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Short-term effects of recombinant human growth hormone and feeding on gluconeogenesis in humans

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Abstract

After a short-term fast, lactating women have increased rates of glucose production but not gluconeogenesis (GNG) despite relative hypoinsulinemia. We explored the effects of non–insulin-dependent increase in glucose utilization and recombinant human growth hormone (rhGH) on glucose production, glycogenolysis, and GNG in both the fed and overnight-fasted condition. Six controls and 7 lactating women were studied twice, in random order, after 7 days of saline or rhGH. Glucose kinetics and GNG were measured using [U-^{13}C]glucose mass isotopomer distribution analysis. The rhGH increased milk production in the lactating women and insulin-like growth factor (IGF) in both groups. Glycogenolysis and GNG were higher in fasting lactating women than controls after either saline or rhGH ($P < .05$). After rhGH administration, GNG remained higher ($P < .02$) in the lactating women than controls. Gluconeogenesis was not suppressed in either group during 5 hours of continuous meal ingestion, despite a 5-fold increase in plasma insulin. Lactating women had similar glucose but lower insulin and C-peptide concentrations than controls after both rhGH and saline treatment ($P < .01$), although rhGH decreased ($P < .01$) insulin sensitivity in both groups ($P < .05$). Gluconeogenesis is not affected by short-term increases in insulin and/or rhGH, which suggests a fundamental rethinking of the role of insulin in acutely regulating GNG.

1. Introduction

Over the past several years, we have studied the impact of lactation on maternal metabolism of glucose and other endogenous substrates. We have demonstrated that fasting lactating women have a 30% higher rate of glucose turnover after 24 hours of fasting and that the primary (but not exclusive) source of milk lactose is plasma glucose [1,2]. In addition, recombinant growth hormone increases milk production in lactating women, as it does in other mammalian species, and thus potentially places further stress on the glucose production of these normal women [3-5].

Much is known about the regulation of glucose production in human under a wide variety of physiologic and nonphysiologic conditions. However, we have not had the investigative tools to partition glucose production into that from glycogenolysis and gluconeogenesis until recently [6-10]. As a result, we know little of the substrate and hormone factors that actually regulate gluconeogenesis in humans. Thus, the lactating woman provides a unique model to explore the regulation of glucose production from gluconeogenesis and glycogenolysis during both feeding and fasting, as well as to determine the effects of recombinant human growth hormone (rhGH) on these processes.

We designed the present studies to explore the effects of feeding and rhGH on gluconeogenesis in healthy women with normal glucose homeostasis and with accelerated non–insulin-mediated increased glucose disposal as result of lactation.

2. Materials and method

2.1. Tracers

Sterile and pyrogen-free [U-^{13}C]glucose (99 atom% ^{13}C; 93.8% $^{13}C_6$) was purchased from Cambridge Isotope Laboratories (Andover, MA) and prepared as previously described [10].

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2.2. Study design

2.2.1. Subjects

After approval by the Institutional Review Board for Human Subjects at Baylor College of Medicine and the General Clinical Research Center (GCRC) Advisory Committee, written consent was obtained from each of the subjects. Seven healthy lactating women (age, 30.7 ± 2.6 years; body mass index, 24.8 ± 1.0 kg/m^2; height, 162.7 ± 2.2 cm; and weight, 65.3 ± 2.5 kg [mean ± SEM]; 6 white and 1 African American and their healthy infants) and 6 healthy, nonpregnant, controls (age, 23.5 ± 2.5 years; body mass index, 23.6 ± 1.0 kg/m^2; height, 161.6 ± 3.0 cm; and weight, 61.3 ± 3.1 kg [mean ± SEM]; 2 white, 2 African American, and 2 American Hispanic) were recruited. All women studied had a negative pregnancy test result at the time of study. Their infants weighed 5.49 ± 0.16 kg at the time of the initial study and weighed 6.01 ± 0.24 kg at the time of the second study. To exclude any pregnancy-related changes in hormones, all lactating women were studied between 6 and 12 weeks postpartum. This is a period during which most women are exclusively breast-feeding their infants and during which milk production is quite constant [11-16]. No attempt was made to study the control women at a specific time in their menstrual cycle. None of the women had a history of gestational diabetes or first-degree relative with diabetes. None was taking any medications that would affect insulin sensitivity.

2.3. Protocol

Each woman (and her infant for lactating subjects) was admitted to the GCRC on the evening before study. At 6:00 PM, 2 intravenous catheters were introduced under Emla cream analgesia, one in the antecubital fossa or forearm vein for isotope infusion and the other in the dorsum of the hand or in a forearm vein of the contralateral arm for blood sampling. Subjects were fed a supper meal of 10 kcal/kg at around 6:00 PM and were subsequently fasted, except for water, overnight. Intravenous fluid of 0.45 normal saline was started at 3:00 AM at the rate of 20 mL/h. At 4:00 AM, baseline breast milk (lactating subjects) and blood samples (7 mL) were obtained after which the subject received a primed constant rate infusion of [U-^{13}C]glucose (60 μmol/kg, 1.04 ± 0.01 μmol kg^{-1} min^{-1} during the fasting period; and 80 μmol/kg, 2.72 ± 0.06 μmol kg^{-1} min^{-1} during the feeding period). Blood samples were collected at 0, 180, 240, 255, 270, 285, and 300 minutes of the study time. Five minutes before blood sampling, the hand was placed in a hot pad to obtain arterialized venous blood [17,18]. At 300 minutes of study time, a Boost High Protein (HP) drink (caloric source is 55% carbohydrates, 20% fat, and 25% protein; Novartis Medical Nutrition U.S., Minneapolis, MN) was taken orally every 15 minutes in the amount of 34 g (36 kcal per drink) for control and 45 g (48 kcal per drink) for lactating women for the following 360 minutes. The difference in caloric intake reflects the estimated need to maintain an isocaloric intake due to the caloric loss for milk production [1]. A total of 36 drinks were given throughout the feeding phase. During the feeding time, blood samples were obtained at 480, 660, 705, 720, 735, 750, 765, 780, 795, 810, 825, and 840 minutes. Infants were fed from both breasts (alternatively) at each nursing and were weighed before and after each feeding. Attempts were made to breast-feed the infants on a 3-hourly schedule. A breast pump was used to empty the breasts and collect milk samples at the end of every feeding.

The women were studied on 2 separate occasions in random order: after 7 days of subcutaneous injections of either isotonic sodium chloride solution or rhGH (0.05 mg kg^{-1} d^{-1}). The rhGH and saline were given by our nurse in the afternoon time. The basis of 7 days was selected because of its proven effects on milk production and glucose homeostasis [4,5,19]. Subjects were blinded to the administration of rhGH or saline. The studies were separated by a "washout" period of 2 weeks.

2.4. Analytical methods

Plasma glucose was measured using a glucose analyzer (YSI Glucose Analyzer, Yellow Springs, OH). Plasma insulin, glucagon, and C-peptide were measured using radioimmunoassay kits (Linco Research, St Charles, MO) (interassay coefficient of variation of replicate analyses, 15% for both). Insulin-like growth factor (IGF)-I concentrations were measured by radioimmunoassay (Esoterix, Calabasas Hills, CA). Free fatty acids (FFA) and β-hydroxybutyrate (β-OHB) were measured using a Cobas Fara II Analyzer (Roche Diagnostic Systems, Montclair, NJ). The pentaacetate derivative of glucose was prepared, and the samples were analyzed using gas chromatography–mass spectometry in the positive chemical ionization mode as described previously [3]. Milk samples were defatted, deproteinized, derivatized, and analyzed as previously described [2].

2.5. Calculations

2.5.1. Total glucose entry and glucose production

The total rate of appearance (R_a) of glucose into the systemic circulation was calculated under near steady-state condition (coefficient of variation ≤10% and a slope not different from zero) using standard isotope dilution equation:

$$R_{a-total} = [Ei/Ep] \times I,$$

where E_i and E_p are the enrichments of the [U-^{13}C]glucose in the infusate and the plasma, respectively, and I is the rate of infusion of the [U-^{13}C]glucose.

In the fasting state, glucose production rate (GPR) was calculated by subtracting the amount of infused [U-^{13}C] glucose from the glucose R_a:

$$GPR = R_{a-total} - I$$

2.5.2. Gluconeogenesis

Using mass isotopomer distribution analysis as described by Haymond and Sunehag [10], the fraction of glucose

derived from gluconeogenesis (GNG %) was calculated using the following formula:

$$GNG\ \% = \left[\sum_{M1-M5} / \sum_{M1-M6}\right] \times \left[\sum_{M1-M5}(^{12}C)\right] / \left[\sum_{M1-M5}(^{13}C)\right],$$

where $M1$ is the glucose molecule with one ^{13}C (labeled carbon) and five ^{12}C (unlabeled carbons) in any position, $M2$ is the glucose molecule with two ^{13}C and four ^{12}C in any position, and so forth. $M6$ is the glucose molecule that is uniformly labeled with ^{13}C and has no ^{12}C.

The rate of gluconeogenesis (GNG) is:

$$GNG = GNG\ \% \times R_{a-total}$$

2.5.3. Glycogenolysis

The rate of glycogenolysis during fasting was determined as:

$$\text{Glycogenolysis}_{fast} = GPR - GNG.$$

During meal ingestion, rates of glycogenolysis were estimated from the carbohydrate content in the Boost drink (~14 g/100 mL). We previously demonstrated that ~75% of orally ingested glucose enters the systemic circulation during both saline and rhGH treatment [19] and that the splanchnic extraction of glucose in lactating and nonlactating women is essentially identical [10]. Thus, knowing the rate of ingestion of glucose and the rate of appearance of dietary glucose into the plasma pool ($R_{a\text{-}meal}$), the rate of glycogenolysis during feeding was estimated as:

$$Glycogenolysis_{feed} = R_{a-total} - (GNG + R_{a-meal})$$

2.5.4. Insulin sensitivity

Insulin sensitivity in the fasting condition was calculated from the averaged baseline insulin and glucose values using the homeostasis model assessment ($HOMA_R$) and quantitative insulin sensitivity check index (QUICKI) methods [20] according to the following formulae:

$$HOMA_R = [\text{glucose(in millimoles per liter)} \times \text{insulin (in microunits per milliliter)}]/22.5$$

$$QUICKI = 1/[\log \text{glucose(in milligrams per deciliter)} \times \log \text{insulin(in microunits per milliliter)}]$$

2.5.5. Milk production

Milk production was calculated by adding the milk volume expressed from the breast to the milk consumed by infant. The latter was determined as the difference in infant's weight just before and immediately after each feeding (assuming milk specific gravity of 1.0). Milk production was expressed as milliliters per kilogram (of infant's weight) per hour of feeding.

2.5.6. Fractional hexoneogenesis

Hexoneogenesis of milk glucose and galactose and the impact of rhGH, feeding, and fasting on this process were measured using the isotopomer distribution (M + 1, M + 2, M + 3, and M + 6) in the milk glucose and galactose vs that of plasma glucose [2].

2.6. Statistical analysis

Values over the steady-state period (240-300 and 780-840 minutes, respectively) were averaged for each subject, and the mean ± SEM was calculated for each group. Data within group were compared using a paired Student t test, and data between groups were compared using a nonpaired t test. Because we hypothesized that rhGH would increase the rate of gluconeogenesis, we used 1-tailed t test in our statistical analyses. Comparisons were made between:

a) Effect of saline vs rhGH in each group of women during feeding and fasting.
b) Difference between lactating women and controls after the administration of either saline or rhGH, and during feeding and fasting.
c) Effect of fasting vs feeding conditions within each group.

3. Results

3.1. Effect of rhGH on IGF-I, milk volume, and hexoneogenesis

Baseline IGF-I concentration was higher in control women than lactating women (223 ± 30 vs 129 ± 9 ng/mL, respectively; $P < .05$). After the administration of rhGH, IGF-I concentration increased ($P < .01$) in both groups to nearly identical concentrations (385 ± 30 vs 375 ± 42 ng/mL, respectively); the increase was greater in lactating women than controls ($P = .03$ using the Δ values). The rhGH increased milk volume from 5.3 ± 0.3 to 6.6 ± 0.2 mL kg^{-1} (of infant's weight) h^{-1} ($P < .01$), a 25% increase.

The isotopomer distribution in plasma glucose was essentially identical to that in milk glucose and galactose after rhGH or saline regardless of feeding and fasting. Thus, we averaged the data obtained after the administration of rhGH and saline studies for each individual. By comparing the M + 6 in milk glucose and galactose with that of plasma glucose (as previously described) [2], the calculated contribution of plasma glucose to milk lactose was 39% ± 4% during fasting and 73% ± 2% during feeding (Table 1).

3.2. Plasma substrate and hormone concentrations

3.2.1. Fasting

After 14 hours of fasting, the plasma glucose, FFA, and β-OHB concentrations were similar in the control and lactating subjects regardless of rhGH or saline treatment (Table 2). In contrast, the plasma insulin and C-peptide

Table 1
The ratios of the isotopomer distribution (M + 1, M + 2, M + 3, and M + 6) in milk glucose and galactose to that in plasma glucose in lactating women after 7 days of either saline or rhGH injections

	Fasting							
	Milk glucose/plasma glucose				Milk galactose/plasma glucose			
	M + 1	M + 2	M + 3	M + 6	M + 1	M + 2	M + 3	M + 6
Saline	1.07 ± 0.02	0.94 ± 0.02	0.52 ± 0.05	0.46 ± 0.09	1.01 ± 0.01	1.02 ± 0.02	0.92 ± 0.09	0.31 ± 0.06
rhGH	1.07 ± 0.02	0.97 ± 0.03	0.60 ± 0.05	0.47 ± 0.05	1.01 ± 0.01	1.05 ± 0.02	0.94 ± 0.05	0.30 ± 0.03
	Fasting							
	Milk glucose/plasma glucose				Milk galactose/plasma glucose			
	M + 1	M + 2	M + 3	M + 6	M + 1	M + 2	M + 3	M + 6
Saline	1.09 ± 0.01	1.10 ± 0.03	0.96 ± 0.12	0.81 ± 0.03	1.10 ± 0.01	1.46 ± 0.09	2.94 ± 0.48	0.60 ± 0.02
rhGH	1.10 ± 0.01	1.11 ± 0.03	1.19 ± 0.09	0.90 ± 0.05	1.13 ± 0.01	1.52 ± 0.03	4.06 ± 0.21	0.65 ± 0.04

Fasting was 14 hours after the last meal at supper, and feeding was after 6 hours of continuous consumption of small feedings of Boost HP.

concentrations were higher in the controls when compared with lactating women after saline injections (insulin: 9.7 ± 1.8 vs 4.7 ± 0.6 μU/mL, respectively, $P < .05$; C-peptide: 1.88 ± 0.14 vs 0.99 ± 0.17 ng/mL, respectively, $P < .05$). The rhGH treatment increased ($P < .05$) the plasma insulin and C-peptide concentrations in both the control and lactating women (insulin: 21.9 ± 5.1 and 11.8 ± 0.9 μU/mL, control vs lactating women, respectively; and C-peptide: 2.94 ± 0.60 vs 2.40 ± 0.28 μU/mL, respectively), and the difference ($P < .05$) between the groups was maintained regarding insulin but not C-peptide (Table 2). No differences were observed in plasma glucagon concentrations between the lactating and control women after either saline or growth hormone (Table 2).

The insulin sensitivity was greater in the lactating women than in the control women (HOMA: 0.96 ± 0.12 vs 2.09 ± 0.41, $P < .01$; QUICKI: 0.84 ± 0.10 vs 0.58 ± 0.07, $P < .02$, respectively). The rhGH treatment decreased insulin sensitivity (HOMA: 2.86 ± 0.22 and 3.38 ± 1.66 for the lactating and control women, respectively, $P < .05$ and $P < .01$, respectively; and QUICKI: 0.47± 0.01 and 0.41 ± 0.04, respectively, $P < .01$ for both), but the relative difference between the lactating and nonlactating women was maintained.

3.2.2. Feeding

Continuous feeding of Boost HP over a 6-hour period increased ($P < .05$) the plasma glucose concentrations in control and lactating women without rhGH and in the lactating women during rhGH treatment (Table 2). However, no significant change was observed during rhGH treatment in the control women. Plasma FFA and β-OHB concentrations were suppressed ($P < .05$) as a result of feeding in both groups of subjects. No differences were observed between the lactating and control women regardless of rhGH treatment (Table 2). In contrast to the plasma glucose concentrations, which increased by only 15% to 20%, plasma insulin and C-peptide concentrations nearly doubled ($P < .05$) with feeding in both groups after saline injections and were again higher in the control women (insulin: 49.3 ±

Table 2
Plasma substrate and hormone concentrations in control and lactating women after 7 days of either saline or rhGH injections

	Control women				Lactating women			
	Fasting		Feeding		Fasting		Feeding	
	Saline	rhGH	Saline	rhGH	Saline	rhGH	Saline	rhGH
Glucose (mmol/L)	4.9 ± 0.1	5.3 ± 0.3	5.6 ± 0.1 [e]	5.7 ± 0.1	4.6 ± 0.1	5.4 ± 0.1 [a]	5.6 ± 0.2 [e]	6.0 ± 0.1 [e]
Insulin (μU/mL)	9.7 ± 1.8	21.9 ± 5.2 [a]	49.3 ± 3.0 [e]	107.5 ± 26.0 [a, e]	4.7 ± 0.6 [c]	11.8 ± 0.9 [a, c]	26.7 ± 3.8 [c, e]	51.9 ± 13.5 [a, c, e]
C-peptide (ng/mL)	1.88 ± 0.14	2.94 ± 0.60 [a]	7.03 ± 0.69	9.19 ± 1.25	0.99 ± 0.17	2.40 ± 0.28 [a]	5.65 ± 1.00	8.04 ± 1.51
Glucagon (pg/mL)	47.7 ± 6.3	38.3 ± 6.6	53.9 ± 4.3	43.5 ± 5.1	53.4 ± 5.4	48.0 ± 6.1	57.6 ± 5.8	56.6 ± 3.4
FFA (mmol/L)	0.49 ± 0.13	0.52 ±0.11	0.05 ± 0.02 [e]	0.06 ± 0.03 [e]	0.46 ± 0.08	0.70 ± 0.06	0.06 ± 0.01 [e]	0.11 ± 0.02 [e]
β-OHB (μmol/L)	0.07 ± 0.01	0.24 ± 0.11	0.04 ± 0.01	0.04 ± 0.01	0.13 ± 0.05	0.19 ± 0.04	0.04 ± 0.01	0.05 ± 0.01 [e]
HOMA	2.09 ± 0.41	5.38 ± 1.66 [a]			0.96 ± 0.12 [d]	2.86 ± 0.22 [b]		
QUICKI	0.68 ± 0.07	0.41 ± 0.04 [b]			0.84 ± 0.10	0.47 ± 0.01 [b]		

Fasting was 14 hours after the last meal at supper, and feeding was after 6 hours of continuous consumption of small feedings of Boost HP.

[a] $P < .05$ rhGH vs saline.
[b] $P < .01$ rhGH vs saline.
[c] $P < .05$ lactating vs control.
[d] $P < .01$ lactating vs control.
[e] $P < .05$ feeding vs fasting.

3.0 vs 26.7 ± 3.8 μU/mL, $P < .05$; C-peptide: 7.03 ± 0.69 vs 5.65 ± 1.00 ng/mL, respectively, $P < .05$) despite a higher rate of carbohydrate ingestion in the lactating women. The rhGH treatment resulted in doubling of the insulin concentrations in both groups (insulin: 107.5 ± 26.0 μU/mL in controls vs 51.9 ± 13.5 μU/mL in the lactating women, respectively, $P < .05$). The plasma C-peptide concentrations were statistically unaffected by rhGH (9.19 ± 1.25 vs 8.04 ± 1.51 ng/mL), but the relative difference ($P < .05$) between the lactating and control women was unchanged (Table 2). Plasma glucagon concentrations were similar in both groups with or without growth hormone (Table 2).

3.3. Glucose kinetics

3.3.1. Fasting

In the postabsorptive 14-hour fasted condition and after saline injection, the rate of glucose production in the lactating women was 30% higher than that in the controls (13.4 ± 0.4 vs 10.2 ± 0.4 μmol kg^{-1} min^{-1}, $P < .05$). In this study of lactating women, the increase in glucose production when compared with that of the control women was the result of a nearly 46 % increase in the rate of glycogenolysis (7.0 ± 0.4 vs 4.8 ± 0.3 μmol kg^{-1} min^{-1}, $P < .05$) and a 23% increase in gluconeogenesis (6.2 ± 0.5 vs 5.0 ± 0.3 μmol kg^{-1} min^{-1}, $P < .05$) (Fig. 1).

The rhGH treatment increased glucose production in the lactating women from 13.4 ± 0.4 to 15.8 ± 0.5 μmol kg^{-1} min^{-1} ($P < .01$) but not in the controls (10.2 ± 0.4 vs 11.8 ± 0.9, $P = .15$). The relatively higher ($P < .05$) rate of glucose production was maintained in the lactating women when compared with control women.

The rhGH treatment had no effect on gluconeogenesis in the control women but increased gluconeogenesis in the lactating women (6.2 ± 0.5 vs 8.2 ± 0.7 μmol kg^{-1} min^{-1}, $P < .05$). The rates of glycogenolysis ($P < .05$) but not gluconeogenesis were lower in the control compared with the lactating women.

3.3.2. Feeding

During meal absorption after saline injection, the total rate of systemic glucose appearance was increased ($P < .01$) to 27.9 ± 0.9 and 36.5 ± 2.4 μmol kg^{-1} min^{-1} in the control and lactating women, respectively. As described above, the lactating women ingested Boost HP at a higher rate than the controls to maintain energy neutrality, which accounted for the higher total glucose rate of appearance in the lactating women. Using previously derived estimates of systemic entry of dietary glucose [19], we estimated the entry rate of dietary glucose into the systemic circulation to be 22.6 ± 0 vs 28 ± 0 μmol kg^{-1} min^{-1} in the control and lactating women, respectively, during both the saline and rhGH studies.

The rates of gluconeogenesis were 8.0 ± 1.3 and 7.7 ± 1.1 μmol kg^{-1} min^{-1} in the control and lactating women, respectively. Thus, the rates of gluconeogenesis did not decrease during meal absorption. They actually increased slightly from the fasted state in the control subjects ($P < .05$), whereas no change was observed in the lactating women (Fig. 1). The rate of glycogenolysis was suppressed ($P < .05$) to values indistinguishable ($P > .40$) from 0 (−1.3 ± 1.9 and 2.3 ± 2.4 μmol kg^{-1} min^{-1} in the control and lactating women, respectively) (Fig. 1).

During meal absorption, rhGH treatment had no effect on the total rate of entry of glucose (27.9 ± 0.9 vs 38.0 ± 1.5 μmol kg^{-1} min^{-1} in the control and lactating women,

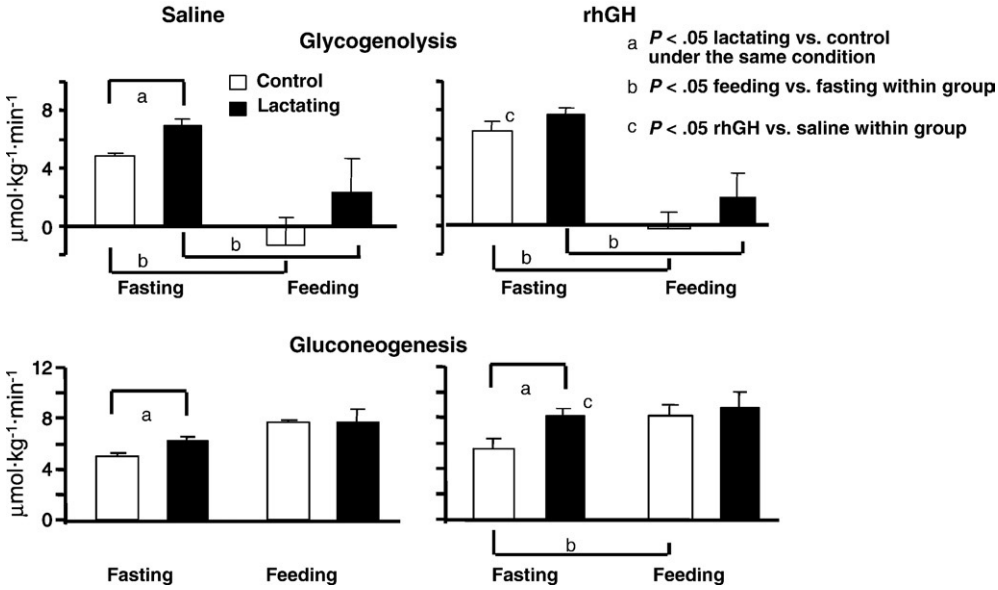

Fig. 1. Effects of 7 days of either saline or rhGH injections on glucose rate of appearance, gluconeogenesis, and glycogenolysis in control and lactating women after 14 hours of fasting or 6 hours of continuous feeding with Boost HP. [a]$P < .05$ rhGH vs saline within group, [b]$P < .05$ feeding vs fasting within group, [c]$P < .05$ lactating vs control under the same treatment condition.

respectively); but the difference between the 2 groups was maintained. The rates of gluconeogenesis were 8.2 ± 1.0 vs 8.8 ± 1.4 μmol kg^{-1} min^{-1} in the control and lactating women, respectively. The calculated rates of glycogenolysis were again suppressed to values indistinguishable from zero (−0.2 ± 1.2 vs 1.8 ± 1.7 μmol kg^{-1} min^{-1} in the control and lactating women, respectively) (Fig. 1).

4. Discussion

Until recently, we have long accepted that glucose production after an overnight fast is almost exclusively from glycogenolysis and that, with fasting, glucose production from gluconeogenesis increases as glycogenolysis decreases [21]. Euglycemic hyperinsulinemic clamp studies have reinforced this assumption because the endogenous rate of glucose production has been reported to be suppressed to zero (or below) [22]. However, with improved techniques, glucose production values during euglycemic hyperinsulinemic clamps are reported to be between 0 and 1 mg kg^{-1} min^{-1} in adult volunteers [22]. No data exist demonstrating whether this residual glucose production is from gluconeogenesis, glycogenolysis, or an artifact of the methodology.

Despite the proposal of the Cori Cycle in 1929 [23], regulation of glucose production from gluconeogenesis has long been inferred from a variety of indirect measurements [24-32]. Because of the interconversion of carbon atoms among the gluconeogenic precursors (lactate, pyruvate, alanine, glutamine, and glutamate) from cycling in and out of the tricarboxylic acid cycle and the lack of an accurate measure of the in vivo precursor pool(s), these isotope dilution methods failed to provide a total integrated rate of gluconeogenesis in vivo [6-8]. We now have a variety of isotopic methodologies that are generally accepted with which to measure gluconeogenesis in vivo [7-10].

Despite these advancements, the measurement of gluconeogenesis in vivo continues to be problematic. Accurate measurement of the fraction of glucose derived from gluconeogenesis (or the change in the fraction derived from gluconeogenesis) requires that the entire glucose pool has come to a new near steady state for both isotopic enrichment and substrate concentration, which under basal conditions take 4 to 5 hours [1]. This excludes the use of non–steady-state conditions such as a single bolus feed for an experimental protocol. Therefore, to achieve near substrate and isotopic steady-state conditions, we used the continuously feed model, which may have blunted the rise in plasma glucose but did result in a 2- to 3-fold increase in glucose turnover and a 5- to 6-fold increase in plasma insulin during the feed portion of these studies.

The present studies using [U-^{13}C]glucose support our original observation using [2-^{13}C]glycerol [1] that gluconeogenesis is not suppressed during meal absorption despite significant hyperinsulinemia. In addition, a week of high-dose subcutaneous rhGH had little effect on gluconeogenesis in either the control or lactating women (Table 1, Fig. 1) [1]. The rate of gluconeogenesis actually increased in the controls during the 6 hours of continuous meal ingestion. Our data are in concordance with those from euglycemic hyperinsulinemic clamps in normal volunteers and subcutaneous insulin injection in individuals with type 1 diabetes mellitus, despite relatively short periods of isotope infusions [33,34]. Thus, we conclude that gluconeogenesis in humans is not affected by acute changes (4 to 6 hours) in plasma insulin. Estimating the fraction of enterally ingested glucose delivered to the systemic circulation [19], we found that glycogenolysis was essentially totally suppressed during meal ingestion. This is in complete agreement with the well-established in vitro effects of insulin on glycogenolysis and glycogen synthesis [35].

As has been known for decades [19,36], acute administration of human growth hormone resulted in insulin resistance. Despite induction of insulin resistance, rhGH had little or no effect on the plasma concentrations of glucose, FFA, or β-OHB after either a 14-hour fast or 6 hours of continuous feeding. Recombinant human growth hormone increased insulin resistance in both groups of subjects but did not affect the relative difference in insulin resistance between the 2 groups. The rhGH-induced increase in insulin resistance in the control women provided a potential explanation for the increase in glycogenolysis observed in the fasting condition but does not explain why a similar effect was not observed in the fasting lactating women. Improved insulin sensitivity during lactation has been reported in rats [37]. However, studies in lactating women with history of gestational diabetes report improved β-cells function without changing insulin sensitivity as measured by frequently sampled intravenous glucose tolerance test [38]. In addition, Kjos and coworkers [39] also reported that lactation, even for a short duration, has beneficial effects on glucose metabolism in women with gestational diabetes.

The increased insulin sensitivity in the lactating women is most likely secondary to the 25% increase in non–insulin-dependent utilization of plasma glucose for milk production. However, it is not clear if this alone could contribute to the striking differences in insulin values or measures of insulin sensitivity using HOMA and QUICKI. The role of milk production and the effects of other factors such as plasma prolactin and/or oxytocin remain to be investigated. The lack of difference in C-peptide concentrations after rhGH in both groups of subjects is interesting. The kidneys [40] clear a substantial proportion of C-peptide, and rhGH increases the glomerular filtration rate [41]. Whether the effect of rhGH on renal glomerular filtration rate is greater in one group of women or the other remains to be determined, but this factor alone complicates the interpretation of the C-peptide data under these conditions. However, the discrepancy in the insulin and C-peptide responses to rhGH in our present study is nearly

identical to the changes observed in a group of normal men and women treated with either saline or rhGH [19].

Recombinant human growth hormone increases milk production in humans [4,5] to a similar extent as that reported with species-specific growth hormone in goats and cows [3]. Every woman receiving rhGH in a blinded fashion in the present studies complained of mild breast engorgement on day 2 to 3 of only rhGH treatment, which resolved by day 4 or 5. Similar isotopomer distribution of ^{13}C in the milk glucose and galactose under all conditions studied suggests that the increased milk production is not the result of increased hexoneogenesis in the mammary epithelium. Thus, rhGH, whether in the lactating breast or the liver, has little effect on the conversion of 3 carbon precursors to glucose (and galactose). The precise biochemical and/or molecular mechanism(s) involved in the rhGH induced increase in milk production remains to be determined.

Our current and previous data [42] and those of others [39,40] suggest a rethinking of the acute effects of insulin and growth hormone on the regulation of glucose homeostasis. During feeding and short-term fasting, gluconeogenesis remains constant despite changes in plasma insulin. As a consequence, the maintenance of euglycemia in the fed condition must be the result of only the balance between decreased glycogenolysis and increased peripheral glucose utilization but not changes in gluconeogenesis. With more prolonged fasting and virtual depletion of glycogen stores, the increased availability of FFA and ketone bodies results in decreased glucose utilization, offsetting the decreasing glucose production. In addition, the metabolic acidosis induced by ketonemia will most likely increase renal gluconeogenesis [43,44]. We have no data on the effects of the prolonged exposure of high or low plasma concentrations of growth hormone, cortisol, epinephrine, or insulin on gluconeogenesis. However, if gluconeogenesis is unaffected by these conditions, as it appears to be during feeding and short-term fasting, we may need to rethink fundamentally our understanding of the glucose homeostasis in a number of medical conditions that are known to cause hyper- or hypoglycemia.

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