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Both Growth Hormone and Exogenous Glutamine Increase Gastrointestinal Glutamine Uptake in Trauma

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Objective
The authors studied the effect of exogenous glutamine (GLN), with and without growth hormone (GH), pretreatment, on gastrointestinal, hepatic, femoral, and renal GLN fluxes.

Summary Background Data
Growth hormone treatment increases gastrointestinal uptake of GLN despite a reduced skeletal muscle and whole body release.

Methods
Piglets were randomized to a GH + GLN group (n = 8), a GLN group (n = 8), a GH group (n = 8), and a control group (CON; n = 8). Genotropin (Pharmacia, Stockholm, Sweden; 24 international units; correspondingly saline in the GLN and the CON group) was given daily 3 days before and at the onset of trauma (surgery). Organ fluxes and whole body release of GLN were determined 1 and 5 hours after surgery. An infusion of GLN 36 μg/kg per minute was started after the first measurement in the GH + GLN and the GLN groups.

Results
Both GH treatment and exogenous GLN increased gastrointestinal GLN uptake ($p = 0.001$ and $p = 0.02$, respectively). Growth hormone treatment reduced hepatic GLN uptake ($p = 0.001$). Hepatic GLN uptake was lower in the GH + GLN group *versus* the GH group ($p = 0.02$), but not in the GLN group *versus* the CON group ($p = 0.98$). Growth hormone treatment reduced femoral and whole-body GLN release ($p = 0.0001$ and $p = 0.02$, respectively). Renal GLN uptake was higher in the two GH-treated groups ($p = 0.003$).

Conclusion
Both exogenous GLN and GH increased gastrointestinal GLN uptake, and the combination was additive. In contrast to exogenous GLN, GH reduced hepatic uptake and consequently facilitated the increased gastrointestinal GLN uptake that occurred despite reduced femoral and whole-body release.

In catabolic states, there is net proteolysis with release of amino acids from skeletal muscle, mainly glutamine and alanine. Glutamine is an important substrate for the intestinal mucosa, intestinal lymphoid tissue, and the pancreas,[1-3] and may be essential for maintenance of gut mucosal barrier function during critical illness.[4] Surgical stress increases uptake of glutamine in the gastrointestinal tract.[5,6]

Growth hormone (GH) treatment reduces skeletal release of glutamine in catabolic states.[7-10] Hence, gastrointestinal substrate depletion is a possible detrimental effect of GH treatment. However, we found increased gastrointestinal uptake of glutamine despite reduced femoral and whole-body release after pretrauma GH treatment.[11] The GH effect was more pronounced when given for 3 days preoperatively than when given at the start of surgery only. The combination of increased gastrointestinal glutamine uptake and reduced skeletal muscle release may suggest that GH treatment improves glutamine economy. The ammonia from glutamine oxidation may enter hepatic glutamine synthesis rather than ureagenesis. The aim of the present study was to address the following questions: Do both GH and exogenous glutamine increase gastrointestinal glutamine uptake? If so, is the combination additive? Does GH facilitate gastrointestinal glutamine uptake by increasing hepatic glutamine synthesis?

MATERIALS AND METHODS

The protocol was approved by the local Committee for Animal Research. Norwegian Landrace piglets, 25 to 30 kg, were randomized to four combinations of GH treatment and exogenous glutamine (GLN) infusion, with eight animals in each group: GH + GLN, GLN, GH, and controls (CON). In the GH + GLN and GH groups, 24 international units of GH (Genotropin, Pharmacia, Stockholm, Sweden) was injected intramuscularly for 3 days before and intravenously at the onset of surgery (correspondingly saline in the GLN and CON group). For the last 24 hours before the experiments, the animals were fasted with free access to water. During the experiments, they received 10 mL/kg per hour of saline. The animals were collected at 8:00 A.M., and the surgical procedure was completed before 11:00 A.M. Organ fluxes of GLN were measured 1 and 5 hours after surgery. An infusion of 36 μg/kg per minute of GLN was started after the first measurement in the GH + GLN and GLN groups.

Surgical Procedure

The animals were sedated with 1 g of ketamine (Ketalar, Parke-Davis, Barcelona, Spain) and 1 mg of atropine sulfate (Atropin, Hydropharma, Oslo, Norway) in the animal department. In the laboratory, anesthesia was induced with 5% isoflurane (Forene, Abbott Laboratories Ltd, UK), 5 mg midazolam (Dormicum, Roche, Switzerland), and 0.1 mg fentanyl (Leptanal, Janssen Pharmaceutica, Belgium). After endotracheal intubation, isoflurane was discontinued. During surgery, the animals were ventilated (Servo Ventilator 900, Elema-Schönander, Sweden) with O_2/N_2O (1:1), and anesthesia was maintained with ketamine 700 mg/hour, fentanyl 0.05 mg/hour, and midazolam 1 mg/hour. During the stabilization period, N_2O was discontinued, and ketamine, fentanyl, and midazolam were reduced by half. Ventilation was adjusted by means of repeated arterial blood gas analysis to achieve an arterial partial pressure of carbon dioxide (pCO_2) within the range 4.0 to 4.5 kiloPascal. Blood temperature was maintained at 38 ± 1 C with a heating pad .

The trauma consisted of a standardized midline laparatomy and right-sided exposure of major vessels of the neck and thigh. Perivascular flow probes (Transonic Animal Research Flowmeters T208, Transonic Systems Inc. Ithaca, NY) were placed around the portal vein (6 mm), the common hepatic artery (3 mm), the right renal artery (2 mm), and the right femoral artery (2 mm). Care was taken to preserve the perivascular nerves. Extraorgan branches were ligated. For infusions, pressure recordings, and collection of blood samples, polytef catheters (Secalon T, Viggo-Spectramed, Swindon, UK) were inserted by direct puncture into the carotid artery and into the jugular, hepatic, portal, renal, and femoral veins. A 5F Edwards Swan-Ganz catheter (Baxter Healthcare Corp., Santa Ana, CA) was positioned in the pulmonary artery for measurement of pressure and cardiac output. The bladder was drained via a cystotomy. Heparin (100 international units/mL) was used to flush catheters.

Glutamine Fluxes and Rate of Appearance

After induction of anesthesia, a primed continuous infusion of U-^{14}C-glutamine (Amersham, Buckinghamshire, UK) was started at a rate of 22 μCi/hour after a bolus of 29 μCi. The HCO_3^- pool was saturated initially with 50 μCi Na-H^{14} HCO_3. Gastrointestinal, hepatic, femoral, and renal fluxes of GLN and GLN rates of appearance were determined 1 hour after surgery (average of 2 consecutive measurements) and 5 hours after surgery (average of 3 consecutive measurements) and analyzed according to Smith and Panico,[12] as described by Jenssen et al.[13] Gastrointestinal flux is portal vein drained splanchnic bed (including spleen and pancreas).

Other Measurements

Serum levels of human GH were determined by two-sided noncompetitive immunometric assay using two

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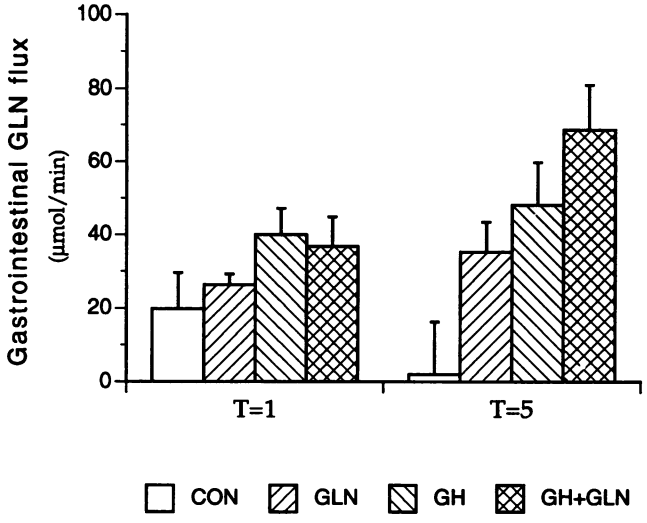

Figure 1. Gastrointestinal glutamine fluxes 1 and 5 hours after surgery (T = 1 and T = 5) in the control group (CON), in the group receiving exogenous glutamine (GLN), in the group pretreated with growth hormone (GH), and in the group pretreated with growth hormone and receiving exogenous glutamine (GH + GLN).

monoclonal antibodies (Medix Biochemica, Kauniainen, Finland).[14] Serum levels of insulin-like growth factor-1 were determined by a radio immunoassay kit (Nichols Instruments, CA).[14] Blood gases and urine pH were analyzed on ABL3 Acid Base Laboratory (Radiometer, Copenhagen, Denmark) and a PHM 80 portable pH-meter (Radiometer), respectively.

Statistics

Statistics were calculated on a Macintosh Quadra 950 using SuperANOVA (Abacus Concepts, Inc., Berkeley, CA). Differences between groups were tested with the multivariate approach to analysis of variance for repeated measures with Greenhouse-Geisser epsilon factor adjusted probability levels. Contrast means comparisons (univariate) between various groups are based on overall analysis of variance for repeated measures that are significant difference between groups. When effects of GH were tested, both time points were included in the contrast means comparison. When effects of GLN and additive effects were tested, only T = 5 was included. All contrasts are based on equal cell weights. Differences concerning one time point only were tested with one-way analysis of variance. When analysis of variance significance occurred, *post hoc* comparisons were performed with Fisher's protected least squared difference (LSD). Net uptake and release (different from zero) were tested with one-sample Student's t test. All statistics are based on type III sum of squares. Assumptions were examined by construction of normal and residual plots. Values are presented as means ± standard error of the mean. Significance level is p = 0.05.

RESULTS

All animals were included in the study. There were no differences in weight (27.3 ± 0.5 kg), gender, and temperature.

Gastrointestinal Glutamine Flux

There was net glutamine uptake in all groups at both time points except for the control group at T = 5 when GLN flux was not significantly different from zero (Fig. 1).

Glutamine uptake was higher in the two GH treated groups *versus* the two nontreated groups (p = 0.001). Glutamine uptake was higher in the two GLN-receiving groups *versus* the two nonreceiving groups (p = 0.02). Also, GLN uptake was higher in the GH + GLN group *versus* the GH and the GLN group (p = 0.003).

Hepatic Glutamine Flux

There was net glutamine uptake in all groups at T = 1, with no difference between groups. At T = 5, the GLN flux was not different significantly from zero in the GH group, and there was a net release in the GH + GLN group. Growth hormone treatment reduced GLN uptake in both GH treated groups *versus* the two nontreated groups (p = 0.001). Exogenous GLN reduced GLN uptake in the GH + GLN group *versus* the GH group (p = 0.02), but not in the GLN group *versus* the control group (Fig. 2).

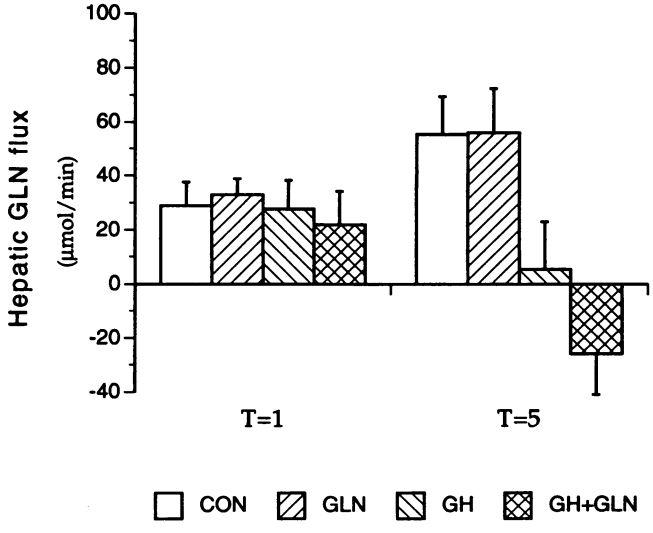

Figure 2. Hepatic glutamine fluxes 1 and 5 hours after surgery (T = 1 and T = 5) in the control group (CON), in the group receiving exogenous glutamine (GLN), in the group pretreated with growth hormone (GH), and in the group pretreated with growth hormone and receiving exogenous glutamine (GH + GLN).

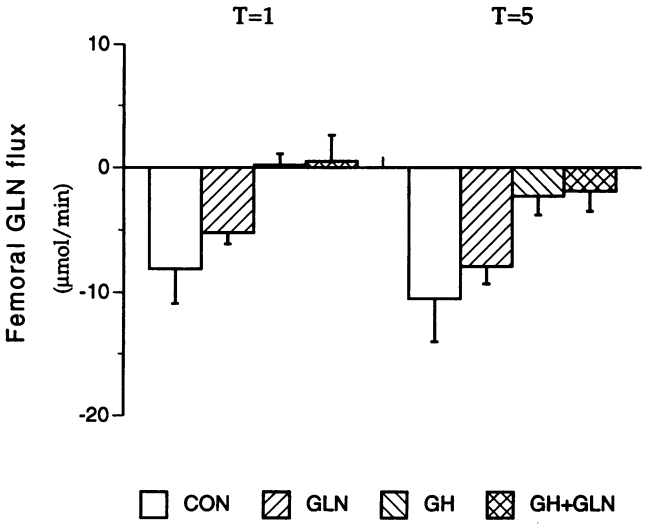

Figure 3. Femoral glutamine fluxes 1 and 5 hours after surgery (T = 1 and T = 5) in the control group (CON), in the group receiving exogenous glutamine (GLN), in the group pretreated with growth hormone (GH), and in the group pretreated with growth hormone and receiving exogenous glutamine (GH + GLN).

Femoral Glutamine Flux

Growth hormone treatment abolished femoral release in the two GH treated groups at T = 1. Glutamine release was lower in the two GH-treated groups *versus* the two nontreated groups (p = 0.0001). Glutamine flux was not different in the two GLN-receiving groups *versus* the two nonreceiving groups (Fig. 3).

Renal Glutamine Flux

Growth hormone treatment induced a higher GLN uptake in the two GH-treated groups *versus* the two nontreated groups (p = 0.003). Glutamine flux was not different in the two GLN-receiving groups *versus* the two nonreceiving groups (Fig. 4).

Glutamine Rate of Appearance

Growth hormone treatment induced a lower GLN rate of appearance in the two GH-treated groups *versus* the two nontreated groups (p = 0.02). Glutamine rate of appearance was not different in the two GLN-receiving groups *versus* the two nonreceiving groups.

When gastrointestinal and hepatic fluxes were added, there was no difference between groups. Plasma concentrations of GLN concentrations in the aorta and the hepatic vein were not different. Plasma concentrations in the femoral vein were lower in the GH-treated groups *versus* the nontreated groups (p = 0.04). Plasma concentrations in the portal vein were lower in the GH-treated groups *versus* the nontreated groups (p = 0.02) at T = 5.

Hemodynamics

There were no differences in cardiac output and aortic and pulmonary artery pressure. There was a tendency toward higher renal artery flow in the GH-treated groups (p = 0.06) and lower portal vein flow (p = 0.07). Within both groups, portal and femoral flow declined, whereas renal and hepatic artery flow was maintained.

Other Measurements

Growth hormone levels were higher in the GH-treated groups (35.2 ± 6.6 *vs.* 0.22 ± 0.02 µg/L at T = 1 and 4.6 ± 1.7 *vs.* 0.21 ± 0.01 %g/L at T = 5; p = 0.0001). Insulin-like growth factor-1 levels were higher in the GH-treated groups (4.7 ± 0.5 *vs.* 1.2 ± 0.2 nmol/L at T = 1 and 4.9 ± 0.8 *vs.* 1.1 ± 0.2 nmol/L at T = 5; p = 0.0001). Aortic concentrations of insulin were not different and did not change within groups. Aortic concentrations of glucose, lactate and free fatty acids are presented in Table 1. Free fatty acid levels were higher in the GH-treated groups (p = 0.08). Urine pH and base excess were lower in the GH-treated groups. Diuresis was not different. Urinary urea and nitrogen excretion were lower in the GH-treated groups.

DISCUSSION

Our study demonstrates that a glutamine infusion started 1 hour after surgery increased gastrointestinal glutamine uptake 5 hours after surgery.

Pretreatment with GH also increased gastrointestinal glutamine uptake. Whereas exogenous glutamine influ-

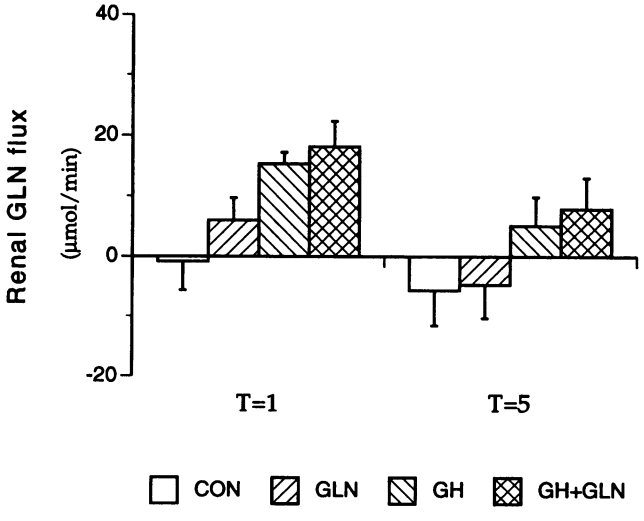

Figure 4. Renal glutamine fluxes 1 and 5 hours after surgery (T = 1 and T = 5) in the control group (CON), in the group receiving exogenous glutamine (GLN), in the group pretreated with growth hormone (GH), and in the group pretreated with growth hormone and receiving exogenous glutamine (GH + GLN).

Table 1. AORTIC LEVELS OF GLUCOSE, LACTATE, AND FREE FATTY ACIDS IN PIGLETS PRETREATED WITH GROWTH HORMONE AND RECEIVING GLUTAMINE, PRETREATED WITH GROWTH HORMONE ONLY, RECEIVING GLUTAMINE ONLY, AND IN NONTREATED NONRECEIVING CONTROLS

	1 hr After Surgery				5 hr After Surgery			
	GH + GLN	GH	GLN	Controls	GH + GLN	GH	GLN	Controls
Glucose (mmol/L)	6.3 ± 0.4	6.1 ± 0.3	5.7 ± 0.5	5.2 ± 0.3	4.9 ± 0.28	5.2 ± 0.2	4.4 ± 0.3	4.2 ± 0.4
Lactate (mmol/L)	4.7 ± 0.7	5.2 ± 0.5	4.0 ± 0.4	3.6 ± 0.7	2.0 ± 0.3	2.7 ± 0.5	2.1 ± 0.2	1.9 ± 0.3
FFAs (nmol/L)	293 ± 43	301 ± 35	263 ± 39	256 ± 42	241 ± 51	239 ± 44	208 ± 24	229 ± 29

GH = growth hormone; GLN = glutamine; FFAs = free fatty acids.
Values are mean ± standard error of the mean.

enced gastrointestinal uptake only, GH also reduced hepatic uptake, increased renal uptake, and reduced femoral and whole-body release of glutamine. The combination of exogenous glutamine and GH pretreatment had an additive effect on gastrointestinal glutamine uptake.

Increased gastrointestinal uptake of glutamine may be of clinical importance. Lack of glutamine causes atrophy of intestinal mucosa[15] and may make the mucosa more susceptible to translocation of bacteria and bacterial products. Insulin-like growth factor-1, the main mediator of GH, has been reported to reduce translocation of endotoxin in burned rats.[16] In the present study, insulin-like growth factor-1 levels were increased in the GH-treated animals. This counteracts the diabetogenic effects of GH (no difference in glucose and insulin levels) while the lipolytic effect (higher free fatty acid levels) is present. In another study, we found increased hepatic and reduced gastrointestinal oxidation of oleic acid after an acute GH injection (unpublished data), indicating again that GH influence the gut–liver interaction in stress states. A reduced gastrointestinal oleic acid oxidation may reflect the availability of glutamine as an energy source whereas increased hepatic oxidation may reflect increased energy-demanding activity, such as synthesis of glutamine. Increased gastrointestinal glutamine uptake may be essential in protecting the mucosa against bacterial translocation, and insulin-like growth factor-1 could be the mediator of this effect. It is interesting that the effects of GH pretreatment apparently are enhanced in the course of trauma.

Souba et al.[5] found increased skeletal muscle release and increased gastrointestinal uptake of glutamine after surgical stress. In accordance, we found increasing femoral release in the two groups without GH treatment. As reported previously,[11] GH abolished this release. In contrast to Souba's finding,[5] gastrointestinal glutamine uptake did not increase in the control group. It decreased to a value not significantly different from zero 5 hours after surgery. However, our study was performed earlier in trauma, in the ebb phase. Theoretically, an earlier increase in gastrointestinal glutamine uptake may be favorable. Using Welbourne's concepts,[17] one may hypothesize a delay from source to sink, that a certain amount of glutamine must become available before this is reflected in increased uptake. In accordance is the finding that when exogenous glutamine was added to the femoral release, gastrointestinal uptake increased, and the increase corresponded almost exactly to the amount of exogenous glutamine given.

Growth hormone increased gastrointestinal uptake. Growth hormone has been shown to enhance luminal glutamine uptake by increasing membrane carriers.[18] Theoretically, uptake of circulating glutamine also could be influenced similarly. In this study, our intention was to study the hepatic response to changes presented by the portal vein. Therefore "gastrointestinal" fluxes represent the portal vein drained splanchnic bed. After GH pretreatment, there was increased gastrointestinal uptake despite reduced femoral and whole-body release. This was made possible by reduced hepatic uptake of glutamine, changing to a net release when exogenous glutamine was given in addition to GH treatment. Growth hormone treatment appears to influence the gut–liver interaction. Gut glutamine uptake becomes counterbalanced by the liver. A reduction in hepatic glutamine uptake may be caused by reduced glutamine degradation or increased glutamine synthesis, or a combination of the two. Increased gastrointestinal uptake corresponded to increased glutamine oxidation,[11] and the resulting ammonia load may enter ureagenesis (periportally) or lead to increased hepatic glutamine synthesis (perivenously). Growth hormone has been reported to induce hepatic glutamine synthetase.[19] The assumption that this effect may contribute is supported by the finding of a more pronounced GH effect after 3 days of preoperative GH treatment when GH was given at the start of surgery only.[11] But there also was a tendency

toward metabolic acidosis in the GH-treated groups. The pH-sensitive hepatic glutaminase is an important amplifier of ureagenesis.[20] Acidosis inhibit ureagenesis by switching off the amplifier, thereby shuttling more ammonia to glutamine synthesis. The lower urinary excretion of urea in the GH-treated groups supports this assumption. Hence, both a direct GH effect and an indirect effect due to reduction in pH may contribute to the observed reduction of hepatic uptake that counterbalanced the increased gastrointestinal uptake in the GH-treated groups. Correspondingly, exogenous glutamine alone increased gastrointestinal uptake, but this was not reflected in the hepatic handling of glutamine. However, when exogenous glutamine was given in combination with GH, the enhanced increase in gastrointestinal uptake was reflected in the hepatic handling of glutamine, resulting in a net hepatic release.

The assumption that increased gastrointestinal glutamine consumption is made possible by increased hepatic glutamine release (reduced uptake) is supported by the lower portal vein plasma levels of glutamine in the GH-treated groups, combined with no differences in hepatic vein and arterial glutamine concentrations. When gut and liver fluxes were added, there was no difference between groups.

The increased renal uptake of glutamine may reflect the GH-induced metabolic acidosis because the kidney use glutamine to handle excess acid. The lower urinary pH in the GH-treated groups may reflect this.

CONCLUSION

Both exogenous glutamine and GH treatment induced increased gastrointestinal glutamine uptake, but the mechanisms appears to be principally different. Exogenous glutamine alone selectively increased gastrointestinal uptake without influencing other organs. Growth hormone reduced the hepatic uptake of glutamine and consequently facilitated the increase in gastrointestinal uptake that occurred despite reduced femoral and whole-body release. The liver becomes an "adjusting" organ. The combination of GH and glutamine seem to have an additive effect on gastrointestinal glutamine uptake, with a resultant net hepatic glutamine release.

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