Review

MOLECULAR METABOLISM

The challenge of correctly reporting hormones content and secretion in isolated human islets

Jean-Claude Henquin

ABSTRACT

Background: An increased access of research laboratories to isolated human islets has improved our understanding of the biology of the endocrine pancreas and hence the mechanisms causing diabetes. However, in vitro studies of human islets remain technically challenging, and optimal use of such precious material requires a minimum of rigor and coordination to optimize the reliability and share of the information. A detailed report of the demographics of pancreas donors and of the procedures of islet handling after isolation is important but insufficient. Correct characterization of islet basic functions (a token of quality) at the time of experimentation is also crucial.
Scope of review: I have analyzed the literature reporting measurements of insulin and glucagon in the human pancreas or isolated human islets. The published information is often fragmentary. Elementary features such as islet size, insulin content, or rate of hormone secretion are either unreported or incorrectly reported in many papers. Although internal comparisons between control and test groups may remain valid, comparisons with data from other laboratories are problematic. The drawbacks, pitfalls and errors of common ways of expressing hormone content or secretion rates are discussed and alternatives to harmonize data presentation are proposed.
Major Conclusions: Greater coherence and rigor in the report of in vitro studies using human islets are necessary to ensure optimal progress in our understanding of the pathogenesis of diabetes.
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Keywords Isolated human islets; Insulin; Glucagon; Hormone content; Secretion; Diabetes

1. INTRODUCTION

Metabolic homeostasis largely relies on pancreatic hormones, in particular insulin and glucagon, which primarily ensure the stability of blood glucose. Deficient insulin secretion by β-cells is the *sine qua non* condition for the development of diabetes, and abnormal regulation of glucagon secretion by α-cells exacerbates hyperglycemia. Thorough knowledge of the biology of the islet of Langerhans is thus imperative to clarify the pathogenesis of the disease. Rodent islets have been and remain invaluable experimental models in that challenging endeavor because they are easily accessible and lend themselves to genetic, molecular, and physiological manipulations that are impossible or difficult in human islets. However, it is necessary to ascertain that findings obtained with these models can be extrapolated to the human species.
The first in vitro study of human islets was published in 1970. It showed that islets isolated by collagenase digestion of the pancreas of five fetuses did not secrete insulin in response to glucose or tolbutamide [1]. One year later, islets isolated from the pancreas of a child with idiopathic hypoglycemia were reported to be glucose-responsive [2]. Thereafter, functional studies of human islets remained rare for two decades, with only one to three papers a year. It was in the mid 1990s that such studies really took off. This acceleration was initially made possible through collaborations with centers launching programs of islet transplantation and, more recently, by the establishment of human islets distribution networks. It is noteworthy, however, that only a small minority of papers published during the last 20 years are based on studies performed mainly or entirely with human islets.
A recent review rightly pointed out the challenges raised by the increasing use of isolated human islets and emphasized the need of adequate and standardized information on the characteristics of each preparation, in particular on the attributes of pancreas donors [3]. These recommendations are sound but insufficient because they mainly concern the donor demographics, much less the features of the isolated islets. There is no doubt that human islets display an intrinsic variability (between and within donors), but the reported heterogeneity is exaggerated by variable laboratory practices. It is also disappointing that the reliability of many conclusions is undermined by the lack of elementary information on the function of the islets. In this contribution, I review the literature reporting measurements of insulin and glucagon in the human pancreas or isolated human islets and discuss the pitfalls and limits of all modes of data expression. I also call attention to inconsistencies in the report of in vitro hormone secretion, which can make evaluation of the results problematic and comparisons with other laboratories impossible. Finally, simple recommendations are made to harmonize the presentation of results obtained with human islets.

2. HOW MUCH INSULIN IN A HUMAN PANCREAS?

Leo Pollak, a Czech doctor working in Vienna, pioneered measurements of insulin in the human pancreas more than 90 years ago [4].

Unit of Endocrinology and Metabolism, Faculty of Medicine, University of Louvain, Brussels, Belgium

E-mail: Jean-Claude.Henquin@uclouvain.be.

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His findings of a lower insulin activity in pancreatic extracts from diabetic patients were confirmed by the seminal study of Scott and Fischer [5], who developed the method of extraction in acid-ethanol that is still used by most laboratories. First, it seems relevant to draw attention to the faulty use of the term "content" while "concentration" is measured. Extraction of pancreatic fragments yields an insulin concentration (in µg per g or ml pancreas) from which the insulin content of the pancreas (µg per pancreas) can be calculated if the total weight of the organ is known. Unfortunately, some published studies are uninformative, because results were expressed in meaningless units.

Fifteen studies correctly reporting measurements of insulin in at least 8 human pancreases could be identified (Table 1). The four older ones (1938—1963) used bioassays to measure insulin [5—8] while the eleven others (1967—2017) used radio-immunoassays [9—19]. The analysis of results obtained in each study is based on means, medians, and weighted means to account for differences in the number (8—46) of studied pancreases. Because the range of values is not always available, the variability of pancreatic insulin concentrations is computed as coefficient of variation (CV). Within individual studies, this CV reflects the inter-subject variability; between studies, this CV reflects the inter-center variability.

Although the results based on bioassays (Table 1A) compare reasonably well with those based on radio-immunoassays (Table 1B), only the latter will be used for further calculations and comparisons with measurements in isolated islets. The inter-center variability is relatively small (CV = 35% and the delta max/min values = 3.6x). It is lower than the inter-subject variability (CV = 54% and delta max/min = 7.1x). For the 11 studies using radio-immunoassays, the insulin concentration averages 112 µg per g of pancreas. As the weight of the pancreas was known in only some of these studies, a standard weight of 90 g was used to calculate a total content of 112 µg × 90 g = 10.1 mg insulin per pancreas, which corresponds to ∼300 IU. Such stores are sufficient for 5—7 days in a normal individual. Note that 15—20% higher values are obtained on the basis of the 8 most recent studies [12—19]: ∼12 mg insulin per pancreas.

Table 1 — Insulin concentration in human pancreas.

Ref	First Author	Year	Region Pancreas	Cases (n)	Insulin (µg/g)	Variability	
						CV (%)	Max/Min
A Bioassays							
[5]	Scott	1938	HBT	14	60	49	6.3 x
[6]	Wrenshall	1952	B	52	82	43	—
[7]	Jorpes	1953	HBT	13	127	13	1.4 x
[8]	Steinke	1963	?	10	69	42	3.5 x
				89			
Mean					**85** (CV35%)	**37%**	**3.7 x**
Weighted mean					**84**		
Median					**76**		
B Radioimmunoassays							
[9]	Kimmel	1967	HBT	29	86	—	—
[10]	Rastogi	1973	HBT	32	54	70	13 x
[11]	Creutzfeldt	1973	*	21	69	46	9.4 x
[12]	Sutherland	1976	?	46	140	86	—
[13]	Hayashi	1977	?	15	199	39	6.6 x
[14]	Gersell	1979	HBT	13	122	50	5.9 x
[15]	Tasaka	1981	?	8	90	36	—
[16]	Tomita	1985	T	8	103	71	—
[17]	Tasaka	1986	T	21	110	57	—
[18]	Rahier	2008	B	25	126	44	3.9 x
[19]	Henquin	2017	B	39	130	40	3.8 x
				257			
Mean					**112** (CV35%)	**54%**	**7.1 x**
Weighted mean					**112**		
Median					**110**		

Original data expressed in nmol/g or IUnits/g were converted into µg/g: (1 nmol Insulin = 5.8 µg) (1 IU Insulin = 34.7 µg).
HBT: Head, Body, Tail of pancreas. *Samples taken in healthy pancreas near insulinoma. ?: Unknown region
CV: Coefficient of variation = SD x 100: mean (in percent).
Weighted mean: mean weighted for the number of cases in each study.

3. HOW MANY ISLETS AND ISLET EQUIVALENTS IN A HUMAN PANCREAS?

It may seem surprising that the total number of islets in a human pancreas remains uncertain 150 years after their first description by Langerhans [20]. However, calculations are complicated by the markedly skewed distribution of islet sizes. Much depends on whether small endocrine-cell clusters (<40—50 µm in diameter) are counted as "islets" although they contribute very little to the total islet mass [21—23]. In transplantation programs, it is standard practice to quantify isolated islets by normalization to spherical structures of 150 µm of diameter [24]. Such a sphere or "Islet Equivalent" (IEQ) has a volume of 1.77 nl. From the total islet volume in an intact pancreas, one can thus calculate how many IEQs the organ can contain. Although purely theoretical, the result will be useful to assess the significance and reliability of measurements made in isolated islets.

Morphometric studies of autopsy pancreases permit determination of the total islet volume if the weight or volume of the pancreas is known (Supplemental Table 1). In a first series of reports, the volume density of whole islets, not only of a specific cell type, was measured relative to both epithelial and mesenchymal (vessel walls, stroma, e.g.) tissues [21,22,25—31]. In a second series, the volume density of β- and α-cells was measured and used to calculate the respective cell masses [32—39]. The sum of these β- and α-cell masses was augmented by 30% to account for the mass of other endocrine cells and islet interstitial tissue. Results collected in 17 studies are presented in Supplemental Table 1. Remarkably, the inter-center variability is again relatively small (CV = 35%) and similar to that of insulin concentrations (Table 1). The average islet volume amounts to ∼1.50 ml of pancreas, which corresponds to 845000 theoretical IEQs (diameter of 150 µm). If one assumes that the "average" islet has a diameter of 120 µm, a total number of ∼1.65 million islets can be estimated.

4. HOW MANY β-CELLS IN ONE ISLET EQUIVALENT?

That number cannot be directly derived from the widely measured relative areas occupied by the different endocrine cell types in islet sections because these cell types differ in volume. Furthermore, measurements rarely take the space occupied by non-endocrine cells and mesenchymal tissue into account. If we accept that β-cells occupy 55% of a standard islet volume [40—42], we obtain: 1.77 nl x 0.55 = 0.98 nl per IEQ. With a diameter of 12.2 µm [43,44] and thus a volume of 0.95 pl per β-cell, one calculates ∼1025 β-cells per IEQ. The result of this rough calculation is in keeping with more direct evaluations. A detailed morphological quantification has counted 1560 cells per IEQ, of which 1140 are β-cells and 420 non-β-cells (endocrine and non-endocrine) [42]. With another technique, based on dispersion of islets in single cells, a total of 1120 cells (all cells types) per IEQ were counted [45]. The reasonable approximation of 1000 β-cells per IEQ will be used in further discussion.

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5. HOW MUCH INSULIN IN ONE THEORETICAL ISLET EQUIVALENT?

From the measured insulin content of 10–12 mg per pancreas (Table 1) (section 2) and the calculated number of 845000 IEQs, one obtains a content of 12–14 ng insulin for a theoretical standard islet in situ. This value should be regarded as indicative and translated to IEQs of isolated islets with caution. Thus, the commonly used dithizone technique overestimates the actual size of isolated islets, mainly of the larger ones that are not perfectly spherical [46–48]. It is also unlikely that an islet retains the very same size as in situ after isolation. A decrease in islet size after a few days of culture has been attributed to collapse of vascular spaces [42]. Some loss of cells may also occur.

6. HOW MUCH INSULIN HAS BEEN MEASURED IN ISOLATED HUMAN ISLETS?

Several studies published in established journals regrettably used incorrect, uninformative units to report islet insulin content (Supplemental Table 2). To be clear, these incorrect modes of data expression do not invalidate comparisons between groups within the study but preclude objective evaluation of the islets in reference to those tested in other laboratories. In too many papers, the islet insulin content is not given although insulin secretion is expressed as a percentage of content or it is normalized without providing absolute values for the reference. After an extensive search of the literature and omission of duplicated reports of similar cohorts, about 40 studies could be identified in which the insulin content of islets isolated from at least 4 distinct donors was reported in a usable way (Tables 2 and 3). Even in these papers, the information was often difficult to retrieve, because it was given as a single mean in the Methods section or as a single column lost within a multi-paneled figure, when it was not relegated to supplemental material.

In the studies listed in Tables 2 and 3, all donors were non-diabetic adults but no distinction was made for sex or BMI. Only eight of these studies included measurements in islets from more than 20 donors. Insulin was consistently extracted by the acid-ethanol method, using highly variable numbers of islets per sample. Whenever that number was <100, the islets were handpicked. The islets were virtually always cultured (in different types of medium containing 5–6 mM glucose), but the length of the culture period was not always mentioned or was difficult to estimate when islets were tested at distance of the isolation center. These variables may have influenced the results. Five correct units have been used to express the insulin

Table 2 — Insulin content of isolated human islets.

A Values unrelated to islet size

Ref	First Author	Year	Donors (n)	Islets/Sample	Insulin (ng/islet)	CV (%)	Isolation center
[49]	Sandler	1997	8	30	~19	~35	Brussels (VUB)
[50]	Conget	1997	7	10	32.4	19	Barcelona
[51]	Straub	1998	12	5	44	63	Leicester
[52]	Hadjivasiliou	1998	8	5	16.2	23	Leicester
[53]	Bjorklund	2000	12	3–5	13.9	105	Brussels (VUB)
[54]	Marchetti	2004	10	30	4.0	27	Pisa
[55]	Henquin	2015	22	>300	17.8	45	Brussels (UCL), Lille
[56]	Manning Fox	2015	56	20	24.3	71	Edmonton
[57]	Brandhorst	2017	34	?	10.3	124	Geneva, Uppsala, Oxford
[58]	Li	2017	12	?	~60	~35	Several Centers USA
[59]	Thomsen	2018	16	15	~46	~55	Oxford, Edmonton
[60]	Lorza-Gil	2019	13	5	~21	~65	Geneva, Lille, Milan
			210				
Mean					**25.7** (CV65%)	56%	
Weighted mean excluding 2 most outliers					**22.8**		
Median					**20.0**		

B Values normalized to IEQ

Ref	First Author	Year	Donors (n)	Islets/sample	Insulin (ng/IEQ)	CV (%)	Isolation center
[61]	Jahr	1983	7	5[b]	~17[a]	~20	Karlsburg-Greifswald
[62]	Brandhorst	2003	54	?	5.0	115	Giessen
[63]	Nano	2005	12	1000	6.5	–	Milan
[64]	Guthalu	2010	5	15	~14.5	~35	Several centers USA
[65]	Dai	2012	5	?	~16	~25	Several centers USA
[66]	Lefebvre	2012	6	40	~12.5	~40	Lille
[67]	Zuellig	2017	4	?	~23[a]	–	Geneva, Milan, Giessen
[68]	Qi	2018	10	400	~10	~65	Duarte
[69]	Henquin	2018	43	>300	18.4	43	Brussels (UCL), Lille
[70]	Haliyur	2019	6	?	~10	~75	Pittsburg
			152				
Mean					**13.3** (CV42%)	52%	
Weighted mean excluding 2 most outliers					**14.6**		
Median					**13.5**		

Original data in pmol/islet or µUnits/islet were converted into ng/islet (1 pmol Insulin = 5.8 ng) (1 µU Insulin = 34.7 pg).
The sign ~ indicates that the value was read from a Figure in the original publication.
?: Unknown number of islets per sample.
[a] Value obtained after recalculation of published data.
[b] Fresh islets.

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Table 3 — Insulin content of isolated human islets normalized per DNA or protein.

A Values normalized to islet DNA

Ref	First Author	Year	Donors (n)	DNA (ng/islet)	Insulin (ng/ng DNA)	CV (%)	Isolation center
[75]	Eizirik	1994	10	28.7	1.6	53	Brussels (VUB)
[76]	Beattie	1997	8	–	0.25†	–	Miami
[77]	Bonner-Weir	2000	4	–	0.92*†	37	Boston (Harvard)
[78]	Dubois	2004	5	–	∼1.7	–	Lille
[79]	Bikopoulos	2008	4	–	17.6	65	Edmonton
[80]	Rosengren	2012	41	–	0.034	57	Lund, Oxford
[81]	Friberg	2012	68	–	2.5	110	Uppsala
[82]	Brandhorst	2016	24	–	5.8	92	Uppsala
[83]	Nacher	2016	6	∼25	∼1.1	∼87	Barcelona
[68]	Qi	2018	10	∼20/IEQ	0.50**	–	Duarte
			180				
Mean					**3.2** (CV166%)	72%	
Weighted mean excluding 2 most outliers					**2.59**		
Median					**1.35**		

B Values normalized to islet proteins

Ref	First Author	Year	Donors (n)	Proteins (μg/islet)	Insulin (ng/μg prot)	CV (%)	Isolation center
[85]	Squires	2000	6	0.40	12	51	London (King's)
[86]	Hanley	2010	5	–	∼54	∼45	Montreal (McGill)
[87]	Chowdhury	2013	5	–	∼35	∼30	Uppsala
[88]	Oh	2014	5	–	41	–	Several centers USA
[89]	Xie	2015	4	–	∼840	–	Edmonton
[90]	Yuan	2017	4	–	∼260	∼80	Several centers USA
[68]	Qi	2018	10	0.45/IEQ	∼22**	–	Duarte
			39				
Mean					**181** (CV167%)	52%	
Weighted mean excluding 2 most outliers					**31**		
Median					**41**		

Original data in pmol/islet or μUnits/islet were converted into ng/islet as in Table 2.
Islets were *90% and **80% pure. †Fresh islets.
The sign ∼ indicates that the value was read from a Figure in the original publication.

content of islets: per islet, per IEQ, per islet DNA, per islet protein and per islet dry weight (Supplemental Table 2). All have drawbacks.

6.1. Insulin content in ng per islet

Table 2A shows the islet insulin content reported by 12 studies totaling over 200 donors [49–60]. Remarkably, the inter-subject variability is similar to that observed for measurements of the insulin concentration of the undigested pancreas (CV = 56% vs 54%), whereas the inter-center variability is greater (CV = 65% vs 35%) (delta max/min = 15x vs 3.6x). Distinct laboratory procedures of islet isolation and handling must underlie this increased variability. The average content of ∼22 ng insulin per islet (Table 2A) exceeds the theoretical value of 12–14 ng per IEQ calculated above. Although commonly used, this easy mode of expression suffers from one serious drawback: the size of the studied islets is not known and likely to be larger than IEQs, particularly when islets are handpicked.

6.2. Insulin content in ng per islet equivalent

Table 2B shows the insulin content of isolated islets reported in 10 studies after normalization to IEQ [61–70]. The inter-subject variability (CV = 52%) is not modified, whereas the inter-center variability is decreased (CV = 42%) (delta max/min = 4.6x). The average of ∼14 ng insulin per IEQ (Table 2B) is similar to the theoretical value of 12–14 ng per IEQ.

Normalization to IEQ, however, is fraught with two problems. As already mentioned the traditional technique based on dithizone staining is approximate and overestimates the islet size [48]. A second drawback is that it does not inform on the actual size of tested islets. The same number of IEQs can be computed by counting different numbers of small and big islets: a volume of 10 IEQs corresponds to 6 islets with a diameter of 180 μm and 25 islets with a diameter of 110 μm. The problem can readily be solved by providing the "islet size index" (ratio of the calculated number of IEQs to the actual number of counted islets) [71]. An "islet size index" above 1.0 is computed when tested islets have an average diameter greater than 150 μm. The underlying issue is the relationship between islet size and hormone content or secretion. The insulin content normalized per IEQ is independent of islet size in *non-selected* preparations with "islet size index" between 0.41 and 1.69, which corresponds to average islet diameters of 110 and 180 μm [69]. However, the situation may be different when smaller and larger islets are compared after selection by handpicking [67,72], probably because the proportion of β-cells decreases with the increase in islet size [35,73,74].

6.3. Insulin content in ng per ng islet DNA

Reference to DNA (i.e. cell number) indirectly normalizes to islet size. Table 3A shows that this type of normalization tends to increase the inter-subject variability (CV = 72%) and markedly augments the inter-center variability (CV = 166%) [68,75–83]. Even after omission of the lowest and highest values, which are not plausible, the variability between centers remains very high (delta min/max = 23x) partly because impure preparations were tested. At best, one can suggest that the islet insulin content ranges between 1 and 3 ng per ng DNA. Another problem is that the DNA content of tested islets is only rarely reported. Like other somatic human cells, β-cells contain ∼6.7 pg DNA [47,84]. An IEQ containing 1200–1500 cells would thus be

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Table 4 — Insulin and glucagon in human pancreas and isolated islets.

A Insulin and glucagon in pancreas

Ref	First Author	Year	Cases (n)	Insulin (μg/g)	Glucagon (μg/g)	Ratio Glcg/Ins (%)	Pancreas content (mg) Glcg/Ins
[13]	Hayashi	1977	15	199	16.4	8.2	–
[14]	Gersell	1979	13	122	11.4	9.3	–
[15]	Tasaka	1981	8	90	16.9	18.7	–
[16]	Tomita	1985	8	103	7.2	7.0	–
[19]	Henquin	2017	39	130	9.3	7.6	0.81/11.3
			83				
Mean				**129** (CV33%)	**12.2** (CV35%)	**10.1**	
Weighted mean				**135**	**11.4**	**9.0**	
Median				**122**	**11.4**	**8.2**	

B Insulin and glucagon in islets

Ref	First Author	Year	Donors (n)	Insulin (ng/islet)	Glucagon (ng/islet)	Ratio Glcg/Ins (%)	Isolation center
[94]	Anderson	1976	6	4.8	2.6	56.0	Uppsala
[92]	Bedoya	1986	6	46/μg dry wt	7.0/μg dry wt	15.0	Microdissection[a]
[97]	Walker	2011	6	32	0.59	1.8	Oxford
[98]	Marchetti	2012	3	4[b]	0.26	6.5	Pisa
[99]	Vilches	2016	4	∼7.5	∼0.5	6.7	London (King's)
[58]	Li	2017	12	60	1.4	2.3	Several centers USA
[100]	Brissova	2018	7	3.87/IEQ	0.21/IEQ	5.4	Pittsburg
[70]	Haliyur	2019	6	∼10/IEQ	∼0.70/IEQ	7.0	Pittsburg
			50				
Mean						**12.6**	
Weighted mean after exclusion of one outlier						**5.8**	
Median						**6.6**	

The sign ∼ indicates that the value was read from a Figure in the original publication.
[a] Fragments of islets microdissected from freeze-dried thick sections of pancreas; original data per μg dry weight.
[b] Insulin value taken from another study (Ref. [54] in Table 2A).

expected to contain ∼8–10 ng DNA, much less than the few published values (Table 3A). Measurements of 25–29 ng DNA/islet indicate that the tested islets were either very large or not pure.
In fact, normalization to islet DNA is not a logical option. First, the technique requires handpicking of islets to eliminate as many contaminating exocrine cells as possible. Second, a substantial fraction of measured DNA corresponds to non-β endocrine cells and non-endocrine cells, the proportion of which may vary between islets, in particular with size, and between individuals, diabetic or not. There is thus no real advantage over normalization to IEQ. Normalization to DNA, however, may be useful in comparisons between native islets and insulin-producing cell lines or pseudo-islets reconstituted from dispersed islet cells. The caveat is that expressing insulin concentration or secretion per DNA inevitably overestimates (by almost 2-fold) the performance of homogenous cell lines compared to islets that contain several cell types.

6.4. Insulin content in ng per μg islet protein

This mode of expression is less common and has sometimes led to publication of aberrant results (Table 3B) [68,85–90]. When extremely high values are omitted one obtains an average of 31 ng of insulin per μg protein, which is plausible if the islet protein content (rarely reported) is in the order of 0.45 μg. This value should however be compared to the islet dry weight (section 6.5). Normalization to islet protein is not more logical than to DNA for the same reasons. A major source of error is contamination by exogenous proteins from the culture or incubation medium. If the latter contains 1% albumin, 1 μl of medium carries the same amount of proteins as about 20 islets.

6.5. Insulin content in ng per μg dry weight

Three studies have used the dry weight of human islets as a reference and reached discordant results. The value of 8.5 ng insulin/μg measured in isolated islets [91] is much lower than the 46–55 ng/μg found after microdissection of islet cores in freeze-dried pancreatic slices [92,93]. An islet dry weight of 0.8 μg was measured in preparations from 7 donors [94]. For comparison, the calculated dry weight of one IEQ (assuming a ratio dry/wet of 0.3) is $1.77 \times 0.3 = 0.53$ μg. Normalization to dry weight is not more specific of β-cells than normalization to DNA or proteins. It is not popular and has been abandoned.

7. HOW MUCH INSULIN IN ONE β-CELL?

From an average β-cell mass of 0.750 g or ml (Supplemental Table 1) and a β-cell volume of 0.95 pl (section 4), one calculates that a pancreas contains about 800 million β-cells. For 10–12 mg insulin in a pancreas, one obtains 12.5–15.0 pg of insulin per single β-cell. Since this calculated average content is certainly unequally apportioned among individual cells, measurements in purified β-cells might not be truly representative of the whole population if the sorting procedure is sensitive to the number of insulin granules.
Calculations based on 10000 granules, each containing 8–9 fg insulin, have yielded a total of 85 pg insulin per single human β-cell [95]. If such a high value were correct, the insulin content of one IEQ would be greater than ever measured (85 ng for 1000 β-cells). Direct measurements of insulin in dispersed islet cells have yielded a more realistic content of 14.4 pg insulin per β-cell [96].

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8. HOW MUCH GLUCAGON IN ONE PANCREAS, ONE ISLET AND ONE α-CELL?

Five studies have measured glucagon and insulin concentrations in the same pancreases, thereby permitting direct comparisons (Table 4A) [13—16,19]. Both inter-subject and inter-center variabilities are similar for glucagon and insulin. The average concentration of 11.4 μg glucagon/g corresponds to a content of ∼1 mg glucagon in a pancreas of 90 g. On a weight basis, the glucagon-to-insulin ratio averages ∼8.5% (Table 4A). Molar ratios have been presented elsewhere [19].

Data on the glucagon content of isolated human islets are scanty and, owing to different modes of expression, difficult to analyze precisely (Table 4B) [58,70,92,94,97—100]. A content of 0.5—1.0 ng glucagon per islet would seem plausible with a ratio glucagon-to-insulin of ∼6%, close to that found in the whole pancreas (Table 1).

Single human α-cells have been reported to contain 3.5 pg of glucagon versus 14.4 pg of insulin in single β-cells, which yields a ratio of 24% [96]. At first sight this ratio appears inconsistent with that found in islets. However, a correct comparison must take into account the relative *numbers* of cells, which is a tricky issue. Owing to the smaller size of α-cells compared to β-cells, neither measurements of volume densities nor counting of nucleated cells in pancreatic sections provide accurate values for reasons discussed elsewhere [42]. In addition, the ratio of α- to β-cell volume densities increases with the islet size [34,35,74]. Further studies should combine determination of the α-to β-cell ratio and measurements of the glucagon-to-insulin ratio in islets from the same pancreas, freshly after isolation and after a few days of culture.

9. HOW MUCH INSULIN AND GLUCAGON IN PANCREAS AND ISLETS FROM TYPE 2 DIABETIC SUBJECTS?

Eight studies have measured the insulin concentration in the pancreas of 169 subjects with type 2 diabetes (T2D) and found it to be about 55% that of non-diabetic (ND) controls (Table 5A). Notably, the type of diabetes was not always firmly established in studies published more than 50 years ago and in which the lowest values were found. In the most recent reports, insulin concentration [17—19] and insulin content [19] were ∼35% lower in T2D than ND pancreas. The pancreas is infrequently sampled in organ donors with T2D, which explains why the insulin content of their islets has been reported in relatively few cases (n = 68) [54,80,86,93,97,101—104]. It averages 64% that of ND controls (Table 5B).

How can we interpret the similar decrease of insulin content in the whole pancreas and in isolated islets (∼35%) of T2D subjects when their β-cell mass is also ∼40% lower than that of ND controls? A decrease in the number of β-cells within each islet appears to be the major contributor. Thus, a decrease in islet size has been measured in situ [26,105] and after islet isolation [106]. It accompanies the loss of β-cells but may vary between islets depending on the increase in fibrotic tissue and presence of amyloid deposits [26]. Since the α-cell mass is unchanged [35,105], a significant decrease in the number of

Table 5 — Insulin in pancreas and isolated islets of ND and T2D subjects.

A Insulin in pancreas

Ref	First Author	Year	Cases	Insulin concentration (μg/g)			Insulin content (mg)
			ND/T2D	ND	T2D	T2D/ND (%)	T2D/ND (ratio)
[5]	Scott	1938	14/12[a]	60	19[b]	32	—
[6]	Wrenshall	1952	52/57[a]	82	35	48	2.2/7.1 (31%)
[8]	Steinke	1963	10/7[a]	69	38	55	—
[9]	Kimmel	1967	29/13[a]	86	29	35	—
[10]	Rastogi	1973	32/11	54	4—225	—	—
[17]	Tasaka	1986	21/24	110	63	57	—
[18]	Rahier	2008	25/27	126	88	70	—
[19]	Henquin	2017	39/18	130	97	75	7.4/11.3 (65%)
			222/169				
Mean						**53%**	
Median						**55%**	

B Insulin in islets

Ref	First Author	Year	Donors	Insulin (ng/islet)			Isolation center
			ND/T2D	ND	T2D	T2D/ND (%)	
[93]	Gepts	1970	8/9	56/μg dry wt	28/μg dry wt	50	Microdissection[c]
[101]	Lohmann	1980	5/7	∼9	∼7	78	Karlsburg-Leipzig
[54]	Marchetti	2004	10/6	4	2.5	63	Pisa
[86]	Hanley	2010	5/5	∼54/μg prot	∼26/μg prot	48	Montreal (McGill)
[97]	Walker	2011	6/6	32	23	72	Oxford
[80]	Rosengren	2012	41/12	0.034/ng DNA	0.024/ng DNA	71	Lund, Oxford
[102]	Taneera	2012	?	10.6	8.8	83	Uppsala
[103]	Lyon	2016	65/20	—	—	65	Edmonton
[104]	Daneshpajooh	2018	3/3	∼8.7	∼4.3	49	Uppsala
			143/68				
Mean						**64%**	
Median						**65%**	

ND: non-diabetic, T2D: Type-2 diabetic, ?: Unknown number.
The sign ∼ indicates that the value was read from a Figure in the original publication.
[a] Inclusion of subjects with type1 diabetes in the oldest studies is not excluded.
[b] All subjects were on insulin.
[c] Fragments of islets microdissected from freeze-dried sections of pancreas; original data per μg dry weight.

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islets is unlikely, with the caveat that small islets (<50 μm) escape counting by certain methods. From a practical point of view, normalization of islets from T2D subjects by size only (IEQ) or cell number (DNA) may introduce bias in comparisons with ND islets. A morphologic assessment of their cellular composition would secure such comparisons.

The glucagon concentration in the pancreas of T2D subjects has been measured in only one study (18 subjects) and, in keeping with the lack of change in the α-cell mass [35,105], was found to be unchanged compared to that of ND subjects [19]. Pancreatic glucagon content averaged 0.76 mg in T2D versus 0.81 mg in ND subjects [19]. The glucagon content of T2D islets was also found unchanged in two studies (9 donors) [98,107] but was tripled in another one based on 6 donors [97]. This doubtful result may have been caused by islet selection.

10. HOW SHOULD ONE EXPRESS IN VITRO INSULIN AND GLUCAGON SECRETION?

Insulin (or glucagon) secretion must be reported as a rate: an amount of insulin per unit of tissue and per unit of time. It is incorrect to report an amount or a concentration only. This statement may seem obvious to the many investigators who correctly express their results. Unfortunately, this is far from the rule. More than 20 different ways of expressing insulin secretion by isolated human islets have been identified in recent papers (Supplemental Table 3). Many are scientifically incorrect and should no longer be used.

Expressing secretion on a per islet basis, as is frequently done, is of limited value unless the islets are characterized; some kind of normalization is necessary. Normalization to size may be misleading. As already discussed, determination of IEQs is not very accurate and does not inform on the actual size of the islets, which should always be provided by the "islet size index". Measuring islet DNA informs on the size, but the reference is not β-cell specific, particularly when control and test islets possibly contain different proportions of β-cells. The problem is the same with islet total proteins. It is more logical to use a β-cell specific reference, insulin itself, and to express insulin secretion rates as a *percentage of the islet insulin content*. This fractional insulin secretion is independent of the actual insulin content of the islets [69]. When content and secretion are measured in the same sample (e.g. islets in a perifusion chamber) the reference accounts for possible differences in islet size and number between samples. This mode of expression also permits unbiased comparisons of secretion rates in primary β-cells and insulin-secreting cell lines. Finally, it tells whether secretion rates measured in isolated islets are physiologically relevant. During perifusion with 8, 10, and 15 mM glucose, human islets secrete about 0.03, 0.04 and 0.05% of their insulin content per min [55,108]. Similar fractional rates can be calculated (for 10–12 mg insulin per pancreas) from secretion rates computed by C-peptide deconvolution during hyperglycemic clamps at these concentrations [109–111].

One note of caution: expressing secretion as a percentage of insulin content may be sufficient when β-cell function is studied in a single group of islet donors. However, when the aim is to compare islet function in different groups, a second mode of expression is necessary: secretion per IEQ. Islets with similar fractional rates of secretion show different rates of secretion per IEQ if the insulin content is substantially different, as in Type1 diabetes [100] or after long-term culture in high glucose [112].

In summary, prospective authors are invited to measure the actual size of the islets that they use in their experiments. Relying on values provided by the isolation center may no longer be accurate after several days of culture. In the absence of easy alternatives, the standard determination of IEQs remains acceptable if it is complemented by the "islet size index" (obtained whenever IEQs are computed), and would optimally be completed by a measure of DNA per IEQ. The insulin content per IEQ is a necessary piece of information in all studies. In classic static incubations, insulin secretion should be expressed as % of islet content per min or h of incubation (Supplemental Table 3). In dynamic perifusion experiments, secretion must also be expressed in % of islet content per min. Finally, the use of relatively large batches of unselected islets is recommended to obtain responses that are representative of the whole pancreas. If islet handpicking is deemed necessary to remove excessive contaminating cells and damaged islets, selection by size should be avoided.

11. CONCLUSIONS

Compared to other endocrine cells, α- and β-cells are difficult to study because of their localization, in variable proportions, within a multitude of micro-organs spread in the potentially hostile exocrine pancreas. Rodent islets will remain essential models because of the limited availability, inescapable variability, and limitations in experimental use of human islets. It is therefore imperative that in vitro experiments using painstakingly isolated human islets be conducted with great rigor and that the collected information be conveyed completely in a correct way. This review of basic features of the human pancreas and of isolated human islets will hopefully serve as a resource for future investigations. The theoretical calculations provide benchmarks to assess the plausibility of personal or published experimental results. The suggested ways to harmonize the report of results represent an easy but necessary step for the progress of human islet research.

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CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

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