Title
Relationship between fractional pancreatic beta cell area and fasting plasma glucose concentration in monkeys

Permalink
https://escholarship.org/uc/item/65q33744

Journal
Diabetologia: Clinical and Experimental Diabetes and Metabolism, 53(1)

ISSN
1432-0428

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Publication Date
2010

DOI
10.1007/s00125-009-1552-z

Peer reviewed
Abstract

Aims/hypothesis We sought to establish the relationship between fasting plasma glucose concentrations and pancreatic fractional beta cell area in adult cynomolgus monkeys (Macaca fascicularis).

Methods Fasting plasma glucose and pancreatic fractional beta cell area were measured in 18 control and 17 streptozotocin-treated adult primates (17.0±1.2 vs 15.4±1.2 years old).

Results Fasting plasma glucose was increased (12.0±2.0 vs 3.4±0.1 mmol/l, \( p<0.01 \)) and fractional beta cell area was decreased (0.62±0.13% vs 2.49±0.35%, \( p<0.01 \)) in streptozotocin-treated monkeys. The relationship between fasting plasma glucose and pancreatic fractional beta cell area was described by a wide range of beta cell areas in controls. In streptozotocin-treated monkeys there was an inflection of fasting blood glucose at \( \sim 50\% \) of the mean beta cell area in controls with a steep increase in blood glucose for each further decrement in beta cell area.

Conclusions/interpretation In adult non-human primates a decrement in fractional beta cell area of \( \sim 50\% \) or more leads to loss of glycaemic control.

Keywords Beta cell mass · Cynomolgus monkey · Streptozotocin · Type 1 diabetes · Type 2 diabetes

Abbreviations

FPG Fasting plasma glucose
STZ Streptozotocin

Introduction

In health, the blood glucose concentration is regulated by insulin secreted by pancreatic beta cells. Diabetes arises when insufficient insulin is secreted to maintain glycaemic control. In type 1 and type 2 diabetes this is at least in part due to a deficit in beta cell mass [1–3]. There is inconsistency in the published literature as to what constitutes sufficient beta cell mass to sustain glycaemic control. The surgical literature has suggested as much as an 80% pancreatic resection can be tolerated without diabetes [4, 5]. However, more comprehensive metabolic studies of humans and dogs after a partial pancreatectomy have suggested that \( \sim 50\% \) pancreatectomy results in impaired fasting glucose and glucose intolerance in the short term [6] and diabetes in some individuals over the next few years [7, 8].

The latter data are consistent with cross-sectional studies of the relationship between fasting blood glucose concentration and pancreatic fractional area in humans [3]. In obese individuals, this relationship is curvilinear, with an inflection of blood glucose with a deficit of \( \sim 50\% \) or more.
beta cells compared with controls. On the other hand, in another study of dogs it was suggested that insulin secretion was minimally effected with an ∼50% pancreatic resection [9].

Here we report the relationship between fasting plasma glucose (FPG) and fractional pancreatic beta cell area in adult cynomolgus monkeys (Macaca fascicularis).

Methods

Animals A total of 35 male cynomolgus monkeys (Macaca fascicularis, age: 16.2±0.9 years, range 5 to 27 years; body weight: 5.8±0.3 kg, range 3.4 to 9.8 kg) were studied. Monkeys were originally imported from Institut Pertanian (Bogar, Indonesia) and were pair-housed at the Comparative Medicine Clinical Research Center at Wake Forest University School of Medicine, Winston-Salem, NC, USA. Among them, 18 monkeys (nine control and nine streptozotocin [STZ]-treated monkeys) had been included in a previous study reported elsewhere [10]. Monkeys were randomly assigned to either the control (n=18) or the STZ (n=17) group. Control and STZ groups were matched for age (17.0±1.2 vs 15.4±1.2 years, p=0.3) and body weight (6.1±0.4 vs 5.6±0.3 kg, p=0.3). All procedures involving animals were conducted in compliance with state and federal laws, standards of the US Department of Health and Human Services, and guidelines established by the Wake Forest University Institutional Animal Care and Use Committee.

Induction of diabetes To induce diabetes, freshly prepared STZ (Zanosar; SICOR Pharmaceuticals, Irvine, CA, USA) was given intravenously in a single bolus (30 mg/kg) to monkeys in the STZ group. To monitor the effect of STZ, the fasting blood glucose concentration was measured on alternate days for a week, (Accu-Check Glucose Monitor; Boehringer Mannheim, Indianapolis, IN, USA). Of the 17 monkeys in the STZ group, six required a second STZ injection 1 week after the first and three monkeys required a third STZ injection 1 week after the second to induce diabetes. The criterion for diabetes was a fasting blood glucose>8 mmol/l 48 h post STZ injection. To avoid ketosis in hyperglycaemic monkeys, insulin therapy (Novolin 70/30; Novo Nordisk USA, Princeton, NJ, USA) was started at a dosage of 0.4 U/kg twice daily and then the dose was gradually increased to a target fasting blood glucose concentration of 8 to 14 mmol/l.

Fasting blood samples were collected under ketamine sedation 4 weeks (nine control and nine STZ monkeys) or 14 weeks (nine control and eight STZ monkeys) after the STZ (or saline) injection. To avoid the effect of exogenous insulin, monkeys were treated with the same dose of regular insulin (Novolin R; Novo Nordisk USA) instead of Novolin 70/30 on the evening prior to blood sampling. Plasma glucose concentration was determined by the glucose oxidase method by using a Glucose Analyzer 2 (Beckman Instruments, Brea, CA, USA).

Tissue collection After collection of the blood samples (i.e. 4 or 14 weeks after the STZ [or saline] treatment), the animals were killed by overdose with intravenous pentobarbital, and the pancreases were harvested intact, immediately weighed, cut into 3–4 mm sections and fixed in ice-cold neutral-buffered 10% (vol/vol.) formalin for 24 h. The pancreases were then transferred into 70% (vol/vol.) alcohol for another 24 h at 4°C prior to processing and embedding in paraffin for the subsequent procedure.

Morphometric analysis For immunohistochemistry, 4 µm sections of pancreas containing a minimum of 100 islets were stained with guinea pig anti-insulin antibody, 1:100 (Zymed Laboratories, San Francisco, CA, USA) and haematoxylin. Then the entire pancreatic section stained for insulin was imaged at ×40 magnification (×4 objective). The ratio of the beta cell area/total pancreas parenchymal area was digitally quantified using Image Pro Plus software (Media Cybernetics, Silver Springs, MD, USA) as previously described [2].

Statistical analysis Data are presented as mean ± SEM. The Student’s t test was used for comparisons. The relation between the fasting plasma glucose (y in mmol/l) and the beta cell area (x in %) was described with the model

Fig. 1 Curvilinear relationship between the percentage of pancreas occupied by beta cells and fasting plasma glucose in cynomolgus monkeys. Circles, control monkeys; triangles, STZ-treated monkeys. The solid line is derived from non-linear regression analysis (r²=0.59; p<0.0001). The dashed vertical lines indicate the mean fractional pancreatic beta cell area in the control group (2.5%, right) and ∼50% of that value (1.3%, left)
proposed by Ritzel et al. [3], describing a mono-exponential decay plus plateau:

\[ y = \text{plateau} + \text{span} \times e^{-k \times x} \]  

(1)

where plateau (mmol/l) was the FPG value related to elevated beta cell areas, span (mmol/l) was the increase above plateau corresponding to a beta cell area equal to zero, and k (\%/min) was the constant rate of the mono-exponential function.

Model variables (i.e. plateau, span and k) were estimated, together with a measure of their precision (coefficient of variation), by non-linear least squares [11] using SAAM II software [12].

**Results**

As expected, the fasting plasma glucose concentrations measured shortly before death and necropsy were higher in STZ-treated diabetic monkeys compared with control monkeys (12.0±2.0 vs 3.4±0.1 mmol/l, \( p < 0.01 \)). Also, the pancreatic fractional beta cell area was decreased in STZ-treated diabetic monkeys compared with control monkeys (0.62±0.13% vs 2.49±0.35%, \( p < 0.01 \)).

The relationship between the fasting plasma glucose concentration and the pancreatic fractional insulin area was well described by the mono-exponential model relationship in equation 1 (Fig. 1). The model variables were estimated with good precision: plateau=3.5±1.3 mmol/l (CV 37%), span=19.4±3.1 mmol/l (CV 16%), k=2.3±0.7 %/min (CV 33%). There was a wide range of fractional beta cell areas in control monkeys. Blood glucose concentrations increased steeply once the pancreatic fractional beta cell area decreased to 50% or less that of the mean of that in control monkeys.

**Discussion**

We report that the relationship between the pancreatic fractional beta cell area and the fasting plasma glucose concentration in cynomolgus monkeys is reminiscent of that in humans with and without type 2 diabetes [3]. The relationship is also comparable with that observed previously in a porcine [13] and rat model of diabetes [14] compared with their respective non-diabetic controls.

In each of these models there is a wide range of fractional beta cell areas in the non-diabetic controls [3, 13, 14]. It is conceivable that this is due to variation in pancreas volume [15]. However, a wide range of beta cell masses in non-diabetic humans has also been reported [16].

Alternatively, the wide range in fractional beta cell areas in non-diabetic individuals represents adaptation to insulin sensitivity. However, this seems unlikely, at least in humans, as in morbidly obese humans the adaptive increase in beta cell mass is only ~50% compared with that in lean individuals [2, 16–18]. Another possible explanation is that maternal nutrition [19], placental function and both nutrition and genetic regulation during postnatal expansion of beta cell mass may define the adult beta cell mass [20].

The relationship between fractional beta cell area and the fasting blood glucose concentration in adult STZ-treated monkeys reported here is also comparable with that in models of type 2 diabetes in adult rats [14] and pigs [13]. In these models, once the fractional beta cell area is decreased by approximately 50% or more of that in non-diabetic individuals, glycaemic control is lost. After a loss of 50% of beta cells in each of these models, there is steep rise in fasting blood glucose concentration with each further decrement in beta cell area. This curvilinear relationship reported in adult rodents, pigs and non-human primates reproduces that previously observed in humans [3]. Moreover, a similar relationship has recently been reported in humans with pancreatitis [21].

The mechanisms responsible for the loss of regulation of glycaemic control after a decrease in pancreatic beta cell area of 50% or more is unknown. One plausible explanation is that with declining beta cell mass the immediately secrutable insulin pool [22] is depleted leading to an impaired insulin pulse mass, as previously documented after 50% pancreatectomy [6]. Since the pattern of insulin delivery to the liver influences insulin sensitivity [23, 24], we postulate that once the immediately secrutable insulin pool is depleted, impaired pulsatile insulin leads to insulin resistance that further confounds the defect in insulin secretion. Once hyperglycaemia develops, the additional confounding adverse effects of hyperglycaemia on beta cell function and insulin sensitivity presumably contribute to the further declining glycaemic control.

In conclusion, in adult non-human primates, a decline of approximately 50% or more in pancreatic beta cells leads to loss of glycaemic control with a relationship comparable with that in adult humans. The cynomolgus monkey treated with sufficient STZ to induce this deficit in beta cells is therefore a useful model for type 2 diabetes.

**Acknowledgement** This study was supported by funding from the NIH (DK059579, DK077967), the Juvenile Diabetes Research Foundation (7-2005-1152), the Larry L. Hillblom Foundation and the Manpei Suzuki Diabetes Foundation. We are grateful to our colleagues in the Larry Hillblom Islet Research Center for their excellent suggestions.

**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.
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