SUPPLEMENTARY MATERIAL

Insulin model and calculation of total insulin extraction and clearance

We assumed the insulin kinetics to be described by a 1 pool model (1), where the input is pre-hepatic insulin secretion rate (ISR) estimated from the deconvolution of C-peptide following the 2-pool model proposed by Van Cauter (2).

The equation that describes insulin kinetics is

$$\frac{dI(t)}{dt} \times V_{Ins} = -K(t) \times I(t) \times V_{Ins} + ISR(t) = -Rd_{Ins}(t) + ISR(t) \qquad \text{eq.1}$$

where

- V_{Ins} is the volume of distribution of insulin (assumed constant) which was previously estimated as 141 ml/kg (3-5)
- $K(t)=k_p+k_L(t)$ is the fractional insulin extraction that is composed of peripheral insulin extraction (k_p, constant according to (3-5)) and hepatic insulin extraction ($k_L(t)$, that might change over time).

- *Calculation of Total Insulin Extraction $Rd_{Ins}(t)$*

From eq.1 we can estimate insulin extraction (Rd_{Ins}) as:

$$Rd_{Ins}(t) = ISR(t) - dI(t)/dt \times V_{Ins} \qquad \text{eq.2}$$

The total insulin extraction during OGTT can be calculated from the integral from 0 to 120 min (i.e. the end of the test) of eq. 2, as

$$\int_0^{120} Rd_{Ins}(t)\,dt = AUC\ Rd_{Ins\ 0\text{-}120} = \int_0^{120} \left[ISR(t) - \frac{dI(t)}{dt} \times V_{Ins}\right] dt =$$

$$= \int_0^{120} ISR(t)dt - V_{Ins} \times \int_0^{120} \frac{dI(t)}{dt} dt = AUC\ ISR_{0\text{-}120} - (I_{120} - I_0) \times V_{Ins} \qquad \text{eq.3}$$

- *Calculation of Total Insulin Clearance MCR_I (t)*

$$MCR_I(t) = K(t) \text{ x } V_{Ins} = [k_p + k_L(t)] \text{ x } V_{Ins} \qquad \text{eq.4}$$

Where k_p and $k_L(t)$ are the fractional extraction rates in peripheral tissue and liver, respectively

From eq.1 we can estimate $MCR_I(t)$ from eq.1 dividing insulin extraction by insulin concentrations at each time point, i.e., as Rd(t)/I(t):

$$MCR_I(t) = \frac{Rd_{Ins}(t)}{I(t)} = \frac{ISR(t)}{I(t)} - \frac{\frac{dI(t)}{dt}}{I(t)} \text{ x } V_{Ins} \qquad \text{eq.5}$$

The insulin clearance during OGTT can be calculated from the integral from 0 to 120 min of eq.5 (that can be numerically solved calculating the AUC) from ISR and insulin concentration:

$$\int_0^{120} MCR_I(t)\, dt = AUC\, [MCR_I]_{0\text{-}120} = \int_0^{120} \frac{Rd_{Ins}(t)}{I(t)} dt$$

$$= \int_0^{120} \frac{ISR(t) - \frac{dI(t)}{dt} \text{x } V_{Ins}}{I(t)} dt = \int_0^{120} \frac{ISR(t)}{I(t)} dt - \int_0^{120} \left[V_{Ins} \times \frac{\frac{dI(t)}{dt}}{I(t)} \right] dt \qquad \text{eq.6}$$

Thus

$$AUC\, [MCR_I]_{0\text{-}120} = AUC \left[\frac{ISR(t)}{I(t)} \right]_{0-120} - V_{Ins} \times \int_0^{120} \frac{\frac{dI(t)}{dt}}{I(t)} dt \qquad \text{eq.7}$$

The formula previously used by Jung (3) and Smith (6):

$$AUC\, MCR_{I\ 0\text{-}120} = \frac{AUC\, ISR_{0-120}}{AUC\, I_{0-120}} - \frac{(I_{120} - I_0) \text{ x } V_{Ins}}{AUC\, I_{0-120}} \qquad \text{eq.8}$$

is different from the formula used by us i.e. equation 7 (see eq.11 for the solution of the integral). We found that the calculation of eq. 8 by Jung (3) and Smith (6) is not correct since it assumes that the integral of the ratio of 2 functions (i.e., Rd(t) and I(t) in eq.7) while

$$\int_0^{120} \frac{Rd(t)}{I(t)} dt \neq \frac{\int_0^{120} Rd(t)dt}{\int_0^{120} I(t)dt}$$

The first part of eq.6 can be calculated as

$$\int_0^{120} \frac{ISR(t)}{I(t)} dt = AUC \left[\frac{ISR(t)}{I(t)}\right]_{0-120} \qquad \text{eq.9}$$

The second part of the eq.6 gives as a result

$$V_{Ins} \times \int_0^{120} \frac{\frac{dI(t)}{dt}}{I(t)} dt = [\ln(I_{120}) - \ln(I_0)] \text{ x } V_{Ins} \qquad \text{eq.10}$$

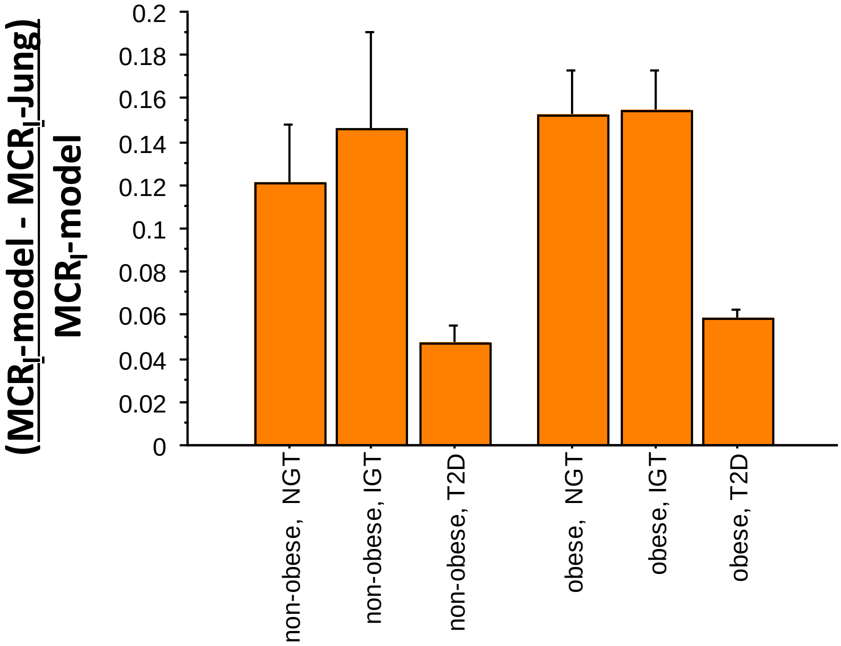
while Jang and Smith calculated the first part of eq.6 as $\frac{AUC\ ISR_{0-120}}{AUC\ I_{0-120}}$ and the second part as $\frac{(I_{120} - I_0) \text{ x } V_{Ins}}{AUC\ I_{0-120}}$ due to the erroneous assumption that $\left[\int_0^{120} \frac{Rd(t)}{I(t)} dt\right]$was equal to $\left[\frac{\int_0^{120} Rd(t)dt}{\int_0^{120} I(t)dt}\right]$.

The correct formula that should be used to calculate total MCR_I is

$$AUC\ MCR_{I\ 0\text{-}120} = AUC \left[\frac{ISR(t)}{I(t)}\right]_{0-120} - [\ln(I_{120}) - \ln(I_0)] \text{ x } V_{Ins} \qquad \text{eq.11}$$

In our cohort, we have compared the results obtained using the model estimations versus the formula (eq.8) used by Jung (3) and Smith (6). We found that the formula in eq.8 underestimates the MCR in all groups by ~15% in the non-diabetics and be ~5% in T2D, with a p<0.0001 in all groups (see suppl. Figure 1 below)

It should be noted that, whether one employs the model estimation (as used by us) or the Jung formula (as used by Smith et al (6)), the difference (5-15%) has little impact on the qualitative interpretation of the results although the quantitative interpretation will differ slightly.



Supplementary Figure 1: % difference between the estimate of MCR-I during OGTT estimated by the model (MCR_I-model) vs the Jung formula (MCR_I-Jung) calculated as (MCR_I-model - MCR_I-Jung)/ MCR_I-model

References

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