The Review of DIABETIC STUDIES

ORIGINAL DATA

How to Use Image Analysis for Islet Counting

Peter Girman, Zuzana Berkova, Eva Dobolilova and Frantisek Saudek

Diabetes Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.
Address correspondence to: Peter Girman, e-mail: pegi@medicon.cz

Manuscript submitted April 15, 2008; resubmitted May 22, 2008; accepted May 25, 2008.

■ Abstract

AIM: Assessment of islet mass before islet transplantation requires a reliable technique to enable exact analysis of islet volume. This study aimed to test the applicability of digital image analysis (DIA) for evaluation of samples of purified and non-purified islets. METHODS: Pancreatic islets were isolated from 10 Lewis rats. Samples of purified (n = 10) and non-purified islets (n = 30) were counted conventionally and by using a computerized method. The equipment for the computerized counting consisted of a digital camera installed on a stereomicroscope and connected to a personal computer. Images of 2272x1704 pixels were processed using a previously described non-commercial program originally developed for this purpose. Islets were converted to equivalents using globe and ellipsoid models. The insulin content of purified islets was assessed using radioimmunoassay and was correlated to the absolute and standardized islet number. RESULTS: Mean absolute numbers of purified islets ± SD were 908 ± 130 and 1049 ± 230 (manually and DIA respectively). Mean insulin content ± SD obtained from purified islets was 161 ± 45 mU. The mean equivalents of purified islets (1589 ± 555 for globe and 1219 ± 452 for ellipsoid) significantly correlated with insulin content. However, this correlation was not significant when absolute islet numbers were used, counted using either method. There was no significant difference in absolute non-purified islet numbers assessed by manual and computerized methods (average ± SD in 50 μl samples; 12.6 ± 4.1 and 13.3 ± 5.3 respectively; $p = 0.22$). The manual method showed a significantly higher yield of islet equivalents (IE; $p < 0.001$ for both globe and ellipsoid). CONCLUSION: The computer-based system for islet counting correlated better to insulin content than conventional islet estimation and prevented overestimation. Reproducibility and ease of assessment make it potentially applicable to clinical islet transplantation.

Keywords: diabetes · islet counting · islet equivalent · islet mass · imaging · islet transplantation · insulin content

Introduction

The mass of transplanted islets is undoubtedly recognized as the basic predictive factor for successful islet transplantation [1] Several different techniques were developed for evaluation of the islet yield, i.e. the amount of islets obtained from donor tissue. Basically, they can be divided into light-based optical methods, laser scanning methods, biochemical procedures, fluorescence sorting and electrozone sensing.

Biochemical methods are based on the observation that beta-cells contain certain substances in significantly higher concentrations. Insulin and zinc content are investigated and compared to islet volume. Hesse *et al.* proposed an ingenious method of estimating islet mass by using insulin/amylase ratios [2]. According to this method, insulin represents endocrine tissue, amylase content defines the degree of exocrine contamination. Most centers, however, did not adopt this technique as it contains some flaws. It is not clear, whether insulin represents islet-insulin alone, because some in-

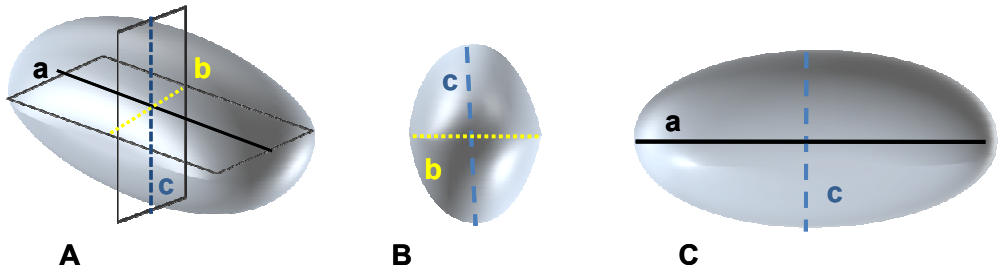

Figure 1. Basic diameters of islets. Three basic diameters could be located on 3D regular objects depending on their position in space. Difference in islet volume counted from three diameters increases significantly as the formula for volume uses a third diameter power.

sulin is released during the isolation process. The activity of amylase can be affected by interaction with other enzymes during incubation. In addition, measuring insulin by radioimmunoassay takes time and the results are not available before transplantation. Another substance, zinc, is a trace element situated mostly in beta-cells, though small amounts were found in alpha-cells and brain cells. The standard exact approach for measuring zinc content has been simplified by Jindal *et al.* using fluorescence staining [3]. They demonstrated a high correlation to islet yield, even in the presence of up to 50% exocrine tissue, and a significant shortening of the measuring time. However, in order to remove additional fluorescence, the solutions must have an absolute purity, excluding zinc and other metals that could interfere. In addition, thresholds corresponding to islet equivalents should be defined and tested on human samples.

Electrozone sensing is based on a device called Coulter Multisizer [4]. For this method, islets are suspended in a conductive liquid and passed through an orifice between electrodes, by which they generate electric impulses. The magnitude of electric pulse is proportional to the particle volume. Compared to the manual approach, this method offers the advantages of a significantly shorter counting time, lower intra- and inter-observer variability, significantly lower islet mass and different size distribution. At present, it seems to be the only technique applied in practice for direct measurement of islet size and volume, which avoids adverse effects of the operator's subjectivity. However, the accuracy of the counting depends on the purity of samples. The presence of any amount of exocrine impurities could mean that results are misinterpreted.

Fluorescence-activated cell sorting seemed to be an attractive possibility combining islet purification and islet counting [5]. In an experiment reported by Gray and co-workers, rat islets stained by neutral red were separated by the sorter from unstained exocrine tissue and subsequently counted. The authors reported that islet numbers correlated negatively with purity. However, in clinical practice, it is inadequate to evaluate islet yield in absolute numbers. This should be determined by islet equivalents (IE), the internationally standardized unit for the determination of islet tissue volume. One IE corresponds to an islet with a diameter of 150 μm.

Three-dimensional information about islets can be obtained by laser scanning confocal microscopy [6]. In a study, single islets were scanned in a series of optical sections and input data were further processed using defined image algorithms. In practice, this method was used to determine the viability of islet cells, to investigate the spatial distribution of single cell types within islets and to analyse cell damage [7]. Although calculation of islet volume was simple (from the number of sections, the thickness of sections and the pixels in each section), it emerged that no more than one islet could be analyzed in this way. With an increasing amount of islets in the optical field, exact analysis might prove difficult.

Conventional assessments of islet yield use light-based optical techniques. Islets are counted in three small samples taken from whole suspension. The islets on the eyepiece are divided according to their estimated diameter into 5 categories under an optical microscope using a calibrated grid. The number of islets in each category is multiplied by a factor that converts the number of islets to IE. The total sum of equivalents represents the islet volume in one sample. The final yield is evaluated as the mean volume of three samples recounted on the whole suspension volume [8]. The accuracy of the calculation depends on four basic factors:

1. **The subjective judgement of the operator, who estimates islet diameters.** Islets are often irregularly shaped and take variable positions in the three dimensional space. The situation is simple when islets are globe-shaped. In this case, there is only one estimated diameter independent of islet positions. Changing the shape to ovoid brings about 3 different possible diameters in a 2-dimensional picture depending on islet positions (Figures 1A-1C).
2. **The ratio of the sample to the whole suspension volume.** Taking small samples from the large volume of islet suspension makes mistakes more likely when evaluating islet mass.
3. **Size of the optical grid.** The smaller the size of the microscope reticule eye, the more accurate the estimate of islet diameters.
4. **Purity of samples.** With an increasing volume of impurities, the exocrine tissue overlapping the islets can increase errors when calculating islet volumes.

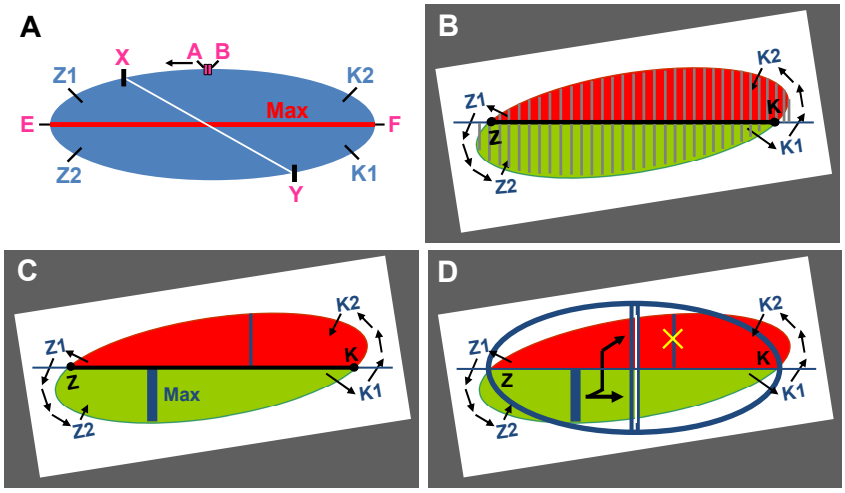

Figure 2. Screening of the axes. A: Screening for the major axis starts from base points A and B and then continues to base points E and F. The endpoints of the major axis will be located on the opposite site, while circumferential distance between base point and endpoint will be one half of object circumference. Once the major axis is defined (EF), the algorithm looks for further conditions. The first, area of ellipse, should be equivalent to area of object. The second, distances from one side of circumferential points, should be equivalent to distances from the opposite site of the major axis. These two conditions could be fulfilled by an ellipse defined by the major axis after first screening. But the algorithm will test all axes from the first one within the range of 1/16 of object circumference (axes from Z1K1 to Z2K2). **B**: The sum of distances from the circumferential points to the major axis, which are counted from both sides (green and red), should be zero. **C**: Finding the minor axis as the longest distance from the circumference perpendicular to major axe. **D**: One of the ellipses that will be considered. Only the ellipse which fulfills the defined conditions will considered to be the best.

An optimal light-based method should compensate for all these disadvantages. Computer vision may represent an approach that provides valuable outcomes.

Computer vision is a scientific discipline studying ways to simulate human vision. At a high level, computer vision applies complex techniques involving artificial intelligence. At a lower level, commonly known as digital image analysis, 2-dimensional data is processed to obtain quantitative information about real objects [9]. The basic steps involved in digital imaging are:

1. Taking the picture and saving it on adequate media.
2. Preprocessing data to cut off signal noise.
3. Segmenting the image.
4. Describing the objects.
5. Classifying the objects described.

Stegemann *et al.* demonstrated digital imaging to be rapid, consistent and objective in islet quantification. Islet volume significantly correlated with both extractable insulin and DNA content [10]. In contrast, manual methods were found to overestimate the total amount of endocrine tissue with poor correlation with insulin or DNA concentrations [11]. Other authors presented similar techniques with comparable software, which resulted in digital imaging of comparable effectiveness [12-14].

We previously reported that a digital counting method using an optical microscope connected to a digital camera was useful for evaluating purified islets

[15]. The algorithm of the software developed in our laboratory differed from the applications used by other authors in several ways. Firstly, we tried to define an appropriate 2-dimensional mathematical model for islet shape; we chose the ellipse and globe. Both are applicable to islet shape and similar formulas are involved for calculating related 3-dimensional objects (ellipsoid and globe). An ellipse is defined by formula where the main longer axis and shorter minor axis form a 90° angle. The algorithm used to find a suitable ellipse started by screening the longest diameter of the object recognized. (Figure 2A). The ellipse was then defined after finding the longest minor axis perpendicular to the main diameter (Figure 2C). This first screening step continued to look for ellipses that might have better parameters than the first one, This means that they should fulfill other conditions. The area of the object (islet) should be equivalent to the area of the ellipse, a condition that could lead to the shortening of the main diameter, which should not fall below 95% of the diameter found after the first screening (Figure 2D). The second condition was that the sum of the distances from the main axis to single circumferential points should be zero. If the sum of distances from one side of the axis has a positive value and the sum of distances from the other side has a negative value, then the sum of all distances will be zero in the case of the optimal ellipse (Figure 2B). The volumes of related three-dimensional objects were then calculated using general formulas for the ellipsoid and globe.

The techniques of digital image analysis mentioned above were generally tested on samples of purified islets. However, in a practical situation, final islet volume differs considerably and usually contains an unpredictable number of exocrine components. Therefore, the aim of this study was to test the applicability of the method for counting islet samples before purification. Using the manual and computerized method, we assessed islet distribution according to their diameters and we evaluated islet loss during the purification process using a density gradient.

Methods

Animals and islet isolation

Pancreatic islets were isolated from 8-10 week old Lewis rats (Charles River, Germany) as previously described [16]. Briefly, after an intraductal collagenase injection (1 mg/ml, Sevapharma, Czech Republic) the distended pancreas was removed and digested at 37°C for 25 min. Islets were separated from the exocrine tissue by centrifugation in discontinuous Ficoll gradient (Sigma-Aldrich, USA). After washing in Hank's balanced salt solution (HBSS, Sigma-Aldrich, USA), the islets were resuspended in HBSS and counted by both methods, conventional and computerized counting.

The protocol was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and the experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Conventional counting

Samples of purified or unpurified islets were placed into separate Petri dishes and put on the stage of a stereomicroscope (Olympus SZH 10, Japan). Using the 50x magnification and the calibrated grid, islets in each sample were counted and sorted according to their approximate diameter in 50 μm increments ranging from 50 to 300 μm. The number of islets in each group was multiplied by the volume of a globe with its respective diameter. The result was obtained by dividing total volume by the volume of an IE [8].

Computer based counting (digital image analysis, DIA)

The apparatus for digital imaging consisted of a digital camera (Olympus C3030) installed on an Olympus stereomicroscope (SZH 10, Japan) and connected directly to a personal computer. Images of 2272 × 1704 pixels were processed by the previously described non-commercial program originally developed for this purpose [15]. The islets were identified by the software as regular, irregular, incomplete or small objects according to a preset definition of both the shape and pixel values. Border pixels were determined according to the operator's preset limits for background and object. If the pixel value fell into the range between object and background limits, it was marked as a pixel belonging to an object. The object was defined when several neighbor pixels are grouped together to form the object. Small objects containing only 2 pixels were automatically deleted. Only objects evaluated as "regular" were counted and converted into IE.

Although most islets have an ovoid and irregular form, their volume is usually calculated as the volume of a perfect globe. We assumed that islet shape is closer to an ellipse than to a perfect circle in two dimensional pictures. Therefore, regular objects were converted to IE as volumes of ellipsoids using the formula of an ellipse (formula: $4/3a^2b$, where a is the major half axis and b the minor half axis). To calculate the volume of a globe, the longest diameter of the ob-

ject is generally used (formula $4/3\pi r^3$, where r is the half of longest diameter).

In order to enhance the contrast between background and islets, microscopic examination of purified islets was performed in a dark field. Islets in non-purified tissue samples were stained red by dithizone, which made them recognizable from surrounding tissue. Then the pictures were taken by the camera in black-and-white mode and processed as described above (Figures 3A-3C). To avoid operator-to-operator bias, each of the methods was performed by a different subject.

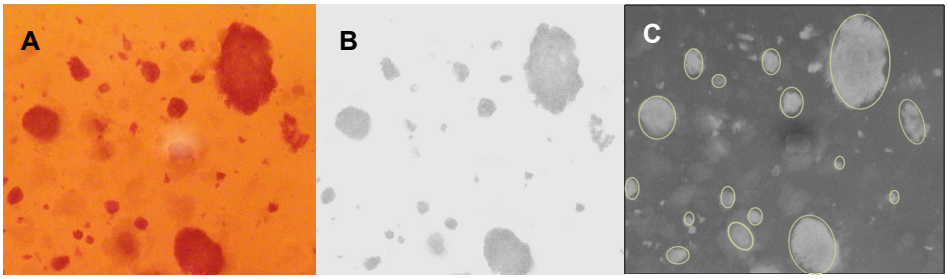

Figure 3. The principle of counting unpurified islets. A: Dithizone staining for recognition of islets from exocrine tissue. **B**: Segmentation of the picture to the background and objects. **C**: Definition of the objects and finding suitable ellipses.

Insulin content quantitative analysis

Purified islets from each pancreas were counted and homogenized. The tissue was resuspended in 1ml of acetic alcohol solution. The samples were sonicated and centrifuged for 10 min at 15 000 rpm. The insulin content in the supernatant was assessed with radioimmunoassay (I^{125} Insulin Ria Kit, ICN Pharmaceutical, USA) [17] and was correlated to absolute and standardized islet numbers.

Experiment 1: counting of purified islets

Freshly isolated purified islets from 10 donors were counted manually and by using the computerized method. Insulin content was subsequently assessed. Absolute islet numbers were counted by both methods. Standardized islet numbers (expressed in IE), the total area of all objects expressed in pixels and the total volume of all islets (expressed in μm^3) were evaluated by the computerized method. All parameters were correlated with insulin content.

Experiment 2: counting of non-purified islets

Absolute islet number and amount of non-purified islet equivalents were estimated in samples stained by dithizone. After digestion, 3 samples from each of 10 islet suspensions (n = 30) containing the endocrine and exocrine tissue were placed into Petri dishes and evaluated using both the manual and computerized method.

Experiment 3: estimation of purification effectiveness

The volume of non-purified islets obtained from each pancreas was counted as the average value of three 50 µl samples taken from a 10 ml suspension. Islet loss during purification was assessed as the difference between the numbers of non-purified and purified islets and IE. The distribution of islets according to their diameter was expressed in histograms before and after purification. Only the digital imaging approach was used in this experiment.

Statistical methods

The correlation between measuring methods and insulin content was tested in terms of the Pearson correlation coefficient. The paired t-test was applied to analyze data obtained by both manual and computerized methods in purified samples. Agreement between both techniques used in counting of non-purified islets was evaluated by calculating the mean difference (high value minus low value) and standard deviation (SD) of the difference. The values indicate how far the results of the manual method is over (upper limit) or below (lower limit) the results obtained by computerized counting. Upper and lower limits were counted as mean difference ± 2 times SD [18].

Results

Experiment 1: counting of purified islets

The mean absolute numbers of purified islets (± SD) per pancreas were 908 ± 130 and 1049 ± 230 (manually and DIA respectively). The mean insulin content (± SD) obtained from purified islets was 161 ± 45 mU. Equivalents of purified islets, total area of objects and total volume of objects (all determined by DIA) correlated significantly with insulin content.

However, this correlation was not significant when absolute islet numbers were used, counted either by the computerized method or the manual approach. Islet equivalents counted manually did not correlate with insulin content ($r = 0.74$, p = ns, Table 1). The mean times ± SD for counting purified islets manually and by the computerized method were 68 ± 7 and 19 ± 6 min respectively (paired t-test, $p \leq 0.001$).

Table 1. Exactness of computerized islet counting evaluated by means of insulin content

Parameter	Mean (n = 10)	SD	Cor. coeff.	p
Absolute number of islets (DIA)	1049.9	203.2	0.49	ns
Absolute number of islets manually	908.0	130.0	-0.03	ns
IEs counted as globes (DIA)	1589.1	555.8	0.89	< 0.05
IEs counted as ellipsoids (DIA)	1219.8	452.5	0.86	< 0.05
Total area of all objects (pixels)	1,9181,982.1	5,412,533.5	0.85	< 0.05
Total volume of counted objects (μm^3)	2,155,852,761.0	800,014,608.8	0.86	< 0.05
IEs counted manually	1008.6	400.5	0.75	ns

Legend: Absolute number, islet quantity and islet area on the image counted in pixels and total volume of islets were expressed as mean ± SD and correlated to insulin content. All parameters measured by the computerized method (DIA - digital image analysis) correlated significantly with insulin amount. IE: islet equivalent.

Experiment 2: counting of non-purified islets

There was no significant difference (using paired t-test) in absolute non-purified islet numbers assessed by both manual and computerized methods (average ± SD in 50 µl samples; 12.6 ± 4.1 and 13.3 ± 5.3 respectively; $p = 0.22$). The manual method showed a significantly higher yield of IE ($p < 0.001$ for both globe and ellipsoid), as is demonstrated in Table 2. The use of mean differences to test for agreement between both approaches has shown that the absolute number of islets counted manually can be 6 islets lower or 7 islets higher than that measured by the computerized method. This difference became more evident after conversion to IE, as is shown in Table 2. The mean times ± SD for counting unpurified islets manually and by using the computerized method were 14 ± 2 and 4 ± 1 min, respectively (paired t-test, $p \leq 0.001$).

Experiment 3: estimation of purification effectiveness

The mean absolute numbers of non-purified islets per pancreas (manually and DIA) were 2526 ± 622 and 2680 ± 810 respectively. No statistical difference, examined by paired t-test, was found ($p = 0.65$). When quantified in IE, significantly lower mean islet numbers were counted by DIA (globe: 3290 ± 1885, ellipsoid: 2300 ± 2520) than by the conventional method (6786 ± 3259; $p = 0.004$ for both globe and ellipsoid), suggesting a significant overestimation by the conventional method.

Mean islet losses, caused by purification and assessed by the computerized method were 61%. The average losses of IE, counted on the basis of globe or ellipsoid, were 51% for globe and 47% for ellipsoid respectively. The distribution of islets and IE were analyzed according their diameter in 50 µm increments (Figures 4 and 5). We concluded from the histograms that even if large islets are far less common than small ones, they contribute significantly to tissue volume. Islet equivalents presented in the histograms were counted from a mathematical globe-shaped model. The ellipsoid model gave a similar pattern of results even if absolute numbers were lower.

Discussion

The general approach to evaluating islet numbers is based on manual counting. Using an optical microscope with a calibrated grid, the islets are counted one by one in a representative sample and sorted according to estimated diameter. The number of islets is multiplied by a coefficient corresponding to diameter range and endocrine mass is assessed from representative samples of all tissue [8].

The recently reported simple computer-based method uses software that recognizes islets from a background, counts them and expresses their quantity in IE. This method has been shown to give results that correspond to manual counting of small samples of highly purified islets. We verified this technique on larger samples of rat purified islets and then tested it on samples of non-purified tissue. Computerized counting was done on a single microscopic field with magnification of 15x and image discrimination of 2272 × 1704 pixels. One pixel corresponded to 6.4 µm and the program performed the measurements with an error of ± 6.4 µm per pixel. The manual method used an optical grid on a microscope, which had an eye of 50 × 50 µm.

The error involved with manual counting, therefore, is ± 50 μm. The implication is that with this optical grid islets with a diameter of multiple of 50 μm only can be precisely measured. For instance, the size of an islet with a diameter of 164 μm can only be estimated not measured, as its diameter lies between 150 and 200 μm.

Table 2. Mean absolute numbers and islet equivalents counted by both methods in 30 non-purified samples

Method	Mean ± SD	p
Absolute number of islets per sample (n = 30)		
1. Computerized method	13.3 ± 5.3	ns
2. Manual method	12.6 ± 4.1	
Difference (1 - 2)	0.7 ± 3.7	
Lower limit (mean - 2SD)	-6.4	
Upper limit (mean + 2SD)	7.9	
IE per sample (n = 30)		
1. Computerized method, globe	16.9 ± 14.3	< 0.001
3. Manual method	33.9 ± 24.7	
Difference (1 - 3)	16.9 ± 17.8	
Lower limit (mean - 2SD)	-18.7	
Upper limit (mean + 2SD)	52.5	
2. Computerized method, ellipsoid	11.5 ± 12.6	< 0.001
3. Manual method	33.9 ± 24.7	
Difference (2 - 3)	11.6 ± 12.6	
Lower limit (mean - 2SD)	-13.6	
Upper limit (mean + 2SD)	36.8	

Legend: Results are compared using the paired t-test, and agreement between both methods is expressed as mean difference ± SD. Upper and lower limits indicate how far the results obtained by one method could be above or below those obtained by the other. IE: islet equivalent.

Parameters measured included islet number, islet equivalents, area of recognized objects and the total volume of recognized objects. As we expected, the parameters obtained from the computerized system correlated highly with insulin amounts in comparison with the poor correlation obtained when absolute numbers and islet equivalents were measured by the routine method. Similar results were reported by Stegemann *et al.* [11]. Islet amounts, when obtained by the use of the ellipsoid model, were obviously lower than those counted when using the globe model. Basic formulas involve a discrepancy, when the volume of the ellipsoid is a function of the product of 2 longer and one shorter diameter, while the volume of the globe is a function of the third power of the longer diameter. Nevertheless, the results based on both models correlated with insulin amounts in a similar way. The distribution of islets according to diameter confirmed that even if found in larger absolute numbers, small islets did not significantly contribute to total tissue volume.

Exocrine tissue has similar optical density to the islets, which preclude their recognition using a standard optical microscope. Staining of beta-cells by dithisone increased the contrast between islets and exocrine impurities, so that the computer was able to detect them even in non-purified samples and estimate their volume. Unpurified tissue was evaluated by the conventional method and then by digital image analysis. As both approaches estimate the same parameters (diameter, number and volume), correlation did not usually provide information about agreement between methods. Therefore, we analyzed data using mean difference and standard deviation. As shown in Table 2, values measured manually could differ by 6 islets above or 7 islets below the value obtained by the computerized method in a 50 μl sample. This discrepancy became significant when tissue was converted to islet equivalents. The 40 % overestimation obtained with conventional counting was reported by Pisania *et al.* in comparison with electron microscopy examination of samples [19], which is similar to the findings made by our group. When evaluating the effectiveness of purification by digital imaging, we can only assume that this process is highly unpredictable. In our experimental setting, it ranged from 23 to 70%, despite using a standard purification procedure.

Bock *et al.* reported on a method for unbiased estimation of the total number of beta-cells in the rodent pancreas. The process consisted of dividing a pancreas into several pieces, random sampling of the pieces and then performing a histological examination of the random sections. The mean volume of beta-cells in rat pancreas was 3.15 mm^3, which corresponded to 1789 islet equivalents [20]. Inuwa *et al.* used the method of random sampling to investigate beta-cell volume and number in weaning Wistar rats with data showing the same volume as in a later investigation [21]. As beta-cells constitute approximately 80% of the islet volume, these results are similar to our estimate of non-purified rat islet volume.

Manual image counting represents a combination of islet imaging using a software-generated grid and conventional counting of islets on this image. Kissler *et al.* studied digital image analysis on 15 human islet samples and demonstrated an excellent correlation with both manual image count and insulin content [13].

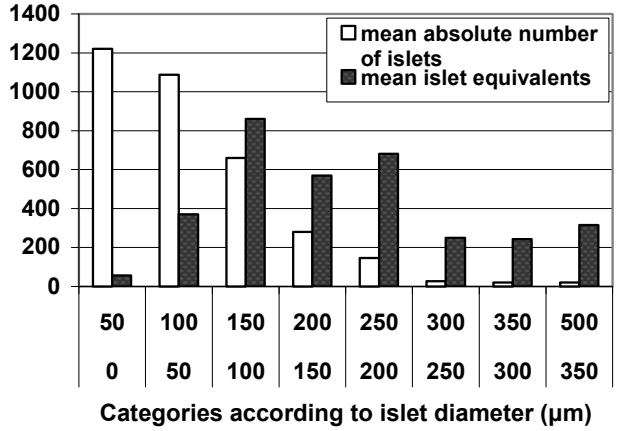

Figure 4. Distribution of non-purified islets and IE. Non- purified islets and IE were distributed according their diameter in 50 µm increments. Islet equivalents were counted from a model of a globe.

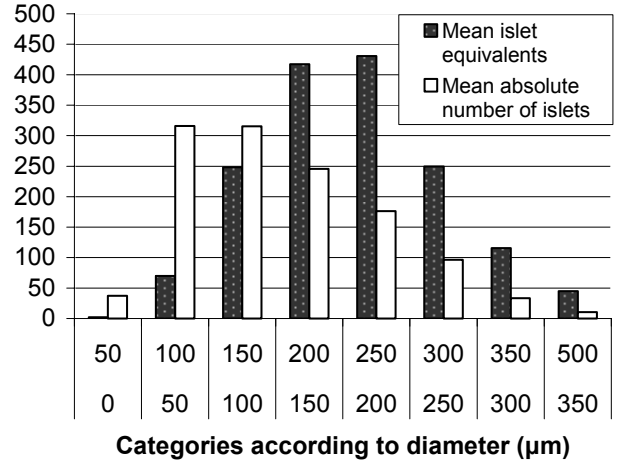

Figure 5. Distribution of purified islets and IE. Distribution of islets and IE was analyzed according their diameter in 50 µm increments. Large islets were far less common than small ones, but they contributed significantly to tissue volume. Islet equivalents were counted from a mathematical globe-shaped model.

The evaluation of 3-dimensional objects from 2-dimensional data represents the main limitation of our method. Pancreatic islets are ovoid in shape and can be further deformed by variable processes [22]. Although the shape of rat islets seems to be more regular than in other species including humans, the globe or ellipsoid may not be sufficiently exact models for estimation of volume. In this case, the other parameter, the area of 2-dimensional objects can be used to estimate amount as it correlated well with insulin content. A larger amount of exocrine tissue could overlap islets and cause important discrepancies when counting. In this connection, an adequate dilution should be used before evaluating a sample. The removal of operator bias and shortening of evaluation time represent the most important advantages.

New approaches including fluorescence microscopy, positron emission tomography and magnetic resonance are effective ways of visualizing islet mass in vivo. In combination with digital imaging, they could provide more comprehensive information on islet status before and immediately after transplantation [23-25]. On the other hand, their use is much more complex, difficult and expensive.

In conclusion, we report on a simple computer-based system for islet counting, which correlates better to insulin content than conventional estimation of islet mass, and prevents overestimation. Reliability, reproducibility and ease of assessment when applied to purified as well as to non-purified islet mass, indicate its potential for monitoring islet mass during the human islet isolation process. At present, this system is routinely used to assess human islet yield in our laboratory. However, further research is needed into whether this approach correlates with other techniques used to estimate islet mass in clinical islet transplantation.

Acknowledgments: The work was supported by grant no. VZ 00023001.

References

1. **Ryan EA, Lakey JR, Rajotte RV, Korbutt GS, Kin T, Imes S, Rabinovitch A, Elliott JF, Bigam D, Kneteman NM, et al.** Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 2001. 50(4):710-719.
2. **Hesse U, Sutherland DE, Florack G, Field J, Ward S, Najarian JS.** Comparison of two methods of islet preparation and transplantation in dogs. *Diabetes* 1986. 35(10):1109-1118.
3. **Jindal RM, Taylor RP, Gray DW, Esmeraldo R, Morris PJ.** A new method for quantification of islets by measurement of zinc content. *Diabetes* 1992. 41(9):1056-1062.
4. **Lehmann R, Fernandez LA, Bottino R, Szabo S, Ricordi C, Alejandro R, Kenyon NS.** Evaluation of islet isolation by a new automated method (Coulter Multisizer Ile) and manual counting. *Transplant Proc* 1998. 30(2):373-374.
5. **Gray DW, Gohde W, Carter N, Heiden T, Morris PJ.** Separation of pancreatic islets by fluorescence-activated sorting. *Diabetes* 1989. 38(Suppl 1):133-135.
6. **Merchant FA, Diller KR, Aggarwal SJ, Bovik AC.** Viability analysis of cryopreserved rat pancreatic islets using laser scanning confocal microscopy. *Cryobiology* 1996. 33(2):236-252.
7. **Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, Powers AC.** Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J Histochem Cytochem* 2005. 53(9):1087-1097.
8. **Ricordi C, Gray DW, Hering BJ, Kaufman DB, Warnock GL, Kneteman NM, Lake SP, London NJ, Socci C, Alejandro R, et al.** Islet isolation assessment in man and large

animals. *Acta Diabetol Lat* 1990. 27(3):185-195.

9. **Fetterhoff TJ, Wile KJ, Coffing D, Cavanagh T, Wright MJ.** Quantitation of isolated pancreatic islets using imaging technology. *Transplant Proc* 1994. 26(6):3351.
10. **Stegemann JP, O'Neil JJ, Nicholson DT, Mullon CJ, Solomon BA.** Automated counting and sizing of isolated porcine islets using digital image analysis. *Transplant Proc* 1997. 29(4):2272-2273.
11. **Stegemann JP, O'Neil JJ, Nicholson DT, Mullon CJ.** Improved assessment of isolated islet tissue volume using digital image analysis. *Cell Transplant* 1998. 7(5):469-478.
12. **Friberg A, Stahle M, Svensson A, Korsgren O, Brandhorst D.** Digital image analysis of islet samples yields consistent, reproducible results. CTS-IPITA-IXA 2007 Joint Conference, Minneapolis, 2007.
13. **Kissler HJ, Zhang X, Chen X, Kaufman DB.** Software digital image analysis to assess the islet yield of human islet products. *The 2006 World Transplant Congress, Abstract 1529,* Boston, MA, 2006.
14. **Niclauss N, Morel PH Sgroi A, Baertschiger R, Wojtusciszyn A, Berney T, Bosco D.** Computer-assisted digital image analysis of human islets. 27th Workshop of the AIDPIT Study Group, 2nd European Diabetes Technology and Transplantation Meddting (EuDDT), Igls, 2008.
15. **Girman P, Kriz J, Friedmansky J, Saudek F.** Digital imaging as a possible approach in evaluation of islet yield. *Cell Transplant* 2003. 12(2):129-133.
16. **Saudek F, Cihalova E, Karasova L, Kobylka P, Lomsky R.** Increased glucagon-stimulated insulin secretion of cryopreserved rat islets transplanted into nude mice. *J Mol Med* 1999. 77(1):107-110.
17. **Kriz J, Saudek F, Girman P, Novota P.** Enhancement of rat islet tolerance with bone marrow transplantation using a non-myeloablative procedure II: failure despite the presence of lymphocyte microchimerism in the fully allogeneic Lewis/Brown-Norway model. *Int J Tissue React* 2004. 26(3-4):75-83.
18. **Bland JM, Altman DG.** Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986. 1(8476):307-310.
19. **Pisania A, Weir CG, Colton CK.** Comparison of light and electron microscopy for estimating islet fraction and enumeration of islet equivalents with nuclei counting. *The 2006 World Transplant Congress, Boston, MA*, 2006.
20. **Bock T, Svenstrup K, Pakkenberg B, Buschard K.** Unbiased estimation of total beta-cell number and mean beta-cell volume in rodent pancreas. *Apmis* 1999. 107(8):791-799.
21. **Inuwa IM, El Mardi AS.** Correlation between volume fraction and volume-weighted mean volume, and between total number and total mass of islets in post-weaning and young Wistar rats. *J Anat* 2005. 206(2):185-192.
22. **Goldstein MB, Davis EA Jr.** The three dimensional architecture of the islets of Langerhans. *Acta Anat (Basel)* 1968. 71(2):161-171.
23. **Kriz J, Jirak D, Girman P, Berkova Z**, **Zacharovova K**, **Honsova E**, **Lodererova A**, **Hajek M**, **Saudek F.** Magnetic resonance imaging of pancreatic islets in tolerance and rejection. *Transplantation* 2005. 80(11):1596-1603.
24. **Moore A, Bonner-Weir S, Weissleder R.** Noninvasive in vivo measurement of beta-cell mass in mouse model of diabetes. *Diabetes* 2001. 50(10):2231-2236.
25. **Toso C, Zaidi H, Morel P, Armanet M**, **Wojtusciszyn A**, **Mai G**, **Baertschiger R**, **Buhler L**, **Berney T.** Assessment of 18F-FDG-leukocyte imaging to monitor rejection after pancreatic islet transplantation. *Transplant Proc* 2006. 38(9):3033-3034.

