Originals

Diabetes mellitus in *Macaca mulatta* monkeys is characterised by islet amyloidosis and reduction in beta-cell population

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Summary. Diabetes mellitus in *Macaca mulatta* rhesus monkeys is preceded by phases of obesity and hyperinsulinaemia and is similar to Type 2 (non-insulin-dependent) diabetes mellitus in man. To relate the progression of the disease to quantitative changes in islet morphology, post-mortem pancreatic tissue from 26 monkeys was examined. Four groups of animals were studied: group I – young, lean and normal ($n = 3$); group II – older (> 10 years), lean and obese, normoglycaemic ($n = 9$); group III – normoglycaemic and hyperinsulinaemic ($n = 6$); group IV – diabetic ($n = 8$). Areas of islet amyloid, beta cells and islets were measured on stained histological sections. Islet size was larger in animals from groups III ($p < 0.01$) and IV ($p < 0.0001$) compared to groups I and II. The mean beta-cell area per islet in μm^2 was increased in group III ($p < 0.05$) and reduced in group IV ($p < 0.001$) compared to groups I and II. Mean beta-cell area per islet correlated with fasting plasma insulin ($r = 0.76$,

$p < 0.001$) suggesting that hyper- and hypoinsulinaemia are related to the beta-cell population. Amyloid was absent in group I but small deposits were present in three of nine (group II) and in four of six (group III) animals, occupying between 0.03–45 % of the islet space. Amyloid was present in eight of eight diabetic animals (group IV) occupying between 37–81 % of the islet area. Every islet was affected in seven of eight diabetic monkeys. There was no correlation of degree of amyloidosis with age, body weight, body fat proportion or fasting insulin. Islet amyloid appears to precede the development of overt diabetes in *Macaca mulatta* and is likely to be a factor in the destruction of islet cells and onset of hyperglycaemia.

Key words: Type 2 (non-insulin-dependent) diabetes mellitus, *Macaca mulatta*, islet of Langerhans – pathology, amyloid, islet amyloid polypeptide, beta cells, obesity.

Amyloid deposits in islets of Langerhans are associated with Type 2 (non-insulin-dependent) diabetes mellitus in man [1–3] but the role of amyloid formation in the pathogenesis of the disease is unclear. Amyloid may be a primary factor in the pathophysiology of Type 2 diabetes [4] or may arise secondarily to beta-cell dysfunction. Progressive amyloid formation contributes to increased severity of the disease in man [5].

The major protein constituent of islet amyloid is a 37 amino acid peptide, islet amyloid polypeptide (IAPP) [6, 7], which is co-localized with insulin in secretory granules of the beta cell [8, 9] and co-secreted following a glucose [10, 11] or amino acid stimulus [12]. Although IAPP is present in all species examined so far [13], diabetes-associated islet amyloid has been found only in man, monkeys and cats [14]. This restriction may be related to a combination of species-specific variations in the sequence of IAPP and, as yet unknown, causative factors in the conversion of soluble IAPP into insoluble fibrils [15]. The IAPP sequence in *Macaca mulatta* has not been determined but in

another macaque species, *Macaca nemestrina*, the sequence differs from human IAPP in only three amino acids [16].

The development of diabetes in *Macaca mulatta* [17, 18] is associated with obesity and insulin resistance and it appears to be very similar to Type 2 diabetes in man. This is unlike development of diabetes in *Macaca nigra* which is characterised by lack of obesity and hypoinsulinaemia. [19, 20]. In this latter animal model, diabetes is associated with amyloid deposition [21].

In *Macaca mulatta* the age of onset of diabetes ranges from 10 to 29 years and most commonly commences between 15 and 25 years ("middle age" for monkeys). A series of phases have been identified in the progression of the disease [18]; animals, older than 7 years with free access to food often become obese and some obese monkeys develop insulin resistance and increased basal and stimulated insulin secretion. This phase is followed by glucose intolerance and impaired beta-cell responses with basal insulin levels declining toward or below normal. Hyper-

Table 1. Physiological data

Group	Monkey	Age (years)	Weight (kg)	Body fat (%)	Pancreas (g)	FPG (mmol/l)	IRI (pmol/l)
I	A	2.1	2.6	–	5.8	2.6	201
	B	2.2	2.7	–	6.5	2.7	187
	Z	5.4	7.4	5.0	1.3	3.8	431
II	C	17.5	9.8	–	–	3.6	215
	G	21.3	10.1	–	–	3.3	194
	V	13.0	9.8	–	–	3.1	115
	T	13.2	10.6	21.9	11.4	3.8	345
	P	29.8	7.6	16.3	–	4.4	101
	L	14.5	15.5	34.0	9.4	3.0	338
	S	15.7	15.1	35.8	12.2	3.6	273
	E	12.0	11.8	21.5	16.5	3.6	416
	Q	29.3	7.8	39.8	7.6	3.4	618
III	M	18.6	13.2	31.0	18.0	3.4	1034
	H	19.5	22.9	39.5	14.6	3.6	1206
	K	20.3	12.0	28.9	12.4	4.4	1479
	J	22.8	17.1	41.7	14.5	6.4	2230
	Y	18.0	10.8	–	15.4	3.9	1213
	O	14.0	10.3	–	8.9	6.6	847
IV	I	24.2	11.3	30.5	8.0	8.4	136
	F	24.4	13.9	27.7	18.5	11.0	136
	W	20.7	10.3	17.8	7.8	10.2	228
	D	24.3	12.2	29.3	–	14.9	345
	N	21.6	14.3	11.1	21.9	15.2	187
	X	13.9	7.7	16.7	9.5	23.9	151
	U	25.9	9.8	46.5	10.2	9.1	65
	R	26.8	13.1	37.4	8.9	15.9	172

I = lean, normoglycaemic, normoinsulinaemic monkeys, less than 10 years old ($n = 3$). II = lean and obese, normoglycaemic, normoinsulinaemic monkeys, more than 10 years old ($n = 9$). III = normoglycaemic, hyperinsulinaemic monkeys (fasting immunoreactive insulin (IRI) > 700 pmol/l) ($n = 6$). IV = diabetic monkeys (fasting plasma glucose (FPG) > 7.8 mmol/l) ($n = 8$)

glycaemia (fasting plasma glucose (FPG) > 7.8 mmol/l) develops in association with declining pancreatic islet function. In the later phases, insulin-mediated glucose uptake during hyperinsulinaemic euglycaemic clamps is decreased. In addition, overtly diabetic monkeys develop complications similar to those seen in humans including neuropathy and dyslipidaemia. In the present study the relationships between amyloid deposition, beta-cell population, islet size, endocrine density and physiological data were examined in a cross section of lean, obese, hyperinsulinaemic and diabetic animals.

Materials and methods

Physiological data

Twenty-six *Macaca mulatta* monkeys were selected for this study. Fasting plasma immunoreactive insulin (IRI) and glucose concentrations were obtained at regular intervals together with measurements of body weight and proportion of body fat [18]. The last measurements for these parameters obtained within 2 months 'pre-mortem' are shown in Table 1. Monkeys were assigned to four groups according to their physiological status and age. Group I comprised lean, normoglycaemic and normoinsulinaemic monkeys less than 10 years of age. Group II comprised lean and obese, normoglycaemic, normoinsulinaemic monkeys older than 10 years of age. Group III comprised obese, hyperinsulinaemic (IRI > 700 pmol/l),

normoglycaemic animals. Diabetic monkeys were included in group IV, with hyperglycaemia (FPG > 7.8 mmol/l) and low or normal concentrations of fasting plasma insulin.

Tissue preparation and immunostaining

Anaesthetised animals were killed and the pancreas was removed, trimmed of surplus fat and weighed (Table 1). Specimens from the body and tail region (regions VI and VIII [22]) were taken and fixed in 10% formaldehyde in 0.9% sodium chloride. These tissue samples were processed for wax embedding. Histological sections were cut from each block and stained using the indirect peroxidase technique. Beta cells were identified by use of antisera to insulin (Miles Scientific, Bucks., Slough, UK), diluted 1:1000, and peroxidase-conjugated anti-guinea pig antiserum (Miles Scientific). Amyloid was stained with Congo red and identified by use of a rabbit polyclonal antiserum to synthetic $IAPP_{19-37}$, diluted 1:1000, and a peroxidase conjugated anti-rabbit antiserum (Dako Ltd, High Wycombe, UK). Specificity of the insulin and IAPP antisera was confirmed by loss of immunoreactivity after preabsorption of the antisera with human insulin (10 μg/ml) and human $IAPP_{1-37}$ (10 μg/ml), respectively. As the IAPP fragment included the sequence 27–37 which is similar to that found in calcitonin gene related peptide (CGRP), preabsorption with $CGRP_{27-37}$ was performed. Immunoreactivity persisted thus excluding cross reactivity with this fragment of CGRP.

Morphometry

The stained slides were examined by quantitative morphometry with the investigator (EJPdK) unaware of the physiological status of the monkeys. Morphometry was performed using a manual optical picture image analyser (Model MOP-01, Kontron, Messergeraete, FRG) on a projected image of the histological section at a magnification of 630×. The projected image was analysed in a rectangular field which corresponded to 11.5×10^3 μm^2 of the histological section. The entire section was examined in sequential fields and the size of the tissue area was determined from the number of fields; non-acinar tissue such as ducts, vascular bundles and adipose depositions were not included as part of the tissue area. All islets were measured on slides immunolabelled for insulin and IAPP. The boundary of the islet area (ISL) was identified adjacent to surrounding exocrine tissue and capillaries. Other measurements included islet beta-cell area (B), on the slides immunolabelled for insulin, and islet amyloid area (AM) on the slides immunolabelled for IAPP. A clear distinction could be made between amyloid deposits and intracellular IAPP-immunoreactivity since staining for IAPP with this antiserum in beta cells was low or absent in all monkeys and round nuclei or cellular outlines were absent from amyloid deposits. Only islet sections with an area greater than 100 μm^2 were included in the study, thus excluding single beta cells located outside islets and small groups of beta cells often found near capillaries.

The sum of islet areas (ΣISL), beta-cell areas (ΣB) and amyloid areas (ΣAM) were determined to calculate the mean proportion of islet beta-cell area (B%) and islet amyloid area (AM%), respectively. Reproducibility of the measurements was estimated in five specimens with a coefficient of variation of less than 10%. The mean beta-cell area per islet and amyloid area per islet, expressed in μm^2, were calculated through division of ΣB and ΣAM, respectively, by the number of islets. The islet area proportion (ISL%) of the tissue area was determined from ΣISL and tissue area for each specimen.

Statistical analysis

Data are presented as geometric mean or median and range. Islet area, beta-cell area and amyloid area measurements were logarithmically transformed since the distributions of these data were markedly skewed. This enabled comparison of data between individual monkeys and between different groups of animals. Animals in
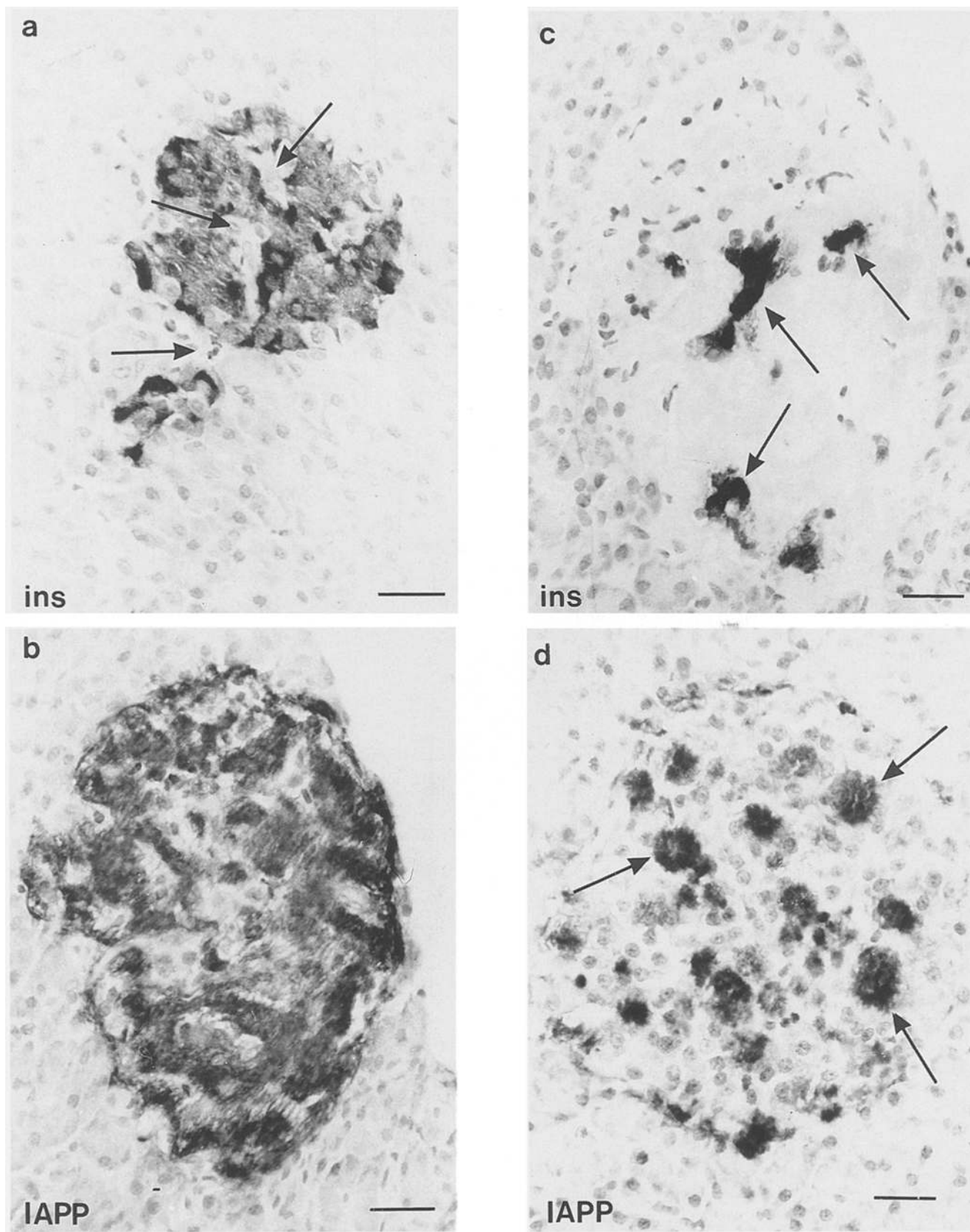
Fig. 1 a–d. Immunoperoxidase labelled pancreatic islets. (a) Immunoreactivity for insulin in a non-diabetic animal (monkey Z). The beta cells occupy a large proportion of the islet. Unstained cells (arrows) line a capillary. This irregularly shaped islet is typical of primates. (b and c) Islet from a diabetic monkey (monkey W). Immunoreactivity for insulin (c) is restricted to small clusters of cells (arrows). An adjacent section through the same islet (b) shows large deposits of amyloid immunoreactive for IAPP. (d) Islet from a normoglycaemic hyperinsulinaemic monkey (monkey J) showing small amyloid deposits immunoreactive for IAPP (arrows). Scale bar = 50 μm
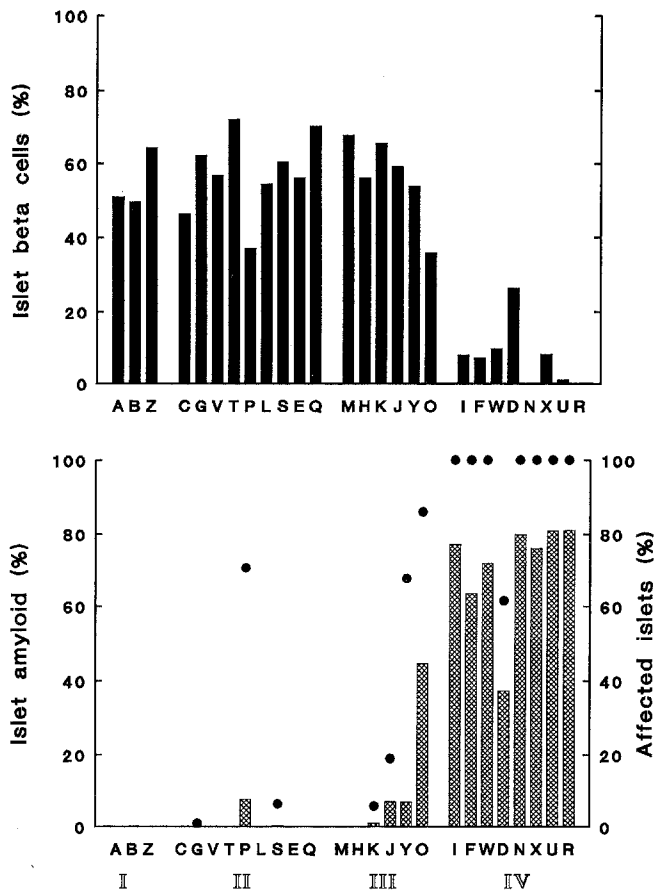
Fig. 2. Proportion of islet beta-cells (■) (*top*) and islet amyloid deposits (▦) with proportion of islets affected (●) (*bottom*). Each animal is indicated by a single lettercode and assigned to one of four groups according to the physiological status at time of death. I = lean, normoglycaemic, normoinsulinaemic monkeys, less than 10 years old (*n* = 3). II = lean and obese, normoglycaemic, normoinsulinaemic monkeys, more than 10 years old (*n* = 9). III = normoglycaemic, hyperinsulinaemic monkeys (*n* = 6). IV = diabetic monkeys (*n* = 8). The proportion of islet beta cells is not different in groups I, II and III but markedly reduced in group IV. All diabetic animals have large amyloid deposits and every islet was affected in seven of eight animals

groups I and II were considered as one population for group analysis since they had been separated only on the basis of age. Correlations were tested by use of Spearman Rank and group differences determined by non-parametric analyses of variance tests (Van der Waerden 3 sample test). Probability values are given with $p < 0.05$ considered to be significant.

Results

Pancreatic tissue from these monkeys was obtained immediately after death at necropsy. The specimens showed little evidence of autolysis and exhibited strong immunoreactivity for islet peptides (Fig. 1 a). All areas showing birefringence in polarised light following Congo red staining were immunoreactive for IAPP (Fig. 1 b). In this study more than 5000 islets were examined in pancreatic specimens from 26 *Macaca mulatta* monkeys. All islets in a tissue section were included in the analysis. Islets were irregular in shape and 170 (59–430) (median and range) islets were examined per animal. There were no statistical differences
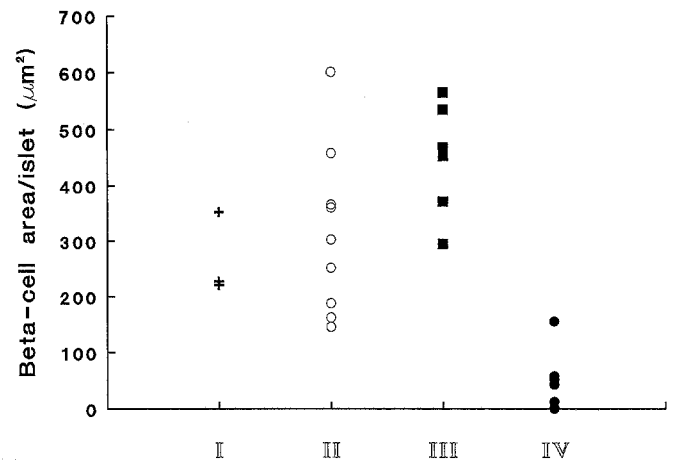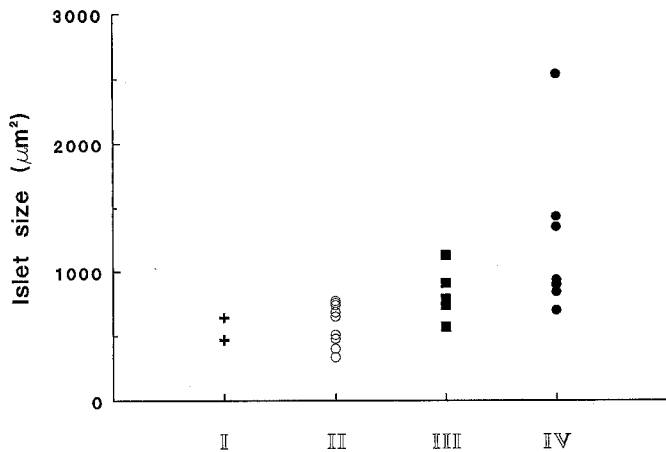

Fig. 3. Beta-cell area per islet. I = lean, normoglycaemic, normoinsulinaemic monkeys, less than 10 years old (*n* = 3). II = lean and obese, normoglycaemic, normoinsulinaemic monkeys, more than 10 years old (*n* = 9). III = normoglycaemic, hyperinsulinaemic monkeys (*n* = 6). IV = diabetic monkeys (*n* = 8). There is a significant increase in beta-cell area per islet in group III compared to group I and II ($p < 0.05$). The reduction in beta-cell area per islet in group IV is highly significant compared to group I and II ($p < 0.0001$) and group III ($p < 0.0001$). These data suggest that a reduction to a critical amount of beta-cell mass is associated with diabetes

in data calculated from measurements made on specimens from the body and specimens from the tail regions of the pancreas in any single animal. Therefore the data from the two regions were combined for each monkey.
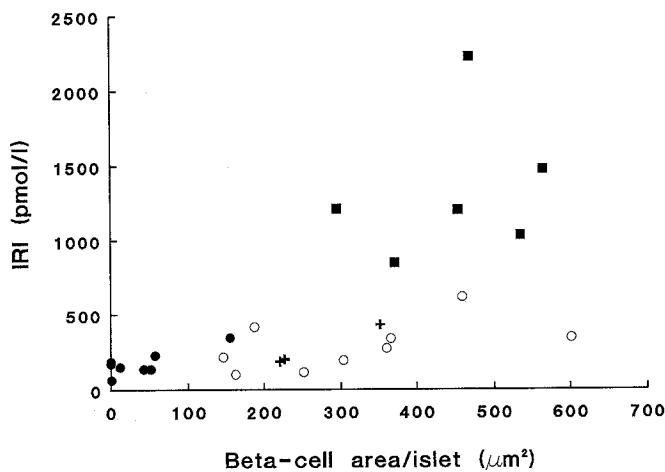
Beta-cell area. Insulin immunoreactive cells were present throughout the islets in non-diabetic animals (Fig. 1 a) and located as isolated cells or in small groups in amyloid deposits (Fig. 1 c). B% for each monkey in groups I–IV is depicted in Figure 2. The mean beta-cell area per islet was increased in group III ($p < 0.05$) and much reduced in diabetic monkeys ($p < 0.0001$) compared to animals from groups I and II (Table 1 and Fig. 3).

Islet amyloid. Islet amyloid was immunoreactive for IAPP. Central areas of the large deposits in some diabetic animals showed less IAPP immunoreactivity and contained extensive calcification. Amyloid deposits were found in all of the eight diabetic monkeys (group IV) (Fig. 1 b) but were absent in the three young non-diabetic animals (group I). In groups II and III amyloid was present in three of nine and four of six animals, respectively. Large amounts of amyloid were present in each islet examined in seven of the diabetic animals, occupying more than 63 % of islet space (Fig. 2). Islet amyloidosis was less severe in one diabetic animal (monkey D) in which 62 % of islets were affected. Amyloid deposits in normoglycaemic animals occupied less islet space and fewer islets were affected compared to animals of the diabetic group (Fig. 2). These smaller deposits either appeared circular in cross section scattered throughout the islet or were adjacent to islet capillaries (Fig. 1 d)

Islet size. Islet size, expressed as the geometric mean of islet areas, varied in different groups. Islet size was in-

Fig. 4. Islet size. I = lean, normoglycaemic, normoinsulinaemic monkeys, less than 10 years old ($n = 3$). II = lean and obese, normoglycaemic, normoinsulinaemic monkeys, more than 10 years old ($n = 9$). III = normoglycaemic, hyperinsulinaemic monkeys ($n = 6$). IV = diabetic monkeys ($n = 8$). Islet size is significantly larger in group III ($p < 0.01$) and Group IV ($p < 0.0001$) compared to groups I and II

Fig. 5. Relationship between fasting plasma immunoreactive insulin (IRI) levels and beta-cell area per islet. Each symbol represents a different group. Group I (+) = lean, normoglycaemic, normoinsulinaemic monkeys, less than 10 years old. Group II (o) = lean and obese, normoglycaemic, normoinsulinaemic monkeys, more than 10 years old. Group III (■) = normoglycaemic, hyperinsulinaemic monkeys. Group IV (●) = diabetic monkeys. A significant correlation ($r = 0.76$, $p < 0.001$) exists between physiological and morphological data

creased in groups III ($p < 0.01$) and IV ($p < 0.0001$) compared to groups I and II. (Table 2 and Fig. 4).

Islet area proportion. ISL% was between 0.9–5.5% in animals of groups I and II. This proportion was significantly increased in animals of groups III ($p < 0.05$) and IV ($p < 0.0001$) compared to groups I and II such that the islet area formed up to 27% of the tissue area in diabetic animals (Table 2).

Relationships of morphological and physiological parameters. Elevated plasma IRI was associated with an increase in the beta-cell population of the islets ($n = 26$; $r = 0.77$, $p < 0.001$) (Fig. 5). This correlation also existed

Table 2. Morphometric data

Group	Monkey	Beta-cell area/islet (μm^2)	Amyloid area/islet (μm^2)	Islet size (μm^2)	Islet area proportion (%)
I	A	227.0	0.0	467.5	2.3
	B	221.0	0.0	471.2	1.5
	Z	352.2	0.0	642.7	2.6
II	C	145.9	0.0	402.3	1.7
	G	303.1	0.3	509.8	2.4
	V	251.8	0.0	479.4	1.0
	T	601.7	0.0	772.5	3.3
	P	162.5	18.9	651.0	3.2
	L	366.2	0.0	755.5	4.3
	S	360.7	0.4	739.2	2.8
	E	188.0	0.0	335.9	0.9
	Q	458.8	0.0	684.1	5.5
III	M	535.5	0.0	911.0	3.7
	H	453.9	0.0	788.4	3.4
	K	565.6	0.4	1129.5	5.6
	J	469.0	0.9	739.4	5.4
	Y	295.3	9.0	572.8	2.6
	O	371.7	112.6	762.1	6.2
IV	I	43.0	2253.8	2540.3	13.0
	F	52.3	889.3	1352.1	5.9
	W	57.8	616.0	937.4	7.5
	D	155.3	23.3	842.7	3.0
	N	0.4	1179.9	1432.5	16.8
	X	12.4	754.0	895.6	10.2
	U	1.0	272.0	904.1	27.3
	R	0.3	620.3	699.7	16.2

I = lean, normoglycaemic, normoinsulinaemic monkeys, less than 10 years old ($n = 3$). II = lean and obese, normoglycaemic, normoinsulinaemic monkeys, more than 10 years old ($n = 9$). III = normoglycaemic, hyperinsulinaemic monkeys (fasting immunoreactive insulin (IRI) > 700 pmol/l) ($n = 6$). Group IV = diabetic monkeys (fasting plasma glucose (FPG) > 7.8 mmol/l) ($n = 8$)

within the normoglycaemic animals (Groups I, II and III) ($n = 18$; $r = 0.68$, $p < 0.002$). Similarly, there was a strong correlation between the amount of islet amyloid (AM%) and FPG ($r = 0.83$, $p < 0.001$) but there was no relationship between the degree of islet amyloidosis and age, body weight, body fat proportion or IRI.

Discussion

There is a high incidence of diabetes in spontaneously obese *Macaca mulatta* monkeys. Each animal in our cross-sectional study had been assigned to a phase in the progression of the disease on the basis of results of physiological tests. Longitudinal studies showed that the diabetic animals in Group IV had passed through successive phases of obesity and hyperinsulinaemia before the onset of hyperglycaemia [18]. These monkeys form a very appropriate animal model for the study of the aetiology and pathophysiology of the disease which is related to obesity as is the case for many patients with Type 2 diabetes. This is unlike the disease process in *Macaca nigra*; these animals do not develop obesity and the first sign of the disease process is impaired insulin secretion [20]. Howard et al. [21] found a reduction in beta-cell numbers and islet amyloid deposition in this unique form of primate diabetes.

The endocrine cell population of islets in the body and tail regions of the non-diabetic *Macaca mulatta* pancreas in which insulin-containing beta cells occupy up to 72 % of the islet space is similar to that of man [2]. The remainder of the islet comprises cells containing glucagon, pancreatic polypeptide, somatostatin, capillaries and, in some normal animals, small amyloid deposits. The body and tail regions of the pancreas were selected for analysis since cellular composition of the islets in these regions is more homogeneous than that of islets in the pancreatic head [23]. Islet amyloid is restricted to those islets derived from the dorsal pancreatic primordium in man [24]. The earliest phases in the aetiology of diabetes in *Macaca mulatta* has been considered from physiological measurements to be primarily related to the spontaneous development of obesity and insulin resistance [17]. However, the dramatic reduction in the islet beta-cell population found in diabetic animals suggests that the disease process, at its latest stages, is also characterized by a major deficiency in islet beta cells. Furthermore, fasting plasma insulin levels in these animals were closely related to the beta-cell population in the islets. It is interesting to note that the monkey with the highest fasting IRI in the diabetic group (monkey D) had the highest beta-cell area per islet and the least severe amyloidosis. Previous longitudinal physiological studies on this monkey showed that fasting plasma IRI levels had been falling from the time of onset of diabetes (B.C.Hansen, unpublished data). Although diabetic animals had a highly significant reduction in beta-cell population, the low fasting insulin levels were not different from those found in animals of groups I and II. This suggests that the low number of residual insulin-producing cells in islets of the diabetic animals must be hyperactive. Similar high levels of synthesis and release have been found in a streptozotocin (STZ)-induced model of diabetes in baboons with a 42 % reduction in beta-cell mass [25]. However, there is no clearly defined minimum beta-cell mass associated with hyperglycaemia. Previous morphometric studies in Type 2 diabetes in man have shown no change [23] or 25 %–40 % reduction in beta-cell population [2, 26] whereas a more severe reduction (> 50 % in beta-cell area per islet) was found in the present study.

Islet amyloidosis appears to precede the onset of overt diabetes in spontaneously diabetic *Macaca mulatta* as in *Macaca nigra* [19, 21] and spontaneously diabetic cats [27]. Small scattered amyloid deposits were found in pancreatic islets of obese and hyperinsulinaemic normoglycaemic animals but fewer islets were affected in these monkeys than in the diabetic animals. The small amyloid deposits were not associated with a significant reduction in beta-cell area and it is unlikely that the observed amount of amyloid would interfere physically with insulin secretion. The process of polymerization of amyloid peptides is progressive and once started the mechanism is continuous [28]. Association of amyloidosis and progression to diabetes is suggested from the data of one hyperinsulinaemic, normoglycaemic animal (monkey O); the moderately sized amyloid deposits were associated with a higher fasting plasma glucose level (6.6 mmol/l) and a lower plasma insulin level (847 pmol/l) than that found in the other hyperinsulinaemic animals. The correlation be-

tween the percentage of the islet occupied by amyloid and reduction of beta-cell area implicates amyloidosis in destruction of insulin-producing cells. A dramatic reduction in beta cells and little change in glucagon and somatostatin cells has been observed in the large islets studied in *Macaca nigra* [21].

Amyloid in pancreatic islets in man had long been thought to be a feature of aging but we found no relationship between the amount of amyloid and age. Two very old monkeys in group II (monkeys P and Q) remained normoglycaemic although one of them had small amyloid deposits indicating that the disease process, as in man, is very heterogeneous.

The increased islet size found in hyperinsulinaemic animals may represent either hypertrophy of beta cells or an increase in the number of beta cells in these islets. Islet size is increased in adult rats by infusion with glucose [29] and in genetically obese rats [30] and mice [31]. The possibility that animals with predetermined factors for the onset of diabetes have larger islets cannot be excluded. However, increased islet size associated with amyloidosis has been reported in Type 2 diabetic patients [32] and *Macaca nigra* [21], possibly related to progressive deposition of extracellular amyloid deposits.

Care should be taken about interpretation and measurement of islet size; separate areas may represent transection of the same islet at different positions due to the irregular shape of primate islets; similarly, single beta cells or small groups of beta cells may represent the outer border of larger islets. The irregularity of islet shape precluded use of the Weibel transform for conversion of data to volume density [33]. Equally, calculation of islet volume from measured diameters is inappropriate with irregular shaped primate islets [34].

In the diabetic group, a relatively larger proportion of the pancreatic tissue was occupied by islets (up to 27 % of tissue area). Although increased islet size would contribute to this phenomenon it is unlikely to be the only factor. Severe intralobular lipomatosis, pancreatitis or fibrosis of exocrine tissue, as has been observed in Type 2 diabetes [35], was not found in this study. Thus severe islet amyloidosis is not only associated with endocrine dysfunction but also with a dramatic reduction in exocrine proportion. As interlobular fat depositions were present in some animals but, by definition, not included in the tissue area in this study, calculations of islet and exocrine volume using pancreatic weight were unreliable. It has been shown that diminished exocrine secretory products and reduced exocrine volume are recognized features of Type 1 (insulin-dependent) diabetes and, to a lesser degree, Type 2 diabetes in man [36, 37]. The requirement of a high intrapancreatic insulin level for maintenance of exocrine function has been proposed [38].

The important question that remains is whether islet amyloid is merely a secondary feature of beta-cell dysfunction or whether its deposition contributes to the development of the diabetic syndrome. Amyloid deposits may present a physical barrier in the islet and cause impaired beta-cell function, or may be part of a process of beta-cell deterioration. The massive accumulations of amyloid associated with beta-cell loss are likely to contribute to the in-

creased severity of the disease in *Macaca mulatta*. Similar progressive islet amyloidosis in man has been associated with the eventual need for insulin therapy [4]. In this animal model an extensive amount of islet amyloid appears to be a sensitive pathological indicator for diabetes.

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