A Novel Index to Identify Steady-State Glucose Infusion Rates during a Clamp

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BACKGROUND

In clinical research, a hyperinsulinemic-euglycemic clamp is considered the gold standard to evaluate insulin sensitivity, however there is no agreement on the duration of a clamp as they can range from 2-8 hours. The outcome measure of this method is the glucose infusion rate (GIR). At a constant insulin infusion, GIR rises in a monoexponential fashion until it reaches a plateau [1]. A flat GIR curve, suggestive of a steady-state condition, is then used for insulin sensitivity determinations. Currently, an arbitrary timeframe; the last hour or 40 min of the protocol designates steady state. However, this strategy may not be ideal as results can greatly differ depending on the length of the elected steady-state phase [2,3].

AIMS

- To precisely identify GIR flatness during the steady state by applying the CONGIR (Continuous Overall Net GIR) calculation, a formula originally developed to examine glucose excursions for continual glucose monitoring [4] and previously applied to an insulin time action profile [5].
- To compare GIR flatness and insulin sensitivity after 2 hours (short protocol) and 3 hours (longer protocol) from the start of the insulin infusion.

STUDY DESIGN

- Healthy adult males and females were recruited to participate in a 6-hour two-step (10 and 40 mU/m²*min⁻¹) hyperinsulinemic-euglycemic clamp study. The protocol was approved by an ethics research board and written informed consent was obtained from each subject.
- Screening assessments included a 2-hour oral glucose tolerance test (OGTT) to exclude subjects with diabetes (2h OGTT glucose >200 mg/dL). Subjects were asked to remain on a stable diet for 3 days prior to the clamp and instructed to fast the evening before the procedure.
- Plasma glucose samples were measured every 5 min. At time 0 min, Humalog® (Insulin lispro) U-100 was infused at a constant rate of 10 mU/m²*min⁻¹ for 180 min, after which insulin infusion was increased to 40 mU/m²*min⁻¹ until 360 min. Dextrose 20% (w/v) was infused to maintain target blood glucose of 90 mg/dL (Figure 1).

RESULTS

Table 1. Subject Characteristics and Anthropometric Results.
Female subjects were significantly older than males, yet both groups were matched for BMI as well as glucose and insulin parameters, and therefore male and female results were pooled for subsequent analysis.

Parameter	Male (n=6)	Female (n=9)	Group (n=15)
Age (years)	25.8±3.9	30.8±3.3*	28.8±4.2
BMI (kg/m²)	21.2±1.7	22.9±2.3	22.2±2.2
FASTING			
Cholesterol (mg/dL)	151.0±19.8	167.8±27.1	161.1±25.2
Glucose (mg/dL)	85.3±4.4	87.2±4.4	86.5±4.3
Insulin (µU/ml)	5.58±1.91	7.70±2.24	6.85±2.31
2h OGTT			
Glucose (mg/dL)	100.5±31.2	104.3±12.8	102.8±21.1
Insulin (µU/ml)	54.19±22.92	77.35±65.54	63.45±44.41

Data represented as mean±SD where *p<0.05 for male vs. female subjects.

Figure 1. Typical Average GIR Results for a Healthy Cohort. Average (black line) and smoothed (dotted line) GIR over the course of the hyperinsulinemic-euglycemic clamp protocol is displayed in Figure 1. Insulin infusion rate of 10 mU/m²*min⁻¹ started at 0 min and was increased to 40 mU/m²*min⁻¹ at 180 min. Subsequent analysis compared the short (60-120 min; 240-300 min) and longer (120-180min; 300-360min) protocols for low and high insulin infusion respectively. Plasma glucose was maintained at a concentration of 90 mg/dL over the 6-hour protocol (grey line, right y-axis).

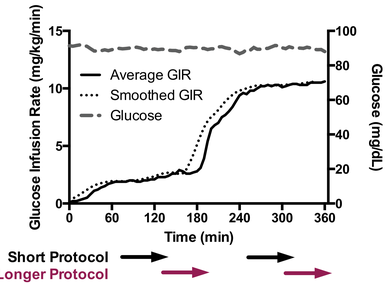


Figure 2. CONGIR Calculates the Standard Deviation of the Change in GIR (*G*) over a Given Time Period (*k*). The formula and a descriptive schematic are shown. A lower CONGIR indicates a flatter segment in the curve.

$$CONGIR = \sqrt{\frac{\sum_{t_1}^{t_k}(D_t - \bar{D})^2}{k-1}} \qquad \bar{D} = \frac{\sum_{t_1}^{t_k} D_t}{k} \qquad D_t = G_t - G_{t-n}$$

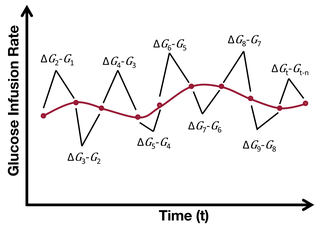
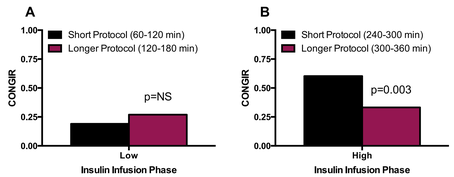
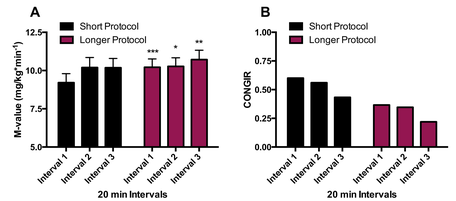


Figure 3. CONGIR Calculation Shows that a Longer Protocol Yields a Flatter GIR. CONGIR calculation for a short (black bars) vs. longer (purple bars) period is shown during the (**A**) low (10 mU/m²*min⁻¹) and (**B**) high (40 mU/m²*min⁻¹) insulin infusion phase.



CONGIR was calculated over the low (60-180 min) and high (240-300 min) insulin infusion phases. This period was partitioned to examine the difference between a short (2-hour) vs. longer (3-hour) protocol. During low insulin infusion, CONGIR was not significantly different between the 60-120 min vs. 120-180 min interval (Figure 3A), suggesting both regions of the curve are equally flat. At a high insulin infusion rate, CONGIR was significantly lower over 300-360 min (longer) vs. 240-300 min (short), indicating greater flatness during the last hour of the protocol (Figure 3B).

Figure 4. Insulin Sensitivity is Significantly Different between the Two Curve Segments. (**A**) Average M-value and (**B**) CONGIR over three 20 min intervals from each curve segment is shown during the high insulin infusion phase. Intervals 1,2,3 refer to 240-260 min, 260-280 min, 280-300 min and 300-320 min, 320-340 min, 340-360 min for the short (black bars) and longer (purple bars) protocol respectively. *p<0.05, **p<0.01 and ***p<0.001 vs short protocol.



By convention, the M-value is determined over a 20 min period [6]. A higher M-value signifies greater peripheral glucose metabolism and insulin sensitivity. Overall, a flatter curve region, 300-360 min, yields a significantly greater M-value.

Table 2. CONGIR is More Sensitive than %Coefficient of Variation (CV) in Identify Curve Flatness.
CONGIR and %CV difference among two segments of the GIR curve over 60 min or 40 min intervals is shown during the high insulin infusion period.

Interval	Curve Segments	CONGIR	CONGIR p-value	%CV	%CV p-value
60 min	240-300 vs. 300-360 min	0.60 0.33	p=0.003	7.8% 4.3%	p=0.008
40 min	260-300 vs. 320-360 min	0.54 0.30	p=0.007	3.8% 2.8%	p=0.03

CONCLUSIONS

- To our knowledge, this is the first application of CONGIR to a hyperinsulinemic-euglycemic clamp.
- CONGIR was more sensitive than %CV in distinguishing flatness between two segments of the GIR curve.
- Our findings highlight the need for longer clamp duration to ensure a true steady state is achieved.
- Since hyperinsulinemic-euglycemic clamps are widely used in early phase clinical research to evaluate diabetes drug efficacy, a novel measure of GIR flatness is a valuable pharmacodynamic tool for ensuring the integrity of insulin sensitivity determinations.

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