The effect of portal and peripheral insulin delivery on carbohydrate and lipid metabolism in a miniature pig model of human IDDM

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Summary A pig model of insulin-dependent diabetes was used to examine the importance of the portal-systemic insulin gradient for whole-body metabolic control. Six pigs had jugular vein, portal vein, and carotid artery cannulae implanted before being made diabetic (150 mg kg⁻¹ streptozotocin). Each animal received 4 weeks of portal and 4 weeks of peripheral insulin delivery in random order. The blood glucose target range was 5–10 mmol · l⁻¹, and serum fructosamine and fasting and postprandial blood glucose concentrations were not different between peripheral and portal insulin infusion. Insulin requirement was not different between the 4 week infusion periods, but fasting peripheral insulin levels after peripheral delivery (124 ± 16 (mean ± SEM) pmol · l⁻¹) were significantly higher ($p < 0.05$) than in portally infused (73.8 ± 5.4 pmol · l⁻¹) or pre-diabetic control animals (68.4 ± 3.6 pmol · l⁻¹). Basal hepatic glucose output was also higher ($p < 0.05$) in peripherally (4.2 ± 0.4 mg · kg⁻¹ · min⁻¹) than in portally infused animals

(2.9 ± 0.4 mg · kg⁻¹ · min⁻¹) or controls (3.0 ± 0.3 mg · kg⁻¹ · min⁻¹). Clamp glucose metabolic clearance rate was, however, not different between the peripheral and portal insulin delivery routes (8.1 ± 1.0 vs 9.0 ± 0.7 ml · kg⁻¹ · min⁻¹), although both were significantly lower ($p < 0.05$) than that measured in prediabetic control animals (11.7 ± 1.0 ml · kg⁻¹ · min⁻¹). Lipid profiles and subfractions were similar in all three groups. It is concluded that the portal route of delivery is superior to the peripheral in maintaining more appropriate insulin concentrations and control of hepatic glucose output, although in the absence of euglycaemia it is still associated with significant metabolic abnormalities. [Diabetologia (1997) 40: 1125–1134]

Keywords Type 1 diabetic model, minipig, streptozotocin, portal vein, insulin, glucose clearance, hepatic glucose production, lipids.

The normal portal-peripheral insulin gradient is absent in patients with insulin-dependent diabetes mellitus (IDDM) managed on conventional insulin

therapy. The effect of this on intermediary metabolism has been studied widely in man and animals. However, results have been conflicting and considerable uncertainty still exists regarding the importance of the gradient for the maintenance of overall metabolic homeostasis.

In animal models the metabolic importance of the portal-systemic gradient has been examined using a variety of techniques including direct cannulation combined with exogenous insulin infusion, islet and pancreatic transplantation, and diversion of pancreatic venous drainage [1–21]. In some studies control of hepatic glucose output or blood glucose concentrations was superior with the portal route of insulin delivery, [3, 8, 15, 16, 18, 20] while in others no

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; IVGTT, intravenous glucose tolerance test; NEFA, non-esterified fatty acids; VLDL, very low density lipoproteins, LDL, low density lipoproteins; HDL, high density lipoproteins; MCR, metabolic clearance rate; HGO, hepatic glucose output; SA, specific radioactivity.

advantage was found [2, 6, 9, 11, 14, 21]. Indeed some acute experiments have shown that peripherally delivered insulin is more effective than the same dose delivered portally [1, 5]. A number of investigations have supported the concept that only portal insulin delivery can sustain normal insulin responsiveness [13, 18, 20]. Others, however, have yielded contradictory findings [2, 4, 7, 11, 14, 17, 19].

In humans the importance of maintaining the portal-peripheral insulin gradient has been investigated mainly by comparing intraperitoneal (i.p., where the gradient is largely re-established [22]) with subcutaneous (s.c.) insulin delivery [23–28]. Metabolic control after pancreas transplantation and in chronic liver disease, when the portal-systemic gradient is lost, has also been the subject of investigation [29–40]. Intraperitoneal studies have yielded somewhat conflicting results, with either improved [23, 24, 26, 29] or unchanged [25, 27, 28] insulin sensitivity. Similarly pancreas transplantation has been shown by many [30–32, 34–37], but not all groups [33, 38] to cause a deterioration in insulin responsiveness.

The contradictory results of animal investigations may largely reflect differences in the experimental protocols employed, such as whether the animals had normal or impaired glucose tolerance, whether their diabetes was surgically or chemically induced, and whether the studies were short or long term. The actual species employed will also have influenced the experimental outcome, not least in determining their relevance to man. In this respect the pig is superior to the more commonly employed rodent or dog models by virtue of its greater endocrine, nutritional and anatomical similarity to man.

It is clear that for the full therapeutic potential of advances in islet and pancreatic transplantation and artificial pancreas technology to be realised, any uncertainties concerning which site of insulin delivery is most metabolically efficacious should be resolved. The aims of the present study were to develop a miniature-pig model of human IDDM. The pig because of its aforementioned physiological homology to man provides an excellent model with which to examine the significance of the portal-systemic insulin gradient for various aspects of metabolic control. Using this carefully defined model the relative impact of longer-term portal-vein compared to peripheral-vein insulin delivery on blood glucose control, intermediary and lipid metabolism, and glucose turnover was examined.

Materials and methods

Animals. Male Göttingen mini-pigs, specific pathogen-free, were purchased (Ellegaard, Dalmose, Denmark) at 12 weeks of age and housed under controlled conditions (21°C room temperature and a 12-h light cycle from 07.00 to 19.00 hours).

Feeding was programmed twice daily at exactly 10.00 and 16.00 hours and on each occasion consisted of 250 g of a standard mini-pig diet (SMPZN; SDS, Witham, Essex, UK). United Kingdom regulations and guidelines concerning the care and use of experimental animals were strictly followed.

Study design. At approximately 52 weeks of age and with a body weight of 35 ± 4.0 kg (mean $\pm$ SD) animals underwent surgery (see following) to implant chronically indwelling carotid artery, jugular vein and portal vein cannulae. From 2–6 weeks post-surgery a series of diagnostic and experimental protocols were performed to obtain baseline values for a range of haematological, endocrine and biochemical measures. Whole body insulin sensitivity and rates of glucose turnover were also determined in each animal at this time (see insulin sensitivity and glucose turnover determinations below). A total of six animals were then made diabetic (see introduction of diabetes below) and managed with s.c. injections of insulin up to the start of infusion studies (see following). Eight to 50 weeks following induction of diabetes each animal underwent 28 days of programmed insulin delivery via either the jugular vein (peripherally), or the portal vein, using portable infusion pumps (see intravenous insulin treatments below). The initial route was randomly determined and after each 28-day study a further period of s.c. treatment ensued, lasting at least 10 days, before initiation of a further 4 weeks of insulin infusion by the alternate route. During the last week of each 4-week treatment interval the diagnostic and experimental protocols performed before diabetes was induced were repeated.

Insulin therapy, whether subcutaneous or venous, was adjusted with the aim of keeping whole blood glucose within the range of 5.0 – 10.0 mmol $\cdot$ l $^{-1}$ in each animal throughout the study. Prior to induction of diabetes and before and after each infusion study an intravenous glucose tolerance test (IVGTT, see following) was carried out to establish reference values in normal animals and ensure that the streptozotocin-treated animals remained C-peptide negative, and therefore accurately modelled human IDDM throughout the study.

Surgery. Anaesthesia was induced in the minipigs after an overnight fast, by intramuscular injection of ketamine (10.0 mg $\cdot$ kg $^{-1}$; Ketalar, Parke Davis, Eastleigh, UK) and diazepam emulsion (2.0 mg $\cdot$ kg $^{-1}$; Diazemuls, Dumex, Aylesbury, UK) and was maintained by inhalation of 1–2% isoflurane (Abbot, Maidenhead, UK) in oxygen (6 – 8 litres $\cdot$ min $^{-1}$). Under anaesthesia, polyurethane catheters (16 G, 150 mm; Secalon Universal Ohmeda, Swindon, UK) were inserted into the carotid artery and jugular and portal veins using established procedures [41]. Cefuroxime (Zinacef 250 mg, i.v.; Glaxo, Greenford, UK) was administered immediately before and for 48 h post-operatively. Post-surgery analgesia consisting of buprenorphine (0.005 mg $\cdot$ kg $^{-1}$, i.v.; Temgesic, Rekitt & Colman, Hull, UK) and Flunixin (2 mg $\cdot$ kg $^{-1}$, i.v.; Finadyne, Schering-Plough Animal Health, Welwyn Garden City, UK) was provided. Catheters were kept patent by daily flushing with 0.15 mol $\cdot$ l $^{-1}$ NaCl in water and were left filled with heparin (Leo, Aylesbury, UK) diluted to 1000 U $\cdot$ ml $^{-1}$ with 0.15 mol $\cdot$ l $^{-1}$ NaCl.

Induction of diabetes. Following an overnight fast, pigs were administered 150 mg $\cdot$ kg $^{-1}$ streptozotocin (Sigma, Poole, UK) [42] in 50 ml of 0.15 mol $\cdot$ l $^{-1}$ NaCl via the jugular vein catheter. Commencing the following morning and prior to feeding each morning and afternoon (see above) throughout the experiments a blood sample (500 μ l) was obtained from the arterial cannula and whole blood glucose concentration measured on a portable glucose meter (One Touch II; Lifescan,

Abingdon, UK). The insulin dose was adjusted accordingly on the basis of these readings. Subcutaneous insulin treatment consisted of twice daily injections, given immediately before feeding, of a mixture of unmodified (Velosulin; Novo Nordisk, Crawley, UK) and NPH (Insulatard; Novo Nordisk) porcine insulins. A subcutaneous dose of between 4.8 and 7.2 nmol · kg⁻¹ was typically required to maintain blood glucose within the required 5.0–10.0 mmol · l⁻¹ range.

Intravenous insulin treatments. Insulin was delivered to the jugular or portal vein from a mini-infusion pump (Disetronic Panomat V; Applied Medical Technology, Cambridge, UK) carried in a specially "in-house" designed harness worn on the animal's back. The pump delivered pre-programmed doses (5–20 µl in 3-min pulses) of unmodified porcine insulin (Velosulin) diluted in polygeline (Haemacel; Hoechst, Hounslow, UK) containing 250 · U l⁻¹ ml of heparin. The concentration of insulin delivered by the pump (20.4–26.4 nmol · ml⁻¹) and the flow rate settings employed (100–400 µl · h⁻¹) were individually titrated in each animal based upon the prevailing blood glucose levels. A typical infusion profile consisted of insulin being delivered throughout the 24-h period at a flow rate of 120 µl · h⁻¹ except for the 4 h immediately following the morning and afternoon meals when the infusion rate was increased by 233 % above basal for the first 2 h and then by 192 and 25 % above basal for the third and fourth hours after eating, respectively.

Insulin sensitivity and glucose turnover determinations. Measurements were made in each pig prior to diabetes induction and on day 28 of each infusion period, using the hyperinsulinaemic glucose clamp technique [43]. Through pre-conditioning, experiments could be performed on unstressed, fully conscious animals lying in purpose built holding crates.

Each pig was fasted for 17 h prior to the study which began at 08.30 hours, designated time 0, with a primed (< 1.3 MBq, depending on basal glucose levels) [44] continuous infusion (0.67 MBq · min⁻¹) of D(3-³H)-glucose (514 Gbq · mmol⁻¹; Amersham, Amersham, UK) in 0.15 mol · l⁻¹ NaCl. To avoid an excessive rise in specific activity the initial continuous infusion rate of 8 ml · h⁻¹ was reduced, by 50 % on each occasion, at 150, 180, and 210 min during the clamp. Blood samples (500 µl) were taken at 5-min intervals from the arterial line throughout the study for the measurement of whole blood and plasma glucose concentrations using the glucose oxidase technique on a glucose analyser with an interassay coefficient of variation (CV) of 3 % (Yellow Springs Instruments, Yellow Springs, Ohio, USA). A 2-h equilibration period was employed and basal glucose turnover was measured over the ensuing 30 min. Arterial blood samples (5 ml) were taken at 0, 120, 130, 135, 140 and 150 min for the measurement of insulin and intermediary metabolite concentrations and plasma glucose specific radioactivity. In the diabetic pigs the portable insulin infusion pump was then disconnected, and at 155 min in all animals an infusion via the jugular catheter of unmodified porcine insulin (Velosulin) diluted in Haemacel was begun at a rate of 300 pmol · kg⁻¹ · h⁻¹. Plasma blood glucose was then clamped at baseline levels by the variable infusion of 200 g · l⁻¹ glucose containing [3-³H]-glucose (0.1 MBq · ml⁻¹) [45]. After a 130-min equilibration period, stimulated glucose turnover and whole body insulin sensitivity were measured between 300 and 330 min. Arterial blood samples (5 ml) were therefore obtained at 300, 310, 320 and 330 min for the determination of insulin and metabolite concentrations and glucose specific radioactivity.

Intravenous glucose tolerance tests (IVGTTs) were performed before and after the studies (see above). After an

overnight fast, 0.5 kg⁻¹ of 500 g · l⁻¹ glucose was administered via the jugular vein catheter over 1 min followed by a 30 ml flush of 0.15 mol · l⁻¹ · NaCl. Arterial blood samples (5 ml) were taken for glucose, insulin, and C-peptide measurement at 2-min intervals until 10 min and then at 5-min intervals until 60 min post-injection.

Lipid measurements. A 20 ml fasting blood sample was obtained from each animal prior to diabetes induction and during the last week of peripheral and hepatic insulin delivery studies. Samples were allowed to clot and following centrifugation (800 × g for 15 min at 4°C) serum was divided into 3-ml aliquots and added to 50 ml of a preservative "cocktail" consisting of 1.0 mmol · l⁻¹ · Na₂-EDTA (BDH, Lutterworth, UK), 1.0 mmol · l⁻¹ phenylmethylsulfonylfluoride (BDH) in dimethylsulfoxide (BDH), and 0.1 mmol · l⁻¹ butylated hydroxytoluene (Sigma) dissolved in absolute ethanol (BDH). Samples were stored at 4°C and were assayed less than 24 h after being obtained.

Analytical procedures. Glucose specific activity was measured in plasma samples deproteinized with ZnSO₄ and Ba(OH)₂ by the method of Somogyi [46]. The supernatant was freeze-dried (Edwards Modulyo; Edwards, Crawley, UK), redissolved in water and counted in Optiphase Hisafe II scintillant (LKB, Turku, Finland) on a liquid scintillation counter (Beckman).

Blood for lactate, alanine, pyruvate, glycerol and 3-hydroxybutyrate assays was collected into chilled perchloric acid (0.77 mol · l⁻¹). Measurement was by fluorometric assay (inter-assay CV 6 %) adapted for centrifugal analysis [47]. The NEFA concentrations were colourimetrically determined using a commercially available kit (Wako Chemicals, Neuss, Germany) on a centrifugal analyser (interassay CV 3 %). Serum insulin was measured by double step [48] radioimmunoassay (RIA) (interassay CV 7.5 %). Porcine C-peptide was determined using an RIA kit employing a double antibody (interassay CV 6.1 %; Linco, St. Louis, Mo., USA). Serum fructosamine, an index of blood glucose control over the preceding 2 weeks [49] was measured colourimetrically using a diagnostic kit (interassay CV 1.5 %; Sigma).

Lipoprotein fractions were separated by sequential flotation ultracentrifugation [50] in a Beckman 70.1 Ti fixed angle rotor (Beckman, Palo Alto, Calif., USA) at 20°C and 100000 × g. Separation of very low density lipoproteins (VLDL; *d* < 1.006 kg · l⁻¹), intermediate density lipoproteins (IDL; 1.006–1.019 kg · l⁻¹), low density lipoproteins (LDL; 1.019–1.063 kg · l⁻¹) and high density lipoproteins (HDL; 1.063–1.21 kg · l⁻¹) was performed in 24-h steps as described previously [51, 52]. Total and free cholesterol, triglyceride and phospholipid in total serum and lipoprotein fractions were measured colourimetrically using cholesterol CII, triglyceride N and phospholipid kits, respectively, from Wako on a Cobas Bio centrifugal analyser (Roche, Welwyn Garden City, UK). In the serum and lipoprotein subfraction, the interassay CV was 2.2 and 3.2 % for total cholesterol and triglyceride, respectively. The interassay CV for phospholipid ranged from 0.22 % when measured in serum and the LDL fraction to 2.9 % when measured in the HDL fraction. Free cholesterol interassay CV ranged from 0.70 % when measured in serum and the LDL fraction to 7.2 % when measured in the VLDL fraction. Protein concentration was measured by a modified Lowry technique [53] on a Cobas Bio centrifugal analyser. The interassay CV was 5.7 %.

Calculations. The metabolic clearance rate (MCR) of insulin (ml · kg⁻¹ · min⁻¹) was calculated for the basal period of the clamp (0–150 min) from the ratio of the basal peripheral or

Table 1. Body weight, pre- and postprandial whole blood glucose, 24 h insulin dose, serum fructosamine concentration and 30-min post-IVGTT (0.5 g/kg) serum C-peptide in six animals

before induction of diabetes and during the last week of peripheral and portal insulin delivery

	Baseline	Peripheral infusion	Portal infusion	Infusion group mean difference
Body weight (kg)	34.8 ± 1.6	37.8 ± 1.0	37.4 ± 0.9	0.47 ± 0.71
Stimulated C-peptide (nmol · l ⁻¹)	0.97 ± 0.1	0.04 ± 0.004 ^c	0.04 ± 0.008 ^c	-0.001 ± 0.009
Blood glucose (mmol · l ⁻¹)				
Fasting	3.1 ± 0.1	4.9 ± 0.3 ^a	5.0 ± 0.5 ^a	-0.22 ± 0.42
Postprandial	3.5 ± 0.2	6.4 ± 0.4 ^b	6.0 ± 0.8 ^a	0.35 ± 0.92
Serum fructosamine (mmol · l ⁻¹)	1.3 ± 0.1	2.1 ± 0.1 ^c	2.0 ± 0.1 ^c	0.03 ± 0.09
Insulin dose (nmol · kg ⁻¹ · day ⁻¹)	–	2.4 ± 0.1	2.6 ± 0.3	-0.24 ± 0.36

Mean ± SEM

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ compared to baseline

portal insulin infusion rate and the corresponding mean arterial serum insulin level. Towards the end of the clamp period the MCR was similarly calculated except that the infusion rate of the peripherally administered insulin ($300 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was used. The rates of glucose appearance (R_a) and glucose disappearance (R_d) were isotopically determined during the basal (120–150 min) and stimulated (300–330 min) periods of the clamp using the isotope-dilution equation $R_a = F/SA$; where F is the infusion rate of tritiated glucose ($\text{dpm min}^{-1} \cdot \text{kg}^{-1}$) and SA is the specific radioactivity of glucose ($\text{dpm} \cdot \text{mg}^{-1}$). Hepatic glucose output (HGO) was taken to be equivalent to R_a during the basal clamp period and was calculated for the insulin stimulated period of the clamp by subtraction of the glucose infusion rate from the R_a . The MCR of glucose ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was calculated during the basal clamp period from the ratio of R_d and the corresponding mean plasma glucose value and during the stimulated period by the ratio of mean glucose infusion rate and the corresponding mean plasma glucose value.

Statistical analysis. Unless otherwise stated, all results are presented as the mean ± SEM for variables measured in six pigs. Differences between and within groups were assessed by Student's two-tailed paired t -test.

Results

Validity of model. Routine biochemical and haematological examinations performed on the pigs throughout the experimental period were consistent with the animals being in good health during the studies. The body weights of diabetic animals after 4 weeks of peripheral ($37.8 \pm 1.0 \text{ kg}$) or portal ($37.4 \pm 0.9 \text{ kg}$) insulin delivery were not significantly different and did not differ from those measured prior to streptozotocin administration ($34.8 \pm 1.6 \text{ kg}$) (Table 1).

Serum C-peptide levels were highest in control animals ($0.97 \pm 0.11 \text{ nmol} \cdot \text{l}^{-1}$) 15 min after glucose infusion, but were close to undetectable in diabetic animals ($0.04 \pm 0.00 \text{ nmol} \cdot \text{l}^{-1}$, $p < 0.001$) (Table 1), and at all other times tested (results not shown). Pre- and postprandial blood glucose concentration was maintained within the 5.0 – $10.0 \text{ mmol} \cdot \text{l}^{-1}$ target range from week 1 through to week 4 of each study.

The results for week 4 are given in Table 1. While glucose concentrations in the diabetic animals were significantly ($p < 0.001$) higher than controls at 10.00 and 16.00 hours, the mean blood glucose concentrations were comparable in the two insulin infusion groups (Table 1). At week 4 serum fructosamine levels were significantly ($p < 0.001$) higher than pre-diabetic values ($1.3 \pm 0.1 \text{ mmol} \cdot \text{l}^{-1}$) in both the peripherally ($2.1 \pm 0.1 \text{ mmol} \cdot \text{l}^{-1}$) and portally ($2.0 \pm 0.1 \text{ mmol} \cdot \text{l}^{-1}$) infused groups, but with no difference between these groups (Table 1).

Insulin dose and serum concentration. The daily insulin dose requirement at around $2.4 \text{ nmol} \cdot \text{kg}^{-1}$ was similar in the two groups of diabetic animals (Table 1). The dose was not different between the groups from week 1 to week 4 (results not shown) and incorporated basal insulin infusion rates of 0.96 ± 0.2 and $1.1 \pm 0.1 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the peripheral and portal infused animals, respectively. However, the fasting arterial insulin concentration with peripheral insulin infusion at 4 weeks was almost two times ($p < 0.05$) higher than after 4 weeks of portal insulin infusion 124 ± 16 vs $73.8 \pm 5.4 \text{ pmol} \cdot \text{l}^{-1}$, $p < 0.05$) (Table 2). The fasting levels in the peripherally infused animals were also around 70 % higher than those measured in the control state ($68.4 \pm 3.6 \text{ pmol} \cdot \text{l}^{-1}$, $p < 0.01$) (Table 2). The MCR of insulin during the basal period was significantly higher ($p < 0.05$) in the portally infused than in the peripherally infused pigs (15.9 ± 2.0 vs $8.5 \pm 1.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (Table 2). By the final 30 min of the clamps jugular vein insulin infusion resulted in serum insulin concentrations which were similar in the normal control ($381 \pm 44 \text{ pmol} \cdot \text{l}^{-1}$), previously portally infused ($388 \pm 66 \text{ pmol} \cdot \text{l}^{-1}$) and previously peripherally infused ($349 \pm 44 \text{ pmol} \cdot \text{l}^{-1}$) animals (Table 2). Similar insulin MCRs of approximately $15 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ were calculated for the last 30 min of the clamp in each of the three groups (Table 2).

Glucose appearance and disposal. Plasma glucose levels during both the basal and insulin-stimulated

Table 2. Serum insulin concentration and MCR, serum NEFA and triglyceride and whole-blood metabolite concentration at basal (0–150 min) and insulin stimulated (300–330 min) steady

states during the clamp in normal and diabetic pigs after receiving 4 weeks of insulin peripherally or hepatically

	Baseline	Peripheral infusion	Portal infusion	Infusion group mean difference
Serum insulin (pmol · l ⁻¹)				
Basal	68.4 ± 3.6	124 ± 16 ^{a,c}	73.8 ± 5.4	50.4 ± 29
Clamp	381 ± 44	349 ± 44	388 ± 66	-37.2 ± 60
Insulin MCR (ml · kg ⁻¹ · min ⁻¹)				
Basal	–	8.5 ± 1.1 ^a	15.9 ± 2.0	-7.4 ± 2.2
Clamp	14.0 ± 1.6	15.9 ± 2.7	14.4 ± 1.9	1.4 ± 2.0
Pyruvate (μmol · l ⁻¹)				
Basal	45.5 ± 6.6	45.8 ± 4.9	55.5 ± 5.4	5.3 ± 9.3
Clamp	60.5 ± 5.6	53.3 ± 3.9	61.3 ± 3.8	-9.7 ± 11
Lactate (μmol · l ⁻¹)				
Basal	505 ± 45	508 ± 38	533 ± 45	-32.3 ± 74
Clamp	636 ± 58	651 ± 36 ^d	736 ± 62 ^e	-24.8 ± 70
Alanine (μmol · l ⁻¹)				
Basal	196 ± 27	178 ± 21	199 ± 23	-48.5 ± 34
Clamp	168 ± 23	122 ± 9 ^{b,d}	150 ± 19	-21.1 ± 37
Glycerol (μmol · l ⁻¹)				
Basal	44.2 ± 9.7	69.8 ± 26	72.2 ± 26	-33.3 ± 26
Clamp	24.0 ± 7.3	51.7 ± 21	49.7 ± 22	-2.4 ± 31
3-hydroxybutyrate (μmol · l ⁻¹)				
Basal	13.5 ± 1.6	20.3 ± 3.1	16.4 ± 2.9	1.2 ± 4.1
Clamp	9.7 ± 2.4 ^d	14.3 ± 1.4	11.4 ± 3.5	3.9 ± 4.6
Serum NEFA (mmol · l ⁻¹)				
Basal	0.37 ± 0.06	0.26 ± 0.04	0.22 ± 0.05	-0.08 ± 0.02
Clamp	< 0.05	< 0.05	< 0.05	< 0.05
Serum triglyceride (mmol · l ⁻¹)				
Basal	0.22 ± 0.03	0.22 ± 0.02	0.24 ± 0.02	-0.01 ± 0.03
Clamp	0.13 ± 0.01 ^e	0.13 ± 0.01 ^f	0.17 ± 0.02	-0.04 ± 0.01

Mean ± SEM

^a $p < 0.05$ vs portal infusion; ^b $p < 0.05$, ^c $p < 0.01$ vs baseline; ^d $p < 0.05$, ^e $p < 0.01$, ^f $p < 0.001$ basal vs stimulated

periods of the clamp were comparable in the experimental groups, but approximately 3.0 mmol · l⁻¹ higher than in control animals (Table 3). The CV for plasma glucose over the time of measurement of glucose disposal during the clamps was 8.4 ± 1.0%. The plasma glucose specific radioactivity (SA) in each of the three study groups did not change significantly over the clamp period (Fig. 1) mean basal SA being 886 ± 72 dpm · mmol⁻¹ and CV 6.2 ± 1.3%, while clamp SA was 724 ± 43 dpm · mmol · l⁻¹ with a CV of 5.4 ± 0.8%.

After 4 weeks of portally infused insulin, measured fasting HGO was not different from that determined in the pre-streptozotocin treated animals (3.0 ± 0.3 vs 2.9 ± 0.5 mg · kg⁻¹ · min⁻¹) (Table 3). The fasting HGO measured in the group previously peripherally infused was, however, more than 25% ($p < 0.05$) higher than that measured in the group previously portally infused or in normal control animals (Table 3). No significant difference existed between the three experimental groups in the basal MCR of glucose (Table 3). By the end of the clamp, HGO had fallen by a similar amount in the non-diabetic animals (3.9 ± 0.8 mg · kg⁻¹ · min⁻¹), the peripherally

infused animals (4.5 ± 0.9 mg · kg⁻¹ · min⁻¹) and the portally infused animals (3.9 ± 0.5 mg · kg⁻¹ · min⁻¹). Suppressed HGO at the end of the clamp was not significantly different between the three groups (Table 3).

Glucose requirement over the last 30 min of the clamp was not different in the peripherally infused (9.6 ± 1.0 mg · kg⁻¹ · min⁻¹) and normal control animals (8.1 ± 0.3 mg · kg⁻¹ · min⁻¹). In the portally infused group the glucose requirement (11.4 ± 0.6 mg · kg⁻¹ · min⁻¹) was higher ($p < 0.01$) than in controls but not significantly different from that in the peripherally infused group (Table 3). Compared with pre-diabetic values glucose MCR were lower ($p < 0.05$) in the diabetic animals after 28 days of either peripheral (-30%) or portal (-23%) insulin delivery (Table 3).

Blood intermediary metabolites. The steady-state fasting (0–150 min) and end-of-clamp (300–330 min) concentrations of whole-blood pyruvate, lactate, alanine, glycerol and 3-hydroxybutyrate and serum NEFAs and triglycerides are shown in Table 2. There were no significant differences in the fasting

Table 3. Isoglycaemic hyperinsulinaemic clamp data in normal and diabetic pigs after 4 weeks of peripheral and hepatic insulin delivery

	Control	Peripheral infusion	Portal infusion	Infusion group mean difference
Plasma glucose (mmol/l)				
Basal	4.1 ± 0.1	7.5 ± 0.3 ^c	6.2 ± 0.9	2.0 ± 1.4
Clamp	3.9 ± 0.4	7.0 ± 0.3 ^b	7.1 ± 0.4	-0.1 ± 0.5
Hepatic glucose output (mg · kg ⁻¹ · min ⁻¹)				
Basal	3.0 ± 0.3	4.2 ± 0.4 ^{a,d}	2.9 ± 0.4	1.4 ± 0.5
Clamp	-0.9 ± 0.6 ^c	-0.3 ± 0.8 ^e	-1.2 ± 0.5 ^f	0.9 ± 0.5
Glucose requirement (mg · kg ⁻¹ · min ⁻¹)	8.1 ± 0.3	9.6 ± 1.0	11.4 ± 0.6 ^b	-0.9 ± 1.3
Glucose MCR (ml · kg ⁻¹ · min ⁻¹)				
Basal	4.1 ± 0.4	3.2 ± 0.4	3.1 ± 0.9	1.5 ± 0.5
Clamp	11.7 ± 1.0	8.1 ± 1.0 ^a	9.0 ± 0.7 ^a	-1.8 ± 1.1

Mean ± SEM

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ compared to control;^d $p < 0.05$ compared to portal infusion;^e $p < 0.01$, ^f $p < 0.001$ compared to baseline

concentrations of these substrates between pre-streptozotocin treated animals and diabetic animals receiving insulin peripherally or portally (Table 2). Similarly, after 4 weeks of insulin infusion treatment, the route of insulin delivery did not appear to affect fasting intermediary metabolite concentrations (Table 2).

However, end of clamp alanine levels in the peripherally insulin-treated animals were significantly lower than those measured in animals before streptozotocin treatment ($p < 0.05$) (Table 2). No other significant differences were found between the three study groups with respect to substrate levels during this period of hyperinsulinaemia (Table 2). In the normal control animals, pyruvate and lactate concentrations tended to increase during the clamp while those of alanine, glycerol, 3-hydroxybutyrate, triglyceride and NEFA tended to fall, but only for serum NEFA and triglyceride was this change statistically significant ($p < 0.05$, Table 2). Following peripheral insulin delivery whole blood lactate concentrations increased significantly during the clamp ($p < 0.05$), alanine, triglycerides and NEFA decreased ($p < 0.05$) and pyruvate, 3-hydroxybutyrate and glycerol remained unchanged (Table 2). After portal insulin infusion, lactate levels also increased during the clamps ($p < 0.05$), those of NEFA decreased ($p < 0.05$), while those of serum triglyceride and blood alanine, glycerol, pyruvate and 3-hydroxybutyrate were not detectably altered (Table 2).

Serum lipoproteins. The results obtained in the lipoprotein fractionation experiments along with the serum lipid levels for the non-diabetic and two diabetic study groups are shown in Table 4. The total fasting serum cholesterol, triglyceride, free cholesterol and phospholipid concentrations in the two insulin treated groups were not significantly different from one another or from those in normal pigs (Table 4). Lipid levels in the lipoprotein subfractions were also not significantly different, but there was a consistent

trend for total and free cholesterol, phospholipid and total protein in the LDL fraction to be higher after 4 weeks of peripherally infused insulin (Table 4). However, the total protein content of the HDL subfraction of these animals was significantly higher ($p < 0.05$) than in animals receiving portally infused insulin (96.1 ± 3.5 vs 86.3 ± 3.2 mg · dl⁻¹) (Table 4).

Discussion

The pig by virtue of its considerable dietary, metabolic, endocrine and anatomical similarity to man is eminently suited as a model in which to examine the importance of the restoration of the portal-systemic insulin gradient for the continuance of normal intermediary and lipid metabolism and glucose turnover. The negative C-peptide response to glucose confirmed that the animals were rendered completely insulin deficient by streptozotocin and remained so throughout the experiments. Blood glucose control was equivalent in the groups of diabetic animals regardless of the route of insulin delivery. The similar serum fructosamine levels obtained would be equivalent (on the basis of a normal mean of 1.3 mmol/l) to an HbA_{1c} of approximately 7.5% on the Diabetes Control and Complications trial (DCCT) assay [54].

Despite similar insulin requirements, the fasting insulin levels in the peripherally infused group were almost twice those of the controls or the portally infused animals. Hence basal insulin clearance rates in the portally infused animals were higher than in those peripherally infused. In contrast during the hyperinsulinaemic clamp period, when insulin was always peripherally infused, clearance rates were approximately the same in controls and in the two infusion groups. The lower rates calculated in the peripherally infused animals are consistent with the infused insulin escaping 'first-pass' hepatic degradation which

Table 4. Serum and lipoprotein lipids in normal and diabetic pigs during the fourth week of peripheral or hepatic insulin delivery

		Lipoprotein subfraction			
		Total serum	VLDL	LDL	HDL
Cholesterol (mmol^{-1})	Control	1.7 ± 0.1	0.07 ± 0.01	0.60 ± 0.12	0.50 ± 0.04
	Peripheral infused	1.9 ± 0.1	0.09 ± 0.01	0.82 ± 0.08	0.57 ± 0.04
	Portal infused	1.8 ± 0.2	0.09 ± 0.01	0.67 ± 0.01	0.53 ± 0.02
	Infusion group mean difference	0.08 ± 0.15	0 ± 0	0.15 ± 0.08	0.03 ± 0.05
Triglycerides (mmol^{-1})	Control	0.30 ± 0.04	0.13 ± 0.03	< 0.05	< 0.05
	Peripheral infused	0.32 ± 0.06	0.14 ± 0.04	< 0.05	< 0.05
	Portal infused	0.33 ± 0.06	0.13 ± 0.04	< 0.05	< 0.05
	Infusion group mean difference	-0.02 ± 0.08	0.16 ± 0.04	0 ± 0	0 ± 0
Free cholesterol ($\text{mg} \cdot \text{dl}^{-1}$)	Control	17.3 ± 1.6	0.70 ± 0.22	8.4 ± 0.15	6.5 ± 0.70
	Peripheral infused	17.7 ± 1.2	0.39 ± 0.16	9.2 ± 0.79	5.4 ± 0.47
	Portal infused	17.7 ± 1.6	0.53 ± 0.14	8.3 ± 1.0	4.9 ± 0.48
	Infusion group mean difference	0.03 ± 1.9	-0.22 ± 0.27	0.9 ± 1.1	0.5 ± 0.76
Phospholipid ($\text{mg} \cdot \text{dl}^{-1}$)	Control	74.1 ± 5.0	1.7 ± 0.39	13.0 ± 2.4	36.0 ± 2.3
	Peripheral infused	78.2 ± 6.6	1.5 ± 0.42	17.6 ± 2.0	37.5 ± 4.4
	Portal infused	73.2 ± 6.4	1.7 ± 0.40	14.7 ± 1.8	29.5 ± 2.5
	Infusion group mean difference	5.0 ± 3.4	-0.13 ± 0.63	2.8 ± 2.0	8.0 ± 5.0
Protein ($\text{mg} \cdot \text{dl}^{-1}$)	Control	–	1.4 ± 0.89	18.4 ± 2.8	89.6 ± 6.1
	Peripheral infused	–	1.9 ± 0.60	25.4 ± 2.4	96.1 ± 3.5^a
	Portal infused	–	3.0 ± 0.92	22.8 ± 1.8	86.3 ± 3.2
	Infusion group mean difference	–	-0.23 ± 1.0	2.6 ± 2.5	9.8 ± 3.7

Mean $\pm$ SEM^a $p < 0.05$ compared with portal infusion **Fig. 1.** ^3H Glucose specific radioactivity before (150 min) and during the insulin stimulated clamp period (300–330 min) in control animals ($\blacktriangle$) and in animals following 4 weeks of either peripheral ($\blacksquare$) or portal vein ($\bullet$) insulin infusion. $p < 0.05$ intravenous vs portal insulin infusion at 150 min

can remove between 50 and 75% of insulin in the fasting state [55].

A lack of agreement exists among previous animal investigations, performed in rodents or dogs, concerning whether or not insulin delivery by the peripheral route results, as in our study, in raised [3, 7, 9, 13, 17–21] or unchanged [2, 6, 11, 14–16] peripheral insulin levels. Studies in man comparing intraperitoneal and subcutaneous insulin delivery have similarly yielded conflicting results relating to the extent of systemic insulinisation [23–28, 56]. However,

the systemic delivery of insulin which accompanies human pancreas transplantation has generally been found to lead to fasting peripheral hyperinsulinaemia [29–37].

Traditionally it has been assumed that hepatic glucose production is controlled by a direct effect of insulin on the liver and that adequate control using conventional therapy can only be achieved by raising peripheral insulin to supranormal concentrations. Despite peripheral hyperinsulinaemia in the peripherally infused group, however, in conjunction with equivalent blood glucose control, HGO remained considerably higher than in either the prediabetic animals or those on portal insulin delivery. Thus the levels of insulin achieved in the peripherally infused group may still not have been high enough to effectively control HGO. Indeed HGO was successfully switched-off when insulin levels were further raised during the clamps.

The importance of peripheral insulin concentrations in indirectly controlling HGO has steadily gained recognition [1, 5, 57–60]. In the present study fasting three-carbon substrate and NEFA concentrations were not dissimilar in the three groups. These results lend support to the concept that the long term control of HGO is not solely through a peripheral action of insulin on gluconeogenic substrate flux. It is possible nonetheless that the hyperinsulinaemia in the peripherally infused animals may have altered the activity and/or concentration of some other 'peripheral messenger' thereby contributing to the increases in HGO observed.

In the present study using the stimulated MCR of glucose as a measure of extrahepatic insulin responsiveness, it was found that neither route of insulin infusion was capable of restoring this variable to pre-diabetic values. However, by using a single relatively high dose of insulin in these clamp experiments without employing an additional low dose infusion, subtle differences between the two infusion groups in insulin sensitivity may have been missed. The lowered glucose clearance rate in both the portal and peripheral infusion groups is likely to be a result of the chronically raised glucose levels in both groups. This may involve defective GLUT 4 intrinsic activity and/or translocation. Indeed abnormal transporter translocation has been previously documented in diabetic rats [61]. Had it been possible to employ a more sophisticated insulin infusion algorithm capable of normalising blood glucose concentrations it is likely that normal insulin sensitivity would have been restored. A recent study in rats found that islet grafts providing normal insulin supply to the portal vein restored insulin sensitivity to normal, something which was not observed in rat islet grafts with peripheral venous drainage [62].

Studies in humans which have examined the relative impact of subcutaneous compared to intraperitoneal insulin administration on lipid metabolism have generally [26, 63–65], although not always [24, 66], found the intraperitoneal administration to be superior in normalising lipid metabolism. The characteristic dyslipidaemia of IDDM patients involves hypertriglyceridaemia and lowered HDL cholesterol concentrations [67]. However, the only significant difference between the two diabetic groups observed in this study was in HDL protein levels. Raised HDL cholesterol levels have previously been recorded in intensively treated IDDM patients [68], but no differences in the Apo A-1 lipoprotein content which constitutes 70 to 80% of total HDL protein (the predominant pig HDL lipoprotein has a similar structure and function to human Apo A-1 [69] were observed in a subsequent study [70]. Serum LDL cholesterol content is generally normal in well controlled IDDM patients although increases have been observed in poorly controlled patients [67]. Hyperinsulinaemia is generally associated with increased LDL catabolism [71] although it is likely this effect could be modulated by nonenzymatic glycation of LDL. The mechanisms responsible for the apparent rise in LDL cholesterol, protein and phospholipid in the peripherally infused animals are currently under study.

In conclusion, the present study found that when compared in the same six pigs, peripheral and portal insulin delivery were equally effective in maintaining relative control of blood glucose, intermediary metabolite and lipid concentrations. However, this was accompanied by higher fasting insulin and HGO levels in the peripherally infused animals. Neither infusion

route was capable of restoring normal rates of glucose clearance. The results therefore indicate that in this diabetic model the portal-peripheral insulin gradient is a prerequisite for the maintenance of both normal hepatic metabolism and peripheral insulin levels during the fasting period. Furthermore, even relatively mild hyperglycaemia has detrimental metabolic effects regardless of the route of insulin infusion.

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