In vivo kinetic studies in inborn errors of metabolism : expanding insights in (patho)physiology

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Chapter 2

Endogenous glucose production after an overnight fast in humans in relation to age and estimated brain weight

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Submitted

Abstract

Objectives: to study the relation between endogenous glucose production (EGP) and age in order to construct a model for EGP in humans after an overnight fast. In addition, the relation between EGP and estimated brain weight is delineated.

Methods: data from our centre on EGP in 16 healthy children, aged 2.5 to 17.1 yrs, and 35 healthy adults, aged 19.7 to 58.3 yrs, were combined to construct a regression model. In all subjects EGP was quantified with the [6,6-$^2H_2$]glucose isotope dilution method after overnight fasting. Since EGP was quantified during isotopic steady-state, peripheral glucose uptake equalled EGP. A second model on EGP after overnight fasting was constructed based on data from literature (N=24). Furthermore, our data on EGP were correlated to estimated brain weight and age after correction for body composition.

Results: regression analysis of our data yielded the following age-dependent model for EGP:

$$\text{EGP } (\mu\text{mol/kg·min}) = 36.04 \cdot e^{-0.1396 \cdot \text{age (y)}} + 10.27 \quad (R^2\ 0.92,\ S_{y.x}\ 2.47)$$

EGP as predicted by the model constructed with data from literature was higher in young children, but the 95% confidence intervals of both models overlapped. Linear regression analysis revealed almost no correlation between EGP per kg estimated brain weight and age after correction for body composition (R^2 0.08, $S_{y.x}$ 3.89, P = 0.043).

Conclusion: our regression model accurately estimates EGP and whole body glucose uptake after an overnight fast, thus representing minimal glucose requirement during resting conditions in healthy subjects. EGP per kg estimated brain weight after correction for body composition appeared to be almost independent of age.

Abbreviations

EGP	endogenous glucose production
R_a	rate of appearance
TTR	tracer/tracee ratio

Introduction

The human brain depends on glucose for its energy metabolism. During fasting glucose is provided through glycogen breakdown and gluconeogenesis, the latter requiring, among other substrates, muscular proteolysis to supply amino acid precursors. To minimize glucose utilization and thus protein breakdown during fasting, insulin dependent glucose uptake in peripheral tissues is inhibited (1), fatty acid oxidation for energy production is augmented and ketone bodies are produced as an alternative energy source for glucose (2). However, neuronal cells cannot oxidize fatty acids and oxidation of ketone bodies can not replace glucose as the major substrate for cerebral energy metabolism (3). Therefore, cerebral glucose utilization is a major contributor to whole body glucose utilization during resting conditions (4;5). As the ratio of brain weight to body weight decreases from infancy towards adulthood glucose requirement per kg body weight is much higher in young children. Therefore, in order to maintain normoglycemia during fasting young children have to produce more glucose per kg body weight to meet cerebral glucose demands. This is emphasized in the seminal paper by Bier *et al* who were the first to show that endogenous glucose production (EGP) in humans decreases with age and has a linear relationship with estimated brain weight (6). However, in their study only limited data on adults were included. Since then, EGP has been studied extensively in adults, but data on EGP in children remain scarce. More knowledge about age related glucose turn-over in children could help to optimize glucose supplementation protocols.

In this paper we describe an age-dependent regression model for EGP in humans based on glucose turn-over data quantified with the [6,6-$^2H_2$] glucose isotope dilution method (7) in children and adults (N=51), covering an age range from 2.8 to 58.3 yrs. Moreover, the relation between EGP, estimated brain weight and age is further delineated.

Materials and Methods

Subjects and study design

Data on 16 children, aged 2.5 yrs to 17.1 yrs, four females and twelve males, were included in this study. All children had a body mass index between 14.7 and 21.4 kg/m^2. The children were studied during a standardized fasting test (8), in order to evaluate fasting tolerance because of a history of ketotic hypoglycemia (9;10). In all subjects, extensive metabolic evaluation, including organic acid analysis in urine, plasma acylcarnitine profiling and plasma amino acid analysis, as well as a full endocrinologic evaluation did not reveal a metabolic or endocrine disorder. During the fasting test glucose kinetics (i.e. the rate of glucose appearance and the rate of glucose disappearance in plasma) were quantified using the [6,6-$^2H_2$]glucose isotope dilution method (7). These pediatric data

obtained during normoglycemia (plasma glucose 4.0 – 5.5 mmol/L) after an overnight fast (14 – 17 hrs of fasting) were combined with data on glucose kinetics after overnight fasting obtained in 35 healthy adult male volunteers, aged 19.7 to 58.3 yrs, with normal body composition, acquired during previous studies by our research group (11-15). The combined data were used to create a non-linear regression model for EGP and glucose uptake after an overnight fast. Data from literature on EGP after an overnight fast in healthy children and adults were used to validate the regression model (3;16-35). All subjects or their parent(s)/legal guardian(s) gave informed consent prior to the studies. All studies were approved by the Institutional Review Board.

Study protocol

All children were admitted one day before the fasting test; adults were admitted between 6 and 7 am on the day of study. An intravenous catheter was inserted into an antecubital vein of each arm after topical application of lidocaine cream. One catheter was used for administration of [6,6-$^2H_2$]glucose and the other for blood sampling. A baseline blood sample was collected to determine the background enrichment of [6,6-$^2H_2$]glucose in plasma. Fasting was started after the consumption of a regular evening meal. All subjects remained fasted throughout the whole test and kept bed rest. They were allowed to drink water *ad libitum*. At 8 a.m. the next day, after 12 to 14 hrs of fasting, a primed continuous infusion of [6,6-$^2H_2$]glucose (>99% pure; Cambridge Isotope Laboratories, Cambridge, MA) was started (bolus: 17.6-52.7 µmol/kg, continuous: 0.22-0.67 µmol/kg·min, both depending on the age of the patient in order to reach 1-2 % plasma enrichment). After two hours of [6,6-$^2H_2$]glucose infusion isotopic steady-state was assumed to be present and three blood samples were collected at 5 – 10 min. intervals. Blood samples were centrifuged at 3000 rpm for 10 min, after which the plasma was collected and stored at -20°C until analysis.

Analytical methods

Plasma glucose concentration: plasma glucose levels were analyzed with the hexokinase method on a Roche MODULAR P800 analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Plasma [6,6-$^2H_2$]glucose enrichment: plasma glucose enrichments were determined as described previously (11). Briefly, plasma was deproteinized with methanol and evaporated to dryness. The extract was derivatized with hydroxylamine and acetic anhydride (36). The aldonitrile pentaacetate derivative of glucose was extracted into methylene chloride and evaporated to dryness. The extract was reconstituted in ethylacetate and injected into a gas chromatograph/mass spectrometer (HP 6890 series GC system and 5973 Mass Selective Detector, Agilent Technologies, Palo Alto, CA, USA). Separation was achieved on a J&W DB17 column (30 m x 0.25 mm, d_f 0.25 µm; J&W Scientific, Folsom, CA). Glucose ions were monitored at *m/z* 187, 188 and 189. The isotopic enrichment of

glucose was determined by dividing the peak area of *m/z* 189 by the peak area of *m/z* 187, after correction for background enrichment of [6,6-$^2H_2$]glucose.

Calculations and statistical analysis

Rates of appearance and disappearance of glucose: the rate of appearance of glucose in plasma (R_a glucose), which reflects whole body endogenous glucose production (EGP) in the post-absorptive state, was calculated with Steele's steady-state equation in the presence of isotopic steady-state (37):

[1] $R_a \text{ glucose} = I / TTR_{plasma}$

in which I is the infusion rate of [6,6-$^2H_2$]glucose in μmol/kg·min and TTR_{plasma} (tracer/tracee ratio) is the ratio of [6,6-$^2H_2$]glucose over unlabelled glucose in plasma, as determined by gas chromatography/mass spectrometry. Because our glucose tracer appeared to be >99% pure we did not include tracer purity in the calculation. During steady-state conditions the rate of disappearance of glucose from plasma (R_d glucose) equals R_a glucose and reflects the rate of peripheral glucose uptake.

Relation between EGP, estimated brain weight and age: total EGP was calculated by multiplying R_a glucose with bodyweight (kg). Total EGP was then divided by estimated brain weight, as calculated by following equation based on weight measurements of 4736 brains in the fresh condition and without any pathological lesions (38):

[2] estimated brain weight (kg) = 1.449 – 3.62 / bodyweight (kg)

This yielded EGP expressed as μmol/kg_{brain}·min. To correct for differences in body composition between children and adults EGP (μmol/kg_{brain}·min) was divided by body mass index (BMI), yielding EGP expressed as μmol/kg_{brain}·min per kg/m^2.

Statistical analysis: Data were analyzed with GraphPad Prism version 3.03 (GraphPad Software, San Diego, USA) and SPSS version 12.0.2 (SPSS Inc., Chicago, Illinois). Non-linear regression analysis was done using a one-phase exponential decay model with age as independent variable. The data on R_a glucose obtained in children combined with the data on R_a glucose in adults from previous studies performed by our research group were used to construct our model (Table 1). A second model was constructed using data on mean R_a glucose from healthy, non-obese children and adults after overnight fasting as reported in literature (Table 2). Linear regression analysis was done between EGP (μmol/kg_{body}·min) and estimated brain weight (kg), and between EGP (μmol/kg_{brain}·min per kg/m^2) and age (y).

Table 1. Rate of appearance of glucose (μmol/kg_{body}·min) after an overnight fast in all subjects

Age (y)	R_a glucose	Age (y)	R_a glucose	Age (y)	R_a glucose	Age (y)	R_a glucose
2.5	35.9	9.1	23.2	21.8	9.7‡	29.3	10.3‡
2.5	28.4	11.5	21.4	22.0	11.3#	31.9	9.3§
2.8	36.9	17.1	12.3	23.1	13.5¶	32.0	12.7‡
3.3	39.7	19.7	13.1*	23.5	11.3¶	32.3	10.2§
3.7	31.7	19.9	13.6*	23.8	11.1*	35.1	11.4†
3.9	38.6	20.1	11.2*	24.1	12.1¶	39.8	10.5§
4.2	28.2	20.1	12.1*	24.5	14.2‡	40.2	11.3†
4.4	29.9	20.2	12.3*	24.8	10.7‡	43.0	9.1§
5.0	25.4	20.5	12.0*	24.9	13.7#	49.3	9.6*
6.2	22.7	21.0	12.5¶	25.8	16.0¶	54.3	10.9†
6.5	22.2	21.1	11.0#	26.8	10.3†	57.6	8.3*
6.7	24.8	21.2	13.3#	27.2	11.1‡	58.3	9.0*
7.4	19.3	21.4	10.4*	28.8	9.3§		

* Unpublished data † Ref. 11 ‡ Ref. 12 § Ref. 13 # Ref. 14 ¶ Ref. 15

Results

Rate of appearance of glucose and age distribution

Data on R_a glucose at various ages obtained from different studies by our research group are presented in Table 1.

Relation between EGP and age

The data on R_a glucose as described in Table 1 were used to construct an age-dependent regression model for EGP, expressed either as μmol/kg_{body}·min or as mg/kg_{body}·min, in humans after an overnight fast (Figure 1). The equations of the model are as follows:

[3] $\text{EGP } (\mu\text{mol/kg}_{body}\cdot\text{min}) = 36.04 \cdot e^{-0.1396\cdot\text{age (y)}} + 10.27$ (R^2 0.92, $S_{y.x}$ 2.47)

or

[4] $\text{EGP } (\text{mg/kg}_{body}\cdot\text{min}) = 6.49 \cdot e^{-0.1397\cdot\text{age (y)}} + 1.85$ (R^2 0.92, $S_{y.x}$ 0.44)

To assess the validity of this regression model data from the literature on R_a glucose in both healthy children and adults (Table 2) were plotted and analysed in the same way (Figure 1). This yielded the following regression equations:

[5] $\text{EGP } (\mu\text{mol/kg}_{body}\cdot\text{min}) = 95.23 \cdot e^{-0.2168\cdot\text{age (y)}} + 11.26$ (R^2 0.85, $S_{y.x}$ 3.28)

or

[6] $\text{EGP } (\text{mg/kg}_{body}\cdot\text{min}) = 17.80 \cdot e^{-0.2229\cdot\text{age (y)}} + 2.05$ (R^2 0.85, $S_{y.x}$ 0.59)

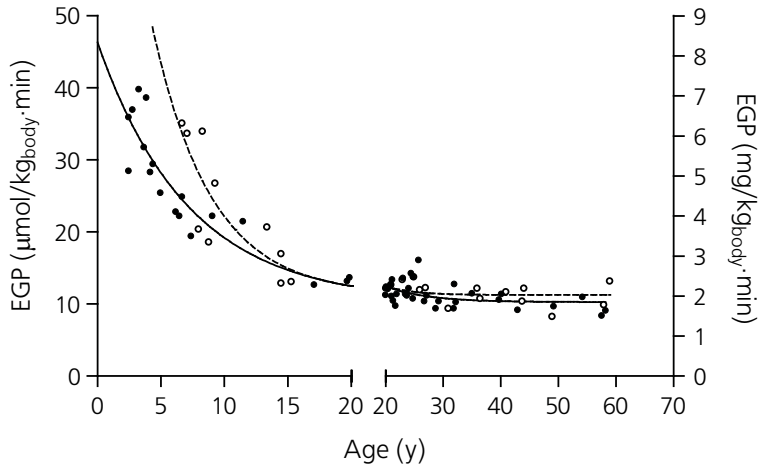

Figure 1. Relation between EGP (µmol/kg$_{body}$·min or mg/kg$_{body}$·min) and age (yrs). EGP data from our group (●; Table 1), EGP data from literature (○; Table 2). Regression models based on our data (continuous line) and based on data from literature (dotted line) are shown. The x-axis was divided into two segments in order to clearly show the EGP curves at age 0 to 20 yrs.

The 95 % confidence intervals of the variables in the regression equations between the regression model based on our data and the model based on data from literature overlapped (Table 3), but the model based on data from literature predicted EGP to be higher in young children.

Relation between EGP, estimated brain weight and age

Linear regression analysis between EGP (µmol/kg$_{body}$·min or mg/kg$_{body}$·min) and estimated brain weight (kg) yielded the following equations (Figure 2):

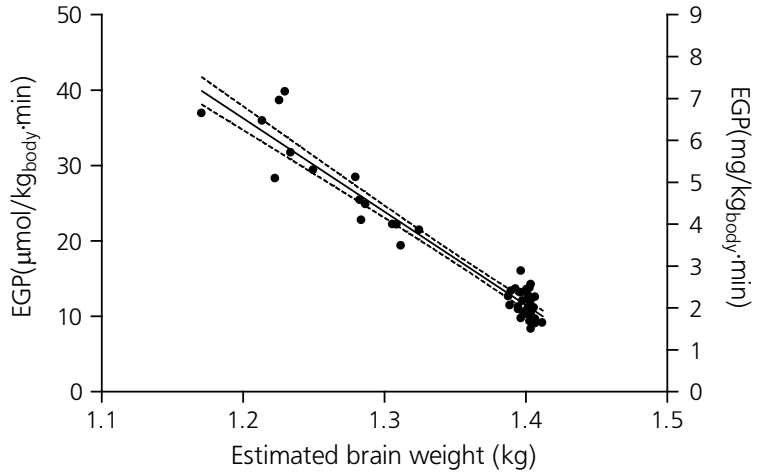

Figure 2. Relation between EGP (µmol/kg$_{body}$·min or mg/kg$_{body}$·min) and estimated brain weight (kg). Linear regression analysis with the 95% confidence interval is shown.

Table 2. Reference data on R_a glucose (μmol/kg_{body}·min) after an overnight fast in humans

Age*	N	R_a glucose	Tracer	Reference
6.7	5	35.0	[6,6-$^2H_2$]glucose	Haymond et al. Am. J. Physiol. 245: E373-378, 1983.
7.1	8	33.6	[6,6-$^2H_2$]glucose	Haymond et al. J. Clin. Invest. 80: 398-405, 1978.
8.0	12	20.3†	[1-^{13}C]glucose	Sunehag et al. J. Clin. Endocrinol. Metab. 87: 5168-5178, 2002.
8.3	4	33.9	[6,6-$^2H_2$]glucose	Haymond et al. Neurology 28: 1224-1231, 1978.
8.8	4	18.5	[1-^{13}C]glucose	Sunehag et al. Pediatr. Res. 50: 115-123, 2001.
9.3	5	26.7	[6,6-$^2H_2$]glucose	Le Stunff et al. Am. J. Physiol. 271: E814-E820, 1996.
13.4	6	20.6	[6,6-$^2H_2$]glucose	Bourneres et al. Diabetes 38: 477-483, 1989.
14.5	7	16.9	[6,6-$^2H_2$]glucose	Austin et al. J. Clin. Endocrinol. Metab. 79: 80-85, 1994.
14.5	12	12.8†	[1-^{13}C]glucose	Sunehag et al. J. Clin. Endocrinol. Metab. 87: 5168-5178, 2002.
15.3	4	13.0	[1-^{13}C]glucose	Sunehag et al. Pediatr. Res. 50: 115-123, 2001.
21	6	12.6‡	[3-^{3}H]glucose	Corssmit et al. Metabolism 43: 1503-1508, 1994.
23	8	13.3	[3-^{3}H]glucose	Corssmit et al. J. Clin. Endocrinol. Metab. 81: 3265-3269, 1996.
25	6	13.7	[3-^{3}H]glucose	Devlin et al. Metabolism 36: 697-702, 1987.
26	8	11.9	[3-^{3}H]glucose	Nielsen et al. Am. J. Physiol. Endocrinol. Metab. 286: E102-E110, 2004.
27	13	12.2	[3-^{3}H]glucose	Jensen et al. Am. J. Physiol. Endocrinol. Metab. 254: E700-E707 1988.
31	6	9.3	[6,6-$^2H_2$]glucose	Roden et al. Diabetes 49: 701-707, 2000.
36	6	12.1	[3-^{3}H]glucose	Corssmit et al. Metabolism 43: 1503-1508, 1994.
36.5	6	10.7	[3-^{3}H]glucose	Chen et al. J. Clin. Invest. 103: 365-372, 1999.
41	21	11.6§	[3-^{3}H]glucose	Gastaldelli et al. J. Clin. Endocrinol. Metab. 89: 3914-3921, 2004.
43.8	8	10.3	[6-^{3}H]glucose	Karlander et al. Diabetologia 29: 778-783, 1986.
44.1	7	12.1	[6,6-$^2H_2$]glucose	Nygren et al. Clin. Nutr. 17: 65-71, 1998.
49.0	8	8.2	[U-^{13}C]glucose	Radziuk et al. Diabetologia 49: 1619-1628, 2006.
58	7	9.8	[3-^{3}H]glucose	Boden et al. Am. J. Physiol. Endocrinol. Metab. 280: E23-E30, 2001.
59	6	13.1	[6,6-$^2H_2$]glucose	Nygren et al. Am. J. Physiol. Endocrinol. Metab. 275: E140-E148, 1998.

* Mean age of study subjects † Mean of both dietary protocols
‡ Mean of women measured in both the follicular and luteal phase of their menstrual cycle
§ Converted to μmol/kg_{body}·min from μmol/$kg_{fat\ free\ mass}$·min

Table 3. 95 % confidence intervals of the variables in the regression model based on our data and the model based on data from literature. The regression equations are built up as follows:
$EGP = Span \cdot e^{Constant \cdot age} + Plateau$

Parameter EGP	Constructed model		Literature model	
	μmol/kg_{body}·min	mg/kg_{body}·min	μmol/kg_{body}·min	mg/kg_{body}·min
Span	31.03 – 41.05	5.60 – 7.32	12.61 – 177.8	1.93 – 33.67
Constant	0.1007 – 0.1785	0.1008 – 0.1786	0.1018 – 0.3317	0.1046 – 0.3411
Plateau	8.96 – 11.58	1.62 – 2.09	9.27 – 13.26	1.69 – 2.40

[7] EGP (μmol/kg_{body}·min) = 185.0 (±6.11) – 124.0 (±4.49) · estimated brain weight (kg) (R^2 0.94, $S_{y.x}$ 2.16, P <0.0001)

or

[8] EGP (mg/kg_{body}·min) = 33.29 (±1.10) – 22.30 (±0.81) · estimated brain weight (kg) (R^2 0.94, $S_{y.x}$ 0.39, P <0.0001)

Linear regression analysis between EGP (μmol/kg_{brain}·min per kg/m^2) and age (y) yielded the following equations (Figure 3):

[9] EGP (μmol/kg_{brain}·min per kg/m^2) = 29.02 (±1.00) – 0.0792 (±0.038) · age (y) (R^2 0.08, $S_{y.x}$ 3.89, P = 0.043)

or

[10] EGP (mg/kg_{brain}·min per kg/m^2) = 5.22 (±0.18) – 0.0142 (±0.0068) · age (y) (R^2 0.08, $S_{y.x}$ 0.70, P = 0.043)

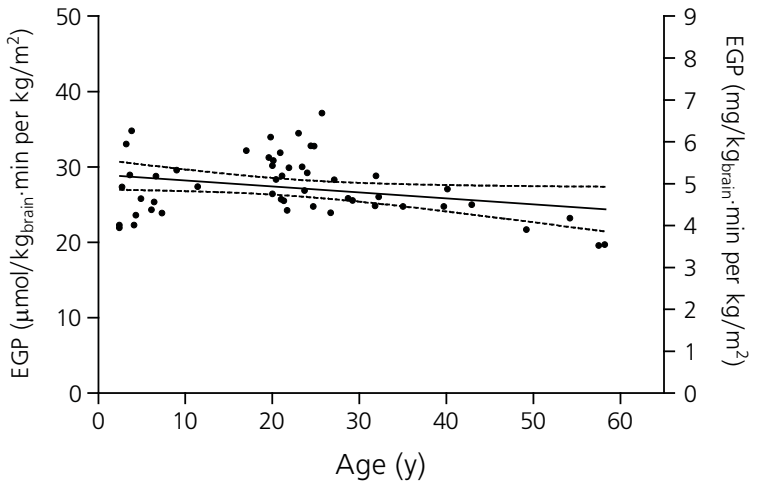

Figure 3. Relation between EGP (μmol/kg_{brain}·min per kg/m^2 or mg/kg_{brain}·min per kg/m^2) and age after correction for body composition. Linear regression analysis with the 95% confidence interval is shown.

Discussion

We were able to construct an age-dependent regression model that accurately describes endogenous glucose production (EGP) as well as peripheral glucose uptake after an overnight fast in humans. The model showed a good fit to the data points, as expressed by the high correlation coefficient and the low $S_{y.x}$. The data on R_a glucose were obtained using the [6,6-$^2H_2$]glucose isotope dilution method, which yields 'true' rates of EGP since

[6,6-$^{2}H_2$]glucose is considered to be a non-recycling glucose tracer (7). All blood samples from both children and adults were analysed in the same laboratory using the same equipment, thereby limiting analytical variation. Since all data on EGP used in our model were acquired during isotopic steady-state of the [6,6-$^{2}H_2$]glucose tracer and during normoglycemia, EGP was essentially the same as peripheral glucose uptake.

To validate our regression model, data from literature on EGP after overnight fasting in both healthy children and adults were combined to construct a second regression model. The fit of this model was not as precise as in our model. However, this model was based on mean EGP data as reported in literature, whereas our model was based on individual EGP data of the subjects in our studies. Furthermore, different glucose tracers were used to determine EGP in the reference studies, which may have contributed to the scatter in this model because of differences in potential tracer recycling of the different glucose tracers. However, it has been established that R_a glucose can be accurately determined with dilution of [6,6-$^{2}H_2$]glucose, [1-^{13}C]glucose and [U-^{13}C]glucose isotopes (7).

Although a significant overlap was detected between the regression equations based on our own data and data retrieved from literature, EGP was predicted to be much higher in young children in the model based on data from literature. There are two possible explanations. Firstly, no data on EGP in children below the age of 6 yrs could be included in the model based on literature. Although Bier *et al* did publish data on EGP in young children, they only reported a range in EGP of 5 to 8 mg/kg·min in children from 1 month to 6 years of age (6). Secondly, EGP in term neonates was predicted to be very high in the model based on data from literature. Although several studies quantified EGP in term newborns, the results could not be included in the literature model because of differences duration of fasting and concomitant intravenous glucose supplementation, resulting in large variations in reported EGP. However, none of these studies reported EGP to be as high as was predicted by the model based on literature, whereas our model predicted EGP in neonates to be in the reported range (6;39;40).

Since all studies were performed during resting conditions both peripheral glucose uptake and glucose production were driven predominantly by cerebral glucose utilization (4;5;41). When rates of EGP as predicted by our model were compared with the estimated rates of cerebral glucose utilization at various ages as reported by Kalhan and Kilic (41), a remarkable resemblance was observed: newborns: 8.3 mg/kg·min (this study) vs. 8.0 mg/kg·min (Kalhan and Kilic), 1 year olds: 7.5 mg/kg·min (this study) vs. 7.0 mg/kg·min (Kalhan and Kilic), 5 year olds: 5.1 mg/kg·min (this study) vs. 4.7 mg/kg·min (Kalhan and Kilic), adolescents: 2.6 mg/kg·min (this study) vs. 1.9 mg/kg·min (Kalhan and Kilic) and adults: 1.9 mg/kg·min (this study) vs. 1.0 mg/kg·min (Kalhan and Kilic). This is in line with the previous reported high correlation between EGP and estimated brain weight (6), which is also confirmed in this study (Figure 2), and supports the accuracy of our model.

Body composition changes significantly towards adulthood, mainly as the result of an increase in muscle and fat mass in relation to brain mass. Therefore, the contribution of peripheral glucose uptake by muscle and fat tissue will increase towards adulthood. This

is illustrated by the increasing difference seen towards adulthood between whole body glucose uptake as estimated by our model on the one hand and the estimated rates of cerebral glucose utilization on the other hand (see above). In order to study EGP and peripheral glucose uptake in relation to estimated brain weight and age independent of body composition, a correction for BMI was done in the subjects. This indeed demonstrated that after correction for body composition EGP is directly correlated with estimated brain weight with only a minor contribution of age (Figure 3). This strongly suggests that cerebral glucose uptake per kg estimated brain weight after overnight fasting only slightly decreases with age. PET data on local cerebral metabolic rates of glucose (LCMRglc) showed that cerebral glucose consumption is about twice as high in children aged 4 to 10 yrs compared to adults (42). However, this study only quantified LCMRglc in cortical and basal ganglia and therefore did not include most of the cerebral white matter. Since the ratio between grey and white matter decreases more than two-fold towards adulthood (43), and white matter does contribute substantially to cerebral glucose utilization (44), cerebral glucose uptake per kg estimated brain weight may indeed be almost independent of age.

In summary, we conclude that our regression model accurately estimates EGP and peripheral glucose uptake after overnight fasting in healthy subjects during resting conditions. This model can be used to assess minimal glucose requirement in healthy, non-obese children after an overnight fast and could help to optimize glucose supplementation protocols. Furthermore, the relation between EGP, peripheral glucose uptake and estimated brain weight after correction for body composition appears to be remarkably constant with age.

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