Comparability of HOMA estimates on Insulin sensitivity and B-cell function depends on bias of insulin assays

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Abstract

Introduction: Homeostasis Model Assessment (HOMA) is widely used in research studies and in routine clinical practice to estimate steady state insulin sensitivity (%S) and B-cell function (%B) from fasting plasma glucose (FPG) and insulin measurements. These estimates may however be affected by differences in insulin assays, since the ADA showed in 1996 that results from different insulin assays varied by a factor of 2. **Aim:** Comparison of %S and %B from HOMA using 12 insulin assays, currently available in UK and US. **Methods:** 99 fasting blood samples were collected in a hospital setting from 14 healthy subjects (HS), 7 IFG, 11 IGT and 67 patients with type 2 diabetes (T2D). Patients with type 1 diabetes or T2D requiring insulin or insulin antibody positive were excluded. Glucose was measured within 10 minutes of sampling by a glucose oxidase method using a Yellow Springs analyser. Twelve commercially available insulin assays were compared: 6 manual/6 automated; 11 immunometric (1 cross-reacting 54% with intact proinsulin) and 1 specific RIA. The RIA-HOMA Calculator (version 2.2) was used to calculate %S and %B. **Results:** Subjects were aged, mean (1SD), HS 47.1(13.8), IFG 63.3 (9.5), IGT 60.9(11.4), T2D 59.8(9.5) years; BMI 27.9(4.8), 33.1 (5.8), 30.3(4.1), 31.8(5.8) kg m⁻²; FPG 5.5(0.8), 6.4 (0.3), 6.1(0.5), 8.4(2.2) mmol l⁻¹. Differences for %B and %S were compared by plotting the difference between each insulin assay against the mean of all 12 insulin assays. The mean differences for %S (1SD) in fasting samples ranged from -27.0 (26.2)% to +25.2 (28.7)%. For %B the differences ranged from -15.8 (10.3)% to +19.6 (12.7)%. **Conclusion:** Estimates of %S and %B from 12 insulin assays currently available differ significantly both statistically and clinically. This may mean that direct comparison of HOMA results obtained using different insulin assays will be difficult to interpret without assay comparison data. Data from multi-assay comparisons such as this will permit legitimate comparability of HOMA estimates.

AIM

To compare %S and %B values obtained from HOMA analysis using 12 insulin assays, currently commercially available in UK and US

INTRODUCTION

A review by the ADA in the 1990s showed that results from different insulin assays varied by a factor of two (Robbins et al 1996, Diabetes 45: 242-256). Since then, newer immunoassay techniques, (some automated) with low cross-reactivity with proinsulin(s), have been introduced for measurement of insulin. Homeostasis Model Assessment (HOMA) is widely used in research studies and in routine clinical practice to estimate steady state insulin sensitivity (%S) and B-cell function (%B) from fasting plasma glucose (FPG) and insulin measurements. These estimates may be affected by differences in the insulin assays

PROTOCOL

- 99 fresh blood samples were collected from fasting individuals. Glucose was measured in the organising laboratory
- Aliquots of serum were sent to 7 routine laboratories in UK and 2 research laboratories in US for measurement of insulin on 5 separate occasions
- RIA-HOMA calculator (ver 2.2) was used to calculate %S and %B (www.dtu.ox.ac.uk)

SAMPLES

- Plasma glucose was measured within 10 minutes of sampling by glucose oxidase method using a Yellow Springs analyser (YSI 2300)
- Blood samples for insulin assay were collected either via vacutainer or iv cannula into plain tubes (serum) which were allowed to clot for 30 minutes and then centrifuged for 5 minutes at 3000rpm at 4°C
- 0.5 ml aliquots were prepared and stored at –20°C initially and then transferred to –70°C. *Samples with obvious haemolysis were discarded*
- Samples and QCs were shipped to centres on dry ice by a courier

DATA ANALYSIS

- Data was analysed using Bland Altman difference plots for all 99 samples with the mean of all 12 assays as the x-axis

INCLUSION CRITERIA

Ethical approval was obtained prior to collecting samples. All subjects gave written informed consent

Inclusion: Male or females aged 18 – 75 years
Exclusion: Persons with Type 1 diabetes, persons with Type 2 diabetes requiring insulin treatment or having known levels of insulin antibodies

SUBJECTS – mean (1SD)

	Age (Years)	BMI (kg m⁻²)	FPG (mmol l⁻¹)	n
Healthy Subjects	47.1 (13.8)	27.9 (4.8)	5.5 (0.8)	14
IFG	63.3 (9.5)	33.1 (5.8)	6.4 (0.3)	7
IGT	60.9 (11.4)	30.3 (4.1)	6.1 (0.5)	11
T2D	59.8 (9.5)	31.8 (5.8)	8.4 (2.2)	67
			Total	**99**

INSULIN ASSAYS

|		
A	Abbott	Axsym Insulin
B	Bayer	ADVIA Centaur Insulin Assay
C	Dako	Insulin Assay
D	DPC	Immulite 1000
E	Linco	ELISA
F	Linco	RIA
G	Medgenix	Insulin EASIA
H	Mercodia	Iso-Insulin Assay
I	MLT	Insulin Assay
J	Roche	Insulin Elecsys
K	Tosoh (UK)	Tosoh ST AIA-Pack IRI
L	Tosoh (US)	as above with modification to calibration

Table 1: Insulin Assay Details

	A	B	C	D	E	F	G	H	I	J	K	L
Detection	E	C	EL	C	EL	RIA	E	EL	C	ELC	E	E
Method	A	U	A	M	A	M	M	M	M	A	A	A
CV (%)	3.9	5.5	5.1	6.7	2.5	4.7	3.8	5.6	1.4	4.5	6.4	5.6

E = Enzyme; C = Chemiluminometric; EL = ELISA: RIA = Radioimmunoassay
ELC = Electrochemiluminometric; A = Automated; M = Manual

RESULTS

Difference plots for insulin sensitivity (%S) by assay

Difference plots for B-cell function (%B) by assay
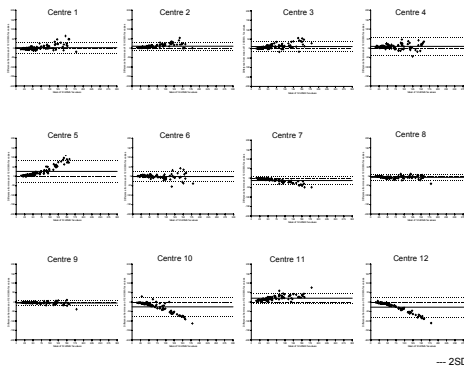
--- 2SD of difference; —— Origin;— Mean difference

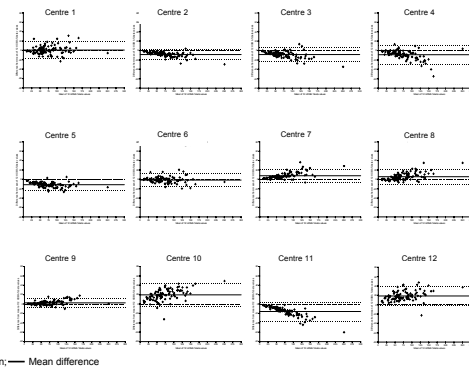**Differences in insulin sensitivity (%S) from mean of 12 assays**

Assay	Mean difference	Min	Max	SD
1	2.6	-20.0	63.5	14.4
2	9.9	-2.7	53.1	10.1
3	11.4	-17.8	55.2	13.4
4	9.1	-44.8	186.9	23.0
5	25.2	-3.4	124.1	28.7
6	-2.1	-55.4	41.0	12.8
7	-12.7	-49.2	2.5	11.3
8	-6.3	-40.5	11.9	7.2
9	-4.9	-38.8	10.1	6.1
10	-25.2	-113.9	29.2	26.2
11	19.8	-0.1	75.5	12.7
12	-27.0	-111.7	4.6	26.2

Mean differences (1SD) in %S calculated from fasting samples range from - 27.0 (26.2) to + 25.2 (28.7)

Differences in B-cell function (%B) from mean of 12 assays

Assay	Mean difference	Min	Max	SD
1	0.8	-23.5	31.2	9.5
2	-8.4	-29.7	3.7	5.3
3	-8.7	-34.8	13.4	7.4
4	-9.0	-55.2	13.2	10.3
5	-11.6	-27.2	4.9	5.6
6	-1.5	-20.0	20.1	6.0
7	7.6	-6.9	36.3	7.1
8	5.7	-7.0	34.8	8.3
9	2.6	-10.3	17.8	5.2
10	19.6	-32.5	48.9	12.7
11	-15.8	-60.4	-2.1	10.3
12	18.7	-23.1	47.3	10.7

Mean differences (1SD) in %B calculated from fasting samples range from - 15.8 (10.3) to + 19.6 (12.7)

CONCLUSION

- Differences in estimates of %S and %B from the 12 different insulin assays in this study are either off-sets or proportional to the HOMA parameter
- Direct comparison of HOMA estimates from different insulin assays is not advisable
- Data from this multi-assay comparison for insulin will assist in comparison of HOMA estimates of %B and %S