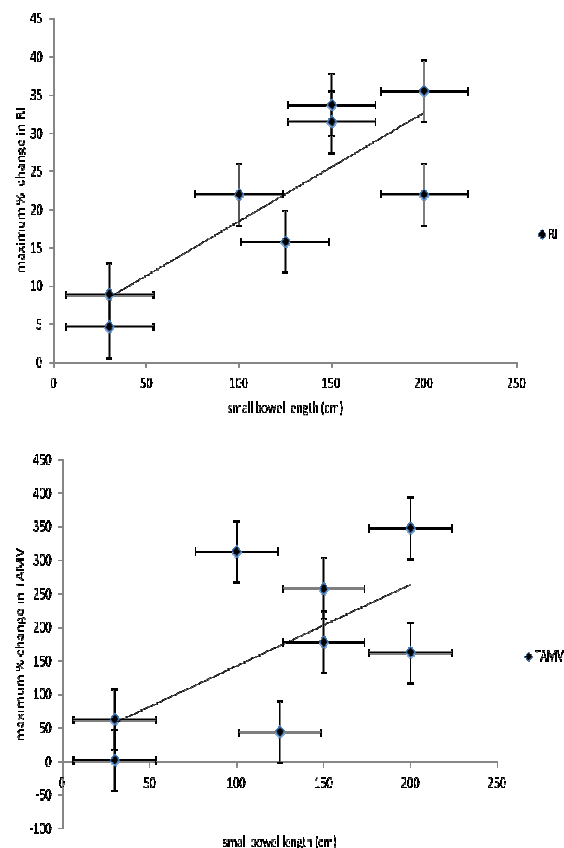


Figure 13 a and b. Correlation between changes in blood flow change measured as resistance index (RI) respectively time averaged maximal velocity (TAMV) and length of remaining intestine.

Cardiovascular parameters

GLP-2 induced a small but significant increase in HR compared to baseline. HR increased from 75 ± 13 beats per minute (bpm) at 0 minutes to 78 ± 14 bpm at 25 min ($P < 0.05$) (Figure 14).

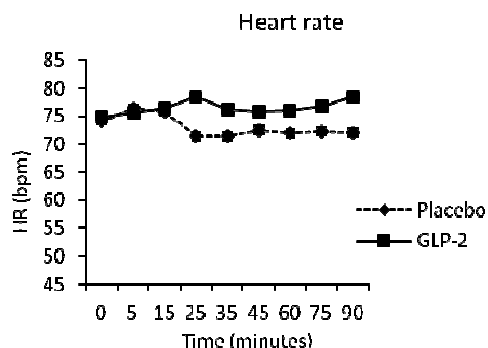


Figure 14: Heart rate (HR); GLP-2 compared to placebo (NS). HR; GLP-2 compared to baseline: ($p < 0.05$). Shown are means \pm SE.

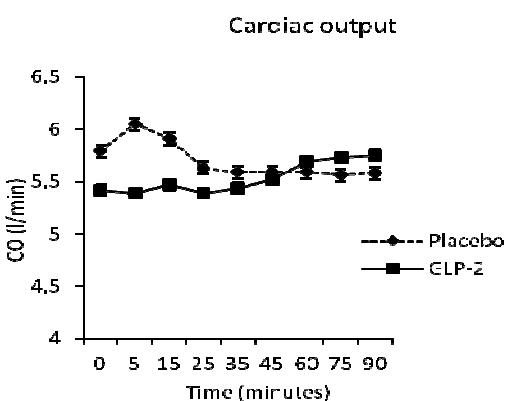
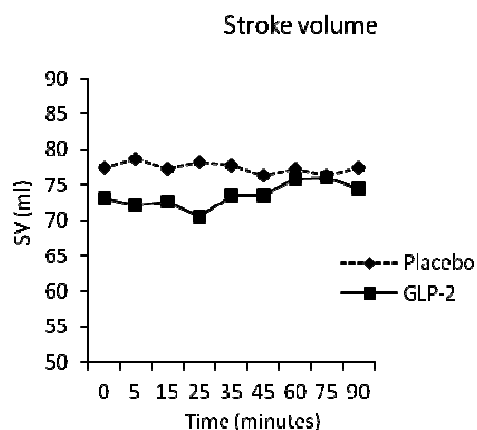


Figure 15 a and b. Change in stroke volume and cardiac output comparing subcutaneous injection of GLP-2 with placebo (isotonic saline) expressed as mean \pm SE.

There was a numerical but not significant increase in HR compared to the placebo arm. A trend towards an increase in SV and

CO was seen in the GLP-2 group, both compared to baseline and placebo (figure 15). SV increased compared to baseline from 73.07 ± 12.54 ml at 0 min to 76.02 ± 14.93 ml at 75 min ($P < 0.7$). CO increased compared to baseline from 5.42 ± 1.05 l/min at 0 min to 5.75 ± 0.90 l/min at 90 min ($P < 0.3$).

There were no significant changes in any other cardiac parameter, neither compared to baseline nor to placebo. This included blood pressure as illustrated in figure 16. All cardiac parameters are illustrated in table 6.

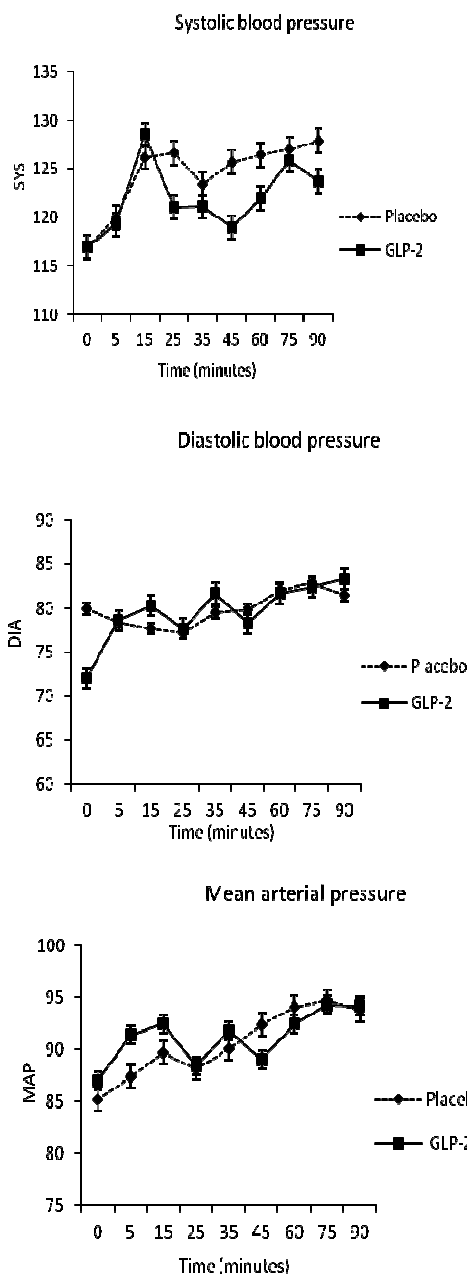


Figure 16 a, b and c. Change in blood pressure values comparing subcutaneous injection of GLP-2 with placebo (isotonic saline) expressed as mean \pm SE. There were no significant changes.

Plasma GLP-2 concentrations

Fasting plasma GLP-2 concentrations before GLP-2 or saline administration were similar. Placebo (saline) did not elicit changes in plasma GLP-2 concentrations at baseline, 30 or 60 min (11 ± 9 pmol/l, 11 ± 5 pmol/l and 12 ± 8 pmol/l, respectively) (NS change). SC GLP-2 induced a significant increase in plasma GLP-2 concentrations from 11 ± 3 pmol/l at baseline to 3743 ± 3285 and 5043 ± 3390 pmol/l after 30 and 60 min respectively ($p < 0.01$). Illustrated in figure 17.

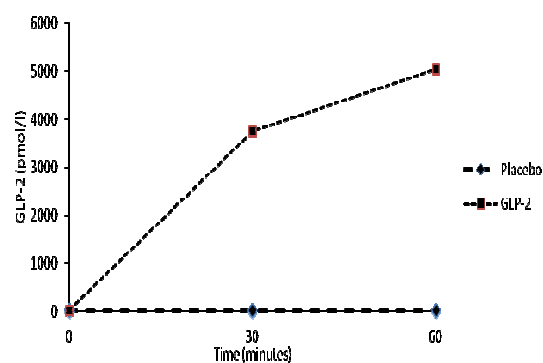


Figure17. Plasma GLP-2 concentrations

Table 6: Cardiac data

GLP-2, mean values (\pm SD)

Time	HR	\pm SD	SYS	\pm SD	DIA	\pm SD	MAP	\pm SD	SV	\pm SD	CO	\pm SD
Minutes	(bpm)		(mmHg)		(mmHg)		(mmHg)		(ml)		(l/min)	
0	74.84	12.83	117.20	9.93	75.58	13.78	86.96	13.08	73.07	12.54	5.42	1.05
5	75.62	12.63	119.27	9.66	79.50	8.03	90.64	7.93	72.20	12.88	5.39	0.93
15	76.35	13.46	122.54	10.65	80.33	8.08	92.48	8.95	72.57	13.30	5.47	1.00
25	78.42	13.97	117.78	12.83	77.67	9.81	88.37	11.06	70.54	15.48	5.39	1.04
35	76.19	14.07	121.06	11.87	81.68	10.13	91.89	10.46	73.58	12.71	5.53	0.97
45	75.82	14.20	118.93	11.13	78.32	7.22	89.01	7.32	73.63	12.59	5.52	1.03
60	75.94	14.53	121.98	16.35	81.67	13.24	92.51	14.07	75.89	11.96	5.69	1.02
75	76.76	15.12	125.88	15.53	82.40	11.46	94.28	13.17	76.02	14.93	5.73	1.06
90	78.35	13.51	123.73	12.93	83.28	10.17	94.24	10.04	74.48	10.75	5.75	0.90

Placebo, mean values (\pm SD)

Time	HR	\pm SD	SYS	\pm SD	DIA	\pm SD	MAP	\pm SD	SV	\pm SD	CO	\pm SD
Minutes	(bpm)		(mmHg)		(mmHg)		(mmHg)		(ml)		(l/min)	
0	74.07	8.99	117.14	7.16	75.46	11.24	85.24	9.66	77.47	18.98	5.39	1.77
5	76.31	11.36	119.99	14.26	75.67	15.96	86.83	15.90	78.58	17.72	5.69	1.93
15	75.82	9.56	124.08	14.27	77.74	17.77	89.66	17.99	77.23	15.02	5.55	1.69
25	71.37	8.32	120.17	12.44	77.17	17.35	88.18	17.20	78.17	15.28	5.28	1.56
35	71.44	7.29	123.37	12.34	79.50	17.02	90.94	17.08	77.68	14.54	5.25	1.43
45	72.46	8.65	125.70	17.81	79.91	19.96	92.36	20.97	76.40	12.24	5.27	1.45
60	71.95	8.21	126.39	10.70	82.12	7.51	94.14	8.50	77.18	12.49	5.26	1.42
75	72.33	7.73	127.04	10.63	82.76	6.88	94.69	8.18	76.38	12.84	5.29	1.35
90	72.05	6.45	127.89	12.74	81.50	8.18	93.75	9.01	77.43	12.77	5.32	1.17

Abbreviations: HR; heart rate, SYS; systolic blood pressure, DIA; diastolic blood pressure, MAP; mean arterial blood pressure, SV; stroke volume, CO; cardiac output, TPR; total peripheral resistance

DISCUSSION

Study 1: Glucagon-like peptide-2 increases mesenteric blood flow in humans

The main findings of this study were the significant changes in SMA blood flow, as a response to GLP-2 administration, both IV and SC. The changes in blood flow mimicked the blood flow changes seen in response to a standard meal(64). This indicates that the known meal induced blood flow changes are mediated by GLP-2. The changes in blood flow occurred immediately, as previously shown with rodents and piglets (28), and reached a maximum approximately 40 min. after SC injection. The significant effects on blood flow, even after the low IV dose, which produced plasma levels which may be seen under physiological circumstances such as meal ingestion, suggest that GLP-2 may influence intestinal blood flow via an endocrine pathway, reaching the target cells via the blood stream. However, there was a striking similarity between the blood flow response to the standard meal, and the response to the SC injection (figure 1). Clearly the concentrations of GLP-2 after SC injection by far exceeded the concentrations elicited after meal ingestion. However, it is possible that endogenous GLP-2 might also exert a paracrine activity on intestinal blood flow.

There was not as expected a significant change in blood pressure. We could not reproduce the previously observed decrease in blood pressure (unpublished data). An explanation for this is probably that the test subjects (healthy volunteers) were very young, and therefore have a high degree of cardiac compensation, even when a significant amount of the blood volume is shunted to the intestines. This was supported by our findings in study 2 and 3 where blood pressure remained unchanged, but cardiac parameters as CO and SV were increased.

Study 2: The effect of glucagon-like peptide-2 on arterial blood flow and cardiac parameters

The study investigated possible GLP-2 mediated changes on arterial blood flow in intestinal and extra intestinal vessels, and cardiac parameters. The study demonstrated significant and rapid changes in the SMA blood flow after SC GLP-2 injection, confirming our previous findings(65) in healthy volunteers.

GLP-2 did not induce significant changes in the flow or resistance in the CA compared to baseline and placebo. This as previous mentioned in agreement with CA blood flow estimates based on fluorescent microsphere deposition performed in piglets(30), where GLP-2 infusion showed no increase in blood flow, and also with the findings of Deniz et al. in rodents showing no GLP-2 mediated increase in CA blood flow(28). This strengthens the assumption that the increased blood flow to the gut is confined to the SMA supplying the duodenum (from the papilla Vateri, mid-gut) jejunum, ileum and partly the proximal colon, which is consistent with the GLP-2R localization in the small intestine.

No significant changes were seen in the extra intestinal sites (RA and CCA), the mammalian trials showing increased flow in the CCA(28) could not be confirmed.

Measurements of effect of GLP-2 on renal blood flow have not previously been examined in any trials. Iwao et al. demonstrated that intake of food was accompanied by renal vasoconstriction in normal humans(66). The vasoconstriction was inversely related to the increase in splanchnic blood flow, and was interpreted by the authors as part of the baro reflex mediated hemodynamic response to splanchnic vasodilatation. The hormonal response to meal stimulation is complex, and other vasoactive substances besides GLP-2 are released. Others have failed to demonstrate renal vasoconstriction after a meal. In this study, no renal hemodynamic changes could be detected after SC GLP-2 injection despite marked increases in splanchnic blood flow, and evidence of baro reflex sympathetic activation (see below).

Also in this study a possible cardiovascular response to GLP-2 was investigated. Compared to our first study, we introduced two novel techniques; continuous noninvasive recording of HR and BP (by finger plethysmography), and CO, SV and TPR (by impedance cardiography). The techniques are as previously mentioned highly reproducible, and equal to thermo dilution which is considered "gold" standard for measuring CO(60;62). We found that GLP-2 did not change blood pressure values, neither SYS nor DIA, but CO, SV and HR increased significantly after GLP-2. This most likely represents a baro reflex response to the redistribution of the blood flow to the intestine. Compared to the placebo group, the changes in these parameters were insignificant, possibly due to the limited size of the groups. In the placebo group, the observed changes in blood pressure probably represent the effect of supine relaxation for 90 minutes. We were unable to detect any response from non-mesenteric sites (renal and carotic) by the non-invasive Doppler technique. In this technique determination of blood flow is carried out using flow probes applied directly on the artery wall. This technique may be more sensitive, and differences in methodology could therefore play a role, but considering that the given GLP-2 dose in our study was in the pharmacological range, we find it unlikely that we have overlooked any effects with physiological significance.

Study 3: The effect of Glucagon-Like Peptide-2 on mesenteric blood flow and cardiac parameters in end jejunostomy short bowel patients

The findings on intestinal blood flow in this study were similar to our previous findings, showing significant rapid increase in blood flow evaluated by RI and TAMW, after SC infusion of GLP-2. The study was partly prompted by previous observations of increases in stoma nipple size, immediately after GLP-2 and teduglutide treatment in end-jejunostomy SBS patients. But the blood flow increase, calculated as TAMV, in the short bowel patients was lower than that observed in healthy subjects(65;67)illustrated in figure 18. We found furthermore that the mean baseline SMA blood flow was higher in the SBS patient study group than in

healthy subjects, and in patients with chronic pancreatitis and exocrine insufficiency(64;65;67). We also noted that the patients with the highest basal flow values had the lowest GLP-2-mediated flow increases, which might indicate a chronic elevated basal blood flow in this fraction of the patients.

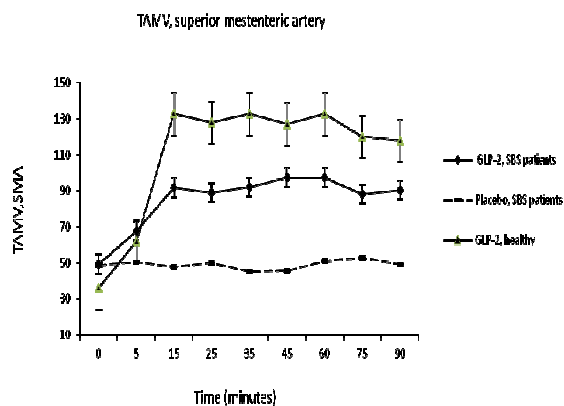


Figure 18. Time averaged maximal velocity measured in the superior mesenteric artery (SMA) under GLP-2 administration (short bowel syndrome (SBS) patients and healthy subjects) compared to placebo, the number of subjects were; 8 short bowel syndrome patients and 10 healthy subjects. Shown are means \pm SE.

Furthermore we analysed the correlation between change in blood flow and length of remaining intestine, and found a significant correlation in one of the two calculated blood flow modalities (RI) and a strong trend in TAMV change. Again as the material is small, even one patient can have impact on this calculation.

Common discussion points

All three studies have shown rapid changes in mesenteric blood flow after administration of SC GLP-2 in identical doses. The changes have been the same both in regards to time to maximum changes (increase) and relatively close in regards to maximum extent of change even though the changes in the SBS population were less pronounced than the healthy population. These findings leaves no doubt about, that GLP-2 is a potent regulator of upper splanchnic blood flow. The study findings also support the notion that the observed increased mesenteric blood flow, isolated to the SMA is secondary to the metabolic responses to GLP-2, and that it is likely due to a paracrine action, by an effect on GLP-2R bearing cells as enteric neurons, by expression of NO. Several factors support this argument: First; in all the trials the response has been limited to the SMA. Second; the response is very rapid. Third; there was a correlation between blood flow change and remaining small bowel length, suggesting that the response is linked more directly to the intestine. Fourth; the SBS patients seem to have a reduced response to GLP-2, also suggesting a correlation between change in blood flow and remaining small bowel length. Fifth; comparing basal flow values between healthy subjects and SBS patients indicates a chronic elevated blood flow, perhaps reflecting that the segment of intestine still remaining is

constantly operating at a maximum digestive and absorptive capacity.

CONCLUSION

The thesis concludes that:

GLP-2 increases mesenteric blood flow in healthy subjects and in SBS patients

GLP-2 increases mesenteric blood flow equivalent to a standard meal

Increase in mesenteric blood flow is dose dependent

GLP-2 does not increase blood flow at other arterial vascular sites

GLP-2 does not acutely alter blood pressure

GLP-2 increases, probably compensatory, pulse rate and cardiac out put

GLP-2 induced vascular response in the superior mesenteric artery is flow correlated with the length of remaining intestine in SBS patients. The effect is therefore likely to reflect the metabolic activity in the tissue rather than direct effect on the vascular system.

METHODOLOGICAL CONSIDERATIONS

Blood flow measuring

When measuring by Doppler ultrasound, the examinations have to be performed by a highly experienced sonographer, in order to argue for trustworthy reproducibility. We strongly believe that this has been the case in the studies, but the method has the clear weakness of being operator dependent.

Cardiac values

In the initial study, the blood pressure was measured by conventional NIBP method. This method is clearly a weaker measuring tool, compared to invasive measuring and the beat to beat measuring used in study 2 and 3.

Also the used beat to beat measuring is a poorer measuring tool than arterial invasive blood pressure measuring. The use of thoracic impedance, for estimation of cardiac parameters requires, immobile test subjects, and we observed that especially the group of SBS patients had problems with full compliance.

Experimental design

Although a placebo group was included in study 2 and 3, only study three was carried out double blinded and with a numeric identical placebo group. We do not believe that this is likely to have a major influence on the results.

The number of study participants was limited to 8-10 per study; of course the statistic strength would have been better with a larger group. But we believe that the number have been large enough to prove the major findings in the studies.

FUTURE

Our group has planned to investigate the effect of GLP-2 on mesenteric blood flow and cardiac parameters in patients with chronic pancreatitis with exocrine insufficiency. The aim of the study is to evaluate the effect of GLP-2 administered SC, compared to

synthetic pancreatic enzymes and placebo, the study will be matched against a healthy control group and will be carried out in a double blinded placebo controlled matter. The study protocol has been approved, and funding has been provided(68;69).

SUMMARY

The 33 amino acid peptide hormone GLP-2 is produced by enteroendocrine L-cells, the density of which is highest in the ileum and the colon, in response to the presence of nutrients in the lumen. The biological effect of GLP-2 is mediated by activation of a G-protein-coupled 7-transmembrane receptor. GLP-2 receptors are expressed in the brainstem, lungs, stomach, small intestine and colon, but not in the heart.

It has been shown in several animal studies that GLP-2 infusion increases intestinal blood flow and that this increase is confined to the small intestine.

The aim of the three studies, on which the thesis is based, was to investigate basic physiological effects of GLP-2, in healthy volunteers and in SBS patients, with focus on the effects on mesenteric blood flow, blood flow at other vascular sites and effects on cardiac parameters. These parameters have been evaluated after both meal stimulation and GLP-2 administration.

The studies showed the following results:

Blood flow: In all three studies, blood flow changes in the SMA after GLP-2 administration were similar regarding changes over time and degree of change. Blood flow changes were similar to changes seen after a standard meal. Only RI changes were registered in all three studies, but the TAMV changes in study 2 and 3 had similar characteristics.

Cardiovascular parameters: In all three studies no significant changes in blood pressure were registered in relation to GLP-2 administration. In study two and three, where cardiac parameters also were registered by impedance cardiography, increases in CO and SV were seen.

Plasma GLP-2: There were, as expected,, supra physiological GLP-2 plasma levels after SC administration.

All three studies have shown rapid changes in mesenteric blood flow after administration GLP-2. The changes have been the same both in regards to time to maximum changes (increase) and relatively close in regards to maximum extent of change. The changes in the SBS patients were less than in the healthy test subjects. The findings leave no doubt about that GLP-2 is a potent regulator of upper splanchnic blood flow. The study findings also support the notion that the observed increased mesenteric blood flow, isolated to the SMA, is secondary to the metabolic responses to GLP-2, and that these are likely due to a paracrine action by GLP-2 acting on GLP-2R bearing cells such as enteric neurons, probably expressing NO.

In conclusion GLP-2 increases mesenteric blood flow in healthy subjects and in SBS patients, the increase is equivalent to a standard meal and dose dependent. The blood flow is not increased at other arterial vascular sites. GLP-2 does not acutely alter blood pressure, but increases, probably as compensation, pulse rate and cardiac output. GLP-2 induced vascular response in the superior

mesenteric artery is related with the length of remaining intestine in SBS patients. The effect is therefore likely to reflect the metabolic activity in the tissue rather than direct effect on the vascular system

ABBREVIATIONS

GLP-2, glucagon-like peptide 2
 GLP-1, glucagon-like peptide 1
 GLP-2R, glucagon-like peptide 2 receptor
 TPN, total parenteral nutrition
 IV, intravenously
 SC, subcutaneously
 SMA, superior mesenteric artery
 IMA, inferior mesenteric artery
 CA, Celiac artery
 RA, Renal artery
 CCA, Common Carotid artery
 MAP, mean arterial pressure
 TFM, Task Force Monitor
 RI, resistance index
 IGF-1, Insulin-Like Growth Factor-1
 TAMV, time averaged maximal velocity
 DIA, diastolic pressure
 SYS, systolic pressure
 CO, cardiac output
 MAP, mean arterial pressure
 SV, stroke volume
 HR, heart rate
 TPR, total peripheral resistance
 DPP-4, dipeptidyl peptidase-4
 SBS, short bowel syndrome
 BMI, body mass index
 SE, standard error
 US, ultrasound scanning
 NO, nitric oxide
 GI, gastro intestinal
 SEMF, subepithelial myofibroblasts

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