SUPPLEMENTARY DATA

Calculations of glucose kinetics and liver intermediate fluxes.

Efficiency of detritiation of [2-^{3}H[-G-6-P by exchange of [^{3}H] of [2-^{3}H]-G-6-P with [H^+] of bulk water mediated by hexose isomerase.

Assuming that glycogen synthesis occurred at a constant rate during the test period, then fractional detritiation of [2-^{3}H]-G-6-P ($D_{[2\text{-}3H]}$), which occurs by exchange of [^{3}H] of [2-^{3}H]-G-6-P with [H^+] of bulk water mediated by hexose isomerase should be stable during the test period and the ratio of [2-^{3}H] to [3-^{3}H]-glucose incorporated into glycogen would then approximate that of the G-6-P pool. The efficiency of detritiation was thereby calculated as the ratio of [2-^{3}H]-glucose to [3-^{3}H]-glucose incorporated into glycogen.

Incorporation of [2-^{3}H]- and [3-^{3}H]-glucose into hepatic and skeletal muscle glycogen

To estimate the amount of incorporated [2-^{3}H]- and [3-^{3}H]-glucose into hepatic and skeletal muscle glycogen (GLY-[2-^{3}H]-PG and GLY-[3-^{3}H]-PG, respectively) during the test period were calculated as follows:

$$\text{GLY-[2-}^3\text{H]-PG} = \frac{[2\text{-}^3\text{H}] \text{ radioactivity in hepatic glycogen (dpm/g liver)}}{([2\text{-}^3\text{H}]\text{SA-PG}_{30}+[2\text{-}^3\text{H}]\text{SA-PG}_{60}+[2\text{-}^3\text{H}]\text{SA-PG}_{90}+[2\text{-}^3\text{H}]\text{SA-PG}_{120}+[2\text{-}^3\text{H}]\text{SA-PG}_{150}+[2\text{-}^3\text{H}]\text{SA-PG}_{180})/6}$$

$$\text{GLY-[3-}^3\text{H]-PG} = \frac{[3\text{-}^3\text{H}] \text{ radioactivity in hepatic glycogen (dpm/g liver)}}{([3\text{-}^3\text{H}]\text{SA-PG}_{30}+[3\text{-}^3\text{H}]\text{SA-PG}_{60}+[3\text{-}^3\text{H}]\text{SA-PG}_{90}+[3\text{-}^3\text{H}]\text{SA-PG}_{120}+[3\text{-}^3\text{H}]\text{SA-PG}_{150}+[3\text{-}^3\text{H}]\text{SA-PG}_{180})/6}$$

Where $([2\text{-}^3\text{H}]\text{SA-PG}_{30}+[2\text{-}^3\text{H}]\text{SA-PG}_{60}+[2\text{-}^3\text{H}]\text{SA-PG}_{90}+[2\text{-}^3\text{H}]\text{SA-PG}_{120}+[2\text{-}^3\text{H}]\text{SA-PG}_{150}+[2\text{-}^3\text{H}]\text{SA-PG}_{180})/6$ and $([3\text{-}^3\text{H}]\text{SA-PG}_{30}+[3\text{-}^3\text{H}]\text{SA-PG}_{60}+[3\text{-}^3\text{H}]\text{SA-PG}_{90}+[3\text{-}^3\text{H}]\text{SA-PG}_{120}+[3\text{-}^3\text{H}]\text{SA-PG}_{150}+[3\text{-}^3\text{H}]\text{SA-PG}_{180})/6$ are the averages of [2-^{3}H]SA-PG and [3-^{3}H]SA-PG of plasma glucose at 30, 60, 90, 120, 150 and 180 min, respectively. The amount of incorporated [2-^{3}H]- and [3-^{3}H]-glucose were measured at the end of the clamp period.

A fractional detritiation of [2-^{3}H]-G-6-P ($D_{[2\text{-}3H]}$) by exchange of [^{3}H] of [2-^{3}H]-G-6-P with [H^+] of bulk water mediated by hexose isomerase was calculated as

$$D_{[2\text{-}3H]} = \frac{\text{GLY-[2-}^3\text{H]-glucose}}{\text{GLY-[3-}^3\text{H]-glucose}}$$

This calculation is based on the assumption that the ratio of [2-^{3}H] to [3-^{3}H]-glucose incorporated into glycogen approximates that of the G-6-P pool.

Fluxes toward G-6-P from plasma glucose, gluconeogenic precursors and others (glycogen and glycerol).

This method is based on two assumptions 1) the label profile of UDPG is reflective that of G-6-P given rapid equilibrium between G-6-P and G-1-P, which is the sole source of UDPG and 2) a steady flux of infused [U-^{14}C] alanine through the gluconeogenic pathway (from PEP to G-6-P) is established before making measurements. The incorporation of [^{14}C] into plasma glucose and liver glycogen needs to be minimized, because [^{14}C] incorporation into G-6-P from plasma glucose by glucose cycling and into G-1-P from glycogen by the glycogen cycle may cause overestimates of contributions from the gluconeogenic pathway to glucose production and glycogen synthesis. In our current study, [U-^{14}C] alanine was infused for 10 min prior to sampling liver. During these 10 min, the [^{14}C]-labeled glucose in plasma was increased progressively and at the end of 10 min, a substantial incorporation of [^{14}C] was found in liver glycogen (data not shown). Further, [^{14}C]SA of UDP-galactose, N-acetyl-glucosamine and

N-acetyl-galactosamine were similar with that of UDPG. These observations imply that a steady flux of [^{14}C] from PEP to G-6-P was established.

The percent contribution of plasma glucose to form UDPG via the direct pathway (UDPG-PG) was calculated as

$$\text{UDPG-PG} = \frac{[^3\text{H}]\text{SA-UDPG} \times [\text{GLY-}[3\text{-}^3\text{H}]\text{-PG}/(\text{GLY-}[2\text{-}^3\text{H}]\text{-PG} + \text{GLY-}[3\text{-}^3\text{H}]\text{-PG})}{[3\text{-}^3\text{H}]\text{SA-PG}_{180}}$$

Where [^{3}H]SA-UDPG × [GLY-[3-^{3}H]-PG/(GLY-[2-^{3}H]-PG + GLY-[3-^{3}H]-PG) is [3-^{3}H]SA of UDPG.

The percent contribution of PEP to form UDPG (UDPG-PEP) were calculated as the ratio of [^{14}C]SA in hepatic UDPG to [^{14}C]SA in hepatic PEP:

$$\text{UDPG-PEP} = \frac{[^{14}\text{C}]\text{SA-UDPG} - [^{14}\text{C}]\text{SA-PG}_{180} \times \text{UDPG-PG}}{2 \times [^{14}\text{C}]\text{SA-PEP}}$$

Where [^{14}C]SA-PG$_{180}$ × UDPG-PG is the fraction of [^{14}C]SA-UDPG from [^{14}C]-plasma glucose.

Rates of glucose appearance, glucose disappearance and endogenous glucose production

Rates of [2-^{3}H]- and [3-^{3}H]-glucose determined unlabeled glucose appearance ([2-^{3}H]Ra and [3-^{3}H]Ra) were calculated using Steele's equation (1) which is based on a one-pool model and on the initial assumption of instant mixing of glucose in its entire space. Proietto et al. (2) reported that the one-pool model gave the best results given the effective volume of distribution of glucose was 50% of glucose distribution volume in obese *fa/fa* rats and their lean littermates. It can be written as follows:

$$[2\text{-}^3\text{H}]\text{Ra} = \frac{[2\text{-}^3\text{H}]\text{GI*} - \{0.5 \cdot \text{V}_\text{D} \cdot (\text{PG}_1 + \text{PG}_2)/2 \cdot ([2\text{-}^3\text{H}]\text{SA-PG}_2 - [2\text{-}^3\text{H}]\text{SA-PG}_1)/(t_2 - t_1)\}}{([2\text{-}^3\text{H}]\text{SA-PG}_2 + [2\text{-}^3\text{H}]\text{SA-PG}_1)/2}$$

$$[3\text{-}^3\text{H}]\text{Ra} = \frac{[3\text{-}^3\text{H}]\text{GI*} - \{0.5 \cdot \text{V}_\text{D} \cdot (\text{PG}_1 + \text{PG}_2)/2 \cdot ([3\text{-}^3\text{H}]\text{SA-PG}_2 - [3\text{-}^3\text{H}]\text{SA-PG}_1)/(t_2 - t_1)\}}{([3\text{-}^3\text{H}]\text{SA-PG}_2 + [3\text{-}^3\text{H}]\text{SA-PG}_1)/2}$$

where [2-^{3}H]GI* and [3-^{3}H]GI* equal the infusion rate of [2-^{3}H]-glucose and [3-^{3}H]-glucose, respectively, PG$_1$ and PG$_2$ equal plasma glucose concentration at time t_1 and t_2, [2-^{3}H]SA-PG$_1$ and [3-^{3}H]SA-PG$_2$ equal the [2-^{3}H] and [3-^{3}H] specific activities of plasma glucose at times t_1 and t_2. V$_D$ is glucose distribution volume (ml/kg) and {0.5·V$_D$·(PG$_1$ + PG$_2$)/2} is the effective fraction of glucose pool.

SUPPLEMENTARY DATA

Rates of [2-^{3}H]- and [3-^{3}H]-glucose determined unlabelled glucose disappearance ([2-^{3}H]Rd and [3-^{3}H]Rd, respectively) according to Steele's equation (1):

$$[2\text{-}^3\text{H}]\text{Rd} = [2\text{-}^3\text{H}]\text{Ra} - 0.5 \cdot V_D \cdot [(PG_2 - PG_1)/(t_2 - t_1)]$$

$$[3\text{-}^3\text{H}]\text{Rd} = [3\text{-}^3\text{H}]\text{Ra} - 0.5 \cdot V_D \cdot [(PG_2 - PG_1)/(t_2 - t_1)]$$

Endogenous glucose production rate was determined as the difference between [3-^{3}H]Ra and exogenous glucose infusion rates.

Glucose cycling.

Glucose cycling is defined as input of extracellular glucose into the G-6-P pool followed by exit of plasma-derived G-6-P back into the extracellular pool. Assuming 100% exchange of $D_{[2\text{-}3H]}$, the minimum estimation of unlabelled glucose cycling was assessed as the difference between [2-^{3}H]Rd and [3-^{3}H]Rd.

The maximum estimation of unlabelled glucose cycling was assessed by dividing the minimal estimation by the efficiency of detritiation of [2-^{3}H] G-6-P ($D_{[2\text{-}3H]}$).

Glucokinase flux.

Glucokinase flux was estimated as the sum of glucose cycling rate and the rate of incorporation of plasma glucose into glycogen via the direct pathway. This calculation might underestimate glucokinase flux due to the possibility that a portion of phosphorylated glucose was utilized in glycolytic and pentose phosphate pathways.

Glucose-6-phosphatase flux.

The *in viv*o flux through glucose-6-phosphatase (G-6-Pase) was assessed as the sum of glucose cycling and endogenous glucose production rates.

Gluconeogenesis was calculated as

$$\text{G-6-Pase Flux-PEP} = \text{G-6-Pase flux} \times \text{UDPG-PEP}$$

Glucose production from glycogen was calculated as

$$\text{G-6-Pase flux-Gly} = \text{G-6-Pase flux} \times [1 - (\text{UDPG-PG} + \text{UDPG-PEP})]$$

Rates of glycogen synthesis via the direct and indirect pathways.

The amount of newly synthesized glycogen from plasma glucose via the direct pathway (GLY[3-^{3}H]: glucose $\rightarrow$ G-6-P $\rightarrow$ glycogen) during the test period was calculated as the division of GLY-[3-^{3}H]-PG by the test period (180 min).

Total amount of newly synthesized glycogen (GLY) during the study in the liver was measured as

$$\text{GLY} = \text{GLY-}[3\text{-}^3\text{H}]\text{-PG/UDPG-PG}.$$

SUPPLEMENTARY DATA

Estimation of percent contribution of gluconeogenic precursors to glucose production and glycogen synthesis was based on the percent contribution of plasma glucose via the direct pathway and phosphoenolpyruvate to form UDPG.

The amount of newly synthesized glycogen from PEP (GLY-PEP) was calculated as

$$\text{GLY-PEP} = \text{GLY} \times \text{UDPG-PEP}$$

The glycogen cycling rate was calculated as

$$\text{GLY-Gly} = \text{GLY} \times [1 - (\text{UDPG-PG} + \text{UDPG-PEP})]$$

While there was substantial glucose cycling in both ZCL and all groups of ZDF, the portion of [^{14}C] in UDPG derived from glucose was calculated using the ratio of [^{14}C]-glucose to [3-^{3}H]-glucose in plasma and [3-^{3}H] in UDPG. The extent of glycogen cycling with the whole glycogen pool has been reported in various ranges (3; 4). If glycogen cycling occurs with the whole glycogen pool, radio-labeled G-1-P/UDPG pool could be diluted by cold G-1-P generated from non-labeled glycogen. In our current study, >95% of UDPG was labeled with [3-^{3}H] or [^{14}C], suggesting that glycogen cycling with the whole glycogen pool low. Muller et al. (5) showed that only a minor portion of hepatic glycogen was actually participating in glycogen turnover in sedentary obese subjects. They postulated that only a peripheral portion of the glycogen molecules incorporated a labeled glycosyl unit and that this same portion is the likely source of any glucose released during the experiments. If true for our study, then glycogen cycling would generate through glycogenolysis a portion of [3-^{3}H]- and [^{14}C]-UDPG and contribute toward an overestimate of both glucose phosphorylation and gluconeogenesis to G-6-P formation with underestimation of glycogenolysis. If this error occurred, it was not corrected, because the true extent of cycling of glycogen could not be assessed in our current study.

During hyperinsulinemic hyperglycemic clamps in ZCL rats, we obtained a negative value for endogenous glucose production and as a result, a lower rate of G-6-Pase flux relative to glucose cycling. The discrepancy is due to the paradox that tracer-derived glucose disposal rates are less than the exogenous glucose infusion rates. The tracer-derived glucose disposal rate can be equal to but never less than the exogenous glucose infusion rate because the tracer-derived glucose disposal rate represents total glucose flux. This discrepancy, however, has been reported by numerous investigators and the potential mechanism was extensively discussed by Argoud GM et al. (6) and Bell PM et al. (7). The precise mechanism has yet to be determined. If the paradox occurs with [3-^{3}H]-glucose but not with [2-^{3}H]-glucose, then the glucose cycling rate calculated as the difference between [2-^{3}H]Rd and [3-^{3}H]Rd would be over-estimated.

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