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Between and within subject variation of the first phase insulin response to intravenous glucose

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Summary. Eight normal subjects underwent two intravenous glucose tolerance tests to determine the between and within subject variation of the first phase insulin response. Variability was represented by the coefficient of variation. The between subject variation for the incremental 0-10 min insulin area was 58%, and the within subject variation was 22% (median value), range 3-55%. The variation of the first phase response expressed in four different ways was compared. The total and incremental (above fasting levels) 0-10 min areas provided less variable results (variation 52 and 58%) than the 1+3 min insulin levels (variation 72%) or mean of the incremental 3-5 min insulin levels (variation 66%). The ratio of the incremental 0-10 min insulin to glucose areas was as variable (variation 53%) as the insulin responses alone. The variability of insulin responses to intravenous glucose severely limits their value as early predictors of B-cell failure.

Key words: Variability, insulin, glucose.

Loss of the first phase insulin response to intravenous glucose is the earliest detectable metabolic abnormality in subjects who later develop Type 1 (insulin-dependent) diabetes [1], and the intravenous glucose tolerance test (IVGTT) is used to complement immunological markers [2] in identifying individuals at risk.

The insulin response to the IVGTT is already known to be highly variable [3], but we have concentrated on variability of the first phase insulin response, since this appears to have the greatest prognostic value [1]. We have investigated the between and within subject variation of the first phase insulin response because these seem crucial for interpretation of cross-sectional or longitudinal data.

Subjects, materials and methods

Eight healthy non-obese Caucasian subjects, 4 male and 4 female, aged 21 to 35 years (mean 29 years) were examined. There was no family history of diabetes. All were on a normal diet and on no medication; smoking was prohibited for 12 h prior to the test. All gave written informed consent before taking part in the study which had been approved by St. Bartholomew's Hospital Ethical Committee. The tests were performed after an overnight fast starting between 08.00 and 09.30 hours.

Each subject underwent 2 IVGTT's (tests A and B) over an 8 week period. The IVGTT was administered as 25 g dextrose in a 50% solution infused over 75 s at 20 g/min (Sage syringe pump, model 351, Arnold R. Horwell Ltd, London, England). Zero time was the point midway through the infusion and baseline blood samples were taken 10 and 5 min before, and 1, 3, 5, 7, 10, 15, 20, 30, 45 and 60 min after the infusion.

Whole blood glucose was determined by a glucose oxidase method (YSI model 23AM glucose analyser, Yellow Springs Instrument Co., Yellow Springs, Ohio, USA) on the same day as the test. Heparinised blood was spun immediately at 4 °C and stored at −20 °C until analysis for insulin was performed. Serum immunoreactive insulin (IRI) was determined by a double antibody technique. All the samples from each subject were analysed in the same assay. The interassay coefficient of variation (cv) at 41 mU/l was 8.2% and the intra-assay cv at 38 mU/l was 3.9%.

Statistical analysis

The first phase insulin response was estimated using four different methods:

1. the total 0-10 min area under the curve (0-10′ area; $mU \cdot l^{-1} \cdot min^{-1}$),
2. the incremental 0-10 min area above fasting concentrations [4], (Δ 0-10′ area; $mU \cdot l^{-1} \cdot min^{-1}$)
3. the sum of the 1 and 3 min concentrations [1], (1′+3′ insulin; mU/l)
4. the mean of the incremental 3 to 5 min values above fasting concentrations [5], (Δ 3-5′ insulin; mU/l).

The first phase glucose response was also expressed as the incremental 0-10 min area above fasting concentrations; this allowed us to calculate the ratios of the incremental 0-10 min insulin to glucose areas.

The Wilcoxon signed rank test was used to see whether there was any difference between Tests A and B. The standard deviation and

Table 1. First phase insulin and glucose responses plus Δ 0-10 min insulin/glucose (I/G) area ratios in tests A and B

Subject	Δ 0-10 min insulin areas ($mU \cdot l^{-1} \cdot min^{-1}$)		Δ 0-10 min glucose areas ($mmol \cdot l^{-1} \cdot min^{-1}$)		Δ 0-10 min I/G ratio (mU/mmol)	
	A	B	A	B	A	B
1	227	516	65	78	3.5	6.6
2	352	451	75	81	4.7	5.6
3	366	325	56	56	6.5	5.8
4	722	492	124	130	5.8	3.8
5	504	288	92	83	5.5	3.5
6	981	1025	89	73	11	14
7	154	291	99	103	1.6	2.8
8	436	387	116	121	3.8	3.2
Mean	468	472	90	91	5.3	5.7
SD	270	240	24	25	2.8	3.7
cv (%)	58	51	27	27	53	65

Table 2. Between and within subject variation of the first phase insulin response determined by four different methods

	Between subject cv%	Within subject cv% (range %)
0-10 min area (mU/l/min)	52	24 (3.9- 47)
Δ 0-10 min area (mU/l/min)	58	22 (3 - 55)
1+3 min insulin (mU/l)	72	36 (11 -110)
mean Δ 3-5 min insulin (mU/l)	66	30 (1 - 66)

coefficient of variation (cv) was used as a measure of between and within subject variation. The cv was calculated: cv = SD/mean × 100%; where SD = standard deviation.

Results

Comparison of the first and second IVGTTs

Before assessing the between and within subject variation for the first phase insulin response we established that there was no difference in insulin or glucose response (0-10 and 10-60 min areas) between the first and second IVGTTs (tests A and B); $p > 0.3$ for each parameter.

Between and within individual variation for the first phase insulin response

First phase insulin and glucose responses and their respective insulin to glucose ratios are shown in Table 1. The between subject variation was greater for insulin (cv 58% and 51%, tests A and B) than for glucose (cv 27% and 27%). This variability of insulin response was not reduced when glucose levels were accounted for by calculating the ratios of the Δ 0-10 min insulin to glucose areas (cv 53% and 65%).

The within subject variation expressed as the median cv (range) was: 22% (3-55%) for the Δ 0-10 min insulin area, 4.4% (0-14%) for the Δ 0-10 min glucose area and 17% (8.4-44%) for the Δ 0-10 min insulin/glucose area ratios.

For comparison, fasting insulin concentrations were 7.1 ± 2.8 mU/l (mean ± SD) producing a cv between subjects of 39%. The within subject variation was 12% (range 0.2-22%).

Comparison of four different methods of expressing the first phase insulin response

We compared the between and within subject variation of the first phase insulin response determined by four different methods (Table 2). Between subject variation is shown for test A only. Calculation of the 0-10 min area (either above baseline or above fasting concentrations) provides the least variable estimate of the first phase response.

Discussion

There is considerable evidence to suggest that an autoimmune process develops in the pancreas many years before the development of Type 1 diabetes [2], and that metabolic decompensation occurs slowly. Loss of the first phase insulin response to glucose may occur years before overt diabetes [1], and episodic hyperglycaemia may precede oral glucose intolerance and symptomatic diabetes [6]. The slow progressive nature of this process would allow scope for intervention, provided that subjects predisposed to diabetes could be identified accurately.

The potential of IVGTTs to identify early abnormalities of carbohydrate metabolism depends on their ability to discriminate normal from abnormal results. This in turn is largely dependent on the degree of between and within subject variation. Ganda et al. [3] demonstrated wide variability of IVGTT results and this study is helpful when considering such tests for epidemiological work, but is unsatisfactory when applied to an individual.

Our concern is to study high risk groups cross-sectionally, and to follow some subjects longitudinally. For this purpose we have determined the between and within subject variation for the first phase insulin response in normal subjects. Emphasis was placed upon the absolute difference between repeated tests, as expressed by the standard deviation and coefficient of variation in normal subjects.

Comparable and wide between and within subject variations were found in normal subjects. The first phase insulin response proved more variable than the fasting insulin concentrations and this variability was not improved by use of an insulinogenic index (ratio of the Δ 0-10 min insulin/glucose areas). It is not known

whether subnormal test results would show the same variability.

A linear loss of the first phase insulin response to intravenous glucose prior to the development of overt diabetes has been described using IVGTT results from 9 individuals (1). However, using the same method of calculating the first phase response (1+3 min values), we found that normal subjects were extremely variable (between subject cv 72%, within subject cv 36%). This wide variability could be improved to some extent by using the 0–10 min area, but will still be very variable, suggesting that prospective interpretation of results from a given individual which fall within the normal range is of limited value. In contrast, a clearly subnormal first phase insulin response appears to carry a very high risk of progression to diabetes.

In conclusion we hope that awareness of the wide between and within subject variation of these tests will lead to appropriate restraint in their interpretation. Claims should not be made which exceed the capabilities of the test.

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