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Differences in glucose-stimulated insulin secretion *in vitro* of islets from human, nonhuman primate and porcine origin

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Abstract

Background—Porcine islet xenotransplantation is considered a potential cell-based therapy for type 1 diabetes. It is currently being evaluated in diabetic nonhuman primates (NHP) to assess safety and efficacy of the islet product. However, due to a variety of distinct differences between the respective species, including the insulin secretory characteristics of islets, the suitability and predictive value of the preclinical model in the extrapolation to the clinical setting remains a critical issue.

Methods—Islets isolated from human (n=3), NHP (n=2), adult pig (AP, n=3) and juvenile pig (JP, n=3) pancreata were perfused with medium at basal glucose (2.5mM) followed by high glucose (16.7mM) concentrations. The total glucose-stimulated insulin secretion (GSIS) was calculated from generated insulin secretion profiles.

Results—NHP islets exhibited GSIS 3-fold higher than human islets, while AP and JP islets exhibited GSIS 1/3 and 1/16 of human islets, respectively. The insulin content of NHP and AP islets was similar to that of human islets, whereas that of JP islets was 1/3 of human islets.

Conclusion—Despite the fact that human, NHP, and AP islets contain similar amounts of insulin, the much higher GSIS for NHP islets than for human, AP and JP islets suggests the need for increased dosing of islets from JP and AP in pig-to-NHP transplantation which may be substantially higher than that required for humans. Finally, porcine islet xenotransplantation to humans may require significantly higher dosing given the lower GSIS of AP islets compared to human islets.

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Conflict of interest: Michael J Taylor PhD, is Vice President Research and Development of Cell & Tissue Systems, Inc, a company with the mission “Preservation of biological materials for restoration of patient health”.

Keywords

human; islets; nonhuman primate; nutrient-stimulated insulin secretion; pig; species incompatibility

Introduction

The potential of islet cell replacement therapies to cure type 1 diabetes has created a considerable demand for transplantable islet preparations. There are over 3 million Americans with type 1 diabetes and only about 4,000 human donor pancreata available each year for organ and islet transplantation [1]. Of these, about 20–30% (or 1,300) of the available pancreata are accepted and utilized for pancreas transplantation [2,3]. This low acceptance rate is related to the (poor) quality of most donor organs. Taking into account the failures in islet transplantation, and the number of donor organs per transplant, annually about 50 patients receive an islet transplant [4]. It is thus clear that alternate sources of islets are needed to supply a constant stream of therapeutic cells.

Porcine-derived islets are being investigated for their safety and possible potency and function in human xenotransplantation. Porcine islet xenotransplantation perhaps offers the most immediate solution due to natural similarities in pancreas size, islet numbers, and insulin protein between pig and human species. Regarding preclinical evidence for a therapeutic benefit, a number of groups have reported long-term diabetes reversal and porcine islet graft function in diabetic non-human primate (NHP) recipients of porcine islets [5–12].

Diabetic NHPs represent a good model for studying the immunological aspects associated with islet xenotransplantation. However, NHPs present key metabolic differences when compared to pigs which can significantly influence the outcome of pig-to-NHP islet transplants. Studies of glucose metabolism in cynomolgus (*Macaca fascicularis*) monkeys indicate that monkeys have high circulating C-peptide and insulin levels associated with low serum glucose levels [13,14]. In response to glucose stimulation *in vitro*, NHP islets are also able to quickly produce high amounts of insulin [15]. In contrast with NHPs, normal serum C-peptide and insulin levels are lower in the pig, and pig islets are widely known to have a poor insulin secretory response to glucose stimulation *in vitro* [15]. While porcine and human islets display similar glucose clearance capacities, the glucose clearance in NHPs occurs at a much faster rate, which is supported by a higher basal serum insulin and lower basal serum glucose level. Due to this seemingly higher insulin demand in NHPs, further study into the metabolic characteristics of pig, NHP, and human islets is needed to determine how to best design preclinical islet xenotransplantation studies.

The present study examines the insulin secretion rate of equal numbers of porcine, NHP, and human islets after nutrient stimulation by glucose, a mixture of glutamine and leucine, and potassium chloride (KCl) in a dynamic perfusion system. The results are normalized to the DNA content of the islets used to account for possible differences in islet size and cell packing density within islets [16].

Materials and Methods

Islet source

Human islets were obtained through the Integrated Islet Distribution Program (n =1) or from islet isolations of local clinical grade pancreata using the standard protocol for the Clinical Islet Transplant Consortium (n=2) [17]. Donors were 39, 24 and 46 years old with a body mass index of 26.9, 21.9 and 29.2, respectively. Pancreata were obtained from two male rhesus monkeys aged 7.3 and 8.1 years weighing 10.4 and 13.0 kg, respectively: these pancreata were subjected to islet cell preparation using a modified Ricordi method [18]. Pancreata were obtained from three Landrace sows aged 3.2, 3.5, and 6.4 years, respectively, and weighing 243, 246, and 255 kg, respectively. Islets were isolated from these pancreata (designated AP) using the standard protocol used at the Schulze Diabetes Institute at the University of Minnesota [5]. Juvenile porcine (JP) islets were obtained from donors less than 3 months of age from Cell and Tissue Systems, South Carolina. A total of 3 juvenile islet products were used, either freshly prepared after procurement (n=1), or after 24-hour hypothermic machine perfusion preservation (n=2) [19]. This difference in preservation had no apparent effect on the outcome of isolation, so that data were combined.

Media and culture

Following purification, human and NHP islets were cultured in CMRL medium (Mediatech, Manassas, VA), supplemented with heparin (10U/ml, APP Pharmaceuticals, LLC, Schaumburg, IL); for human islets supplementation was performed with human albumin (25% HSA, 10% v/v, Grifols, Los Angeles, CA), and for NHP islets supplementation was done with heat-inactivated fetal bovine serum (10% v/v, Mediatech). Culture was done in gas-permeable culture devices (GRex100, Wilson-Wolf Manufacturing, New Brighton, MN) or T-flasks, in humidified air with 5% CO₂, 95% air for 1 day at 37°C and thereafter at 22°C. AP and JP islets were cultured in ME199 culture medium (Mediatech) with 10% heat-inactivated porcine serum (Gibco, Auckland, NZ), L-glutamine (Mediatech) and heparin (10U/ml, APP Pharmaceuticals) in GRex100 at 37°C, in humidified air without CO₂ for 6–9 days.

Islet assessments

Macroscopic assessment was performed on a sample of islets stained with dithizone and viewed under the microscope as described previously for visual counting, purity and quality score assessment. The purity of the three human preparations ranged between 70 and 90%, for the two NHP preparations purity ranged between 75 and 90%, for the three AP preparations it was 90% and for the four JP preparations it ranged between 45 and 85% (Table 1).

Tissue quantity was assessed using the DNA assay and tissue viability was measured by oxygen consumption rate (OCR) as described previously [16,20–23]; samples for OCR were normalized to DNA (OCR/DNA).

Islet products were assayed *in vitro* for dynamic insulin secretory function (GSIS) using various stimulating factors with the following rationale: at first glucose because this is the

physiologic initiator of insulin secretion by pancreatic β -cells; then glucose with a combination of amino acids leucine and glutamine because amino acids play a role in glucose homeostasis and insulin release *in vivo*; and finally potassium chloride (KCl) because this acts as non-specific membrane stimulator. The method used is described elsewhere [24], with a modification to allow a larger number of islets (approximately 75–80 IEs for human islets, and 300 IEs for NHP, AP and JP islets). The base media used was Krebs Ringer's bicarbonate. The basal glucose concentration was maintained at 2.5 mM, with a glucose stimulus consisting of a single-step increase in glucose concentration to 16.7 mM. After reduction to 2.5 mM glucose, a second stimulation was performed with a combination of 2.5 mM glucose with amino acids 10 mM leucine and 4 mM glutamine. Finally, after reduction to 2.5 mM glucose, a final stimulation was done with 30 mM KCl alone. The supernatant was assessed for insulin concentration using an enzyme-linked immunosorbent assay (ELISA) for human insulin (80-INSHU-E01, ALPCO Diagnostics, Salem, NH) for human and NHP islets or porcine insulin (80-INSPO-E01, ALPCO Diagnostics) for porcine islets. Most measurements were performed in triplicate. Insulin levels in the supernatant were normalized to DNA. The area under the curve (AUC) was calculated using the linear trapezoidal rule. Insulin content measurement was determined by ELISA.

Statistical analysis

Data are presented as mean \pm SD. Statistical analysis for differences between groups was analyzed using the parametric t-test. Differences were considered statistically significant in case of $p < 0.05$.

Results

The reproducibility in the yield of islet preparations was acceptable, enabling the evaluation of a small number of preparations per group. The same was observed regarding the quality assessments. Fractional viability (\pm SD) measured by OCR/DNA in the four groups was 121 ± 12 nmol/min.mg DNA in the human islet preparations, 188 ± 3 nmol/min.mg DNA in the JP preparations, 221 ± 57 nmol/min.mg DNA in the NHP preparations, and 265 ± 25 nmol/min.mg DNA in the AP islet preparations. The difference between individual groups was statistically significant, except for the difference between NHP and JP islet preparations, and between NHP and AP islet preparations (Table 1).

The results on the insulin secretory function showed such a low variation in each individual group (Table 1), that the combined evaluation of a small number of samples per group gave informative data. A representative graph of the profiles of insulin secretion upon nutrient stimulation for each species is shown in Fig. 1. The first stimulation at 16.7 mM insulin induced a biphasic response, a first immediate and high response peaking at around 9–10 min followed by a lower response peaking at around 25 min. This was most clear for NHP and AP islets, but also apparent for human islets. The combination of leucine and glutamine stimulation resulted in a quick insulin secretory response, which was in the same range or somewhat lower than the first peak after glucose stimulation. Finally, KCl stimulation

resulted in a quick insulin secretory response that peaked at a higher level than any of the preceding responses.

The graphs also show a relevant difference between the four species. The highest insulin secretory response was seen for islets from NHP, followed by the response of human islets. Islets from AP islets showed a much lower response, while the response from JP islets was very low. These differences between the groups are shown for the AUC values of all individual islet preparations in Fig. 2. Highest values are evident for the NHP islets, followed by human islets: for the 2nd phase glucose stimulation and KCl stimulation values for NHP islets were similar to those of human islets (Table 1). The outcome was lower for AP islets and lowest for JP islets. In statistical analysis the difference in AUC values between JP islets and each of the other groups was significant. The difference between AP islets and human islets reached statistical significance for AUC values of the glucose 1st stimulation phase and glutamine/leucine stimulation, but not for AUC values of glucose 1st stimulation phase and KCl. The difference between NHP and human islets reached statistical significance for the AUC values of glucose 1st stimulation phase but not for any other AUC value.

The data were also expressed in ratios of glucose (1st and 2nd phase combined) over glutamine/leucine stimulation and KCl stimulation, and for the ratio between glutamine/leucine stimulation and KCl stimulation (Table 1). Regarding the glucose over glutamine/leucine stimulation ratio, the difference between AP and NHP islets was statistically significant, and regarding the glucose over KCl or glucose or glutamine/leucine over KCl ratio the difference between JP islets and all other groups was statistically significant. Considering the very low AUC values of JP islets, this latter observation might have little physiological relevance.

There were some differences in purity between the various islet preparations, but differences between groups did not reach statistical significance. This aside, we repeated the statistical evaluation after correction of the AUC values for purity of the islets. The outcome of this evaluation was the same as that of evaluation of AUC values without this correction for purity: this included the significant differences in insulin secretory responses. This indicates that there was no unequivocal effect of purity on the difference in outcome between the various groups. It should be noted that lowest purity of islet preparations was observed for the preparations from juvenile pigs (63%, compared with purity between 83 and 90% for preparations from the other three sources).

Finally, the data on insulin content of the various preparations are presented in Fig. 3. The levels were in the same range for islets from NHP, human and AP pancreata (differences were not statistically significant), but significantly lower in islet preparations from JP pancreata ($P < 0.02$ or less).

Discussion

Despite the fact that AP and NHP islets contain similar amounts of insulin, the GSIS for NHP islets is much higher than that of AP and JP islets. These differences *in vitro* agree

with published data regarding *in vivo* incompatibilities between species in insulin secretion after stimulation with either glucose or arginine [13,14]: we have summarized these data in the Introduction and have described these in detail elsewhere [14]. This may indicate the need for increased numbers of islets from JP and AP in pig-to-NHP transplantation. Since GSIS from NHP islets is 2-fold higher than that of human islets, as evidenced by the AUC comparison of the 1st phase of glucose stimulation, the islet dosing requirement in NHPs may be substantially higher than what is required for humans. The differences in insulin secretory capacity between porcine and NHP islets may explain in part the higher porcine islet dose requirement for diabetes reversal in NHP (25,000 IE/Kg BW) [5] as opposed to 5,000–10,000 IE/Kg BW for islet allotransplantation in either human or NHP [14,18]. The very low insulin secretory activity of JP islets could in part be ascribed to the lower purity of the preparations (63% versus 83–90%), but even more by the more immature status of pancreatic β -cells in juvenile pigs.

These results are also relevant in the area of tissue engineering for islet xenotransplantation where current research is being pursued towards constructs which would allow for islets transplanted in biocompatible, immunoisolating implantable devices [25,26]. The size, as determined by the surface area of the device needed for efficacy, is a function of the number of islets placed in the device (IE/cm²), which in turn is strongly dependent on the oxygen consumption rate and insulin secretory capacity of the islets [25–30]. Here we report that porcine islets secrete half as much insulin with twice as much demand for oxygen (OCR) than human islets – this difference is more pronounced when compared with NHP islets. These differences are expected to have a profound impact on the design of devices for tissue engineering applications such that substantially larger devices will be required, given that everything else is constant, if porcine (xeno) instead of allo islets were to be used in humans or NHPs, as well as the choice of the animal model used.

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Abbreviations

AP	adult pig
AUC	area under the curve
ELISA	enzyme-linked immunosorbent assay
GSIS	glucose-stimulated insulin secretion
IE	islet equivalent
JP	juvenile pig
NHP	nonhuman primate
OCR	oxygen consumption rate

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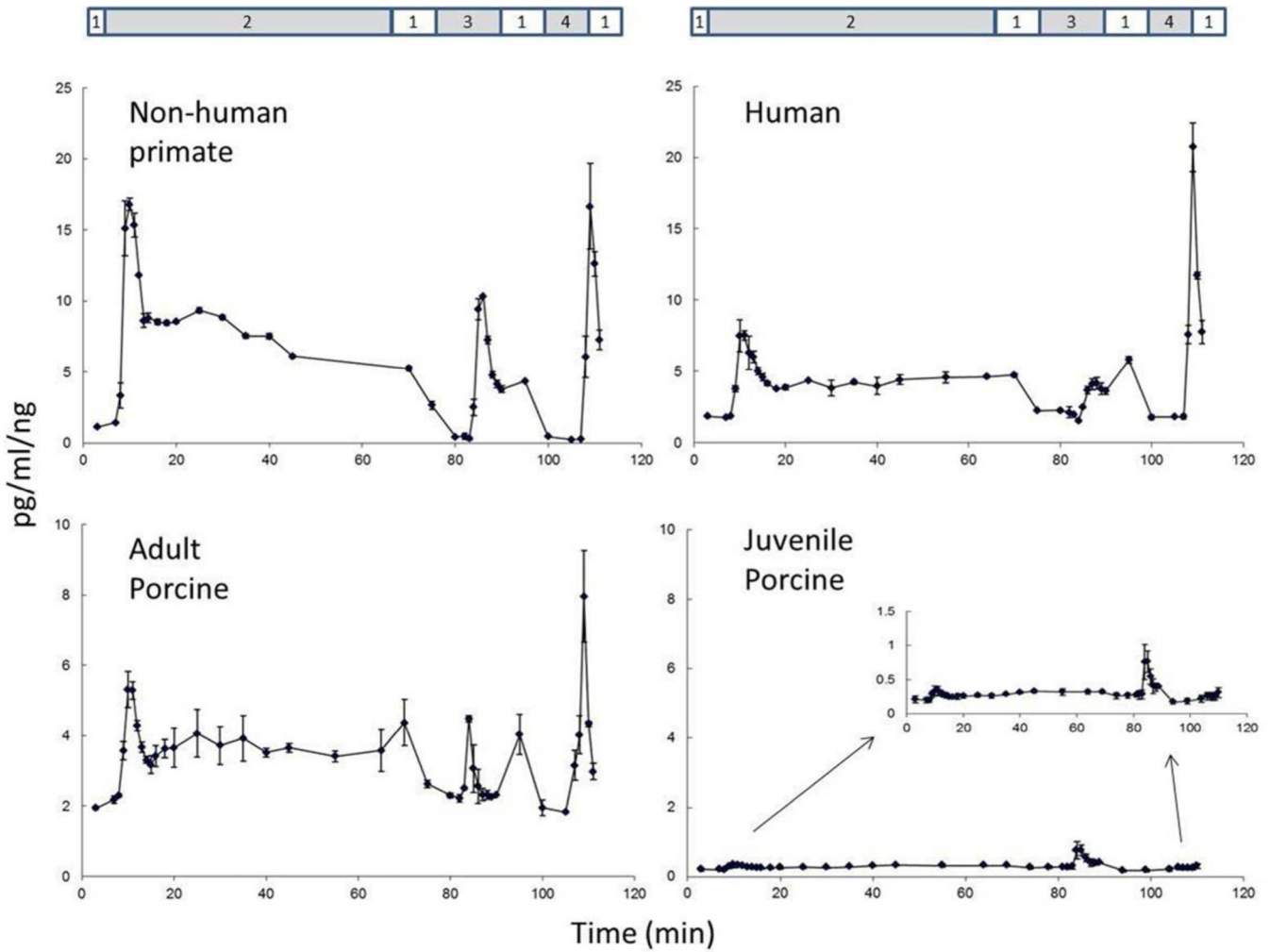


Fig 1. Representative glucose-stimulated insulin secretion profiles in islet preparations from nonhuman primates, human, adult porcine and juvenile porcine pancreata. Stimulation is done in successive order with (1) 2.5mM glucose (basal), (2) 16.7mM glucose (1st and 2nd phase), (3) leucine+glucine, and (4) potassium chloride.

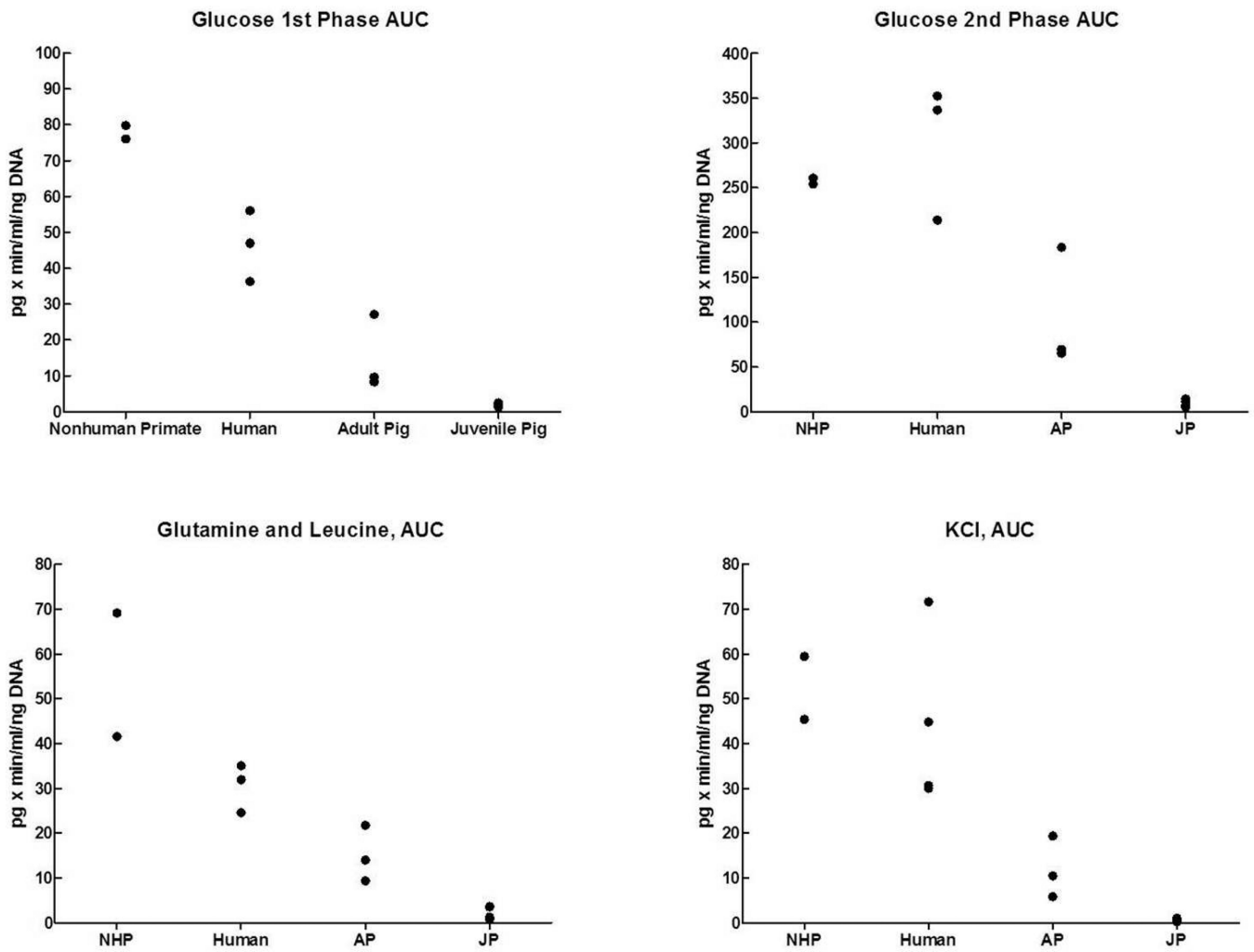


Fig. 2. Responses of islets preparations from nonhuman primates, human, adult pig and juvenile pig pancreata during the different phases of the glucose-stimulated insulin secretion assay. AUC, area-under-the curve.

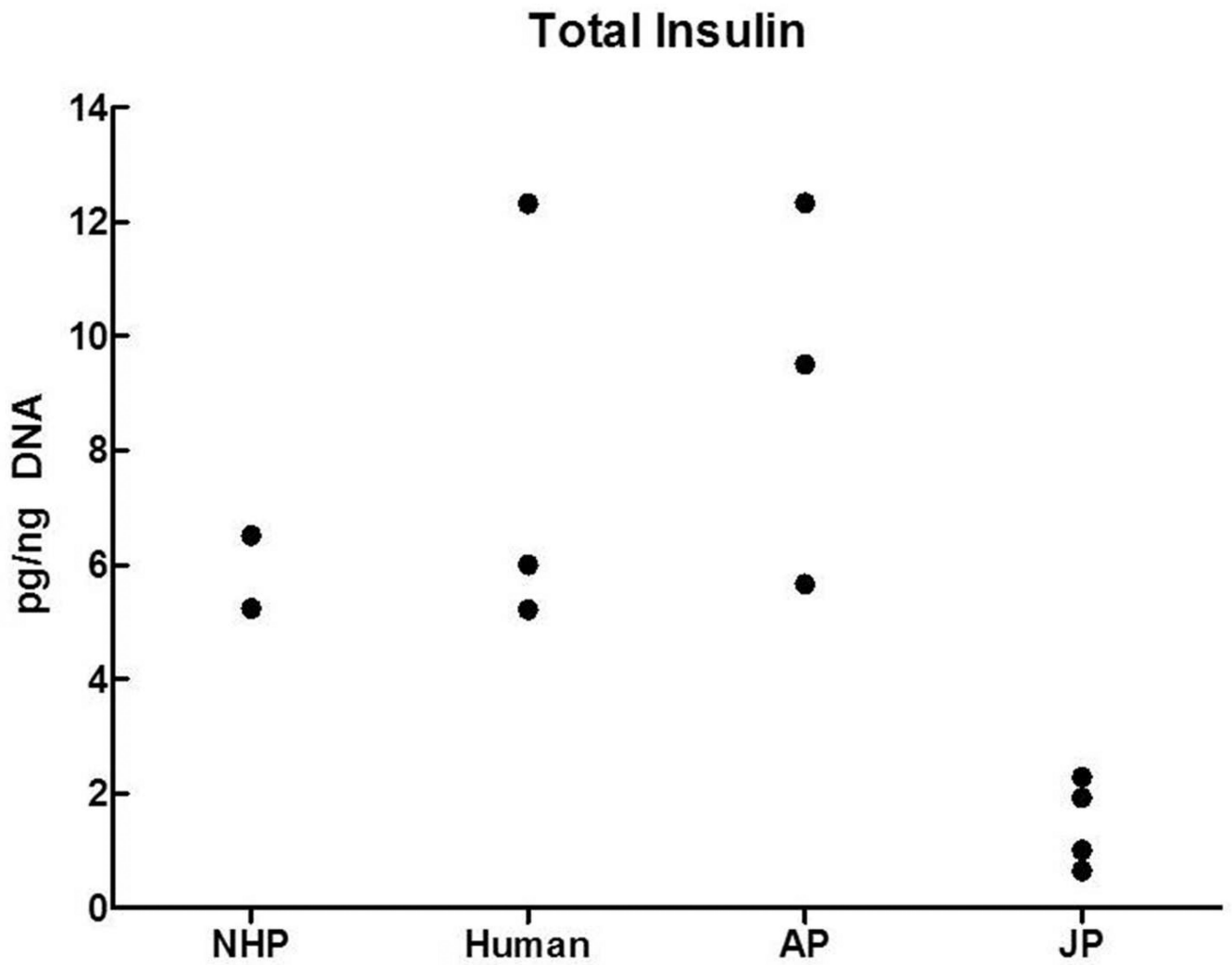


Fig. 3. Insulin content of islet preparations from nonhuman primates (NHP), human, adult pig (AP) and juvenile pig (JP) pancreata.

Table 1

Characteristics of islet preparations and outcome of dynamic insulin function assay

	NHP n=2	Human n=3	AP n=3	JP n=3
<i>Characteristics of islet preparations</i>				
Purity (%, dithizone stain)	83 ±11	85 ±13	90 ±0	63 ±19
OCR/DNA (nmol/min.mgDNA)	221 ±57	121 ±12	265 ±25	188 ±3
Total insulin (pg/ng DNA)	5.9 ±0.9	7.8 ±3.9	9.2 ±3.4	1.5 ±0.8
<i>Insulin secretory function (AUC , pg/ml.min.ngDNA)</i>				
Glucose 1 st phase	77.8 ±2.6	46.5 ±9.8	15.1 ±10.5	1.9 ±0.6
Glucose 2 nd phase	258 ±5	301 ±76	106 ±67	9.5 ±3.8
Glutamine and Leucine	55.4 ±19.5	30.5 ±5.4	15.1 ±6.3	2.3 ±1.5
KCL	52.4 ±9.9	49.1 ±20.8	11.9 ±6.9	0.6 ±0.3
<i>Ratio of phases in dynamic insulin secretion</i>				
Glucose 1 st +2 nd phase/ glutamine and Leucine	6.5 ±2.4	11.3 ±1.3	7.7 ±2.1	6.0 ±2.3
Glucose 1 st +2 nd phase/ KCL	6.5 ±1.4	7.9 ±4.0	10.4 ±2.7	19.1 ±3.7
Glutamine and leucine/ KCL	1.04 ±0.18	0.71 ±0.38	1.35 ±0.24	3.47 ±1.23

Data are presented as arithmetic means values ±SD