Islet Morphometry in the Diabetic Pancreas of Man

KEN SAITO, TOHRU TAKAHASHI, NOBUHISA YAGINUMA and NORIYUKI IWAMA

The First Department of Pathology, Tohoku University School of Medicine, Sendai 980


--- Quantitative changes of the pancreatic islets in diabetes mellitus were analyzed by a stereological method. 26 maturity-onset and 5 growth-onset diabetics, and 37 nondiabetics including 9 hypertensives were selected from autopsy materials and the pancreases were subjected to histometry. The total islet volume \( V_i \) was 0.974 cm\(^3\) in the control, whereas it was only 0.596 and 0.255 cm\(^3\) in the maturity-onset and growth-onset diabetic groups, respectively. The hypertensive group gave almost the same value as the control. There was an obvious negative correlation between \( V_i \) and the maximum blood sugar level during glucose tolerance test, whether the case was diabetic or not. Moreover, in the diabetic group \( V_i \) diminished with descending age of onset. These findings indicate the importance of \( V_i \) in the pathophysiology of diabetes and support the classical concept of insulin deficiency as the primary pathogenetic role. On the other hand, the total islet number \( N_i \) decreased with increasing mean radius \( \bar{r} \), and the diabetic and control cases shared a common regression of \( N_i \) on \( \bar{r} \). The diabetic pancreas was not characterized by \( N_i \), \( \bar{r} \) or by the distribution pattern of \( r \).

In studying the pathogenesis of diabetes mellitus in man, it is important to correlate the development of the disease to some morphological changes of the pancreatic islets. Especially useful is information about their quantitative behavior. About a couple of decades ago, Maclean and Ogilvie (1955) and Gepts (1958) reported that the total islet volume was significantly small in the diabetic pancreas. Further they discussed changes in the number and size of islets in diabetes, principally on the basis of mean area of islet sections (Maclean and Ogilvie 1955, 1959; Gepts 1965). Their methods, however, do not seem to be pertinent. As already stressed by some authors (Warren et al. 1966), the two quantities cannot be estimated immediately from measurements of islet sections on a histological slide.

In the foregoing paper (Saito et al. 1978), the authors reported an application of the stereological method of Suwa et al. (1976) to islet morphometry in the nondiabetic human pancreas. The present report is a further extension of the

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quantitative analysis to diabetic insular changes. The study involves comparative examinations of the diabetics and nondiabetics in terms of several quantitative parameters such as the total volume and number of islets, mean islet radius and the distribution pattern of islet radii. The results are correlated to the laboratory data of glucose tolerance.

Besides, nondiabetic cases of sustained hypertension were included in the examination, because the disease sometimes produces ischemic pancreatic injuries which may influence the quantity of insular tissues.

**MATERIAL AND METHODS**

The material consists of the pancreases from the autopsy cases of 15 growth-onset and 26 maturity-onset diabetics and 28 nondiabetic patients (Table 1). Diagnosis of diabetes mellitus was established on the basis of urinalysis, fasting blood sugar measurement (FBS) and 50 g oral glucose tolerance test (GTT). The data of GTT were available for 45 cases and were evaluated according to the criteria recommended by the Committee of Japanese Diabetes Society (Kuzuya et al. 1970), in which the diabetic type was characterized by venous blood sugar levels higher than 160 and 130 mg/100 ml 1 and 2 hr after glucose administration, respectively. The diabetics in the present series exhibit varying impairment of carbohydrate metabolism, but none of them was chemical diabetic. The control cases were so selected as to match the diabetic groups in age composition (Table 1). Besides, 9 pancreases were obtained from nondiabetic, hypertensive autopsy cases in which systolic blood pressure was persistently higher than 180 mmHg.

After 10% formalin fixation the volume of the pancreas was measured by means of water replacement; then each one sagittal slice was taken at the caudal one third and one fourth portions where the size and density of islets were most stable and uniform in the whole organ (Saito et al. 1978). The slices were embedded in paraffin. A 3 μm thick section was prepared from each block and stained with the modified Masson’s trichrome method.

The principle of morphometrical procedure is as follows: If a test line of sufficient length is randomly drawn on a histological slide, a number of chords are delivered by intersection of the line with islet sections. By using the horizontal line of the eye-piece

**Table 1. Overall examined cases**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases (M:F)</th>
<th>Age at death (years)</th>
<th>Age diagnosis (years)</th>
<th>Body weight* (mg/100 ml)</th>
<th>FBS (mg/100 ml)</th>
<th>BS&lt;sub&gt;max&lt;/sub&gt; (mg/100 ml) during GTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic Control</td>
<td>28 (18:10)</td>
<td>51.1</td>
<td>91.4</td>
<td>75.6</td>
<td>130.8</td>
<td></td>
</tr>
<tr>
<td>Hypertensive</td>
<td>9 (5: 4)</td>
<td>49.6</td>
<td>96.2</td>
<td>88.9</td>
<td>148.7</td>
<td></td>
</tr>
<tr>
<td>Diabetic Growth-onset</td>
<td>5 (2: 3)</td>
<td>37.6</td>
<td>19.4</td>
<td>85.0</td>
<td>181.5</td>
<td>311.0</td>
</tr>
<tr>
<td>Maturity-onset</td>
<td>26 (17: 9)</td>
<td>61.3</td>
<td>55.1</td>
<td>96.5</td>
<td>158.2</td>
<td>290.9</td>
</tr>
</tbody>
</table>

* Percentage of ideal body weight (=100) calculated on the basis of Table by Japanese Welfare Ministry in 1962. In the four different groups arithmetic means of the percentage did not differ significantly.
Fig. 1. Photomicrograph of an islet section illustrating histometrical procedures. Perpendicularly crossed scales of eye-piece are superimposed on an islet section. By using the horizontal line as a test line, and by sliding a histological section along the direction of the line, the length of chords delivered is successively measured. It is seen in the figure that a chord of 44 divisions is generated by the horizontal line. The vertical scale is available to determine the interval of the parallel scanning lines. Modified Masson’s trichrome stain. × 200. Reproduced from Tohoku J. exp. Med., 1978, 124, 177-186.

shown in Fig. 1 as the test line, and by sliding the histological section in the direction of the line, the length of chords was successively measured under 200-fold magnification. When measurement along a test line was finished, the histological section was shifted in the vertical direction at an interval of 0.3 mm, and the sampling was continued along the new test line. In this way the whole pancreatic section was scanned with parallel test lines. Chord lengths obtained from the histological slides of two different parts of the pancreas were pooled for each case. The number of chords measured was 200 to 500 in most cases. Occasional hyalinized islets in some diabetic cases were also included in the measurement. Even in the case with the highest incidence of insular hyalinization, the number of chords generated from the hyalinized islets was only 21 out of 233, and the exclusion of such chords would not influence the results significantly.

The shape of the islets was simulated by spheres of different radii \( r \), and Weibull function was assumed for the distribution of \( r \). Weibull function is expressed as:

\[
N(r) = N_{vo}(m/r_0)(r/r_0)^{-m-1} \exp\left[-(r/r_0)^m\right]
\]

where \( N_{vo} \), \( m \) and \( r_0 \) are the number of islets per unit volume, geometrical parameter of distribution pattern and scalar parameter, respectively, and each parameter was determined from the chord length distribution. It was reported in the foregoing papers (Suwa et al. 1976; Saito et al. 1978) that this function fits the islet radius distribution excellently. The detailed mathematical treatments were also described in those reports. The volume ratio of islets was calculated from the sum of chord lengths per unit length of test line.

**RESULTS**

Quantitative changes of islets with aging

Influence of age on various quantities of the pancreatic islets was examined in 28 control cases. There is no decrease in the total islet volume \( V_i \) with advancing age (Fig. 2).

Geometrical parameter \( m \) which primarily determines the pattern of islet radius distribution, however, rises with age (Fig. 3). The regression equation is given by:

\[
m = 0.0048x + 0.7931,
\]
Fig. 2. The total islet volume $V_i$ and the age in the control cases. No senile decrease of $V_i$ is noted.

Fig. 3. The pattern estimator $m$ of the islet radius distribution rises steadily with age in the control group. The regression equation of $m$ on the age $x$: $m = 0.0048x + 0.7931$ (correlation coefficient $= 0.411$, $p < 0.05$).

where $x$ is the age in years. On the other hand, $m$ is closely correlated with the scalar parameter $\tau_0$, as expressed by:

$$m = 34.01\tau_0 + 0.4032.$$  

The correlation coefficient is 0.932, which is statistically significant at 1% level. These relations determine the shape of expected distribution curve of islet radii for different ages. The curves calculated for 25, 50 and 75 years of age are shown in Fig. 4 in which all the curves show a remarkably skew pattern with the highest incidence in the smallest range of $r$. But there is a definite age-dependent change of the pattern, and the ratio of larger islets increases with aging. The mean radius $\bar{r}$
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Fig. 4. Expected patterns of the islet radius distribution calculated for three different ages in the control group. 1, for 25 years of age; 2, for 50 years; 3, for 75 years. Note a relative increase of larger $r$ with advancing age.

TABLE 2. Group means of estimated quantities*

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>$V_p$(cm³)</th>
<th>$V_i$(cm³)</th>
<th>$N_i$(10⁶)</th>
<th>$\bar{r}$(mm)</th>
<th>$m$</th>
<th>$r_a$(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>28</td>
<td>58.18</td>
<td>0.974</td>
<td>8.091</td>
<td>0.0184</td>
<td>1.036</td>
<td>0.0186</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±5.67</td>
<td>±0.090</td>
<td>±2.115</td>
<td>±0.0022</td>
<td>±0.085</td>
<td>±0.0027</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>9</td>
<td>49.31</td>
<td>0.839</td>
<td>8.426</td>
<td>0.0170</td>
<td>0.983</td>
<td>0.0169</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±12.79</td>
<td>±0.222</td>
<td>±5.005</td>
<td>±0.0041</td>
<td>±0.117</td>
<td>±0.0047</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth-onset</td>
<td>5</td>
<td>31.60</td>
<td>0.255</td>
<td>4.867</td>
<td>0.0165</td>
<td>1.059</td>
<td>0.170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±15.54</td>
<td>±0.177</td>
<td>±8.127</td>
<td>±0.0076</td>
<td>±0.251</td>
<td>±0.0090</td>
</tr>
<tr>
<td>Maturity-onset</td>
<td>26</td>
<td>50.42</td>
<td>0.596</td>
<td>5.980</td>
<td>0.0173</td>
<td>1.025</td>
<td>0.175</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±7.54</td>
<td>±0.096</td>
<td>±1.509</td>
<td>±0.0023</td>
<td>±0.067</td>
<td>±0.0026</td>
</tr>
</tbody>
</table>

* The confidence limits were calculated using t-distribution ($\alpha=0.05$).

Statistical significance (t-distribution)

- $V_p$: control, hypertensive, maturity-onset diabetic>growth-onset diabetic ($\alpha<0.05$)
- $V_i$: control, hypertensive>maturity-onset diabetic>growth-onset diabetic ($\alpha<0.02$; $\alpha<0.01$, respectively)
- (control>maturity-onset diabetic, $\alpha<0.001$).

of islets also increases slightly with age and the islet number $N_{v_o}$ per unit volume decreases, but these differences are not substantiated by statistical tests ($0.05<\alpha<0.10$).

In the diabetic group, the age of death did not influence the estimated quantities.

Comparison of the estimated quantities between diabetic and nondiabetic groups

Group means of the estimated quantities are listed in Table 2 and are graphi-
The total islet volume $V_i$ in the four different groups. The mean of $V_i$ is indicated by a short vertical line for each group. Note obvious diminution of $V_i$ in the two diabetic groups. See also Table 2.

The total islet number $N_i$ in the four groups. No significant reduction of $N_i$ is noticed in the diabetic groups. See also Table 2.

The mean islet radius $\bar{r}$ in the four groups. Numerically compared among the four groups in Figs. 5, 6 and 7. The volume $V_p$ of the pancreas is significantly smaller in the growth-onset diabetic group than in the other three groups ($\alpha<0.05$).

There is a prominent difference in the total islet volume $V_i$ between the groups, and the mean is 0.974 cm$^3$ for the control, 0.596 cm$^3$ for the maturity-onset diabetic and 0.255 cm$^3$ for the growth-onset diabetic group. The difference between any two of the values is statistically significant (Table 2; Fig. 5). The hypertensive group gives the mean of 0.839 cm$^3$, which does not differ significantly from that of the control.

The total islet number $N_i$ is slightly smaller in the diabetic groups than in the control, but the difference is not statistically significant (Table 2; Fig. 6). No significant difference between the four groups can be established with regard to the other indices, namely, the mean islet radius $\bar{r}$, the geometrical parameter $m$ and the scalar parameter $\tau_0$ of the distribution of $r$ (Table 2; Fig. 7). The islets in the diabetic pancreas are not characterized by these quantitative-morphological parameters.
Relation between the total islet number and the mean radius

It is found that the total islet number $N_i$ becomes evidently smaller with increasing mean radius $\bar{r}$ when all the examined cases are pooled (Fig. 8). There appears to be a definite linear regression of $N_i$ on $\bar{r}$ if these values are expressed in logarithmic scales. The regression is common to the diabetic and nondiabetic groups, and is expressed by the equation:

$$\log_{10} N_i = -2.569 \log_{10} \bar{r} + 2.183$$

which can be rewritten on Cartesian scales as:

$$N_i = 152.4 \bar{r}^{-2.569}.$$  

The above equation is drawn in Fig. 8 as the regression curve.

The total islet volume $V_i$ and the clinical history of diabetes

In the diabetic group, an obvious positive correlation is noticed between the total islet volume $V_i$ and the age at which the diagnosis of diabetes was made (Fig. 9). The correlation coefficient between $V_i$ and the age in years at diagnosis is 0.594, which is significant at 1% level.

Another notable finding is a negative correlation between $V_i$ and the duration of diabetes (Fig. 10). If the duration of diabetes is expressed in years, the correlation coefficient is $-0.461$, which is significant at 2% level. However, this relation becomes obscure when only the senescence-onset cases are taken into consideration. Within the 16 patients whose diabetes was first diagnosed after the
Fig. 9. The total islet volume $V_i$ decreases progressively with descending age of onset in the diabetic group. $\Delta$, maturity-onset diabetic; $\triangleright$, growth-onset diabetic. The regression equation of $V_i$ on the age of onset $x$: $V_i = 0.0095x + 0.0786$ (correlation coefficient $= 0.594$, $\alpha < 0.01$). One growth-onset diabetic (asterisked) presented a clinical course of insulin-independent, maturity-onset type of the disease. For details see text.

Fig. 10. The total islet volume $V_i$ vs. duration of diabetes. The same symbols as in Fig. 9 are used.

age of 50, no correlation can be obtained between $V_i$ and the diabetes duration.

$V_i$ versus levels of FBS and maximum blood sugar during GTT

In the diabetic group the level of fasting blood sugar (FBS) rises progressively with decreasing total islet volume $V_i$ (Fig. 11). The regression equation was determined in a linear form between logarithmic values of FBS and $V_i$, and the correlation coefficient is $-0.583$, being significant at 1% level. The regression equation is rewritten on Cartesian scales, and is shown in Fig. 11.

In the present study the data of GTT have been available for 19 nondiabetic and 26 diabetic cases. The maximum blood sugar level or BS$_{\text{max}}$ during GTT
Fig. 11. The level of fasting blood sugar gradually increases with decreasing total islet volume \( V_i \) in the diabetic group. The same symbols as in Fig. 9. The regression curve is given by: 
\[
FBS = 107.7 V_i^{-0.413}
\]
For calculation of the equation see text.

Fig. 12. The total islet volume \( V_i \) vs. maximum blood sugar level during GTT. 
\( \triangle \), maturity-onset diabetic; \( \blacktriangle \), growth-onset diabetic; \( o \), control; \( \bullet \), hypertensive. The regression equation: 
\[
BS_{\text{max}} = 140.6 V_i^{-0.994}
\]
Note a continuous transition between the diabetics and nondiabetics.

**DISCUSSION**

It has been reported by several authors that the aging by itself implies a potential impairment of carbohydrate metabolism (Silverstone et al. 1957; Pozefsky et al. 1965; Crockford et al. 1966). Wrenshall et al. (1952) demonstrated a downward trend of insulin extractable from autopsy pancreas after the sixth decade of life. In the present study, however, impaired carbohydrate metabolism of aged individuals is not suggested, because control subjects do not reveal a
senile decrease in the total islet volume $V_i$.

On the other hand, worthy of note is a relative increase of larger islets with advancing age in the control group, as revealed by the gradual increase of $m$ (Figs. 3 and 4). The reason for the prominence of larger islets in the senile subjects is unknown. An explanation may be that the islet is normally subjected to a structural turnover of very slow advancement, which becomes impeded with aging. Continuous growth of cells with protracted breakdown of islet may cause partial enlargement of islets. The mechanism can be compared with the enlargement of hepatocellular nuclei in senile livers, which has been well-known as an aging phenomenon (Tauchi and Sato 1962).

The group mean of the total islet volume $V_i$ is 0.947 cm$^3$ in the control, whereas it is only 0.596 cm$^3$ and 0.255 cm$^3$ in the maturity-onset and growth-onset diabetic groups, respectively. The difference between any two of the means is statistically significant. Maclean and Ogilvie (1955, 1959), Gepts (1958) and Westermark and Wilander (1977) reported similar values calculated from the areal ratio of islet sections to the pancreatic section. In recent years, a possible contribution of glucagon to diabetogenesis has been emphasized (Unger and Orci 1975). However, according to the reports by Maclean and Ogilvie and Gepts, the total weight of A cells also reduced in diabetes, although less pronouncedly than that of B cells. Further, Sherwin et al. (1976) have demonstrated that continuous glucagon infusion does not affect blood glucose and ketone levels in insulin-treated diabetics. On the other hand, a series of hypotheses on insulin antagonism have been presented since the observation of Yalow and Berson (1960), but the recent progress in the analysis of blood glucose-insulin interrelation gradually reveals that the insulin response is obviously impaired even in early diabetes and prediabetes (Perley and Kipnis 1966; Cerasi and Luft 1967a; Seltzer et al. 1967; Goto et al. 1971; Fujita et al. 1975). All these observations indicate the importance of insulin deficiency in the pathogenesis of diabetes mellitus.

Correlation analysis with the laboratory data further emphasizes the importance of $V_i$ as an index of glucose tolerance. In the present series, GTT values were available in 45 cases including both nondiabetics and diabetics. The maximum blood sugar level during GTT rises progressively with decreasing $V_i$ (Fig. 12), and it deserves special notice that the negative correlation is consistent even within the nondiabetic group. There are nondiabetics that exhibit relatively small $V_i$ and correspondingly lower glucose tolerance, and these cases are mixed with the diabetic cases in a rather broad range of $V_i$ from 0.6 to 1.0 cm$^3$.

The overlapping of the nondiabetic and diabetic groups in the above range presents a feasible definition of prediabetes on a morphological basis. Clinical observations have also revealed that there is a continuous transition between the individuals of carbohydrate tolerance and intolerance (Cerasi and Luft 1967a, b; Fajans and Conn 1959). In the present series, nondiabetic cases of $V_i$ of 0.6 to 1.0 cm$^3$ may be regarded as being susceptible to extrainsular diabetogenic influences on account of lower carbohydrate tolerance. It seems likely that they tend to
manifest diabetic syndrome and are in the prediabetic condition.

In the diabetic group, the total islet volume depends upon the age at diabetes onset. The younger the age at diagnosis, the value of $V_i$ (Fig. 9) is the smaller. Maclean and Ogilvie (1955) noticed a similar trend in the total weight of B cells. The age-dependence of $V_i$ may correspond to the well-known difference in the severity of diabetes between the growth-onset and maturity-onset types, as exhibited by the more severe fasting hyperglycemia in the former type (Goto et al. 1976). At the same time, Fig. 9 shows a continuous transition of $V_i$ between the growth-onset and maturity-onset diabetics. An example of intermediate case is presented by a woman of 60 years (asterisked in Fig. 9). Her diabetes was found at as early as 21 years of age, but the clinical course thereafter was that of the insulin-independent, maturity-onset type, until the patient died of coronary heart disease. $V_i$ in this case was 0.416 cm$^3$, a value which is not so minimized as in ordinary growth-onset cases. Diabetes of a similar type was also reported by Fajans et al. (1971).

$V_i$ also diminished along with the duration of diabetes, but not significantly in the patients whose diabetes had been found after the age of 50 years. The above diminution would be attributable to the pronounced reduction of $V_i$ in the younger diabetics.

In the present study the analysis of cell composition in insular tissues was not performed. The total volumes of A and B cells will be estimated and correlated to the clinical data in the forthcoming report.

It seems rather surprising that the diabetic pancreas is hardly characterized by other morphometrical indices, such as the total islet number $N_i$, the mean radius $r$ or the pattern estimator $m$ (Table 2). The results are not compatible with an assumption that islet changes, such as maturity suppression, compensatory neogenesis or hypertrophy underlie diabetes. However, there is one notable relation. $N_i$ decreases with increasing $r$, forming a hyperbola-like regression curve (Fig. 8). The close correlation seems to have some bearing on the dynamics of a supposed cycle of neogenesis, growth and breakdown of islets, but its exact meaning remains to be clarified. The figure simultaneously exhibits a complete overlapping of diabetics and nondiabetics on $N_i$-$r$ coordinates.

All the subjects in the hypertensive group had high systolic blood pressures over 180 mmHg. Histological examination disclosed multiple hypertensive arteriolar lesions with subsequent necroses and scarring in the pancreatic tissue. In spite of the pronounced vascular lesions, however, significant difference is not found between the hypertensive and control groups in any morphometrical indices of the islets (Table 2). It appears likely that the islets are so located in the pancreatic vascular structure as to escape hypertensive circulatory disturbances.

References


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