Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
Department of Clinical Sciences

A porcine type 1 Diabetes Mellitus model, for non-invasive *in vivo* imaging of the glucagon-like peptide-1 receptor in the pancreas, using [^{68}Ga]Ga-DO3A-VS-Cys40-conjugated synthetic exendin-4 in PET-CT

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Kan betacellsmassan i *pankreas* bestämmas med hjälp av PET-CT? En studie av GLP-1R/exendin-4-komplexet hos gris

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SUMMARY

Diabetes mellitus is a rising epidemic throughout the world and there is currently great interest in quantifying the beta-cell mass (BCM) *in vivo* non-invasively. In the present experiment, the feasibility of *in vivo* imaging of the glucagon-like peptide-1 receptor (GLP-1R) in beta-cells was examined, using the positron emission tomography (PET) tracer [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 as a marker, in native pancreatic beta-cells of a porcine diabetic animal model and healthy controls.

Eight Swedish high-health domestic pigs were randomly assigned to be either controls or made diabetic using streptozotocin (STZ). The experiment proceeded during eight weeks, starting with an acclimatisation period. Once the pigs had been socialised they underwent surgery for the insertion of a jugular vein catheter, allowing induction of diabetes with STZ, intravenous (i.v.) injections and stress-free blood sampling. Development of diabetes was confirmed by clinical examinations, blood glucose values and insulin-staining of pancreatic sections *post mortem*.

The diabetic pigs were insulin treated and responded well. PET-CT (PET-computed tomography) examinations were performed on healthy controls and insulin-treated diabetic pigs. At the beginning of the PET-CT scan, oxygen-15 labelled water ([^{15}O]WAT) was injected i.v. to measure tissue perfusion in the pancreas and kidneys. The specific binding of [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 to the GLP-1R *in vivo* was assessed by i.v. administration of the tracer compound giving a baseline image. This was followed by the administration of a competing high dose of synthetic exendin-4 and a new imaging sequence. The pigs were humanely euthanised 0–6 days after the PET-CT examination and full *post mortem* examinations were performed in all pigs.

Diabetes was successfully induced, confirmed by immunohistochemical (IHC) staining for insulin. An important incidental finding, during PET-CT examination, was that the tracer and synthetic exendin-4 immediately induced a significant tachycardia in all pigs both at low and high dose. PET scans showed a reduced tissue perfusion in the pancreas and kidneys of the diabetic pigs. GLP-1R-mediated uptake of the tracer was detected in the pancreas of both healthy controls and diabetic pigs and surprisingly, the uptake of the tracer did not differ between the two groups. Thus, the pancreatic tracer uptake of [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 was not significantly reduced by selective destruction of beta-cells in diabetic pigs and the GLP-1R is not a suitable target for imaging of native pancreatic beta-cells in pigs. Additionally, this experiment shows how the pig can be made diabetic, insulin-treated and properly anaesthetised for several hours, which makes the pig a suitable animal model for further diabetic research.

SAMMANFATTNING

Diabetes mellitus är en växande epidemi världen över. Att på ett icke-invasivt sätt kunna uppskatta betacellsmassan *in vivo* skulle vara av stor betydelse för diabetesforskningen. I detta experiment utvärderades om den glucagon-lika peptid-1 receptorn (GLP-1R) kan detekteras på betaceller *in vivo*, icke-invasivt, genom positron emission tomografi (PET) och tracern [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4, hos friska och diabetes-inducerade grisar.

Åtta svenska serogrisar (av rasen Yorkshire x Svensk lantras x Hampshire) från samma kull randomiserades till att antingen vara kontroll eller att bli diabetiker med hjälp av streptozotocin (STZ). Experimentet pågick under åtta veckor och inleddes med en acklimatiseringsperiod. När grisarna hade blivit socialiserade genomgick de kirurgi då en jugulär venkateter placerades, för att möjliggöra diabetesinduktion med STZ samt intravenösa (i.v.) injektioner och blodprovstagningar under stressfria förhållanden. Genom klinisk undersökning, blodglukosvärden och insulin-färgning av bukspottskörtelvävnad *post mortem* konfirmerades att grisarna blivit diabetiska.

De diabetiska grisarna insulinbehandlades, med gott resultat. PET-CT-undersökning utfördes på både friska och insulinbehandlade diabetiska grisar. Först genomfördes en PET-CT-scan med radioaktivt vatten ([^{15}O]WAT) för att mäta vävnadsperfusionen i bukspottskörteln och njurarna. För att påvisa specificiteten *in vivo* av tracern [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 till GLP-1R injicerades först tracern ensamt varpå en omgång bilder togs, följt av i.v. administration av en kompetetiv högdos syntetiskt exendin-4 och en ny sekvens bilder. Grisarna avlivades några dagar efter PET-CT-undersökningen.

Ett viktigt bifynd under PET-CT-undersökningarna var att samtliga grisar drabbades av kraftig takykardi omedelbart efter injektion av tracer och syntetiskt exendin-4. Vävnads-perfusionen i bukspottkörteln och njurarna var signifikant lägre hos de diabetiska grisarna jämfört med de friska kontrollerna. Vidare visade PET-CT-undersökningarna receptorspecifik bindning av tracern till GLP-1R, men upptaget skiljde sig inte väsentligt mellan de två grupperna. Detta visar att upptaget av [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 inte påverkas av selektiv destruktion av betaceller hos diabetiska grisar och att GLP-1R inte är en biomarkör för levande betaceller i *pankreas* hos gris. Studien visar dock att grisen med gott resultat kan göras diabetisk och insulinbehandlas, för att i sitt diabetiska insulinbehandlade tillstånd sövas under flera timmar i sträck. Detta gör grisen till ett lämpligt modelldjur för vidare diabetesforskning.

TABLE OF CONTENT

INTRODUCTION

Diabetes mellitus is a rising epidemic throughout the world. Prevalence studies show that diabetes is a growing burden for the global society and there is currently no peak in sight (Whiting *et al.*, 2011; King *et al.*, 1998; Amos *et al.*, 1997; King & Rewers, 1993).

This master degree project is part of a larger collaboration between various professions (medical doctors, mathematicians, chemists and physicists) from the PET preclinical platform at Uppsala University and veterinarians from the Department of Clinical Sciences at the Swedish University of Agricultural Sciences.

Studies to quantify the glucagon-like peptide-1 receptor (GLP-1R) in the pancreas using positron emission tomography-computed tomography (PET-CT) have previously been performed on a rodent diabetic model and healthy cynomolgus primates (Selvaraju *et al.*, 2013), but there is currently no published study on the pig as an animal model using this technology and tracer.

The aim of this study was to examine the feasibility of *in vivo* imaging of the GLP-1R in beta-cells, using the PET tracer [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 as a marker, in native pancreatic beta-cells of a porcine diabetic animal model. The hypothesis was that this could be a possible method for quantifying the beta-cell mass.

LITERATURE REVIEW

Prevalence of Diabetes Mellitus

Humans

There are two types of diabetes mellitus described in humans – type 1 diabetes mellitus (T1D) and type 2 (T2D). Since data sources from the World Health Organisation (WHO) do not separate these two types, the presented prevalence numbers include both T1D and T2D (Wild *et al.*, 2004).

Wild *et al.* (2004) estimated that the prevalence of diabetes, in all age-groups worldwide, was 2.8% in year 2000 and predicted it would be 4.4 % in 2030. The total number of people with diabetes was estimated to 171 million in year 2000 and it was predicted to be 366 million in year 2030. Another study, including prevalence numbers from 91 countries, estimated that the world prevalence of diabetes among adults were 286 million (6.4%) in 2010 and would be 439 million (7.7%) in 2030 (Shaw *et al.*, 2010). In the most recently published article, data from 1980 to April 2011 was reviewed. The authors say that previous estimates of the global burden of diabetes have been too conservative and Whiting *et al.* say that in 2011 there were 366 million people with diabetes, a number which they expect to increase to 522 million people by 2030 (Whiting *et al.*, 2011).

Based on data from WHO the global mortality, that can be attributed to diabetes, in year 2000 was estimated to be 2.9 million deaths, corresponding to 5.2% of all deaths that year (Roglic *et al.*, 2005).

Cats, dogs and pigs

Prevalence estimates of diabetes in cats vary between 0.25 to 2.0% (Rand *et al.*, 2004). A U.K. study that included 14 030 insured cats found a prevalence of 0.43%, with Burmese cats significantly more likely (odds ratio 3.7) to become diabetic (McCann *et al.*, 2007). In cats, diabetes mellitus is one of the endocrinopathies with highest prevalence (Zini *et al.*, 2010).

The incidence of diabetes in a Swedish study in 180 000 dogs was 13 cases per 10 000 dog-years at risk with significant breed-specific sex- and age-differences (Fall *et al.*, 2007).

In pigs, spontaneous diabetes is extremely rare, and only one case has been reported. However, the pig is a suitable animal model for diabetes in humans, since they are similar in terms of physiology and metabolism. Thus, for studies in the diabetic pig, diabetes needs to be chemically induced (Jensen-Waern *et al.*, 2009; Larsen & Rolin, 2004).

Non-invasive imaging of the beta-cell mass

Diabetes mellitus is a serious illness with major implications to the patient as well as the global economy. There are several imaging techniques, which may be used for examining the pancreatic tissue as a whole. Currently, the beta-cell function and glycaemic control may be analysed through different metabolic tests. The pancreatic tissue can only be examined *post mortem* and by *in vivo* biopsy sampling for partial tissue analysis. However, a non-invasive *in vivo* method to examine and quantify the endocrine pancreatic beta-cells has not yet been developed (Leibiger *et al.*, 2012; Malaisse & Maedler, 2012; Nauck, 2009; Robertson, 2007; Souza *et al.*, 2006a).

Having the possibility to *in vivo* image the beta-cell mass (BCM) would provide further insight into the pathogenesis and changes of BCM throughout different diabetic stages. It would be a helpful diagnostic tool and useful when distinguishing the two types of diabetes as well as a valuable tool for monitoring medical therapy or to follow-up islet transplantation. Furthermore, the ability to accurately image and quantify the insulin-producing beta-cells is of high importance to further islet transplantation and therapeutic research (Yang *et al.*, 2013; Andralojc *et al.*, 2012; Arifin & Bulte, 2011; Virostko & Powers, 2009; Paty *et al.*, 2004).

Imaging methods

Positron emission tomography – computed tomography and single photon emission computed tomography

Positron emission tomography (PET) is a nuclear imaging method that uses a radiolabelled biological compound (tracer). The examined subject is placed in the PET scanner tunnel and is surrounded by detectors. The tracer is injected and is then distributed following its pharmacological properties in the body of the subject, where the tracer results in high energy γ-photons being emitted. The detectors convert the high-energy γ-photons emitted from the patient into an electronic signal. Repeated image sequences are acquired to measure the radioactive concentration in specific tissues as a function of time. Based on the photons that are emitted as a result of the tracer and using mathematical correction algorithms, a three-dimensional image showing the quantity of radioactivity in a specific region is provided

(Andralojc *et al.*, 2012; Saha, 2010; Alessio & Kinahan, 2006). To relate the tracer signal with a correct anatomical localisation in the patient a CT is used in combination with the molecular imaging technique. The addition of a CT greatly enhanced the PET technology, but also resulted in increased radiation dose to the patient (Dobrucki & Sinusas, 2005; Beyer *et al.*, 2000).

Single photon emission computed tomography (SPECT) and PET are based on different physical methods. In PET, the radioactive isotope decays with the emission of a positron. The positron collides with atomic electrons of the surrounding tissues; these collisions cause the positron to lose energy and it finally annihilates with an electron. This annihilation results in *two* high-energy photons being emitted in opposite directions, which are then simultaneously registered by the detectors of the PET. The position of origin of the photons in the volume of the examined subject can then be calculated. On the other hand, in SPECT, the technology is based on using a radioactive isotope which emits single photons instead (Chatziioannou, 2005). SPECT-CT is not as widely used as PET-CT, due to high cost in relation to the number of clinical indications for SPECT-CT (Rahmim & Zaidi, 2008). The implications of the difference in the basics of image technology between PET and SPECT will be discussed below.

SPECT has several similarities with PET. PET-CT and SPECT are both highly sensitive nuclear imaging modalities, measuring picomolar radioactivity. However, PET has an advantage over SPECT in terms of sensitivity, i.e. PET detects a higher proportion of emitted tracer signals (~ two to three times as many) (Andralojc *et al.*, 2012; Rahmim & Zaidi, 2008). Furthermore, PET can quantify the tracer signal in absolute terms (Selvaraju *et al.*, 2013), which is not possible with SPECT (Rahmim & Zaidi, 2008; Chatziioannou, 2005).

Through multiple-energy windows dual tracers can be used in SPECT; this cannot be performed using PET (Rahmim & Zaidi, 2008). Moreover, with SPECT, there is a possibility to widen the observational time window to follow biological events over a longer period of time (hours to days) compared to PET, due to longer half-lives of single photon emitters used in SPECT (Meikle *et al.*, 2005). However, this also prolongs the radiation load to the patient. Moreover, the short half-life of radionuclides used in PET imaging improves sensitivity, since these tracers may be injected at higher radioactivity (of course to a certain extent), consequently increasing the detectable radiation over a shorter period of time to the same radiation load as a tracer with longer half-life but lower radioactivity (Rahmim & Zaidi, 2008).

An important issue with PET and SPECT is photon attenuation. This refers to the fact that emitted radiation interacts with the tissue when it passes through the body of the subject, which makes the photon change direction, ultimately leading to loss of energy and scattering and thus reducing the signal in the image. There are different approaches to perform photon attenuation correction, one of which is to use a CT to map the attenuation of a body region and use this data to correct for attenuation in the PET image. Attenuation correction is easier achieved with PET than with SPECT, due to the fact that the origin of the tracer signal can be acquired in PET since the photons are emitted in pairs, while in SPECT the origin of the

signal needs to be well known since it cannot be calculated. This is a great challenge for SPECT and results in lower sensitivity compared to PET (Saha, 2010; Rahmim & Zaidi, 2008; Bailey *et al.*, 2005; Chatziioannou, 2005). However, there may be inaccuracies in attenuation correction followed by discrepancies between the PET and CT images (Mawlawi & Townsend, 2009; Pan *et al.*, 2005). Additionally an imaging artefact called random coincidences occurs in PET (not an issue in SPECT). This occurs when two photons are detected within the same coincidence time frame but the photons do not come from the same origin. This results in incorrect location of the origins of those photons in the PET-image. Trying to accurately correct for these random coincidences is subject for ongoing research in PET imaging (Rahmim & Zaidi, 2008).

The spatial resolution (i.e. the detail of the image) of both PET and SPECT is relatively low, and visualising individual pancreatic islets is not possible. PET has superior spatial resolution compared to SPECT (2 – 4 mm versus 8 – 10 mm), but even so a highly specific tracer which emits a great amount of radioactivity or a dense amount of target on the beta-cells is required for imaging of the BCM due to the small size of the islets (Andralojc *et al.*, 2012). Higher spatial resolution causes less spread of the tracer signal. The spatial resolution is determined both by the features of the scanner (crystal and pixel size, for example) as well as the mathematical algorithms used for image reconstruction (Soret *et al.*, 2007).

Furthermore, both PET-CT and SPECT underestimate tracer signals from targets smaller than the scanner's spatial resolution. This is called the "partial-volume-effect" (PVE) (Andralojc *et al.*, 2012). The PVE is an issue when imaging targets that are < 2.5 times the spatial resolution of the imaging system used and causes inaccurate images due to blurring. Other factors that may affect the PVE are the shape of the target, the background activity concentration and the mathematical algorithm used to reconstruct the PET image (Mawlawi & Townsend, 2009; Soret *et al.*, 2007). The PVE ultimately leads to underestimation of the standardized uptake (SUV) of the target due to dilution of tracer signals in the background/surrounding signals (Blomberg *et al.*, 2013), consequently leading to underestimation of the BCM (Eriksson & Alavi, 2012).

It has been estimated that the uptake of a tracer signal needs to be 100 times higher in beta-cells compared to surrounding cells in a healthy individual to overcome the shortcomings of PET/SPECT spatial resolution. The purpose of imaging the BCM is to detect small changes in BCM. Since the proportion of beta-cells in a diabetic patient may be as low as 0.2% of the pancreatic tissue (Weir *et al.*, 1990), as to normal 2%, the tracer uptake has to be 1000–fold in diabetics. Finding a tracer that meets those demands is not an easy task (Sweet *et al.*, 2004a; Sweet *et al.*, 2004b).

However, quantifying the BCM does not necessarily require identifying individual beta-cells, but could instead be performed by detecting the radioactive signal associated with the total amount of beta-cells throughout the pancreas, which can be possible since the pancreas is larger than the spatial resolution of the PET system (Selvaraju *et al.*, 2013). Still, when imaging a structure as small as the beta-cells, the target-to-background-ratio has to be very high (Di Gialleonardo *et al.*, 2012).

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a less sensitive technique than PET or SPECT, but has higher spatial resolution and exquisite soft tissue contrast. High-field MRI has high resolution which is needed for individual islet imaging, but makes it more susceptible to motion artefacts due to longer acquisition times. Breathing motion artefacts, but not gastrointestinal motion, can be corrected for (Andralojc *et al.*, 2012; Lamprianou *et al.*, 2011; Smirnov *et al.*, 2008).

There is no radiation exposure caused by the MRI, which makes it suitable for repeated imaging sessions, although examination times can be long and the environment noisy. Moreover, patients with pacemakers and some types of metal implants cannot be examined (Gotthardt *et al.*, 2013).

Using MRI technology to non-invasively image native beta-cells is not yet possible. This is due to it being a technology with lower sensitivity compared to PET and lack of available tracers meeting the requirements of beta-cell-to-background contrast (Andralojc *et al.*, 2012; Medarova & Moore, 2009). When using MRI, a specific contrast reagent is needed to distinguish exocrine from endocrine pancreas, which can at this point in time only be performed through *ex vivo* staining before performing islet transplantation. Recent studies using MRI for BCM imaging, are still in the very early stages and further investigation is needed to provide appropriate tracers for MRI technology (Yang *et al.*, 2013; Lubag *et al.*, 2011; Antkowiak *et al.*, 2009).

PET/MRI

Combining the qualities of PET and MRI could be a promising tool for examination of the BCM. There is ongoing research on hybrid PET/MR imaging (Torigian *et al.*, 2013; Pichler *et al.*, 2010; Wu & Kandeel, 2010).

Requirements of the imaging method, tracers and targets

There are several requirements of an appropriate imaging method for *in vivo*, non-invasive imaging of the BCM. The beta-cells are dispersed throughout the pancreatic tissue, which only consists of 1–2% of beta-cells in a healthy pancreas. In a diabetic state, this proportion of beta-cells is most likely decreased. Moreover, healthy pancreatic islets are very small (~ 50–400 µm) (Katsumichi & Pour, 2007). To overcome the difficulties in accurately imaging a target as sparse as the beta-cells, the imaging method needs to have a high spatial resolution and requires a highly specific tracer molecule as well as a highly specific target for the beta-cells only. The tracer must not bind to the exocrine pancreas or the surrounding tissues (high signal-to-background ratio and high signal-to-noise ratio) and the imaging method needs to discriminate endocrine from exocrine tissue. The target needs to stay unaffected in the diabetic state (Andralojc *et al.*, 2012; Brom *et al.*, 2010; Ley, 2006).

The result is also dependant of the expression rate of the target, which has to be highly expressed by the beta-cells to be detectable by the techniques available. To reach detectable tracer signal rates, some tracer doses needs to be relatively high. This is an issue if using a biologically active compound or when considering the radiation to the patient. The stability of the tracer molecule is also important. *In vivo*, the tracer has to avoid degradation by

endogenous peptidases, to stay active throughout the whole imaging session (Andralojc *et al.*, 2012; Kwee *et al.*, 2011).

Furthermore, there are issues concerning the size of the tracer molecule. *In vitro* imaging may prove good results showing a molecule with high affinity and specific binding to the beta-cells comparing to the exocrine tissue, although if the tracer molecule is too large it may remain in the circulation *in vivo* rather than migrating to the beta-cell target, consequently leading to a low target-to-background ratio and difficulties in detecting the beta-cells due to the slow clearance. A similar problem occurs if a large amount of tracer is accumulated in the excretion route, i.e. the kidneys, liver or intestinal organs (Andralojc *et al.*, 2012).

At this point in time, there is still no tracer and imaging method meeting all the above mentioned needs for imaging the BCM.

Suggested targets and ligands

To quantify the BCM, several receptors and biological molecules have been proposed as targets. Some of the targets and ligands (binding to the actual target) that are or have been under evaluation for imaging of the BCM are mentioned below.

The vesicular monoamine transporter type 2 (VMAT2) has been proposed being a promising target, since it is expressed by beta-cells but not by exocrine pancreatic cells (Maffei *et al.*, 2004; Anlauf *et al.*, 2003). Several studies have been published, where VMAT2 has been targeted with dihydrotetrabenazine (DTBZ) as a PET tracer, with the aim to quantify the BCM. However, VMAT2 is not an optimal target, due to nonspecific binding in exocrine pancreatic tissue (Singhal *et al.*, 2011; Virostko *et al.*, 2011; Eriksson *et al.*, 2010; Fagerholm *et al.*, 2010; Goland *et al.*, 2009; Kung *et al.*, 2008; Souza *et al.*, 2006b). It has been observed that VMAT2 is expressed in pancreatic polypeptide cells and hence, is not beta-cell specific (Saisho *et al.*, 2008).

The dopamine D_2-like receptor was suggested an appropriate target, since it was detected on beta-cells and mediated insulin-secretion (Rubí *et al.*, 2005). However, BCM could not be determined using this target since exocrine pancreas uptake also occurred (de Lonlay *et al.*, 2006).

The neurotransmitter acetylcholine seems to play a central part in the insulin secretion via muscarinic receptors expressed by the beta-cells (Gilon & Henquin, 2001; Ahrén, 2000). Preliminary data from a small study indicate excellent uptake in the pancreatic tissue of mice, human and monkeys, using [^{18}F]4-fluorobenzylatrozamicol as a tracer targeting muscarinic receptors. However, a significant uptake could also be detected in salivary gland, gall bladder and liver (Clark *et al.*, 2004). High uptake in these tissues may hamper signals coming from tracer uptake in the pancreas.

Another target that has been considered for beta-cell imaging is the sulfonylurea receptor 1 (SUR1), which is an ATP-regulated K^+-channel (Wängler *et al.*, 2004a; Bernardi *et al.*, 1988). The SUR1 has been targeted using sulfonureas, such as glibenclamide and repaglinide, which are glucose-lowering drugs used in T2D patients (Brom *et al.*, 2010; Hansen *et al.*, 2005;

Henquin, 1992), but a high uptake has been observed in the liver, kidneys and small intestines, while the uptake in the pancreas was rather low (Schmitz *et al.*, 2004; Wängler *et al.*, 2004b). In a study using immunostaining, SUR1 was shown to be expressed all over the pancreatic islet, i.e. in alpha-, beta- and delta-cells, which makes it an unsuitable target for BCM determination (Suzuki *et al.*, 1999).

D-mannoheptulose is transported into the beta-cells mainly through GLUT2 transporters, which has been confirmed *in vitro*. However, this target is not completely beta-cell-specific (Ladrière *et al.*, 2001). Moreover, D-mannoheptulose elevates blood glucose levels by inhibition of insulin secretion, which makes this substance inappropriate for use in diabetic patients (Yang *et al.*, 2013).

Since beta-cells have high zinc content which is co-released with insulin (Søndergaard *et al.*, 2003; Dodson & Steiner, 1998), it has been attempted to stain beta-cells using ^{125}I-labelled dithizone, a zinc chelator which binds to beta-cells. Unfortunately, the compound is rapidly degraded *in vivo* (Andralojc *et al.*, 2012; Brom *et al.*, 2010), thus it was conjugated with histamine (dithizone-[^{131}I]-histamine) to enhance the stability of the tracer. The stability was increased, but resulted in high background activity in liver and kidneys (Garnuszek *et al.*, 2000; Garnuszek *et al.*, 1998). It has also been shown that dithizone may be toxic to beta-cells (Clark *et al.* 1994; Toroptsev & Eshchenko, 1983), which gives it a limited use as a tracer for *in vivo* imaging. Another tracer, gadolinium-DOTA-diBPEN, has also been used to detect the Zn^{2+}-release by the beta-cells *in vivo* using MRI, with promising results (Lubag *et al.*, 2011; Esqueda *et al.*, 2009).

Manganese-enhanced MRI is another technique presently under evaluation. Instead of targeting the beta-cell surface, the technique aims to detect intracellular manganese incorporated into the beta-cells by Ca^{2+}-channels activated by glucose. Only beta-cells with a proper glucose metabolism will incorporate the Mn^{2+}, since Mn^{2+}-uptake is glucose-dependent (Malaisse & Maedler, 2012; Gimi *et al.*, 2006). Studies with promising result have been performed *in vivo* in mice (Antkowiak *et al.*, 2012; Antkowiak *et al.*, 2009), but there are concerns about cytotoxicity caused by manganese (Dobson *et al.*, 2004; Olanow, 2004; Roth & Garrick, 2003). It has been shown that manganese concentrations > 300 µM causes apoptosis in neuronal cells (Latchoumycandane *et al.*, 2005), which may limit its use *in vivo*. However, much lower doses are applied for the use in MRI, and further studies using Mn^{2+}-enhanced MRI are currently ongoing (Malaisse & Maedler, 2012; Lamprianou *et al.*, 2011; Silva *et al.*, 2004).

The monoclonal islet cell surface IgM antibody IC2 has been used as a tracer targeting beta-cells to image the BCM in a T1D mouse model. The tracer was highly specific to the beta-cells (Moore *et al.*, 2001; Aaen *et al.*, 1990; Buschard *et al.*, 1988), but the large size of this tracer molecule, being an immunoglobulin, lead to a slow clearance, a low beta-cell-to-background ratio and may limit its role as a tracer (Schneider, 2008). Furthermore, this IgM antibody could lead to an immune reaction in humans since it is of rat origin (Di Gialleonardo *et al.*, 2012) and it has been proposed that the corresponding beta-cell antigen is not expressed constantly by the beta-cells (Buschard *et al.*, 1988). Another suggestion has been single-chain

antibodies which are highly specific to beta-cells and exhibit fast blood clearance. The tracer signal has shown a linear correlation with the BCM *in vitro* and *in vivo* in rats (Ueberberg *et al.*, 2010; Ueberberg *et al.*, 2009). Another monoclonal antibody (8/9-mAb), targeting transmembrane protein 27 (TMEM27) has also been used to detect BCM *in vivo* in mice using PET. TMEM27 is observed to be beta-cell specific in both mouse and human pancreas using fluorescence. This makes TMEM27 a promising target for further research (Vats *et al.*, 2012; Akpinar *et al.*, 2005).

Previous studies have also mentioned the glucagon-like peptide-1 receptor (GLP-1R) as a promising target for *in vivo* imaging, for example tumour imaging such as insulinomas and pheochromocytomas (Kiesewetter *et al.*, 2012; Gao *et al.*, 2011; Wild *et al.*, 2008; Körner *et al.*, 2007; Wild *et al.*, 2006). The GLP-1R is expressed on the beta-cell surface (Tornehave *et al.*, 2008) and an antagonist of the GLP-1R has been used to quantify the BCM in rats, but it was shown that kidney and liver uptake was relatively high, which may hamper the image in this small animal model (Mukai *et al.*, 2009). The glucagon-like peptide-1 (GLP-1), an agonist of the GLP-1R, is rapidly inactivated *in vivo*, why it was conjugated with albumin to prolong its activity (Kim *et al.*, 2003). Unfortunately, this lead to a slower clearance and ultimately an attenuated pancreas-to-background ratio (Andralojc *et al.*, 2012).

Synthetic exendin-4 (exenatide), a GLP-1 mimetic which is less prone to rapid degradation (Kazafeos, 2011), has been used for targeting GLP-1R in insulinomas (Sowa-Staszczak *et al.*, 2013; Christ *et al.*, 2009; Wicki *et al.*, 2007; Wild *et al.*, 2006), on beta-cells in mice and rats (Selvaraju *et al.*, 2013; Reiner *et al.*, 2011; Gotthardt *et al.*, 2006), in a human and mice beta-cell graft (Wu *et al.*, 2011; Pattou *et al.*, 2010) and in nonhuman primates (Selvaraju *et al.*, 2013), with promising result. By conjugating exenatide with DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) or DO3A (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid), it is possible to label the molecule with a positron emitter, which is needed for PET imaging (Brom *et al.*, 2010).

In rats and mice, by using IHC and autoradiography exendin-4 has shown to bind specifically to insulin-producing cells (Wu *et al.*, 2013; Connolly *et al.*, 2012). The high tracer excretion through the kidneys causes heavy spill-over and makes it impossible to properly analyse the acquired images in a small animal model, such as the rodent. This was, however, not considered an issue in nonhuman primates (Selvaraju *et al.*, 2013). Further studies have been requested, with the pig as a suggested animal model (Selvaraju *et al.*, 2013; Simonsen *et al.*, 2006), thus the present study was performed.

The glucagon-like peptide-1 receptor

The receptor and its expression

The GLP-1R is a transmembrane G-protein coupled receptor (GPCR) in family B of GPCR (Doyle & Egan, 2007; Josefsson, 1999). It has sequence homologies with other GPCR, i.e. the receptors for secretin, calcitonin and parathyroid hormone (Thorens, 1992).

In human tissue, GLP-1R protein expression was detected, by autoradiography, at low concentration in the brain, intestine, pancreas, kidney, blood vessels in the lung and in the

breast tissue, whilst no receptors could be found in the spleen, liver, adrenal glands, heart, skeletal muscle, prostate, hypophysis, adipose tissue or lymph nodes. The absolute highest amounts of receptors were found in the neurohypophysia. In the pancreas, GLP-1 receptors were observed in both islets and acini, although with a higher receptor density in the islets (about twice as high). Islets were receptor positive at lesser extent if the tissue had endured chronic pancreatitis (Körner *et al.*, 2007). In another study, using another technique, mRNA from the GLP-1R was found in the human heart, brain, kidney, lung and stomach, where it had the same amino acid sequence as the pancreatic form (Wei & Mojsov, 1995). Furthermore, in humans, *in vitro* GLP-1R autoradiography shows that GLP-1R mRNA is present in many parts of the brain, such as the cerebral cortex, hypothalamus, hippocampus and thalamus (Alvarez *et al.*, 2005).

In the human pancreas, it was strongly indicated by Tornehave *et al.* (2008) that GLP-1R has the highest expression on the surface of the beta-cells, facing the endothelium. Despite the use of a more sensitive technique than previous studies, according to the authors, they were unable to observe GLP-1R activity in alpha- and delta-cells. However, immunoreactivity was found in some of the large pancreatic ducts (Tornehave *et al.*, 2008). *In vitro*, GLP-1 can stimulate delta-cells to secrete somatostatin, which may indicate that delta-cells also express GLP-1R, although it has been suggested that the somatostatin-secretion following GLP-1 exposure may be due to paracrine effects (Tornehave *et al.*, 2008; Fehmann *et al.*, 1995; Fehmann & Habener, 1991).

In human cell lines, GLP-1 receptors have been found on bone marrow stromal cells (Sanz *et al.*, 2010) and on immature osteoblasts (Pacheco-Pantoja *et al.*, 2011; Walsh & Henriksen, 2010). No GLP-1R have been found on mature osteoblasts (Bollag *et al.*, 2000).

In rats, GLP-1 receptors are expressed in the lungs and brain. The binding affinity by GLP-1 is rapid, reversible and high, specifically in the hypothalamus and brain stem (Rodriquez de Fonseca *et al.*, 2000; Kanse *et al.*, 1988). mRNA transcripts for GLP-1R have also been found in the pancreas, intestine, stomach, liver, lung, brain, kidney, smooth muscle cells and cardiomyocytes of mice/rats, (Pyke & Knudsen, 2013; Ban *et al.*, 2008; Bullock *et al.*, 1996; Campos *et al.*, 1994) and in tubular cells of the porcine kidney (Schlatter *et al.*, 2007), although it was proposed by Bullock *et al.* (1996) that the receptor found in rodent kidneys and heart may be a structural variant of the pancreatic GLP-1R (i.e. not being completely identical). Furthermore, in rats, GLP-1R gene expression was found in the nodose ganglion, an important junction for *nervus vagus* (Nakagawa *et al.*, 2004). Through light microscopic autoradiography in rat brain, GLP-1R was observed in the subfornical organ and *area postrema* (AP), which are in close neuroanatomical connection with vagal nerves and important hypothalamic regions involved in water and food intake as well as the sensation of emesis. Through its anatomical situation, AP is an interface between the brain and the peripheral blood. Furthermore, AP has an insufficient blood-brain barrier to large polar molecules (Orskov *et al.*, 1996a; Miller & Leslie, 1994).

Significant species differences have been demonstrated, with high expression of GLP-1R in rodent thyroid c-cells while human and primates had low expression of GLP-1R in this tissue.

Similar results were demonstrated concerning lung tissue, where high receptor density was found in rodent lung tissue while it was remarkably low in human lung tissue in comparison (Bjerre Knudsen *et al.*, 2010; Körner *et al.*, 2007).

Ligands

The binding of the peptide GLP-1 to its receptor is highly specific. Glucagon also binds to the GLP-1R, although with much less affinity than GLP-1. This has low relevance since glucagon never reaches plasma levels at which it would bind to the receptor instead of GLP-1 (Doyle & Egan, 2007; Graziano *et al.*, 1993). A study in dogs has shown that a metabolite of truncated GLP-1, the glucagon-like peptide-1-(9-36)-amide, is an antagonist of the GLP-1R but has low binding affinity to the receptor (Knudsen & Pridal, 1996).

As mentioned, exendin-4 is also an agonist of the GLP-1 receptor and truncated exendin-(9-39)-amide is an antagonist (Schirra *et al.*, 1998; Göke *et al.*, 1993; Thorens *et al.*, 1993).

Intracellular effect of GLP-1 receptor binding

The receptor is stimulated by GLP-1 binding when glucose is elevated**.** This causes an intracellular reaction to take place, where adenylyl cyclase is being activated and consequently cAMP formatted (Graziano *et al.*, 1993; Drucker *et al.*, 1987). The increase of cAMP induces the activation of protein kinase A and cAMP-regulated guanine nucleotide exchange factor II (Epac2) (Shibasaki *et al.*, 2007; Renström *et al.*, 1997), which in turn leads to membrane potential being shifted and intracellular Ca^{2+} rising inside the beta-cells (Tsuboi *et al.*, 2003). This reaction sequence ultimately leads to an increase of readily releasable insulin secretory vesicles and their exocytosis (Vilsbøll, 2009; Holst & Gromada, 2004; Renström *et al.*, 1997).

The glucagon-like peptide-1

Production of GLP-1

The glucagon-like peptide-1 (7-36)-amide (GLP-1) is a gastrointestinal hormone in the group of incretin hormones. GLP-1 is produced by, and secreted from, intestinal endocrine L-cells (Doyle & Egan, 2007; Holst, 2007) which are mainly located in the distal parts of the small intestine (Theodorakis *et al.*, 2006; Mortensen *et al.*, 2003). They are epithelial endocrine cells with direct contact with the lumen of the intestine and thereby in contact with the nutrients passing by their apical surface (Baggio & Drucker, 2007).

The GLP-1 secretion is stimulated by several factors, meal ingestion in particular (Orskov *et al.*, 1996b; D'Alessio *et al.*, 1995; Herrmann *et al.*, 1995). Studies have shown that oral administration of glucose stimulates secretion of incretin hormones, as a function of the glucose load (Nauck *et al.*, 1986b; Unger *et al.*, 1968). It has also been demonstrated that leptin, an adipose tissue hormone involved in the regulation of satiety, stimulate the secretion of GLP-1 (Anini & Brubaker, 2003; Friedman & Halaas, 1998). There is a rapid GLP-1 increase 10–15 minutes following meal intake, and since the majority of the L-cells are located in the distal part of the small intestines it is unlikely that the GLP-1 secretion is due to stimulation by nutrients in contact with the L-cells only (Baggio & Drucker, 2007; Herrmann

et al., 1995). There is most likely a neuroendocrine stimulation involved in the GLP-1 secretion as well (Balkan & Li, 2000; Rocca & Brubaker, 1999; Balks *et al.*, 1997) and it has been proposed that GLP-1 is being locally produced within the CNS, thereby modulating neurotransmission (Holst *et al.*, 2011).

Regulatory mechanisms of GLP-1 levels

Once GLP-1 is secreted it is rapidly inactivated. The half-life of its bioactive form in the circulation is < 2 minutes (Baggio & Drucker, 2007; Simonsen *et al.*, 2006; Deacon *et al.*, 1995). The mechanism of the rapid inactivation of GLP-1 remained unknown until dipeptidyl-peptidase 4 (DPP-IV) was found on the surface of the endothelial cells in the capillaries that supply and drain the intestinal mucosa, adjacent to the L-cells. DPP-IV, also known as CD26, is a serine protease with specific action, cleaving dipeptides at the N-terminal of peptides containing an alanine or proline at a certain site (Baggio & Drucker, 2007; Hansen *et al.*, 1999; Deacon *et al.*, 1996; Mentlein *et al.*, 1993). GLP-1 is rapidly cleaved and metabolised into GLP-1(9-36) amide, and thereby inactivated, by DPP-IV (Hansen *et al.*, 1999; Mentlein *et al.*, 1993). As a consequence, half of the GLP-1 entering the *vena portae* has already been inactivated before reaching the systemic circulation (Hansen *et al.*, 1999). DPP-IV is omnipresent and can be found in several tissues throughout the body including the kidney, lung, adrenal gland, liver, spleen, testis, CNS, pancreas, intestine and also on the surface of lymphocytes and macrophages (Baggio & Drucker, 2007; Elovson, 1980). In rats, over 50% of GLP-1 amounts injected by intravenous (i.v.) infusion was degraded within two minutes, while in knock-out mice, lacking the functional gene producing DPP-IV, GLP-1 remained intact (Baggio & Drucker, 2007; Marguet *et al.*, 2000).

In rats, the cleaved metabolite of GLP-1 is excreted through the kidneys within approximately five minutes (Ruiz-Grande *et al.*, 1993) In a study, comparing renal failure patients with healthy controls, it was shown that GLP-1 metabolites were elevated in the renal patients, while the levels of the bioactive form of GLP-1 did not differ from the controls. This indicates that the kidneys are important for the elimination of GLP-1 metabolites in humans as well (Meier *et al.*, 2004). However, in pigs, Simonsen *et al.* (2006) shows that the liver and peripheral tissues also play a role in the excretion of GLP-1.

There are limited studies regarding the inhibition of GLP-1 production, although there are a few studies that indicate that insulin, somatostatin as well as the neuropeptide galanin may inhibit GLP-1 secretion from the L-cells (Baggio & Drucker, 2007; Lim & Brubaker, 2006; Chisholm & Greenberg, 2002; Hansen *et al*. 2000; Fehmann *et al.*, 1995).

The function of GLP-1

The incretin effect

Oral administration of glucose is associated with a greater increase in insulin levels, than when given as an i.v. glucose infusion, which has brought upon the expression “the incretin effect” (Holst, 2007; Nauck *et al.*, 1986b; Perley & Kipnis, 1967; Elrick *et al.*, 1964).

Being an incretin hormone, GLP-1 promotes the synthesis of insulin, increases glucose-dependent insulin secretion and improves beta-cell responsiveness to glucose. GLP-1

stimulates an elevation of insulin mRNA levels as well as insulin content in the beta-cells (Vilsbøll, 2009; Farilla *et al.*, 2003; Zander *et al.*, 2002; Wang *et al.*, 1995; Holz *et al.*, 1993; Nathan *et al.*, 1992; Drucker *et al.*, 1987; Mojsov *et al.*, 1987). In one study, diabetic rats were injected with GLP-1 during two days. The rats' insulin secretion increased and consequently the plasma glucose levels were reduced (Farilla *et al.*, 2002). This has also been shown in humans, following subcutaneous (s.c.) injection of GLP-1. At high dose GLP-1, the human subjects felt unwell and 50% experienced nausea/vomiting (Ritzel *et al.*, 1995).

It has been proposed that an intact sensory afferent system is essential to GLP-1-mediated insulin secretion, since low doses of GLP-1 administered i.v. to mice increase glucose-dependent insulin secretion in control subjects but not in sensory denervated mice (Ahrén, 2004).

In obese adults and patients with T2D levels of GLP-1 seem to be attenuated and the incretin effect diminished (Kjems *et al.*, 2003; Toft-Nielsen *et al.*, 2001; Verdich *et al.*, 2001; Vilsbøll *et al.*, 2001; Ranganath *et al.*, 1996; Nauck *et al.*, 1986a; Perley & Kipnis, 1967), although elimination rates of GLP-1 are equal compared to healthy individuals (Vilsbøll *et al.*, 2003a). Therefore it has been proposed that the reduction in GLP-1 levels in obese and T2D patients may be due to lowered secretion of GLP-1 (Vilsbøll *et al.*, 2003b; Ranganath *et al.*, 1996) or due to leptin resistance, since obese people often exhibit leptin resistance (Anini & Brubaker, 2003; Friedman & Halaas, 1998; Halaas *et al.*, 1997; Considine *et al.*, 1996). However, there are T2D patients exhibiting a proper incretin effect following GLP-1 exposure (Vollmer *et al.*, 2008; Elahi *et al.*, 1994; Nauck *et al.*, 1993) and GLP-1 levels have been shown to not differ between diabetic subjects and healthy controls (Knop *et al.*, 2007) thus this is a debated topic. Some authors mean that a deterioration of the incretin effect is more likely secondary to other metabolic causes, such as hyperglucagonaemia (Nauck *et al.*, 2011; Vollmer *et al.*, 2008); this hypothesis could perhaps be confirmed by a study where four weeks of intensive insulin treatment significantly improved beta-cell responsiveness to GLP-1 threefold in T2D subjects (Højberg *et al.*, 2009) and these results are in line with a study showing that GLP-1R expression is down-regulated by hyperglycaemia (Xu *et al.*, 2007). Concluding, this topic is complex, with several factors that may affect the observed attenuated incretin response, for example high BMI, genetic background and duration of the T2D, thus further studies are required (Gjesing *et al.*, 2012; Herzberg-Schäfer *et al.*, 2012; Nauck *et al.*, 2011; Vollmer *et al.*, 2008).

GLP-1 also reduces post-prandial glycaemic excursions. This has been shown in T2D patients following both short-term (Rachman *et al.*, 1997) as well as after three and six weeks of GLP-1 exposure (Zander *et al.*, 2002; Todd *et al.*, 1998). A study in T1D patients demonstrated that GLP-1 reduces glycaemic excursions in this group as well (Dupre *et al.*, 1995).

Delaying gastric emptying and body weight reduction

Delayed gastric emptying has been observed in response to GLP-1 (Meier *et al.*, 2006; Meier *et al.*, 2003; Zander *et al.*, 2002; Flint *et al.*, 2001; Näslund *et al.*, 1999; Wishart *et al.*, 1998; Nauck *et al.*, 1997; Willms *et al.*, 1996). Willms *et al.* (1996) suggested that the delayed gastric emptying in itself most likely contributes to the glucose lowering effect of GLP-1. In a

6-week pilot study of T2D human subjects, treatment with GLP-1 resulted in an average weight loss of 1.9 kg (Zander *et al.*, 2002).

Regulating beta-cell mass

GLP-1 inhibits beta-cell apoptosis *in vitro*, in human islets of Langerhans. GLP-1 preserved morphology and function of the beta-cells and prevented apoptosis when the beta-cells were exposed to oxidative stress by glucotoxicity, lipotoxicity and both in combination (Buteau *et al.*, 2004; Robertson *et al.*, 2004; Farilla *et al.*, 2003; Hui *et al.*, 2003).

Pancreatic ductal cells from rats cultured during GLP-1 exposure underwent re-distribution of the cell cycle and differentiation towards endocrine insulin producing cells (Bulotta *et al.*, 2002). A similar result was demonstrated when pancreatic tumour (AR42J) cells (Zhou *et al.*, 1999) and progenitor cells in pancreatic islets and ducts were exposed to GLP-1 (Abraham *et al.*, 2002) and proliferation of acinar and ductal rat cells has been observed in another study (Perfetti *et al.*, 2000). Diabetic rats were injected with GLP-1 during two days. Four days later autopsy and immunostaining concluded there was an increase in cell proliferation of both endocrine and exocrine segments of pancreas and in parallel a decrease in apoptotic processes (Farilla *et al.*, 2002). Thus, GLP-1 seems to regulate BCM by stimulating proliferation and by inhibiting apoptotic processes.

Other endocrine effects

It has been demonstrated that GLP-1 inhibits the secretion of glucagon in both healthy and T2D human patients. The exact mechanism(s) for this effect remains undetermined (Meier *et al.*, 2003; Nauck *et al.*, 2002; Nauck *et al.*, 1997; Creutzfeldt *et al.*, 1996; Ritzel *et al.*, 1995; Nauck *et al.*, 1993; Fehmann & Habener, 1991).

GLP-1 can also stimulate secretion of somatostatin from delta cells. This effect may either be due to direct stimulation of the pancreatic delta cells or possibly through paracrine pathways (Chisholm & Greenberg, 2002; Hansen *et al.*, 2000; Fehmann *et al.*, 1995; Fehmann & Habener, 1991).

In one study, mesenchymal human bone marrow stem cells were exposed to GLP-1, which promoted the cell proliferation and inhibited the differentiation into adipocytes. Since GLP-1 receptors have been located on bone marrow stromal cells and osteoblasts, this topic demands further studies on long-term effects of incretin-based therapies (Phillips & Prins, 2011; Sanz *et al.*, 2010).

Another study has demonstrated that GLP-1 eliminates postprandial elevation of triglyceride concentrations in humans. The authors suggested these findings may be explained by delayed gastric emptying as well as inhibition of lipolysis due to enhanced insulin secretion (Meier *et al.*, 2006).

It has been demonstrated that GLP-1 has a natriuretic effect in both healthy and obese, insulin-resistant men, showing reduced H^+-secretion and enhanced Na^+-excretion (Gutzwiller *et al.*, 2004). Similar results have been seen in rats (Crajoinas *et al.*, 2011; Yu *et al.*, 2003).

Cardiovascular effects

It has been proposed that GLP-1 may have cardiovascular benefits, such as cardioprotective and vasodilatatory effects (Ban *et al.*, 2008; Basu *et al.*, 2007; Sokos *et al.*, 2007; Meier *et al.*, 2006). In a study consisting of T2D patients with coronary heart disease, GLP-1 improved endothelial function (Nyström *et al.*, 2004). In mice, GLP-1 increased the recovery of heart function cardiomyocyte viability and reduced reperfusion injury following ischemia of the myocardium (Ban *et al.*, 2008).

In rats, GLP-1 protected the myocardium against infarction and reperfusion injury. It also increased left ventricular (LV) function and myocardial glucose uptake (Zhao *et al.*, 2006; Bose *et al.*, 2005). In dogs with paced-induced dilated cardiomyopathy, GLP-1 increased cardiac output, improved left ventricular and systemic haemodynamics, in combination with increased myocardial insulin sensitivity and glucose uptake (Nikolaidis *et al.*, 2004a). GLP-1 seems to improve LV function of the human myocardium as well. When GLP-1 was administered as a 72-hour infusion following an acute myocardial infarction in humans, LV function was improved (Nikolaidis *et al.*, 2004b), and beneficial cardiovascular results have also been reported in three other studies (Read *et al.*, 2012; Müssig *et al.*, 2008; Sokos *et al.*, 2006). Interestingly, beneficial cardiac effects have also been observed in GLP-1R knockout mice models when exposed to GLP-1, indicating there being a pathway not mediated through the GLP-1R (Ban *et al.*, 2008). However, no beneficial cardiac result could be seen in a small study of non-diabetic patients with compensated chronic heart failure following 48h GLP-1 infusion (Halbirk *et al.*, 2010).

In rats, GLP-1 increased arterial blood pressure and heart rate (HR) significantly when administered i.v. as well as intracerebroventricularly (i.c.v.). The stimulating effect on arterial blood pressure and HR could be blocked by exendin-(9-39)-amide, administered i.v. or i.c.v. (Barragán *et al.*, 1999). Similar results were shown in another study in rats, where both peripheral and central administration of GLP-1 resulted in increased arterial blood pressure and HR (Yamamoto *et al.*, 2002) and increased HR has also been shown in calves following i.v. GLP-1 infusion (Edwards *et al.*, 1997). Barragán *et al.* (1994) concluded that the stimulating effect on cardiovascular parameters did not seem to be mediated by catecholamines through the α- or β-adrenergic receptors, while Yamamoto *et al* (2002) suggested the opposite – that sympathical innervation is involved. Thus, further research is required.

Central and peripheral neuroendocrine effects

GLP-1 promotes satiety and reduces food and water intake (Gutzwiller *et al.*, 1999a; Gutzwiller *et al.*, 1999b; Toft-Nielsen *et al.*, 1999; Flint *et al.*, 1998; Navarro *et al.*, 1996; Turton *et al.*, 1996). It has been proposed that GLP-1 is locally produced within the CNS (Holst *et al.*, 2011), that peripheral GLP-1 is able to cross the blood-brain barrier (Kastin *et al.*, 2002), as well as that there being vagal-brainstem-hypothalamic pathways involved in the regulation of satiety. The role of GLP-1 in the regulation of feeding is still not fully understood (Phillips & Prins, 2011; Baggio & Drucker, 2007; Abbott *et al.*, 2005; Tang-Christensen *et al.*, 2001; Rodriquez de Fonseca *et al.*, 2000; Näslund *et al.*, 1999). During hyperglycaemic conditions, stimulation of GLP-1R in the CNS has an important role in

whole-body glucose homeostasis by inhibiting muscle glucose use, increasing insulin secretion as well as enhancing storage of hepatic glycogen, through peripheral neural pathways (Knauf *et al.*, 2005).

One study of human subjects demonstrated that GLP-1 induced a significant increase in cortisol and ACTH levels (Ryan *et al.*, 1998). Similar results were achieved in another study, consisting of healthy and T1D subjects who were exposed to GLP-1, resulting in elevated cortisol levels, no matter the glycaemic state of the subject, proposing that GLP-1R agonists may affect the hypothalamus pituitary axis in humans. Although, the pathway of this effect needs to be investigated further (Gil-Lozano *et al.*, 2010).

In vitro, GLP-1 has shown to protect rat hippocampal neurons from apoptosis when exposed to degenerative substances. This indicates that GLP-1 may have a neuroprotective and/or a neurotrophic function (Perry *et al.*, 2002a; Perry *et al.*, 2002b) and similar results were demonstrated *in vivo* in mice. In GLP-1R deficient mice, learning deficits were observed, while improved learning and memory skills were observed in rats over-expressing GLP-1R (During *et al.*, 2003). Furthermore, GLP-1 has been observed to reduce amyloid accumulation *in vivo* in the mouse brain (Perry *et al.*, 2003).

Exenatide – synthetic exendin-4

History

Exendin-4 is a natural GLP-1 receptor agonist, isolated from the venom produced in the salivary glands of the Gila monster (Nielsen *et al.*, 2004; Eng *et al.*, 1992).

Pharmacology of exenatide and field of application

Exenatide, synthetic exendin-4, is a synthetic peptide with an amino acid sequence that is homogenous with the natural GLP-1 to 53%. It is also a GLP-1R agonist and has similar glucoregulatory and insulinotropic effects as endogenous GLP-1, but is ~5 500 times more potent in blood glucose lowering (Vilsbøll, 2009; Alarcon *et al.*, 2006; Nielsen *et al.*, 2004; Kolterman *et al.*, 2003; Young *et al.*, 1999; Göke *et al.*, 1993; Eng *et al.*, 1992). Exenatide has an extended half-life compared to physiological GLP-1, due to amino acid differences at position two of the peptide; exendin-4 has a glycine instead of alanine, which makes it a less suitable substrate for DPP-IV to truncate (Kazafeos, 2011; Vilsbøll, 2009).

Exenatide improves glycaemic control, induces significant weight loss and attenuate haemoglobin A_{1c} (HbA1c) levels, which may be analysed to monitor glycaemic control (Higgins, 2012; Moretto *et al.*, 2008; Amori *et al.*, 2007; Buse *et al.*, 2007; Blonde *et al.*, 2006; DeFronzo *et al.*, 2005; Kendall *et al.*, 2005; Buse *et al.*, 2004; Dupré *et al.*, 2004; Fineman *et al.*, 2003; Edwards *et al.*, 2001; Szayna *et al.*, 2000). There are several studies reporting that GLP-1 and exendin-4 are involved in reducing food intake (affecting satiety) and delaying gastric emptying (Linnebjerg *et al.*, 2008; Abbott *et al.*, 2005; Talsania *et al.*, 2005; Dupré *et al.*, 2004; Kolterman *et al.*, 2003; Edwards *et al.*, 2001; Szayna *et al.*, 2000) and it has been reported that exenatide, as short-term treatment, also results in weight loss in obese, non-diabetic women (Dushay *et al.*, 2012).

Exenatide was approved in 2005 by the Food and Drug Administration (FDA) in the U.S for use in T2D patients that are unable to achieve adequate glycaemic control using metformin and/or sulfonylurea and where weight loss or hypoglycaemia is of concern. It was labelled as *Byetta*® and is administered by s.c. injections twice daily. It is recommended to discontinue after six months of the therapy if HbA1c or the weight has not decreased by at least 1% and 3% respectively during this time (Kazafeos, 2011; Madsbad *et al.* 2011; NICE, 2011; Arnolds *et al.*, 2010; Nathan *et al.*, 2009). In the European Union *Byetta*® was approved in 2006 (EMA, 2013).

In a small study in healthy cats, exenatide stimulated insulin secretion with a mild lowering of blood glucose, although, not to the degree that it greatly increased the cats' abilities to normalise serum glucose levels following an i.v. injection. The authors request further studies to evaluate exenatide treatment in diabetic cats (Gilor *et al.*, 2011).

Several studies *in vitro* and *in vivo* indicate that exendin-4, like GLP-1, affects beta-cell mass and function (Yusta *et al.*, 2006; Nielsen *et al.*, 2004; Fineman *et al.*, 2003; Xu *et al.*, 1999). Furthermore, it has been demonstrated that exendin-4 protects rat hippocampal neurons from apoptosis when exposed to degenerative substances, which indicates that exendin-4 may have a neuroprotective and/or a neurotrophic function. Similar results were seen in a study using a rat animal model for Parkinson's disease, where treatment with exendin-4 promoted adult neurogenesis both *in vitro* and *in vivo*. In Huntington's disease mice treated with exendin-4, motor function was improved and survival time prolonged. These results may be of interest for further research in neurodegenerative processes in the CNS (Martin *et al.*, 2009; Bertilsson *et al.*, 2008; Perry *et al.*, 2002a; Perry *et al.*, 2002b).

Side effects

General side effects

The most reported side effect following treatment with exenatide is nausea, with higher incidence during the first eight weeks of treatment (up to 30% at 10 µg dosage and 15–25% at 5 µg dosage). There has been no correlation between degree of weight loss and duration of nausea, and people not experiencing nausea lost weight as well. Other frequent side effects were vomiting and diarrhoea, which also were reported to a higher extent at the beginning of the treatment (Blonde *et al.*, 2006; DeFronzo *et al.*, 2005; Kendall *et al.*, 2005; Buse *et al.*, 2004).

There have been none or few reported incidences of severe hypoglycaemia following exenatide-treatment during trials. The hypoglycaemic events reported were mild or moderate in severity, although occurring at a significant incidence (from 5.3%, up to 35% at high dose, i.e. 10 µg). Only one withdrawal occurred due to hypoglycaemia. Other reported adverse effects are upper respiratory tract infection and headache (DeFronzo *et al.*, 2005; Kendall *et al.*, 2005; Buse *et al.*, 2004).

The frequency of serious and severe adverse events during trials were low (3–10 %) and evenly distributed between categories (10 µg, 5 µg and placebo) (DeFronzo *et al.*, 2005; Buse *et al.*, 2004). There has been no evidence indicating cardiovascular, pulmonary, hepatic or

renal toxicity during the 30 week trials of exenatide treatment (DeFronzo *et al.*, 2005; Kendall *et al.*, 2005).

Antibody formation

At week 30 of three different exenatide-trials, anti-exenatide antibody titres were detected in 41–49% of subjects. The majority of the antibody titres were in the low range and the presence of antibodies had no connection with outcome or adverse events (Amori *et al.*, 2007; DeFronzo *et al.*, 2005; Kendall *et al.*, 2005; Buse *et al.*, 2004).

Renal side effects

The U.S. FDA has received 78 reports of kidney related problems in patients using exenatide from April 2005 through October 2008. This is a small percentage of the total number of users – nearly 7 million prescriptions of *Byetta*® were dispensed during this period, although, safety information regarding possible kidney problems has been featured the *Byetta*® label information. Health care professionals are recommended not to initiate treatment with *Byetta*® in patients with severe renal impairment and caution should be applied when considering dose increase in patient with moderate renal impairment (Tuttle *et al.*, 2013; U.S. Food and Drug Administration, 2009a; U.S. Food and Drug Administration, 2009b).

Risk of pancreatitis

In non-diabetic rats treated with exenatide, pancreatic acinar inflammation and pyknosis were observed (Nachnani *et al.*, 2010). There have been concerns regarding an excess of pancreatitis cases in exenatide-treated patients, since there have been several case reports published in this matter (Iyer *et al.*, 2012; Ayoub *et al.*, 2010; Tripathy *et al.*, 2008; Denker & Dimarco, 2006). However, patients with T2D have in general nearly a threefold higher risk of pancreatitis compared with non-diabetics (Girman *et al.*, 2010; Noel *et al.*, 2009) and there is conflicting evidence if there is a true increased risk for pancreatitis associated with the use of exenatide (Tatarkiewicz *et al.*, 2013; Elashoff *et al.*, 2011; NICE, 2011; Spranger *et al.*, 2011). A large safety surveillance report, including 27 966 subjects, did not identify any increased risk in patients treated with exenatide (Dore *et al.*, 2009). Similar results were seen in a large cohort-study, including 25 719 subjects (Dore *et al.*, 2011). Despite these results, a warning has been added to the exenatide patient information leaflet not to use exenatide if there is a history of pancreatitis or severe hypertriglyceridaemia, and further studies on this matter are requested (Phillips & Prins, 2011; Spranger *et al.*, 2011).

Pancreatic cancer

Concerns have also been raised regarding a possibly increased risk for pancreatic cancer in patients using exenatide, based on the suggested higher risk of pancreatitis and since chronic pancreatitis has been proposed to increase the risk of pancreatic cancer (Elashoff *et al.*, 2011; Jura *et al.*, 2005). There are also several studies indicating that exendin-4 affects proliferation of pancreatic cells. In one study pancreatic tumour cells (AR42J cells), which were negative for islet hormones and their mRNAs, were exposed to exendin-4. The results demonstrated that the intracellular cAMP levels rose and cells started becoming positive for islet hormones (Zhou *et al.*, 1999). Furthermore, it has been demonstrated that exendin-4 stimulates differentiation of beta-cells in rat ductal progenitor cells, and in a partial pancreatectomy rat

model 15 days of exenatide treatment stimulated regeneration of beta-cell mass and prevented the development of diabetes (Xu *et al.*, 1999). In mice, it was seen that exendin-4 suppressed beta-cell apoptosis in the pancreas after streptozotocin (STZ) injection (Li *et al.*, 2003). However, in a 13 week study in rats exposed to exenatide, no signs of ductal proliferation was observed (Tatarkiewicz *et al.*, 2013). In a recently published review, the authors request further evidence of the drug safety of incretin-based therapies (Butler *et al.*, 2013).

Thyroid effects

In rodents, long-term exposure to another GLP-1R agonist (called liraglutide) resulted in calcitonin release, proliferation of the rodent c-cells and tumour formation. In cynomolgus monkeys, 20 months of exposure to liraglutide did not lead to hyperplasia of the c-cells. Both humans and monkeys demonstrated low expression of GLP-1R in the thyroid, comparing to the rodent model. This strongly indicates species differences, although long-term effects of the usage of GLP-1R agonists in human beings, with regards to the thyroid, demands further investigation (Bjerre Knudsen *et al.*, 2010). Elashoff *et al.* (2011) stated that there was an increased risk of thyroid cancer in humans using exendin-4, although this study has been questioned by other authors (Phillips & Prins, 2011).

Cardiovascular effects

No increased cardiovascular risk has been observed in humans using exenatide, rather a reduction in cardiovascular events has been reported (Best *et al.*, 2011; Ratner *et al.*, 2011). It has been proposed that exendin-4 may have a beneficial effect regarding the development of atherosclerosis (Arakawa *et al.*, 2010). In a porcine animal model of ischemia and reperfusion injury, treatment with exenatide reduced myocardial infarct size (Timmers *et al.*, 2009).

In some studies exendin-4 appears to reduce the systolic blood pressure in mice and humans, with a weak correlation between reduction in blood pressure and weight loss in humans (Gill *et al.*, 2010; Okerson *et al.*, 2010; Hirata *et al.*, 2009; Moretto *et al.*, 2008), although, there is conflicting evidence in this matter. Yamamoto *et al.* states that stimulation of the GLP-1R using exendin-4 increases blood pressure in rats (Yamamoto *et al.*, 2002). In healthy humans no differences in blood pressure was seen following a s.c. injection of 10 µg exenatide, but a heart rate increase of 8.2 beats per minute was observed (Mendis *et al.*, 2012).

In mice, i.c.v. administration of exendin-4 depressed heart rate variability. An increase in heart rate was seen following both acute and chronic i.c.v. administration of exenatide. It was also observed that the stimulation of central GLP-1R reduced the parasympathetic modulation of the heart rate, which led to an increased heart rate (Griffioen *et al.*, 2011). Recently, a systematic review and meta-analysis was published, including 32 studies, concerning the effects of exenatide on heart rate and blood pressure, concluding that there is a connection between GLP-1 analogues and a small increase in heart rate as well as modest lowering of the blood pressure. The authors are welcoming further studies using more accurate means of measuring the heart rate (Robinson *et al.*, 2013).

Other side effects

It has been demonstrated in one study that exendin-4 may affect the hypothalamus-pituitary-axis in humans, resulting in elevated cortisol levels (Gil-Lozano *et al.*, 2010). Furthermore, exenatide has been shown to significantly reduce the plasma concentration and production rate of triglyceride-rich lipoprotein-apolipoprotein B-48, RLP-cholesterol, RLP-triglyceride and apolipoprotein C-III in humans (Xiao *et al.*, 2012; Schwartz *et al.*, 2010; Schwartz *et al.*, 2008), although there is conflicting evidence in this matter (Amori *et al.*, 2007).

The future of exenatide

At this point a long-acting exenatide, with administration once a week, is under evaluation. The overall result until this point is that exenatide given once weekly offers superior glycaemic control, greater reduction in HbA1c-levels and there are less reports regarding nausea. Similar reductions in weight were seen no matter once weekly or twice a day administration (Russell-Jones *et al.*, 2012; Blevins *et al.*, 2011; Drucker *et al.*, 2008; Kim *et al.*, 2007). There may be limitations in once weekly exenatide due to longer time to reach steady state (4-5 weeks). Additionally, a higher rate of antibody formation has been reported and in case of adverse events, such as pancreatitis, it will take weeks for the levels to decline (Madsbad *et al.*, 2011).

To conclude, the long-term effects of incretin-based therapies, such as exenatide, has not yet been explored completely, and continued evaluation regarding long-term adverse events is required (Amori *et al.*, 2007).

AIM OF THE PRESENT EXPERIMENT

The aim of the present experiment was to examine the feasibility of *in vivo* imaging of the GLP-1R in beta-cells, using the PET tracer [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 as a marker, in native pancreatic beta-cells of a porcine diabetic animal model. There is at this point no published study on the pig as an animal model using this technology and tracer.

Previous studies have stated that Swedish high-health herd-certified domestic pigs (Yorkshire x Swedish Landrace) can be made diabetic by i.v. injection of STZ and that pigs have a similar diabetic metabolism as humans. It is therefore a suitable animal model for further diabetic studies (Jensen-Waern *et al.*, 2009; Gäbel *et al.*, 1985).

Quantifying the BCM is of major interest in several areas of diabetic research and medicine. Finding a way to quantify the BCM, *in vivo* and non-invasively, is essential to islet transplantation research and would provide further insight into the pathogenesis and changes of BCM throughout different diabetic stages of both T1D and T2D. It would also be useful at a time of subsequent examination after having initiated a medical treatment and to follow-up viability of transplanted islets (Arifin & Bulte, 2011; Virostko & Powers, 2009; Souza *et al.*, 2006a; Paty *et al.*, 2004). The GLP-1 receptor is known to be highly expressed on the beta-cell surface (Tornehave *et al.*, 2008), thus quantifying the GLP-1 receptor in the pancreas could be an opening towards quantifying the BCM.

In this study, we also wanted to assess the perfusion in the pancreas and kidneys in healthy controls and STZ-induced diabetic pigs. During diabetic disease progression, changes in the islet vasculature can be seen. Currently, *in vivo* studies reflecting the change in perfusion of the pancreatic islets are scarce (Medarova & Moore, 2009).

MATERIAL AND METHODS

Experimental design

The experiment was approved by the Ethical Committee for Animal Experimentation, Uppsala, Sweden.

In this study, eight Swedish high-health herd-certified domestic pigs (Yorkshire x Swedish Landrace x Hampshire) from the same litter were used. The pigs were born February 21st 2013 and bred at Lövsta, SLU, Sweden. Four pigs of each sex were randomly assigned to be either controls (#2 castrate, #4 female, #6 female, #7 castrate) or made diabetic (#1 castrate, #3 female, #5 female, #8 castrate) using STZ. The experiment proceeded during eight weeks, please see Figure 1 below.

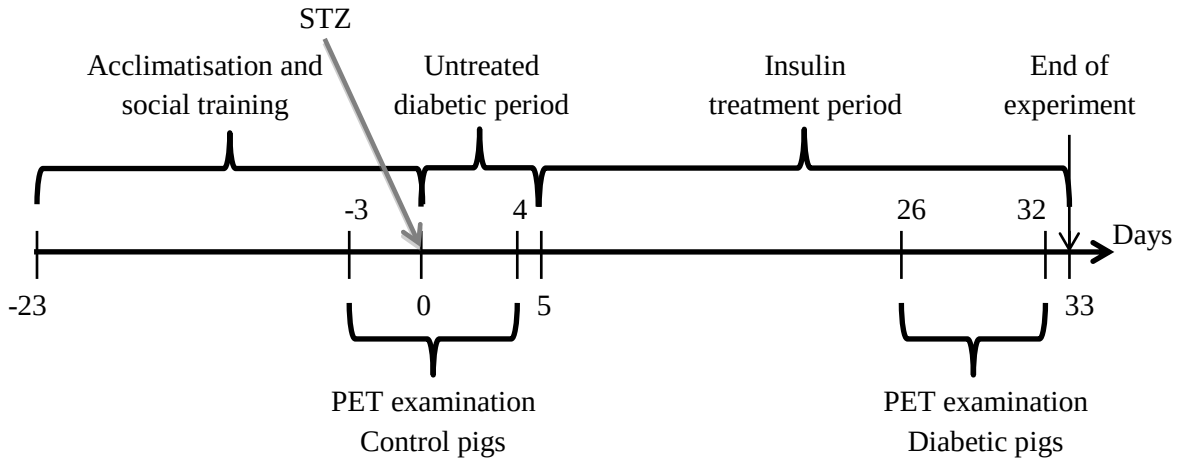

Figure 1. *Experimental design of the present experiment. The pigs were acclimatised and socially trained. Diabetes was induced by STZ-injection and insulin treatment was initiated five days post STZ. PET examination was performed on control pigs and insulin treated diabetic pigs.*

Animals and housing

The pigs were housed individually, within sight and sound of one another, in pens of approximately 3 m^2, at the Department of Clinical Sciences (Swedish University of Agricultural Sciences, SLU, Uppsala, Sweden). In the pen every pig was provided straw and wood shavings as bedding and an infrared lamp in one corner (24h). The pens were cleaned twice a day. The room temperature was 18 ± 2 °C. The pigs were fed a commercial finisher diet with no growth promoters (Solo 330 P SK, Lantmännen Sweden) twice a day (at 07:00 and 18:00), apple as treats and they had free access to water. The pigs were 40 days old upon arrival on April 2nd 2013.

Acclimatisation and social training

Upon arrival at the Department of Clinical Sciences the pigs were immediately moved into their own pen. Initially they were frightened and unsecure in their new environment; this is why they were given two days to adapt. Social training began on day three. The trainer sat down inside the pen, allowing the pigs to get used to humans. At first the pigs were very stressed, but after a few minutes the pigs accepted pieces of apple offered by the trainer. Every day for at least fifteen minutes the pigs were trained to get used to social contact, which included accepting pieces of apple, brushing and cuddling. The pigs were also accustomed to being touched around their ears as preparation for injections in that area. After a week they were greeting us by the door of the pen. Seven days after arrival we also started performing daily clinical examinations, taking their temperature and auscultating heart and lungs.

Figure 2. *The pigs were curious and quickly adapted to people*

Gradually the pigs were introduced to a piece of canvas with Velcro, since the jugular vein catheter, later on placed, would be protected by a canvas pocket sealed with Velcro. At first the pigs were allowed to smell the canvas and later on the Velcro was opened a couple of times at 1-2 meters of distance making the pigs used to the sound. A majority of the pigs were curious about the Velcro but a couple were scared at the sound of it. After a few days one could open the Velcro next to the head of each pig without the pig getting bothered the least.

The pigs were also trained to step on an electronic spring scale (Ecco 101, Farmer Tronic Industries A/S, Vamdrup, Denmark) and were then weighed three times a week throughout the study. At arrival the pigs weighed an average of 15.3 ± 1.4 kg (mean ± SD).

Surgery and anaesthesia – Insertion of a jugular vein catheter

The operational procedure

Once the pigs had been socialised they underwent surgery, (controls on April 17th and the diabetics on April 25th) to insert a jugular vein catheter which later on would be used to perform i.v. injections and sample blood in a stress-free way throughout the study.

The pig was starved over night with water provided *ad lib*. Analgesia and general anaesthesia was induced inside the pen. The pig was moved to a preparation room where an auricular vein catheter was placed and intubation with an endotracheal tube was performed. Throughout the operational procedure the pig was inhaling 30% oxygen in nitrogen and an i.v. infusion of Ringer's acetate (Ringer-acetat, Fresenius Kabi AB, Uppsala, Sweden) was administered. Anaesthesia was maintained either by iterating the same medicaments as for induction or by using a total-intravenous-anaesthesia-protocol (TIVA), as noted below.

The area of surgery was shaved and cleaned with soap and chlorhexidine solution and the hoofs were covered with socks to minimise heat loss. The pig was placed in dorsal recumbency and straps were used for fixation of the legs. An incision was made over the right jugular vein. A silicon catheter (SIL-C70 with rounded tip, Instec Solomon, PA, USA) was

placed in a sterile manner and the incision site was sutured. Thereafter the pig was placed in lateral recumbency and a subcutaneous tunnel was made for the silicone catheter to appear between the scapulae. Canvas was sutured to the back of the pig between the scapulas, to protect the catheter inside of a pocket sealed by Velcro. A catheter (Venflon®, Becton-Dickinson, Helsingborg, Sweden) was inserted at the proximal end of the silicon catheter to facilitate i.v. injections. The duration of the surgical procedure was approximately one hour**.**

Procaine benzyl penicillin 30 mg/kg (Penovet® vet. 300mg/ml, Boehringer Ingelheim Vetmedica, Malmö, Sweden) was administered by an intramuscular (i.m.) injection once a day for three days, starting at the day of surgery.

Anaesthesia

Anaesthesia was induced in the controls (pig #2, #4, #6 and #7) and in two of the destined to be diabetic pigs (#5 and #8) by an i.m. injection of 5 mg/kg tiletamin and zolazepam (Zoletil Forte® vet. 250mg/ml, Virbac, Carros, France), 0.05 mg/kg medetomidine (Domitor® vet. 1 mg/ml, Orion Pharma Animal Health, Sollentuna, Sweden) and 0.1 mg/kg butorphanol (Dolorex® vet. 10 mg/ml, Intervet AB, Sollentuna, Sweden). General anaesthesia was maintained by iterating 25% of the initial dose Zoletil Forte® vet. i.v. when needed.

Anaesthesia was induced in the two other destined to be diabetic pigs (#1 and #3) by an i.m. injection of 4 mg/kg alfaxalone (Alfaxan® 10 mg/ml, Vétoquinol UK Limited, Buckingham, U.K.) and 2 mg/kg midazolam (Dormicum® 5mg/ml, Roche AB, Stockholm, Sweden). Before intubation analgesia was ensured through an i.v. injection of 2 mg/kg fentanyl (Fentanyl B. Braun® 50 µg/ml, B. Braun Medical AB, Danderyd, Sweden).

In one of the control pigs (#6) and the other two destined to be diabetic pigs (#1 and #3) anaesthesia was maintained using a TIVA protocol of the medicaments used for the induction. Infusion rate was adjusted finely whenever needed.

Throughout the anaesthesia the pigs were being monitored with regards of respiration rate (RR), HR, oxygen saturation (SpO_2), end-tidal carbon dioxide ($EtCO_2$), rectal body temperature, ECG, non-invasive blood pressure, tidal volume, minute ventilation and inspired oxygen concentration (FiO_2).

Induction of diabetes

Diabetes was induced under anaesthesia in pig #1, #3, #5 and #8 on April 25th with an i.v. injection of STZ (150 mg/kg, Sigma S0130, Stockholm, Sweden), according to (Jensen-Waern *et al.*, 2009; Gäbel *et al.*, 1985). STZ has a short half-life why it was dissolved in 100 mmol/L disodium citrate buffer solution (pH 4.5), at 80 mg/ml concentration. The injection was performed within five minutes, with an injection rate of approximately 1 ml/second.

Postoperative care

The pigs were moved back to their own pen after surgery and extubated. The pigs were monitored and given food once they were fully awake and could walk without difficulty.

Analgesia was given if required by an i.m. injection of 0.01 mg/kg buprenorphine (Temgesic® 0.3 mg/ml, RB Pharmaceuticals, Berkshire, U.K.).

The indwelling jugular vein catheters were flushed twice daily with 10 ml sterile saline (Natriumklorid Fresenius Kabi® 9 mg/ml, Fresenius Kabi AB, Uppsala, Sweden) and 5 ml 2‰ heparinised saline solution (5000 IU/ml, LEO Pharma AB, Malmö, Sweden) throughout the study. The daily clinical examination now included looking at the incision wound.

Streptozotocin may initially cause hypoglycaemia (Gäbel *et al.*, 1985), thus the affected pigs (#1, #3, #5 and #8) were closely monitored until they were hyperglycaemic. Blood glucose was measured at 3, 5, 7 and 9 hours post STZ injection. If any pig was hypoglycaemic it was given an i.v. glucose infusion.

On the morning of April 30th (five days post STZ) pig #5 was found dead in its pen, thus this pig was not included in any further stages of the experiment. The *post mortem* diagnosis was acute circulatory distress, with acute liver and lung stasis. Focally in the myocardium, a small area of fibrosis was seen, which may be due to a chronic infarct or previous focal myocarditis. The body was pale, but no aetiology to anaemia could be found. Degenerative changes seen in the hepatocytes were estimated to be of reversible nature. The pancreas could not be examined due to autolysis.

Insulin treatment

On May 1st the diabetic pigs (i.e. #1, #3 and #8) were given 3.5 IU Actrapid® Penfill® (100 IU/ml, Novo Nordisk Scandinavia AB, Malmö, Sweden) s.c., which is a fast-acting humane insulin with rapid onset of acting and short duration. Since there is a risk of antibody formation in the pigs against the humane insulin, these injections were a onetime event due to hyperketonaemia.

In the evening of May 2nd (seven days post STZ) insulin treatment was continued, now injecting intermediate-acting porcine insulin s.c. (Caninsulin® vet. 40 IU/ml, Intervet AB, Sollentuna, Sweden), with starting dose 0.8 IU/kg x 2. The dosage was adjusted if needed, aiming towards a blood glucose concentration of 6–15 mmol/L. Injections were performed whilst the pigs were eating.

Blood analyses

Glucose measurements

All glucose values throughout the study were obtained with test strips using a glucometer (Aviva Accu-Chek, Roche Diagnostics, Basel, Switzerland) that had been validated for porcine blood previously at the Department of Clinical Sciences, SLU, Sweden.

Throughout the study blood glucose was measured at least twice daily. Blood was sampled from the jugular vein catheter, which was flushed with sterile saline (Natriumklorid Fresenius Kabi® 9 mg/ml, Fresenius Kabi AB, Uppsala, Sweden) before and after sampling. Before sampling, 3 ml blood was drawn and thrown away.

Ketone bodies

Blood samples were taken for analysis of β-ketone bodies if clinically indicated, using test strips (FreeStyle Precision β-ketone, Abbot/ADC, Alameda, CA, U.S.). Ketone bodies were analysed on May 1st – (see results below in Table 2).

C-peptide and glucagon stimulation test

C-peptide is widely used in human trials and is a means of analysing insulin secretion (Souza *et al.*, 2006a). Several blood samples were drawn to analyse serum C-peptide concentrations during the study. The samples were analysed at Rudbeck Laboratory (Uppsala University, Sweden) using Porcine C-peptide ELISA (Mercodia Uppsala, Sweden). Samples were drawn from all eight pigs, on the day of catheterization/STZ. In the STZ-pigs samples were also drawn on day 4, 15 and 27 post STZ.

A glucagon stimulation test (GST) was performed either on day 28 or 29 post STZ (May 23rd and 24th) by injection of 1 mg glucagon (Glucagon Novo Nordisk 1 mg, Novo Nordisk Scandinavia AB, Malmö, Sweden) s.c. to the diabetic pigs to cause hyperglycaemia and, in case of remaining functional beta-cells in the pancreas, induce release of C-peptide. Blood for analysis of serum C-peptide was sampled 30 minutes after the injection of glucagon.

Haematology and biochemistry

On April 17th and April 25th, i.e. the days of surgery, blood was sampled from all eight pigs to analyse haematology (Hb, EPK, EVF, MCV, MCHC, reticulocytes and morphology) using an electronic cell counter (Advia 2120, Siemens, Erlangen, Germany) and total and differential white blood cell counts using EDTA-preserved blood. Using automated equipment (Architect C4000, Abott, Diagnostics, North Ryde, Australia), serum was analysed for values of total bilirubin, electrolytes (sodium, potassium and chloride**)** and enzyme activity of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyltransferase (GT) and glutamate dehydrogenase (GLDH).

On April 29th (four days post STZ) and May 22nd (27 days post STZ) blood was sampled from the diabetic pigs for the analyse of ALAT, ASAT, GLDH and creatinine.

All blood parameters were analysed at an accredited laboratory at SLU, using methods validated for porcine blood.

Pet-CT and anaesthesia

Preparations

On the day of the examination the pigs were fed and, if diabetic given their regular morning dose of insulin, at least over an hour prior to anaesthesia. Anaesthesia was induced in their own pen at the Department of Clinical Sciences (SLU, Uppsala). A peripheral catheter was inserted into an auricular vein (all pigs) for injections and infusions, and into an ear artery (pig #2) to be able to measure the arterial blood pressure. Two different anaesthetic protocols were used. Anaesthesia was maintained using a TIVA-protocol. Please see a detailed description of the anaesthesia below.

The anaesthetised pig was intubated with an endotracheal tube and transported approximately 15 minutes by car and wired up to a portable monitor device (Mindray) measuring RR, HR, SpO_2 and $EtCO_2$, to the Uppsala University Hospital, where the PET-CT examination was performed. All diabetic pigs were connected to a portable ventilator (Hamilton C2) during the transport or ventilated using a manual resuscitator.

At the Uppsala University Hospital the pig was placed in dorsal recumbency, connected to a Kion ventilator, 30% oxygen in nitrogen was administered and the pig continued being monitored now measuring RR, HR, SpO_2, $EtCO_2$, rectal body temperature, ECG, non-invasive blood pressure, tidal volume, minute ventilation and FiO_2. The pig was mechanically ventilated to ensure $EtCO_2$ was maintained at approximately 5.5 kPa.

Anaesthesia during PET

Two anaesthetic protocols were used, one for controls and one for diabetic pigs. This was due to the fact that medetomidine (used for controls) is not suitable for diabetics, since it increases blood glucose levels and decreases insulin secretion (Ambrisko *et al.*, 2005). The anaesthetic protocol used for controls was evaluated by the anaesthesiologist prior to this study and is the regular protocol used for healthy pigs at the research facility.

The controls were anaesthetised by an i.m. injection of 5 mg/kg tiletamin and zolazepam (Zoletil Forte® vet. 250mg/ml, Virbac, Carros, France), 0.05 mg/kg medetomidine (Domitor® vet. 1 mg/ml, Orion Pharma Animal Health, Sollentuna, Sweden) and 0.1 mg/kg butorphanol (Dolorex® vet. 10 mg/ml, Intervet AB, Sollentuna, Sweden) as analgesia.

Anaesthesia and analgesia was induced in the diabetic pigs using 4 mg/kg alfaxalone (Alfaxan® 10 mg/ml, Vétoquinol UK Limited, Buckingham, U.K.), 2 mg/kg midazolam (Dormicum® 5 mg/ml, Roche AB, Stockholm, Sweden) and 2 µg/kg fentanyl (Fentanyl B. Braun® 50 µg/ml, B. Braun Medical AB, Danderyd, Sweden), given i.v. (#3) or i.m. (#1 and #8). Anaesthesia was induced to pig #1 and #8 by i.m. injections, since their catheters were malfunctioning. In the pigs with malfunctioning catheters, access for venous sampling was then granted through invasive (on site) surgery to the jugular vein (#1) or a catheter reaching the jugular through the auricular vein.

General anaesthesia and analgesia was maintained using two different TIVA-protocols, administered through the auricular vein catheter, where control pigs were given a constant rate infusion (CRI) of 5 mg/kg/h tiletamin and zolazepam, 0.05 mg/kg/h medetomidine and 0.1 mg/kg/h butorphanol. The diabetic pigs were given a CRI of 3 mg/kg/h alfaxalone, 1 mg/kg/h midazolam and 1 µg/kg/h fentanyl at infusion starting dose, continuously adjusted to achieve adequate anaesthetic depth. The anaesthesia was administered through the auricular vein catheter. The duration of the general anaesthesia was approximately five hours.

To maintain the intravenous fluid homeostasis, an i.v. infusion of Ringer's acetate (Ringer-acetat, Fresenius Kabi AB, Uppsala, Sweden) was administered using the indwelling jugular vein catheter.

Perfusion study

At the beginning of the PET examination, oxygen-15 labelled water ([^{15}O]WAT) was injected i.v. in the auricular vein as a bolus (200 MBq) using a contrast pump (Medrad) 0.8 ml/s for ten seconds, followed by 15 ml NaCl and 1.0 ml/s [^{15}O]WAT for 15 seconds. This was performed to measure the perfusion in the pancreas and kidneys by a 10 minute dynamic PET scan (27 frames).

The PET-CT examination

Each pig was positioned with the pancreas in the centre of the 15 cm axial field of view. A full body CT, low dose scout view (140 kV, 10 mAs), was performed to detect the pancreatic area, using a Discovery ST PET-CT scanner (GE Healthcare, Milwaukee, MI, U.S.) and a photon attenuation correction scan was acquired.

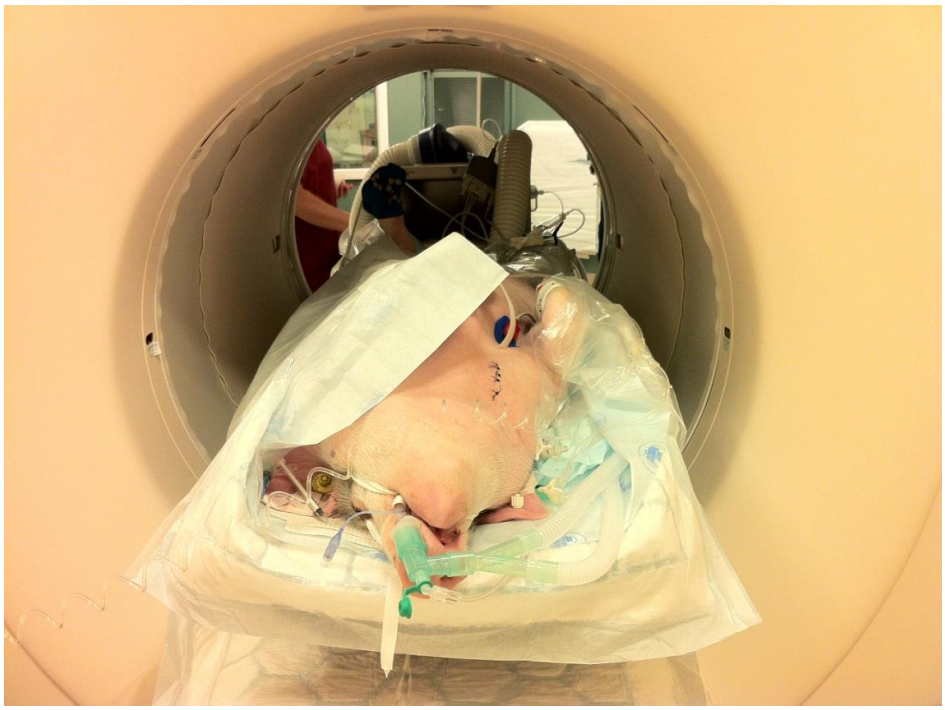

Figure 3. *PET-CT examination. Photograph taken by the author.*

The tracer [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 was injected i.v., 8.08 ± 3.83 MBq (mean ± SD), corresponding to a low dose of 0.025 ± 0.01 µg exenatide peptide/kg, to obtain a baseline PET image (31 dynamic frames). In two controls and two diabetics a full body PET-CT was repeated, to study the full body uptake of the tracer signal. Thereafter, the tracer [^{68}Ga]Ga-DO3A-vs-Cys40-exendin-4 (69.01 ± 26.6 MBq) was injected i.v. together with a high dose of unlabelled exenatide (3.98 ± 1.33 µg/kg), to make the unlabelled high dose exenatide compete with the tracer for the GLP-1R and outnumber the tracer, to obtain a blocking image (31 dynamic frames).

The tracer and unlabelled exenatide were administered through the auricular vein catheter and the indwelling jugular vein catheter was used to analyse blood glucose during PET and to measure the radioactivity of the tracer, for PET image mathematical calculations.

Cystocentesis was performed if the urinary bladder needed to be emptied during the examination. This procedure resulted in a change in patient position, thus a new attenuation correction CT was made after cystocentesis and images were checked, to make sure the pancreas was in the focal area.

PET-CT examination was performed on each pig in the following order (see Table 1). The diabetic pigs were examined three to four weeks after insulin treatment had been initiated.

Table 1. *Dates of PET examinations*

	Controls	Diabetics	Time during the day
April 22nd	#2		Afternoon
April 23rd	#4		Afternoon
April 24th	#6		Morning
April 29th	#7		Afternoon
May 22nd		#3	Afternoon
May 24th		#1	Morning
May 28th		#8	Afternoon

The PET-CT examination lasted for approximately four to five hours. The pig was transported back to the Department of Clinical Sciences in the same manner as it was transported to the Uppsala University Hospital. Extubation was performed in the pen, once the pig could swallow, and the ear vein catheter was removed.

An additional PET examination was performed on the brain of a ninth pig (slightly older than the previous eight pigs), to assess if the tracer could pass the blood-brain-barrier and bind to GLP-1R in the brain.

Euthanasia, *post mortem* examination and tissue sampling

The pigs were euthanised, 0–6 days after PET, with an overdose i.v. injection of pentobarbital sodium (Pentobarbital® vet. 100 mg/ml, Apoteksbolaget, Sweden). Additionally, an intracardiac injection of 20 ml Pentobarbital® was performed, once the pig was deeply anaesthetised.

In all pigs a full *post mortem* examination including histopathology was performed by a veterinary pathologist at SLU or the Veterinary Institute, Uppsala. The autopsy was performed on the same day as the pig was euthanised, except pig #5 where autopsy was performed two days later. Tissue samples from three different regions of the pancreas (duodenal, connective and splenic lobe) were fixed in formalin to perform insulin-staining by IHC.

Statistical analyses

Data is given as mean ± SD. Nonparametric Mann-Whitney rank sum test (GraphPad Prism 5 and GraphPad Instat) was used to test for significance in the perfusion study and for MAP and HR values, considering $p < 0.05$ to be significant.

RESULTS

Surgery and anaesthesia – Insertion of a jugular vein catheter

All pigs were clinically examined before surgery and the general condition was good. The jugular vein catheter was placed without complications in the right side in all the pigs except in pig #1. In pig #1 the catheter was placed into the left jugular vein. Both anaesthetic protocols worked satisfactorily.

Postoperative care

The pigs recovered well and quickly after the anaesthesia and surgery. They were hungry and fed within a couple of hours after being extubated. Twelve hours post surgery the pigs were brisk and curious as usual. Additional analgesia was not required.

The days after the operation all of the pigs had subcutaneous swelling and oedema around the incision site, but no pain reaction was observed when the oedema was palpated. The swelling was reduced substantially within a couple of days. The wounds healed without signs of infection, although the incision wound of pig #1 did not heal completely, which is why the catheter was removed on May 7th (twelve days post surgery).

General appearance, induction of diabetes and insulin treatment

The general appearance was mainly good throughout the study for all pigs, and the control pigs in particular. The pigs showed a daily weight gain of 0.7 ± 0.05 kg/pig during a two week period prior to STZ.

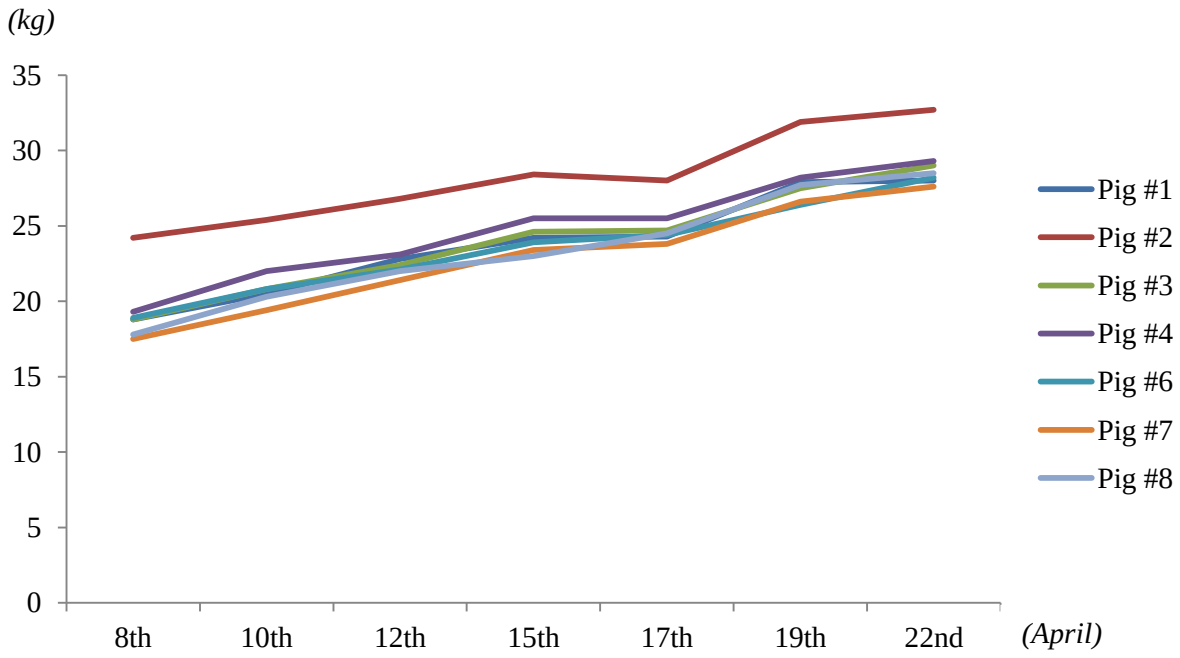

Chart 1. *Weight gain the two weeks prior to diabetes induction by STZ-injection.*

The first 24 hours after the STZ-injection are critical, due to risk of hypoglycaemia (Gäbel *et al.*, 1985), thus blood glucose was closely monitored. Nine hours post STZ all pigs were hyperglycaemic (blood glucose > 11 mmol/L) and after 24h blood glucose was > 25 mmol/L.

Within a couple of days, all three pigs exhibited clinical signs such as polydipsia, polyuria, hyperglycaemia and weight loss, showing diabetes mellitus was successfully induced. The average weight loss was 0.52 ± 0.02 kg/pig/day during the untreated diabetic period, which is shown by Chart 2 below.

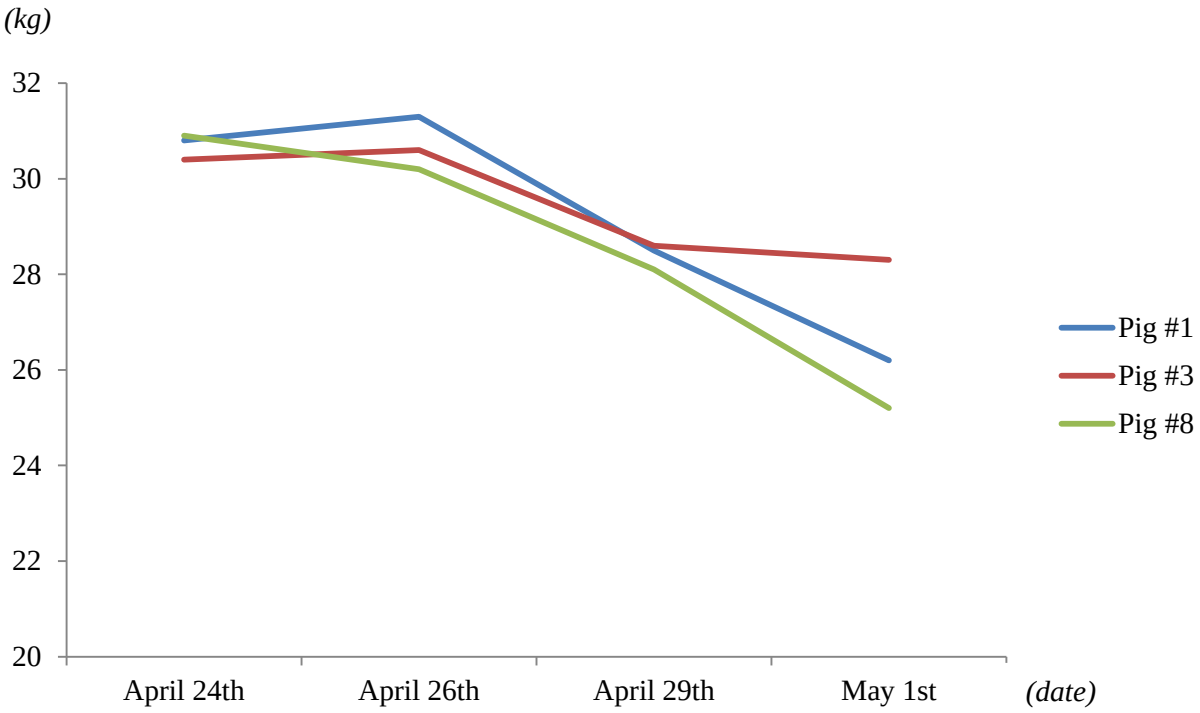

Chart 2. *Weight loss after diabetes induction by STZ-injection on April 25th.*

On the morning of May 1st insulin treatment was initiated using the humane insulin Actrapid® Penfill® (100 IU/ml, Novo Nordisk Scandinavia AB, Malmö, Sweden) since β-ketone bodies were elevated, please see results below in Table 2. The blood concentration of β-ketone bodies was still elevated in one pig three hours later, thus the Actrapid® was iterated. Additional β-ketone tests were then negative.

The following day, May 2nd (seven days post STZ), insulin treatment was continued twice a day injecting 0.8 UI/kg intermediate-acting insulin instead (Caninsulin® vet. 40 IU/ml, Intervet AB, Sollentuna, Sweden), since this is a porcine insulin. The dosage was thereafter adjusted several times, according to blood glucose and weight, and doses varied from 0.3 IU/kg to 1.0 IU/kg twice a day.

The insulin injections were performed whilst the pigs were busy eating and pieces of apples were used as rewards. At the beginning of the insulin treatment the pigs were scared or shaking their head when the injection was given, but after a few days of treatment they had stopped reacting to the injection and it could be carried out quickly and easily.

Proper glycaemic control was hard to achieve, which already is well known (Manell *et al.* 2014), thus slight hyperglycaemia was preferred to avoid hypoglycaemia during night. However, the therapeutic response was good, inasmuch the pigs did not show polydipsia and polyuria to the same extent and started gaining weight (0.53 ± 0.02 kg/pig/day the first week after insulin treatment was initiated) and accumulate subcutaneous fat again (please see Chart

3 below). The weight gain corresponds to that of commercial high-health herd-certified pigs, which is about 0.5 kg/day (Manell *et al.* 2014).

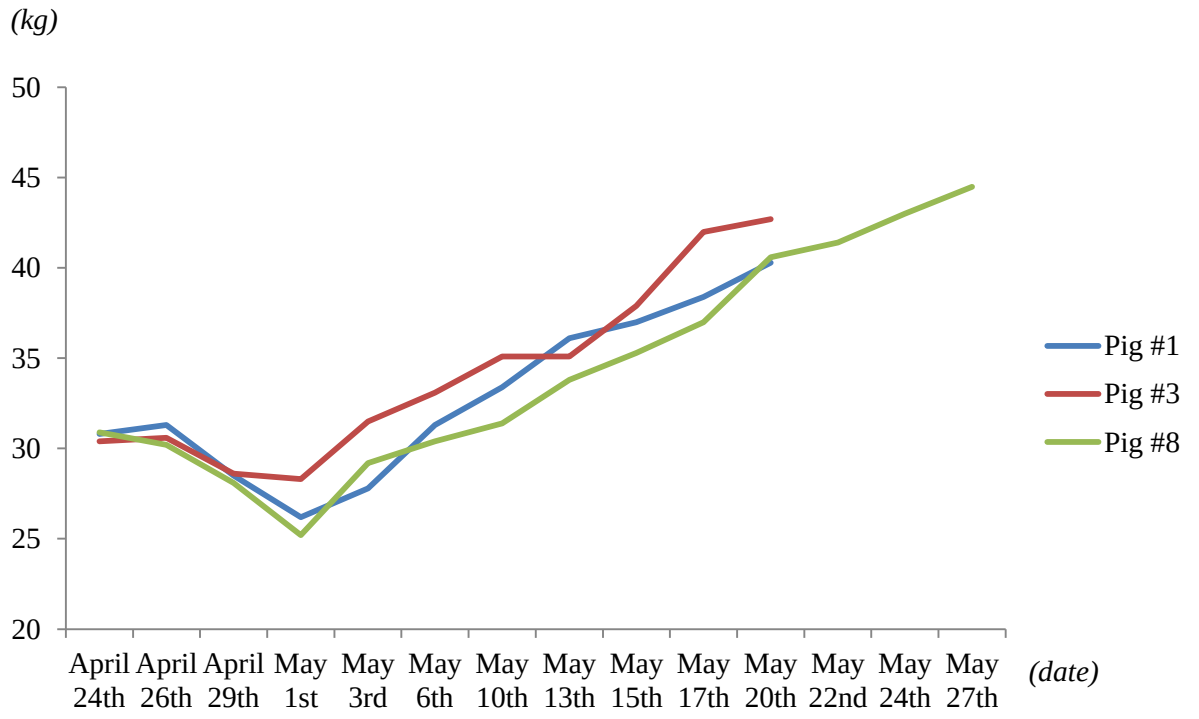

Chart 3. *Weight gain from STZ and after initiated insulin treatment on May 1st until end of study.*

Blood analyses

Glucose measurements

The STZ successfully induced diabetes, resulting in persistent hyperglycaemia in all pigs. Blood glucose was analysed daily to adjust the insulin dose. Proper glycaemic control was hard to achieve which led to slight hyperglycaemia throughout the study. However, the pigs were insulin-treated aiming towards a blood glucose concentration of 6–15 mmol/L, to avoid acute hypoglycaemia.

Ketone bodies

β-ketone bodies in the blood were analysed on May 1st due to poor general condition, and considering the acute death of pig #5. Please see the results below (Table 2). No blood test was performed on pig #1 in the afternoon, due to problems with its indwelling catheter.

Table 2. *Ketone values on May 1st before and after noon*

	Ketone value before noon (mmol/L)	**Ketone value in the afternoon (mmol/L)**
Pig #1	2.4	-
Pig #3	1.4	0.0
Pig #8	3.7	3.4

Results indicated ketonaemia in the morning in two of the diabetic pigs (i.e. elevated β-ketone

bodies) and in the afternoon in one pig. Following tests were β-ketone negative and no more testing was clinically indicated.

C-peptide and glucagon stimulation test

The C-peptide levels remained low in all pigs (fasting levels up to ~ 60 pmol/L), including the controls. The glucagon stimulation test resulted in a slight increase (but still < 120 pmol/L) in one diabetic pig (#3).

Haematology and biochemistry

The test results from April 17th and April 25th, from all eight pigs, were within reference range. Blood samples four days post STZ (April 29th) were within reference range, except for pig #1 which had elevated ASAT and GLDH activities, which most likely was due to toxic effects of the STZ (Dufrane *et al.*, 2006). The same parameters were analysed 27 days post STZ (May 22nd) and all values were then restored to normal.

Complications

On April 23rd pig #2 was squinting with its left eye, most likely due to the fact that it had not been given eye lubricant during surgery the previous day. It was given fucidic acid eye drops (Fucithalmic® vet. 1%, Dechra Veterinary Products A/S, Uldum, Denmark) twice that day in the affected eye. The following day the eye appeared normal and treatment was discontinued.

Pig #1, #3 and #8 had fever and/or affected general condition on April 30th (five days post STZ), and also considering the death of pig #5, they were given procaine benzyl penicillin 30 mg/kg (Penovet® vet. 300mg/ml, Boehringer Ingelheim Vetmedica, Malmö, Sweden) i.m. once daily during one week.

On May 2nd pig #1 still had fever, chills and poor general condition. It was then given 0.1 mg/kg butorphanol (Dolorex® vet. 10 mg/ml, Intervet AB, Sollentuna, Sweden) i.m. once for analgesia at onset of illness and 2.5 mg/kg enrofloxacin (Baytril® vet. 100mg/ml, Bayer A/S, Animal Health Division, Copenhagen, Denmark) i.m. once daily during six days. Pig #1 also showed stereotypical behaviour (persistent licking on a wall and other objects), which could have been a sign of hepatic encephalopathy, since the pig at that point also had elevated activities of liver enzymes (ASAT and GLDH).

The catheters were flushed twice a day, which could be performed easily initially but after a few days it became more difficult to flush them until they stopped functioning one by one. Towards the end of the experiment pig #3 was the only pig with a functioning catheter. This pig was euthanised on May 23rd and the last five days of the study we could not sample blood glucose from the remaining pig (#8), although blood glucose from pig #8 was measured on the day of PET (May 29th).

We experienced problems with the indwelling jugular vein catheter of control pig #6 during PET. It is possible that the catheter was blocked by a thrombus and that forced flushing led to it being flushed into the blood stream. The day after PET the pig's personality and behaviour had changed. Now, it did not want to eat apple, appeared not to understand how to drink from

the water nipple and was very calm (from earlier having been a crazy pig playing and biting on your shoes).

Anaesthesia during PET

Overall, the anaesthetic protocols of both types worked adequately. No muscle relaxant was needed and the pigs lay perfectly still as required for the performance of the PET-CT examination.

During the whole PET anaesthesia, from start, the diabetic pigs had lower mean arterial blood pressure (MAP) than the control pigs. Diabetic pigs had mean MAP values of 54.9 ± 10.8 mmHg and median MAP value of 53.0 mmHg whilst controls had mean MAP values of 93.0 ± 17.8 mmHg and median MAP value of 93.0 mmHg during PET, which is a significant difference ($p < 0.0001$).

Perfusion study

There was a significantly ($p < 0.05$) lower perfusion of Oxygen-15 labelled water ([^{15}O]WAT) in the pancreas and the kidneys in the diabetic pigs compared to controls. Please see Figure 4 below. The perfusion was decreased by 46% in the pancreas and 40% in the kidneys of the diabetic pigs.

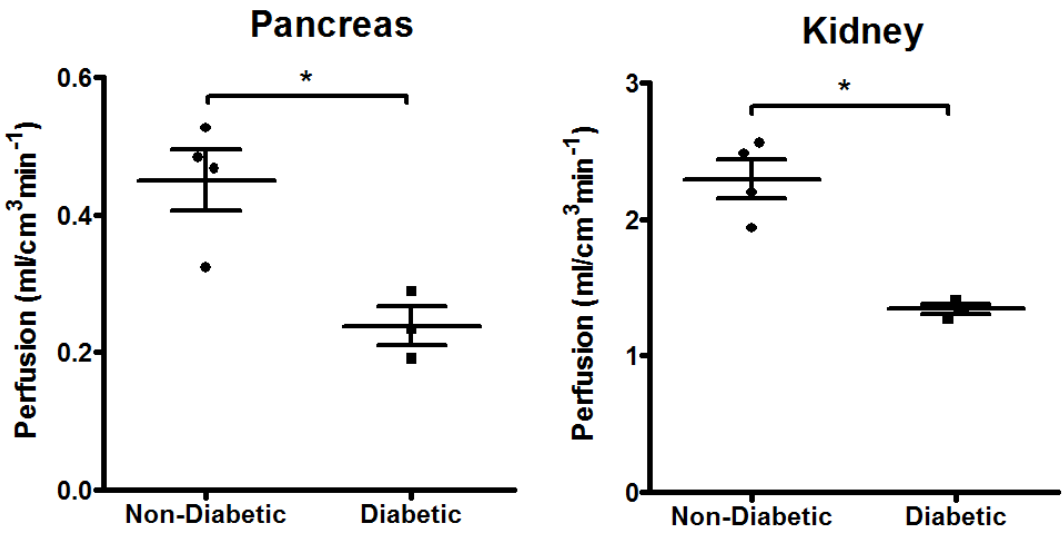

Figure 4. *Perfusion in the pancreas and kidneys in Non-Diabetic (controls) and Diabetic pigs. The asterisk indicates* p < *0.05. The short horizontal bars represent interquartile range and the long horizontal bars represent median values. The figure is acquired by Marie Berglund at the PET preclinical platform.*

PET-CT results

In the control pigs, the PET results showed specific tracer binding to GLP-1R located in the pancreas, by a high uptake of the compound at low dose (baseline), which could be blocked by the unlabelled high dose exenatide as competition. However, the pancreatic uptake of the tracer did not differ significantly ($p = 0.43$) between the control pigs and the diabetic pigs.

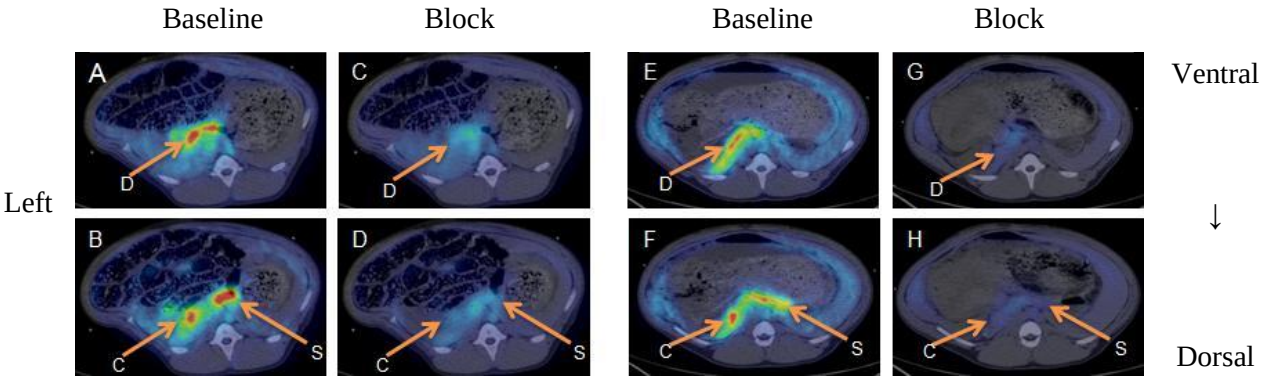

Figure 5. *Transverse PET-CT images of the pancreas using the tracer [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 , showing the duodenal lobe (D), connective lobe (C) and splenic lobe (S), with two controls to the left (panels A – D) and two diabetics to the right (panels E – H). The figure is acquired by Ram K. Selvaraju at the PET preclinical platform.*

In the thoracic view of tracer distribution at low dose, a higher uptake was observed in the lungs of the diabetic pigs compared to control pigs, please see Figure 6. This uptake persisted following high dose exenatide, thus the uptake was not receptor-mediated.

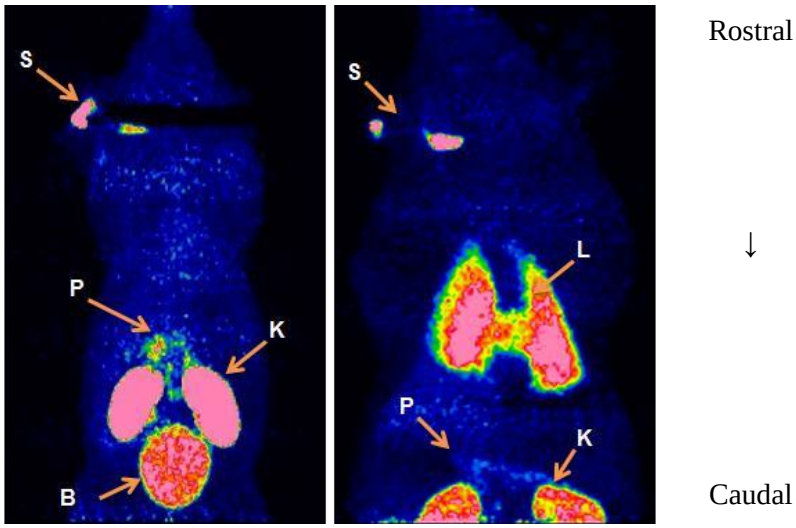

Figure 6. *Dorsal PET images, thoracic and partly abdominal view, showing the high [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 tracer uptake in a diabetic pig lung compared to a control pig. Site of injection (S), pancreas (P), kidneys (K), urinary bladder (B) and lungs (L). The figure is acquired by Ram K. Selvaraju at the PET preclinical platform.*

There was a high uptake in the kidneys and urinary bladder (the main route of tracer excretion) in all pigs. The uptake in other tissues, such as liver and muscle, was negligible in both groups and no detectable receptor binding was seen in the heart. The additional PET examination, concluded there was no tracer uptake in the brain.

Tachycardia

In all pigs, administration of the tracer compound resulted in significant increases in HR, both at low dose (92 ± 14 beats/min to 102 ± 10 beats/min (mean ± SD), median HR of 89 to 100, $p = 0.0337$) and high dose (115 ± 17 beats/min to 217 ± 25 beats/min (mean ± SD), median HR of 108 to 228, $p = 0.0022$). A β-blocker, metoprolol (Seloken® 1 mg/ml, AstraZeneca AB, Södertälje, Sweden) was administered i.v. at a dosages of 1–5 mg in three of the control pigs, but the severe tachycardia persisted.

Pig #4 suffered cardiac arrest during the transport back to SLU. The pig was given 0.3 mg epinephrine (Adrenalin 0.1 mg/ml, Martindale Pharmaceuticals, Romford, Essex, U.K.) i.v. and CPR was performed. The heart started beating after a couple of minutes and the pig began to breathe again. Once extubated at SLU the pig woke up quite quickly and wanted to eat only one hour later.

Three days post PET (April 25th) occasional arrhythmias were auscultated in pig #2. Four days after PET (April 27th) arrhythmia at a frequency of one extra beat approximately every eight regular beats was auscultated in pig #4.

Chart 4 below shows the significant HR increase in all pigs immediately after both low and high dose administration of exendin-4 and tracer (significance calculated based on HR values at 45 minutes compared to 60 and 105 compared to 120 minutes). The HR started to increase after 60 minutes from the start of the PET examination.

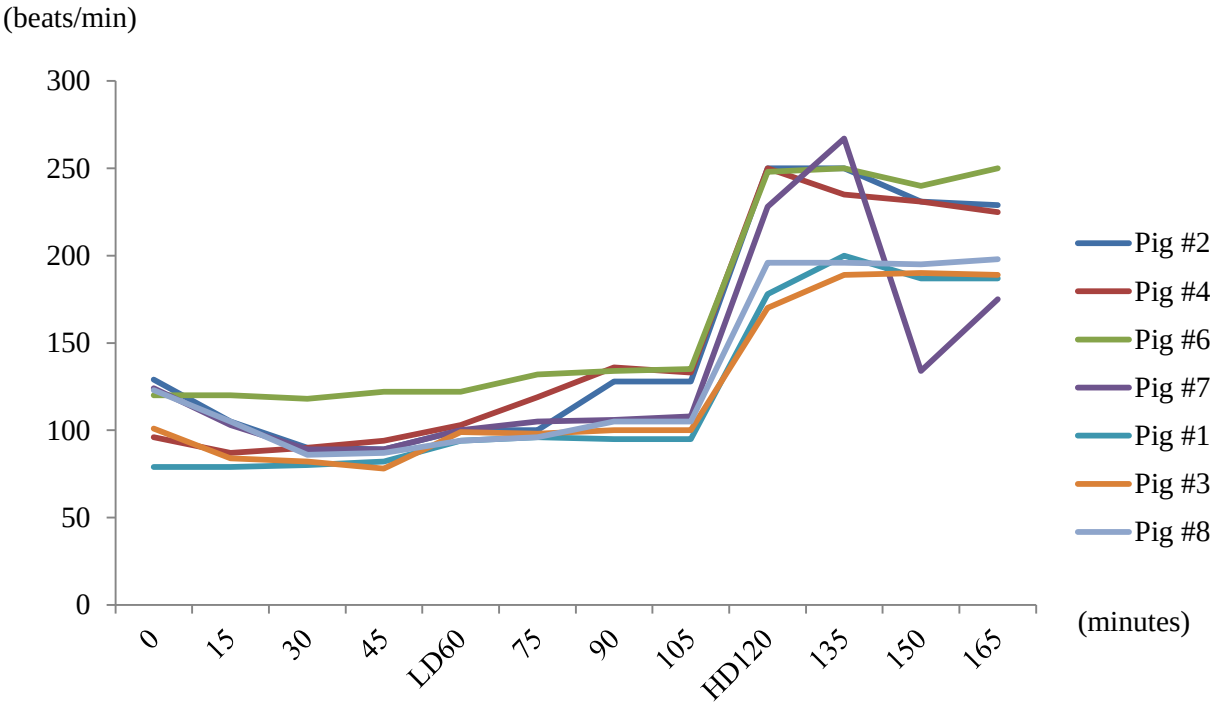

Chart 4. *Heart rate increase following intravenous injection of high dose exendin-4 and tracer. Low dose (LD) given at 60 minutes and high dose (HD) administered at 120 minutes from start of the PET examination.*

The additional pig, on which the PET examination of the brain was performed, had also an elevation of HR following high dose i.v. exenatide injection (maximum HR 160 beats per

minute). This pig was older than both controls and the diabetic pigs. The control pigs, which were the youngest, had a maximum HR of 250–260 beats per minute, whilst the diabetic pigs, which were three to four weeks older than the controls, had a maximum HR of 190–200 beats per minute, after high dose exenatide.

Blood glucose during PET

During PET, two pigs were hypoglycaemic (control #6 and diabetic #1) towards the end of the PET examination, after both low and high dose exenatide. The other five pigs were normo- or hyperglycaemic during PET.

Post-mortem examination and tissue sampling

No *post mortem* findings affecting the quality of the animal model were seen in any of the pigs. The only findings related to the heart were caused by the euthanasia.

The IHC of the pancreas with insulin-staining, showed a normal staining pattern in control pigs, whereas there was a poor to no staining in the STZ-treated pigs.

DISCUSSION

Animal model

The porcine animal model was successful. Following STZ-injection, clinical signs of diabetes were observed, such as hyperglycaemia, polydipsia, polyuria, hyperketonaemia and weight loss. Upon insulin treatment the clinical signs were alleviated, and the pigs started gaining weight and accumulate subcutaneous fat, which is also evidence of a well functioning diabetic animal model. Furthermore, the IHC with insulin-staining of the pancreas showed a markedly low level of staining in the STZ-treated pigs, in comparison to control pigs, thus, confirming that STZ effectively destroyed the vast majority of the beta-cells in the STZ-treated pigs**.**

The C-peptide values were low in all pigs, indicating that analyse of C-peptide may not be a suitable test for hypoinsulinaemia in pigs and has to be further evaluated.

The anaesthetic protocols worked satisfactorily as 1) the animals lay absolutely still during PET-CT examinations, as required, without the use of muscle relaxants, and 2) the same anaesthesia could be used during transportation and examinations.

Perfusion study

A significantly reduced perfusion (almost half) was seen in the pancreas and kidneys of the diabetic STZ-pigs compared to controls. The STZ-pigs had also a lower MAP than the control pigs during PET. It is possible that this lowering of MAP is the cause of the difference in perfusion. The reason why the diabetic STZ-pigs had a lower MAP is not known; however, different anaesthetic protocols, hyperglycaemia and STZ-toxicity may be potential explanations. Furthermore, it cannot be excluded that the age difference between controls and diabetic pigs (three to four weeks) has affected the result.

An anaesthetic study in pigs, shows that Alfaxan®, which was a substantial part of the TIVA used in the diabetic STZ-pigs, causes peripheral vasodilatation (Pfeiffer *et al.*, 2013), while

Zoletil®, an important part of the TIVA used in the control pigs, instead causes an increase in peripheral blood pressure (Lee & Kim, 2012). Thus, the significant difference in pancreatic and kidney perfusion between control and STZ-treated pigs could be due to net MAP differences caused by the different anaesthetic protocols used.

In a rat diabetic model, tissue perfusion was significantly lower in non-insulin-treated diabetic rats compared to insulin-treated rats, two weeks post STZ (Kashiwagi *et al.*, 2012). In another study of STZ-treated rats, a significantly lower systolic blood pressure was seen eight weeks post STZ-treatment. The lowered blood pressure was associated with up-regulation of cardiac muscarinic M_2-receptors. The blood pressure and the M_2-receptor mRNA levels, were restored once glycaemic control was achieved (Liu *et al.*, 2008). Since the pigs of the current experiment were not under optimal glycaemic control, hyperglycaemia could explain the lower MAP seen in the diabetic pigs. Hyperglycaemia causes secondary osmotic diuresis and increased water loss, which together with anaesthesia, might have led to hypovolaemia during the PET examination, and thereby causing the lower MAP and subsequently reduced tissue perfusion (Oliver *et al.*, 2010). Furthermore, hypotension is seen in the initial phase of diabetic disease progression (Maeda *et al.*, 1995).

A known side effect of chronic hyperglycaemia is vascular damage (Son *et al.*, 2004), thus theoretically, hyperglycaemic stress may have caused the difference in perfusion. Vascular dysfunction and oxidative stress parameters were seen in STZ-treated rats six weeks post STZ but could be reversed by insulin treatment (Oelze *et al.*, 2011). In the current experiment, the pigs were only diabetic for three to four weeks and they were insulin treated. Even though they were not under optimal glycaemic control, it is unlikely that vascular damage occurred in that short period of time causing that significant difference in perfusion and there were no signs of vascular damage *post mortem*.

Another explanation to the difference in perfusion is STZ-toxicity**,** which can be seen in the liver and kidneys (Dufrane *et al.*, 2006). However, the STZ-treated pigs in this experiment had no elevated blood parameters indicating kidney damage and there were no *post mortem* findings indicating STZ-toxicity. This strongly indicates that the reduction in blood pressure and reduced tissue perfusion was not due to the STZ-treatment (Oelze *et al.*, 2011).

Still, the reason to the significant difference in perfusion is only speculative and therefore remains unknown. However, in future metabolic studies, it would be wise to use one single anaesthetic protocol in all subjects. Regardless, the PET method to measure perfusion was successful since it was able to detect a significant difference.

PET-CT results

A high uptake of the labelled [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 tracer was seen in the pancreas of the control pigs at low dose, which could be successfully abolished after administration of competitive unlabelled exenatide at high dose, resulting in a blocking image, demonstrating that the uptake in the pancreas was receptor-mediated. This implies that [^{68}Ga]Ga-DO3A-VS-Cys40-exendin-4 may become an important tool for non-invasive evaluation of the GLP-1R system and the pharmacodynamic effects of GLP-1R agonists.

Furthermore, since no uptake of the tracer was seen in the liver, it is plausible that this compound may be useful for *in vivo* imaging of GLP-1R expressed by vivid beta-cells transplanted to the liver.

However, the pancreatic uptake of the tracer at baseline did not differ significantly between the control pigs and the diabetic pigs, suggesting that GLP-1 receptors are expressed in significant amounts in other parts of the pancreas than just beta-cells, which consequently disproves GLP-1R being a suitable target for imaging of native beta-cells and accurate quantification of the BCM.

There are several possible explanations to why a significant difference in tracer uptake is lacking between control and diabetic pigs. It could be due to the fact that beta-cells constitute such a small portion of the total pancreatic tissue, that the PET technology is not sensitive enough to detect a significant difference. Or, theoretically, the GLP-1R could have been up-regulated, i.e. other cells starting to express GLP-1 receptors, in the diabetic pigs. Another explanation may be that the difference in BCM could not be detected due to a more abundant, non-beta-cell-specific uptake in other pancreatic cells, in both controls and diabetic pigs, thereby masking the difference in BCM. The presence of GLP-1R on pancreatic ductal cells has been reported (Tornehave *et al.*, 2008). There are also studies showing that GLP-1R may be present on delta-cells of the pancreas (Fehmann *et al.*, 1995; Fehmann & Habener, 1991) and that GLP-1 can bind to somatostatin subtype 2 receptors on alpha-cells in a perfused rat pancreas (de Heer *et al.*, 2008).

Furthermore, an unspecific uptake (i.e. not receptor-bound) was seen in the lungs of the diabetic pigs. Anaesthetics may affect the uptake and distribution of PET tracers (Hildebrandt *et al.*, 2008; Momosaki *et al.*, 2004), thus the unspecific uptake in the lungs could be due to the different anaesthetic protocols. Alfaxan®, used in the diabetics, is partly metabolised in the lungs (Nicholas *et al.*, 1981), which theoretically implies that a metabolite of Alfaxan® may have bound to the tracer. To be sure of the role of different anaesthetics, healthy pigs should be anaesthetised with alfaxalone and the lungs evaluated by PET after tracer administration. Another theory is that the STZ or the intermittent hyperglycaemia of the diabetic pigs might have damaged the vessels in the lung, leading to leakage and tracer accumulation. However, this is not likely since no signs of vascular injury were found at gross or histopathologic examination *post mortem*.

Towards the end of the PET examination, two pigs were hypoglycaemic. The other five pigs had been normo- or hyperglycaemic during the whole PET examination. The PET of the two hypoglycaemic pigs had been performed before noon, whereas the other pigs after noon. Also, the control pig had not received any breakfast and the diabetic pig had been given insulin just prior to the anaesthesia and following PET. This, together with the known insulinotropic and glucose-lowering effects of exenatide, may have caused the hypoglycaemia. Furthermore, no difference in heart rate or blood pressure was seen during PET between the hypoglycaemic and the other normo-/hyperglycaemic pigs. Thus, the hypoglycaemia observed is considered unlikely to have affected the results in the experiment.

Tachycardia

During PET, all pigs suffered severe tachycardia immediately following high dose i.v. exenatide injection. No detectable receptor binding was seen in the heart. The tachycardia was mediated by the tracer compound, since the HR was affected in all pigs, immediately after tracer administration, regardless of age, STZ-treatment or anaesthetic protocol.

The degree of tachycardia somewhat differed between control pigs, diabetic pigs and the ninth pig on which the brain PET scan was performed, with the highest degree of tachycardia in the youngest pigs and the lowest degree of tachycardia in the oldest, thus it cannot be excluded that the age difference may have affected the result. Still, there was a significant increase in heart rate following i.v. injection of exenatide in all pigs.

Similar effects of exenatide have been seen in other previous studies. In rats, GLP-1 increased arterial blood pressure and heart rate significantly, when administered i.v. as well as i.c.v. The stimulating effect on arterial blood pressure and heart rate could be blocked by exendin-(9-39)-amide, administered through the same route and also i.c.v. if the GLP-1 had been administered i.v. (Barragán *et al.*, 1999). The stimulating effect on cardiovascular parameters seem not to be mediated by catecholamines through the α- or β-adrenergic receptors (Barragán *et al.*, 1994), which is in line with the result in the current experiment, where the tachycardia persisted even though a β-blocker was administered. Similar results were also seen in another study in rats, which were conscious and unrestrained. Both peripheral and central administration of GLP-1 resulted in increased arterial blood pressure and heart rate (Yamamoto *et al.*, 2002). Furthermore, increased heart rate has been observed in calves following i.v. GLP-1 infusion (Edwards *et al.*, 1997). In healthy humans no differences in blood pressure was seen following a s.c. injection of 10 µg exenatide, but a heart rate increase of 8.2 beats per minute was observed (Mendis *et al.*, 2012). A systematic review and meta-analysis published recently concluded there is a connection between GLP-1 analogues and a small increase in heart rate as well as modest lowering of the blood pressure. The authors are welcoming further studies using more accurate means of measuring the heart rate (Robinson *et al.*, 2013).

One study shows how GLP-1 inhibits blood-brain glucose transfer in humans (Lerche *et al.*, 2008), thus a possible theory causing the tachycardia is brain hypoglycaemia in the pigs. Another way to explain the tachycardia could possibly be by the region *area postrema* (AP), involved in nausea sensations. GLP-1 receptors are seen in AP (Orskov *et al.*, 1996a), which much likely explains the nausea seen in subjects exposed to GLP-1 or exenatide (Ritzel *et al.*, 1995). *Area postrema* is in close neuroanatomical connection with vagal nerves and important hypothalamic regions involved in water and food intake as well as the sensation of emesis. Through its anatomical situation, AP is an interface between the brain and the peripheral blood. Furthermore, AP has an insufficient blood-brain barrier to large polar molecules (Miller & Leslie, 1994). In the additional pig no uptake of the tracer could be detected in the brain, indicating that the tachycardia was not mediated by direct effect on receptors located inside the CNS. Still, in mice, i.c.v. administration of exendin-4 depressed heart rate variability. An increase in heart rate was seen following both acute and chronic i.c.v. administration of exenatide. It was also observed that the stimulation of central GLP-1R

reduced the parasympathetic modulation of the HR, which led to an increased HR (Griffioen *et al.*, 2011).

Explaining the reason behind the tachycardia is an intriguing task and additional studies are required to determine the aetiology. The effect on the HR has to be considered if this tracer compound is to be used in a clinical setting.

CONCLUSIONS

The pig is a well functioning animal model for human T1D. In this study we were able to anaesthetise the pigs for long periods of time, with an anaesthetic depth, which was demanded for PET examination. The pigs rapidly recovered from anaesthesia and quickly showed normal behaviour.

The [^{68}Ga]Ga-DO3A-VS-Cys40-conjugated synthetic exendin-4 was not a selective tracer for native beta-cells. Thus, the GLP-1R is not a suitable target for BCM imaging. Furthermore, the pigs showed severe tachycardia as a side-effect of the tracer and exenatide injection, thus this compound should be used with great caution in pigs.

Moreover, a significantly reduced perfusion was seen in the pancreas and kidneys of the STZ-treated pigs. The reason for this remains unknown but it is possible it was a result of the two different anaesthetic protocols, thus only one anaesthetic protocol should be used in future PET-CT studies, to eliminate this factor.

Concluding, the pig is a promising animal model for further diabetic research in the endeavour to quantify the BCM. It would be of high interest to know if the actual insulin production of a patient relies on a few beta-cells with high insulin-productivity or a larger amount of beta-cells with attenuated efficacy, thus research towards the estimation of BCM should strive towards a methodology where both mass and function can be observed.

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REFERENCES

Aaen, K., Rygaard, J., Josefsen, K., Petersen, H., Brogren, C.H., Horn, T. & Buschard, K. (1990). Dependence of antigen expression on functional state of beta-cells. *Diabetes* 39(6), 697-701.

Abbott, C.R., Monteiro, M., Small, C.J., Sajedi, A., Smith, K.L., Parkinson, J.R., Ghatei, M.A. & Bloom, S.R. (2005). The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain Res* 1044(1), 127-31.

Abraham, E.J., Leech, C.A., Lin, J.C., Zulewski, H. & Habener, J.F. (2002). Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. *Endocrinology* 143(8), 3152-61.

Ahrén, B. (2000). Autonomic regulation of islet hormone secretion--implications for health and disease. *Diabetologia* 43(4), 393-410.

Ahrén, B. (2004). Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. *Am J Physiol Regul Integr Comp Physiol* 286(2), R269-72.

Akpinar, P., Kuwajima, S., Krützfeldt, J. & Stoffel, M. (2005). Tmem27: a cleaved and shed plasma membrane protein that stimulates pancreatic beta cell proliferation. *Cell Metab* 2(6), 385-97.

Alarcon, C., Wicksteed, B. & Rhodes, C.J. (2006). Exendin 4 controls insulin production in rat islet beta cells predominantly by potentiation of glucose-stimulated proinsulin biosynthesis at the translational level. *Diabetologia* 49(12), 2920-9.

Alessio, A.M. & Kinahan, P.E. (2006). Improved quantitation for PET/CT image reconstruction with system modeling and anatomical priors. *Med Phys* 33(11), 4095-103.

Alvarez, E., Martínez, M.D., Roncero, I., Chowen, J.A., García-Cuartero, B., Gispert, J.D., Sanz, C., Vázquez, P., Maldonado, A., de Cáceres, J., Desco, M., Pozo, M.A. & Blázquez, E. (2005). The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. *J Neurochem* 92(4), 798-806.

Ambrisko, T.D., Hikasa, Y. & Sato, K. (2005). Influence of medetomidine on stress-related neurohormonal and metabolic effects caused by butorphanol, fentanyl, and ketamine administration in dogs. *Am J Vet Res* 66(3), 406-12.

Amori, R.E., Lau, J. & Pittas, A.G. (2007). Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. *JAMA* 298(2), 194-206.

Amos, A.F., McCarty, D.J. & Zimmet, P. (1997). The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med* 14 Suppl 5, S1-85.

Andralojc, K., Srinivas, M., Brom, M., Joosten, L., de Vries, I.J., Eizirik, D.L., Boerman, O.C., Meda, P. & Gotthardt, M. (2012). Obstacles on the way to the clinical visualisation of beta cells: looking for the Aeneas of molecular imaging to navigate between Scylla and Charybdis. *Diabetologia* 55(5), 1247-57.

Anini, Y. & Brubaker, P.L. (2003). Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes* 52(2), 252-9.

Anlauf, M., Eissele, R., Schäfer, M.K., Eiden, L.E., Arnold, R., Pauser, U., Klöppel, G. & Weihe, E. (2003). Expression of the two isoforms of the vesicular monoamine transporter (VMAT1 and VMAT2) in the endocrine pancreas and pancreatic endocrine tumors. *J Histochem Cytochem* 51(8), 1027-40.

Antkowiak, P.F., Tersey, S.A., Carter, J.D., Vandsburger, M.H., Nadler, J.L., Epstein, F.H. & Mirmira, R.G. (2009). Noninvasive assessment of pancreatic beta-cell function in vivo with manganese-enhanced magnetic resonance imaging. *Am J Physiol Endocrinol Metab* 296(3), E573-8.

Antkowiak, P.F., Vandsburger, M.H. & Epstein, F.H. (2012). Quantitative pancreatic β cell MRI using manganese-enhanced Look-Locker imaging and two-site water exchange analysis. *Magn Reson Med* 67(6), 1730-9.

Arakawa, M., Mita, T., Azuma, K., Ebato, C., Goto, H., Nomiyama, T., Fujitani, Y., Hirose, T., Kawamori, R. & Watada, H. (2010). Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. *Diabetes* 59(4), 1030-7.

Arifin, D.R. & Bulte, J.W. (2011). Imaging of pancreatic islet cells. *Diabetes Metab Res Rev* 27(8), 761-6.

Arnolds, S., Dellweg, S., Clair, J., Dain, M.P., Nauck, M.A., Rave, K. & Kapitza, C. (2010). Further improvement in postprandial glucose control with addition of exenatide or sitagliptin to combination therapy with insulin glargine and metformin: a proof-of-concept study. *Diabetes Care* 33(7), 1509-15.

Ayoub, W.A., Kumar, A.A., Naguib, H.S. & Taylor, H.C. (2010). Exenatide-induced acute pancreatitis. *Endocr Pract* 16(1), 80-3.

Baggio, L.L. & Drucker, D.J. (2007). Biology of incretins: GLP-1 and GIP. *Gastroenterology* 132(6), 2131-57.

Bailey, D.L., Townsend, D.W., Valk, P.E. & Maisey, M.N. (2005). *Positron Emission Tomography: Basic Sciences.* London: Springer. Available from: www.google.se/books. [2013-11-18].

Balkan, B. & Li, X. (2000). Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. *Am J Physiol Regul Integr Comp Physiol* 279(4), R1449-54.

Balks, H.J., Holst, J.J., von zur Mühlen, A. & Brabant, G. (1997). Rapid oscillations in plasma glucagon-like peptide-1 (GLP-1) in humans: cholinergic control of GLP-1 secretion via muscarinic receptors. *J Clin Endocrinol Metab* 82(3), 786-90.

Ban, K., Noyan-Ashraf, M.H., Hoefer, J., Bolz, S.S., Drucker, D.J. & Husain, M. (2008). Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation* 117(18), 2340-50.

Barragán, J.M., Eng, J., Rodríguez, R. & Blázquez, E. (1999). Neural contribution to the effect of glucagon-like peptide-1-(7-36) amide on arterial blood pressure in rats. *Am J Physiol* 277(5 Pt 1), E784-91.

Barragán, J.M., Rodríguez, R.E. & Blázquez, E. (1994). Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7-36) amide in rats. *Am J Physiol* 266(3 Pt 1), E459-66.

Basu, A., Charkoudian, N., Schrage, W., Rizza, R.A., Basu, R. & Joyner, M.J. (2007). Beneficial effects of GLP-1 on endothelial function in humans: dampening by glyburide but not by glimepiride. *Am J Physiol Endocrinol Metab* 293(5), E1289-95.

Bernardi, H., Fosset, M. & Lazdunski, M. (1988). Characterization, purification, and affinity labeling of the brain [3H]glibenclamide-binding protein, a putative neuronal ATP-regulated K+ channel. *Proc Natl Acad Sci U S A* 85(24), 9816-20.

Bertilsson, G., Patrone, C., Zachrisson, O., Andersson, A., Dannaeus, K., Heidrich, J., Kortesmaa, J., Mercer, A., Nielsen, E., Rönnholm, H. & Wikström, L. (2008). Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. *J Neurosci Res* 86(2), 326-38.

Best, J.H., Hoogwerf, B.J., Herman, W.H., Pelletier, E.M., Smith, D.B., Wenten, M. & Hussein, M.A. (2011). Risk of cardiovascular disease events in patients with type 2 diabetes prescribed the glucagon-like peptide 1 (GLP-1) receptor agonist exenatide twice daily or other glucose-

lowering therapies: a retrospective analysis of the LifeLink database. *Diabetes Care* 34(1), 90-5.

Beyer, T., Townsend, D.W., Brun, T., Kinahan, P.E., Charron, M., Roddy, R., Jerin, J., Young, J., Byars, L. & Nutt, R. (2000). A combined PET/CT scanner for clinical oncology. *J Nucl Med* 41(8), 1369-79.

Bjerre Knudsen, L., Madsen, L.W., Andersen, S., Almholt, K., de Boer, A.S., Drucker, D.J., Gotfredsen, C., Egerod, F.L., Hegelund, A.C., Jacobsen, H., Jacobsen, S.D., Moses, A.C., Mølck, A.M., Nielsen, H.S., Nowak, J., Solberg, H., Thi, T.D., Zdravkovic, M. & Moerch, U. (2010). Glucagon-like Peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. *Endocrinology* 151(4), 1473-86.

Blevins, T., Pullman, J., Malloy, J., Yan, P., Taylor, K., Schulteis, C., Trautmann, M. & Porter, L. (2011). DURATION-5: exenatide once weekly resulted in greater improvements in glycemic control compared with exenatide twice daily in patients with type 2 diabetes. *J Clin Endocrinol Metab* 96(5), 1301-10.

Blomberg, B.A., Codreanu, I., Cheng, G., Werner, T.J. & Alavi, A. (2013). Beta-cell imaging: call for evidence-based and scientific approach. *Mol Imaging Biol* 15(2), 123-30.

Blonde, L., Klein, E.J., Han, J., Zhang, B., Mac, S.M., Poon, T.H., Taylor, K.L., Trautmann, M.E., Kim, D.D. & Kendall, D.M. (2006). Interim analysis of the effects of exenatide treatment on A1C, weight and cardiovascular risk factors over 82 weeks in 314 overweight patients with type 2 diabetes. *Diabetes Obes Metab* 8(4), 436-47.

Bollag, R.J., Zhong, Q., Phillips, P., Min, L., Zhong, L., Cameron, R., Mulloy, A.L., Rasmussen, H., Qin, F., Ding, K.H. & Isales, C.M. (2000). Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors. *Endocrinology* 141(3), 1228-35.

Bose, A.K., Mocanu, M.M., Carr, R.D., Brand, C.L. & Yellon, D.M. (2005). Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes* 54(1), 146-51.

Brom, M., Andrałojć, K., Oyen, W.J., Boerman, O.C. & Gotthardt, M. (2010). Development of radiotracers for the determination of the beta-cell mass in vivo. *Curr Pharm Des* 16(14), 1561-7.

Bullock, B.P., Heller, R.S. & Habener, J.F. (1996). Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology* 137(7), 2968-78.

Bulotta, A., Hui, H., Anastasi, E., Bertolotto, C., Boros, L.G., Di Mario, U. & Perfetti, R. (2002). Cultured pancreatic ductal cells undergo cell cycle re-distribution and beta-cell-like differentiation in response to glucagon-like peptide-1. *J Mol Endocrinol* 29(3), 347-60.

Buschard, K., Brogren, C.H., Röpke, C. & Rygaard, J. (1988). Antigen expression of the pancreatic beta-cells is dependent on their functional state, as shown by a specific, BB rat monoclonal autoantibody IC2. *APMIS* 96(4), 342-6.

Buse, J.B., Henry, R.R., Han, J., Kim, D.D., Fineman, M.S., Baron, A.D. & Group, E.-C.S. (2004). Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care* 27(11), 2628-35.

Buse, J.B., Klonoff, D.C., Nielsen, L.L., Guan, X., Bowlus, C.L., Holcombe, J.H., Maggs, D.G. & Wintle, M.E. (2007). Metabolic effects of two years of exenatide treatment on diabetes, obesity, and hepatic biomarkers in patients with type 2 diabetes: an interim analysis of data from the open-label, uncontrolled extension of three double-blind, placebo-controlled trials. *Clin Ther* 29(1), 139-53.

Buteau, J., El-Assaad, W., Rhodes, C.J., Rosenberg, L., Joly, E. & Prentki, M. (2004). Glucagon-like peptide-1 prevents beta cell glucolipotoxicity. *Diabetologia* 47(5), 806-15.

Butler, P.C., Elashoff, M., Elashoff, R. & Gale, E.A. (2013). A critical analysis of the clinical use of incretin-based therapies: Are the GLP-1 therapies safe? *Diabetes Care* 36(7), 2118-25.

Campos, R.V., Lee, Y.C. & Drucker, D.J. (1994). Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology* 134(5), 2156-64.

Chatziioannou, A.F. (2005). Instrumentation for molecular imaging in preclinical research: Micro-PET and Micro-SPECT. *Proc Am Thorac Soc* 2(6), 533-6, 510-11.

Chisholm, C. & Greenberg, G.R. (2002). Somatostatin-28 regulates GLP-1 secretion via somatostatin receptor subtype 5 in rat intestinal cultures. *Am J Physiol Endocrinol Metab* 283(2), E311-7.

Christ, E., Wild, D., Forrer, F., Brändle, M., Sahli, R., Clerici, T., Gloor, B., Martius, F., Maecke, H. & Reubi, J.C. (2009). Glucagon-like peptide-1 receptor imaging for localization of insulinomas. *J Clin Endocrinol Metab* 94(11), 4398-405.

Clark, P.B., Gage, H.D., Brown-Proctor, C., Buchheimer, N., Calles-Escandon, J., Mach, R.H. & Morton, K.A. (2004). Neurofunctional imaging of the pancreas utilizing the cholinergic PET radioligand [18F]4-fluorobenzyltrozamicol. *Eur J Nucl Med Mol Imaging* 31(2), 258-60.

Clark, S.A., Borland, K.M., Sherman, S.D., Rusack, T.C. & Chick, W.L. (1994). Staining and in vitro toxicity of dithizone with canine, porcine, and bovine islets. *Cell Transplant* 3(4), 299-306.

Connolly, B.M., Vanko, A., McQuade, P., Guenther, I., Meng, X., Rubins, D., Waterhouse, R., Hargreaves, R., Sur, C. & Hostetler, E. (2012). Ex vivo imaging of pancreatic beta cells using a radiolabeled GLP-1 receptor agonist. *Mol Imaging Biol* 14(1), 79-87.

Considine, R.V., Sinha, M.K., Heiman, M.L., Kriauciunas, A., Stephens, T.W., Nyce, M.R., Ohannesian, J.P., Marco, C.C., McKee, L.J. & Bauer, T.L. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334(5), 292-5.

Crajoinas, R.O., Oricchio, F.T., Pessoa, T.D., Pacheco, B.P., Lessa, L.M., Malnic, G. & Girardi, A.C. (2011). Mechanisms mediating the diuretic and natriuretic actions of the incretin hormone glucagon-like peptide-1. *Am J Physiol Renal Physiol* 301(2), F355-63.

Creutzfeldt, W.O., Kleine, N., Willms, B., Orskov, C., Holst, J.J. & Nauck, M.A. (1996). Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7-36) amide in type I diabetic patients. *Diabetes Care* 19(6), 580-6.

D'Alessio, D.A., Prigeon, R.L. & Ensinck, J.W. (1995). Enteral enhancement of glucose disposition by both insulin-dependent and insulin-independent processes. A physiological role of glucagon-like peptide I. *Diabetes* 44(12), 1433-7.

de Heer, J., Rasmussen, C., Coy, D.H. & Holst, J.J. (2008). Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. *Diabetologia* 51(12), 2263-70.

de Lonlay, P., Simon-Carre, A., Ribeiro, M.J., Boddaert, N., Giurgea, I., Laborde, K., Bellanné-Chantelot, C., Verkarre, V., Polak, M., Rahier, J., Syrota, A., Seidenwurm, D., Nihoul-Fékété, C., Robert, J.J., Brunelle, F. & Jaubert, F. (2006). Congenital hyperinsulinism: pancreatic [18F]fluoro-L-dihydroxyphenylalanine (DOPA) positron emission tomography and immunohistochemistry study of DOPA decarboxylase and insulin secretion. *J Clin Endocrinol Metab* 91(3), 933-40.

Deacon, C.F., Nauck, M.A., Toft-Nielsen, M., Pridal, L., Willms, B. & Holst, J.J. (1995). Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 44(9), 1126-31.

Deacon, C.F., Pridal, L., Klarskov, L., Olesen, M. & Holst, J.J. (1996). Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am J Physiol* 271(3 Pt 1), E458-64.

DeFronzo, R.A., Ratner, R.E., Han, J., Kim, D.D., Fineman, M.S. & Baron, A.D. (2005). Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 28(5), 1092-100.

Denker, P.S. & Dimarco, P.E. (2006). Exenatide (exendin-4)-induced pancreatitis: a case report. *Diabetes Care* 29(2), 471.

Di Gialleonardo, V., de Vries, E.F., Di Girolamo, M., Quintero, A.M., Dierckx, R.A. & Signore, A. (2012). Imaging of β-cell mass and insulitis in insulin-dependent (Type 1) diabetes mellitus. *Endocr Rev* 33(6), 892-919.

Dobrucki, L.W. & Sinusas, A.J. (2005). Molecular imaging. A new approach to nuclear cardiology. *Q J Nucl Med Mol Imaging* 49(1), 106-15.

Dobson, A.W., Erikson, K.M. & Aschner, M. (2004). Manganese neurotoxicity. *Ann N Y Acad Sci* 1012, 115-28.

Dodson, G. & Steiner, D. (1998). The role of assembly in insulin's biosynthesis. *Curr Opin Struct Biol* 8(2), 189-94.

Dore, D.D., Bloomgren, G.L., Wenten, M., Hoffman, C., Clifford, C.R., Quinn, S.G., Braun, D.K., Noel, R.A. & Seeger, J.D. (2011). A cohort study of acute pancreatitis in relation to exenatide use. *Diabetes Obes Metab* 13(6), 559-66.

Dore, D.D., Seeger, J.D. & Arnold Chan, K. (2009). Use of a claims-based active drug safety surveillance system to assess the risk of acute pancreatitis with exenatide or sitagliptin compared to metformin or glyburide. *Curr Med Res Opin* 25(4), 1019-27.

Doyle, M.E. & Egan, J.M. (2007). Mechanisms of action of glucagon-like peptide 1 in the pancreas. *Pharmacol Ther* 113(3), 546-93.

Drucker, D.J., Buse, J.B., Taylor, K., Kendall, D.M., Trautmann, M., Zhuang, D., Porter, L. & Group, D.-S. (2008). Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. *Lancet* 372(9645), 1240-50.

Drucker, D.J., Philippe, J., Mojsov, S., Chick, W.L. & Habener, J.F. (1987). Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci U S A* 84(10), 3434-8.

Dufrane, D., van Steenberghe, M., Guiot, Y., Goebbels, R.M., Saliez, A. & Gianello, P. (2006). Streptozotocin-induced diabetes in large animals (pigs/primates): role of GLUT2 transporter and beta-cell plasticity. *Transplantation* 81(1), 36-45.

Dupre, J., Behme, M.T., Hramiak, I.M., McFarlane, P., Williamson, M.P., Zabel, P. & McDonald, T.J. (1995). Glucagon-like peptide I reduces postprandial glycemic excursions in IDDM. *Diabetes* 44(6), 626-30.

Dupré, J., Behme, M.T. & McDonald, T.J. (2004). Exendin-4 normalized postcibal glycemic excursions in type 1 diabetes. *J Clin Endocrinol Metab* 89(7), 3469-73.

During, M.J., Cao, L., Zuzga, D.S., Francis, J.S., Fitzsimons, H.L., Jiao, X., Bland, R.J., Klugmann, M., Banks, W.A., Drucker, D.J. & Haile, C.N. (2003). Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat Med* 9(9), 1173-9.

Dushay, J., Gao, C., Gopalakrishnan, G.S., Crawley, M., Mitten, E.K., Wilker, E., Mullington, J. & Maratos-Flier, E. (2012). Short-term exenatide treatment leads to significant weight loss in a subset of obese women without diabetes. *Diabetes Care* 35(1), 4-11.

Edwards, C.M., Edwards, A.V. & Bloom, S.R. (1997). Cardiovascular and pancreatic endocrine responses to glucagon-like peptide-1(7-36) amide in the conscious calf. *Exp Physiol* 82(4), 709-16.

Edwards, C.M., Stanley, S.A., Davis, R., Brynes, A.E., Frost, G.S., Seal, L.J., Ghatei, M.A. & Bloom, S.R. (2001). Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. *Am J Physiol Endocrinol Metab* 281(1), E155-61.

Elahi, D., McAloon-Dyke, M., Fukagawa, N.K., Meneilly, G.S., Sclater, A.L., Minaker, K.L., Habener, J.F. & Andersen, D.K. (1994). The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects. *Regul Pept* 51(1), 63-74.

Elashoff, M., Matveyenko, A.V., Gier, B., Elashoff, R. & Butler, P.C. (2011). Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. *Gastroenterology* 141(1), 150-6.

Elovson, J. (1980). Biogenesis of plasma membrane glycoproteins. Purification and properties of two rat liver plasma membrane glycoproteins. *J Biol Chem* 255(12), 5807-15.

Elrick, H., Stimmler, L., Hlad, C.J. & Arai, Y. (1964). Plasma insulin response to oral and intravenous glucose administration. *J Clin Endocrinol Metab* 24, 1076-82.

EMA (European Medicines Agency) (2013-11-25). Byetta, exenatide. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000698/human_med_000682.jsp&mid=WC0b01ac058001d124 [2013-12-06].

Eng, J., Kleinman, W.A., Singh, L., Singh, G. & Raufman, J.P. (1992). Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem* 267(11), 7402-5.

Eriksson, O. & Alavi, A. (2012). Imaging the islet graft by positron emission tomography. *Eur J Nucl Med Mol Imaging* 39(3), 533-42.

Eriksson, O., Jahan, M., Johnström, P., Korsgren, O., Sundin, A., Halldin, C. & Johansson, L. (2010). In vivo and in vitro characterization of [18F]-FE-(+)-DTBZ as a tracer for beta-cell mass. *Nucl Med Biol* 37(3), 357-63.

Esqueda, A.C., López, J.A., Andreu-de-Riquer, G., Alvarado-Monzón, J.C., Ratnakar, J., Lubag, A.J., Sherry, A.D. & De León-Rodríguez, L.M. (2009). A new gadolinium-based MRI zinc sensor. *J Am Chem Soc* 131(32), 11387-91.

Fagerholm, V., Mikkola, K.K., Ishizu, T., Arponen, E., Kauhanen, S., Någren, K., Solin, O., Nuutila, P. & Haaparanta, M. (2010). Assessment of islet specificity of dihydrotetrabenazine radiotracer binding in rat pancreas and human pancreas. *J Nucl Med* 51(9), 1439-46.

Fall, T., Hamlin, H.H., Hedhammar, A., Kämpe, O. & Egenvall, A. (2007). Diabetes mellitus in a population of 180,000 insured dogs: incidence, survival, and breed distribution. *J Vet Intern Med* 21(6), 1209-16.

Farilla, L., Bulotta, A., Hirshberg, B., Li Calzi, S., Khoury, N., Noushmehr, H., Bertolotto, C., Di Mario, U., Harlan, D.M. & Perfetti, R. (2003). Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 144(12), 5149-58.

Farilla, L., Hui, H., Bertolotto, C., Kang, E., Bulotta, A., Di Mario, U. & Perfetti, R. (2002). Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. *Endocrinology* 143(11), 4397-408.

Fehmann, H.C. & Habener, J.F. (1991). Functional receptors for the insulinotropic hormone glucagon-like peptide-I(7-37) on a somatostatin secreting cell line. *FEBS Lett* 279(2), 335-40.

Fehmann, H.C., Janssen, M. & Göke, B. (1995). Interaction of glucagon-like peptide-I (GLP-I) and galanin in insulin (beta TC-1)- and somatostatin (RIN T3)-secreting cells and evidence that both peptides have no receptors on glucagon (INR1G9)-secreting cells. *Acta Diabetol* 32(3), 176-81.

Fineman, M.S., Bicsak, T.A., Shen, L.Z., Taylor, K., Gaines, E., Varns, A., Kim, D. & Baron, A.D. (2003). Effect on glycemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonylurea treatment in patients with type 2 diabetes. *Diabetes Care* 26(8), 2370-7.

Flint, A., Raben, A., Astrup, A. & Holst, J.J. (1998). Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 101(3), 515-20.

Flint, A., Raben, A., Ersbøll, A.K., Holst, J.J. & Astrup, A. (2001). The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. *Int J Obes Relat Metab Disord* 25(6), 781-92.

Friedman, J.M. & Halaas, J.L. (1998). Leptin and the regulation of body weight in mammals. *Nature* 395(6704), 763-70.

Gao, H., Niu, G., Yang, M., Quan, Q., Ma, Y., Murage, E.N., Ahn, J.M., Kiesewetter, D.O. & Chen, X. (2011). PET of insulinoma using ^{18}F-FBEM-EM3106B, a new GLP-1 analogue. *Mol Pharm* 8(5), 1775-82.

Garnuszek, P., Liciñska, I., Fiedor, P. & Mazurek, A.P. (1998). The synthesis, radioiodination and preliminary biological study of the new carboxylic derivatives of dithizone. *Appl Radiat Isot* 49(12), 1563-71.

Garnuszek, P., Licińska, I., Mrozek, A., Wardawa, A., Fiedor, P.S. & Mazurek, A.P. (2000). Identification of transplanted pancreatic islet cells by radioactive dithizone-[131I]-histamine conjugate. Preliminary report. *Nucl Med Rev Cent East Eur* 3(1), 61-3.

Gil-Lozano, M., Pérez-Tilve, D., Alvarez-Crespo, M., Martís, A., Fernandez, A.M., Catalina, P.A., Gonzalez-Matias, L.C. & Mallo, F. (2010). GLP-1(7-36)-amide and Exendin-4 stimulate the HPA axis in rodents and humans. *Endocrinology* 151(6), 2629-40.

Gill, A., Hoogwerf, B.J., Burger, J., Bruce, S., Macconell, L., Yan, P., Braun, D., Giaconia, J. & Malone, J. (2010). Effect of exenatide on heart rate and blood pressure in subjects with type 2 diabetes mellitus: a double-blind, placebo-controlled, randomized pilot study. *Cardiovasc Diabetol* 9, 6.

Gilon, P. & Henquin, J.C. (2001). Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. *Endocr Rev* 22(5), 565-604.

Gilor, C., Graves, T.K., Gilor, S., Ridge, T.K. & Rick, M. (2011). The GLP-1 mimetic exenatide potentiates insulin secretion in healthy cats. *Domest Anim Endocrinol* 41(1), 42-9.

Gimi, B., Leoni, L., Oberholzer, J., Braun, M., Avila, J., Wang, Y., Desai, T., Philipson, L.H., Magin, R.L. & Roman, B.B. (2006). Functional MR microimaging of pancreatic beta-cell activation. *Cell Transplant* 15(2), 195-203.

Girman, C.J., Kou, T.D., Cai, B., Alexander, C.M., O'Neill, E.A., Williams-Herman, D.E. & Katz, L. (2010). Patients with type 2 diabetes mellitus have higher risk for acute pancreatitis compared with those without diabetes. *Diabetes Obes Metab* 12(9), 766-71.

Gjesing, A.P., Ekstrøm, C.T., Eiberg, H., Urhammer, S.A., Holst, J.J., Pedersen, O. & Hansen, T. (2012). Fasting and oral glucose-stimulated levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are highly familial traits. *Diabetologia* 55(5), 1338-45.

Goland, R., Freeby, M., Parsey, R., Saisho, Y., Kumar, D., Simpson, N., Hirsch, J., Prince, M., Maffei, A., Mann, J.J., Butler, P.C., Van Heertum, R., Leibel, R.L., Ichise, M. & Harris, P.E. (2009). 11C-dihydrotetrabenazine PET of the pancreas in subjects with long-standing type 1 diabetes and in healthy controls. *J Nucl Med* 50(3), 382-9.

Gotthardt, M., Bleeker-Rovers, C.P., Boerman, O.C. & Oyen, W.J. (2013). Imaging of inflammation by PET, conventional scintigraphy, and other imaging techniques. *J Nucl Med Technol* 41(3), 157-69.

Gotthardt, M., Lalyko, G., van Eerd-Vismale, J., Keil, B., Schurrat, T., Hower, M., Laverman, P., Behr, T.M., Boerman, O.C., Göke, B. & Béhé, M. (2006). A new technique for in vivo imaging of specific GLP-1 binding sites: first results in small rodents. *Regul Pept* 137(3), 162-7.

Graziano, M.P., Hey, P.J., Borkowski, D., Chicchi, G.G. & Strader, C.D. (1993). Cloning and functional expression of a human glucagon-like peptide-1 receptor. *Biochem Biophys Res Commun* 196(1), 141-6.

Griffioen, K.J., Wan, R., Okun, E., Wang, X., Lovett-Barr, M.R., Li, Y., Mughal, M.R., Mendelowitz, D. & Mattson, M.P. (2011). GLP-1 receptor stimulation depresses heart rate variability and inhibits neurotransmission to cardiac vagal neurons. *Cardiovasc Res* 89(1), 72-8.

Gutzwiller, J.P., Drewe, J., Göke, B., Schmidt, H., Rohrer, B., Lareida, J. & Beglinger, C. (1999a). Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol* 276(5 Pt 2), R1541-4.

Gutzwiller, J.P., Göke, B., Drewe, J., Hildebrand, P., Ketterer, S., Handschin, D., Winterhalder, R., Conen, D. & Beglinger, C. (1999b). Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut* 44(1), 81-6.

Gutzwiller, J.P., Tschopp, S., Bock, A., Zehnder, C.E., Huber, A.R., Kreyenbuehl, M., Gutmann, H., Drewe, J., Henzen, C., Goeke, B. & Beglinger, C. (2004). Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. *J Clin Endocrinol Metab* 89(6), 3055-61.

Gäbel, H., Bitter-Suermann, H., Henriksson, C., Säve-Söderbergh, J., Lundholm, K. & Brynger, H. (1985). Streptozotocin diabetes in juvenile pigs. Evaluation of an experimental model. *Horm Metab Res* 17(6), 275-80.

Göke, R., Fehmann, H.C., Linn, T., Schmidt, H., Krause, M., Eng, J. & Göke, B. (1993). Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. *J Biol Chem* 268(26), 19650-5.

Halaas, J.L., Boozer, C., Blair-West, J., Fidahusein, N., Denton, D.A. & Friedman, J.M. (1997). Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proc Natl Acad Sci U S A* 94(16), 8878-83.

Halbirk, M., Nørrelund, H., Møller, N., Holst, J.J., Schmitz, O., Nielsen, R., Nielsen-Kudsk, J.E., Nielsen, S.S., Nielsen, T.T., Eiskjaer, H., Bøtker, H.E. & Wiggers, H. (2010). Cardiovascular and metabolic effects of 48-h glucagon-like peptide-1 infusion in compensated chronic patients with heart failure. *Am J Physiol Heart Circ Physiol* 298(3), H1096-102.

Hansen, A.M., Hansen, J.B., Carr, R.D., Ashcroft, F.M. & Wahl, P. (2005). Kir6.2-dependent high-affinity repaglinide binding to beta-cell K(ATP) channels. *Br J Pharmacol* 144(4), 551-7.

Hansen, L., Deacon, C.F., Orskov, C. & Holst, J.J. (1999). Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* 140(11), 5356-63.

Hansen, L., Hartmann, B., Bisgaard, T., Mineo, H., Jørgensen, P.N. & Holst, J.J. (2000). Somatostatin restrains the secretion of glucagon-like peptide-1 and -2 from isolated perfused porcine ileum. *Am J Physiol Endocrinol Metab* 278(6), E1010-8.

Henquin, J.C. (1992). The fiftieth anniversary of hypoglycaemic sulphonamides. How did the mother compound work? *Diabetologia* 35(10), 907-12.

Herrmann, C., Göke, R., Richter, G., Fehmann, H.C., Arnold, R. & Göke, B. (1995). Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 56(2), 117-26.

Herzberg-Schäfer, S., Heni, M., Stefan, N., Häring, H.U. & Fritsche, A. (2012). Impairment of GLP1-induced insulin secretion: role of genetic background, insulin resistance and hyperglycaemia. *Diabetes Obes Metab* 14 Suppl 3, 85-90.

Higgins, T. (2012). HbA(1c)--an analyte of increasing importance. *Clin Biochem* 45(13-14), 1038-45.

Hildebrandt, I.J., Su, H. & Weber, W.A. (2008). Anesthesia and other considerations for in vivo imaging of small animals. *ILAR J* 49(1), 17-26.

Hirata, K., Kume, S., Araki, S., Sakaguchi, M., Chin-Kanasaki, M., Isshiki, K., Sugimoto, T., Nishiyama, A., Koya, D., Haneda, M., Kashiwagi, A. & Uzu, T. (2009). Exendin-4 has an anti-hypertensive effect in salt-sensitive mice model. *Biochem Biophys Res Commun* 380(1), 44-9.

Holst, J.J. (2007). The physiology of glucagon-like peptide 1. *Physiol Rev* 87(4), 1409-39.

Holst, J.J., Burcelin, R. & Nathanson, E. (2011). Neuroprotective properties of GLP-1: theoretical and practical applications. *Curr Med Res Opin* 27(3), 547-58.

Holst, J.J. & Gromada, J. (2004). Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol Endocrinol Metab* 287(2), E199-206.

Holz, G.G., Kühtreiber, W.M. & Habener, J.F. (1993). Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature* 361(6410), 362-5.

Hui, H., Nourparvar, A., Zhao, X. & Perfetti, R. (2003). Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5'-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. *Endocrinology* 144(4), 1444-55.

Højberg, P.V., Vilsbøll, T., Rabøl, R., Knop, F.K., Bache, M., Krarup, T., Holst, J.J. & Madsbad, S. (2009). Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. *Diabetologia* 52(2), 199-207.

Iyer, S.N., Drake, A.J., West, R.L., Mendez, C.E. & Tanenberg, R.J. (2012). Case report of acute necrotizing pancreatitis associated with combination treatment of sitagliptin and exenatide. *Endocr Pract* 18(1), e10-3.

Jensen-Waern, M., Andersson, M., Kruse, R., Nilsson, B., Larsson, R., Korsgren, O. & Essén-Gustavsson, B. (2009). Effects of streptozotocin-induced diabetes in domestic pigs with focus on the amino acid metabolism. *Lab Anim* 43(3), 249-54.

Josefsson, L.G. (1999). Evidence for kinship between diverse G-protein coupled receptors. *Gene* 239(2), 333-40.

Jura, N., Archer, H. & Bar-Sagi, D. (2005). Chronic pancreatitis, pancreatic adenocarcinoma and the black box in-between. *Cell Res* 15(1), 72-7.

Kanse, S.M., Kreymann, B., Ghatei, M.A. & Bloom, S.R. (1988). Identification and characterization of glucagon-like peptide-1 7-36 amide-binding sites in the rat brain and lung. *FEBS Lett* 241(1-2), 209-12.

Kashiwagi, Y., Nodaira, M., Amitani, M., Murase, K. & Abe, K. (2012). Assessment of peripheral tissue perfusion disorder in streptozotocin-induced diabetic rats using dynamic contrast-enhanced MRI. *Magn Reson Imaging* 30(2), 254-60.

Kastin, A.J., Akerstrom, V. & Pan, W. (2002). Interactions of glucagon-like peptide-1 (GLP-1) with the blood-brain barrier. *J Mol Neurosci* 18(1-2), 7-14.

Katsumichi, I. & Pour, P.M. (2007). Diabetes mellitus in pancreatic cancer: is it a causal relationship? *Am J Surg* 194(4 Suppl), S71-5.

Kazafeos, K. (2011). Incretin effect: GLP-1, GIP, DPP4. *Diabetes Res Clin Pract* 93 Suppl 1, S32-6.

Kendall, D.M., Riddle, M.C., Rosenstock, J., Zhuang, D., Kim, D.D., Fineman, M.S. & Baron, A.D. (2005). Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. *Diabetes Care* 28(5), 1083-91.

Kiesewetter, D.O., Gao, H., Ma, Y., Niu, G., Quan, Q., Guo, N. & Chen, X. (2012). 18F-radiolabeled analogs of exendin-4 for PET imaging of GLP-1 in insulinoma. *Eur J Nucl Med Mol Imaging* 39(3), 463-73.

Kim, D., MacConell, L., Zhuang, D., Kothare, P.A., Trautmann, M., Fineman, M. & Taylor, K. (2007). Effects of once-weekly dosing of a long-acting release formulation of exenatide on glucose control and body weight in subjects with type 2 diabetes. *Diabetes Care* 30(6), 1487-93.

Kim, J.G., Baggio, L.L., Bridon, D.P., Castaigne, J.P., Robitaille, M.F., Jetté, L., Benquet, C. & Drucker, D.J. (2003). Development and characterization of a glucagon-like peptide 1-albumin conjugate: the ability to activate the glucagon-like peptide 1 receptor in vivo. *Diabetes* 52(3), 751-9.

King, H., Aubert, R.E. & Herman, W.H. (1998). Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 21(9), 1414-31.

King, H. & Rewers, M. (1993). Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Ad Hoc Diabetes Reporting Group. *Diabetes Care* 16(1), 157-77.

Kjems, L.L., Holst, J.J., Vølund, A. & Madsbad, S. (2003). The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects. *Diabetes* 52(2), 380-6.

Knauf, C., Cani, P.D., Perrin, C., Iglesias, M.A., Maury, J.F., Bernard, E., Benhamed, F., Grémeaux, T., Drucker, D.J., Kahn, C.R., Girard, J., Tanti, J.F., Delzenne, N.M., Postic, C. & Burcelin, R. (2005). Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. *J Clin Invest* 115(12), 3554-63.

Knop, F.K., Vilsbøll, T., Højberg, P.V., Larsen, S., Madsbad, S., Vølund, A., Holst, J.J. & Krarup, T. (2007). Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? *Diabetes* 56(8), 1951-9.

Knudsen, L.B. & Pridal, L. (1996). Glucagon-like peptide-1-(9-36) amide is a major metabolite of glucagon-like peptide-1-(7-36) amide after in vivo administration to dogs, and it acts as an antagonist on the pancreatic receptor. *Eur J Pharmacol* 318(2-3), 429-35.

Kolterman, O.G., Buse, J.B., Fineman, M.S., Gaines, E., Heintz, S., Bicsak, T.A., Taylor, K., Kim, D., Aisporna, M., Wang, Y. & Baron, A.D. (2003). Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 88(7), 3082-9.

Kung, M.P., Hou, C., Lieberman, B.P., Oya, S., Ponde, D.E., Blankemeyer, E., Skovronsky, D., Kilbourn, M.R. & Kung, H.F. (2008). In vivo imaging of beta-cell mass in rats using 18F-FP-(+)-DTBZ: a potential PET ligand for studying diabetes mellitus. *J Nucl Med* 49(7), 1171-6.

Kwee, T.C., Basu, S., Saboury, B., Torigian, D.A., Naji, A. & Alavi, A. (2011). Beta-cell imaging: opportunities and limitations. *J Nucl Med* 52(3), 493; author reply 493-5.

Körner, M., Stöckli, M., Waser, B. & Reubi, J.C. (2007). GLP-1 receptor expression in human tumors and human normal tissues: potential for in vivo targeting. *J Nucl Med* 48(5), 736-43.

Ladrière, L., Leclercq-Meyer, V. & Malaisse, W.J. (2001). Assessment of islet beta-cell mass in isolated rat pancreases perfused with D-[(3)H]mannoheptulose. *Am J Physiol Endocrinol Metab* 281(2), E298-303.

Lamprianou, S., Immonen, R., Nabuurs, C., Gjinovci, A., Vinet, L., Montet, X.C., Gruetter, R. & Meda, P. (2011). High-resolution magnetic resonance imaging quantitatively detects individual pancreatic islets. *Diabetes* 60(11), 2853-60.

Larsen, M.O. & Rolin, B. (2004). Use of the Göttingen minipig as a model of diabetes, with special focus on type 1 diabetes research. *ILAR J* 45(3), 303-13.

Latchoumycandane, C., Anantharam, V., Kitazawa, M., Yang, Y., Kanthasamy, A. & Kanthasamy, A.G. (2005). Protein kinase Cdelta is a key downstream mediator of manganese-induced apoptosis in dopaminergic neuronal cells. *J Pharmacol Exp Ther* 313(1), 46-55.

Lee, J.Y. & Kim, M.C. (2012). Anesthesia of growing pigs with tiletamine-zolazepam and reversal with flumazenil. *J Vet Med Sci* 74(3), 335-9.

Leibiger, I.B., Caicedo, A. & Berggren, P.O. (2012). Non-invasive in vivo imaging of pancreatic β-cell function and survival - a perspective. *Acta Physiol (Oxf)* 204(2), 178-85.

Lerche, S., Brock, B., Rungby, J., Bøtker, H.E., Møller, N., Rodell, A., Bibby, B.M., Holst, J.J., Schmitz, O. & Gjedde, A. (2008). Glucagon-like peptide-1 inhibits blood-brain glucose transfer in humans. *Diabetes* 57(2), 325-31.

Ley, Charles (2006). *Navicular bone location in radiographs and solar scintigrams. The radiographic location of the equine navicular bone in the front legs, the effect of leg positioning, and a method to position a navicular bone region of interest in a solar scintigram.* Swedish University of Agricultural Sciences. Master thesis in Veterinary Medicine.

Li, Y., Hansotia, T., Yusta, B., Ris, F., Halban, P.A. & Drucker, D.J. (2003). Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* 278(1), 471-8.

Lim, G.E. & Brubaker, P.L. (2006). Glucagon-like peptide 1 secretion by the L-cell. *Diabetes* 55(S2), S70-77.

Linnebjerg, H., Park, S., Kothare, P.A., Trautmann, M.E., Mace, K., Fineman, M., Wilding, I., Nauck, M. & Horowitz, M. (2008). Effect of exenatide on gastric emptying and relationship to postprandial glycemia in type 2 diabetes. *Regul Pept* 151(1-3), 123-9.

Liu, I.M., Chang, C.K., Juang, S.W., Kou, D.H., Tong, Y.C., Cheng, K.C. & Cheng, J.T. (2008). Role of hyperglycaemia in the pathogenesis of hypotension observed in type-1 diabetic rats. *Int J Exp Pathol* 89(4), 292-300.

Lubag, A.J., De Leon-Rodriguez, L.M., Burgess, S.C. & Sherry, A.D. (2011). Noninvasive MRI of β-cell function using a Zn2+-responsive contrast agent. *Proc Natl Acad Sci U S A* 108(45), 18400-5.

Madsbad, S., Kielgast, U., Asmar, M., Deacon, C.F., Torekov, S.S. & Holst, J.J. (2011). An overview of once-weekly glucagon-like peptide-1 receptor agonists--available efficacy and safety data and perspectives for the future. *Diabetes Obes Metab* 13(5), 394-407.

Maeda, C.Y., Fernandes, T.G., Timm, H.B. & Irigoyen, M.C. (1995). Autonomic dysfunction in short-term experimental diabetes. *Hypertension* 26(6 Pt 2), 1100-4.

Maffei, A., Liu, Z., Witkowski, P., Moschella, F., Del Pozzo, G., Liu, E., Herold, K., Winchester, R.J., Hardy, M.A. & Harris, P.E. (2004). Identification of tissue-restricted transcripts in human islets. *Endocrinology* 145(10), 4513-21.

Malaisse, W.J. & Maedler, K. (2012). Imaging of the β-cells of the islets of Langerhans. *Diabetes Res Clin Pract* 98(1), 11-8.

Manell, E.A.K., Hedenqvist, P., Jacobson, M. and Jensen-Waern, M. (2014). Insulin treatment of streptozotocin-induced diabetes re-establishes the patterns in carbohydrate, fat and amino acid metabolism in growing pigs. *Laboratory animals*. Accepted 2013, in press 2014.

Marguet, D., Baggio, L., Kobayashi, T., Bernard, A.M., Pierres, M., Nielsen, P.F., Ribel, U., Watanabe, T., Drucker, D.J. & Wagtmann, N. (2000). Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci U S A* 97(12), 6874-9.

Martin, B., Golden, E., Carlson, O.D., Pistell, P., Zhou, J., Kim, W., Frank, B.P., Thomas, S., Chadwick, W.A., Greig, N.H., Bates, G.P., Sathasivam, K., Bernier, M., Maudsley, S., Mattson, M.P. & Egan, J.M. (2009). Exendin-4 improves glycemic control, ameliorates brain and pancreatic pathologies, and extends survival in a mouse model of Huntington's disease. *Diabetes* 58(2), 318-28.

Mawlawi, O. & Townsend, D.W. (2009). Multimodality imaging: an update on PET/CT technology. *Eur J Nucl Med Mol Imaging* 36 Suppl 1, S15-29.

McCann, T.M., Simpson, K.E., Shaw, D.J., Butt, J.A. & Gunn-Moore, D.A. (2007). Feline diabetes mellitus in the UK: the prevalence within an insured cat population and a questionnaire-based putative risk factor analysis. *J Feline Med Surg* 9(4), 289-99.

Medarova, Z. & Moore, A. (2009). MRI in diabetes: first results. *AJR Am J Roentgenol* 193(2), 295-303.

Meier, J.J., Gallwitz, B., Salmen, S., Goetze, O., Holst, J.J., Schmidt, W.E. & Nauck, M.A. (2003). Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *J Clin Endocrinol Metab* 88(6), 2719-25.

Meier, J.J., Gethmann, A., Götze, O., Gallwitz, B., Holst, J.J., Schmidt, W.E. & Nauck, M.A. (2006). Glucagon-like peptide 1 abolishes the postprandial rise in triglyceride concentrations and lowers levels of non-esterified fatty acids in humans. *Diabetologia* 49(3), 452-8.

Meier, J.J., Nauck, M.A., Kranz, D., Holst, J.J., Deacon, C.F., Gaeckler, D., Schmidt, W.E. & Gallwitz, B. (2004). Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes* 53(3), 654-62.

Meikle, S.R., Kench, P., Kassiou, M. & Banati, R.B. (2005). Small animal SPECT and its place in the matrix of molecular imaging technologies. *Phys Med Biol* 50(22), R45-61.

Mendis, B., Simpson, E., MacDonald, I. & Mansell, P. (2012). Investigation of the haemodynamic effects of exenatide in healthy male subjects. *Br J Clin Pharmacol* 74(3), 437-44.

Mentlein, R., Gallwitz, B. & Schmidt, W.E. (1993). Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 214(3), 829-35.

Miller, A.D. & Leslie, R.A. (1994). The area postrema and vomiting. *Front Neuroendocrinol* 15(4), 301-20.

Mojsov, S., Weir, G.C. & Habener, J.F. (1987). Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 79(2), 616-9.

Momosaki, S., Hatano, K., Kawasumi, Y., Kato, T., Hosoi, R., Kobayashi, K., Inoue, O. & Ito, K. (2004). Rat-PET study without anesthesia: anesthetics modify the dopamine D1 receptor binding in rat brain. *Synapse* 54(4), 207-13.

Moore, A., Bonner-Weir, S. & Weissleder, R. (2001). Noninvasive in vivo measurement of beta-cell mass in mouse model of diabetes. *Diabetes* 50(10), 2231-6.

Moretto, T.J., Milton, D.R., Ridge, T.D., Macconell, L.A., Okerson, T., Wolka, A.M. & Brodows, R.G. (2008). Efficacy and tolerability of exenatide monotherapy over 24 weeks in antidiabetic drug-naive patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther* 30(8), 1448-60.

Mortensen, K., Christensen, L.L., Holst, J.J. & Orskov, C. (2003). GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. *Regul Pept* 114(2-3), 189-96.

Mukai, E., Toyoda, K., Kimura, H., Kawashima, H., Fujimoto, H., Ueda, M., Temma, T., Hirao, K., Nagakawa, K., Saji, H. & Inagaki, N. (2009). GLP-1 receptor antagonist as a potential probe for pancreatic beta-cell imaging. *Biochem Biophys Res Commun* 389(3), 523-6.

Müssig, K., Oncü, A., Lindauer, P., Heininger, A., Aebert, H., Unertl, K., Ziemer, G., Häring, H.U., Holst, J.J. & Gallwitz, B. (2008). Effects of intravenous glucagon-like peptide-1 on glucose control and hemodynamics after coronary artery bypass surgery in patients with type 2 diabetes. *Am J Cardiol* 102(5), 646-7.

Nachnani, J.S., Bulchandani, D.G., Nookala, A., Herndon, B., Molteni, A., Pandya, P., Taylor, R., Quinn, T., Weide, L. & Alba, L.M. (2010). Biochemical and histological effects of exendin-4 (exenatide) on the rat pancreas. *Diabetologia* 53(1), 153-9.

Nakagawa, A., Satake, H., Nakabayashi, H., Nishizawa, M., Furuya, K., Nakano, S., Kigoshi, T., Nakayama, K. & Uchida, K. (2004). Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. *Auton Neurosci* 110(1), 36-43.

Nathan, D.M., Buse, J.B., Davidson, M.B., Ferrannini, E., Holman, R.R., Sherwin, R., Zinman, B., Association, A.D. & Diabetes, E.A.f.S.o. (2009). Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 32(1), 193-203.

Nathan, D.M., Schreiber, E., Fogel, H., Mojsov, S. & Habener, J.F. (1992). Insulinotropic action of glucagonlike peptide-I-(7-37) in diabetic and nondiabetic subjects. *Diabetes Care* 15(2), 270-6.

Nauck, M., Stöckmann, F., Ebert, R. & Creutzfeldt, W. (1986a). Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 29(1), 46-52.

Nauck, M.A. (2009). Unraveling the science of incretin biology. *Am J Med* 122(6 Suppl), S3-S10.

Nauck, M.A., Heimesaat, M.M., Behle, K., Holst, J.J., Nauck, M.S., Ritzel, R., Hüfner, M. & Schmiegel, W.H. (2002). Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J Clin Endocrinol Metab* 87(3), 1239-46.

Nauck, M.A., Heimesaat, M.M., Orskov, C., Holst, J.J., Ebert, R. & Creutzfeldt, W. (1993). Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 91(1), 301-7.

Nauck, M.A., Homberger, E., Siegel, E.G., Allen, R.C., Eaton, R.P., Ebert, R. & Creutzfeldt, W. (1986b). Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab* 63(2), 492-8.

Nauck, M.A., Niedereichholz, U., Ettler, R., Holst, J.J., Orskov, C., Ritzel, R. & Schmiegel, W.H. (1997). Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol* 273(5 Pt 1), E981-8.

Nauck, M.A., Vardarli, I., Deacon, C.F., Holst, J.J. & Meier, J.J. (2011). Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia* 54(1), 10-8.

Navarro, M., Rodriquez de Fonseca, F., Alvarez, E., Chowen, J.A., Zueco, J.A., Gomez, R., Eng, J. & Blázquez, E. (1996). Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. *J Neurochem* 67(5), 1982-91.

NICE (National Institute for Health and Clinical Excellence) (2011-08-22). *NICE guidelines. CG87 Type 2 diabetes: newer agents for blood glucose control in type 2 diabetes (partial update of CG66): short guideline.* http://www.nice.org.uk/CG87shortguideline [2013-10-11].

Nicholas, T.E., Jones, M.E., Johnson, D.W. & Phillipou, G. (1981). Metabolism of the steroid anaesthetic alphaxalone by the isolated perfused rat lung. *J Steroid Biochem* 14(1), 45-51.

Nielsen, L.L., Young, A.A. & Parkes, D.G. (2004). Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. *Regul Pept* 117(2), 77-88.

Nikolaidis, L.A., Elahi, D., Hentosz, T., Doverspike, A., Huerbin, R., Zourelias, L., Stolarski, C., Shen, Y.T. & Shannon, R.P. (2004a). Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. *Circulation* 110(8), 955-61.

Nikolaidis, L.A., Mankad, S., Sokos, G.G., Miske, G., Shah, A., Elahi, D. & Shannon, R.P. (2004b). Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation* 109(8), 962-5.

Noel, R.A., Braun, D.K., Patterson, R.E. & Bloomgren, G.L. (2009). Increased risk of acute pancreatitis and biliary disease observed in patients with type 2 diabetes: a retrospective cohort study. *Diabetes Care* 32(5), 834-8.

Nyström, T., Gutniak, M.K., Zhang, Q., Zhang, F., Holst, J.J., Ahrén, B. & Sjöholm, A. (2004). Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab* 287(6), E1209-15.

Näslund, E., Barkeling, B., King, N., Gutniak, M., Blundell, J.E., Holst, J.J., Rössner, S. & Hellström, P.M. (1999). Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord* 23(3), 304-11.

Oelze, M., Knorr, M., Schuhmacher, S., Heeren, T., Otto, C., Schulz, E., Reifenberg, K., Wenzel, P., Münzel, T. & Daiber, A. (2011). Vascular dysfunction in streptozotocin-induced experimental diabetes strictly depends on insulin deficiency. *J Vasc Res* 48(4), 275-84.

Okerson, T., Yan, P., Stonehouse, A. & Brodows, R. (2010). Effects of exenatide on systolic blood pressure in subjects with type 2 diabetes. *Am J Hypertens* 23(3), 334-9.

Olanow, C.W. (2004). Manganese-induced parkinsonism and Parkinson's disease. *Ann N Y Acad Sci* 1012, 209-23.

Oliver, J.A., Clark, L., Corletto, F. & Gould, D.J. (2010). A comparison of anesthetic complications between diabetic and nondiabetic dogs undergoing phacoemulsification cataract surgery: a retrospective study. *Vet Ophthalmol* 13(4), 244-50.

Orskov, C., Poulsen, S.S., Møller, M. & Holst, J.J. (1996a). Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. *Diabetes* 45(6), 832-5.

Orskov, C., Wettergren, A. & Holst, J.J. (1996b). Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day. *Scand J Gastroenterol* 31(7), 665-70.

Pacheco-Pantoja, E.L., Ranganath, L.R., Gallagher, J.A., Wilson, P.J. & Fraser, W.D. (2011). Receptors and effects of gut hormones in three osteoblastic cell lines. *BMC Physiol* 11, 12.

Pan, T., Mawlawi, O., Nehmeh, S.A., Erdi, Y.E., Luo, D., Liu, H.H., Castillo, R., Mohan, R., Liao, Z. & Macapinlac, H.A. (2005). Attenuation correction of PET images with respiration-averaged CT images in PET/CT. *J Nucl Med* 46(9), 1481-7.

Pattou, F., Kerr-Conte, J. & Wild, D. (2010). GLP-1-receptor scanning for imaging of human beta cells transplanted in muscle. *N Engl J Med* 363(13), 1289-90.

Paty, B.W., Bonner-Weir, S., Laughlin, M.R., McEwan, A.J. & Shapiro, A.M. (2004). Toward development of imaging modalities for islets after transplantation: insights from the National Institutes of Health Workshop on Beta Cell Imaging. *Transplantation* 77(8), 1133-7.

Perfetti, R., Zhou, J., Doyle, M.E. & Egan, J.M. (2000). Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 141(12), 4600-5.

Perley, M.J. & Kipnis, D.M. (1967). Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects. *J Clin Invest* 46(12), 1954-62.

Perry, T., Haughey, N.J., Mattson, M.P., Egan, J.M. & Greig, N.H. (2002a). Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. *J Pharmacol Exp Ther* 302(3), 881-8.

Perry, T., Lahiri, D.K., Chen, D., Zhou, J., Shaw, K.T., Egan, J.M. & Greig, N.H. (2002b). A novel neurotrophic property of glucagon-like peptide 1: a promoter of nerve growth factor-mediated differentiation in PC12 cells. *J Pharmacol Exp Ther* 300(3), 958-66.

Perry, T., Lahiri, D.K., Sambamurti, K., Chen, D., Mattson, M.P., Egan, J.M. & Greig, N.H. (2003). Glucagon-like peptide-1 decreases endogenous amyloid-beta peptide (Abeta) levels and protects hippocampal neurons from death induced by Abeta and iron. *J Neurosci Res* 72(5), 603-12.

Pfeiffer, N., Ebner, J., von Thaden, A-K., Schuster, T., Erhardt, W. & Baumgartner, C. (2013). Cardiovascular effects of alfaxalone on hemodynamic function in pigs. *Open Access Animal Physiology* 2013(5), 15-26.

Phillips, L.K. & Prins, J.B. (2011). Update on incretin hormones. *Ann N Y Acad Sci* 1243, E55-74.

Pichler, B.J., Kolb, A., Nägele, T. & Schlemmer, H.P. (2010). PET/MRI: paving the way for the next generation of clinical multimodality imaging applications. *J Nucl Med* 51(3), 333-6.

Pyke, C. & Knudsen, L.B. (2013). The glucagon-like peptide-1 receptor--or not? *Endocrinology* 154(1), 4-8.

Rachman, J., Barrow, B.A., Levy, J.C. & Turner, R.C. (1997). Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM. *Diabetologia* 40(2), 205-11.

Rahmim, A. & Zaidi, H. (2008). PET versus SPECT: strengths, limitations and challenges. *Nucl Med Commun* 29(3), 193-207.

Rand, J.S., Fleeman, L.M., Farrow, H.A., Appleton, D.J. & Lederer, R. (2004). Canine and feline diabetes mellitus: nature or nurture? *J Nutr* 134(8 Suppl), 2072S-2080S.

Ranganath, L.R., Beety, J.M., Morgan, L.M., Wright, J.W., Howland, R. & Marks, V. (1996). Attenuated GLP-1 secretion in obesity: cause or consequence? *Gut* 38(6), 916-9.

Ratner, R., Han, J., Nicewarner, D., Yushmanova, I., Hoogwerf, B.J. & Shen, L. (2011). Cardiovascular safety of exenatide BID: an integrated analysis from controlled clinical trials in participants with type 2 diabetes. *Cardiovasc Diabetol* 10, 22.

Read, P.A., Khan, F.Z. & Dutka, D.P. (2012). Cardioprotection against ischaemia induced by dobutamine stress using glucagon-like peptide-1 in patients with coronary artery disease. *Heart* 98(5), 408-13.

Reiner, T., Thurber, G., Gaglia, J., Vinegoni, C., Liew, C.W., Upadhyay, R., Kohler, R.H., Li, L., Kulkarni, R.N., Benoist, C., Mathis, D. & Weissleder, R. (2011). Accurate measurement of pancreatic islet beta-cell mass using a second-generation fluorescent exendin-4 analog. *Proc Natl Acad Sci U S A* 108(31), 12815-20.

Renström, E., Eliasson, L. & Rorsman, P. (1997). Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells. *J Physiol* 502 (Pt 1), 105-18.

Ritzel, R., Orskov, C., Holst, J.J. & Nauck, M.A. (1995). Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7-36 amide] after subcutaneous injection in healthy volunteers. Dose-response-relationships. *Diabetologia* 38(6), 720-5.

Robertson, R.P. (2007). Estimation of beta-cell mass by metabolic tests: necessary, but how sufficient? *Diabetes* 56(10), 2420-4.

Robertson, R.P., Harmon, J., Tran, P.O. & Poitout, V. (2004). Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes* 53 Suppl 1, S119-24.

Robinson, L.E., Holt, T.A., Rees, K., Randeva, H.S. & O'Hare, J.P. (2013). Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. *BMJ Open* 3(1).

Rocca, A.S. & Brubaker, P.L. (1999). Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology* 140(4), 1687-94.

Rodriquez de Fonseca, F., Navarro, M., Alvarez, E., Roncero, I., Chowen, J.A., Maestre, O., Gómez, R., Muñoz, R.M., Eng, J. & Blázquez, E. (2000). Peripheral versus central effects of glucagon-like peptide-1 receptor agonists on satiety and body weight loss in Zucker obese rats. *Metabolism* 49(6), 709-17.

Roglic, G., Unwin, N., Bennett, P.H., Mathers, C., Tuomilehto, J., Nag, S., Connolly, V. & King, H. (2005). The burden of mortality attributable to diabetes: realistic estimates for the year 2000. *Diabetes Care* 28(9), 2130-5.

Roth, J.A. & Garrick, M.D. (2003). Iron interactions and other biological reactions mediating the physiological and toxic actions of manganese. *Biochem Pharmacol* 66(1), 1-13.

Rubí, B., Ljubicic, S., Pournourmohammadi, S., Carobbio, S., Armanet, M., Bartley, C. & Maechler, P. (2005). Dopamine D2-like receptors are expressed in pancreatic beta cells and mediate inhibition of insulin secretion. *J Biol Chem* 280(44), 36824-32.

Ruiz-Grande, C., Alarcón, C., Alcántara, A., Castilla, C., López Novoa, J.M., Villanueva-Peñacarrillo, M.L. & Valverde, I. (1993). Renal catabolism of truncated glucagon-like peptide 1. *Horm Metab Res* 25(12), 612-6.

Russell-Jones, D., Cuddihy, R.M., Hanefeld, M., Kumar, A., González, J.G., Chan, M., Wolka, A.M., Boardman, M.K. & Group, D.-S. (2012). Efficacy and safety of exenatide once weekly versus metformin, pioglitazone, and sitagliptin used as monotherapy in drug-naive patients with type 2 diabetes (DURATION-4): a 26-week double-blind study. *Diabetes Care* 35(2), 252-8.

Ryan, A.S., Egan, J.M., Habener, J.F. & Elahi, D. (1998). Insulinotropic hormone glucagon-like peptide-1-(7-37) appears not to augment insulin-mediated glucose uptake in young men during euglycemia. *J Clin Endocrinol Metab* 83(7), 2399-404.

Saha, G.B. (2010). *Basics of PET Imaging*. 2. ed. London: Springer. Available from: http://scholar.google.se/. [2013-11-15].

Saisho, Y., Harris, P.E., Butler, A.E., Galasso, R., Gurlo, T., Rizza, R.A. & Butler, P.C. (2008). Relationship between pancreatic vesicular monoamine transporter 2 (VMAT2) and insulin expression in human pancreas. *J Mol Histol* 39(5), 543-51.

Sanz, C., Vázquez, P., Blázquez, C., Barrio, P.A., Alvarez, M.e.M. & Blázquez, E. (2010). Signaling and biological effects of glucagon-like peptide 1 on the differentiation of mesenchymal stem cells from human bone marrow. *Am J Physiol Endocrinol Metab* 298(3), E634-43.

Schirra, J., Sturm, K., Leicht, P., Arnold, R., Göke, B. & Katschinski, M. (1998). Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans. *J Clin Invest* 101(7), 1421-30.

Schlatter, P., Beglinger, C., Drewe, J. & Gutmann, H. (2007). Glucagon-like peptide 1 receptor expression in primary porcine proximal tubular cells. *Regul Pept* 141(1-3), 120-8.

Schmitz, A., Shiue, C.Y., Feng, Q., Shiue, G.G., Deng, S., Pourdehnad, M.T., Schirrmacher, R., Vatamaniuk, M., Doliba, N., Matschinsky, F., Wolf, B., Rösch, F., Naji, A. & Alavi, A.A. (2004). Synthesis and evaluation of fluorine-18 labeled glyburide analogs as beta-cell imaging agents. *Nucl Med Biol* 31(4), 483-91.

Schneider, S. (2008). Efforts to develop methods for in vivo evaluation of the native beta-cell mass. *Diabetes Obes Metab* 10 Suppl 4, 109-18.

Schwartz, E.A., Koska, J., Mullin, M.P., Syoufi, I., Schwenke, D.C. & Reaven, P.D. (2010). Exenatide suppresses postprandial elevations in lipids and lipoproteins in individuals with impaired glucose tolerance and recent onset type 2 diabetes mellitus. *Atherosclerosis* 212(1), 217-22.

Schwartz, S.L., Ratner, R.E., Kim, D.D., Qu, Y., Fechner, L.L., Lenox, S.M. & Holcombe, J.H. (2008). Effect of exenatide on 24-hour blood glucose profile compared with placebo in patients with type 2 diabetes: a randomized, double-blind, two-arm, parallel-group, placebo-controlled, 2-week study. *Clin Ther* 30(5), 858-67.

Selvaraju, R.K., Velikyan, I., Johansson, L., Wu, Z., Todorov, I., Shively, J., Kandeel, F., Korsgren, O. & Eriksson, O. (2013). In vivo imaging of the glucagonlike peptide 1 receptor in the pancreas with 68Ga-labeled DO3A-exendin-4. *J Nucl Med* 54(8), 1458-63.

Shaw, J.E., Sicree, R.A. & Zimmet, P.Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 87(1), 4-14.

Shibasaki, T., Takahashi, H., Miki, T., Sunaga, Y., Matsumura, K., Yamanaka, M., Zhang, C., Tamamoto, A., Satoh, T., Miyazaki, J. & Seino, S. (2007). Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. *Proc Natl Acad Sci U S A* 104(49), 19333-8.

Silva, A.C., Lee, J.H., Aoki, I. & Koretsky, A.P. (2004). Manganese-enhanced magnetic resonance imaging (MEMRI): methodological and practical considerations. *NMR Biomed* 17(8), 532-43.

Simonsen, L., Holst, J.J. & Deacon, C.F. (2006). Exendin-4, but not glucagon-like peptide-1, is cleared exclusively by glomerular filtration in anaesthetised pigs. *Diabetologia* 49(4), 706-12.

Singhal, T., Ding, Y.S., Weinzimmer, D., Normandin, M.D., Labaree, D., Ropchan, J., Nabulsi, N., Lin, S.F., Skaddan, M.B., Soeller, W.C., Huang, Y., Carson, R.E., Treadway, J.L. & Cline, G.W. (2011). Pancreatic beta cell mass PET imaging and quantification with [11C]DTBZ and [18F]FP-(+)-DTBZ in rodent models of diabetes. *Mol Imaging Biol* 13(5), 973-84.

Smirnov, P., Poirier-Quinot, M., Wilhelm, C., Lavergne, E., Ginefri, J.C., Combadière, B., Clément, O., Darrasse, L. & Gazeau, F. (2008). In vivo single cell detection of tumor-infiltrating lymphocytes with a clinical 1.5 Tesla MRI system. *Magn Reson Med* 60(6), 1292-7.

Sokos, G.G., Bolukoglu, H., German, J., Hentosz, T., Magovern, G.J., Maher, T.D., Dean, D.A., Bailey, S.H., Marrone, G., Benckart, D.H., Elahi, D. & Shannon, R.P. (2007). Effect of glucagon-like peptide-1 (GLP-1) on glycemic control and left ventricular function in patients undergoing coronary artery bypass grafting. *Am J Cardiol* 100(5), 824-9.

Sokos, G.G., Nikolaidis, L.A., Mankad, S., Elahi, D. & Shannon, R.P. (2006). Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. *J Card Fail* 12(9), 694-9.

Son, S.M., Whalin, M.K., Harrison, D.G., Taylor, W.R. & Griendling, K.K. (2004). Oxidative stress and diabetic vascular complications. *Curr Diab Rep* 4(4), 247-52.

Soret, M., Bacharach, S.L. & Buvat, I. (2007). Partial-volume effect in PET tumor imaging. *J Nucl Med* 48(6), 932-45.

Souza, F., Freeby, M., Hultman, K., Simpson, N., Herron, A., Witkowsky, P., Liu, E., Maffei, A. & Harris, P.E. (2006a). Current progress in non-invasive imaging of beta cell mass of the endocrine pancreas. *Curr Med Chem* 13(23), 2761-73.

Souza, F., Simpson, N., Raffo, A., Saxena, C., Maffei, A., Hardy, M., Kilbourn, M., Goland, R., Leibel, R., Mann, J.J., Van Heertum, R. & Harris, P.E. (2006b). Longitudinal noninvasive PET-based beta cell mass estimates in a spontaneous diabetes rat model. *J Clin Invest* 116(6), 1506-13.

Sowa-Staszczak, A., Pach, D., Mikołajczak, R., Mäcke, H., Jabrocka-Hybel, A., Stefańska, A., Tomaszuk, M., Janota, B., Gilis-Januszewska, A., Małecki, M., Kamiński, G., Kowalska, A., Kulig, J., Matyja, A., Osuch, C. & Hubalewska-Dydejczyk, A. (2013). Glucagon-like peptide-1 receptor imaging with [Lys40(Ahx-HYNIC- 99mTc/EDDA)NH2]-exendin-4 for the detection of insulinoma. *Eur J Nucl Med Mol Imaging* 40(4), 524-31.

Spranger, J., Gundert-Remy, U. & Stammschulte, T. (2011). GLP-1-based therapies: the dilemma of uncertainty. *Gastroenterology* 141(1), 20-3.

Suzuki, M., Fujikura, K., Kotake, K., Inagaki, N., Seino, S. & Takata, K. (1999). Immuno-localization of sulphonylurea receptor 1 in rat pancreas. *Diabetologia* 42(10), 1204-11.

Sweet, I.R., Cook, D.L., Lernmark, A., Greenbaum, C.J. & Krohn, K.A. (2004a). Non-invasive imaging of beta cell mass: a quantitative analysis. *Diabetes Technol Ther* 6(5), 652-9.

Sweet, I.R., Cook, D.L., Lernmark, A., Greenbaum, C.J., Wallen, A.R., Marcum, E.S., Stekhova, S.A. & Krohn, K.A. (2004b). Systematic screening of potential beta-cell imaging agents. *Biochem Biophys Res Commun* 314(4), 976-83.

Szayna, M., Doyle, M.E., Betkey, J.A., Holloway, H.W., Spencer, R.G., Greig, N.H. & Egan, J.M. (2000). Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. *Endocrinology* 141(6), 1936-41.

Søndergaard, L.G., Stoltenberg, M., Flyvbjerg, A., Brock, B., Schmitz, O., Danscher, G. & Rungby, J. (2003). Zinc ions in beta-cells of obese, insulin-resistant, and type 2 diabetic rats traced by autometallography. *APMIS* 111(12), 1147-54.

Talsania, T., Anini, Y., Siu, S., Drucker, D.J. & Brubaker, P.L. (2005). Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. *Endocrinology* 146(9), 3748-56.

Tang-Christensen, M., Vrang, N. & Larsen, P.J. (2001). Glucagon-like peptide containing pathways in the regulation of feeding behaviour. *Int J Obes Relat Metab Disord* 25 Suppl 5, S42-7.

Tatarkiewicz, K., Belanger, P., Gu, G., Parkes, D. & Roy, D. (2013). No evidence of drug-induced pancreatitis in rats treated with exenatide for 13 weeks. *Diabetes Obes Metab* 15(5), 417-26.

Theodorakis, M.J., Carlson, O., Michopoulos, S., Doyle, M.E., Juhaszova, M., Petraki, K. & Egan, J.M. (2006). Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. *Am J Physiol Endocrinol Metab* 290(3), E550-9.

Thorens, B. (1992). Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci U S A* 89(18), 8641-5.

Thorens, B., Porret, A., Bühler, L., Deng, S.P., Morel, P. & Widmann, C. (1993). Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. *Diabetes* 42(11), 1678-82.

Timmers, L., Henriques, J.P., de Kleijn, D.P., Devries, J.H., Kemperman, H., Steendijk, P., Verlaan, C.W., Kerver, M., Piek, J.J., Doevendans, P.A., Pasterkamp, G. & Hoefer, I.E. (2009). Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. *J Am Coll Cardiol* 53(6), 501-10.

Todd, J.F., Edwards, C.M., Ghatei, M.A., Mather, H.M. & Bloom, S.R. (1998). Subcutaneous glucagon-like peptide-1 improves postprandial glycaemic control over a 3-week period in patients with early type 2 diabetes. *Clin Sci (Lond)* 95(3), 325-9.

Toft-Nielsen, M.B., Damholt, M.B., Madsbad, S., Hilsted, L.M., Hughes, T.E., Michelsen, B.K. & Holst, J.J. (2001). Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 86(8), 3717-23.

Toft-Nielsen, M.B., Madsbad, S. & Holst, J.J. (1999). Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care* 22(7), 1137-43.

Torigian, D.A., Zaidi, H., Kwee, T.C., Saboury, B., Udupa, J.K., Cho, Z.H. & Alavi, A. (2013). PET/MR imaging: technical aspects and potential clinical applications. *Radiology* 267(1), 26-44.

Tornehave, D., Kristensen, P., Rømer, J., Knudsen, L.B. & Heller, R.S. (2008). Expression of the GLP-1 receptor in mouse, rat, and human pancreas. *J Histochem Cytochem* 56(9), 841-51.

Toroptsev, I.V. & Eshchenko, V.A. (1983). Toxic action of dithizone on insulin-producing cells. *Bulletin of Experimental Biology and Medicine* 96(3), 1215-1217.

Tripathy, N.R., Basha, S., Jain, R., Shetty, S. & Ramachandran, A. (2008). Exenatide and acute pancreatitis. *J Assoc Physicians India* 56, 987-8.

Tsuboi, T., da Silva Xavier, G., Holz, G.G., Jouaville, L.S., Thomas, A.P. & Rutter, G.A. (2003). Glucagon-like peptide-1 mobilizes intracellular Ca2+ and stimulates mitochondrial ATP synthesis in pancreatic MIN6 beta-cells. *Biochem J* 369(Pt 2), 287-99.

Turton, M.D., O'Shea, D., Gunn, I., Beak, S.A., Edwards, C.M., Meeran, K., Choi, S.J., Taylor, G.M., Heath, M.M., Lambert, P.D., Wilding, J.P., Smith, D.M., Ghatei, M.A., Herbert, J. & Bloom, S.R. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379(6560), 69-72.

Tuttle, K.R., Heilmann, C., Hoogwerf, B.J., Brown, C. & Anderson, P.W. (2013). Effects of exenatide on kidney function, adverse events, and clinical end points of kidney disease in type 2 diabetes. *Am J Kidney Dis* 62(2), 396-8.

Ueberberg, S., Meier, J.J., Waengler, C., Schechinger, W., Dietrich, J.W., Tannapfel, A., Schmitz, I., Schirrmacher, R., Köller, M., Klein, H.H. & Schneider, S. (2009). Generation of novel single-chain antibodies by phage-display technology to direct imaging agents highly selective to pancreatic beta- or alpha-cells in vivo. *Diabetes* 58(10), 2324-34.

Ueberberg, S., Ziegler, D., Schechinger, W., Dietrich, J.W., Akinturk, S., Klein, H.H. & Schneider, S. (2010). In vitro phage display in a rat beta cell line: a simple approach for the generation of a single-chain antibody targeting a novel beta cell-specific epitope. *Diabetologia* 53(7), 1384-94.

Unger, R.H., Ohneda, A., Valverde, I., Eisentraut, A.M. & Exton, J. (1968). Characterization of the responses of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. *J Clin Invest* 47(1), 48-65.

U.S. Food and Drug Administration, 2 November 2009a. (2013-08-15). *Information for Healthcare Professionals: Reports of Altered Kidney Function in patients using Exenatide (Marketed as Byetta).* http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/DrugSafetyInformationforHeathcareProfessionals/ucm188656.htm [2013-10-09].

U.S. Food and Drug Administration, 2 November 2009b. (2013-04-16). *FDA: Byetta Label Revised to Include Safety Information on Possible Kidney Problems*. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm188708.htm [2013-10-09].

Vats, D., Wang, H., Esterhazy, D., Dikaiou, K., Danzer, C., Honer, M., Stuker, F., Matile, H., Migliorini, C., Fischer, E., Ripoll, J., Keist, R., Krek, W., Schibli, R., Stoffel, M. & Rudin, M.

(2012). Multimodal imaging of pancreatic beta cells in vivo by targeting transmembrane protein 27 (TMEM27). *Diabetologia* 55(9), 2407-16.

Verdich, C., Toubro, S., Buemann, B., Lysgård Madsen, J., Juul Holst, J. & Astrup, A. (2001). The role of postprandial releases of insulin and incretin hormones in meal-induced satiety--effect of obesity and weight reduction. *Int J Obes Relat Metab Disord* 25(8), 1206-14.

Vilsbøll, T. (2009). The effects of glucagon-like peptide-1 on the beta cell. *Diabetes Obes Metab* 11 Suppl 3, 11-8.

Vilsbøll, T., Agersø, H., Krarup, T. & Holst, J.J. (2003a). Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. *J Clin Endocrinol Metab* 88(1), 220-4.

Vilsbøll, T., Krarup, T., Deacon, C.F., Madsbad, S. & Holst, J.J. (2001). Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 50(3), 609-13.

Vilsbøll, T., Krarup, T., Sonne, J., Madsbad, S., Vølund, A., Juul, A.G. & Holst, J.J. (2003b). Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. *J Clin Endocrinol Metab* 88(6), 2706-13.

Virostko, J., Henske, J., Vinet, L., Lamprianou, S., Dai, C., Radhika, A., Baldwin, R.M., Ansari, M.S., Hefti, F., Skovronsky, D., Kung, H.F., Herrera, P.L., Peterson, T.E., Meda, P. & Powers, A.C. (2011). Multimodal image coregistration and inducible selective cell ablation to evaluate imaging ligands. *Proc Natl Acad Sci U S A* 108(51), 20719-24.

Virostko, J. & Powers, A.C. (2009). Molecular imaging of the pancreas in small animal models. *Gastroenterology* 136(2), 407-9.

Vollmer, K., Holst, J.J., Baller, B., Ellrichmann, M., Nauck, M.A., Schmidt, W.E. & Meier, J.J. (2008). Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance. *Diabetes* 57(3), 678-87.

Walsh, J.S. & Henriksen, D.B. (2010). Feeding and bone. *Arch Biochem Biophys* 503(1), 11-9.

Wang, Y., Egan, J.M., Raygada, M., Nadiv, O., Roth, J. & Montrose-Rafizadeh, C. (1995). Glucagon-like peptide-1 affects gene transcription and messenger ribonucleic acid stability of components of the insulin secretory system in RIN 1046-38 cells. *Endocrinology* 136(11), 4910-7.

Wei, Y. & Mojsov, S. (1995). Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett* 358(3), 219-24.

Weir, G.C., Bonner-Weir, S. & Leahy, J.L. (1990). Islet mass and function in diabetes and transplantation. *Diabetes* 39(4), 401-5.

Whiting, D.R., Guariguata, L., Weil, C. & Shaw, J. (2011). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 94(3), 311-21.

Wicki, A., Wild, D., Storch, D., Seemayer, C., Gotthardt, M., Behe, M., Kneifel, S., Mihatsch, M.J., Reubi, J.C., Mäcke, H.R. & Christofori, G. (2007). [Lys40(Ahx-DTPA-111In)NH2]-Exendin-4 is a highly efficient radiotherapeutic for glucagon-like peptide-1 receptor-targeted therapy for insulinoma. *Clin Cancer Res* 13(12), 3696-705.

Wild, D., Béhé, M., Wicki, A., Storch, D., Waser, B., Gotthardt, M., Keil, B., Christofori, G., Reubi, J.C. & Mäcke, H.R. (2006). [Lys40(Ahx-DTPA-111In)NH2]exendin-4, a very promising ligand for glucagon-like peptide-1 (GLP-1) receptor targeting. *J Nucl Med* 47(12), 2025-33.

Wild, D., Mäcke, H., Christ, E., Gloor, B. & Reubi, J.C. (2008). Glucagon-like peptide 1-receptor scans to localize occult insulinomas. *N Engl J Med* 359(7), 766-8.

Wild, S., Roglic, G., Green, A., Sicree, R. & King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27(5), 1047-53.

Willms, B., Werner, J., Holst, J.J., Orskov, C., Creutzfeldt, W. & Nauck, M.A. (1996). Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in type 2 (noninsulin-dependent) diabetic patients. *J Clin Endocrinol Metab* 81(1), 327-32.

Wishart, J.M., Horowitz, M., Morris, H.A., Jones, K.L. & Nauck, M.A. (1998). Relation between gastric emptying of glucose and plasma concentrations of glucagon-like peptide-1. *Peptides* 19(6), 1049-53.

Wu, Z. & Kandeel, F. (2010). Radionuclide probes for molecular imaging of pancreatic beta-cells. *Adv Drug Deliv Rev* 62(11), 1125-38.

Wu, Z., Liu, S., Hassink, M., Nair, I., Park, R., Li, L., Todorov, I., Fox, J.M., Li, Z., Shively, J.E., Conti, P.S. & Kandeel, F. (2013). Development and evaluation of 18F-TTCO-Cys40-Exendin-4: a PET probe for imaging transplanted islets. *J Nucl Med* 54(2), 244-51.

Wu, Z., Todorov, I., Li, L., Bading, J.R., Li, Z., Nair, I., Ishiyama, K., Colcher, D., Conti, P.E., Fraser, S.E., Shively, J.E. & Kandeel, F. (2011). In vivo imaging of transplanted islets with 64Cu-DO3A-VS-Cys40-Exendin-4 by targeting GLP-1 receptor. *Bioconjug Chem* 22(8), 1587-94.

Wängler, B., Beck, C., Shiue, C.Y., Schneider, S., Schwanstecher, C., Schwanstecher, M., Feilen, P.J., Alavi, A., Rösch, F. & Schirrmacher, R. (2004a). Synthesis and in vitro evaluation of (S)-2-([11C]methoxy)-4-[3-methyl-1-(2-piperidine-1-yl-phenyl)-butyl-carbamoyl]-benzoic acid ([11C]methoxy-repaglinide): a potential beta-cell imaging agent. *Bioorg Med Chem Lett* 14(20), 5205-9.

Wängler, B., Schneider, S., Thews, O., Schirrmacher, E., Comagic, S., Feilen, P., Schwanstecher, C., Schwanstecher, M., Shiue, C.Y., Alavi, A., Höhnemann, S., Piel, M., Rösch, F. & Schirrmacher, R. (2004b). Synthesis and evaluation of (S)-2-(2-[18F]fluoroethoxy)-4-([3-methyl-1-(2-piperidin-1-yl-phenyl)-butyl-carbamoyl]-methyl)-benzoic acid ([18F]repaglinide): a promising radioligand for quantification of pancreatic beta-cell mass with positron emission tomography (PET). *Nucl Med Biol* 31(5), 639-47.

Xiao, C., Bandsma, R.H., Dash, S., Szeto, L. & Lewis, G.F. (2012). Exenatide, a glucagon-like peptide-1 receptor agonist, acutely inhibits intestinal lipoprotein production in healthy humans. *Arterioscler Thromb Vasc Biol* 32(6), 1513-9.

Xu, G., Kaneto, H., Laybutt, D.R., Duvivier-Kali, V.F., Trivedi, N., Suzuma, K., King, G.L., Weir, G.C. & Bonner-Weir, S. (2007). Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. *Diabetes* 56(6), 1551-8.

Xu, G., Stoffers, D.A., Habener, J.F. & Bonner-Weir, S. (1999). Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48(12), 2270-6.

Yamamoto, H., Lee, C.E., Marcus, J.N., Williams, T.D., Overton, J.M., Lopez, M.E., Hollenberg, A.N., Baggio, L., Saper, C.B., Drucker, D.J. & Elmquist, J.K. (2002). Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest* 110(1), 43-52.

Yang, L., Ji, W., Xue, Y. & Chen, L. (2013). Imaging beta-cell mass and function in situ and in vivo. *J Mol Med (Berl)* 91(8), 929-38.

Young, A.A., Gedulin, B.R., Bhavsar, S., Bodkin, N., Jodka, C., Hansen, B. & Denaro, M. (1999). Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (ob/ob, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (Macaca mulatta). *Diabetes* 48(5), 1026-34.

Yu, M., Moreno, C., Hoagland, K.M., Dahly, A., Ditter, K., Mistry, M. & Roman, R.J. (2003). Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 21(6), 1125-35.

Yusta, B., Baggio, L.L., Estall, J.L., Koehler, J.A., Holland, D.P., Li, H., Pipeleers, D., Ling, Z. & Drucker, D.J. (2006). GLP-1 receptor activation improves beta cell function and survival following induction of endoplasmic reticulum stress. *Cell Metab* 4(5), 391-406.

Zander, M., Madsbad, S., Madsen, J.L. & Holst, J.J. (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 359(9309), 824-30.

Zhao, T., Parikh, P., Bhashyam, S., Bolukoglu, H., Poornima, I., Shen, Y.T. & Shannon, R.P. (2006). Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. *J Pharmacol Exp Ther* 317(3), 1106-13.

Zhou, J., Wang, X., Pineyro, M.A. & Egan, J.M. (1999). Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. *Diabetes* 48(12), 2358-66.

Zini, E., Hafner, M., Osto, M., Franchini, M., Ackermann, M., Lutz, T.A. & Reusch, C.E. (2010). Predictors of clinical remission in cats with diabetes mellitus. *J Vet Intern Med* 24(6), 1314-21.