Assessment of Isolated Islet Equivalents

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Following a consensus report in 1990 by Ricordi et al[1] on islet isolation assessment the total volume of isolated islets is generally expressed in the number of islet equivalents (IEQs) — defined as islets of 150 µm diameter— and many centers now use the advocated, now classic, international procedure (CIP) of dividing all islets with diameters of ≥ 50 µm in classes of 50 µm increments (ie, 50 to 100, 100 to 150, etc.) for calculation of the number of IEQs. For each class the number of IEQs is calculated by multiplying the islet counts with a conversion factor — based on the mean volume of that class. We previously[2] recorded all islets ≥40µm in 10 µm increments, and routinely categorized the isolated islets in 25-µm classes for similar calculation of IEQs, albeit using slightly differently derived conversion factors, as detailed in what follows (conventional Leiden procedure [CLP]). Because accurate sizing of isolated islets is time-consuming and may interfere with the progress of isolation and quality of the islets, we recenty examined the convenient CIP for calculation of pig islet yields, and noted up to 300 % higher yields compared to our conventional calculations. We, therefore, re-examined the assessment of islet yield in 51 canine, human, and pig islet preparations by an unbiased "control procedure" based on the actual size (with no classification) of the islets vs (i) the CIP; (ii) a modified international procedure (MIP) using 25-µm rather than 50-µm class increments; and (iii) our CLP. In addition, calculation of the volume-average diameter (VAØ) of the islets, and the impact of the VAØ on procedures for calculation of IEQs is reported.

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METHODS

We re-examined the islet yield data obtained during consecutive islet isolations — largely performed as described in detail previously[2] — in digest preparations of the canine splenic (n = 10), duodenal (n = 11), and whole pancreas (n = 6), the tail of human pancreas (n = 4); and also in the digest and purified islet preparations (n = 20) obtained from the body segment of pig pancreas. For morphometry, multiple 25- or 50-µL aliquots of the islet suspensions were stained with dithizone on a microscopic slide, and the mean diameter of each islet ≥50 µm was recorded using an ocular micrometer by microscopy at 100x magnification performed as accurately as possible, that is, in increments of 10 µm (i.e. 50, 60, etc.). As a control procedure the islet equivalent yield was assessed, without the bias introduced by dividing the data into classes, by calculating the volume of each islet based on the actually measured islet diameter (Ø), dividing the islet volume by the volume of 1 IEQ, and summing the data. This corresponds to calculating for each islet $Ø^3/150^3$, and summing the results. Likewise, the volume-average diameter may be calculated by multiplying, for each islet, the islet diameter (Ø) by $Ø^3/150^3$ (ie, take $Ø^4/150^3$), and then summing the results for all islets, and dividing the total by the total number of IEQs in the sample. These control data were used for comparison with three different procedures based on dividing the islets in size classes for calculation of the yield of IEQs as described next, and illustrated in Table 1.

According to the classic international procedure (CIP) islets are categorized in classes of 50-µm increments. For each class, the number of IEQs is calculated by multiplying the number of islets with a conversion factor for that class — derived from the mean volume of the class, divided by the volume of an islet with a 150-µm diameter. Thus, for instance, for the 50- to 100-µm class, the conversion factor is $0.5\ (^4/_3\ \pi\ 25^3 + {}^4/_3\ \pi\ 50^3)\ /\ {}^4/_3\ \pi\ 75^3$, which, using diameters , equals $0.5(50^3 + 100^3)/150^3 = 0.1667$. Based on this we calculated the VAØ of the islets by multiplying the mean diameter of each class by the number of IEQs in that class, divided by the total number of IEQs in the sample, and summing the results.

Likewise, according to our modified international procedure (MIP), islet yield was calculated after dividing the islets into classes of 25-µm intervals. Thus, islets are divided in the classes 50 to 75, 75 to 100, etc, and in, for example, the 50- to 75 µm class, the conversion factor equals: $0.5\ (50^3 + 75^3)/150^3 = 0.081$.

Our conventional Leiden procedure (CLP)[2], is also based on 25-µm classes, but (I) it starts with a lower limit of 37.5 µm (eg, 37.5 to 62.5, etc.); and (ii) the conversion factors are based on the mean diameter (not the mean volume) of a class. Thus, for instance, the conversion factor for the 37.5- to 62.5-µm-diameter class (mean diameter 50 µm) equals: $50^3/150^3 = 0.037$.

Differences between means were analyzed by analysis of variance and Scheffé's procedure for multiple contrasts, and considered not significant (NS) at $P > .05$.

RESULTS AND DISCUSSION

The deviations of the values obtained by the classic international procedure (CIP) from our control values for islet equivalent yield correlated with the volume-average diameter (VAØ) of the islet preparations. The mean yield of human islets (VAØ = 222 ± 115) amounted to 107 ± 19%, 100 ± 2%, and 101 ± 1% of the control values (NS), as calculated by the CIP, the modified international procedure (MIP), and our conventional Leiden procedure (CLP), respectively. For canine splenic (VAØ = 171 ± 46), whole organ (VAØ = 129 ± 37), and duodenal (VAØ = 127 ± 26) pancreatic islets, false high values of 116 ± 12%, 134 ± 22%, and 129 ± 15% respectively, were obtained using the CIP ($P < .0001$), but values obtained using the MIP (100 ± 4, 103 ± 7, 102 ± 7%, respectively) and CLP (101 ± 1%, 101 ± 2%, 102 ± 6%, respectively) did not differ significantly from control values. The on-average small porcine islets (VAØ = 97 ± 34) could clearly not be converted to IEQs using the CIP (183 ± 43% vs control IEQs; $P < .0001$); however, only insignificant deviations from control values were demonstrated using the MIP (120 ± 13%; NS) and the CLP (100 ± 4%; NS). Overall, irrespective of the origin of the islet preparations ($n = 51$), regression analysis demonstrated a strong negative correlation ($r = 0.92$; $P < .0001$) of the VAØ of an islet preparation vs the ratio of IEQs calculated by the CIP and control procedure. Regression demonstrated the CIP to be accurate for preps with a VAØ ≥200µm, but VAØs ≤ 150 µm resulted in false high values ≥ 125%. Thus, for islet preparations with VAØs < 150µm, the ≤25-µm islet-sizing procedures should be used.

REFERENCES

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2. Van der Burg MPM, Guicherit OR, Frölich M, et al: Cell Transplant 3:91, 1994

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Table 1. Comparison of four procedures for assessment of yield, expressed in islet equivalents (IEQs), and the volume-average diameter (VAØ)*

Procedure	Diameter (μm) Mean	From	Through	Calculation of IEQs x Factor	Counts	IEQs	Calculation of VAØ(μm) (Mean Ø x IEQs / TotalIEQs)	
1) Classic International Procedure (CIP)								
	75	50	100	0.167	224	37.33	75 x 37.33 / 56.91 =	49.20
	125	100	150	0.648	25	16.20	125 x 16.20 / 56.91 =	35.59
	175	150	200	1.685	2	3.37	175 x 3.37 / 56.91 =	10.36
					Total IEQs=	56.91	VAØ (= total) =	95.2
2) Modified International Procedure (MIP)								
	62.5	50	75	0.081	187	15.15	62.5 x 15.15 / 39.62 =	23.90
	87.5	75	100	0.211	37	7.79	87.5 x 7.79 / 39.62 =	17.21
	112.5	100	125	0.438	18	7.88	etc.	22.36
	137.5	125	150	0.789	7	5.53		19.18
	162.5	150	175	1.294	1	1.29		5.31
	187.5	175	200	1.979	1	1.98		9.37
	212.5	200	225	2.873	0	0		0
	237.5	225	250	4.002	0	0		0
	262.5	250	275	5.396	0	0		0
	287.5	275	300	7.081	0	0		0
	312.5	300	325	9.086	0	0		0
	337.5	325	350	11.438	0	0		0
	362.5	350	375	14.164	0	0		0
	387.5	375	400	17.294	0	0		0
					Total IEQs =	39.62	VAØ =	97.3
3) Conventional Leiden procedure (CLP)								
	50	37.5	62.5	0.037	151	5.59	50 x 5.59 / 36.77 =	7.61
	75	62.5	87.5	0.125	46	5.75	75 x 5.75 / 36.77 =	11.73
	100	87.5	112.5	0.296	38	11.26	etc.	30.62
	125	112.5	137.5	0.579	9	5.21		17.71
	150	137.5	162.5	1.000	5	5.00		20.40
	175	162.5	187.5	1.588	1	1.59		7.56
	200	187.5	212.5	2.370	1	2.37		12.89
					Total IEQs =	36.77	VAØ =	108.5
4) Control procedure (no classification; but accuracy of sizing in practice limited to 10-μm increments)								
	50			0.037	106	3.93	106 x 50^4/150^3 =	196.3
	60			0.064	45	2.88	45 x 60^4/150^3 =	172.8
	70			0.102	36	3.66	etc.	256.1
	80			0.152	10	1.52		121.4
	90			0.216	15	3.24		291.6
	100			0.296	12	3.56		355.6
	110			0.394	11	4.34		477.2
	120			0.512	7	3.58		430.1
	130			0.651	2	1.30		169.3
	140			0.813	3	2.44		341.5
	150			1.000	2	2.00		300.0
	170			1.456	1	1.46		247.5
	200			2.370	1	2.37		474.1
					Total IEQs =	36.27	VAØ (Sum / Total IEQs) =	105.7

Ø = diameter; ^ = to the power of.

*Representative pig pancreatic digest sample, demonstrating overestimation (157%) of yield by the CIP vs our control procedure; no 160-, 180-190 μm islets were observed.