

## PROTEIN METABOLISM/PEPTIDES

### 0.37 Delayed response to feeding of protein synthesis in human skeletal muscle measured by the flooding dose technique

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The temporal sequence of changes in human protein metabolism with feeding is not well elucidated. Incorporation of a flooding amount of (1-<sup>13</sup>C)leucine enables quantitative and sequential measurements of protein synthesis rate in vivo in individual tissues. Protein synthesis rate in human skeletal muscle was measured 1h and 10h after the commencement of feeding.

Healthy male volunteers (n = 8) were studied by the flooding dose technique in the postabsorptive state and after 1h and 10h of continuous feeding. The normal daily intake was divided into 12 hourly meals consisting of milk and sandwiches. For each measurement percutaneous muscle biopsies were taken from the quadriceps femoris muscle before and 90 min after intravenous injection of (1-<sup>13</sup>C)leucine (0.05 g/kg, 20 atoms %). The rate of muscle protein synthesis was calculated from the increase in enrichment of leucine in protein determined by gas isotope mass spectrometry.

The protein synthesis rate was  $2.07 \pm 0.22\%/24\text{h}$  in the postabsorptive state, compared with  $2.08 \pm 0.23\%/24\text{h}$  after 1h and  $2.84 \pm 0.36\%/24\text{h}$  after 10h of feeding. Thus, no elevation in synthesis rate was detectable at 1h but by 10h protein synthesis had increased significantly ( $p < 0.05$ ). An interindividual variation of response was also observed.

### 0.38 Moderate TPN infusion rates do not stimulate overall whole body protein synthesis in malnourished patients with or without cancer

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**Aim:** To evaluate whether malnourished cancer patients could increase whole body protein synthesis to a similar magnitude as malnourished non-cancer patients after institution of intravenous nutrition.

**Methods:** Twenty-eight patients, 11 females and 17 males, with clinical and biochemical signs of malnutrition were randomized to be included in the study. Twelve of the patients had malignant disease, the others had non malignant disease and served as controls.

The two categories of patients, cancer and non-cancer, were also randomized to receive non protein calories corresponding to either 120% or 200% of measured resting energy expenditure (REE) with 0.2g and 0.33g of nitrogen/kg body weight respectively. These four groups of patients were well matched according to body composition. Whole body protein synthesis was measured by a standard primed constant infusion technique with L-[U-<sup>14</sup>C] tyrosine. All patients were investigated both in the fasted state and after intravenous nutrition. Substrate exchanges across the leg were also measured before and after intravenous nutrition. Blood flow was measured with a strain gauge plethysmograph. The leg exchange of substrates were calculated according to Fick's principle.

**Results:** The cancer patients had elevated resting energy expenditure ( $20.8 \pm 1.4$  vs  $17.6 \pm 0.8$  kcal/kg/day,  $p < 0.05$ ) and increased whole body tyrosine flux in the fasted state ( $631 \pm 39$  vs  $488 \pm 30$  nmol/min/kg,  $p < 0.05$ ) compared to the control patients. Feeding with low rate of TPN (120% of REE with 0.2g N/kg b.w.) did not stimulate whole body protein synthesis neither in the cancer group nor in the control group. The high rate of TPN (200% of REE with 0.33g N/kg b.w.) stimulated protein synthesis in both groups ( $809 \pm 76$  vs  $676 \pm 39$  nmol/min/kg,  $p < 0.05$  TPN vs fasted state cancer patients,  $563 \pm 28$  vs  $456 \pm 29$  nmol/min/kg,  $p < 0.05$  TPN vs fasted state control patients). Both TPN regimens switched an efflux of branched amino acids from the leg to an uptake into the leg in both cancer and control patients.

These effects were not obtained either for tyrosine or phenylalanine, which remained in negative balance across the leg. The leg exchange of glucose was switched to an uptake by the high TPN rate in both groups ( $6.7 \pm 0.4$  vs  $4.6 \pm 0.3$  mmol/l,  $p < 0.05$  TPN vs fasted state cancer patients,  $7.7 \pm 0.7$  vs  $4.7 \pm 0.2$  mmol/l,  $p < 0.05$  TPN vs fasted state control patients).

**Conclusions:** Standard TPN regimens seem to be insufficient to stimulate overall protein synthesis in malnourished cancer and non-cancer patients, while high TPN infusions (200% of REE, 0.33g N/kg body weight) are effective.

### 0.39 Stress hormones initiates changes in muscle free amino acid concentrations characteristic for surgical trauma

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Stress hormones evoke changes in muscle amino acid concentrations similar to those seen after surgery. This amino acid pattern develops gradually in a characteristic manner. It is controversial whether or not elevated concentrations of stress hormones alone may explain these effects. Here surgical trauma was simulated by a 6-h stress hormone infusion and muscle amino acids were determined during 24h.

**Methods:** Healthy male volunteers (n = 8) were given a 6-h triple hormone infusion of adrenaline, cortisol, and glucagon. Percutaneous muscle biopsies were taken before and 6, 12, 24h after the start of the infusion. Muscle free amino acids were analyzed using ion-exchange chromatography.

**Results:** Muscle free amino acids (mmol/kg wet weight muscle tissue)

	0h	6h	12h	24h
GLU	2.103±0.22	0.60±0.09***	1.41±0.15*	1.56±0.13*
GLN	13.15±0.61	12.42±0.82	10.74±0.61**	9.24±0.41**
ALA	1.84±0.24	2.85±0.27*	1.71±0.16	1.75±0.19
BCAA	0.39±0.04	0.17±0.02**	0.35±0.03	0.58±0.02**
ESS	1.66±0.15	0.98±0.07**	1.23±0.08**	1.78±0.09

**Conclusion:** The changes in the pattern of free amino acids in muscle during 24h after a 6-h infusion of stress hormones were identical to those seen during the first day after surgical trauma. These changes were initiated by stress hormones and remained long after the hormone levels in plasma were normalized. Stress hormones alone may thus explain the characteristic alterations in muscle free amino acids seen after surgical trauma.

### 0.40 Glutamine in the human portal vein during and after operation

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Several animal studies have shown that glutamine (GLN) may be categorized as semiessential in all settings where the gut may be affected. However, whether this holds true for the clinical situation is unclear. Moreover, there is a lack of clear biochemical data about GLN in the GI tract. Therefore we took the opportunity to assess GLN in the portal vein of patients who were treated postoperatively with chemotherapy by portal vein perfusion with 5-Fluorouracil.

16 patients (age: 65.6 (56–73.5) years; body weight: 75 (62–82) kg; 10 male, 6 female) operated for colorectal carcinoma were included in this study. Before, during and at each of the first 7 days after operation plasma was drawn from the portal catheter and from the central venous line. Amino acids (AA) were assessed as usual with ion exchange chromatography. Patients were nourished according to a standardized regimen with a complete AA mixture (TPE 1800, Pfrimmer Kabi, Erlangen), thereafter oral supplementation was allowed.

There was a close similarity between the portal vein and the central venous blood in the time course of concentration change of all AA. GLN in the portal blood correlated ( $\rho = 0.6154$ ) significantly to that in the central venous

blood, but this correlation coefficient was distinctly lower than that for all other AA. With conservative non-parametric statistics a highly significant difference ( $P \leq 0.0001$ ) could be shown revealing a median concentration difference of  $-45 \mu\text{mol/l}$  of GLN between portal and central venous blood. With these data, combined with GLN data about arterio-venous concentration difference and about the portal blood flow it is now possible to estimate the GLN-consumption of the portal venous drained GI organs. It amounts to around 20g GLN per day.

#### 0.41 Role of interleukin 1 and tumor necrosis factor on interorgan amino acids metabolism in dogs

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We investigated the effects of intravenous infusion of the recombinant cytokines interleukin 1 (IL-1) and tumor necrosis factor (TNF) on the net rates of amino acids exchange across the gut and liver in awake, unrestrained dogs.

Eighteen dogs underwent laparotomy and the placement of catheter in the aorta, portal vein and hepatic vein. Blood flow of portal vein and hepatic artery was measured with an ultrasonic flow probe. Studies were performed on POD 1 in an awake, fasted condition. After a basal measurement, dogs were administered IL-1 or TNF as a continuous intravenous infusion ( $5 \mu\text{g/kg/h}$ ) for 2h.

	IL-1				TNF					
	0	1hr	2hrs	3hrs	0	1hr	2hrs	3hrs	4hrs	
<b>Liver uptake (<math>\mu\text{mol/kg. min}</math>)</b>										
Glutamine	3.1	4.0	1.7*	1.2*	2.5	2.2	2.0	2.2	2.5	2.0
Alanine	7.0	11.5*	11.0*	9.8*	9.6*	6.7	7.0	7.8	9.2*	8.8
<b>Gut release (<math>\mu\text{mol/kg. min}</math>)</b>										
Glutamine	-1.5	-2.7*	-3.2*	-2.8*	-2.0	-2.2	-2.0	-2.2	-2.5	-2.0
Alanine	1.7	2.0	2.6	2.9*	2.1*	1.5	1.4	1.4	1.9	1.7

Mean: \*  $p < 0.05$  as compared to 0hr

There was a positive correlation between glutamine uptake and alanine release by the gut following IL-1 infusion ( $p < 0.05$ ). In contrast, there were no relations between glutamine uptake and alanine release by the gut during TNF infusion.

These results suggest that IL-1, not TNF, plays an important role on the net rates of amino acids exchange across the gut and liver in this dog model.

#### 0.42 Xylitol normalises the increased capacity of urea synthesis and improves the nitrogen balance in rats with experimental diabetes

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The hepatic conversion of amino nitrogen to urea nitrogen is increased in diabetes, this results in body nitrogen wasting. Xylitol decreases the hepatic nitrogen conversion in normal rats. We studied the effects of xylitol on the hepatic nitrogen conversion in diabetic rats.

Rats of 225g were fed ad lib. Diabetes was induced with streptozotocin 75mg/kg i.v., controls received the vehicle. After 2 weeks the Capacity of the Urea Nitrogen Synthesis (CUNS), determined during i.v. alanine loading to constant amino acid concentration between 7.3–11.6 mmol/l, the alanine metabolism (Mala), calculated as the alanine infusion rate, and the nitrogen balance (N-bal) calculated as the difference between CUNS and Mala, were studied in 3 groups of rats ( $n = 7$ ): Control rats, diabetic rats (DM), and diabetic rats where xylitol were clamped at 1 mmol/l during investigation (DM + Xylil). Mean blood glucose concentration (glucose) was determined during experiments.

	Glucose (mmol/l)	CUNS	Mala ( $\mu\text{mol}/(\text{min } 100\text{g BW})$ )	N-bal
Controls	$5.7 \pm 1.0$	$9.4 \pm 1.1$	$6.7 \pm 0.4$	$-2.7 \pm 1.2$
DM	$27.1 \pm 1.7^*$	$26.6 \pm 1.9^*$	$10.4 \pm 0.7^*$	$-16.3 \pm 2.1^*$
DM + Xylitol	$30.7 \pm 1.8^*$	$11.2 \pm 1.0^\dagger$	$8.6 \pm 0.5^\dagger$	$-3.8 \pm 2.1^\dagger$

\* Higher than control ( $p < 0.001$ ), † Lower than DM ( $p < 0.01$ )

**Conclusion:** Xylitol improves the nitrogen economy of uncontrolled diabetes by decreasing the hepatic nitrogen conversion to normal values.

#### 0.43 Growth hormone after gastrointestinal surgery: effect on skeletal muscle metabolism

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The study was conducted to determine the effect of growth hormone (GH) after abdominal surgery on nitrogen balance and skeletal muscle metabolism in patients receiving total parenteral nutrition (TPN) and epidural analgesia.

Elective patients were randomized to treatment with GH (24 IU s.c. each morning) ( $n = 9$ ) or placebo (PL) ( $n = 10$ ) the first five postoperative days, while receiving TPN and epidural analgesia. Preoperatively, the 2nd, and 4th postoperative days, forearm muscle flux studies were performed; flow was measured with strain gauge technique, arterial and deep venous blood samples analyzed for glutamin (GLN), glutamate, free fatty acids, beta-hydroxybutyrate, glycerol (GLYC), glucose, lactate, and pyruvate (PYR). 24 hourly urine samples were analyzed for nitrogen.

The GH patients retained nitrogen ( $4.1 \pm 1.1 \text{g/m}^2$ , cumulative balance) compared to the PL patients ( $-3.1 \pm 1.8 \text{g/m}^2$ ,  $p = 0.004$ ). GLN flux was less negative ( $p = 0.03$  day 2), GLYC flux was more negative ( $p = 0.01$  day 4), and PYR flux changed from negative to positive in GH patients ( $p = 0.04$  day 2) while it stayed negative in the PL patients. For the other parameters no significant differences were detected.

**Conclusion:** Growth hormone given postoperatively to patients with epidural analgesia and parenteral nutrition induced nitrogen retention, attenuated the glutamine and pyruvate loss, and increased the glycerol loss from skeletal muscle.

#### 0.44 Initial characterization of glutamine transport in human skeletal muscle sarcolemmal vesicles

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Evidence exists that the availability of glutamine for use by the rest of the body is influenced by efflux of glutamine from skeletal muscle. However, no information is available on the mechanisms of membrane transport of glutamine (or any other amino acid) in human skeletal muscle. Our aim was to isolate skeletal muscle sarcolemmal vesicles and study the transport of glutamine into vesicles made from it.

Sarcolemmal vesicles were isolated from 10–15g mixed human leg muscle (vastus lateralis, soleus) using differential centrifugation. The plasma membrane marker ( $5'$ -nucleotidase and KpNPPase) enzyme activity expressed as  $\mu\text{mol.mg}^{-1} \text{protein.h}^{-1}$  was  $\approx 12$  fold higher in sarcolemmal fraction (SL) than in the crude muscle homogenate (CH);  $5'$ -nucleotidase (from  $1.0 \pm 0.3$  in CH to  $13.0 \pm 1.6$  in SL), KpNPPase (from  $0.15 \pm 0.03$  in CH to  $1.8 \pm 0.6$  in SL). There was very little contamination of SL by sarcoplasmic reticulum marker enzymes (CaATPase and G-6-Pase). Transport was assayed using gel-filtration method. The uptake of tracer ( $0.5 \mu\text{M}$ )  $\text{L-}^{[3]\text{H}}$ glutamine was stereospecific and  $\text{Na}^+$ -dependent, the initial rate of uptake in presence of  $0.1 \text{M NaCl}$  (inward) gradient being  $8.5 \pm 1.5 \times 10^{-3} \text{pmol.mg}^{-1} \text{protein.s}^{-1}$  but was only  $1.3 \pm 0.3 \times 10^{-3} \text{pmol.mg}^{-1} \text{protein.s}^{-1}$  in  $0.1 \text{M Choline Cl}$ . Various L-amino acids at  $1 \text{mM}$  showed inhibition of glutamine transport (Gln  $90 \pm 6\%$ , Asn  $93 \pm 2\%$ , His  $80 \pm 2\%$  and Cys  $90 \pm 5\%$ ). MeAIB and Phe at  $1 \text{mM}$  inhibited glutamine transport by  $23 \pm 10\%$  and  $22 \pm 8\%$  respectively. Results are expressed as means  $\pm$  SEM of 3 membrane preparations from seven patients. The transport of glutamine in human sarcolemmal vesicles appears to be similar to that previously characterized in rat sarcolemmal vesicles, so the pathophysiological control of glutamine efflux from human muscle may depend on modulation of transporter activity as demonstrated in the rat. Our method for isolating human sarcolemmal vesicles at high purity should be of great use in investigating transport of amino acids and other solutes in human skeletal muscle.