Paradoxical Changes of Muscle Glutamine Release During Hyperinsulinemia Euglycemia and Hypoglycemia in Humans: Further Evidence for the Glucose-Glutamine Cycle

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Insulin suppresses and counterregulatory hormones increase proteolysis. Therefore, if proteolysis were a major factor determining amino acid fluxes in plasma, one would expect release of glutamine into plasma to be suppressed by insulin under euglycemic conditions and to be stimulated under hypoglycemic conditions. However, release of glutamine into plasma remains unaltered or increases during euglycemic hyperinsulinemia and decreases during insulin-induced hypoglycemia. To investigate the mechanisms for these paradoxical observations and the role of skeletal muscle, we infused overnight fasted volunteers with [U-^{14}C] glutamine and measured release of glutamine into plasma, its removal from plasma, and forearm glutamine net balance, fractional extraction, uptake and release during 4-hour euglycemic (~5.0 mmol/L, n = 7) and hypoglycemic (~3.1 mmol/L, n = 8) hyperinsulinemic (~230 pmol/L) clamp experiments. During the euglycemic clamps, plasma glutamine uptake and release (both $P < .05$) and forearm muscle glutamine fractional extraction ($P < .05$), uptake ($P < .02$) and release ($P < .01$) all increased, whereas forearm glutamine net balance remained unchanged. The increase in muscle glutamine release (from 1.85 ± 0.26 to 2.18 ± 0.30 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) accounted for approximately 60% of the increase in total glutamine release into plasma (from 5.54 ± 0.47 to 6.10 ± 0.64 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and correlated positively with the increase in muscle glucose uptake ($r = 0.80$, $P < .03$). During the hypoglycemic clamps, plasma glutamine uptake and release and forearm glutamine release remained unaltered, but forearm glutamine fractional extraction and uptake decreased approximately 25% (both $P < .01$) so that forearm glutamine net release increased from 0.37 ± 0.06 to 0.61 ± 0.09 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($P < .03$). We conclude that skeletal muscle is largely responsible for the increased release of glutamine into plasma during euglycemic hyperinsulinemia in humans, and that this may be due to increased conversion of glucose to glutamine as part of the glucose-glutamine cycle; during hypoglycemic hyperinsulinemia decreased glutamine uptake by skeletal muscle may be important for providing substrate for increased glutamine gluconeogenesis.

GLUTAMINE IS THE most abundant free amino acid in human plasma and muscle[1] and an important vehicle for interorgan transport of protein-derived carbon. When used as a gluconeogenic substrate, glutamine adds more net carbon to the glucose pool than either lactate or alanine.[2] This is due to the fact that lactate and alanine are largely derived from glucose,[2] whereas glutamine is predominantly derived from the body's free amino acid pool, which depends to a large extent on rates of proteolysis, or from proteolysis directly.[2,3]

Recent studies in humans have found that conversion of plasma glutamine into plasma glucose (glutamine gluconeogenesis) is suppressed by insulin[4] and stimulated by counterregulatory processes.[5] However, contrary to the expectation that insulin would decrease release of glutamine into plasma by suppressing proteolysis,[6] recent studies indicate that release of glutamine into plasma remains unchanged[7] or may actually increase[4] during euglycemic physiologic hyperinsulinemia. Moreover, during counterregulation of hypoglycemia, when one may expect increased release of glutamine into plasma due to stimulation of proteolysis by counterregulatory hormones,[8-11] release of glutamine into plasma was found to be reduced.[7]

The tissues responsible for these unexpected changes in release of glutamine into plasma are unclear. In postabsorptive humans, skeletal muscle has been reported to be the major site for glutamine release accounting for approximately 50% to 70% the total.[3,12] It may seem likely therefore that muscle would be involved in the changes in plasma glutamine release under the euglycemic and hypoglycemic clamp conditions.

To address this issue, we used a combination of isotopic and forearm net balance techniques to determine forearm glutamine release during hyperinsulinemic euglycemic and hypoglycemic clamp experiments in healthy postabsorptive volunteers and extrapolated forearm data to the whole body. In addition, this approach allowed us to examine the effects of hyperinsulinemia euglycemia and hypoglycemia on forearm glutamine net balance, fractional extraction, and uptake, which has not yet been investigated in humans.

MATERIALS AND METHODS

Subjects

Informed written consent was obtained from 15 normal healthy volunteers after the protocol had been approved by the University of Rochester Institutional Review Board. Subjects (9 men, 6 women) were 33 ± 2 years of age and had a body mass index of 25.9 ± 0.9 kg/m^2. All subjects had normal glucose tolerance tests according to World Health Organization criteria[13] and no family history of diabetes mellitus.

Protocol

Subjects were admitted to the University of Rochester General Clinical Research Center between 6 and 7 PM in the evening before

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experiments; they consumed a standard meal (10 kcal/kg, 50% carbohydrate, 35% fat, and 15% protein) between 6:30 and 8 PM and were fasted overnight until experiments were completed.

At approximately 5:30 AM, an antecubital vein was cannulated and a primed-continuous infusion of [U-^{14}C] glutamine (~30 μCi, 0.3 μCi/min) was begun. For other purposes, which have been separately reported,[4,5,14] infusions of [6-^{3}H] glucose (~30 μCi, ~0.3 μCi/min) and [9,10-^{3}H] palmitate (~0.2 μCi/min) were started at approximately 5:30 AM and approximately 9 AM, respectively, and a renal vein was catheterized under fluoroscopy between 8 and 9 AM. Shortly thereafter, a dorsal hand vein was cannulated in a retrograde fashion and kept in a thermoregulated Plexiglass box at 65°C for sampling arterialized venous blood. In the opposite arm, a deep antecubital vein was cannulated in a retrograde fashion for sampling deep venous blood, which originates primarily from muscle. Starting at approximately 10 AM, 3 blood samples were simultaneously obtained from the dorsal hand vein and the deep forearm vein at 30-minute intervals for determination of [^{14}C] glutamine specific activities (SAs) and glucose and glutamine concentrations. Arterial blood samples were also obtained for measuring insulin, glucagon, epinephrine, norepinephrine, growth hormone and cortisol concentrations, and hematocrit. At approximately 11 AM (0 minute), a continuous insulin infusion (0.6 mU $\cdot$ kg^{-1} $\cdot$ min^{-1}) was begun. Subsequently, blood glucose concentrations were either allowed to decrease to approximately 3.2 mmol/L during hypoglycemic clamp experiments (n = 8) or maintained at approximately 5.0 mmol/L in euglycemic (control) experiments (n = 7) using the "hot GINF" technique.[15] During the insulin infusion, arterial blood was collected at 30- to 40-minute intervals throughout the study; arterial and deep venous blood was collected simultaneously at 180, 210, and 240 minutes after a new steady state had been achieved. Forearm blood flow (FBF) was determined following each deep venous blood sampling using electrocapacitance plethysmography[16] after the procedure described by Jackson et al.[17]

Analytical Procedures

Blood samples were collected for glucose and glutamine concentrations and glutamine SAs in oxalate-fluoride tubes. Blood samples were collected for plasma catecholamines in EGTA tubes, and for plasma insulin and glucagon concentrations in EDTA tubes containing the protease inhibitor, aprotinin. Whole blood glucose was immediately determined in quadruplicate with a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). For high-performance liquid chromatography (HPLC) analysis of plasma glutamine concentrations and SAs, an internal standard (25 nmol p-fluoro-phenylalanine) was added to 4 mL of plasma; the pH was adjusted to 4.8 to 5.0, and samples were frozen for later analysis.[18] For other determinations, samples were placed immediately in a 4°C ice bath and plasma was subsequently separated by centrifugation at 4°C. Plasma insulin, glucagon, growth hormone, and cortisol concentrations were determined by standard radioimmunoassays, and plasma epinephrine and norepinephrine concentrations were measured by a radioenzymatic method.[19]

Calculations

Systemic rates of appearance (RA) and disappearance (RD) of plasma glutamine were calculated by Steel's steady-state equation at baseline[20] and subsequently during the infusion of insulin by De Bodo's non–steady-state equation[21] using a pool fraction of 0.75 and a volume of distribution of 430 mL/kg.[22] Forearm plasma flow (FPF) was calculated as FBF $\times$ (1 $-$ hematocrit). Forearm net balance (NB) of glutamine was calculated as (glutamine $conc_{art}$ $-$ glutamine $conc_{deep\ vein}$) $\times$ FPF. Forearm glutamine fractional extraction (Fx) was calculated as (glutamine $conc_{art}$ $\times$ glutamine SA_{art} $-$ glutamine $conc_{deep\ vein}$ $\times$ glutamine $SA_{deep\ vein}$)/(glutamine $conc_{art}$ $\times$ glutamine SA_{art}). Forearm glutamine uptake was calculated as FPF $\times$ glutamine c_{art} $\times$ Fx, and forearm glutamine release was calculated as forearm glutamine uptake $-$ forearm glutamine NB.[12,23] Forearm glucose NB was calculated analogously to forearm glutamine NB except that FBF was used.

Forearm data per 100 mL of tissue were converted to values per kilogram forearm muscle as previously described,[24] assuming that 80% of the measured forearm blood flow perfused muscle[25] and that muscle compromised 60% of the forearm volume.[26] Assuming that forearm muscle was representative of skeletal muscle elsewhere in the body, these values were multiplied by total body skeletal muscle mass, which was calculated from midarm circumference and triceps skinfold thickness using the equation of Heymsfield et al.[27] Arterial glutamine concentrations and plasma glutamine turnover have been previously reported from all subjects who participated in the euglycemic clamps,[4] and arterial glutamine concentrations have been previously reported from 7 of 8 subjects who participated in the hypoglycemic clamps.[5]

Statistical Analysis

Unless stated otherwise, data are expressed as mean $\pm$ SEM. Paired 2-tailed Student's *t* tests were used to compare the average of data obtained from baseline with the mean of data obtained from the last 60 minutes of the 4-hour euglycemic and hypoglycemic clamp experiments. Unpaired 2-tailed Student's *t* tests were used to compare data obtained from hypoglycemic and euglycemic clamp experiments after adjustment of baseline data to zero. A P value less than .05 was considered statistically significant.

RESULTS

Arterial Glucose and Hormone Concentrations

Infusion of insulin increased plasma insulin to comparable physiologic concentrations (~230 pmol/L) in euglycemic and hypoglycemic clamp experiments (Fig 1). Plasma glucose concentrations were maintained at 4.96 $\pm$ 0.06 during the euglycemic experiments and were allowed to decrease to 3.11 $\pm$ 0.05 mmol/L during the hypoglycemic experiments. In the euglycemic experiments, plasma epinephrine, norepinephrine, growth hormone, and cortisol remained unchanged, whereas plasma glucagon decreased. In the hypoglycemic experiments, plasma concentrations of glucagon, epinephrine, norepinephrine, and growth hormone increased significantly by 80 minutes (plasma glucose 3.57 $\pm$ 0.21) (all $P < .05$), whereas plasma cortisol increased significantly by 120 minutes (plasma glucose 3.29 $\pm$ 0.09) ($P < .05$).

Concentrations and Turnover of Plasma Glutamine

In euglycemic experiments, arterial glutamine concentrations decreased from 602 $\pm$ 48 to 530 $\pm$ 33 μmol/L during the last hour of the insulin infusion ($P < .01$) (Fig 2). In hypoglycemic experiments, arterial glutamine concentrations decreased to a greater extent than in euglycemic experiments (28% $\pm$ 3% *v* 11% $\pm$ 2%, $P < .01$) from 608 $\pm$ 33 to 437 $\pm$ 30 μmol/L ($P < .001$).

The greater reduction in arterial glutamine concentrations in hypoglycemic experiments was due to the fact that during the initial approximately 180 minutes of the insulin infusion, uptake of glutamine from plasma exceeded release of glutamine into plasma to a greater extent than in the euglycemic experiments. During these initial 180 minutes, the difference between uptake and release of glutamine averaged 0.27 $\pm$ 0.03 μmol $\cdot$

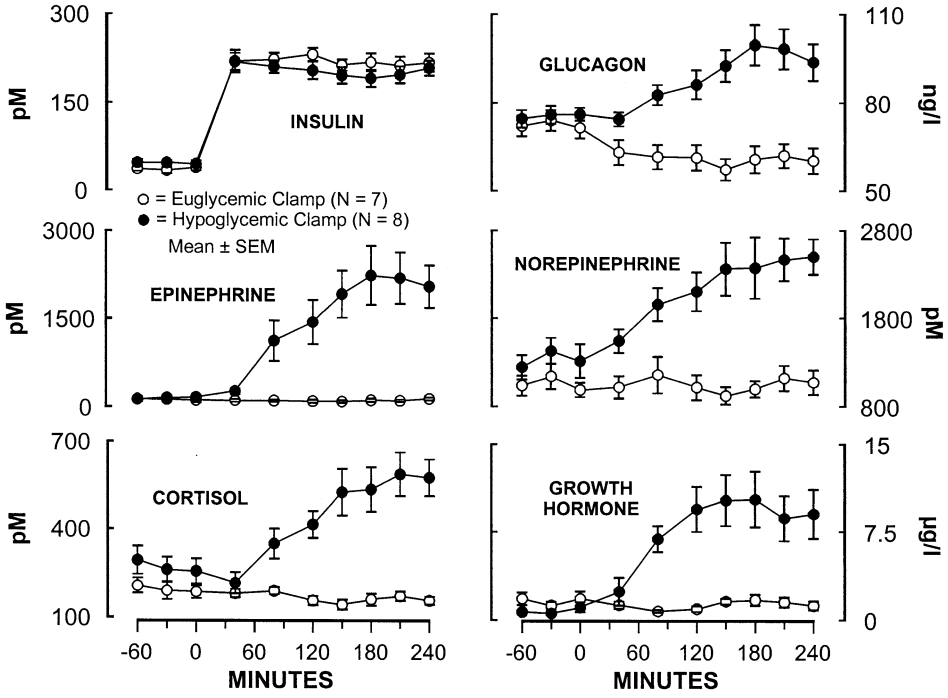

Fig 1. Arterial insulin and counterregulatory hormone concentrations during the hyperinsulinemic euglycemic and hypoglycemic clamp experiments in postabsorptive subjects.

$kg^{-1} \cdot min^{-1}$ in hypoglycemic experiments and 0.08 ± 0.02 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in euglycemic experiments ($P < .001$).

During the last hour of the euglycemic experiments, uptake of glutamine from plasma (6.16 ± 0.62 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and its release into plasma (6.10 ± 0.64 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) were increased approximately 10% above baseline rates (5.54 ± 0.47 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (both $P < .05$); in contrast, uptake and release of glutamine (5.00 ± 0.63 and 4.94 ± 0.64 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively) were unchanged from baseline (5.02 ± 0.54 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) in the hypoglycemic experiments (both $P > .6$).

During the last hour of the insulin infusion, plasma glutamine clearance was increased approximately 20% in euglycemic (from 9.8 ± 1.2 to 12.0 ± 1.3 $mL \cdot kg^{-1} \cdot min^{-1}$, $P < .01$) and approximately 40% in hypoglycemic experiments (from 8.5 ± 1.2 to 11.8 ± 1.7 $mL \cdot kg^{-1} \cdot min^{-1}$, $P < .01$), but percent increments were not significantly different ($P = .16$).

Forearm Muscle Glutamine Metabolism

Insulin infusion increased forearm plasma flow in both sets of experiments (both $P < .01$), but to a greater extent in hypoglycemic than in euglycemic experiments ($P < .04$) (Table 1). As a consequence, in both experiments, the product of forearm plasma flow and arterial glutamine concentrations, ie, forearm glutamine delivery, remained unaltered (both $P > .7$). In euglycemic experiments, forearm glutamine fractional extraction ($P < .05$), uptake ($P < .02$) and release ($P < .01$) all increased so that forearm glutamine net release remained unaltered ($P = .56$). In contrast, in hypoglycemic experiments, forearm glutamine fractional extraction and uptake decreased approximately 25% (both $P < .01$), and forearm glutamine release remained unchanged ($P = .59$) so that forearm glutamine net release increased ($P < .03$).

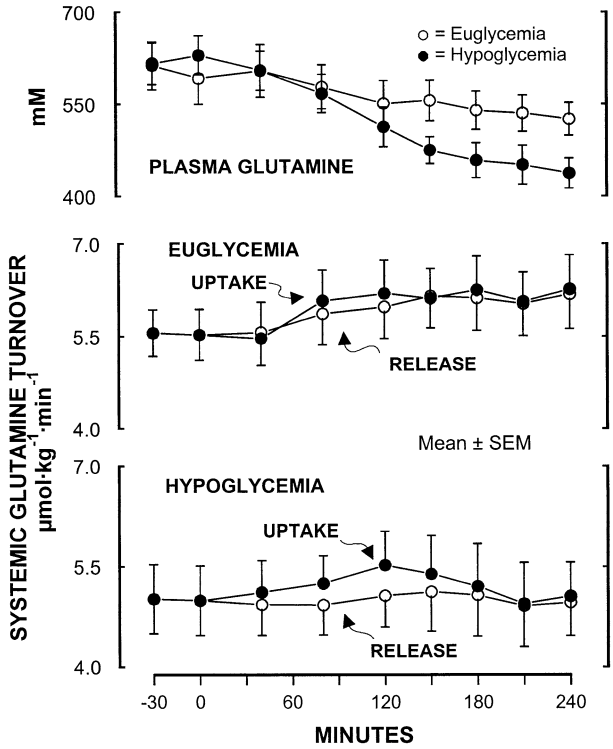

Fig 2. Arterial plasma glutamine concentrations and systemic glutamine uptake and release during the hyperinsulinemic euglycemic and hypoglycemic clamp experiments in postabsorptive subjects.

Table 1. Forearm Glutamine Kinetics During Hyperinsulinemic Euglycemic and Hypoglycemic Clamp Experiments

	Euglycemic Clamp		Hypoglycemic Clamp		
	Baseline	Insulin	Baseline	Insulin	*P*
Forearm plasma flow (mL/min)	1.99 ± 0.12	2.33 ± 0.16§	1.86 ± 0.11	2.60 ± 0.14§	
Change from baseline (%)		17.0 ± 2.8		42.2 ± 9.2	<.04
Glutamine fractional extraction (%)	25.8 ± 2.3	31.6 ± 2.4†	23.6 ± 2.1	18.0 ± 1.8§	
Change from baseline (%)		26.0 ± 9.4		−24.3 ± 5.8	<.001
Glutamine uptake*	0.298 ± 0.031	0.385 ± 0.045‡	0.263 ± 0.019	0.197 ± 0.015§	
Change from baseline (%)		30.1 ± 8.6		−24.4 ± 5.1	<.001
Glutamine release*	0.395 ± 0.027	0.469 ± 0.032§	0.340 ± 0.027	0.326 ± 0.019	
Change from baseline (%)		18.8 ± 2.2		−1.8 ± 6.4	<.02
Glutamine net balance*	−0.097 ± 0.016	−0.084 ± 0.020	−0.078 ± 0.010	−0.129 ± 0.014‡	
Change from baseline (%)		−11.0 ± 27.4		96.6 ± 39.9	<.05

*(μmol · 100 cc^{-1} · min^{-1}).
†$P < .05$, ‡$P < .03$, §$P < .01$ compared with baseline.

Contribution of Skeletal Muscle to Plasma Glutamine Turnover

When forearm muscle data were extrapolated to the whole body, baseline data from both sets of experiments indicate that glutamine uptake and release by skeletal muscle averaged 1.32 ± 0.14 and 1.73 ± 0.16 μmol · kg BW^{-1} · min^{-1}, respectively, which accounted for 26% ± 2% of overall glutamine uptake from plasma and 33% ± 3% of overall glutamine release into plasma. In euglycemic experiments, skeletal muscle glutamine uptake and release increased from 1.41 ± 0.26 to 1.83 ± 0.35 ($P < .03$) and from 1.85 ± 0.26 to 2.18 ± 0.30 μmol · kg BW^{-1} · min^{-1} ($P < .01$), respectively, representing approximately 70% and 60% of the increases in plasma glutamine uptake and release. In hypoglycemic experiments, glutamine uptake by skeletal muscle decreased from 1.23 ± 0.13 to 0.92 ± 0.10 μmol · kg BW^{-1} · min^{-1} ($P < .01$) so that its contribution to glutamine uptake from plasma was reduced from 26% ± 4% to 19% ± 2% ($P < .03$); glutamine release by skeletal muscle (1.54 ± 0.15 *v* 1.60 ± 0.19 μmol · kg BW^{-1} · min^{-1} at baseline, $P = .61$) and its contribution to release of glutamine into plasma (33% ± 3% *v* 34% ± 5% at baseline, $P = .71$) remained unaltered.

Forearm Muscle Glucose Uptake

Forearm glucose uptake increased from a basal rate of 0.48 ± 0.08 to 4.12 ± 0.75 μmol · 100 cc^{-1} · min^{-1} during the last hour of the insulin infusion in euglycemic experiments ($P < .001$), but remained unaltered in hypoglycemic experiments (0.43 ± 0.09 *v* 0.42 ± 0.15 μmol · 100 cc^{-1} · min^{-1} at baseline, $P = .95$). In euglycemic experiments, the increase in forearm glucose uptake correlated significantly with the increase in forearm glutamine release ($r = 0.80$, $P < .03$) (Fig 3), whereas during the hypoglycemic experiments, in which muscle glucose uptake was unaltered, no correlation was found ($r = 0.34$, $P = .42$).

DISCUSSION

In the present study, release of glutamine into plasma and its release from forearm muscle both increased during hyperinsulinemia when euglycemia was maintained, but remained unaltered when plasma glucose concentrations were allowed to decrease to mild hypoglycemia. When forearm muscle data were extrapolated to the whole body, approximately 60 % of the increase in systemic glutamine release during the euglycemic experiment was accounted for by skeletal muscle.

Previous studies indicate that hyperinsulinemia similar to the present studies suppresses proteolysis.[6,7,28] Consequently, if proteolysis were the main factor influencing muscle glutamine release, one would expect muscle glutamine release to decrease during euglycemic hyperinsulinemia. The findings of the present study and those by Battezzati et al,[7] who found that during a hyperinsulinemic euglycemic clamp systemic glutamine release remained unaltered despite suppressed proteolysis, are clearly inconsistent with this notion.

A possible explanation for the increase in muscle glutamine release during euglycemic hyperinsulinemia may be increased conversion of glucose to glutamine in muscle as part of the so-called glucose-glutamine cycle.[2] Under postabsorptive conditions, approximately 12% of glutamine carbons entering the

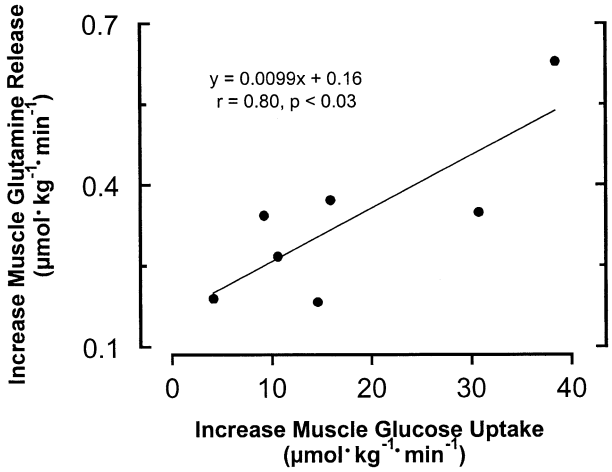

Fig 3. Correlation between increases in muscle glucose uptake and muscle glutamine release during the hyperinsulinemic euglycemic clamp experiments in postabsorptive subjects.

systemic circulation originate from plasma glucose,[2] and the remainder is due to release of glutamine from intracellular pools influenced by the relative rates of protein synthesis and degradation. Assuming that skeletal muscle conversion of glucose to glutamine accounted for approximately 50% of all the glutamine produced from plasma glucose, ie, similar to its contribution to alanine produced from plasma glucose,[2] this would represent approximately 6% of all glutamine released into plasma or approximately 0.32 μmol · kg^{-1} BW · min^{-1} in the present experiments (ie, ~5.3 μmol · kg^{-1} BW · min^{-1} mean systemic glutamine release × 6%). Accordingly, at baseline approximately 18% of muscle glutamine release (ie, ~0.32 μmol · kg^{-1} BW · min^{-1}/~1.73 μmol · kg^{-1} BW · min^{-1} mean muscle glutamine release × 100%) could have been due to operation of the glucose-glutamine cycle and approximately 13% of glucose taken up by muscle (ie ~0.32 μmol · kg^{-1} BW · min^{-1}/~2.1 μmol · kg^{-1} BW · min^{-1} mean muscle glucose uptake × 100% × 5/6) could have been converted to glutamine and subsequently released into the systemic circulation.

In the euglycemic experiments of the present study, muscle glucose uptake increased approximately 8.5-fold. If a comparable proportion of the increased muscle glucose uptake were converted to glutamine as under basal conditions and the resultant glutamine subsequently released, this would have increased muscle glutamine release by about 2.72 μmol · kg^{-1} BW · min^{-1}, ie more than 8 times the observed increase in muscle glutamine release during the euglycemic clamp. On the other hand, hyperinsulinemia comparable to that in the present studies has been reported to reduce forearm muscle proteolysis 25% to 40%.[29] As estimated above, approximately 18% of glutamine release by skeletal muscle could have been due to operation of the glucose-glutamine cycle leaving at most approximately 82% of muscle glutamine release that could have been directly or indirectly due to proteolysis. Assuming that muscle proteolysis was 40% reduced in our studies, this would have suppressed muscle glutamine release by approximately 0.6 μmol · kg^{-1} BW · min^{-1} (ie, 1.73 μmol · kg^{-1} BW · min^{-1} × 82% × 40%). These calculations suggest that the increase in muscle glutamine release during the hyperinsulinemic euglycemic clamps may be the net effect of suppression of proteolysis being more than compensated for by increased conversion of glucose to glutamine due to stimulation of the glucose-glutamine cycle. This concept is further supported by our finding that there was a significant correlation between increases in muscle glucose uptake and glutamine release, and that the slope of the regression (increase muscle glutamine release = 0.0099 × increase muscle glucose uptake + 0.16) was markedly lower than if approximately18% of muscle glutamine release were due to conversion from plasma glucose.

Operation of the glucose-glutamine cycle may also explain the fact that in the present studies muscle glutamine release during hypoglycemia was less than during euglycemia and in fact was not different from baseline. Consistent with previous reports,[30,31] we found that muscle glucose uptake remained unchanged during the hypoglycemic experiments. If a similar proportion of glucose taken up by skeletal muscle were converted to glutamine during hypoglycemia as it was at baseline, this would have left muscle glutamine release unchanged. The unaltered muscle glutamine release during the present hypoglycemic experiments may therefore be the result of suppressed proteolysis by insulin[29] counterbalanced by stimulated proteolysis by counterregulatory hormones.[32]

Regarding glutamine uptake, we found that forearm muscle glutamine uptake increased during euglycemic experiments, but decreased during hypoglycemic experiments. In both experiments, decreases in plasma glutamine concentrations were associated with increases in forearm blood flow so that forearm glutamine delivery actually remained unchanged. Accordingly, changes in forearm glutamine uptake in both experiments were solely due to changes in forearm glutamine fractional extraction.

Skeletal muscle glutamine uptake is primarily mediated by the Nm amino acid transport system.[33] Studies using the perfused rat hindlimb model or isolated myoblasts and myotubes from neonatal skeletal muscle indicate that this transport system is stimulated by insulin and inhibited by glucocorticoids.[34,35] Furthermore, muscle glutamine transport has been shown to be inhibited by growth hormone in humans.[36] Our finding that muscle glutamine fractional extraction was increased during the hyperinsulinemic euglycemic clamp, but decreased during hyperinsulinemic hypoglycemic clamp, is in agreement with such a regulation of muscle glutamine transport and suggests that during hypoglycemia the inhibition of glutamine transport by counterregulatory hormones might have been greater than its stimulation by insulin, whereas during euglycemia, the stimulation was unopposed.

Counterregulation of hypoglycemia largely involves increased gluconeogenesis,[37] which increases the demand of gluconeogenic substrates by liver and kidney. We have previously reported that during identical hypoglycemic clamp conditions in humans systemic conversion of glutamine to glucose was increased approximately 0.6 μmol · kg^{-1} · min^{-1} compared with euglycemic control clamps.[5] In the present studies, extrapolation of forearm muscle data to the whole body indicates that muscle glutamine uptake was decreased approximately 0.7 μmol · kg^{-1} · min^{-1} during the hypoglycemic clamp compared with the euglycemic control clamp, suggesting that the sparing of glutamine uptake by skeletal muscle during hypoglycemia could have wholly accounted for the increased demand of glutamine for gluconeogenesis.

It has been previously reported that glutamine uptake and release by skeletal muscle accounts for approximately 40% to 50% and 50% to 70% of systemic glutamine uptake and release, respectively.[3,12] In contrast, in the present study, the corresponding contributions of skeletal muscle were 26% and 33%, which warrants comment. Essentially all circulating glutamine that is involved in tissue exchange is distributed in the plasma pool in humans.[38-40] Accordingly, glutamine fluxes across human skeletal muscle need to be calculated using plasma flow if glutamine concentrations and specific activities are measured in plasma. However, in the previous studies mentioned above,[3,12] muscle glutamine fluxes were calculated using blood flow despite the fact that glutamine concentrations and specific activities were measured in plasma. This has led to significantly exaggerated values for muscle glutamine fluxes and its contributions to systemic glutamine fluxes. Notably, if in these studies[3,12] the correct calculations had been used, these

values would have been comparable to those of the present study.

In conclusion, the present data indicate that during euglycemic hyperinsulinemia, skeletal muscle is largely responsible for the increased release of glutamine into plasma, and that this may be due to increased conversion of glucose to glutamine as part of the glucose-glutamine cycle. During hypoglycemia, decreased glutamine uptake by skeletal muscle may be an important mechanism to meet the increased demand of glutamine for gluconeogenesis.

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