



Published in final edited form as:

Diabetes Obes Metab. 2018 March ; 20(3): 549–555. doi:10.1111/dom.13106.

Performance of individually-measured vs population-based C-peptide kinetics to assess β -cell function in presence and absence of acute insulin resistance

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Abstract

Aims—Standardized, population-based kinetics of C-peptide distribution and clearance are used to estimate insulin secretion from plasma C-peptide concentrations without direct measurement of C-peptide kinetics. We then compared the performance of population-based kinetics to directly measured C-peptide kinetics when used to calculate β -cell responsivity indices. To ensure that population-based kinetics apply to all conditions where β -cell function is measured, subjects were studied in the presence and absence of acute insulin resistance.

Materials and Methods—Somatostatin was used to inhibit endogenous insulin secretion in 56 nondiabetic subjects. Subsequently, a C-peptide bolus was administered and the changing concentrations used to calculate individual kinetic parameters of C-peptide clearance. In addition, they were studied on 2 occasions in random order using an oral glucose tolerance test (OGTT). On one occasion, free fatty acid (FFA) elevation to cause insulin resistance, was achieved by infusion of intralipid + heparin. Disposition Index (DI) was then estimated by the oral minimal model using either population-based or individual C-peptide kinetics.

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Author contributions: R.T.V. A.S., M.S. researched data and ran the studies; F.P. & M.C.L. undertook mathematical modeling of insulin secretion and action; K.R.B. oversaw the statistical analysis; and C.D.M. supervised mathematical modeling of insulin secretion and action and contributed to discussion and reviewed/edited manuscript; R.A.R. contributed to discussion and reviewed/edited manuscript; C.C. contributed to discussion and reviewed/edited manuscript and A.V. designed the study, oversaw its conduct, researched data, wrote the manuscript. Dr. Adrian Vella is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Disclosure: Dr. Vella has consulted for VTV therapeutics, XOMA, Sanofi-Aventis, Novartis and Bristol-Myers Squibb in the past 5 years. None of the other authors (Drs. Varghese, Laurenti, Piccinini, Dalla Man, Sharma, Shah, Bailey, Cobelli, Rizza) have relevant disclosures.

Conflict of Interest: No potential conflicts of interest.

Results—There were marked differences in the exchange parameters (k_{12} and k_{21}) of the model describing C-peptide kinetics, but smaller differences in the fractional clearance, i.e. the irreversible loss from the accessible compartment (k_{01}), obtained from population-based estimates compared to experimental measurement. Since it is predominantly influenced by k_{01} , DI estimated using individual kinetics correlated well with those estimated using population-based kinetics.

Conclusions—These data support the use of population-based measures of C-peptide kinetics to estimate β -cell function during OGTT.

Keywords

C-peptide fractional clearance; hepatic insulin extraction; insulin secretion; insulin action; β -cell function; acute insulin resistance

Introduction

Defects in insulin secretion are central to the pathogenesis of type 2 diabetes, and are evident early in the development of impaired glucose tolerance and the subsequent transition to type 2 diabetes ¹. As such, accurate and reproducible measurement of insulin secretion is central to understanding disorders of glucose metabolism as well as quantifying the effect of therapies or other interventions on β -cell function ². The Disposition Index ² expresses insulin secretion as a function of the prevailing insulin action and is accepted as a reliable measure of β -cell function ³ producing consistent results in response to different oral challenges ⁴.

Unfortunately, measurement of insulin concentrations before and after a stimulus to β -cell secretion does not accurately reflect insulin secretion into the portal vein, since insulin undergoes hepatic extraction prior to its appearance in the systemic circulation ⁵. Because of this, peripheral insulin concentrations reflect the net sum of two processes; insulin secretion and hepatic extraction ². Indeed, there is evidence that the fraction of insulin extracted across the liver is altered by changes in β -cell function (measured using the disposition index ⁶), or by insulin secretory burst mass during euglycemia or hyperglycemia ⁷. Therefore, differences in hepatic insulin clearance introduce a systematic error in measures based on peripheral insulin concentrations when comparing groups with differing β -cell function ⁶.

C-peptide is co-secreted in an equimolar ratio with insulin and does not undergo hepatic extraction ⁸, therefore C-peptide concentrations in the peripheral circulation would theoretically be more representative of insulin secretion by the β -cell. However, the half-lives of insulin and C-peptide in the circulation differ dramatically so that C-peptide accumulates in the circulation compared to insulin ⁹. Since the circulating concentration of a hormone or substrate represents the balance between secretion and clearance, estimating the rate of insulin secretion from C-peptide concentrations requires knowledge of C-peptide clearance.

We ¹⁰ and others ¹¹ have used measures of β -cell function that depend on the deconvolution of insulin secretion rates from C-peptide concentrations in the systemic circulation. These methods incorporate age-associated changes in C-peptide kinetics that were directly

measured in an experiment undertaken by Van Cauter et al. in 200 subjects, including people with type 2 diabetes and obesity¹². Subsequently, these data were utilized to measure 24 hour profiles of insulin secretion in 36 subjects. Toffolo et al, validated this methodology¹³ by directly comparing these standardized estimates to individually-measured C-peptide kinetics and the effect of these differences on the estimates of β -cell function during an intravenous glucose tolerance test (IVGTT). The authors concluded that the population-based parameters, as proposed by Van Cauter et al, allow for accurate estimates of β -cell function during an IVGTT in 7 subjects. The time course of insulin secretion in response to an intravenous challenge (Intravenous Glucose Tolerance Test – IVGTT) differs significantly from that of an oral challenge¹⁴. Indeed measurement of β -cell responsivity in prediabetes with IVGTT and OGTT can produce discordant results³. It is currently unknown if population and individually-assessed C-peptide kinetics provide comparable assessment of indices of β -cell function in response to an oral challenge because a validation comparable to that performed by Toffolo et al.¹³ has not been undertaken. For a full discussion of the methodology please see².

As part of a series of experiments examining the pathogenesis of prediabetes and the potential role of insulin pulse frequency and amplitude, we measured sequential insulin and C-peptide concentrations after a bolus injection of both peptides in 56 individuals when endogenous insulin (and C-peptide) secretion was inhibited by somatostatin as part of a euglycemic, hyperinsulinemic clamp experiment¹⁵. This enabled derivation of individual C-peptide kinetic parameters. We subsequently compared indices of β -cell function obtained using population-based kinetics to those resulting from the use of individually-derived kinetics in response to an oral challenge during basal conditions and during acute insulin resistance induced by Free Fatty Acid (FFA) elevation¹⁰.

Materials and Methods

Subjects

The subjects in this study represent a subset of those who participated in a series of previously published experiments intended to examine the role of the diabetes-associated genotype at the *TCF7L2* locus in the pathogenesis of prediabetes^{10, 15}. After approval from the Mayo Clinic Institutional Review Board, we identified suitable subjects who provided written, informed consent. At the time of screening, body composition was measured using dual-energy X-ray absorptiometry (iDXA scanner; GE, Wauwatosa, WI).

Experimental Design – Clamp Experiment

On one study day, subjects underwent a euglycemic, hyperinsulinemic clamp over a 375 minute period. The first part of the experiment (0 to 240 minutes) has been described previously¹⁵. During this time an infusion of somatostatin (60ng/kg/min), glucagon (0.65ng/kg/min) and growth hormone (0.25ng/kg/min) was started and maintained for the duration of study. Insulin was also infused at 0.30mU/kg/min. Dextrose was infused to maintain glucose at ~5.5 mmol/l over the period of study. Arterialized venous blood samples were collected to allow measurement of hormone, tracer and substrate concentrations. At 255 min, a bolus of C-peptide (60pmol/kg) was administered over 1 minute and blood

samples collected at 10 minute intervals over the subsequent 2 hours to enable individual calculation of C-peptide clearance.

Experimental Design – Oral Glucose Challenge in the presence (FFA) or absence (GLY) of acute insulin resistance

This experimental design has been described previously^{10, 15}. Briefly, subjects were initially studied on two occasions in random order, two weeks apart. On one occasion, subjects received an infusion of Intralipid and heparin to raise free fatty acid (FFA) concentrations while on the other occasion glycerol (GLY) was infused at a rate of 5 μ mol/kg/min (to match the amount of glycerol present in the Intralipid infused during the FFA study day). The infusions commenced three hours prior to challenge with a glucose drink (1g per kg body weight) and were continued until the end of study (six hours after the start of the meal). Blood samples were obtained at periodic intervals for hormone and substrate measurement over the course of the experiment.

Analytical techniques

Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence assay (Access Assay; Beckman, Chaska, MN). Plasma glucagon and C-peptide were measured by Radio-Immunoassay (Linco Research, St. Louis, MO).

Calculations

Data are presented as Means \pm SEMs. We utilized a two-compartment model of C-peptide kinetics, where the relevant kinetic indices were estimated using anthropometric data as previously described¹². The population-based C-peptide kinetics were then used to estimate insulin secretion and subsequent β -cell responsivity during the oral challenges¹². Separately, observed C-peptide decay curves were used to estimate the kinetic parameters of C-peptide¹⁶ in each individual after bolus injection when endogenous secretion is inhibited, using a Maximum *A Posteriori* (MAP) estimation^{17, 18}. The resulting parameters were then used to derive the β -cell responsivity indices during the oral glucose challenge (both on the GLY and FFA study day) using the oral C-peptide minimal model¹⁹.

The oral C-peptide minimal model assumes that insulin secretion comprises static and dynamic components. The static component, proportional, through the parameter Φ_s , to the delayed glucose concentration, represents the provision of new insulin to the releasable pool. The dynamic component is proportional, through the parameter Φ_d , to the rate of increase of glucose concentrations. An index of total beta-cell responsivity to glucose (Φ) is then derived from both indices²⁰. Net insulin action (S_i) was measured using the oral minimal model as previously described²¹. Disposition indices (DI) were subsequently calculated by multiplying the relevant β -cell responsivity indices (Φ_d , Φ_s and Φ) by S_i .

Statistics

Data are presented in the text as mean \pm SEM. The primary analysis compared individual indices of β -cell responsivity obtained using either population-based or individually-derived C-peptide clearance. The between-group differences in indices of β -cell responsivity

obtained using either population-based or individually-derived C-peptide kinetics were examined by a paired, two-tailed *t*-test or a signed-rank test as warranted by their distribution. In addition, to better understand intra-individual differences we calculated the percentage differences for kinetic parameters and Disposition Indices for each individual derived using individually measured or population-derived data using the formula: -

$$\% \Delta = \frac{[ABS(Individualized - Population)]}{Individualized} * 100$$

To characterize the sensitivity of individual variation in DI to each of the kinetic parameters (k_{01} , k_{12} and k_{21}) we identified the C-peptide minimal model three times for every individual. For each identification, we fixed two of the kinetic parameters to their individually-measured values and the third to its population value. We then calculated the sensitivity of DI to that parameter using the following formula: -

$$\frac{\% \Delta DI}{\% \Delta k_{ij}}$$

where k_{ij} represents one of the coefficients k_{01} , k_{12} and k_{21} .

Statistical analysis was performed in Primer 5 (GraphPad Software, San Diego, CA). Bland-Altman plots (Absolute difference vs. average values) were used to examine differences in indices calculated using the different methods of estimating C-peptide clearance. A multivariate analysis performed in JMP Pro 11 (SAS, Cary, NC) was used to examine the contribution of anthropometric characteristics to the absolute differences in indices ([Population based] – [Individualized kinetics]). A *p*-value < 0.05 was considered statistically significant.

Results

Volunteer Characteristics

We studied 56 subjects (19 men and 37 women), with a mean age of 47 ± 2 years. The Body mass Index (BMI) was 27.8 ± 0.5 Kg/M² and fasting glucose was 4.9 ± 0.1 mmol/l. Serum Creatinine was 0.83 ± 0.02 mg/dl.

C-peptide Concentrations after bolus injection (Figure 1)

C-peptide concentrations (Panel A) rose from 0.10 ± 0.02 nmol/l at baseline to a peak concentration of 0.63 ± 0.02 nmol/l 10 minutes after intravenous injection. They subsequently declined to a nadir concentration of 0.11 ± 0.02 nmol/l at the end of the study.

Comparison of C-peptide kinetic parameters k_{01} , k_{12} and k_{21} and indices of β -cell responsivity calculated from population data or from individual C-peptide clearance (Figure 2)

The kinetic parameter k_{01} (Figure 1, Panel B), was only slightly, albeit significantly, higher when calculated from demographic data (Figure 2, Panel A – 0.059 ± 0.001 vs. 0.055

$\pm 0.001 \text{ min}^{-1}$, $p = 9.0 \times 10^{-4}$). The mean % absolute difference was $13 \pm 2\%$ (Panel B). The kinetic parameter k_{12} (Figure 1, Panel B) was greater when calculated from demographic data (Panel C – 0.049 ± 0.001 vs. $0.032 \pm 0.001 \text{ min}^{-1}$, $p < 1.0 \times 10^{-4}$). The mean % absolute difference was much higher than that for k_{01} (Panel D, $58 \pm 4\%$). k_{21} (Figure 1, Panel B) calculated from demographic data was even higher than those calculated from individual C-peptide clearance (Panel E – 0.058 ± 0.004 vs. $0.020 \pm 0.008 \text{ min}^{-1}$, $p < 1.0 \times 10^{-4}$). The mean % absolute difference between methods was the highest of the three kinetic parameters (Panel F, $242 \pm 24\%$).

Correlation of DI_d , DI_s and DI calculated using population-based and individually-measured C-peptide clearance during Glycerol infusion or FFA elevation (Figure 3)

DI_d (Panels A and B), DI_s (Panel C and D), and DI (Panel E and F) derived using population-based measures of C-peptide kinetics were closely correlated with those calculated using individually-determined rates of C-peptide kinetics during both the GLY and FFA study day. The correlation for all indices of β -cell responsivity did not differ significantly between the GLY and FFA study days with the exception of DI_d ($p = 0.02$). There was no evidence of bias detected by Bland-Altman plots comparing the two methods. Moreover the absolute differences in indices ([Population based] – [Individualized kinetics]) could not be explained by age, sex or weight (data not shown).

Comparison of Intra-individual differences in DI_d , DI_s and DI calculated using population-based and individually-measured C-peptide clearance during Glycerol infusion or FFA elevation (Figure 4)

The % intra-individual difference in DI_d did not differ during the GLY and FFA study days respectively (17 ± 2 vs. $24 \pm 3\%$, $p = 0.11$ – Panel A). The same was true for DI_s (15 ± 2 vs. $16 \pm 2\%$, $p = 0.32$ – Panel B) and DI (16 ± 2 vs. $16 \pm 2\%$, $p = 0.38$ – Panel C).

Sensitivity of DI to intra-individual differences in kinetic parameters of C-peptide clearance during Glycerol infusion or FFA elevation (Figure 5)

The sensitivity of DI to k_{01} did not differ between study days (92 ± 17 vs. $104 \pm 17\%$, $p = 0.10$ – Panel A) but was much higher than for the other kinetic parameters. The sensitivity of DI to k_{12} (9 ± 1 vs. $12 \pm 2\%$, $p = 0.55$ – Panel B) also did not differ between study days. The sensitivity of DI to k_{21} did not differ between study days and was even lower (7 ± 2 vs. $5 \pm 1\%$, $p = 0.14$ – Panel C).

Discussion

We report that, in general, indices of β -cell responsivity to oral glucose calculated using population-based kinetics correlated well with those derived using individually measured C-peptide kinetics. Taken together these data imply that utilization of population-based C-peptide kinetics enables reliable estimation of β -cell function in response to an oral challenge even in situations where insulin action is decreased acutely. This is because DI is relatively insensitive to errors in the estimation of k_{12} and k_{21} but sensitive to errors in the estimation of k_{01} . This is to some extent expected as this parameter represents the fractional clearance rate of C-peptide. A large error in this parameter translates into a large error in

insulin secretion rate and thus in the β -cell responsivity index (Φ) and DI². However, the differences between parameters calculated by either method are lowest for k_{OI} explaining the good correlation of DI calculated using population kinetics with that calculated using individually measured kinetics.

Measurement of β -cell function using the oral C-peptide minimal model depends on of C-peptide kinetics. We^{6, 22, 23} and others^{24, 25}, have utilized C-peptide kinetics data derived by Van Cauter et al¹² where C-peptide kinetic parameters were estimated from direct measurement in a group of 200 subjects of varying age, gender, obesity and glucose tolerance. Although measures of β -cell function using alternative methodology (independent of C-peptide concentrations) can correlate well with those dependent on these measures of C-peptide kinetics, direct comparison of functional indices obtained with or without individually-determined C-peptide kinetics has not been undertaken in the presence of acute change in insulin action during an oral glucose tolerance test. The latter is an important consideration given that insulin secretion in response to an intravenous glucose challenge (where the approach had been validated independently¹³) differs significantly from that in response to an oral challenge^{26, 27}.

To ensure an ability to accurately detect insulin pulses in the hepatic venous circulation and in the systemic circulation (as part of a series of studies examining the pathogenesis of prediabetes), we estimated C-peptide kinetics in 56 subjects (whose body weight varied from 50 to 110kg) during a pancreatic clamp to ensure that C-peptide measurement was not confounded by endogenous β -cell secretion. These data provide an opportunity to compare oral minimal model-derived indices obtained using population-based C-peptide kinetics to those measured experimentally in each individual. Furthermore, prior participation in a paired experiment enabled comparison of the methodology in the presence (FFA) and absence (GLY) of acute insulin resistance¹⁰.

We report that in this population of otherwise healthy adults, utilization of population-based C-peptide kinetics produces disposition indices that correlate well with those calculated using direct measures of C-peptide kinetics. There are small, but significant, differences in the kinetic parameters estimated by the different methods, with the population-based data exhibiting systematic overestimation compared to those calculated from individual C-peptide clearance. However, these data show that estimation of DI using the oral minimal model is more sensitive to the actual hormone and substrate concentrations used to measure β -cell responsivity than it is to these kinetic parameters in otherwise healthy humans – at least in those with intact renal function and demographic characteristics like those of the study population.

Total Disposition Index varied by 16 ± 2 % depending on whether directly-measured C-peptide kinetics or a population-based measure of C-peptide kinetics was used. This is similar to the difference in values of mean insulin secretion, reported as 10-12 % by Van Cauter et.al. depending on the methodology used to estimate C-peptide kinetics¹². In the presence of FFA elevation to cause insulin resistance, a similar variance was also observed. In light of this, it is reasonable to conclude that population-based measures of C-peptide kinetics performed adequately, when compared to individual measurement of C-peptide

clearance. This is of considerable importance, given the increasing use of the oral minimal model for estimating β -cell function and the cost and inconvenience of measuring individual C-peptide kinetics in each subject². However, it is important to underline that, while on average the performance of the population kinetics is satisfactory, the error in individual DI estimation, especially in subjects with low DI, may be far higher than the mean difference of 16%.

While the results from this study are reassuring, it is important to recognize that the calculation of individual C-peptide kinetics was limited by the absence of frequent sampling immediately after the bolus injection of C-peptide – a constraint imposed by both immunoassay cost and blood volume limitations. In order to improve accuracy and precision of parameters and overcome this limitation, a MAP estimation has been used to obtain the individual- measures of C-peptide kinetics, since Bayesian estimation improves model performance in data-poor scenarios^{17, 18}. However, despite this limitation, indices of β -cell responsivity (and DI) were relatively unaffected by the method used to determine C-peptide kinetics.

The other potential limitation is that the cohort studied was recruited on the basis of subjects' *TCF7L2* genotype as previously described¹⁰. This locus has been associated with type 2 diabetes²⁸, impaired insulin secretion²⁹ and impaired suppression of glucagon¹⁰. However, we studied subjects with both the TT (diabetes-associated) and the CC (diabetes-protective) genotype at rs7903146 (in the *TCF7L2* locus) and there was no evidence of an effect of genotype on C-peptide clearance (Data not shown). This concurs with an *a priori* absence of evidence that this locus alters C-peptide clearance, has effects on renal function or on hepatic and extra-hepatic insulin action¹⁵. In this cohort, the genotype at rs7903146 in the *TCF7L2* locus did not alter the relationship between indices calculated using individually-determined C-peptide clearance versus those using population-based data, on either study day (Data not shown).

Taken together, these data support the application of population-based measures of C-peptide kinetics to the measurement of β -cell function using the oral minimal model in non-diabetic subjects with normal renal function.

Acknowledgments

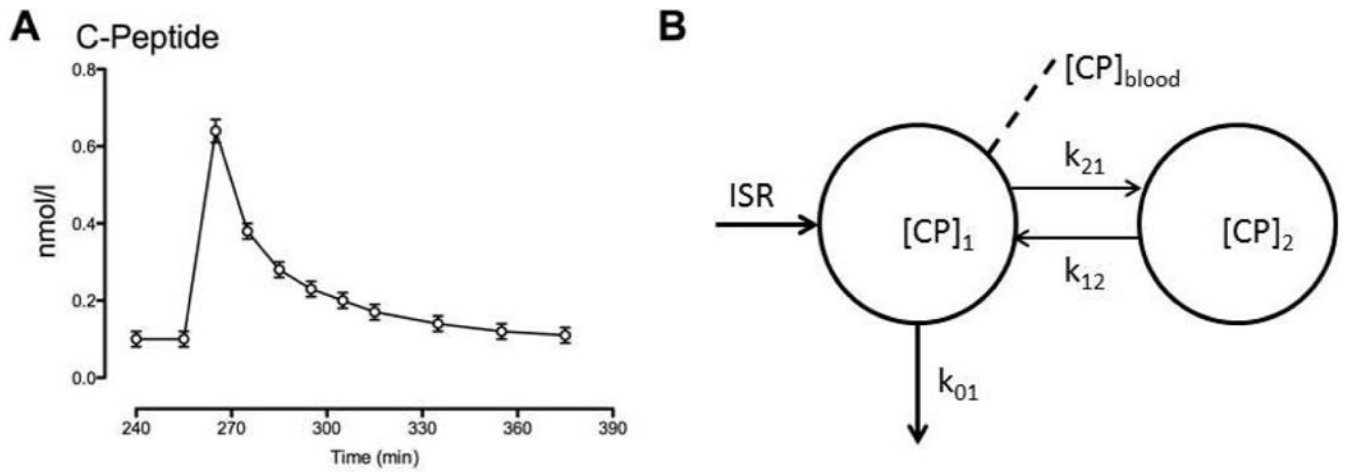
Grant Support: This work was supported by the National Institutes of Health grant numbers UL1 TR000135, R01 DK78646, and 5T32 DK007352-37.

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**Fig. 1.**

C-peptide concentrations after bolus injection in the presence of somatostatin to block endogenous insulin (and C-peptide) secretion (Panel A). Kinetics of C-peptide (Panel B) where $[CP]_1$ and $[CP]_2$ are C-peptide concentrations in the accessible and peripheral compartments respectively; k_{01} , k_{12} , k_{21} are kinetic parameters while ISR represents the insulin secretion rate and $[CP]_{\text{blood}}$ is the C-peptide concentration sampled from the peripheral circulation.

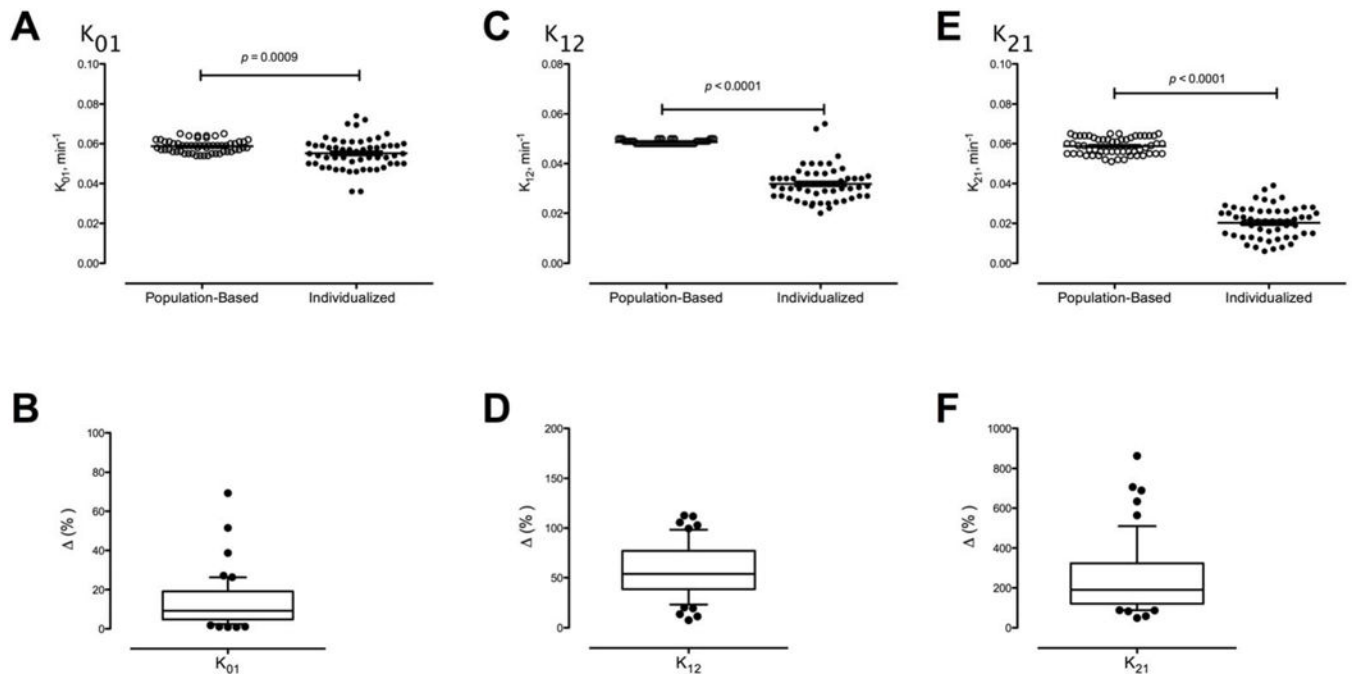


Fig. 2.
Comparison of C-peptide kinetic parameters k_{01} , k_{12} and k_{21} calculated from demographic data or from individual C-peptide kinetics shown as absolute values (Panels A, C and E). The absolute percentage inter-individual difference for each parameter is shown in the lower panels (Panels B, D and F).

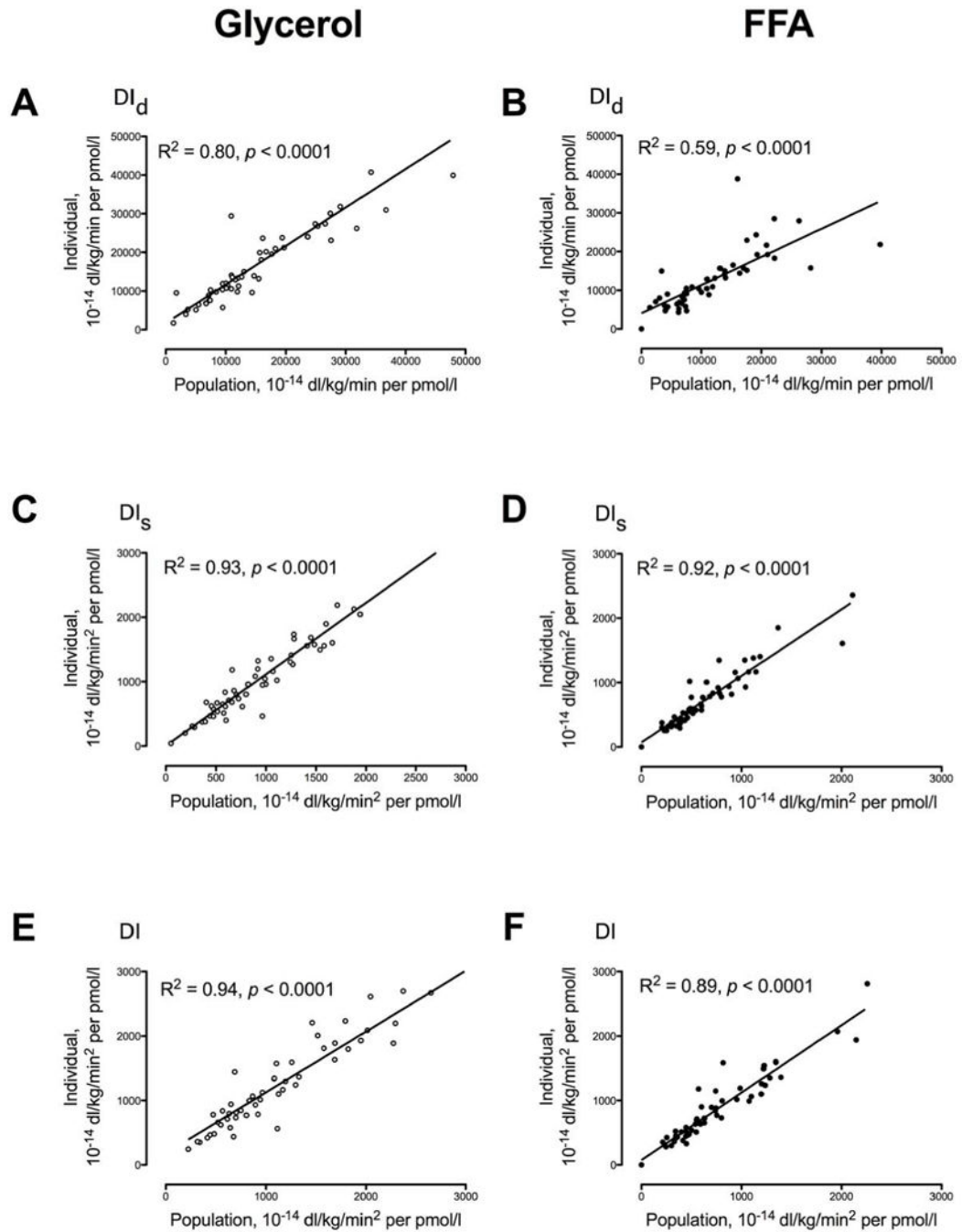


Fig. 3. Relationship of DI_d (Panels A and B), DI_s (Panels C and D) and DI (Panels E and F) calculated using anthropometric characteristics (Population) versus those calculated from experimental data for C-peptide kinetics (Individual) for each subject (open circles) in response to a 1g/Kg body weight glucose challenge with accompanying glycerol infusion or during an identical glucose challenge with free fatty acid (FFA) elevation (solid circles).

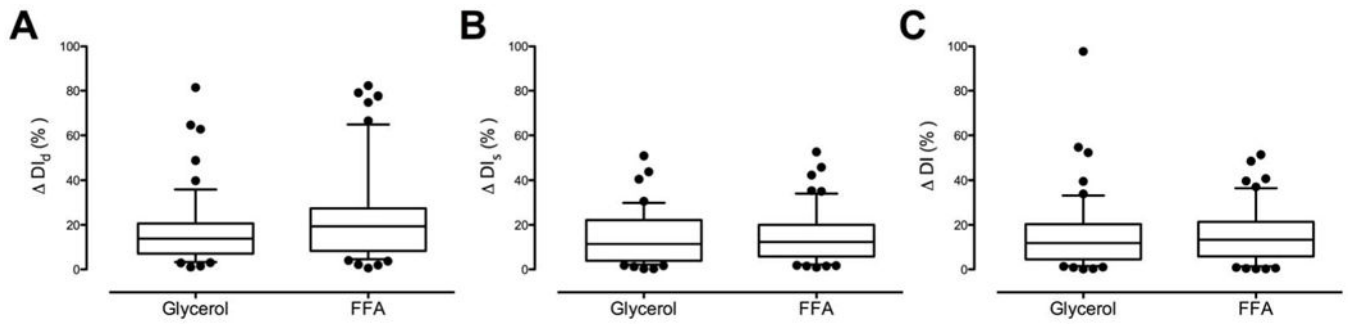


Fig. 4. Comparison of absolute percentage inter-individual difference for DI_d (Panel A), DI_s (Panel B) and DI (Panel C) using two estimates of C-peptide kinetics during the glycerol and FFA study days.

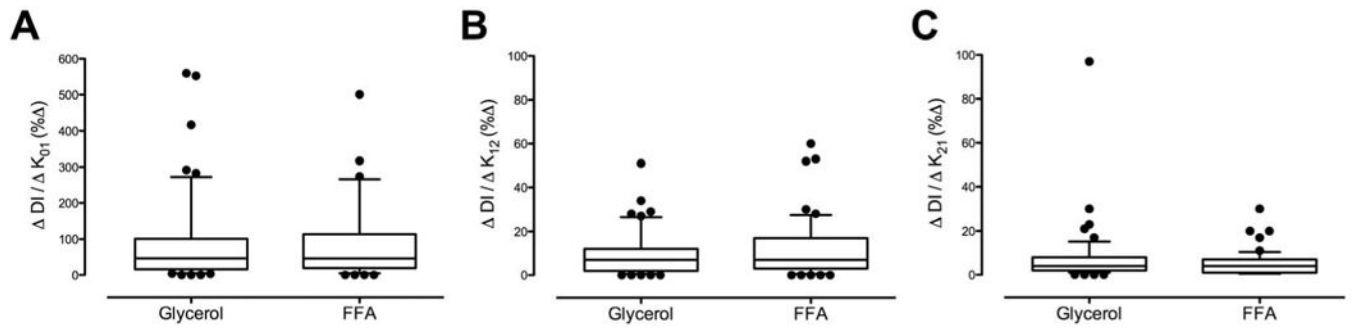


Fig. 5. Sensitivity of DI to inter-individual differences in k_{0I} (Panel A), k_{12} (Panel B) and k_{2I} (Panel C) during the glycerol and FFA study days.