Elevated Hepatic Glucose Production in Children with Cystic Fibrosis

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ABSTRACT

We hypothesized that elevated hepatic glucose output (HGO) may occur in children with cystic fibrosis (CF) as an early sign of declining insulin secretion and that tolbutamide therapy would correct the defect. We studied eight glucose-tolerant CF patients ($\bar{X} \pm \text{SD}$, 9.1 ± 1.9 y) and five healthy controls (9.0 ± 1.6 y). Fasting glucose, insulin, and insulin-connecting peptide concentrations were not different in the CF and control subjects; however, meal stimulation tests in the CF patients suggested that insulin secretion was defective in the fed state. HGO ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was 26% higher in the CF patients (4.2 ± 0.7 versus 3.1 ± 0.6 in HC) ($p = 0.016$). When normalized for fat-free mass ($\text{mg} \cdot \text{kg fat-free mass}^{-1} \cdot \text{min}^{-1}$), HGO was 27% higher in CF (4.9 ± 0.8 versus 3.8 ± 0.5) ($p = 0.015$). However, when expressed as a function of resting energy expenditure ($\text{mg} \cdot \text{kcal}^{-1}$), HGO was not significantly different in CF (121 ± 22) versus healthy controls (116 ± 30). In seven of the CF group, HGO was re-assessed after a 2-h glucose infusion at a rate of $0.90 \pm 0.02 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. HGO was suppressed ($p < 0.05$) by an amount equal to $103 \pm 18\%$ of the glucose infusion rate.

Finally, in five CF patients, HGO was re-measured after 2 wk of oral therapy with tolbutamide (750 mg/d). Tolbutamide did not affect HGO (fasting or during the glucose infusion). In conclusion, fasting HGO was elevated in the CF patients in proportion to energy expenditure. (*Pediatr Res* 37: 600–605, 1995)

Abbreviations

CF, cystic fibrosis

C peptide, insulin-connecting peptide

D₂O, deuterium oxide

F DG, the rate of administration of glucose tracer

GCRC, NIH General Clinical Research Center

HGO, hepatic glucose output

IE DG, isotopic enrichment of glucose produced by deuterated glucose tracer

Ra DG, rate of appearance of glucose measured using deuterated glucose

Vco₂, net rate of excretion of CO₂ (equated to rate of CO₂ production)

Many patients with CF have secondary complications of glucose intolerance, the incidence of which appears to increase with age (1–4). In a retrospective study of clinical data, Lanng *et al.* (5) compared CF patients who developed diabetes mellitus with control CF patients during the previous 6-y period. Those who had developed diabetes demonstrated impaired weight gain (and lower body mass index), worsening pulmonary function, and impaired exocrine pancreatic function from 1 to 4.5 y before the diagnosis of diabetes.

We (6) previously showed that tolbutamide therapy in non-diabetic children with CF is associated with improved growth and accretion of fat-free mass in comparison to a baseline observation period. Subsequently, we found that feeding did not suppress whole body proteolysis in children with CF, suggesting that impaired insulin secretion or insulin action

might limit accretion of protein in this patient population (7). We speculated that impaired nutritional status could limit host defense and pulmonary function resulting in increased infection and worsening prognosis.

As part of a therapeutic trial, we examined the effect of tolbutamide on HGO and its suppressibility by i.v. glucose infusion and also compared fasting HGO in the patients with that in healthy control children. This report describes the results of this study.

METHODS

Subjects. Eight, nondiabetic, prepubertal, CF patients requiring oral pancreatic enzyme supplementation were recruited for this study (Table 1). In addition, we recruited five healthy controls. To participate, subjects had to be free of overt infection, afebrile, and off antibiotics for a minimum of 10 days before being studied. In addition, the CF patients had to be glucose-tolerant as evidenced by plasma glucose concentrations 2 h after a standard meal (*vide infra*) (6). The procedures were explained to the subjects and their parents, and informed

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Table 1. Physical characteristics of the CF patients

Subject no.	Sex	TS	Age (y)	Height (cm)	Weight (kg)	IBW (%)	CF score
CF patients							
2029*	F	1	10.5	132.9	33.6	116	89
2012	M	1	9.5	123.3	24.2	103	92
2031	M	1	11.4	144.0	28.1	78	76
2036	M	1	7.3	118.0	20.5	95	95
2035	M	1	8.0	120.4	20.2	92	73
2038	M	1	11.3	140.1	31.3	94	79
2039	M	1	8.5	130.5	30.6	113	91
2041	M	1	6.4	112.7	19.3	99	N/A
Mean $\pm$ SD			9.1 $\pm$ 1.9†	127.8 $\pm$ 11.5†	26.0 $\pm$ 5.6†	99 $\pm$ 12†	
Healthy controls							
2030	F	1	11.1	139.9	31.9	94	N/A
2032	M	1	6.7	125.7	25.4	102	N/A
2033	F	1	8.8	132.7	36.8	127	N/A
2034	M	1	9.6	133.2	28.0	97	N/A
2037	F	1	9.0	146.5	39.2	103	N/A
Mean $\pm$ SD			9.0 $\pm$ 1.6†	135.6 $\pm$ 7.9†	32.3 $\pm$ 5.8†	105 $\pm$ 13†	

Abbreviations used: TS, Tanner stage (8) used to assess maturation; percent IBW, $100 \cdot (\text{weight} \div \text{median weight for height age})$; CF score, NIH criteria for clinical status of cystic fibrosis; N/A, not available or not applicable.

* Subject no. 2029 was receiving systemic steroid therapy (prednisone 5 mg p.o. every day) at the time of each study.

† There were no significant differences between groups in age, height, weight, or percent IBW.

consent was obtained before participation in the study. The study was approved by the institutions' committees for research on human subjects.

To determine the degree to which HGO in the fasting state was abnormal in the CF patients, we also assessed glucose turnover in five healthy, prepubertal children (8).

Experimental design. Four days before the glucose tracer study, total body water was estimated using the procedure noted below.

As part of our overall study which included measurements of leucine oxidation in the fed and fasted states, subjects were admitted to the General Clinical Research Center (GCRC) for 4 d. During the first 3 d of admission, subjects were provided a diet that matched the energy intake of the typical diet based on 4-d diet records kept by the subjects. Subjects began fasting at 2100 h on d 3 and continued to fast until 1400 h on d 4. During the period, 0900–0950 h on d 4, the V_{CO_2} was measured using the Douglas bag technique (described below). A catheter for blood drawing was inserted into a peripheral vein of an upper extremity on d 3; after warming the vein, a blood sample was drawn from this catheter at 0950 h on d 4 for assessment of background enrichment of plasma glucose. A second catheter in the opposite arm was also inserted on d 3 and used for isotope and glucose infusion. Beginning at 1000 h on d 4, subjects received a prime constant infusion of [6,6- d_2]-glucose (99%, MSD Isotope; Quebec, Canada; prime: $18 \mu\text{mol} \cdot \text{kg} \cdot \text{body weight}^{-1}$; infusion: $0.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ or $0.04 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). After 80 min of infusion, four blood samples were drawn at 10-min intervals. Samples were centrifuged, and plasma was stored at -80°C until analysis of the enrichment of plasma glucose. In addition, plasma samples obtained during the last 40 min of the fasting tracer infusion were pooled and analyzed for glucose, insulin, and C peptide concentrations.

At the end of 2 h, in the CF patients only, while the deuterated glucose infusion continued, an i.v. infusion of unlabeled

glucose at an approximate rate of $1.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($5.6 \mu\text{mol} \cdot \text{kg} \cdot \text{min}^{-1}$) was administered to determine the suppressibility of the hepatic glucose output by glucose (9). Blood samples were collected during the period 80–120 min of the dual infusion, again at 10-min intervals.

After the completion of these fasting studies, the subjects were allowed to eat, and then they were discharged from the GCRC. However, five of the CF patients then began daily therapy with tolbutamide (750 mg/day) for 2 wk after which the tracer studies were repeated according to the protocol just described.

Healthy controls were admitted to the GCRC overnight and studied the next morning at 1000 h, after a 13-h fast.

Estimation of HGO. The Ra DG was calculated as follows (10):

$$\text{Ra DG} = (\text{F DG})/(\text{IE DG})$$

where F DG is the rate of administration of [6,6- d_2]-glucose (molar units, corrected for enrichment) and IE DG is the isotopic enrichment of plasma glucose (expressed as fractional abundance, moles fraction excess). For the data reported here, the rate of appearance of glucose was not corrected for the infusion rate of tracer, because there is evidence that even tracer doses of glucose will suppress endogenous glucose production proportionately (11). However, since there is controversy about the correct approach, we also repeated our calculations using such a correction (11). There was a slightly greater relative increase in HGO in the CF patients using this correction (also see "Results"), but the data describing the effects of the glucose infusions and tolbutamide therapy were virtually identical using this approach. HGO was equated to Ra DG in the fasting state. During the glucose infusion, HGO was calculated by subtracting the rate of glucose infusion from Ra DG.

The HGO data in the resting, baseline state in the two groups were compared when expressed per unit body weight and per

unit fat-free mass, to correct for differences in body composition between the two groups. Several (12–15) but not all (16) studies suggest that resting energy expenditure is abnormally elevated in CF patients. Because energy expenditure per unit cell then may be greater in children with CF compared with healthy children, we also compared fasting HGO data in the two groups as a function of resting energy expenditure estimated from the V_{CO_2} .

GC/MS. The enrichment of plasma glucose (IE DG) and the tracer was analyzed using the pentacetate derivative and electron impact, gas chromatography/mass spectrometry GC/MS (17). The following ions were monitored: 200(M), 201(M + 1), and 202(M + 2). We monitored the 201 ion to correct the 202 enrichment for excess enrichment produced by recycled 203 ion (*i.e.* the original deuterated tracer containing a naturally abundant ^{13}C atom) (18).

Measurement of body composition. Fat-free mass and percent of body fat were estimated from measurements of total body water determined using either ^{18}O -water or D_2O . After collecting a baseline urine sample (and emptying the bladder), subjects ingested either 0.3 g ^{18}O -water $\cdot$ kg body weight $^{-1}$ (or about 15 mmol $\cdot$ kg body weight $^{-1}$) (11.2%, MSD Isotope, Ontario, Canada) or 0.04 g $D_2O \cdot$ kg $^{-1}$ body weight (or about 2 mmol $\cdot$ kg weight $^{-1}$) (99%, MSD Isotope, Ontario, Canada). Urine samples were collected 3 and 4 h after tracer ingestion; the 4-h sample was used to determine the equilibrium enrichment of the body water. To determine enrichment of ^{18}O in the urine, samples were equilibrated with CO_2 for 72 h, and the gas sample was then cryogenically purified and then analyzed by gas isotope ratio mass spectrometry (Delta E, Finnigan MAT, Bremen, Germany) (17). D_2O enrichment was determined also by gas isotope ratio mass spectrometry using the zinc reduction method (19). Total body water was calculated using the deuterium or ^{18}O labeled isotopes after correction for isotopic fractionation (20, 21). An adjustment was made in the calculations for the fact that the D_2O space is about 3% larger than the $H_2^{18}O$ space (17). Using an estimate of the hydration of fat-free mass that was appropriate for sex, ethnicity, and maturation level (22), total body water was converted to fat-free weight.

Measurement of the V_{CO_2} and estimation of energy expenditure. Using a face mouthpiece, 2-way non-re-breathing valve, and Douglas bags, expired air was collected for two, 6-min periods in the fed and fasted state. Subjects, at rest and in a reclining position, were briefly accustomed to the apparatus before actually collecting expired air samples. A 60-ml aliquot was drawn from each bag and set aside for subsequent analysis of the fractional concentration of CO_2 , measured with a Beckman Industrial CO_2 analyzer (Beckman Instruments, Fullerton, CA). The volume of the timed collection was measured with a Rayfield gas meter (Rayfield System, Chicago, IL), which had been validated against a Tissot spirometer. Assuming a room air CO_2 concentration of 0.03%, V_{CO_2} was then calculated. Energy expenditure was estimated by standard equations (23) assuming a respiratory quotient of 0.834 (12).

Measurement of plasma and tracer concentrations of glucose, plasma concentrations of insulin and C peptide, and meal stimulation test. Glucose concentration was measured

using a YSI Glucose Analyzer (model 27, Yellow Springs Instrument Co., Yellow Springs, OH). Plasma insulin and C peptide were measured by radioimmunoassay using previously described methodology (24). The assay sensitivity for insulin was approximately $2.5 \mu U \cdot ml^{-1}$ (or $17.9 pmol \cdot L^{-1}$) and the assay sensitivity for C peptide was about $0.1 ng \cdot ml^{-1}$ (or $0.033 nmol \cdot L^{-1}$).

In the CF patients only, a meal stimulation test was conducted as previously described (6) as part of our initial screening of the subjects. The data were compared with laboratory control data from previous studies in nine healthy, prepubertal children not involved with the tracer studies.

Statistical design. Data are presented as mean $\pm$ SD. A two-sample *t* test was used to determine whether HGO differed between CF patients and healthy controls. A paired *t* test was used to evaluate the suppressibility of HGO when unlabeled glucose was infused and to assess the effect of tolbutamide on HGO. A probability level of 0.05 was selected for statistical significance.

RESULTS

Individual CF patient characteristics are presented in Table 1. The mean ages of the CF patients and healthy controls were respectively 9.1 ± 1.9 y and 9.0 ± 1.6 y. Fasting glucose concentration ($mg \cdot dl^{-1}$ or $mmol \cdot L^{-1}$) in the CF patients (84 ± 19 or 4.7 ± 1.1) was not statistically different from controls (81 ± 7 or 4.5 ± 0.4).

Insulin and C peptide concentrations during the last 30 min of the fasted tracer study (before tolbutamide therapy) were, respectively, $2.2 \pm 2.0 \mu U \cdot ml^{-1}$ ($15.8 \pm 14.4 pmol \cdot L^{-1}$) and $0.8 \pm 0.8 ng \cdot ml^{-1}$ ($0.26 \pm 0.26 nmol \cdot L^{-1}$) in the CF patients ($n = 8$) and $2.6 \pm 1.2 \mu U \cdot ml^{-1}$ ($18.7 \pm 8.6 pmol \cdot L^{-1}$) and $0.9 \pm 0.4 ng \cdot ml^{-1}$ ($0.30 \pm 0.13 nmol \cdot L^{-1}$) in the controls ($p > 0.05$ for both). There also were no differences in the means of the individual ratios for insulin/C peptide between the CF patients (3.4 ± 2.7) and controls (3.1 ± 1.5) or in the means for the fasting glucose/insulin concentration ratio between CF patients (0.025 ± 0.015) and controls (0.032 ± 0.013).

During the meal stimulation test, the mean area under the curve for glucose was not significantly different from that derived from the previously studied, laboratory controls ($15\ 650 \pm 1494$ versus $15\ 430 \pm 1599 mg \cdot dL^{-1} \cdot min$) (869 ± 83 versus $856 \pm 89 mmol \cdot L^{-1}$). The mean area under the curve for insulin was 40% of the control value (1099 ± 534 versus $2734 \pm 1091 \mu U \cdot ml^{-1} \cdot min$) (7885 ± 3831 versus $19\ 616 \pm 7828 pmol \cdot L^{-1} \cdot min$) ($p = 0.002$); the value in all CF patients was below the control mean, and in six of seven was greater than 1 SD below the mean (Fig. 1). The mean area under the curve for C peptide was 60% of the control mean ($p > 0.05$), and in four of seven was greater than 1 SD below the control mean (Fig. 2).

HGO ($mg \cdot kg$ body weight $^{-1} \cdot min^{-1}$ or $\mu mol \cdot kg$ body weight $^{-1} \cdot min^{-1}$) was 26% greater in CF patients (4.2 ± 0.7 or 23.3 ± 3.8) than in healthy controls (3.1 ± 0.6 or 17.2 ± 3.3) ($p < 0.05$) (Fig. 3); factoring in the tracer infusion in the

Figure 1. Insulin response in CF patients during meal stimulation test ($\mu\text{U} \cdot \text{ml}^{-1}$) (equal to $7.175 \text{ pmol} \cdot \text{L}^{-1}$) ($\bar{X} \pm \text{SD}$).

Figure 2. C Peptide response in CF patients during meal stimulation test ($\text{ng} \cdot \text{ml}^{-1}$) (equal to $0.333 \text{ nmol} \cdot \text{L}^{-1}$) ($\bar{X} \pm \text{SD}$).

HGO calculation resulted in a 38% increase in the CF patients ($p \approx 0.01$).

The CF patients seemed to be leaner than the controls although not to a statistically significant extent; percent of body fat was $14 \pm 6\%$ in the CF patients versus $20 \pm 8\%$ in controls ($p < 0.05$). However, when expressed as $\text{mg} \cdot \text{kg} \text{ fat free mass}^{-1} \cdot \text{min}^{-1}$ (or $\mu\text{mol} \cdot \text{kg} \text{ fat-free mass}^{-1} \cdot \text{min}^{-1}$) HGO was 27% higher in the CF group compared to the controls (4.9 ± 0.8 versus 3.8 ± 0.5) (or 27.2 ± 4.4 versus 21.1 ± 2.8) ($p = 0.015$) (Fig. 3). Mean resting energy expenditure ($\text{kcal} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was 29% higher but not significantly different in the CF patients (0.036 ± 0.005) compared with the healthy controls (0.028 ± 0.013). However, when expressed per unit energy expenditure ($\text{mg} \cdot \text{kcal}^{-1}$ or $\mu\text{mol} \cdot \text{kcal}^{-1}$), HGO in CF patients (121 ± 22 or 672 ± 122) was not statistically different from the controls (116 ± 30 or 644 ± 167).

As noted above, in seven of the CF patients, the glucose tracer infusion was continued for an additional 120 min during which a continuous infusion of unlabeled glucose also was administered ($\bar{X} \pm \text{SD}$, $0.90 \pm 0.02 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ or $5.0 \pm 0.11 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). HGO was suppressed by an amount approximately equal ($103 \pm 18\%$) to the glucose infusion (Fig. 4 and Table 2). Oral tolbutamide therapy for 2

Figure 3. Mean ($\pm \text{SD}$) hepatic glucose output ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (equal to $5.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in fasted healthy children (HC) and in children with CF (CF) in the fasted state. Mean $\pm \text{SD}$ values are presented adjusted for body weight and fat-free mass. Expressed per unit body weight, CF patients vs controls: $p < 0.05$; expressed per unit fat-free mass, CF vs controls: $p = 0.015$.

Figure 4. Mean ($\pm \text{SD}$) hepatic glucose output ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (equal to $5.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in patients with cystic fibrosis, in the fasting state and at the end of a two hour infusion of glucose ($\bar{X} \pm \text{SD}$, $0.90 \pm 0.02 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), both before (baseline) and after (Post-Tol) oral therapy with tolbutamide (750 mg/day). Hepatic glucose output was suppressed by an amount approximately equal ($103 \pm 18\%$) to the glucose infusion. There were no significant effects of tolbutamide therapy for 2 wk on either the fasting rate of hepatic glucose output or the degree of suppression by glucose.

wk (750 mg/d) (in 5 subjects only) had no effect on either the fasting rate of HGO or the degree of suppression by glucose (Table 2 and Fig. 4).

DISCUSSION

As can be seen from Figure 4, oral tolbutamide had no effect on the elevated rate of hepatic glucose production in the fasting state and had no effect on the suppression of hepatic glucose output by glucose infusion. The latter observation is not surprising considering our observation that, even in the baseline condition, a glucose infusion of approximately $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ suppressed HGO to an equivalent degree. In view of our earlier observations that tolbutamide therapy

Table 2. Individual data on HGO

Subject no.	Group	HGO (mg · kg ⁻¹ · min ⁻¹)			
		Fasting baseline	i.v. glucose baseline	Fasting post-tol	i.v. glucose post-tol
2029	CF	3.7	2.6	2.4	2.0
2012	CF	5.0	4.3		
2031	CF	5.5	4.6	3.8	2.8
2036	CF	3.5	2.4		
2035	CF	4.2	3.5	5.0	3.7
2038	CF	3.5	2.5	3.6	2.7
2039	CF	3.9	2.9		
2041	CF	4.5		5.4	3.2
$\bar{X} \pm SD$		4.2 ± 0.7*	3.3 ± 9†	4.0 ± 1.2‡	2.9 ± 0.6†‡
2030	HC	2.6			
2032	HC	3.7			
2033	HC	2.7			
2034	HC	3.8			
2037	HC	2.6			
$\bar{X} \pm SD$		3.1 ± 0.6			

Abbreviations used: HC, healthy controls; post-tol, post tolbutamide therapy.

* $p = 0.016$ compared to fasted, control.

† Compared to fasting same treatment: baseline, $p < 0.0001$; post-tol, $p = 0.02$.

‡ $p > 0.05$ fasting post-tol vs fasting baseline; i.v. glucose post-tol vs i.v. glucose baseline.

seemed to enhance accretion of fat-free mass in children with CF (6), the present results suggest that the putative effect of this drug on growth is not related to its possible inhibitory effects on hepatic gluconeogenesis (25). This view coincides with recent thinking that an accelerated rate of gluconeogenesis does not increase whole body proteolysis in trauma (26, 27).

Despite normal clinical tolerance of glucose and normal fasting concentration of glucose, insulin, and C peptide, the CF patients demonstrated higher rates of endogenous glucose production compared with normal children. Because we observed on the basis of the C peptide concentration, no evidence for decreased insulin secretion in the fasting state, we wondered whether the CF patients were demonstrating hepatic insulin resistance. There is evidence in the literature for both decreased (3) and increased (4) peripheral sensitivity of glucose metabolism to insulin in CF patients, but, to our knowledge, hepatic glucose output has not been studied, at least in prepubertal children with the disease. However insulin/C peptide ratios also were not different in the two groups (implying no difference in hepatic insulin extraction), and the glucose infusion was associated with an equivalent suppression of HGO, suggesting normal hepatic sensitivity to insulin (although glucose not insulin was infused) (9). Thus, there was no evidence for hepatic insulin resistance. Moreover, although we have not formally studied peripheral insulin sensitivity in these patients, it is of interest that insulin to glucose ratios were not different from the healthy control subjects.

Normalizing the HGO data for the fat-free mass did not appreciably reduce the percentage difference observed between CF patients and controls, but if the intensity of energy metabolism per unit cell was greater in the CF patients, then elevated HGO would seem to be an adaptive response to the need for

greater glucose uptake. In fact, there is some evidence that interindividual differences in insulin-mediated glucose disposal can best be explained or normalized by using metabolic rate (28). In our study, mean resting energy expenditure was not elevated to a statistically significant degree in the CF patients (despite a 29% higher rate), but when HGO (mg/min) was normalized for resting energy expenditure (kcal/min), a difference in HGO between CF patients and controls was no longer identified. Thus, elevated glucose turnover seems to have been the appropriate response to increased glucose, and perhaps energy, needs. Estimation of energy expenditure was based on an assumed respiratory quotient, but this is unlikely to have caused much error in this value (29).

Although our CF patients were afebrile and clinically stable, it is quite plausible that subclinical or incipient inflammation of the lungs also may have contributed to hormonal stress that would lead to elevated HGO. However, counter-regulatory hormone secretion was not assessed in this study; therefore, we are uncertain about the hormonal chain of events that "permitted" HGO to increase above normal and thus, presumably, meet elevated glucose and energy needs.

In summary, glucose tolerant CF patients, with abnormally diminished insulin secretion in the fed state, demonstrated an increase in fasting, steady-state HGO that was balanced by increased glucose uptake and proportional to an increase in energy expenditure. HGO was apparently normally regulated by exogenous glucose intake (via insulin presumably). Future exploration of the mechanism responsible for the observed increase in HGO might include detailed study of counter regulatory hormone levels and peripheral insulin sensitivity.

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REFERENCES

1. Lannig S, Thorsteinsson B, Erichsen G, Nerup J, Koch C 1991 Glucose tolerance in cystic fibrosis. *Arch Dis Child* 66:612-616
2. Handwerger S, Roth J, Gorden P, Di Sant'Agnese P, Carpenter DF, Peter G 1969 Glucose intolerance in cystic fibrosis. *N Engl J Med* 281:451-461
3. Andersen O, Garne S, Heilmann C, Petersen KE, Petersen W, Koch C 1988 Glucose tolerance and insulin receptor binding to monocytes and erythrocytes in patients with cystic fibrosis. *Acta Paediatr Scand* 77:67-71
4. Ahmad T, Nelson R, Taylor R 1994 Insulin sensitivity and metabolic clearance rate of insulin in cystic fibrosis. *Metabolism* 43:163-167
5. Lannig S, Thorsteinsson B, Nerup J, Koch C 1992 Influence of the development of diabetes mellitus on clinical status in patients with cystic fibrosis. *Eur J Pediatr* 151:684-687
6. Zipf WB, Kien CL, Horswill CA, McCoy KS, O'Dorisio T, Pinyard BL 1991 Effects of tolbutamide on growth and body composition of nondiabetic children with cystic fibrosis. *Pediatr Res* 30:309-314
7. Zipf WB, Kien CL, Horswill CA, McCoy KS 1993 Effects of feeding on daily protein turnover balance (DPTB) in cystic fibrosis. *FASEB J* 7:A178
8. Tanner JM 1962 Growth at Adolescence, 2nd Ed. Blackwell Scientific Publications, Oxford, pp 28-36
9. Wolfe RR, Allsop JR, Burke JF 1979 Glucose metabolism in man: responses to intravenous glucose infusion. *Metabolism* 28:210-220

10. Catalano PM, Tyzbir ED, Wolfe RR, Calles J, Roman NM, Amini SB, Sims EAH 1993 Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. *Am J Physiol* 264:E60–E67
11. Jahoor F, Peters EJ, Wolfe RR 1990 The relationship between gluconeogenic substrate supply and glucose production in humans. *Am J Physiol* 258:E288–E296
12. Spicher V, Roulet M, Schutz Y 1991 Assessment of total energy expenditure in free-living patients with cystic fibrosis. *J Pediatr* 118:865–872
13. Grunow JE, Azcue MP, Berall G, Pencharz PB 1993 Energy expenditure in cystic fibrosis during activities of daily living. *J Pediatr* 122:243–246
14. Tomezsko JL, Stallings VA, Scanlin TF 1989 Resting energy expenditure (REE) in children with cystic fibrosis (CF). *Pediatr Res* 25:126A (abstr)
15. Vaisman N, Pencharz PB, Corey M, Canny GJ, Hahn E 1987 Energy expenditure of patients with cystic fibrosis. *J Pediatr* 111:496–500
16. Fried MD, Durie PR, Tsui L-C, Corey M, Levison H, Pencharz PB 1991 The cystic fibrosis gene and resting energy expenditure. *J Pediatr* 119:913–916
17. Wolfe RR 1992 Radioactive and stable isotope tracers in biomedicine. In: *Principles and Practice of Kinetic Analysis*. John Wiley & Sons, New York, pp 425–426
18. Weber JM, Klein S, Wolfe RR 1990 Role of the glucose cycle in control of net glucose flux in exercising humans. *J Appl Physiol* 68:1815–1819
19. Wong WW, Lee LS, Klein PD 1987 Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva and human milk. *Am J Clin Nutr* 45:905–913
20. Wong WW, Cochran WJ, Klish WJ, Smith EO'B, Lee LS, Klein PD 1988 *In vivo* isotope-fractionation factors and the measurement of deuterium- and oxygen-18-dilution spaces from plasma, urine, saliva, respiratory, water vapor, and carbon dioxide. *Am J Clin Nutr* 47:1–6
21. Schoeller DA, Dietz W, Van Santen E, Klein PD 1982 Validation of saliva sampling for total body water determination by $H_2^{18}O$ dilution. *Am J Clin Nutr* 35:591–594
22. Boileau RA, Lohman TG, Slaughter MH, Ball TE, Going SB, Hendrix MK 1984 Hydration of the fat-free body in children during maturation. *Hum Biol* 56:651–666
23. Black AE, Prentice AM, Coward WA 1986 Use of food quotients to predict respiratory quotients for the doubly-labeled water method of measuring energy expenditure. *Hum Nutr Clin Nutr* 40C:381–391
24. Osei K, Holland GC 1987 Altered C-peptide/insulin molar ratios and glucose turnover rates after stimulation in nondiabetic offsprings of type II diabetic patients. *Metabolism* 36:122–127
25. Cabello MA, Rodriguez-Tarduchy G, Ortega JL, Samper B, Feliu JE 1991 Inhibition of gluconeogenesis by tolbutamide in isolated rat hepatocytes: modulation of glucose-6-phosphate substrate cycle. *Metabolism* 9:934–940
26. Jahoor F, Shangraw RE, Miyoshi H, Wallfish H, Herndon DN, Wolfe RR 1989 Role of insulin and glucose oxidation in mediating the protein catabolism of burns and sepsis. *Am J Physiol* 257:E323–E331
27. Wolfe RR, Jahoor F, Hartl WH 1989 Protein and amino acid metabolism after injury. *Diabetes Metab Rev* 5:149–164
28. Lillioja S, Bogardus C 1988 Obesity and insulin resistance: lessons learned from the Pima Indians. *Diabetes Metab Rev* 4:517–540
29. Elia M 1991 Energy equivalents of CO_2 and their importance in assessing energy expenditure when using tracer techniques. *Am J Physiol* 260:E75–E88