Use of the hyperinsulinemic euglycemic clamp to assess insulin sensitivity in guinea pigs: dose response, partitioned glucose metabolism and species comparisons

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Abstract

The guinea pig is an alternate small animal model for the study of metabolism, including insulin sensitivity. However, only one study to date has reported the use of the hyperinsulinemic-euglycemic clamp (HEC) in anaesthetized animals in this species, and the dose-response has not been reported. We therefore characterized the dose-response curve for whole-body glucose uptake using recombinant human insulin in the adult guinea pig. Inter-species comparisons with published data showed species differences in maximal whole body responses (guinea pig ≈ human < rat < mouse), and the insulin concentrations at which half-maximal insulin responses occurred (guinea pig > human ≈ rat > mouse). In subsequent studies, we used concomitant D-[3-3H]-glucose infusion to characterize insulin sensitivities of whole body glucose uptake, utilization, production, storage and glycolysis in young adult guinea pigs at human insulin doses that produced ~half (7.5 mU.min⁻¹.kg⁻¹) and near-maximal whole body responses (30 mU.min⁻¹.kg⁻¹). Although human insulin infusion increased rates of glucose utilization (up to 68%) and storage, and at high concentrations increased rates of glycolysis in females, glucose production was only partially suppressed (~23%), even at high insulin doses. Fasting glucose, metabolic clearance of insulin and rates of glucose utilization, storage and production during insulin stimulation were higher in female than male guinea pigs (P<0.05), but insulin sensitivity of these and whole body glucose uptake did not differ between sexes. This study establishes a method for measuring partitioned glucose metabolism in chronically catheterized conscious guinea pigs, allowing studies of regulation of insulin sensitivity in this species.

Key words: guinea pig, insulin sensitivity, sex differences, species differences
Introduction

Insulin secretion and its actions on glucose metabolism in vivo have been characterized in a range of species, using chronically catheterized preparations to reduce the impact of the stress of anesthesia and surgery. The hyperinsulinemic-euglycemic clamp (HEC, 8) is considered the reference standard method for measurement of insulin sensitivity (39). Concomitant administration of radio-labelled glucose during the HEC also allows the metabolic fate of glucose and insulin actions to be partitioned between central and peripheral actions. This approach has been used extensively in small mammalian species, such as the mouse and rat, to investigate the insulin axis under normal and pathophysiological conditions (1, 20, 29, 43, 52). An alternative animal model is the guinea pig, which resembles the human in its susceptibility to diabetes (32, 33) and diet-induced elevated circulating LDL cholesterol and atherosclerosis (12). However, to date, use of the HEC has only been reported in a single study in guinea pigs, investigating the effects of the chemotherapeutic agent cisplatin on insulin sensitivity (57). Only males were used in this study and they were assessed at a single insulin dose and under general anesthesia (57), which reduces whole body and hepatic insulin sensitivity in the rat (5). Validation of the methodology for the HEC and characterization of both whole body and partitioned insulin action on glucose metabolism in conscious male and female guinea pigs is required to enable further study of insulin sensitivity and its regulation in this species.

The sequence of insulin in hystricomorph mammals such as guinea pig is relatively divergent from that of other mammals, sharing ~65% homology across the entire sequence, whereas the sequence of the A- and B-regions of insulin are tightly conserved (90-95%) in other mammals (61). This difference in sequence translates to lower activity of guinea pig insulin compared to that of other species. For example, guinea pig insulin has only ~10% of the potency of bovine insulin as a stimulus for glucose oxidation in rat tissues (64), and is ~3% as potent as porcine, bovine or recombinant human insulin in stimulating glucose oxidation in rat adipocytes (25). In contrast, the insulin receptors of guinea pigs, humans and rats share similar binding affinities for chicken and porcine
insulin (37), suggesting that responses to a single type of insulin may be similar across species. In vivo metabolic responses to human insulin have not been compared between guinea pig and other species, and whether the guinea pig responds sufficiently to human insulin to allow assessment of changes in insulin receptor number and downstream signaling has not been established.

Hence, the aim of this study was to characterize the human insulin dose-response curve for whole body glucose metabolism in chronically catheterized guinea pigs using the HEC, and compare that to dose responses to human insulin in other species. Secondly, we measured whole body glucose utilization, production, glycolysis and storage, and their responsiveness to human insulin in chronically catheterized young adult guinea pigs and compared these between males and females.

**Materials and Methods**

**Animals**

All animal studies were approved by the Animal Ethics Committee of the University of Adelaide (Approval number M56/96). Adult male guinea pigs (n=8, 3-4 months of age, Institute of Medical and Veterinary Science Tri-coloured, Gilles Plains Resource Centre, Gilles Plains South Australia) were used for the initial insulin dose response study. Management of animals used for all additional experiments was as described previously (18). Nulliparous female guinea pigs (3-4 months of age) were entered into a mating program, and their progeny were studied as young adults at 100 days of age. All animals were housed under 12:12 hour light:dark conditions and had *ad libitum* access to a commercial guinea pig/rabbit ration modified with an increased content of vitamin E (165 mg.kg⁻¹), except when fasted for HEC studies as described below, and free access to tap water supplemented with ascorbic acid (400 mg.L⁻¹).

**Catheterization**
A total of 84 guinea pigs underwent surgery for the insertion of vascular catheters. Body weight in the males ranged from 678 g to 942 g (Mean ± SEM, 806 ± 11 g, n=45) and in the females from 562 g to 806 g (686 ± 10 g, n=39). Male and female animals were reproductively intact and females were spontaneously cycling. Catheters were inserted into the right jugular vein (Silastic tubing, 0.51 mm ID, 0.94 mm OD, sleeved onto polyvinyl, 0.5 mm ID, 1.00 mm OD) and right carotid artery (Polyvinyl tubing, 0.4 mm ID, 0.8 mm OD, sleeved into polyvinyl, 0.58 mm ID, 0.96 mm OD) under general anesthesia induced by ketamine (75 mg kg⁻¹ body weight, intraperitoneal) and xylazine (6 mg kg⁻¹ body weight, intramuscular). Both carotid and jugular catheters were successfully implanted in 80 animals (95%). Catheters were maintained by flushing daily with heparinized saline (500 U ml⁻¹).

Patent carotid and jugular catheters were present in 78%, 68%, 58% and 40% of the guinea pigs at 5, 7, 10 and 14 days after surgery, respectively. HEC studies commenced a minimum of 5 days after surgery and were successfully completed in 66 of the 80 catheterized guinea pigs.

Hyperinsulinemic-Euglycemic Clamps (HEC)

Guinea pigs were fasted for 16 h prior to each HEC, and HEC commenced at 0900 h. Extension lines, made from polyvinyl tubing, were attached to the catheters and exteriorized through the top of the cage allowing guinea pigs to remained unrestrained in their home cage during the experiment. Blood samples during the clamp were collected via the arterial catheter. Infusions of insulin and glucose were delivered via the jugular vein catheter.

Recombinant human insulin (Actrapid, Novo Nordisk, A/S, Denmark) was diluted in 0.9% NaCl to the required concentration and infused intravenously at a rate of 25 μl min⁻¹ for 120 minutes. In a subset of eight males, the effect of increasing the rate of insulin infusion was examined by performing separate, sequential HEC at insulin infusion rates of 7.5 (n=8), 15 (n=8), 30 (n=3) and 60 (n=4) mU min⁻¹ kg⁻¹. At least 3 days recovery was allowed between each HEC. Remaining HEC were then performed using insulin infusions of either 7.5 mU min⁻¹ kg⁻¹ (n=31 male, n=22 female) or 30 mU min⁻¹ kg⁻¹ (n=16 male, n=19 female). Twenty-five of these animals had HEC performed at both 7.5 and 30
mU.min\(^{-1}\).kg\(^{-1}\) insulin infusion rates. For these animals, doses were randomized, and at least 3 days recovery was allowed between clamps. These included nineteen animals in which partitioned glucose infusion was measured as described below.

Blood glucose was measured by glucometer (HemoCue AB, Sweden) in fasting samples collected 20, 15, 10, 5 and 0 minutes prior to the start of the insulin infusion, and in blood (50 - 100 μl) collected every 5 minutes throughout the HEC. Intravenous infusion of glucose (10% glucose, Baxter Healthcare, NSW, Australia) commenced 15 minutes after the start of the insulin infusion. The glucose infusion rate (GIR) was adjusted based on the blood glucose measurements to restore and maintain euglycemia, which was defined as the mean fasting blood glucose concentration. Glucose infusion rates were determined using a modified version (46) of the algorithm described by De Fronzo et al. (8).

A subset (n=19) of the animals were co-infused with D-[3-\(^{3}\)H]-glucose (Amersham Pharmacia Biotech, Buckinghamshire, England) to determine insulin sensitivity of hepatic and peripheral components of whole body glucose metabolism (peripheral glucose utilization and endogenous glucose production) during the 7.5 and 30 mU.min\(^{-1}\).kg\(^{-1}\) clamps. D-[3-\(^{3}\)H]-glucose, diluted in 0.9% NaCl, was administered as a priming bolus (14.5 μCi.kg\(^{-1}\)) followed by a continuous intravenous infusion (0.45 μCi.min\(^{-1}\).kg\(^{-1}\) at 25 μl.min\(^{-1}\)) for 120 minutes prior to the clamp. Intravenous infusion of D-[3-\(^{3}\)H]-glucose at 0.45 μCi.min\(^{-1}\).kg\(^{-1}\) then continued throughout the 2 h of the clamp, with the D-[3-\(^{3}\)H]-glucose included in the insulin infusion.

Larger arterial blood samples (500 μl) were collected at -20, -15, -10, -5, 0, 60, 75, 80, 85, 90, 95, 105 and 120 minutes from the start of the insulin infusion for subsequent analysis of radio-labelled metabolites, and human and guinea pig insulin. Blood was centrifuged at 3000 rpm for 15 minutes and plasma was removed and stored at -20°C. The total blood volume removed from each guinea pig during the experiment was approximately 7.5 ml (~12% of blood volume in a young adult guinea pig).
The average fluid volume infused throughout the 2 hours of the clamp was 9 ml in the 7.5 mU.min⁻¹.kg⁻¹ clamps and 11.5 ml for the 30 mU.min⁻¹.kg⁻¹ clamps.

Comparative whole-body insulin sensitivity of glucose metabolism

To identify studies in which the whole-body insulin sensitivity of glucose metabolism had been assessed, PubMed was searched ("dose response"[All Fields] AND ("insulin"[MeSH Terms] OR "insulin"[All Fields]) AND ("glucose"[MeSH Terms] OR "glucose"[All Fields]) AND clamp[All Fields]), initially on 24 March 2016 and updated on 12 October 2016, which identified a total of 497 sources. A subsequent more refined search in Web of Science on 16 October 2016 ("dose response" OR "dose curve") AND insulin AND glucose AND ("hyperinsulinemic euglycemic clamp" OR "hyperinsulinaemic euglycaemic clamp") identified 30 sources, including duplicates. Abstract screening from both searches was used to identify 139 papers that potentially described in vivo evaluation of insulin sensitivity using two or more doses of insulin, and that were written in English. An additional 18 sources were identified for full-text screening from reference lists of these papers. Subsequent full-text screening restricted papers to those that described in vivo whole-body net glucose uptake (glucose infusion rate) on a body weight basis in response to at least two doses of recombinant human insulin assessed by hyperglycemic euglycemic clamp in conscious, fasted, healthy adolescent or adult animals, and excluding states of perturbed or manipulated insulin sensitivity such as pregnancy, lactation, obesity and pharmacological treatments. Data for circulating insulin concentrations and net glucose uptake (glucose infusion rate) in the fasted (basal) state and at each insulin infusion rate were extracted and used to plot insulin dose responses for each study. Study characteristics of the 24 papers that met these criteria are summarized in Supplementary Table 1.

Analysis of human and guinea pig insulin

Human insulin concentrations were analyzed using a commercially available kit (Insulin-CT, CIS Bio International, France). Cross-reactivity of guinea pig insulin in this assay was <2%. Guinea pig insulin concentrations were measured by radioimmunoassay (14, 23), in plasma samples collected prior to (-
Guinea pig insulin was iodinated with Na\(^{125}\)I (Amersham Pharmacia-Biotech, Sydney, NSW, Australia) and chloramine T to specific activities of 35-50 Ci.g\(^{-1}\), and separated from reaction components by chromatography on Sephadex G50 (Amersham Pharmacia-Biotech, Sydney, NSW, Australia). Guinea pig insulin was measured in duplicate samples of guinea pig plasma and standards (0.1225 to 31.25 ng.ml\(^{-1}\)). The amount of guinea pig insulin that inhibited radioligand binding by 50% averaged 485 pg, while the CV for the same sample assayed on different occasions was 9.6% within assays, and 5.3% between assays.

*Plasma D-[\(3\)-\(3^H\)]-glucose and \(3^H\)\(_2\)O for partitioned glucose metabolism*

The specific activities of D-[\(3\)-\(3^H\)]-glucose and of \(3^H\)\(_2\)O were measured in plasma samples collected prior to and during the HEC (-20, -15, -10, -5, 0, 60, 75, 80, 85, 90, 95, 105, 120 minutes), using methods based on previous studies in the rat (26, 27). Samples were deproteinised using 0.3N Ba(OH)\(_2\) and 0.3N ZnSO\(_4\). Duplicate 40 μl aliquots of the deproteinised supernatant were dried at 55°C for 60 minutes, and reconstituted in an equivalent volume of water. The \(3^H\) content of duplicate, 40 μl undried aliquots of supernatant and the dried aliquots was measured by β-scintillation counting (Beckman Coulter Inc., Fullerton CA), following overnight equilibration with 1 ml NCS- II Tissue Solubilizer 0.5N (Amersham International Arlington Heights, IL) and 10 ml Ready Organic Scintillant (Beckman Coulter Inc.). The DPM of the dried (radioactivity of D-[\(3\)-\(3^H\)]-glucose) vials was subtracted from that of the undried vials (radioactivity of D-[\(3\)-\(3^H\)]-glucose + \(3^H\)\(_2\)O) to give the DPM of \(3^H\)\(_2\)O alone. Plasma glucose concentrations were measured in samples collected at the same time points by colorimetric enzymatic analysis on a COBAS Mira automated centrifugal analyser (Glucose HK, Roche).

*Calculations*

Basal glucose concentrations were calculated by averaging values in samples collected -20, -15, -10, -5, and 0 minutes prior to commencing the insulin infusion. Steady-state plasma concentrations of
guinea pig and human insulin were calculated as the average of concentrations measured every 15 minutes throughout the final hour of the HEC (60-120 minutes). Steady state glucose infusion rate (ssGIR) was calculated as the average GIR during the final hour of the clamp. Whole body insulin sensitivity was calculated by dividing ssGIR by steady-state plasma concentrations of human insulin. The post-hepatic metabolic clearance rate (MCR) of human insulin was calculated as the insulin infusion rate during the HEC divided by steady-state plasma concentrations of human insulin.

**Glucose utilization:** A steady state plateau of plasma D-[3-3H]-glucose specific activity was achieved during the last 20 to 40 minutes of D-[3-3H]-glucose infusion in the basal state and during HEC at insulin infusion of 7.5 and 30 mU.min⁻¹.kg⁻¹, similar to the timing reported in HEC studies in the rat (28). The rate of glucose utilization was calculated by dividing the D-[3-3H]-glucose infusion rate by the steady-state plasma D-[3-3H]-glucose specific activity during this steady-state period (26, 27).

**Glucose production:** In the basal state, the rate of whole body glucose utilization is equal to the rate of endogenous glucose production. In the insulin-stimulated state, the rate of whole body glucose utilization equals the rate of endogenous glucose production plus the exogenous glucose infusion. Therefore, endogenous glucose production was calculated as the rate of whole body glucose utilization minus exogenous glucose infusion (26, 27).

**Glycolysis and glucose storage:** The ³H in the C-3 position of D-[3-³H]-glucose is lost selectively to H₂O during glycolysis (50). Rates of whole body glycolysis were determined from the rate of increase in ³H₂O multiplied by the total body water mass and divided by the D-[3-³H]-glucose specific activity. Plasma H₂O was assumed to be 93% of plasma volume, and the mass of H₂O in the body was assumed to be 65% of total body weight, based on estimates in rodents (26, 27). The rate of whole body glycolysis was determined during the last 15 minutes of D-[3-³H]-glucose infusion in the basal state, and the last 60 minutes of insulin infusion. Glucose storage in each state was calculated as the difference between total glucose utilization and glycolysis.

**Statistical analysis**
Data were analyzed using SPSS 24.0 for Windows (IBM, Armonk, USA) with individual guinea pig as the unit of analysis. Due to inclusion of different animals in studies at each insulin dose, the effect of sex was determined by analysis of variance separately at each dose. The effects of recombinant human insulin infusion on plasma guinea pig insulin concentrations, and on components of glucose metabolism were determined using paired two-tailed t-tests. Significance was accepted at $P < 0.05$, and results are expressed as mean ± SEM.

**Results**

**Insulin dose response of whole body glucose metabolism**

Blood glucose levels during the steady state period of insulin infusion were similar to fasting blood glucose levels at all doses of insulin studied (Table 1). The coefficient of variation of blood glucose during the 60-120 minute period of the clamp ranged from 6.5% to 10.5% at differing insulin doses (Table 1). Circulating human insulin concentration and steady state glucose infusion rate increased with increasing infusion rate of human insulin up to doses of 30 mU.min$^{-1}$.kg$^{-1}$ (Table 1, Figure 1). Compared to fasting values, the circulating concentrations of guinea pig insulin were reduced by infusion of human insulin at doses of 7.5 and 60 mU.min$^{-1}$.kg$^{-1}$ ($P < 0.05$, Table 1) and a similar trend was observed at 15 mU.min$^{-1}$.kg$^{-1}$ ($P < 0.1$, Table 1). Insufficient samples were available for analysis of guinea pig insulin in animals infused with 30 mU.min$^{-1}$.kg$^{-1}$ insulin. Infusion rates of 7.5 and 30 mU.min$^{-1}$.kg$^{-1}$ were chosen for further study to measure insulin responses at around half and near maximal human insulin doses.

**Comparative whole-body insulin sensitivity of glucose metabolism**

Dose-response data for the net glucose uptake in response to human insulin (Figure 1A) was available for male or mixed-sex populations, in human (2-4, 6, 15, 16, 31, 34, 36, 40, 41, 44, 45, 51, 56, 58), rat (13, 17, 47, 48, 55), mouse (1, 52) and pig (62). Data in other species was only available for states of physiological challenge such as pregnancy or lactation, which were not included in the
comparative analysis. Both the maximal glucose uptake rates and the concentrations of circulating insulin at which maximal uptake was evident differed between studies (Figure 2A). In humans, dose responses were similar across multiple studies in male and mixed-sex adult populations (Figure 2A). To allow between-species comparisons, dose response curves were fitted to data from papers describing responses in intact adult male mice (1, 52), Wistar rats (13, 47, 48, 55) and humans (2, 6, 15, 31, 44), and the dose-response for guinea pig from the present study added. Maximal rates of net glucose uptake, measured as glucose infusion rate required to maintain euglycemia were similar in guinea pigs (~85 μmol.kg⁻¹.min⁻¹) as in humans (~80 μmol.kg⁻¹.min⁻¹), but occurred at far higher circulating insulin concentrations (~14 000 μU.ml⁻¹ compared to ~1000 μU.ml⁻¹, Figure 2B). On a per bodyweight basis, rates of maximal whole body glucose uptake responses to insulin were ~160% higher in rats than guinea pigs, and maximal responses were more than double in mice compared to rats (Figure 2B). The concentration of insulin at which half-maximal glucose uptake responses occurred was ~150% higher in guinea pigs (~185 μU.ml⁻¹) compared to humans or rats (~71 μU.ml⁻¹ and ~73 μU.ml⁻¹ respectively, Figure 2B) and ~200% higher compared to mice (~61 μU.ml⁻¹).

Whole body insulin sensitivity and partitioned glucose metabolism at ~half-maximal insulin dose (7.5 mU.min⁻¹.kg⁻¹)

In the cohort of guinea pigs in which whole body insulin sensitivity was assessed at 7.5 mU.min⁻¹.kg⁻¹, fasting glucose was higher in females than males (P<0.05, Table 2). The co-efficient of variation of blood glucose averaged 5.9% between 60-120 minutes of insulin infusion. Steady state human insulin concentrations achieved during insulin infusion at 7.5 mU.min⁻¹.kg⁻¹ averaged 238 ± 13 μU.ml⁻¹ overall, and were higher in males than females (P<0.007, Table 2), and the metabolic clearance rate of human insulin was higher in females (P<0.02, Table 2). Steady state glucose infusion rates averaged 47.8 ± 2.5 μmol.min⁻¹.kg⁻¹ during the HEC, and ssGIR and whole body insulin sensitivity did not differ between males and females (Table 2).
Similarly, in the cohort of guinea pigs in which partitioned glucose metabolism was measured during clamps at 7.5 mU.min⁻¹.kg⁻¹ insulin, fasting blood glucose was higher in females than males (P<0.001, Table 2), and steady state human insulin concentrations and ssGIR did not differ between sexes (Table 2). Basal rates of glucose production and utilization tended to be higher in females compared to males prior to the 7.5 mU.min⁻¹.kg⁻¹ HEC (P<0.075 for both). Insulin infusion at 7.5 mU.min⁻¹.kg⁻¹ suppressed endogenous glucose production (P<0.001, Table 2) and enhanced whole body glucose utilization in both males and females (P<0.001, Table 2). The insulin-stimulated rates of glucose utilization at half-maximal insulin dose were higher in females than males (P<0.03, Table 2), and endogenous glucose production under insulin-stimulated conditions tended to be higher in females than males (P=0.08, Table 2). Basal levels of whole body glycolysis and glucose storage did not differ between sexes prior to the HEC. Insulin infusion at 7.5 mU.min⁻¹.kg⁻¹ tended to enhance the rate of whole body glycolysis in males (P<0.08), but not females (Table 2). Whole body glucose storage was increased by insulin infusion in males (P<0.001) and females (P<0.005), and insulin-stimulated rates of glucose storage were higher in females than males (P<0.05, Table 2). Overall, glycolysis accounted for 43 ± 6% (males: 50 ± 9%; females: 35 ± 7%) of glucose utilization in the fasting state and 37 ± 4% (males: 43 ± 7%; females: 32 ± 4%) during infusion of human insulin at 7.5 mU.min⁻¹.kg⁻¹ (n=19). Therefore, glucose storage accounted for 57 ± 6% (males: 50 ± 9%; females: 65 ± 7%) of glucose utilization in the fasting state, and 63 ± 4% (males: 58 ± 7%; females: 68 ± 4%) during infusion of 7.5 mU.min⁻¹.kg⁻¹ human insulin. The proportions of glucose utilization accounted for by glycolysis and glucose storage did not differ between males and females.

Whole body insulin sensitivity and partitioned glucose metabolism at near maximal insulin dose (30 mU.min⁻¹.kg⁻¹)

In the cohort of guinea pigs in which whole body insulin sensitivity was assessed at 30 mU.min⁻¹.kg⁻¹ (n=38), fasting glucose was higher in females than males (P<0.05, Table 3). The co-efficient of variation of blood glucose averaged 6.7% during 60-120 minutes of insulin infusion. Steady state human insulin concentrations achieved during insulin infusion at 30 mU.min⁻¹.kg⁻¹ averaged 3199 ±
156 μU.ml⁻¹ overall, and were higher in males than females (P<0.01, Table 3); however, metabolic clearance rate of human insulin did not differ between sexes. Steady-state glucose infusion rates averaged 65.4 ± 3.0 μmol.min⁻¹.kg⁻¹ during this HEC, and ssGIR and whole body insulin sensitivity did not differ between males and females (Table 3).

In the guinea pigs in which partitioned glucose metabolism was measured at 30 mU.min⁻¹.kg⁻¹ (n=19), fasting blood glucose was also higher in females than males (P<0.05) and steady state human insulin concentrations and glucose infusion rates did not differ between sexes (Table 3). Insulin infusion at 30 mU.min⁻¹.kg⁻¹ suppressed endogenous glucose production in males (P<0.001) and females (P<0.05) and enhanced whole body glucose utilization to a similar degree in both sexes (P<0.001 for both, Table 3). Glucose production during insulin infusion at 30 mU.min⁻¹.kg⁻¹ was higher in females than males (P<0.04), but the insulin-stimulated decrease in endogenous glucose production did not differ between the sexes. Insulin infusion at 30 mU.min⁻¹.kg⁻¹ did not alter whole body glycolysis in males, but enhanced glycolysis in females (P<0.01, Table 3), and therefore the insulin-stimulated change in glycolysis from basal levels was higher in females than males (P<0.04, Table 3). Insulin-stimulated rates of glycolysis during the clamp also tended to be higher in females than males (P<0.06, Table 3). Prior to the 30 mU.min⁻¹.kg⁻¹ HEC, basal rates of glucose storage were higher in females than males (P=0.05). Insulin infusion at 30 mU.min⁻¹.kg⁻¹ increased whole body glucose storage in males (P<0.001) and females (P<0.04), and insulin-stimulated glucose storage was similar in males and females (Table 3). In the fasting state, prior to insulin infusion at 30 mU.min⁻¹.kg⁻¹, glycolysis accounted for 43 ± 6% of glucose utilization and glucose storage accounted for 57 ± 6%.

Glycolysis accounted for a higher percentage of glucose utilization in males than females (males: 54 ± 9%; females: 31 ± 6%, P<0.04) and conversely glucose storage accounted for a lower percentage of utilization in males (males: 46 ± 9%; females: 69 ± 6%, P<0.04). In the insulin infused state, at 30 mU.min⁻¹.kg⁻¹ insulin, glycolysis accounted for 38 ± 3% of glucose utilization (males: 36 ± 4%; females: 40 ± 5%) and glucose storage accounted for 62 ± 3% (males: 64 ± 4%; females: 60 ± 5%), and there were no differences between males and females.
This study has characterized whole body glucose metabolism and its response to insulin in the euglycemic state in the chronically catheterized guinea pig, and includes the first dose response study for insulin action in this species. Although maximal whole-body glucose uptake responses to insulin were similar on a per bodyweight basis in guinea pig as human, adult male guinea pigs were less insulin sensitive than humans, rats or mice. Human insulin increased glucose utilization and suppressed endogenous glucose production in the guinea pig, and the insulin sensitivity for suppression of endogenous glucose production was also lower in the guinea pig than reported previously in human (7, 30, 31, 49), or in other small experimental species, such as rats and mice (1, 26, 27, 52, 54). Nevertheless, these studies have demonstrated that HEC using human insulin can be used for quantitative assessment of insulin sensitivity in the guinea pig. As the guinea pig resembles the human in its susceptibility to diabetes (32, 33), atherosclerosis and in aspects of cholesterol homeostasis (12), this establishes methodology that will allow this species to be used for further investigation of the mechanistic basis underlying perturbations of glucose metabolism and insulin action.

While the HEC is well established and used in rats and mice (1, 26, 27, 29, 52), the volume of blood that can be sampled, and the number of experiments and analyses possible is limited in these species, particularly in younger animals. The guinea pig, in contrast, ranges in weight from 60 to 140 g at birth and attains a body weight of 400 g by 3-4 weeks of age, thus avoiding some of the limitations related to size and body weight in other small animal models. This greater size and the ability to maintain catheters in this species also permitted repeat HEC studies to be performed in the chronically catheterized young adult guinea pig in the present study. An additional potential advantage of the guinea pig is the ability to differentiate infused and endogenous insulin, and directly measure suppression of insulin secretion. Infusion of human insulin suppressed circulating guinea pig
insulin in the present study, providing direct evidence that human insulin does not cross-react in this guinea pig insulin assay.

When compared to insulins of other species, guinea pig insulin has a very low affinity for many mammalian insulin receptors, including its own (37), and reflecting the low homology of guinea pig insulin with those of most other mammals (61). Bolus doses of guinea pig insulin are only about 1/3 as potent as bovine insulin in lowering blood glucose in the guinea pig (64). The guinea pig appears to compensate for this through an increased plasma insulin concentration and possibly an increased tissue abundance of insulin receptors (14, 23, 37, 64). Although the sequence of insulin has diverged between species, the receptor for insulin is functionally conserved and binds chicken and porcine insulins \textit{in vitro} with similar affinity as receptors from humans and rats (37). This study reports the first \textit{in vivo} dose response study for insulin in the guinea pig, and establishes insulin infusion rates and concentrations at which \textasciitilde{half-maximal} and maximal whole-body glucose uptake responses are obtained. Our results were somewhat inconsistent with those reported in a previous study utilizing the HEC in male guinea pigs. In that study, infusion of human insulin at 3 mU.kg\(^{-1}\) generated a plateau plasma insulin concentration of 100 ± 5 μU.ml\(^{-1}\) in their control groups, and glucose infusion rates of \textasciitilde{72-83} μmol.min\(^{-1}\).kg\(^{-1}\) were required to maintain euglycemia (57). These ssGIR resemble the rates of 65.4 μmol.min\(^{-1}\).kg\(^{-1}\) required to maintain glycaemia at human insulin concentrations of \textasciitilde{3200} μU.ml\(^{-1}\) in the current study. A number of factors may have contributed to the discrepancies in apparent insulin sensitivity between these two studies. Guinea pigs were anesthetized during the HEC in the previous study (57); however, in rats anesthesia reduces insulin sensitivity (5), suggesting that anesthesia may not explain the differences. In addition, the body weight of animals studied by Szilvassy et al. (57) ranged from 350-400 g, a considerably lighter weight than the male guinea pigs in the current study, and animals were fasted for 24 hours, compared to 16 hours used in this study. In mice, fasting time can influence insulin sensitivity assessed by HEC, with insulin sensitivity increasing following an 18 hour compared to a 5 hour fast (1). Furthermore, details of the assay used to measure plasma insulin in the previous study were not provided (57). Hence, while the previous
study (57) reported a higher ssGIR at a lower steady-state insulin concentration in the guinea pig than we observed, differences in experimental design may explain this. Based on the dose response study we performed in adult male guinea pigs, the sensitivity of whole-body glucose metabolism to human insulin is lower in guinea pig than in adult male human (2, 6, 15, 31, 44), Wistar rat (13, 47, 48, 55) and mouse (1, 52), despite similar receptor binding of mammalian insulin previously reported in vitro (37). Maximal rates of net glucose uptake were similar in guinea pigs as humans, but less than half of those evident in rats, and <25% of rates of glucose uptake in mouse, when expressed on a body weight basis, while half-maximal glucose uptake was achieved at insulin concentrations ~150% higher in guinea pigs than humans or rats.

Similar to species-differences in the insulin sensitivity of whole-body glucose uptake, tissue-specific glucose metabolism and its regulation by insulin differed between humans and guinea pigs as well as compared to rodents. In the guinea pig, as in other species, glucose is utilized predominantly for oxidative purposes and carbon dioxide production, with much of the remainder directed to hepatic glycogen storage in the fasted state or to lipid storage in adipose tissue in the fed state (38). In the present study, fasting (basal) rates of endogenous glucose production in the guinea pig were high (e.g. 61 μmol.min⁻¹.kg⁻¹ prior to the 7.5 mU.min⁻¹.kg⁻¹ clamps) when compared to rates reported for healthy adult humans (11-16 μmol.min⁻¹.kg⁻¹, 7, 30, 31, 49), rats (27-38 μmol.min⁻¹.kg⁻¹, 19, 26, 54) or mice (83-216 μmol.min⁻¹.kg⁻¹, 1, 52). We have now shown that hepatic insulin sensitivity also differs in this species from many others, and is less insulin-responsive than glucose utilization in the guinea pig. In the present study, human insulin infusion at 7.5 mU.min⁻¹.kg⁻¹ suppressed endogenous glucose production by 20% and stimulated glucose utilization by 39% in the guinea pig, whilst infusion of human insulin at 30 mU.min⁻¹.kg⁻¹ suppressed glucose production by an average of 23% and increased glucose utilization by 68%. In contrast, in humans, maximal suppression of endogenous production is achieved at lower insulin concentrations than those required to stimulate maximal rates of glucose utilization. For example, in dose response studies using porcine insulin (49) half-maximal suppression of endogenous glucose production occurred at insulin concentrations of 29 ± 2
μU.ml⁻¹, whereas half-maximal stimulation of glucose utilization occurred at 55 ± 7 μU.ml⁻¹. Similar results are reported in the rat and mouse, with lower plasma insulin concentrations required to achieve maximal suppression of endogenous glucose production, compared to those required for stimulation of glucose utilization (1, 26, 54). In rats, endogenous glucose production is suppressed by >90% and glucose uptake is stimulated ~5-fold at insulin concentrations of 124 μU.ml⁻¹ during HEC with porcine insulin (54). Endogenous glucose production is completely suppressed by elevated insulin concentrations (37 μU.ml⁻¹), and glucose uptake is increased >5-fold at higher insulin concentrations (183 μU.ml⁻¹) during HEC with human insulin in mouse (1). Similarly, in humans, maximal and complete suppression of glucose production occurs at an insulin concentration of 57 μU.ml⁻¹ and a ~5-fold increase in glucose utilization occurs at plateau plasma insulin levels of 678 μU.ml⁻¹ (49). In the guinea pig, a 37% suppression of endogenous glucose production and 68% increase in glucose utilization during the 30 mU.min⁻¹.kg⁻¹ HEC were observed at human insulin concentrations of >3000 μU.ml⁻¹, suggestive of lower activity of human insulin in guinea pig liver and peripheral tissues, when compared to its actions in human tissues.

This study provides the first evidence of sex-differences in glucose metabolism in the young adult guinea pig. Compared to males, female guinea pigs had higher fasting glucose, metabolic clearance of insulin and rates of glucose utilization, storage and production during insulin stimulation. Whole body insulin sensitivity and the insulin sensitivity of endogenous glucose production and glucose utilization did not differ between the sexes, although the insulin-stimulated increase in glycolysis was greater in females than males at high-dose insulin. Metabolic clearance of insulin is greater in women than men (21), similar to the sex-differences that we observed in guinea pigs. Whole-body insulin sensitivity of glucose metabolism is generally higher in women than men, as in female than male rodents (reviewed by 35, 59), although in some studies greater whole-body insulin sensitivity in women is only seen when expressed per unit muscle mass, rather than per unit body weight (63). This greater whole-body insulin sensitivity of glucose uptake in women is partly due to greater insulin sensitivity of skeletal muscle, which was 47% higher for femoral muscle in women than men when
directly measured using labelled glucose and positron emission tomography (42). We have reported that relative weights of fat depots and muscles are similar in male and female guinea pigs at similar ages as the animals in the present study (18), suggesting that sex differences in insulin sensitivity are unlikely to reflect sex-differences in body composition in these young adult guinea pigs. Greater rates of glucose utilization and storage in female than male guinea pigs therefore imply that in guinea pigs, as in humans, the insulin sensitivity of peripheral tissue is higher in females than males. The direction of sex differences in hepatic insulin sensitivity in humans is also consistent with those for whole-body responses. In the basal (fasting) state, sex differences vary between studies, with either similar or ~20% higher rates of endogenous glucose production in women than men (reviewed by 35, 59). Our finding of greater basal endogenous glucose production in female than male guinea pigs (29% and 25% sex difference before 7.5 and 30.0 mU.min⁻¹.kg⁻¹ dose insulin clamps respectively) is thus not inconsistent with reports in humans. In young adult guinea pigs, endogenous glucose production remained higher in females than males at insulin doses producing approximately half-maximal and near-maximal increases in net glucose uptake, and the decrease from basal rates did not differ between sexes at either dose. This contrasts with humans where suppression of endogenous glucose production is greater in women than men at low, but not at high, doses of insulin (reviewed by 35). Differences in relative adiposity and lean muscle mass, and the potential actions of steroid hormones are suggested as potential factors contributing to sex-specific differences in insulin sensitivity and glucose metabolism, including risk of T2D, which is usually diagnosed at younger ages and lower BMI in men than women (22, 35, 59, 63). Our finding of sex-differences in insulin regulation of glucose metabolism in the guinea pig reinforce the need to include animals of both sexes in studies investigating perturbations of insulin sensitivity of glucose metabolism in the guinea pig.

Perspectives and Significance
This study has validated the HEC in chronically catheterized male and female guinea pigs, and has confirmed that concomitant tracer infusions can also be utilized to investigate partitioned glucose metabolism in the un-anesthetized guinea pig. Human insulin stimulated glucose utilization and suppressed endogenous glucose production in the guinea pig, although the relative magnitude of these changes differed from those reported during HEC in the human, rat and mouse. This species is likely to be a useful additional small animal model for biomedical research, in part due to its relatively large size enabling studies early in life and the similarities between guinea pig and human cholesterol metabolism. The smaller litter size of the guinea pig compared to rats, often used to investigate effects of perturbations during pregnancy and lactation (9, 10, 53, 60), and the similarly precocial developmental patterns in guinea pig as humans, including presence of ~10% body fat at term birth (11) are likely to make this species particularly valuable for studies of developmental programming. We have already reported that restricted fetal growth due to maternal feed restriction or large litter size in guinea pigs programs postnatal appetite, growth and adiposity (18), and perturbs cholesterol metabolism and glucose tolerance (23, 24) and establishment of methods for HEC in guinea pigs will enable future studies of programming of glucose metabolism by perinatal exposures in this species. Finally, a unique feature of guinea pigs is the ability to differentiate endogenous guinea pig insulin from infused (heterologous) insulin (the reason why antibodies used to detect insulin from other species are largely raised in guinea pig), enabling investigation of insulin suppression of its own secretion in this species.

Acknowledgements

We thank Dr Arkadi Katsman, Ms Melissa Walker and Ms Patricia Grant for assistance with aspects of in vivo studies and assays. Purified guinea pig insulin and rabbit anti-guinea pig insulin used in analyses of guinea pig insulin were kindly donated by Prof. C.C. Yip (University of Toronto, Canada).

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Disclosures

The authors have no conflicts of interest to disclose.
References


Table 1. Metabolic responses to differing insulin infusion rates during the hyperinsulinemic euglycemic clamp in the guinea pig.

<table>
<thead>
<tr>
<th>Insulin infusion rate (mU.min⁻¹.kg⁻¹)</th>
<th>7.5</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Bodyweight (g)</td>
<td>698 ± 27</td>
<td>695 ± 29</td>
<td>795 ± 53</td>
<td>685 ± 25</td>
</tr>
<tr>
<td><strong>Fasting state outcomes (20 min prior to HEC)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting whole blood glucose (mmol.l⁻¹)</td>
<td>7.02 ± 0.29</td>
<td>7.51 ± 0.22</td>
<td>7.22 ± 0.48</td>
<td>7.60 ± 0.46</td>
</tr>
<tr>
<td>Fasting plasma guinea pig insulin (ng.ml⁻¹)</td>
<td>5.91 ± 0.96</td>
<td>6.06 ± 1.51</td>
<td>nd</td>
<td>6.73 ± 0.88</td>
</tr>
<tr>
<td><strong>Steady state outcomes (60-120 min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose (mmol.l⁻¹)</td>
<td>7.06 ± 0.34</td>
<td>7.41 ± 0.23</td>
<td>7.15 ± 0.54</td>
<td>7.50 ± 0.45</td>
</tr>
<tr>
<td>Blood glucose %CV</td>
<td>6.5 ± 1.4</td>
<td>6.4 ± 0.8</td>
<td>10.5 ± 6.0</td>
<td>8.1 ± 1.6</td>
</tr>
<tr>
<td>Glucose infusion rate (μmol.min⁻¹.kg⁻¹)</td>
<td>53.7 ± 3.9</td>
<td>69.4 ± 8.7</td>
<td>82.9 ± 11.1</td>
<td>88.1 ± 12.0</td>
</tr>
<tr>
<td>Glucose infusion rate %CV</td>
<td>20.3 ± 5.6</td>
<td>19.5 ± 2.6</td>
<td>23.5 ± 13.4</td>
<td>23.3 ± 6.6</td>
</tr>
<tr>
<td>Plasma human insulin (μU.ml⁻¹)</td>
<td>291 ± 33</td>
<td>1419 ± 175</td>
<td>10557 ± 2124</td>
<td>15724 ± 1897</td>
</tr>
<tr>
<td>Plasma guinea pig insulin (ng.ml⁻¹)</td>
<td>4.16 ± 0.49*</td>
<td>3.60 ± 0.48</td>
<td>nd</td>
<td>3.74 ± 0.29*</td>
</tr>
<tr>
<td>Whole body insulin sensitivity (μmol.ml.μU⁻¹.min⁻¹.kg⁻¹)</td>
<td>0.211 ± 0.037</td>
<td>0.064 ± 0.020</td>
<td>0.0086 ± 0.002</td>
<td>0.0057 ± 0.0007</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Steady state outcomes are averaged during 60-120 minutes of insulin infusion. *P<0.05 compared to fasting levels. nd= not determined.
Table 2. Whole body insulin sensitivity and partitioned glucose metabolism at ~half-maximal insulin dose in the guinea pig (7.5 mU.min\(^{-1}\).kg\(^{-1}\)).

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Basal (fasting)</th>
<th>Hyper-insulinemia</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td><strong>Whole body insulin sensitivity measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (n)</td>
<td>31</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>Bodyweight (g)</td>
<td>796 ± 15</td>
<td>661 ± 12***</td>
<td>n/a</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol.l(^{-1}))</td>
<td>6.77 ± 0.13</td>
<td>7.31 ± 0.19*</td>
<td>n/a</td>
</tr>
<tr>
<td>Glucose infusion rate (μmol.min(^{-1}).kg(^{-1}))</td>
<td>n/a</td>
<td>n/a</td>
<td>48.7 ± 3.6</td>
</tr>
<tr>
<td>Plasma human insulin (μU.ml(^{-1}))</td>
<td>n/a</td>
<td>n/a</td>
<td>266 ± 18</td>
</tr>
<tr>
<td>Whole body insulin sensitivity (μmol.ml.μU(^{-1}).min(^{-1}).kg(^{-1}))</td>
<td>n/a</td>
<td>n/a</td>
<td>0.204 ± 0.019</td>
</tr>
<tr>
<td>MCR human insulin (ml.min(^{-1}).kg(^{-1}))</td>
<td>n/a</td>
<td>n/a</td>
<td>32.9 ± 2.7</td>
</tr>
<tr>
<td><strong>Partitioned glucose metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol.l(^{-1}))</td>
<td>6.41 ± 0.22</td>
<td>7.52 ± 0.18**</td>
<td>n/a</td>
</tr>
<tr>
<td>Glucose infusion rate (μmol.min(^{-1}).kg(^{-1}))</td>
<td>n/a</td>
<td>n/a</td>
<td>36.6 ± 3.4</td>
</tr>
<tr>
<td>Plasma human insulin (μU.ml(^{-1}))</td>
<td>n/a</td>
<td>n/a</td>
<td>203 ± 21</td>
</tr>
<tr>
<td>Metabolic Component</td>
<td>Value 1 ± SEM</td>
<td>Value 2 ± SEM</td>
<td>Value 3 ± SEM</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Hepatic glucose production (μmol.min⁻¹.kg⁻¹)</td>
<td>53.7 ± 5.9</td>
<td>69.3 ± 5.7</td>
<td>39.6 ± 5.7</td>
</tr>
<tr>
<td>Whole body glucose utilization (μmol.min⁻¹.kg⁻¹)</td>
<td>53.7 ± 5.9</td>
<td>69.3 ± 5.7</td>
<td>74.7 ± 7.3</td>
</tr>
<tr>
<td>Whole body glycolysis (μmol.min⁻¹.kg⁻¹)</td>
<td>23.5 ± 3.7</td>
<td>25.7 ± 4.9</td>
<td>29.9 ± 4.4</td>
</tr>
<tr>
<td>Whole body glucose storage (μmol.min⁻¹.kg⁻¹)</td>
<td>30.2 ± 7.3</td>
<td>43.6 ± 4.6</td>
<td>44.8 ± 8.2</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Glucose infusion rate, plasma human insulin, whole-body insulin sensitivity and metabolic clearance rates (MCR) are averaged during steady-state conditions from 60-120 minutes of insulin infusion. For whole body insulin sensitivity, human insulin concentrations were available for n=30 males and 20 females. §P<0.10, *P<0.05, **P<0.01, ***P<0.001 compared to value for males. †P<0.05, ††P<0.01, †††P<0.001 compared to basal level prior to the clamp within each sex. n/a: not applicable.
### Table 3. Whole body insulin sensitivity and partitioned glucose metabolism at an insulin dose induced near-maximal response in the guinea pig (30 mU.min⁻¹.kg⁻¹).

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Basal (fasting)</th>
<th>Hyper-insulinemia</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>Number (n)</td>
<td>Bodyweight (g)</td>
<td>Fasting blood glucose (mmol.l⁻¹)</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>785 ± 19</td>
<td>6.7 ± 0.2</td>
</tr>
<tr>
<td>Whole body insulin sensitivity measures</td>
<td>19</td>
<td>658 ± 15***</td>
<td>7.3 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>n/a</td>
<td>66.7 ± 4.3</td>
</tr>
<tr>
<td>Partitioned glucose metabolism</td>
<td>Number</td>
<td>Fasting blood glucose (mmol.l⁻¹)</td>
<td>Glucose infusion rate (μmol.min⁻¹.kg⁻¹)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6.3 ± 0.3</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>7.0 ± 0.2*</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>66.0 ± 6.3</td>
<td>61.5 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>n/a</td>
<td>n/a</td>
<td>3679 ± 451</td>
</tr>
<tr>
<td>--------------------------------</td>
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<td>-----------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>Plasma human insulin (μU.ml⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hepatic glucose production (μmol.min⁻¹.kg⁻¹)</strong></td>
<td>51.9 ± 5.8</td>
<td>65.1 ± 6.0</td>
<td>24.3 ± 6.1**</td>
</tr>
<tr>
<td><strong>Whole body glucose utilization (μmol.min⁻¹.kg⁻¹)</strong></td>
<td>51.9 ± 5.8</td>
<td>65.1 ± 6.0</td>
<td>89.7 ± 7.0***</td>
</tr>
<tr>
<td><strong>Whole body glycolysis (μmol.min⁻¹.kg⁻¹)</strong></td>
<td>25.6 ± 2.5</td>
<td>19.6 ± 4.0</td>
<td>30.7 ± 3.1</td>
</tr>
<tr>
<td><strong>Whole body glucose storage (μmol.min⁻¹.kg⁻¹)</strong></td>
<td>26.3 ± 6.7</td>
<td>45.5 ± 6.3*</td>
<td>59.1 ± 7.2***</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Glucose infusion rate, plasma human insulin, whole-body insulin sensitivity and metabolic clearance rates (MCR) are averaged during steady-state conditions from 60-120 minutes of insulin infusion. For whole body insulin sensitivity, human insulin concentrations were available for n=13 males and 18 females. For partitioned glucose metabolism, human insulin concentrations were available for n=6 males and 5 females. §P<0.10, *P<0.05, **P<0.01, ***P<0.001 compared to value for males. †P<0.05, ‡P<0.01, ††P<0.001 compared to basal level prior to the clamp within each sex. n/a: not applicable.
Figure legends

Figure 1. Insulin dose response curve for glucose infusion rates during hyperinsulinemic-euglycemic clamps in the guinea pig. Plasma human insulin concentrations (log μU.ml⁻¹) and glucose infusion rates are mean values between 60 and 120 minutes of insulin infusion, with the insulin infusion dose in mU.min⁻¹.kg⁻¹ shown next to each data point. Data are mean ± SEM.

Figure 2. Whole body sensitivity of glucose metabolism to human insulin in conscious animals. A. Data from individual studies. Data points from the same study are joined. B. Dose-response curves fitted by 3-parameter logistic regression to combined data for intact male mice, Wistar rats, human and guinea pigs. Symbol fills indicate sex (black: males; mixed/not-specified: grey) and symbol shapes indicate species (circle: human; upwards triangle: rat; downwards triangle: mouse; square: pigs; diamonds: guinea pig).
Figure 1