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Repeatability characteristics of insulin response patterns and measures of insulin resistance

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Background: Hyperinsulinaemia is emerging as an independent risk factor for metabolic disease, but diagnostic measures are limited. It is plausible that insulin resistance measures, such as homeostatic model assessment (HOMA) type 2 variants, may model hyperinsulinaemia, but repeatability data are limited. Kraft and Hayashi insulin response patterns may not only add value in diagnosing hyperinsulinaemia, but also lack suitable repeatability data.

Aim: The aim of this study was to investigate the repeatability of insulin response patterns, and fasting and dynamic measures of insulin resistance, and to determine whether these latter measures can predict the insulin response pattern.

Setting: This study was conducted at Auckland University of Technology Millennium Institute's sports performance laboratories.

Methods: Oral glucose (100 g) tolerance tests were conducted weekly on eight people. Six people completed four tests, while two completed at least two tests. Each test assessed insulin resistance and response patterns. Insulin resistance measures included fasting tests (HOMA2, McAuley Index) and a dynamic test (oral glucose insulin sensitivity [OGIS]). The insulin response patterns were assessed with both Kraft and Hayashi methodologies. Repeatability characteristics of ordinal variables were assessed by Bland and Altman methods, while Fleiss' κ was applied to categorical variables.

Results: Fasting measures of insulin resistance recorded poor repeatability (HOMA2) or poor sensitivity (McAuley Index) compared to the dynamic measure (OGIS). Kraft insulin response patterns were more repeatable compared to Hayashi patterns, based on a combination of Fleiss' κ (0.290 vs. 0.186,) *p*-value (0.15 vs. 0.798) and 95% confidence intervals.

Conclusions: Both hyperinsulinaemia and insulin resistance should be dynamically assessed with a multi-sampled oral glucose tolerance test. Further investigations are required to confirm a preferred methodology.

Keywords: insulin resistance; hyperinsulinaemia; Kraft Patterns; Hayashi Patterns; HOMA; OGIS; McAuley Index; insulin response pattern.

Introduction

Insulin resistance is recognised as being a significant risk factor for type 2 diabetes and other metabolic diseases. Yet, insulin resistance measures do not add value to disease risk calculations.[1,2] People with insulin resistance generally have chronic hyperinsulinaemia to compensate for poor glucose uptake rates. This compensatory hyperinsulinaemia is an independent risk factor for metabolic disease,[3] and may be one of the earliest indicators of incipient disease that precedes changes to blood glucose levels,[4,5] and potentially also obesity[4] and hypertension.[6] This suggests that there is a need to accurately quantify hyperinsulinaemia in people with normal glucose tolerance to include the measure as a public health screening tool.

Because hyperinsulinaemia coexists with insulin resistance, it is plausible that insulin resistance measures may also predict hyperinsulinaemia. The gold-standard method for assessing insulin resistance is the hyperinsulinaemic-euglycaemic clamp (HIEG). However, this method is often impractical, especially in clinical settings or with large cohorts, so alternative methods are often used that model the HIEG. These alternative methods include fasting tests such as homeostatic model assessment (HOMA) and the McAuley Index. 'Dynamic' methods are derived from a combination of fasting and post-prandial testing during an oral glucose tolerance test (OGTT) and include oral glucose insulin sensitivity (OGIS).

Despite being widely used, there is limited information regarding population normative values of either hyperinsulinaemia or insulin resistance, with many studies defining insulin resistance as a

quantile of the population under investigation, especially quartiles as recommended by the World Health Organization.[7] This method limits the generalisability of results from different studies and may confound results depending on the population under investigation.

One explanation for insulin resistance measures reporting a poor risk predictive value is that many of these measures, including HOMA, are based on a single sample of fasting insulin. Unlike many other biomarkers, insulin is a hormone that is secreted in response to potentially rapidly changing needs as well as the body's natural oscillations, stress, food and exercise to maintain glycaemic control.[8] This means that blood insulin levels, especially fasting insulin, are highly variable.

It is theorised that using a dynamic method for assessing insulin resistance may yield better disease predictive values, but there is limited information to support their use. Previous research has also suggested using insulin response patterns following a multiple-sampled OGTT to predict disease risk. Kraft described five distinct insulin patterns formed during a 3–5-h OGTT on the basis of magnitude and timing of the peak plasma insulin level and rate of decay.[9] A normal insulin response (Kraft I pattern) was considered to be a fasting insulin ≤ 30 μU/mL, with a moderate peak 30–60 min after the glucose load and a rapid rate of decay. Independently, in their sample of 400 Japanese American men, Hayashi and colleagues determined that an insulin peak at 120 min during a 2-h OGTT (Hayashi pattern 4 and 5) significantly increased the risk of developing type 2 diabetes over the following 10 years.[5]

Assessing insulin response patterns is expensive as they require four to five blood samples over a 2–3-h time period. It is plausible that insulin resistance methods may be able to predict hyperinsulinaemia given the two conditions are intertwined. However, to have clinical utility, tests need to have low variability. There are concerns about the variability of insulin resistance measures, and the repeatability of insulin response patterns is unknown.

There are a number of statistical methods used to assess the variability of a measure. One of the most common is the coefficient of variation (CV), which is the ratio of the standard deviation to the mean (Equation 1). Coefficient of variations indicate the extent of variability in relation to the mean of the population or sample; the bigger the number, the more variable the sample.

Coefficient of variation:

$$CV = \frac{sd}{\mu} \times 100 \qquad \text{[Eqn 1]}$$

Test–retest reliability, also known as repeatability, is the closeness of the agreement between the results of successive measurements of the same variable taken under the same conditions.[10] For two tests to be in agreement, then the two resulting measures should lie within the repeatability coefficient (RepCoef) (Equation 2).

Test–retest reliability:

$$Test\ 1 \approx Test\ 2 \pm repeability\ coefficient \qquad \text{[Eqn 2]}$$

Assuming the data are normally distributed, it is expected that 95% of the results will lie within 2 standard deviations from the mean. The 95% RepCoef can then be calculated (Equation 3) according to the methods of Bland and Altman, where s_w is the within-subject variance.[10]

Repeatability coefficient:

$$\text{RepCoef} = 1.96\sqrt{2s_w^2} \qquad \text{[Eqn 3]}$$

This means that, for clinical tests, if a subsequent test differs from the former by an amount smaller than the RepCoef, it suggests biological variation. However, if greater, it suggests that there is a change to the clinical condition. The RepCoef may be expressed either as a discreet figure (e.g. 0.5 mmol/L) or as a percentage relative to the grand mean of the sample (or mean of the sample population). The latter may be more useful where population norms are less well known, or when a co-existent clinical condition defines sample mean; for example, there may be different RepCoef of HOMA depending on the underlying glucose tolerance status.[11]

For example, HOMA and HOMA2 variants have both a high CV (25% – 50%) and a large RepCoef relative to the population mean (89% – 135%).[11] This may reflect the known variability of fasting insulin.[12] There is limited repeatability data for other measures. The McAuley Index has one study, showing a CV of 15% taken over two visits.[13] The dynamic insulin resistance measure, OGIS, has a lower degree of variability as indicated by CV (7% – 8%) and small RepCoef proportional to the population mean (22%).[11,14] No studies have assessed the repeatability of either the Kraft or Hayashi patterns.

The aims of this study are twofold: firstly, to assess the test–retest repeatability of fasting and dynamic insulin resistance measures, and that of dynamic insulin response patterns, and, secondly, to determine whether measures of insulin resistance can predict hyperinsulinaemia.

Methods

Subjects and study design

We recruited 10 healthy participants aged 20–55 years (six male, four female) for four repeated multiple-sampled OGTTs with insulin assays. 'Healthy' was defined as no acute or chronic injury or illness requiring medical attention in the previous 3 months and a current HbA_{1c} < 40 mmol/mol (5.8%). These tests were conducted according to the protocols outlined by Kraft[9] and standard oral glucose tolerance testing procedures, including an overnight fast and no vigorous exercise on the morning of the test. Participants were also required to maintain the consumption of at least 150 g carbohydrate per day for at least 14 days prior to the first test

and throughout the testing period.[9] Participants maintained their normal habits, especially physical activity patterns, throughout the 2-week lead-in and 4-week study period. A deliberate decision was made to not formally assess diet or physical activity to make the results more representative of those that would be seen in clinical practice. The overall design including the clinical criteria and short study time meant it would be unlikely that an underlying clinical condition would influence insulin responses and confound the results.

On each test occasion, after an overnight fast, each subject had a cannula inserted into their antecubital fossa and provided fasting venous blood samples before consuming 100 g glucose (400 mL Carbotest™ solution). The glucose was consumed within 10 min of test commencement (0 min). With the exception of water, no further food or drink was permitted until the end of the test. Further venous samples were drawn at 30, 60, 120 and 180 min. Vein patency was maintained by flushing with saline before and after each collection, with the first 2 mL of blood collected being discarded. Blood samples were collected into plasma separator tubes (PST) vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ) for glucose and insulin analysis. Plasma was extracted from the PST tubes after centrifugation (1500 × *g* at 4°C for 10 min), and then frozen at -20°C within 2 h of collection. This protocol was repeated weekly for a total of four tests. On the first test occasion, height, weight and waist girth (smallest girth between iliac crest and the lowest rib) were measured. On the initial testing occasion, an additional fasting venous blood sample was collected into an Ethylenediaminetetraacetic acid (EDTA) vacutainer (Becton, Dickinson and Company) for HbA_{1c} analysis.

Analysis

Sample analysis

Prior to analysis, plasma samples were allowed to warm to room temperature and centrifuged (10 000 × *g* at 20°C for 30 s) to remove any protein precipitants. Samples were batch-analysed by participant to reduce intermediate precision. All plasma samples were quantitated on the Roche Diagnostics cobas Modular Analytics E170. Insulin was quantitated on the E module via electrochemiluminescence (intermediate precision 2.5% – 4.9%). All other analytes were quantitated on the P module: Glucose was quantitated via the hexokinase enzymatic method (intermediate precision 1.7% – 1.9%) and triglycerides via an enzymatic colorimetric method (intermediate precision 1.8% – 2.4%). Where possible automated haemolysis index measured quantified haemolysed samples. Samples were excluded from further analysis if significant haemolysis was present. The whole blood EDTA samples were analysed for HbA_{1c} (Roche Cobas C111, tubidimetric inhibition immunoassay with interbatch CV of 1.32% – 2.36%).

Calculations and statistical analysis

Statistical analysis and calculations were performed with either SPSS 22.0 (Armonk, NY) or Microsoft Excel 2013 (Redmond, WA).

HOMA2 variants (HOMA2 %B, HOMA2 %S, HOMA2 IR) and OGIS were calculated using their respective downloadable calculators.[15,16] The McAuley Index, which assesses glucose disposal rate, corrected for fat-free mass (Mffm/I), was calculated as per the formula in Equation 4.[17] Glucose tolerance testing followed World Health Organization (WHO) protocols.[18] Hayashi patterns were derived according to their protocol,[5] and Kraft patterns were derived according to the 2014 protocol.[4]

McAuley Index:

$$Mffm/I = \exp[2.63 - 0.28 \ln(\textit{fasting insulin}) - 0.31 \ln(\textit{fasting triglycerides})]. \quad \text{[Eqn 4]}$$

Two group comparisons were made with two-tailed independent *t* tests. Missing data were imputed as according to the most likely clinical scenario for pattern reconstruction and for Fleiss's κ calculations only. Insulin and glucose response curves collected over repeat visits were summarised by plotting point-wise arithmetic mean concentrations for each participant.

Test–retest repeatability measures

Fleiss's κ was calculated as a means of assessing pattern repeatability for both Kraft and Hayashi patterns (1971). As there is no standard interpretation of κ, significant agreement for the pattern was considered to be a combination of Landis and Koch's recommendations,[19] significance of κ and whether the 95% confidence intervals (CIs) crossed zero.

For insulin resistance measures, repeatability was quantified by estimating RepCoef.[10] As this method assumes a non-significant mean-variance relationship, within-subject means were plotted against within-subject standard deviations to determine if there was a mean–variance relationship. Scatterplots and ordinary least squares regression was used to assess the strength of such relationships. If the slope coefficient (SC) was significant at the 0.05 significance level, the process was repeated for the mean and standard deviation of the natural log of the variable.

If a significant mean–variance relationship was determined, participants were divided into sub-groups according to test results. The intent of these sub-groups was to reduce the mean–variance relationship and therefore the risk of bias in the RepCoef at each end of the range while maintaining a clinically meaningful result.

The 95% RepCoef were derived by taking the square root of the residual mean square errors (s_w) from one-way analyses of variance with subjects as factors fitted to the raw or logged responses for each outcome variable (Equation 3).[10,20]

Ranges within which two repeat measurements could be expected to fall were defined as Equation 2 for non-log-transformed data or as Equation 5 for log-transformed data.

Test–retest reliability for log-transformed data:

$$Test\ 1 \approx Test\ 2 \underset{\div}{\overset{\times}{}} e^{\mathrm{RepCoef}} \quad \text{[Eqn 5]}$$

CV was derived from the RepCoef using Equation 6, where $\hat{\mu}$ represents the grand mean of the sample.

Coefficient of variation derived from RepCoef:

$$CV = \frac{\left(\mathrm{RepCoef} \middle/ 1.96 \cdot \sqrt{2}\right)}{\hat{\mu}} \approx \frac{\mathrm{RepCoef}}{2.77 \cdot \hat{\mu}} \quad \text{[Eqn 6]}$$

Ethical consideration

This study was granted ethical approval by Auckland University of Technology Ethics Committee (AUTEC) on 16 December 2014 (reference no. 14/363).

Results

Ten participants consented to the study, but only eight participants completed at least two tests; results are included for the latter eight participants. The baseline characteristics of these eight participants are displayed in Table 1[3]. Six participants completed all four tests, one participant (K10) could not attend on one occasion and one participant (K5) was unable to adhere to fasting requirements on two occasions.

Figure 1[3] displays the mean insulin and glucose response curves for each participant. A higher peak and/or delayed rate of decay can be observed for participants K2, K5 and K6.

Repeatability coefficient for insulin resistance measures

Mean–variance relationships could only be detected for fasting glucose, fasting insulin and glucose at 180 min. After the removal of participant K4 from the data set, a mean–variance relationship could no longer be detected for either fasting glucose or glucose at 180 min. Log-transformation of fasting insulin did not remove the mean–variance relationship. No mean–variance relationship could be detected for fasting insulin for the subset of hyperinsulinaemic participants (K2, K5 and K6).

Table 2[3] displays the RepCoef for all time points for insulin and glucose, the McAuley Index, all HOMA measures and OGIS. With the exception of glucose 0 min, there was no practical difference in the RepCoef for glucose when participant K4 was excluded. Among the fasting models of insulin resistance, the McAuley Index had the lowest RepCoef compared to the grand mean of the sample (17.4%).

Repeatability of insulin response patterns

Table 3[3] presents the distribution of each test per participant for both Kraft and Hayashi insulin response patterns. The most common Kraft pattern was pattern I, recorded by five of the eight participants on at least one occasion, while the most common Hayashi pattern was pattern 3, which was recorded by every participant on at least one occasion. No participant recorded a Kraft IV or V pattern, or a Hayashi pattern 5.

Three participants (K5, K6 and K10) were initially excluded from κ calculations as they did not have four eligible tests for both pattern responses. However, small participant numbers meant that missing data decreased the power of the study. Therefore, we replicated the repeatability calculations after imputing the clinically most likely, or most frequent, outcome for participants with missing data (K5, K6 and K10), as shown in Table 4[3] and Table 5[3].

The inclusion of the imputed data did not cause a qualitative change in the overall results, as shown in Table 6[3]. Estimated κ for the Kraft patterns was higher than for Hayashi patterns (0.290 vs. 0.186), but only the κ for the Kraft patterns was significantly different from zero (95% CIs, 0.515–0.798 and –1.238 to 1.610 for Kraft and Hayashi, respectively).

Characteristics of insulin resistance measures compared to insulin response patterns

Table 7[3] displays the participants' insulin resistance measures when dichotomised into normal (Kraft I) and hyperinsulinaemic (Kraft IIA, IIB, III) insulin response patterns.[9] Following a two-sample *t* test (defined by Kraft pattern), statistically significant differences can be noted for HOMA2 measures and for OGIS, but not for the McAuley Index.

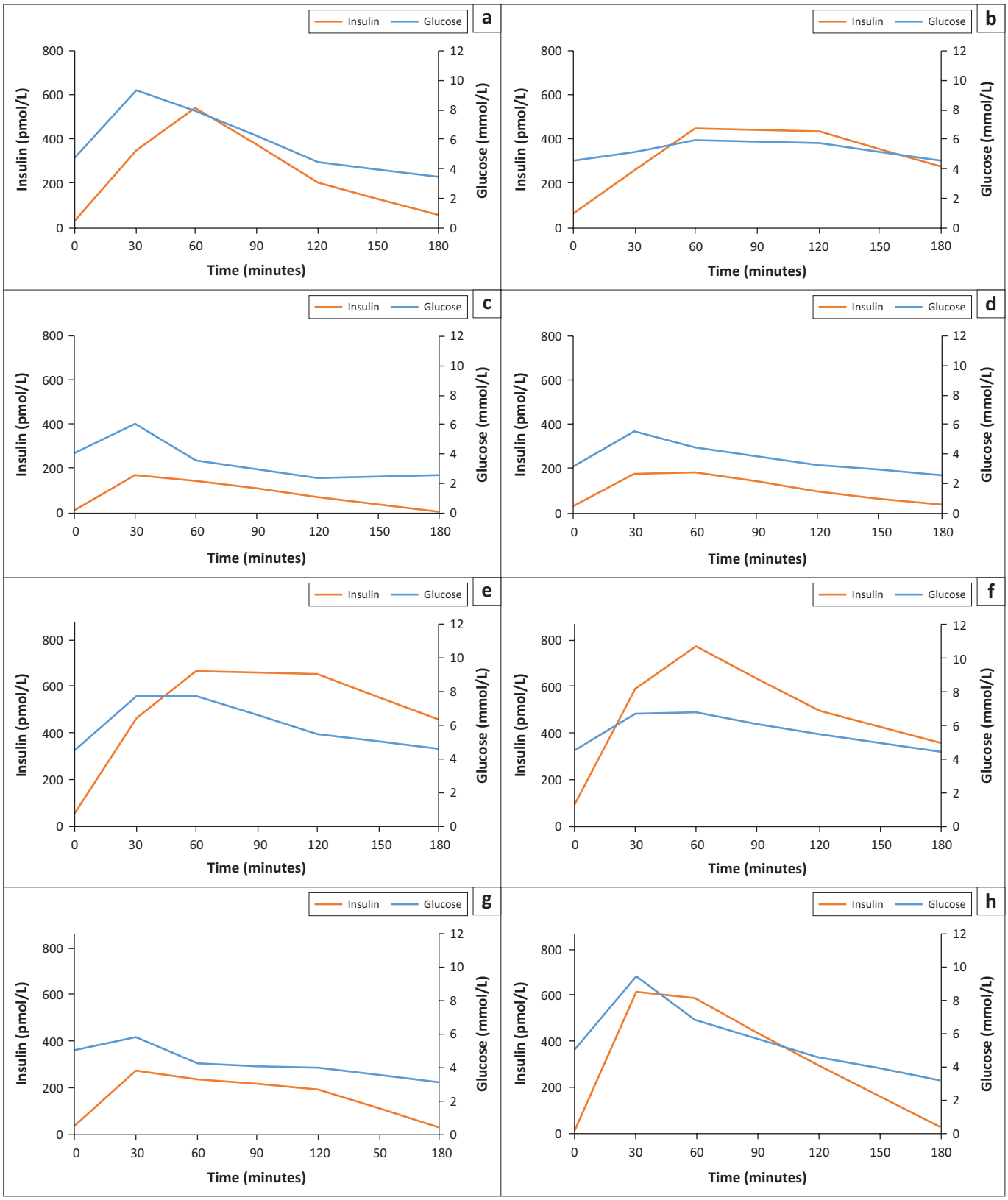
Discussion

Numerous tests are available for assessing insulin resistance and may be either based on fasting measures or dynamically modelled from multiple-sampled OGTTs. Tests based on

TABLE 1: Participant characteristics.

Code	Sex	Age (years)	Height (m)	Weight (kg)	Body mass index (kg/m²)	Waist (m)	Waist:height	HbA1c (mmol/mol)
K1	M	47	1.744	81.8	26.9	0.872	0.50	32.4
K2	M	53	1.737	81.8	27.1	0.956	0.55	35.4
K3	M	44	1.726	74.0	24.8	0.810	0.47	35.8
K4	F	29	1.721	71.0	24.0	0.792	0.46	37.5
K5	F	39	1.515	60.0	26.1	0.755	0.50	36.5
K6	M	30	1.634	65.9	24.7	0.832	0.51	34.2
K9	M	31	1.852	91.6	26.7	0.823	0.44	32.8
K10	M	27	1.774	76.7	24.4	0.804	0.45	35.8

Source: Crofts C. Understanding and diagnosing hyperinsulinaemia. PhD thesis. Auckland: Auckland University of Technology; 2015. [cited n.d.]. Available from: http://aut.researchgateway.ac.nz/handle/10292/9906



Source: Crofts C. Understanding and diagnosing hyperinsulinaemia. PhD thesis. Auckland: Auckland University of Technology; 2015. [cited n.d.]. Available from: http://aut.researchgateway.ac.nz/handle/10292/9906

Note: Participants K4 and K6 both had week 1 results excluded. K4, aberrant 60-min glucose result from unknown origin (asymptomatic for hypoglycaemia); K6, extensive haemolysis of the 60-min sample.

FIGURE 1: Point-wise arithmetic mean insulin (pmol/L) and glucose (mmol/L) concentrations for each participant: (a) K1; (b) K2; (c) K3; (d) K4: weeks 2-4; (e) K5: two test; (f) K6: weeks 2-4; (g) K9; (h) K10: three tests.

TABLE 2: Repeatability coefficients for all participants.

Variable	s_w	±RepCoef	$\hat{\mu}$	$\frac{RepCoef}{\hat{\mu}}$ %	CV %
Glucose, 0 min (mmol/L)	0.27	0.74	4.81	15.4	5.5
Glucose, 0 min† (mmol/L)	0.20	0.56	4.86	11.5	4.2
Glucose, 30 min (mmol/L)	1.02	2.81	7.43	37.8	13.7
Glucose, 60 min (mmol/L)	1.83	5.08	6.00	84.7	30.5
Glucose, 120 min (mmol/L)	1.33	3.68	4.94	74.5	26.9
Glucose, 180 min (mmol/L)	0.80	2.23	3.94	56.6	20.4
Insulin, 0 min‡ (pmol/L)	11.00	31.00	44.42	68.9	24.8
Insulin, 30 min (pmol/L)	101.00	279.00	348.94	80.0	28.9
Insulin, 60 min§ (pmol/L)	178.00	494.00	415.16	119.0	42.9
Insulin, 120 min (pmol/L)	102.00	282.00	294.38	95.8	34.6
Insulin, 180 min (pmol/L)	71.00	197.00	152.83	129.0	46.5
McAuley Index (Mffm/I)	0.35	0.98	5.62	17.4	6.3
HOMA2 %B	14.20	39.50	95.66	41.3	14.8
HOMA2 %S	26.10	72.40	129.56	55.9	20.1
HOMA2 IR	0.24	0.67	0.89	75.4	27.1
OGIS (mL/min/m^2)	27.50	76.10	514.19	14.8	5.3

Source: Crofts C. Understanding and diagnosing hyperinsulinaemia. PhD thesis. Auckland: Auckland University of Technology; 2015. [cited n.d.]. Available from: http://aut.researchgateway.ac.nz/handle/10292/9906

s_w, residual mean square error; RepCoef, repeatability coefficient; $\hat{\mu}$, grand mean; CV, coefficient of variation; OGIS, oral glucose insulin sensitivity.

†, *Excluding K4, week 1 because of an aberrant result.*

‡, *Significant mean–variance relationship.*

§, *Excluding K6, week 1 because of haemolysis.*

TABLE 3: Observed Kraft and Hayashi pattern frequencies on eight participants over four visits per participant.

Participant	Kraft pattern						Hayashi pattern				
	I	IIA	IIB	III	IV	V	1	2	3	4	5
K1	4	-	-	-	-	-	-	-	4	-	-
K2	-	-	2	2	-	-		-	2	2	-
K3	4	-	-	-	-	-	3	-	1	-	-
K4	3	-		1	-	-	2	-	1	1	-
K5	-	-	1	1	-	-	-	-	1	1	-
K6†	-	1	3	-	-	-	-	-	3	-	-
K9	4	-	-	-	-	-	-	2	2	-	-
K10	2	1	-	-	-	-	1	-	2	-	-

Source: Crofts C. Understanding and diagnosing hyperinsulinaemia. PhD thesis. Auckland: Auckland University of Technology; 2015. [cited n.d.]. Available from: http://aut.researchgateway.ac.nz/handle/10292/9906

†, K6: The week 1, 60-min result was extensively affected by haemolysis. Although this did not affect Kraft patterning, the Hayashi pattern could not be determined.

TABLE 4: Kraft and Hayashi pattern frequencies on eight participants over four visits per person after imputation.

Participant	Kraft pattern						Hayashi pattern				
	I	IIA	IIB	III	IV	V	1	2	3	4	5
K1	4	-	-	-	-	-	-	-	4	-	-
K2	-	-	2	2	-	-	-	-	2	2	-
K3	4	-	-	-	-	-	3	-	1	-	-
K4	3	-	-	1	-	-	2	-	1	1	-
K5	-	-	2	2	-	-	-	-	2	2	-
K6	-	1	3	-	-	-	-	-	4	-	-
K9	4	-	-	-	-	-	-	2	2	-	-
K10	3	1	-	-	-	-	2	-	2	-	-

Source: Crofts C. Understanding and diagnosing hyperinsulinaemia. PhD thesis. Auckland: Auckland University of Technology; 2015. [cited n.d.]. Available from: http://aut.researchgateway.ac.nz/handle/10292/9906

fasting insulin such as HOMA and HOMA2 variants are popular, as they require fewer resources compared to those based on dynamic testing (e.g. OGIS). As both insulin resistance and hyperinsulinaemia are becoming increasingly recognised as independent disease risk predictors, there is a need for an effective diagnostic test. However, a lack of repeatability testing for both insulin resistance and hyperinsulinaemia measures precludes their clinical use. This study assessed the repeatability characteristics of the fasting measures (HOMA2 variants and McAuley Index) and the dynamic measure (OGIS) by comparing each RepCoef to the cohort grand mean. We also assessed the repeatability of the two insulin response patterns, Kraft and Hayashi patterns using Fleiss's κ.

Repeatability of insulin resistance measures

Of the insulin resistance measures (HOMA2, McAuley and OGIS), only the McAuley Index (fasting measure) and OGIS (dynamic measure) demonstrated a low RepCoef relative to the grand mean of the sample population with a change of 17.4% and 14.8%, respectively. By contrast HOMA2 variants had a greater degree of change (HOMA2 %B = 41.3%, HOMA2 %S = 55.9% and HOMA2 IR = 75.4%). These HOMA2 findings are comparable to our previous research in a population of people with normal glucose tolerance.[11]

Most studies assess repeatability using CV. Although it may not be possible to directly compare the repeatability of the original HOMA model with the HOMA2 model, our findings (HOMA2 %B = 14.8%, HOMA2 %S = 20.1% and HOMA2 IR = 27.1%) align with CVs reported from the original model including that of Mather and colleagues, who reported HOMA IR having a CV of 24%.[20] Coefficient of variation data for the McAuley Index is limited with one study reporting a CV of 15.1%.[13] This is higher than our finding of 6.3%.

Repeatability of insulin response patterns

There is limited data on the repeatability of the OGTT, yet it is a very common clinical test.[21] Few studies have investigated

TABLE 5: Kraft and Hayashi pattern frequencies on eight participants over four visits per person after imputation.

Participant	Kraft pattern explanation	Participant	Hayashi pattern explanation
K5	One test each added to pattern IIB and III as there was previously a 50:50 split.	K5	One test each added to patterns 3 and 4 as there was previously a 50:50 split.
-	-	K6	The week 1, 60-min result was extensively affected by haemolysis. Extrapolation of the raw data suggested a 60-min peak was the most likely scenario, therefore pattern 3.
K10	One test added to pattern I as this was (1) the most common pattern, and (2) the pattern IIA was associated with a sub-acute change to normal clinical state (mild cold).	K10	Unable to extrapolate from raw data whether a pattern 1 or 3 was most likely. Both scenarios run, with negligible difference to κ.

Source: Crofts C. Understanding and diagnosing hyperinsulinaemia. PhD thesis. Auckland: Auckland University of Technology; 2015. [cited n.d.]. Available from: http://aut.researchgateway.ac.nz/handle/10292/9906

TABLE 6: Fleiss' κ calculations before and after imputation.

Variable	Kraft patterns		Hayashi patterns	
	Before	After	Before	After
p	0.015	< 0.001	0.798	0.347
κ	0.290	0.417	0.186	0.451
95% CI	0.267–0.622	0.230–0.532	-1.23 to 1.61	-0.49 to 1.39

Source: Crofts C. Understanding and diagnosing hyperinsulinaemia. PhD thesis. Auckland: Auckland University of Technology; 2015. [cited n.d.]. Available from: http://aut.researchgateway.ac.nz/handle/10292/9906

CI, confidence interval.

TABLE 7: Insulin resistance measures compared to insulin response patterns.

Variable	Kraft I (*n* = 5)		Kraft IIA, IIB, III (*n* = 3)		*p*
	Mean	SD	Mean	SD	
McAuley Index (Mffm/I)	4.99	0.82	4.51	0.46	0.095
HOMA2 %B	73.87	19.70	121.11	16.09	< 0.001
HOMA2 %S	183.93	52.96	82.43	20.34	< 0.001
HOMA2 IR	0.58	0.21	1.28	0.35	< 0.001
OGIS (mL/min/m^2)	547.49	52.86	450.92	28.18	< 0.001

Source: Crofts C. Understanding and diagnosing hyperinsulinaemia. PhD thesis. Auckland: Auckland University of Technology; 2015. [cited n.d.]. Available from: http://aut.researchgateway.ac.nz/handle/10292/9906

SD, standard deviation.

the repeatability or reproducibility of insulin response curves; of those that have, no significant differences in $AUC_{insulin}$ have been noted.[21,22] There are no published studies that have assessed the repeatability of insulin response patterns, namely the Kraft and Hayashi patterns. Our study demonstrated that the Kraft pattern methodology had a higher reproducibility and was more likely to be consistent following repeated OGTT when compared to the Hayashi patterning method. Kraft patterns account for both the magnitude of the insulin response and rate of decay as well as the timing of the insulin peaks. By contrast, the Hayashi pattern algorithm is based solely on the timing of the insulin peaks. As there is little long-term data associating insulin response patterns to health outcomes, we suggest that insulin response patterns should be categorised using a combination of factors, including the magnitude and timing of the insulin peak and rate of decay.

Consistency among insulin response patterns was more common for participants who were predominantly Kraft I pattern (*n* = 5). Two participants deviated from Kraft I pattern for one of the four tested occasions: K4 (week 1) and K10 (week 4). It is unknown why these deviations occurred. Changes to insulin responses can occur for a variety of reasons, including sub-acute illnesses, menstrual cycle, stress or even poor sleep patterns.[8,23,24] This suggests that insulin response patterning should only be conducted during times of stable clinical condition or with an understanding of these caveats. Concurrent assessment of inflammatory markers such as c-reactive protein or cortisol could also be considered.

For those participants who never exhibited a Kraft I pattern (*n* = 3), consistency among patterns was lower. Two participants exhibited a 50:50 split between patterns IIB and III, while the third was predominately pattern IIB, with one occasion of pattern IIA. Unlike the participants who deviated from a predominant Kraft I pattern, there was no clear plausible clinical indication for these variations. These fluctuations may indicate that hyperinsulinaemic states are more transitory than a normal insulin response (Kraft I) and they support previous findings that demonstrate that people with hyperinsulinaemia have lower repeatability rates with OGTT.[21] Assessing insulin pattern repeatability in a large cohort of people with known hyperinsulinaemia is required to understand these variations. This study suggests that Kraft patterns are sufficiently reproducible to dichotomise patients into a Kraft I pattern or 'normal' insulin status, or a hyperinsulinaemic status (Kraft patterns II–III); however, a larger study is required to confirm these results.

Variation was higher within the Hayashi patterns. Every participant exhibited a Hayashi 3 pattern at least once. Most (75%) also exhibited either a Hayashi 1 or 2 pattern, or a Hayashi 4 pattern. With one exception, no participant had both a Hayashi 1 or 2 pattern and a Hayashi 4 pattern. Although this increased variation within the Hayashi patterns suggests that Kraft patterns should be preferred to Hayashi patterns in future research, it must also be noted that Kraft patterns, to date, do not have any longitudinal outcome data.

Using insulin resistance measures to assess insulin response patterns

Using the definition of normal insulin tolerance as Kraft I pattern,[9] the McAuley Index was unable to distinguish between normal and hyperinsulinaemic sub-groups. This contrasts to the HOMA2 variables and OGIS, which all had clear delineations between the normal and hyperinsulinaemic sub-groups. Returning a similar value across a range of Kraft patterns, HOMA2 and OGIS values suggests the McAuley Index is less sensitive to changes of physical state than the other measures.

Although HOMA2 variants clearly delineated between normal and hyperinsulinaemic states, high variability decreases the sensitivity of the test. Only OGIS had both

sensitivity and repeatability. This further questions the value of fasting tests, especially for assessing compensatory hyperinsulinaemia. Our previous research found a poor association between a fasting insulin < 30 μU/mL and a delayed insulin peak.[11]

Limitations

We recognise that our study had a number of limitations, especially with respect to participant dropout rates and small sample size. However, sample sizes of 10 participants are common in repeatability studies for insulin resistance.[21,25] Nevertheless, this study may be the first to assess the test–retest repeatability of insulin response patterns. Future research for diagnosing insulin resistance should focus on a dynamic test based on an OGTT. There are concerns about using methodologies based on the oral glucose tests because of previous reports of poor repeatability or variable glucose absorption rates. However, our study has shown that dynamic tests have a higher degree of repeatability compared to those based on fasting models. The lower rate of repeatability from models based on fasting tests may be because of the natural lability of insulin, which, our study shows, has a CV of 25% – a figure consistent with previous reports.[26]

Although previous research has focused on diagnosing insulin resistance for the early diagnosis of many metabolic diseases, hyperinsulinaemia is an emerging field.[3,27] Although there is an accepted association between insulin resistance and hyperinsulinaemia, the direction of causality is unknown and there are multiple plausible aetiologies that could start with either condition.[3,27] It is also becoming accepted that hyperinsulinaemia may be corrected while insulin resistance is maintained. Given the high degree of overlap between the conditions, it is also plausible that diagnostic tests for hyperinsulinaemia and insulin resistance may overlap. Given the variability of fasting insulin, this study suggests that dynamic insulin or glucose modelling or insulin response patterning may be more effective in diagnosing hyperinsulinaemia and/or insulin resistance, and this is where future research should be focused.

Conclusion

Hyperinsulinaemia may indicate metabolic disease earlier than conventional measures, but a lack of a consistent testing process hampers ongoing research. As hyperinsulinaemia is closely associated with insulin resistance, assessing the latter may also diagnose hyperinsulinaemia. Fasting insulin resistance measures are not suitable either because of a lack of repeatability (HOMA2 variants) or sensitivity (McAuley Index). Dynamic testing, either using OGIS or insulin response patterns, should be further investigated for assessing hyperinsulinaemia, but the latter should consider both the magnitude and timing of the insulin peaks.

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Competing interests

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Authors' contributions

C.A.P.C. was the project lead and writer and was responsible for concept and design, sample collection and analysis, and data analysis and interpretation. M.C.W. was the reviewer, was responsible for concept and design, and provided statistical expertise. C.Z. was the reviewer and performed data analysis and interpretation. F.M. was the reviewer and provided expertise in sample analysis and results interpretation. G.S. was the reviewer and performed data analysis and interpretation.

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References

1. Samaras K, McElduff A, Twigg SM, et al. Insulin levels in insulin resistance: Phantom of the metabolic opera? Med J Aust. 2006;185(3):159.
2. Schmiegelow MD, Hedlin H, Stefanick ML, et al. Insulin resistance and risk of cardiovascular disease in postmenopausal women: A cohort study from the Women's Health Initiative. Circ Cardiovasc Qual Outcomes. 2015;8(3):309–316. https://doi.org/10.1161/CIRCOUTCOMES.114.001563
3. Crofts C. Understanding and diagnosing hyperinsulinaemia. PhD thesis. Auckland: Auckland University of Technology; 2015. [cited n.d.]. Available from: http://aut.researchgateway.ac.nz/handle/10292/9906
4. Crofts C, Schofield G, Zinn C, Wheldon M, Kraft J. Identifying hyperinsulinaemia in the absence of impaired glucose tolerance: An examination of the Kraft database. Diabetes Res Clin Pract. 2016;118:50–57. https://doi.org/10.1016/j.diabres.2016.06.007
5. Hayashi T, Boyko EJ, Sato KK, et al. Patterns of insulin concentration during the OGTT predict the risk of type 2 diabetes in Japanese Americans. Diabetes Care. 2013;36(5):1229–1235. https://doi.org/10.2337/dc12-0246
6. Kashiwabara H, Inaba M, Maruno Y, et al. Insulin levels during fasting and the glucose tolerance test and Homa's index predict subsequent development of hypertension. J Hypertens. 2000;18(1):83–88. https://doi.org/10.1097/00004872-200018010-00012
7. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva: World Health Organization; 1999.
8. Crofts C, Neill A, Campbell A, Bartley J, White DE. Sleep architecture, insulin resistance and the nasal cycle: Implications for positive airway pressure therapy. J Insulin Resistance. 2018;3(1):6. https://doi.org/10.4102/jir.v3i1.34
9. Kraft JR. Detection of diabetes mellitus in situ (occult diabetes). Lab Med. 1975;6(2):10–22. https://doi.org/10.1093/labmed/6.2.10
10. Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res. 1999;8(2):135–160. https://doi.org/10.1177/096228029900800204

11. Crofts C, Wheldon MC, Zinn C, et al. Assessing the test–retest repeatability of insulin resistance measures: Homeostasis model assessment 2 and oral glucose insulin sensitivity. J Insulin Resistance. 2017;2(1):9. https://doi.org/10.4102/jir.v2i1.27
12. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004;27(6):1487–1495. https://doi.org/10.2337/diacare.27.6.1487
13. Sarafidis P, Lasaridis A, Nilsson P, et al. Validity and reproducibility of HOMA-IR, 1/HOMA-IR, QUICKI and McAuley's indices in patients with hypertension and type II diabetes. J Hum Hypertens. 2007;21(9):709–716. https://doi.org/10.1038/sj.jhh.1002201
14. Mari A, Pacini G, Murphy E, Ludvik B, Nolan J. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care. 2001;24(3):539–548. https://doi.org/10.2337/diacare.24.3.539
15. Diabetes Trials Unit. HOMA Calulator [homepage on the Internet]. 2015 [cited 2015 Apr 02]. Available from: http://www.dtu.ox.ac.uk/homacalculator/
16. Mari A. OGIS: Insulin sensitivity from the oral glucose test [homepage on the Internet]. n.d. [cited 2013 Jun 06]. Available from: http://webmet.pd.cnr.it/ogis/index.php
17. McAuley KA, Williams SM, Mann JI, et al. Diagnosing insulin resistance in the general population. Diabetes Care. 2001;24(3):460–464. https://doi.org/10.2337/diacare.24.3.460
18. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Geneva: World Health Organization; 2006.
19. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics. 1977;33:363–374. https://doi.org/10.2307/2529786
20. Mather KJ, Hunt AE, Steinberg HO, et al. Repeatability characteristics of simple indices of insulin resistance: Implications for research applications. J Clin Endocrinol Metab. 2001;86(11):5457–5464. https://doi.org/10.1210/jcem.86.11.7880
21. Gordon B, Fraser S, Bird S, Benson A. Reproducibility of multiple repeated oral glucose tolerance tests. Diabetes Res Clin Pract. 2011;94(3):e78–e82. https://doi.org/10.1016/j.diabres.2011.08.025
22. Utzschneider KM, Prigeon RL, Tong J, et al. Within-subject variability of measures of beta cell function derived from a 2 h OGTT: Implications for research studies. Diabetologia. 2007;50(12):2516–2525. https://doi.org/10.1007/s00125-007-0819-5
23. Björntorp PE, Rosmond R. Hypothalamic origin of the metabolic syndrome x. Ann N Y Acad Sci. 1999;892(1):297–307. https://doi.org/10.1111/j.1749-6632.1999.tb07803.x
24. Sheu WHH. Alteration of insulin sensitivity by sex hormones during the menstrual cycle. J Diabetes Investig. 2011;2(4):258–259. https://doi.org/10.1111/j.2040-1124.2011.00116.x
25. Le DS, Brookshire T, Krakoff J, Bunt JC. Repeatability and reproducibility of the hyperinsulinemic-euglycemic clamp and the tracer dilution technique in a controlled inpatient setting. Metabolism. 2009;58(3):304–310. https://doi.org/10.1016/j.metabol.2008.09.029
26. Widjaja A, Morris RJ, Levy JC, et al. Within- and between-subject variation in commonly measured anthropometric and biochemical variables. Clin Chem. 1999;45(4):561–566.
27. Kelly CT, Mansoor J, Dohm GL, et al. Hyperinsulinemic syndrome: The metabolic syndrome is broader than you think. Surgery. 2014;156(2):405–411. https://doi.org/10.1016/j.surg.2014.04.028