Glucose Production, Gluconeogenesis, and Insulin Sensitivity in Children and Adolescents: An Evaluation of Their Reproducibility

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ABSTRACT

The prevalence of overweight and obese children has doubled, and the incidence of type 2 diabetes in children (0–19 y) has increased 4-fold during the past several decades. As a result we can anticipate an increased number of metabolic studies in children. There are few data on measures of glucose metabolism in normal children, and virtually none relating to their reproducibility. The aims of this study were 1) to provide new data on energy expenditure and glucose, lipid, and protein metabolism in nonobese, healthy children and adolescents; 2) to evaluate their reproducibility; and 3) on the basis of these data, to perform power calculations for metabolic studies. Eight nonobese subjects (8–16 y) were studied on two occasions, preceded by 7 d of a diet with identical energy content and macronutrient distribution. Gluconeogenesis, measured by deuterium oxide, accounted for 50% of glucose production. Insulin sensitivity, measured by the labeled minimal model, averaged 4.9×10^{-4} mL(mU·min)⁻¹. Glucose appearance rate was significantly higher ($p < 0.01$) in the children than in the adolescents. Furthermore, we demonstrated that for energy intake and expenditure, plasma concentrations of glucose and C-peptide, and rates of appearance of glucose and leucine, a 10% difference can be

detected in fewer than five subjects with a power of 80% and a type I error of 5%. Insulin concentration, gluconeogenesis, insulin secretory indices, insulin sensitivity, and glucose effectiveness were more variable, but with the above power a difference of 25% could be detected in 7–11 subjects using a paired study design. (*Pediatr Res* 50: 115–123, 2001)

Abbreviations

BMI, body mass index
BMR, basal metabolic rate
CV, coefficient of variation
GC, gas chromatography
GCMS, gas chromatography mass spectrometry
GPR, glucose production rate
HMT, hexamethylenetetramine
IVGTT, intravenous glucose tolerance test
KIC, α -ketoisocaproic acid
RQ, respiratory quotient
Ra, rate of appearance
S_I, insulin sensitivity
S_G, glucose effectiveness

For the past several decades, we have experienced a dramatic increase in the incidence of obesity, insulin resistance, and type 2 diabetes in children and adolescents in the United States. The prevalence of overweight children (BMI > 85th percentile) has increased from approximately 15% to 22% and the prevalence of obesity (BMI > 95th percentile) from 5% to 11% (1). Concomitant with this increase in weight, the inci-

dence of type 2 diabetes in a pediatric population (birth to age 19 y) increased from approximately 4% before 1992 to approximately 16% in 1994 (2). It has been recently estimated that 30% of the individuals presenting with diabetes in the second decade of life have type 2 diabetes (3). As a result of these frightening trends, we can anticipate an increased number of metabolic studies conducted in children and adolescents.

A variety of tools and techniques have been developed during the past decades to evaluate a wide spectrum of measures of glucose metabolism (4–11). These tools will become of increasing importance as clinical research is focused on the etiology and pathogenesis of obesity and type 2 diabetes. Because of the difficulty in studying normal children for technical and ethical reasons, few measurements of GPR and other metabolic variables in normal children have been used (4), and there are virtually no studies relating to the reproducibility of

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such measures in children. Although gluconeogenesis has been estimated in premature infants and adults (6, 8, 12–15), no information is available about the fraction of glucose production derived from gluconeogenesis in children. The majority of studies on S_I in children has been conducted using the unlabeled minimal model IVGTT or the euglycemic hyperinsulinemic clamp technique (16–20). The stable, labeled IVGTT (9) is more informative than the unlabeled IVGTT in that it distinguishes between the effect of insulin on hepatic glucose production and peripheral glucose uptake. However, we are aware of only one study using the labeled minimal model in children (21).

In designing research protocols, it is essential to make a power calculation to estimate the number of subjects needed for a particular study. The present study was conducted to obtain intra- and intersubject reproducibility measures on energy expenditure, glucose production, gluconeogenesis, insulin secretion, S_I , S_G , lipolysis, and proteolysis in children and adolescents for just such a purpose.

METHODS

Subjects

The protocol was approved by the Institutional Review Board for Human Subject Research for Baylor College of Medicine and affiliated hospitals. The subjects were recruited by local advertisement. Attempts were made to recruit from all ethnic groups. Several potential subjects were excluded on the basis of body fat criteria, resulting in the ultimate recruitment of six white, one African-American, and one Asian subjects. All children signed assent and their parents, informed consent. Eight children, four prepubertal (Tanner pubertal stage 1) (22) and four postpubertal (Tanner pubertal stage 5) (22), two boys and two girls in each group, were studied on two occasions (Table 1). We did not include any early or mid-pubertal children, because puberty is associated with decreased S_I . Thus, advancing puberty would have confounded our ability to compare data over time and among individuals. All children were healthy as determined by a standard medical history, a physical examination, and standard blood chemistry. They were all selected for normal weight-for-height (23) and for normal body composition, defined as <28% body fat determined by dual-energy x-ray absorptiometry (Hologic QDR 2000, Hologic, Inc., Waltham, MA, U.S.A.) (Table 1) and a BMI between 18 and 26 kg/m² (24). The dual-energy x-ray

absorptiometry allows for analysis of total and regional lean tissue mass, bone mineral content, and fat mass. Fat-free mass is defined as the sum of lean tissue mass and bone mineral content. Subjects were excluded if they had obese parents (BMI > 28 kg/m²) or a first-degree relative with diabetes.

Study Design

The children were admitted to the metabolic research unit at the Children's Nutrition Research Center for 2 d on two occasions separated by 2–8 wk. For the 7 d before each study occasion, the children received a constant diet of known composition, 60% carbohydrate, 15% protein, and 25% fat (Table 2). The total energy intake and distribution of energy from carbohydrate, fat, and protein were analyzed using the Minnesota Database System (version 2.8 NDS, Minneapolis, MN, U.S.A.). According to reported values of physical activity, total energy intake for each individual subject was set as a multiple of 1.7 to 2.0 × BMR, predicted from weight and age according to Schofield (25). Three meals and two snacks per day were weighed, prepacked, and sent to the subject's home by the research kitchen. Unconsumed food was returned to the kitchen and examined for constituents, and the energy and macronutrient composition of the consumed food was calculated by difference (Table 2). A dietitian was in daily telephone contact with the families to evaluate the children's compliance with the diet.

Following the 7-d diet period at home, the subjects were admitted to the metabolic research unit. In the afternoon of the day of admission (d 1), the subjects were placed in a room calorimeter to assess 24-h energy expenditure. The calorimeter design characteristics have been previously described in detail (26). Three electrodes were applied to the child's skin, and the heart rate was recorded by telemetry at 1-min intervals (DS-3000, Fukuda Denshi, Tokyo, Japan). Two 20-min exercise sessions were conducted on stationary bicycles (CombiCycle Ex80, COBI, Co, Ltd, Tokyo, Japan) at workloads approximating 40 and 60% of the child's peak oxygen consumption. After fasting overnight (12 h), the subject was awakened at 0630 h (on d 2), asked to void, and returned to sleep. The child was again awakened about 30 min later, and after confirmed awake, BMR was measured for 40 min, beginning at 0720 h. The child was monitored both visually and by the activity sensor to confirm that he or she was lying still (<50 counts) for the entire measurement period. BMR, sleeping metabolic rate,

Table 1. Subjects

Subject	Age	Sex	Tanner stage	Weight (kg)	Height (cm)	DXA % fat
1	9	F	1	27.0	127.6	20.8
2	9	F	1	27.2	139.5	15.8
3	8	M	1	36.7	140.7	24.5
4	9	M	1	36.4	140.5	23.9
5	16	M	5	70.0	176.6	6.6
6	14	F	5	46.8	160.8	23.0
7	15	F	5	64.2	171.7	18.1
8	16	M	5	64.5	172.7	12.1
Mean ± SEM	12 ± 2			46.7 ± 6.2	153.8 ± 6.7	18.1 ± 2.2

DXA, dual-energy x-ray absorptiometry.

and 24-h energy expenditure were calculated using the de Weir equation (27).

During the calorimeter tests, the children were fed a diet of equal composition as during the week preceding the study, except the total energy content was decreased to 1.4 to 1.5 $\times$ BMR to adjust for a lower activity level (Table 2). Net fat and carbohydrate utilization was computed using 24-h excretion rates of urinary nitrogen. After completion of the calorimeter test (in the afternoon on study d 2), the subjects were served dinner at 1700 h and a snack at 2000 h and were subsequently fasted until termination of the study on d 3. Subjects were allowed water *ad libitum* throughout the study. In the evening on study d 2, two i.v. catheters were placed under Emla cream analgesia, one in an antecubital vein for infusion and one in the opposite antecubital vein or a vein on the opposite hand for blood sampling.

Tracer Infusions

Tracers. Deuterium oxide (99% atom % ^2H), [$^2\text{H}_5$]glycerol (99% atom % [^2H]; 93.5% [$^2\text{H}_5$]), [$1\text{-}^{13}\text{C}$]glucose (99% atom % [^{13}C]); [6,6- $^2\text{H}_2$]glucose (99% [^2H]; 98% [$^2\text{H}_2$]), and [$^2\text{H}_3$]leucine (99% [^2H]; 97% [$^2\text{H}_3$]) were purchased from Cambridge Isotope Laboratories (Andover, MA, U.S.A.). The isotopes were tested for sterility and pyrogenicity by the investigation pharmacy at Texas Children's Hospital. The infusates were filtered through a Gelman (Pall Gelman Laboratory, Ann Arbor, MI, U.S.A.) syringe filter (2 μm) and were stored at 4°C for 24–48 h before administration.

On study d 2, the subjects received a total of 3 g/kg of deuterium oxide, administered in five doses at 1400 h, 1600 h, 1800 h, 2000 h, and 2200 h, to measure gluconeogenesis. On study d 3, between 0600 h and 1300 h, the subjects received a simultaneous, primed (60 $\times$ the minute infusion rate), constant rate i.v. infusion of [$1\text{-}^{13}\text{C}$]glucose, [$^2\text{H}_5$]glycerol, and [$^2\text{H}_3$]leucine. The [$1\text{-}^{13}\text{C}$]glucose was infused at a rate of $0.66 \pm 0 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (mean $\pm$ SEM) in prepubertal children and $0.33 \pm 0 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in adolescents, [$^2\text{H}_5$]glycerol

was infused at $0.29 \pm 0.01 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $0.14 \pm 0 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in the two groups, respectively, and leucine at $0.06 \pm 0 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in both groups. At 0900 h, on study d 3 (after the 0-min blood sample, see below), a bolus injection of glucose, $0.30 \pm 0 \text{ g/kg}$ containing 10% [6,6- $^2\text{H}_2$]glucose, was administered for 90–120 s.

Blood sampling. Blood samples (2.5 mL each) were obtained at the following times: 1) at screening, *i.e.* weeks before any tracer infusions (prebaseline); 2) before start of the tracer infusions, *i.e.* 7 h after the last dose of deuterium oxide (baseline); 3) every 10 min between 2.5 and 3 h after start of the tracer infusions; and 4) beginning exactly with the completion of the bolus injection (0 min) and thereafter at 2, 3, 4, 5, 8, 10, 18, 20, 28, 32, 40, 60, 120, 180, and 240 min (9), *i.e.* a total of 20 blood samples of 2.5 mL, resulting in a blood loss of 50 mL per study occasion. The samples were analyzed as described in detail below.

Analyses

Plasma glucose concentrations were measured by a glucose analyzer (YSI 2300 Stat plus, Yellow Springs Instrument Co Inc., Yellow Springs, OH, U.S.A.). Plasma insulin and C-peptide concentrations were analyzed using commercially available RIA kits (Linco, St. Charles, MO, U.S.A.). The detection limit for this RIA is 0.2 $\mu\text{U/mL}$ for insulin and 0.1 ng/mL for C-peptide.

Plasma Isotopic Enrichments

The pentaacetate derivative of glucose was prepared as previously described (28, 29), and the [$1\text{-}^{13}\text{C}$]glucose enrichment was analyzed by GC-combustion-isotope ratio mass spectrometry using an HP5890 GC (GC column: DB 1701, 25 m $\times$ 0.25 mm $\times$ 0.25 μm ; J&W Scientific Inc., Folsom, CA, U.S.A.) combined with an Orchid combustion oven and a 20–20 analyzer from Europa Scientific (Franklin, OH, U.S.A.). In addition, the (M+1) and (M+2) isotopomers of glucose were analyzed by GCMS (HP5890/5989B; GC column DB 1701, 25 m $\times$ 0.25 mm $\times$ 0.25 μm) using the electron impact mode and a fragment from which glucose carbon-1 is cleaved. Thus, the isotopic contribution from [$1\text{-}^{13}\text{C}$]glucose is minimized. The ions monitored were 242, representing unlabeled glucose, 243, representing glucose derived from deuterium oxide, and 244, derived from 1) the [6,6- $^2\text{H}_2$]glucose tracer, 2) [$^2\text{H}_5$]glycerol *via* gluconeogenesis, and 3) deuterium oxide. One hydrogen/deuterium atom is lost in the conversion of glycerol-3-phosphate to dihydroxyacetone phosphate, a second in the formation of fructose-1,6-diphosphate from glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, and a third in the conversion of fructose-6-phosphate to glucose-6-phosphate. Therefore, the [$^2\text{H}_5$]glycerol tracer results in an (M+2) isotopomer of glucose when glycerol is converted to glucose *via* the gluconeogenic pathway.

The ^2H -enrichment in glycerol was measured using a modification of the previously described triacetate derivative (28, 29), substituting propionic anhydride for acetic anhydride. An internal standard ([2- ^{13}C]glycerol) (99 atom % ^{13}C ; Cambridge Isotope Laboratories) was added to the plasma samples to

Table 2. Energy intake and distribution at home (EI_H) and in the calorimeter (EI_C), energy expenditure in the calorimeter (EE_C), substrate utilization, nonprotein RQ, and BMR*

	Study 1	Study 2
EI_H (EI_C) (kcal/d)	2252 $\pm$ 255 (1864 $\pm$ 160)	2311 $\pm$ 246 (1857 $\pm$ 156)
Energy distribution % of EI_H (% of EI_C)		
Protein	15.2 $\pm$ 0.3 (15.2 $\pm$ 0.3)	15.4 $\pm$ 0.3 (15.6 $\pm$ 0.2)
Fat	24.9 $\pm$ 0.3 (25.3 $\pm$ 0.4)	25.0 $\pm$ 0.4 (25.6 $\pm$ 0.5)
Carbohydrate	61.7 $\pm$ 0.6 (60.8 $\pm$ 0.6)	61.4 $\pm$ 0.5 (59.9 $\pm$ 0.5)
EE_C (kcal/d)	1835 $\pm$ 145	1878 $\pm$ 160
Substrate utilization % of EE_C		
Protein	19.0 $\pm$ 1.5	18.7 $\pm$ 1.2
Fat	28.2 $\pm$ 2.9	30.7 $\pm$ 2.0
Carbohydrate	65.0 $\pm$ 3.7	62.1 $\pm$ 2.6
Nonprotein RQ	0.89 $\pm$ 0.01	0.88 $\pm$ 0.01
BMR (kcal/min)	0.89 $\pm$ 0.07	0.91 $\pm$ 0.07

* Values are mean $\pm$ SEM.

No comparison between study 1 and 2 was significantly different.

measure glycerol concentration using reverse isotope dilution. The ^2H - and ^{13}C -glycerol enrichments were analyzed by GCMS (HP6890/5973; GC column, SP 17: 25 m $\times$ 0.25 m $\times$ 0.25 μm ; Supelco, Bellefonte, PA, U.S.A.) in the electron impact mode by selective monitoring of m/z 173, unlabeled glycerol; m/z 174, representing the internal standard; and m/z 176, representing $^2\text{H}_3$ -enriched glycerol as a result of the loss of carbon 1 with its attachments during the fragmentation in the mass spectrometer. Leucine turnover was calculated from the $[\text{H}_3]$ enrichment of KIC (the intracellular metabolite of leucine). The oxime-terbutyldimethylsilyl derivative of KIC was prepared, and the $[\text{H}_3]$ KIC enrichment was measured by GCMS (HP6890/5973; GC column, SP 1701: 25 m $\times$ 0.25 m $\times$ 0.25 μm ; Supelco), using the electron impact mode with selective monitoring of m/z 316, unlabeled KIC; 319, $[\text{H}_3]$ -labeled KIC; and 323, representing the internal standard $[\text{H}_7]$ KIC (used to measure plasma concentrations of KIC).

Deuterium incorporation at carbon 6 of glucose after ingestion of deuterium oxide was determined using the HMT derivative as described by Kalhan *et al.* (30). The Hickman still (Weaton, Millville, NJ, U.S.A.) was, however, modified by a glass blower, who enlarged the middle part of the neck of the still. This modification prevents the solution to be distilled from "bumping" into the collection portion of the still and enables us to successfully distill approximately 20 samples simultaneously. The deuterium enrichment in HMT was analyzed by GCMS (HP6890/5973; GC column, HP 5: 25 m $\times$ 0.25 mm $\times$ 1.0 μm , Hewlett Packard Co, Palo Alto, CA, U.S.A.) in the electron impact mode with selected monitoring of m/z 140 and 141. HMT enrichments were converted to the corresponding glucose enrichments using a standard curve prepared from $[1\text{-}^2\text{H}_1]\text{glucose}$ (99 atom % ^2H , Cambridge Isotope Laboratories) after conversion to sorbitol (31). Deuterium enrichment in body water (represented by plasma water) was measured by isotope ratio mass spectrometry (Finnigan Delta-E, Finnigan MAT, San Jose, CA, U.S.A.) after reduction to hydrogen gas according to accepted methods (10, 32). The potential influence of the $[1\text{-}^{13}\text{C}]\text{glucose}$ tracer on the measurements of gluconeogenesis was evaluated in five additional adolescents (14.0 ± 0.5 y; 62.5 ± 3.5 kg; 169.1 ± 1.2 cm; 21.9 ± 1.3 kg/m 2 ; $16.1 \pm 1.4\%$ body fat). These subjects were studied twice. On one occasion they received $[1\text{-}^{13}\text{C}]\text{glucose}$ alone and on the other both $[1\text{-}^{13}\text{C}]\text{glucose}$ and deuterium oxide. The HMT was prepared from the samples obtained at the two study occasions, and fractional gluconeogenesis was calculated (30). The results show that the $[1\text{-}^{13}\text{C}]\text{glucose}$ contribution to the (M+1) enrichment of HMT resulted in an overestimation of gluconeogenesis by $2.9 \pm 0.7\%$ (range, 1.2–5.1%). We consider this error to be clinically insignificant.

Calculations

During the period after 2.5 to 3 h of isotope infusion, approximate steady state (defined by a CV of $\leq 10\%$ with a slope not different from 0) was achieved for plasma concentrations and isotopic enrichments of the substrates analyzed (steady-state measurement period). Substrate appearance rates were calculated from the average enrichment obtained for each

substrate during this period using conventional tracer dilution techniques (33, 34). Thus, plasma glucose Ra were calculated from the ^{13}C enrichments of glucose, plasma glycerol Ra from the $[\text{H}_3]\text{glycerol}$ enrichment (12), and plasma leucine Ra from the $[\text{H}_3]\text{KIC}$ enrichment (12). Plasma concentrations of glycerol and KIC were calculated from the ion current ratio 174/173 and 323/316, respectively, using reverse isotope dilution.

Gluconeogenesis as a fraction of glucose Ra (equivalent to GPR) was calculated from the mean of the deuterium enrichments at the sixth carbon of glucose during the steady-state measurement period according to Kalhan *et al.* (30).

The gluconeogenic contribution from glycerol was calculated from the average fractions of the (M+2) isotopomer of glucose obtained during the steady-state measurement period after subtracting the fraction of (M+2) of glucose measured in the baseline sample (representing the contribution from deuterium oxide). This estimate represents the minimal gluconeogenic contribution from glycerol. The maximal contribution would correspond to $1/2 \times \text{glycerol Ra}$ assuming that all glycerol generated *via* lipolysis was converted to glucose. S_1 and S_1^* (the sensitivity of glucose disposition to insulin) and S_G and S_G^* (the effect of glucose *per se* on its own disposition at basal insulin concentrations) were calculated both using the unlabeled minimal model (11) based on glucose and insulin concentrations of the samples obtained after the glucose bolus, and the labeled minimal model (35) based on tracer, *i.e.* exogenous glucose, and insulin data, respectively. We define, as in previous work (36–38), tracer as the exogenous glucose (g), tracee as the endogenous glucose (G_{end}), and tracer to tracee ratio (ttr) as their ratio. Tracer glucose for the minimal model identification can be easily derived from total glucose measurements (G) and ttr, as from

$$G = G_{\text{end}} + g \quad (1)$$

and

$$\text{ttr} = \frac{g}{G_{\text{end}}} \quad (2)$$

it follows that

$$g = G \frac{\text{ttr}}{(1 + \text{ttr})} \quad (3)$$

To calculate ttr during a stable labeled IVGTT, in Cobelli *et al.* (38), we used the formula derived in Cobelli *et al.* (36, 37), based on isotope ratios $r^{(2)}$ (*i.e.* the ratio between $[6,6\text{-}^2\text{H}_2]\text{glucose}$ and $[6,6\text{-}^1\text{H}_2]\text{glucose}$) and $r^{(1)}$ (*i.e.* the ratio between $[6\text{-}^2\text{H}_1, 6\text{-}^1\text{H}_1]\text{glucose}$ and $[6,6\text{-}^1\text{H}_2]\text{glucose}$). Here, a different approach is used, which combines the relation between ttr and isotope ratios used in Cobelli *et al.* (38) with the relation between isotope ratios and the original mass spectrometry measurements, *i.e.* ion current ratios, to express ttr directly from ion current ratios:

$$\text{ttr} = \frac{(p^{(2)} - p_{\text{ss}}^{(2)})}{(p_i^{(2)} - p^{(2)})} \times \frac{(1 + r_i^{(1)} + r_i^{(2)})}{(1 + r_n^{(1)} + r_n^{(2)})} \quad (4)$$

In Eq. 4, $p^{(2)}$ is the ion current ratio 244/242 in a sample taken during the experiment. To eliminate interference from any (M+2) glucose isotopomer generated by deuterium oxide and $[^2\text{H}_5]\text{glycerol}$, $p_{ss}^{(2)}$ is represented by the average value of the ion current ratio 244/242 obtained during the steady-state measurement period. The error introduced by using this value for $p_{ss}^{(2)}$ was evaluated in the five additional adolescents who received deuterium oxide and $[^2\text{H}_5]\text{glycerol}$ as described above in the study design, in which the labeled glucose bolus was replaced by an unlabeled glucose bolus. This enabled us to follow the course of the (M+2) of glucose during the IVGTT. During the period 2–8 min after infusion of the IVGTT bolus, the concentrations of glucose and insulin are at their peak values. As a consequence, during this period, the suppression of glucose production and gluconeogenesis should reach a maximum, and the (M+2) of glucose derived from deuterium oxide or $[^2\text{H}_5]\text{glycerol}$ via the gluconeogenic pathway should reach its lowest value. The results from our control study demonstrate that during the period 2–8 min after the glucose bolus, the (M+2) of glucose was lower than that obtained during the steady-state period immediately preceding the IVGTT glucose bolus. During the remaining 232 min of the IVGTT sampling period, the (M+2) was similar to that obtained during the steady-state period. Thus, using the steady-state value for $p_{ss}^{(2)}$ would underestimate 244/242 by at most 2.5% during the 2- to 8-min period after the IVGTT bolus. An error of this magnitude would not significantly interfere with the calculations of S_I and S_G . $p_i^{(2)}$ is the ion current ratio 244/242 in the tracer; $r_n^{(1)}$ and $r_n^{(2)}$ are tracee isotope ratios to be evaluated theoretically, from knowledge of the natural abundance (for $[6,6-^2\text{H}_2]\text{glucose}$, $r_n^{(1)} = 0.0003$; $r_n^{(2)}$ is negligible); $r_i^{(1)}$ and $r_i^{(2)}$ are tracer isotope ratios, to be calculated from natural and tracer ion current ratios 244/242 ($p_n^{(2)}$, $p_i^{(2)}$) and 243/242 ($p_n^{(1)}$, $p_i^{(1)}$) by using the following relations:

$$\begin{aligned} r_i^{(1)} &= r_n^{(1)} + p_i^{(1)} - p_n^{(1)} \\ r_i^{(2)} &= r_n^{(2)} - (p_n^{(1)} - r_n^{(1)})(p_i^{(1)} - p_n^{(1)}) + p_i^{(2)} - p_n^{(2)} \end{aligned} \quad (5)$$

The unlabeled minimal model cannot separate glucose production from glucose disposal. Therefore, this model describes the net hepatic glucose balance as a function of plasma glucose and insulin action, and S_I and S_G measure the effects of glucose and insulin on both glucose production (inhibition) and glucose disposal (stimulation). This limitation does not apply to the labeled minimal model, which measures the effect of glucose and insulin on glucose disposal alone (9, 35). β -Cell sensitivity indices during the IVGTT, related to the control of glucose on

first ($\Phi 1$) and second ($\Phi 2$) phase secretions were estimated by using the minimal model C-peptide secretion and kinetics (39).

Model Identification (labeled and unlabeled minimal models)

Variables of the minimal models, together with their precision, were estimated by using the SAAMII software (40). Measurement errors have been assumed to be independent and to have a Gaussian distribution and a zero mean. Additional assumptions are that glucose concentration data have a constant CV equal to 2%; tracer glucose concentrations (within the range 0.05–7.2 mM) have an experimentally determined CV with a typical range of 4–8%; and C-peptide data have a constant SD which has been estimated *a posteriori*.

Statistical Methods

Data are presented as mean $\pm$ SEM. Differences between values obtained on the two study occasions were tested by paired, two-tailed *t* test. Differences between groups (glucose Ra and gluconeogenic rate) were tested by nonpaired, two-tailed *t* test. Power calculations were performed using the STAT PLAN software (Version 4.0: June 1993, Brown BW, Brauner C, Chan A, Gutierrez D, Herson J, Lovato J, Polsey J (eds), University of Texas, M.D. Anderson Cancer Center, Houston, TX, U.S.A.).

RESULTS

Subject characteristics, including age, weight, height, BMI, and percent fat, are depicted in Table 1, and the dietary intakes in Table 2. The consumed diet was equal on each study occasion both with regard to energy intake and distribution of carbohydrate, fat, and protein calories. In accordance with the study design, the calorimeter diet corresponded to $86 \pm 5\%$ (study 1) and $83 \pm 4\%$ (study 2) of the home diet, although the composition of the diet consumed in the calorimeter was virtually identical to the home diet (Table 2). The energy expenditure corresponded well to the energy intake in the calorimeter (Table 2). The results from the 24-h calorimeter test (Table 2) also show that RQ, BMR, and oxidation and utilization of carbohydrate, fat, and protein were not statistically different on the two study occasions.

Plasma concentrations of glucose, glycerol, insulin, and C-peptide during the steady-state measurement period (study hour 2.5 to 3) are depicted in Table 3, demonstrating that there were no differences between the two study occasions in either group.

Table 3. Plasma concentrations of substrates and hormones during steady state (study h 2.5 to 3)

Study group	[Glucose] (mM)		[Glycerol] (μM)		[Insulin] ($\mu\text{U/mL}$)		[C-peptide] (ng/mL)	
	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2
Prepubertal children	4.8 ± 0.1	4.8 ± 0.1	63 ± 5	68 ± 4	6.3 ± 0.2	6.7 ± 0.2	1.3 ± 0.1	1.3 ± 0.1
Adolescents	4.7 ± 0.1	4.8 ± 0.1	61 ± 4	43 ± 2	6.9 ± 0.7	6.9 ± 0.5	1.8 ± 0.3	1.9 ± 0.3
All subjects	4.7 ± 0.1	4.8 ± 0.1	62 ± 6	56 ± 8	6.8 ± 0.9	6.7 ± 0.7	1.6 ± 0.2	1.7 ± 0.2

Table 4. Isotopic enrichments during steady state (study h 2.5 to 3) for the whole study group ($n = 8$)

Tracer	Enrichment (%)	
	Study 1	Study 2
[1- ¹³ C]glucose	3.0 ± 0.1	3.0 ± 0.1
[² H ₅]glycerol	5.7 ± 0.6	6.7 ± 0.7
[² H ₃]KIC	3.1 ± 0.1	3.1 ± 0.1
² H in body water	0.40 ± 0.01	0.41 ± 0.01
² H in glucose C6	0.40 ± 0.01	0.40 ± 0.01

Isotopic enrichments for [1-¹³C]glucose, [²H₅]glycerol, [²H₃]KIC, and ²H in body water and glucose carbon 6 are depicted in Table 4. These enrichments were used to calculate glucose Ra (or GPR), glycerol Ra, leucine Ra, fractional gluconeogenesis, and gluconeogenic rate, respectively. These results are reported in Table 5. There were no significant differences between the values obtained on the two study occasions for any of these variables. Total plasma glucose Ra was significantly higher ($p < 0.05$) in the adolescents, $789 \pm 43 \mu\text{mol}/\text{min}$ (mean of studies 1 and 2 in the adolescent group) than in the prepubertal children, $582 \pm 30 \mu\text{mol}/\text{min}$ (mean of studies 1 and 2 in the prepubertal group). However, when expressed per kilogram of body weight, glucose production was significantly higher ($p < 0.01$) in the prepubertal children compared with the adolescents, 18.5 ± 0.9 versus $13.0 \pm 0.6 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, (mean of studies 1 and 2 within the two groups). Gluconeogenic rates expressed on a body weight basis were also significantly higher ($p < 0.05$) in the prepubertal children ($10.0 \pm 0.6 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ on both study occasions) when compared with those of the adolescents ($6.7 \pm 0.6 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, study 1 and $6.1 \pm 0.6 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, study 2; Table 5).

Estimating gluconeogenesis from the deuterium enrichment at glucose carbon 6 represents gluconeogenesis from pyruvate and does not include the contribution from glycerol (12, 30). However, the glycerol contribution was calculated from the data obtained using the [²H₅]glycerol tracer (see Methods). Thus, glycerol contributed a minimum of $2.4 \pm 0.3\%$ and $2.0 \pm 0.3\%$ of glucose production on study occasion 1 and 2, respectively (NS). Assuming that all glycerol was converted to glucose, the maximal glycerol contribution to glucose production via gluconeogenesis would be $11.3 \pm 1.2\%$ (study 1) and $9.4 \pm 1.2\%$ (study 2; NS). Thus, the minimal total gluconeogenesis (pyruvate + glycerol) would be $54 \pm 3\%$ (study 1) and $53 \pm 3\%$ (study 2; NS), and maximal total gluconeogenesis, 63 ± 2 (study 1) and $60 \pm 4\%$ (study 2; NS). Figure 1 displays the

concentrations of glucose, tracer glucose, insulin, and C-peptide after the IVGTT bolus in studies 1 and 2. The ability of the minimal model to describe glucose, tracer glucose, and C-peptide data is also shown in Figure 1, demonstrating that the values predicted by the model correspond well to the actual data. Table 6 demonstrates S_1^* and S_1 and S_G^* and S_G calculated from the labeled and unlabeled models, respectively, and first- and second-phase insulin secretion indices (Φ_1 and Φ_2) calculated from the C-peptide model. The average precision with which S_1^* and S_1 were estimated, expressed as their CVs, were 22 and 47%, respectively, for S_G^* and S_G , the CVs were 7 and 82%, respectively, and for Φ_1 and Φ_2 , 7 and 16%.

We have expressed the reproducibility of the above variables as 1) the mean $\pm$ SD ($n = 8$) of the differences between the values obtained on each of the two study occasions in percent of the mean of these values (Table 7), and 2) the correlation between the values from study 1 and 2 (Table 7). Furthermore, based on the SD for the percent difference of the mean, we have performed power calculations (assuming a power of 80% and a type I error of 5%) for each variable, i.e. we have calculated the number of subjects required to detect differences of 10 and 25%, respectively, for each variable (Table 3). Thus, a 10% difference could be detected in five or fewer subjects for energy intake and expenditure, plasma concentrations of glucose and C-peptide, glucose Ra, and leucine Ra, whereas a 25% difference could be detected in 11 or fewer subjects for plasma insulin concentration, fractional gluconeogenesis, S_1^* , S_G^* , S_G , Φ_1 , and Φ_2 . Glycerol turnover and S_1 (unlabeled minimal model) are more variable and required 16 and 18 subjects, respectively, to detect a 25% difference.

DISCUSSION

To our knowledge, this is the first study to report intraindividual reproducibility data for measures of glucose and insulin metabolism in children. Our results demonstrate that, using a prepacked meal strategy and frequent contacts with a dietitian, dietary macronutrient and energy intake can be controlled for a 7-d period in children in a free-living setting, and after a period of 2 to 8 wk the dietary composition can be reproduced. Under these study conditions, in a sample of eight children, differences as small as 2–7% could be detected with a power of 80% and a type I error of 5% for measurements of energy intake and expenditure, plasma concentrations of glucose and C-peptide, rates of glucose appearance (and thus disappearance), and proteolysis (represented by leucine turnover) (Table 7). The fraction of glucose derived from gluconeogenesis,

Table 5. Glucose Ra (equivalent to GPR), gluconeogenesis, glycerol Ra, and leucine Ra during steady state (study h 2.5 to 3)

Study group	GPR ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)		GNG rate ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)		GNG % GPR		Glycerol Ra ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)		Leucine Ra ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	
	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2
Prepubertal children	18.6 ± 0.7*	18.4 ± 1.0*	10.0 ± 0.6**	10.0 ± 0.6**	52 ± 1	53 ± 3	4.3 ± 0.6	3.8 ± 0.5	1.9 ± 0.1	1.9 ± 0.1
Adolescents	12.9 ± 0.4	13.1 ± 0.8	6.6 ± 0.7	6.1 ± 0.5	51 ± 6	47 ± 5	2.8 ± 0.3	2.1 ± 0.1	1.9 ± 0.1	1.8 ± 0.1
All subjects	15.8 ± 1.2	15.7 ± 1.2	8.2 ± 0.7	7.9 ± 0.8	52 ± 3	50 ± 3	3.5 ± 0.5	3.0 ± 0.4	1.9 ± 0.1	1.9 ± 0.1

* $p < 0.01$, ** $p < 0.05$ prepubertal children vs adolescents.
GNG, gluconeogenesis.

Figure 1. Concentrations of glucose, tracer glucose, insulin, and C-peptide and the minimal model fit after the IVGTT bolus in studies 1 and 2.

plasma concentrations of insulin, S_I (as determined by the labeled minimal model), S_G , and indices of insulin secretion were somewhat more variable; however, even for these measures, differences of 15–25% could be detected in only eight subjects (Table 7). S_I determined by the unlabeled minimal model and lipolysis requires a larger number of subjects, 16 and 18, respectively, to detect a difference of 25% at the power described above. With the exception of lipolysis and insulin secretory index Φ_2 , the correlation coefficient between the values obtained on the two study occasions was high, ranging between 0.72 and 0.996 (Table 3). The greater variability for measurements of glycerol factors is not surprising, as glycerol Ra is increased dramatically with small changes of plasma catecholamine concentrations (41). Therefore, special precautions should be taken to achieve stable measurements of lipolysis (such as a quiet room and minimal disturbance of the subject).

The labeled minimal model provides information on the effect of insulin on peripheral glucose uptake in response to an i.v. glucose bolus, whereas the unlabeled minimal model cannot distinguish between the hepatic (suppression of glucose production) and peripheral effects (increasing the glucose uptake) of insulin. Our results also demonstrate that the labeled minimal model provides more precise and consistent results than the unlabeled model. Despite these advantages of the labeled model, we are only aware of one report on S_I using the stable labeled minimal model in children (21). Hoffman and

Armstrong (21) compared S_I in lean and obese children between 5 and 12 y of age. They reported significantly higher S_I in the lean children compared with the obese. Our S_I values were within the range reported by Hoffman and Armstrong (21), although our mean value was somewhat lower. Decreased S_I has been demonstrated in adolescents compared with prepubertal children (16–19). In the present study, S_I was somewhat (but not significantly) higher in prepubertal children. However, the lack of significance may be owing to the small number of subjects in each group.

The brain weight of a 6- to 9-year-old is similar to that of an adolescent (1300 g in the children and 1400 g in the adolescents) (42). Thus, assuming similar glucose utilization per 100 g of brain tissue (approximately 20 $\mu\text{mol}/100\text{ g}$ brain weight per minute) (43), total glucose utilization from brain metabolism would be similar in adolescents and children, 280 and 260 $\mu\text{mol}/\text{min}$, respectively. However, the greater brain to body weight ratio in the young children (4% compared with 2% in the adolescents) explains the greater glucose Ra in children when compared with adolescents when expressed on a body weight basis. The greater absolute rate of glucose Ra (in micromoles per minute) in the adolescents is most likely because of a larger nonbrain tissue mass (*e.g.* fat and muscle).

To our knowledge, these studies are the first to demonstrate that the rate of gluconeogenesis, on a body weight basis, is greater in children between the ages of 8 and 9 y than in adolescents between the ages of 14 and 16 y, whereas the fraction of glucose production derived from gluconeogenesis was essentially identical between the two groups of subjects.

The deuterium oxide method with assessment of deuterium enrichment at carbon 6 of glucose does not include the gluconeogenic contribution from glycerol (12, 30). However, using the information obtained by the [$^2\text{H}_5$] glycerol tracer, we could calculate the glycerol turnover rates, an indicator of lipolysis, as well as the minimal and maximal estimates of the fraction of glucose Ra derived from glycerol *via* the gluconeogenic pathway. These estimates were also highly reproducible. Using these minimal estimates would result in a minimal estimate of total fractional gluconeogenesis of approximately 53%. A maximal estimate would be approximately 60%, assuming that 100% of glycerol Ra was converted to glucose.

We could not demonstrate any significant relationships between measures of glucose and lipid metabolism and percent body fat, energy intake and expenditure, oxidation rates, or RQ. This is not surprising, as the subjects were all healthy and nonobese, and they were studied under strict dietary and experimental conditions. We did not compare boys and girls because of the small sample size (prepubertal children, two boys, two girls; adolescents, two boys, two girls). Except for the differences in glucose Ra and gluconeogenic rate described above, we could not demonstrate any significant differences for metabolic variables between prepubertal children and adolescents. This may be related to the small group sizes (four subjects in each group), but the study was not designed to compare the two groups.

In summary, we demonstrate, using a prepacked meal strategy as described, that it is possible to maintain a consistent dietary energy intake and composition for a period of 7 d with

Table 6. S_I^* and S_I (labeled and unlabeled models, respectively), S_G^* and S_G (labeled and unlabeled models, respectively), and $\Phi 1$ and $\Phi 2$ insulin secretion indices (C-peptide model)

Study group	$S_I^* \times 10^{-4}$ (min^{-1})/($\mu\text{U mL}^{-1}$)		$S_I \times 10^{-4}$ (min^{-1})/($\mu\text{U mL}^{-1}$)		$S_G^* \times 10^{-2}$ (min^{-1})		$S_G \times 10^{-2}$ (min^{-1})		$\Phi 1$ $\times 10^{-9}$	$\Phi 1 \times 10^{-9}$	$\Phi 2 \times 10^{-9}$ min^{-1}	$\Phi 2 \times 10^{-9}$ min^{-1}
	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2
Prepubertal children	5.5 $\pm$ 0.7	6.0 $\pm$ 0.8	5.5 $\pm$ 1.5	5.6 $\pm$ 1.8	1.4 $\pm$ 0.1	1.4 $\pm$ 0.1	2.1 $\pm$ 0.1	2.3 $\pm$ 0.1	210 $\pm$ 33	224 $\pm$ 38	12.2 $\pm$ 0.7	11.9 $\pm$ 0.8
Adolescents	3.4 $\pm$ 1.3	4.3 $\pm$ 1.3	4.9 $\pm$ 1.2	3.7 $\pm$ 1.0	0.9 $\pm$ 0.1	0.8 $\pm$ 0.2	1.4 $\pm$ 0.2	1.2 $\pm$ 0.3	298 $\pm$ 84	292 $\pm$ 62	13.3 $\pm$ 0.3	19.4 $\pm$ 1.5
All subjects	4.5 $\pm$ 0.8	5.2 $\pm$ 0.8	5.7 $\pm$ 0.9	4.6 $\pm$ 0.8	1.2 $\pm$ 0.1	1.1 $\pm$ 0.1	1.6 $\pm$ 0.3	1.8 $\pm$ 0.3	254 $\pm$ 45	258 $\pm$ 36	12.8 $\pm$ 0.4	5.7 $\pm$ 1.6

Table 7. Calculations of population size required detecting a 10 and 25% difference at a power of 80% and a type I error of 5%

Variable	Mean of studies 1 and 2	Difference (% of mean) [†]	$\Delta\%$ detected if $n = 8$	Sample size to detect		Correlation study 1 vs 2
				$\Delta 10\%$	$\Delta 25\%$	
Energy intake (home) (kcal/d)	2280	2.9 $\pm$ 3.6	4.2	4	3	0.996
Energy intake (calorimeter) (kcal/d)	1860	0.1 $\pm$ 3.5	4.0	4	3	0.99
Energy expenditure (calorimeter) (kcal/d)	1857	2.0 $\pm$ 5.6	6.5	5	3	0.97
[Glucose] (mM)	4.8	2.4 $\pm$ 5.6	6.5	5	3	0.76
[Insulin] ($\mu\text{U/mL}$)	6.8	1.0 $\pm$ 19.8	23.0	33	7	0.79
[C-peptide] (ng/mL)	1.7	1.0 $\pm$ 1.8	2.0	3	<1	0.97
Glucose Ra ($\mu\text{mol/min}$)	686	0.1 $\pm$ 5.3	6.1	5	3	0.97
Gluconeogenesis (%)	51	3.6 $\pm$ 12.4	14.0	14	4	0.72
Glycerol Ra ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	3.3	19.0 $\pm$ 33.0	38.0	40	16	0.51
Leucine Ra ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	1.9	3.0 $\pm$ 4.1	4.7	4	3	0.94
Insulin sensitivity (S_I^*) $\times 10^{-4}$ ($\text{min}^{-1}/\mu\text{U mL}^{-1}$) (SLIVGTT)	4.9	20.0 $\pm$ 24.0	28.0	47	10	0.94
Insulin sensitivity (S_I) $\times 10^{-4}$ ($\text{min}^{-1}/\mu\text{U mL}^{-1}$) (unlabeled model)	5.1	11.4 $\pm$ 35.0	40.0	98	18	0.94
Glucose effectiveness (S_G^*) $\times 10^{-2}$ (min^{-1}) (SLIVGTT)	1.2	8.0 $\pm$ 18.0	21.0	28	7	0.92
Glucose effectiveness (S_G) $\times 10^{-2}$ (min^{-1}) (unlabeled model)	1.7	13.8 $\pm$ 20.4	23.1	34	7	0.92
β -Cell sensitivity indices						
$\Phi 1 \times 10^{-9}$	256	3.7 $\pm$ 22.2	25.6	41	9	0.79
$\Phi 2 \times 10^{-9}$ (min^{-1})	14.3	18.2 $\pm$ 26.3	30.3	57	11	0.53

[†] $100 \times [(\text{study 1} - \text{study 2})/(\text{mean of studies 1 and 2})]$.
SLIVGTT, stable labeled IVGTT.

the children carrying out their normal activities. Furthermore, using our study design, it is possible to provide highly reproducible data on a number of measures of glucose metabolism. The power calculations performed on the basis of these data will enable us and other investigators to more precisely design studies to evaluate the impact of various treatments and dietary intervention strategies in children (e.g. children and adolescents with obesity and/or type II diabetes).

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