Appendix 1: Computational Model development

The model consisted of three processes:

1. The import of glucose from the gastrointestinal compartment into the blood with rate v_1;

2. The elimination of glucose from the plasma into the tissues and its subsequent storage or metabolism with rate v_2; and

3. The metabolism or storage of glucose before it enters into the plasma pool, indicated as loss, with rate v_L.

Rates v were expressed in $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

The rates of these processes were described by first-order kinetics, with the following rate equations for the tracer:

$$v_1(q_1) = k_1 \cdot q_1 \quad (1)$$

$$v_L(q_1) = k_L \cdot q_1 \quad (2)$$

$$v_2(c_2) = k_2 \cdot c_2 \cdot Vol \quad (3)$$

All rate constants k were expressed in min^{-1}. The conversion factor Vol in $mL \cdot kg^{-1}$ is the apparent distribution volume of the plasma compartment and was introduced to convert the concentration c_2 (mM) into a pool size ($\mu mol \cdot kg^{-1}$), such that the rate v_2 is also expressed in $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

Analogous rate equations for the unlabeled glucose were defined by:

$$v_1(Q_1) = k_1 \cdot Q_1 \quad (4)$$

$$v_L(Q_1) = k_L \cdot Q_1 \quad (5)$$

$$v_2(C_2) = k_2 \cdot C_2 \cdot Vol \quad (6)$$

Since tracer and unlabeled glucose exhibit chemically identical behavior, the rate constants k_1, k_2, and k_L were assumed to be identical for both and were derived from the tracer kinetics.

In addition, unlabeled glucose is produced mainly by the liver, but also by the kidney and intestine. This process was quantified by the time-dependent Endogenous Glucose Production, EGP*. Taken together, this led to the following set of ordinary differential equations (ODE):

$$\frac{dq_1}{dt} = -(k_1 + k_L) \cdot q_1 \quad (7)$$

$$\frac{dc_2}{dt} = \frac{k_1 \cdot q_1}{Vol} - k_2 \cdot c_2 \quad (8)$$

$$\frac{dQ_1}{dt} = -(k_1 + k_L) \cdot Q_1 \quad (9)$$

$$\frac{dC_2}{dt} = \frac{k_1 \cdot Q_1}{Vol} - k_2 \cdot C_2 + EGP^*(t) \quad (10)$$

Time t was expressed in min and EGP* in mM·min^{-1}. The asterisk in EGP* denotes that it differs from the more commonly used EGP in µmol·kg^{-1}·min^{-1}.

The analytical solution of ODEs 7 and 8 gives (derived in the Supp. Material S2):

$$c_2(t) = C \cdot \left(e^{-k_2 t} - e^{-k_a t}\right) \quad (11)$$

in which:

$$k_a = k_1 + k_L \quad (12)$$

The tracer time-course data were fitted to equation 11, yielding C, k_2 and k_a. Identifiability and constraints are addressed in the Supp. Material S3.

To estimate the apparent volume of distribution of the tracer (*Vol,* the plasma compartment in the model), the bioavailability F is required. F is defined as:

$$F = \frac{k_1}{k_1 + k_L} \quad (13)$$

It can be derived (Supp. Material S2) that:

$$Vol = \frac{q_1(0)}{C} \cdot \frac{k_a \cdot F}{k_a - k_2} = \frac{q_1(0)}{C} \cdot \frac{k_1}{k_a - k_2} \quad (14)$$

in which $q_1(0)$ represents the amount of tracer administered ($1.6 \cdot 10^3$ µmol·kg^{-1} in the present study).

The basal rate of disappearance for glucose (R_d^{OGTT}) in µmol·kg^{-1}·min^{-1} was calculated according to the equation below:

$$R_d^{OGTT} = k_2 \cdot C_2(0) \cdot Vol \quad (15)$$

EGP calculation

The endogenous glucose production EGP* in mM·min^{-1} was computed by rearranging equation 10 as follows:

$$EGP^*(t) = -\frac{k_1 \cdot Q_1(t)}{Vol} + \frac{dC_2(t)}{dt} + k_2 \cdot C_2(t) \quad (16)$$

The components of the EGP can be determined as follows. From equation 9 it is derived that:

$$Q_1(t) = Q_1(0) \cdot e^{-(k_1+k_L)t} = Q_1(0) \cdot e^{-k_a t} \quad (17)$$

in which $Q_1(0)$ is the amount of unlabeled glucose administered ($3.9 \cdot 10^3$ µmol·kg^{-1} in the present study). The compartment model presented here does not include transit compartments, since the absorption phase was too fast to capture more detail with our experimental setup.

Although k_1 and *Vol* in equation 16 depend on the choice of F (eq. 13-14), the ratio k_1/Vol does not and by rearranging equation 14, it can be written as:

$$\frac{k_1}{Vol} = \frac{(k_a - k_2) \cdot C}{q_1(0)} \quad (18)$$

Finally, $C_2(t)$ in equation 16 is obtained by fitting the time course of the unlabeled glucose concentration to:

$$C_2(t) = C_{0u} + C_{1u} \cdot e^{-k_{1u}t} + C_{2u} \cdot e^{-k_{2u}t} \quad (19)$$

in which u denotes 'unlabeled' and the parameters C_{0u}, C_{1u}, k_{1u}, C_{2u}, and k_{2u} are considered phenomenological constants without a direct relation to the underlying rate equations. Therefore, unique identifiability of the time parameters is of less relevance than a good fit of the data. Nevertheless, to aid the identification of biologically pertinent fits, k_{1u} was fixed at the same value obtained for k_a per animal based on tracer fits. Finally, taking the time derivative of equation 19 gives:

$$\frac{dC_2(t)}{dt} = -C_{1u} \cdot k_{1u} \cdot e^{-k_{1u}t} - C_{2u} \cdot k_{2u} \cdot e^{-k_{2u}t} \quad (20)$$

To compute the EGP* in mM·min^{-1}, equation 16 can be rewritten in full, which is independent of the bioavailability F:

$$EGP^*(t) = C_{1u} \cdot (k_2 - k_{1u}) \cdot e^{-k_{1u}\cdot t} - \frac{(k_a - k_2) \cdot C}{q_1(0)} \cdot Q_1(0) \cdot e^{-k_a \cdot t}$$

$$+C_{2u} \cdot (k_2 - k_{2u}) \cdot e^{-k_{2u} \cdot t} + k_2 \cdot C_{0u} \quad (21)$$

To convert EGP* (mM·min^{-1}) to EGP (µmol·kg^{-1}·min^{-1}), EGP* was multiplied by *Vol*, which is a function of the bioavailability *F* (equation 14).

Steady-state intravenous infusion (SS-IV)

Experiments were performed according to the protocol described by van Dijk et al. (1). Animals were fasted overnight for 9h. On the day of the experiment, animals were placed in cages without access to the RW. Animals were continuously infused with [U-^{13}C$_6$]-glucose for 6 hours, and blood glucose was sampled every hour. Glucose and tracer measurements were conducted as described above. Calculation of $R_d^{SS\text{-}IV}$ and $EGP^{SS\text{-}IV}$ was conducted as described below:

$$\frac{dc_2(SS\text{-}IV)}{dt} = \frac{v_{infusion} - v_{consumption}}{Vol} \quad (22)$$

$$v_{infusion} = R_{inf} \cdot M_I \quad (23)$$

$$v_{consumption} = R_d^{SS\text{-}IV} \cdot M_C \quad (24)$$

Where R_{inf} represents the glucose infusion rate in µmol·kg^{-1}·min^{-1}, M_I is the fractional abundance of tracer in the infusion solution, M_C is the fractional abundance of tracer measured in the circulation after correction for natural abundance, and $R_d^{SS\text{-}IV}$ is the steady-state rate of disappearance of total glucose in µmol·kg^{-1}·min^{-1}. At steady state:

$$\frac{dc_2(SS\text{-}IV)}{dt} = 0 \quad (25)$$

$$\therefore R_d^{SS\text{-}IV} = \frac{R_{inf} \cdot M_I}{M_C} \quad (26)$$

Following the same logic, the rates of appearance *($EGP^{SS\text{-}IV} + R_{inf}$)* and disappearance *($R_d^{SS\text{-}IV}$)* for total glucose (unlabeled + tracer) should be equal to each other at steady state, resulting in:

$$EGP^{SS\text{-}IV} = R_d^{SS\text{-}IV} - R_{inf} \quad (27)$$

in which all rates, including *$EGP^{SS\text{-}IV}$*, are in µmol·kg^{-1}·min^{-1}.

Insulin sensitivity indices

HOMA-IR was calculated according to (2):

$$HOMA\text{-}IR = \frac{C_2(0) \cdot INS(0)}{14.1} \quad (28)$$

in which $C_2(0)$ represents fasting glucose levels in mM, $INS(0)$ fasting insulin levels in mU·L^{-1}, and 14.1 the reference value for mice in mM· mU·L^{-1}.

Based on the OGTT data, the insulin sensitivity index for peripheral tissues (IS-P) was defined as:

$$IS\text{-}P = \frac{k_2}{INS_{OGTT}} \quad (29)$$

in which INS_{OGTT} is the average insulin level over all time points during the OGTT time frame, calculated for each animal separately.

The adapted liver insulin sensitivity (IS-L) was defined as:

$$IS\text{-}L = \frac{\overline{EGP} \times \overline{INS_{OGTT}}}{EGP \times INS_{OGTT}} \quad (30)$$

in which EGP is the time-averaged EGP from 5 to 120 min. The denominator represents a value calculated per specific group, whereas the numerator is the average EGP times average INS_{OGTT} (over all animals in all 16 experimental groups). With this, the mean was centered and a dimensionless index was obtained.

All indices were obtained by using the mean $C_2(0)$, k_2, EGP, $INS(0)$ and INS_{OGTT} values per experimental group. To obtain the distribution (SD and SEM) of the calculated indices, the distribution of the product or ratio of the means was calculated assuming normal distribution of the data.

References

1. van Dijk TH, Boer TS, Havinga R, Stellaard F, Kuipers F, Reijngoud D-J: Quantification of hepatic carbohydrate metabolism in conscious mice using serial blood and urine spots. Analytical Biochemistry 2003;322:1-13
2. van Dijk TH, Laskewitz AJ, Grefhorst A, Boer TS, Bloks VW, Kuipers F, Groen AK, Reijngoud DJ: A novel approach to monitor glucose metabolism using stable isotopically labelled glucose in longitudinal studies in mice. Lab Anim 2013;47:79-88