FREE AMINO ACID EXCRETION IN TETRAPLEGIC PATIENTS¹

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Introduction

PATIENTS with traumatic tetraplegia have a progressive loss of muscle and collagen. As water retention occurs often, weight is not a good index of these losses. Creatinuria is a better index for muscle (Cardus, 1972), except during periods of inadequate food intake (Haverberg, 1975). Hydroxyproline and hydroxylysine being hydroxylated on the amino acid chains reflect collagen metabolism. They are mostly excreted incorporated into polypeptides, but a small fraction is excreted free. The amino acid 3-methylhistidine is a constant, non age-related component of actin and myosin in all muscles except the heart; it is released in constant proportion with amounts of actin and myosin being broken down; it is not incorporated in new protein, and 92 per cent is excreted in the urine (Long, 1975). In rats fed a low protein food, the fall in 3-methylhistidine is far larger than weight loss or reduction in creatinuria (Haverberg, 1975).

The aim of this experiment was to find out, first, if the excretion of the free hydroxyproline and hydroxylysine reflects collagen catabolism and can be utilised for evaluating any beneficial effects of therapy toward reducing the collagen losses; and second, if the absolute amounts of urine 3-methylhistidine, but not those per milligram of creatinine, are progressively reduced after onset of tetraplegia and are low in tetraplegics of long standing, in comparison with values found in healthy subjects. If such changes occur, the values of urine 3-methylhistidine can be used to calculate the rate of muscle loss by using the tables of Young *et al.* (1975) in order to assess the efficiency of adequate nutrition and physical therapy in maintaining muscle mass.

Method

To this day, ten patients and six healthy subjects participated in the study. Their characteristics and their grouping are shown in Table I. While they were in the hospital, the urine was collected daily to make 7-day pools. In the clinic and out of the hospital, urine for patients and healthy subjects was collected for 24 hours.

Chromatographic fingerprint of the free amino acids was carried out in a 119 Beckman amino acid analyser, on a column of cross-linked sulfonated styrene copolymer resin which was eluted with a lithium citrate buffer.

The excretion of the amino acids related to bone and muscle metabolism were compared between the three groups by variance analysis.

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TABLE I
Description of the subjects studied

Group	Subject identi- fication	Sex	Age at study time (yrs)	Weight (kg) at study time	Origin of trauma	Level of injury	Duration of injury at study time (wks, yrs)	Patient status
I. Early tetraplegics	HUR	M	22	67	car accident	C4	9 w	No complication, wheel- chair sitting, 8 hrs
							13 w	Same
							19 w	Patient is disturbed; autonomic dysreflexia
							20 w	Patient doing well
	GRAD	M	25	75	pipe fall	C4	9 w	Pneumonia, skin ulcers
	DUP	M	17	47	diving	C5	9 w 10 w	Autonomic dysreflexia Same
	ASH	M	18	50	diving	C4	10 w	No wheelchair; autonomic dysreflexia
	STE	M	22	63	mountain climbing	C6	9 w	Low pulmonary function, wheelchair sitting, 6 hrs
							12 w	No complication
							16 w	Same
Mean and SE							18 w	Same
							23 w	Autonomic dysreflexia
							26 w	Same
			20.8 ± 1.46	61 ± 4.3			14.5 ± 1.6	
	STE†	M	22	not known	see above		30 w	Sweating profusely
							34 w	Doing well
							42 w	Doing well

II. Late tetraplegics		GRAV	M	21 22	75 76	football	C4	5 y 6 y	No complication, clinic Same
18/3 — B		LUN	M	25	54	car accident	C6	3 y	Decubiti and back pain, kidney stones
		WIL	M	21	72	car accident	C6	4 y	Urinary infection and bladder stones
		WLE	M	30	65	diving	C5	12 y	Bedfast for 2 yrs
		STR	M	32	65	car accident	C4	13 y	No complication, clinic
	Mean and SE			25.2 ± 2.0	67 ± 3.0			7.2 y + 1.7	
III. Healthy		ROD	M	48	76				
		GAR	M	26	66				
		SAN	M	29	75				
		MUL	M	30	71				
		MCD	M	30	82				
		HUB	M	28	80				
Mean and SE				31.8 ± 3.3	75 ± 2.4				
difference between									
	I and II			-4.4 y	-6 kg-ns				
	I and III			-11 y*	-10 kg*				
	II and III			-6.6 y	-4 kg				

* Significant at p = 0.05.
† The patient STE was at home and urine was mailed to the laboratory. The results are not included in the mean for Group I.

Results

(a) Comparison of mean results in the three groups (Table II)

Only one subject in each group does excrete small amounts of the free proline. *Total amino acid* and the *lysine* excretion are similar in all tetraplegics, but less than in healthy subjects. Daily excretion of 3-methylhistidine is identical in early and late patients, but much lower than in healthy subjects; the excretion per milligram of creatinine is similar in the tetraplegic and the healthy group (0.23 ± 0.01 v. 0.26 ± 0.03 μ moles).

(b) Effect of the duration of paralysis on individual excretion of 3-methylhistidine in two patients

The data shown in Table III do not disclose a relationship between injury duration and 3-methylhistidine excretion.

Discussion

The results show that the free urine amino acids which are the precursors and the metabolites of collagen are not different in healthy or tetraplegic subjects, whereas hydroxyproline polypeptides and hydroxylysine glucosides are increased in early tetraplegia (Claus-Walker, 1975 and 1977). Evaluation of these amino acids in polypeptidic and glycosidic linkage needs to be carried out on special columns, or after extensive hydrolysis, as free amino acids on another type of column. Daily urinary 3-methylhistidine is much higher in healthy than in tetraplegic subjects; there is no difference between early and late tetraplegics and in two patients no difference in urine collected at various periods after onset. The amount of 3-methylhistidine per milligram of creatinine was identical in all study subjects, which confirms that urine 3-methylhistidine, similarly to creatinine, depends in part upon muscle mass. The absence of visible changes in 3-methylhistidine excretion during 11 weeks in one patient and 32 weeks in another, indicate that other factors that decrease in muscle mass, influence the excretion rate.

It has been shown that patients with traumatic injury and no increase in blood ketones have twice more urine 3-methylhistidine than those with hyperketonemia because they have an excessive muscle protein breakdown (Williamson, 1977). However, Hoover-Plow (1978) found that in rats, after surgery without ketosis, the breakdown occurs in muscle sarcoplasm, where the protein metabolism, mostly catabolism, is augmented, and in myofibrils, where the protein anabolism is depressed. These changes are the result of complex hormonal interplay (Munro, 1979). It is likely that 3-methylhistidine, which fails to be incorporated in myosin, is excreted.

Conclusions

As none of the early patients studied had ketosis, it could be concluded that part of their urinary 3-methylhistidine was related to the metabolic response to injuries and part was related to the decreasing daily turnover of myosin and actin. In the late patients and in the healthy subjects, the urine 3-methylhistidine was related only to the turnover of myosin and actin. The dual origin of 3-methylhistidine during trauma suppresses its usefulness to assess the efficiency of adequate nutrition and physical therapy in maintaining muscle mass.

TABLE II
Group means, standard error, and differences between groups

	Hydroxyproline μmoles		Lysine μmoles		Hydroxylysine μmoles		Creatinine mg		3-methylhistidine μmoles		Total amino acids μmoles	
	per day	per kg	per day	per kg	per day	per kg	per day	per kg	per day	per kg	per day	per kg
<i>Group I</i> 5 patients 14 samples	154 ± 34	5.2 ± 2.5	91 ± 14	1.3 ± 0.2	52 ± 222	0.9 ± 0.4	846 ± 131	14 ± 1.4	210 ± 29	3.3 ± 0.4	5115 ± 465	85 ± 9
<i>Group II</i> 5 patients 6 samples	55 ± 22	0.7 ± 0.3	98 ± 30	1.5 ± 0.5	18 ± 8	0.3 ± 0.1	750 ± 92	11 ± 1.7	198 ± 17	3.0 ± 0.2	4823 ± 451	73 ± 7
difference between I and II	-99ns	-4.5ns	+3ns	+0.2ns	-34ns	-0.6ns	-116ns	-3ns	-12ns	-0.3ns	-292ns	-8ns
<i>Group III</i> 6 subjects 6 samples	28 ± 5	0.4 ± 0.2	240 ± 60	3.2 ± 0.8	42 ± 14	0.6 ± 0.2	1793 ± 306	24.5 ± 5.1	481 ± 103	6.6 ± 1.7	12120 ± 2947	166 ± 46
difference between I and III	+126ns	+4.8ns	-149***	-3***	+10ns	+0.3ns	-937*	10.5*	-271***	-3.3**	-7005***	-81**
difference between II and III	+27ns	+0.2ns	-142*	1.7ns	-24ns	-0.3ns	-1043*	-13.5*	-283**	-3.6**	-7297**	-93*

* significant at p = 0.05; ** p = 0.01; *** p = 0.001; ns, not significant.

TABLE III

Effect of the duration of paralysis on the individual excretion of 3-methylhistidine in two patients

Patient's identification	Duration of paralysis (wks)	3-methylhistidine μ moles/day
HUR	9	325
	13	308
	19	208
	20	378
STEG urine collected in hospital	9	150
	12	276
	16	87
	18	72
	23	91
	26	239
urine collected at home*	30	474
	34	137
	42	146

* Not included in Table II calculations for group values.

SUMMARY

Free amino acid chromatographic fingerprints obtained in automated analysers in 24-hour urines may help to quantitate muscle and collagen changes occurring after cervical spinal cord injury.

The technique was used to compare the urine of six healthy and ten tetraplegic men, 18 to 25 years old, three of whom became paralysed in diving, one in a mountain fall, one in the fall of a pipe, four in car accidents, and one in a football game. Nine weeks to 14 years after injury, the tetraplegics excreted less total free amino acids, lysine, and 3-methylhistidine than the controls, whereas hydroxyproline and hydroxylysine were similar in both groups. Values of urine 3-methylhistidine, measured 9, 13, 19 and 20 weeks post onset in one patient, and 9, 12, 16, 18, 23, and 26 weeks post onset in another, were not related to the duration of paralysis. These results indicate that collagen and muscle metabolism may not be quantitated by measuring urine free amino acids.

RÉSUMÉ

Le profile chromatographique des analyseurs automatiques d'acides aminés libres est peut-être intéressant pour évaluer les changements des muscles et du collagène qui suivent les lésions à la moelle épinière cervicale. Cette technique fut utilisée pour comparer l'urine de 6 hommes témoins et de 10 hommes tétraplégiques, dont trois furent paralysés par des plongeurs, un par une chute en montagne, un par la chute d'un tuyau, quatre par des accidents de voiture et un, dans un jeu de football.

De 9 semaines à 14 ans après la lésion, les tétraplégiques excrétaient moins d'acides aminés totaux, de lysine, et de 3-méthyl-histidine, que les témoins, tandis que l'excrétion de l'hydroxyproline et l'hydroxylysine sont similaires dans les 2 groupes. Les valeurs de la 3-méthylhistidine urinaire mesurée 9, 13, 19 et 20 semaines après la lésion chez un tétraplégique et 9, 12, 16, 18, 23 et 26 semaines après la lésion chez un autre, n'ont pas de rapport avec la durée de la paralysie. Ces résultats montrent que les changements du

metabolism du collagène et du muscle ne peuvent pas être évalués quantitativement par la mesure des acides aminés libres de l'urine.

ZUSAMMENFASSUNG

Freie Aminosäurewerte (mittels automatischer chromatographischer Analyse bestimmt) in 24-Stunden Urinabscheidung mag uns quantitative Auskunft ergeben betr. Muskel- und Collagen Veränderungen, die nach hohen (Halswirbelsäule) Rückenmarksverletzungen stattfinden.

Diese Technik wurde benutzt, um die Urine von 6 gesunden und 10 totalgelähmten Männern, 18 bis 25 Jahre alt, zu vergleichen. Drei dieser Gelähmten erlitten ihre Verletzung als Resultat eines Falles vom Sprungbrett, 1 während einer Bergbesteigung, 1 durch den Fall eines schweren Rohres auf den Nacken, 4 in Automobilunfällen und 1 durch Fussball-Unfall. Neun Wochen bis 14 Jahre nach der Verletzung haben die Total-Gelähmten weniger freie Aminosäure, Lysin und 3-methylhistidin ausgeschieden als ihre Kontrollen, wogegen die Hydroxyprolin- und Hydroxylysin-Werte in beiden Gruppen ähnlich waren. Die Urin-Werte von 3-methylhistidin, die 3, 13, 19 und 20 Wochen nach dem Unfall in einem Patienten bestimmt wurden, und 9, 12, 16, 18, 23 und 26 Wochen nach dem Unfall in einem anderen Patienten, waren von der Zeit seit des Unfalles unabhängig.

Die Resultate zeigen, dass Collagen- und Muskel-Stoffwechsel *nicht* mittels Messung des freien Aminosäure Spiegels quantitativ bestimmt werden kann.

REFERENCES

- CARDUS, D. & McTAGGART, W. G. (1972). Total creatine in patients with extensive muscular paralysis estimated by radioisotope tracer method. Project Report No. 10, SRS 16 P 56813/6-09, Project 122.
- CLAUS-WALKER, J., SPENCER, W. A., CARTER, R. E., HALSTEAD, L. S., MEIER, R. H. III & CAMPOS, R. J. (1975). Bone metabolism in quadriplegia: dissociation between calciuria and hydroxyprolinuria. *Arch. Phys. Med. Rehabil.*, **54**, 327-332.
- CLAUS-WALKER, J., SINGH, J., LEACH, C. S., HATTON, D. V., HUBERT, C. W. & DiFERRANTE, N. (1977). The urinary excretion of collagen degradation products by quadriplegic patients and during weightlessness. *J. Bone and Joint Surg.*, **59A**, 209-212.
- HAVERBERG, L. N., DECKELBAUM, L., BILMAZES, C., MUNRO, H. N. & YOUNG, V. R. (1975). Myofibrillar protein turnover and urinary *N* τ -methylhistidine output. *Biochem. J.*, **152**, 503-510.
- HOOVER-PLOW, J. & CLIFFORD, A. J. (1978). The effect of surgical trauma on muscle protein turnover in rats. *Biochem. J.*, **176**, 137-142.
- LONG, C. L., HAVERBERG, L. N., YOUNG, V. R., KINNEY, J. M., MUNRO, H. N. & GEIGER, J. W. (1975). Metabolism of 3-methylhistidine in man. *Metabolism*, **24**(8), 929-935.
- MUNRO, H. N. (1979). Hormones and the metabolic response to injury. *N. Engl. J. Med.*, **300**(1), 41-42.
- WILLIAMSON, D. H., FARRELL, R., KERR, A. & SMITH, R. (1977). Muscle-protein catabolism after injury in man, as measured by urinary excretion of 3-methylhistidine. *Clinical Science and Molecular Medicine*, **52**, 527-533.
- YOUNG, V. R. & MUNRO, H. N. (1978). *N* τ -methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. *Federation Proc.*, **37**, 2291-2300.