Quantitative importance of non-skeletal-muscle N^{τ} -methylhistidine and creatine in human urine*

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The excretion of N^{τ} -methylhistidine and creatinine was determined in a totally paralysed patient with neither macroscopic nor microscopic detectable skeletal-muscle tissue. In this subject, it was possible for the first time to measure the basal non-skeletal-muscle-dependent excretion of N^{τ} -methylhistidine and creatinine per 24 h and per kg of non-muscular body weight, 1.15 μmol (N^{τ} -methylhistidine) and 35 μmol (creatinine) respectively. For the calculation of myofibrillar protein breakdown and skeletal-muscle mass on the basis of N^{τ} -methylhistidine and creatinine excretion, the values have to be corrected for non-muscular sources. Our data show that skeletal-muscle tissue is the major contributor of N^{τ} -methylhistidine in urine, since it contributes as much as 75% to the urinary excretion.

N^{τ} -Methylhistidine excretion has widely been used as an index of myofibrillar protein breakdown (Ward & Buttery, 1978; Young & Munro, 1978). This amino acid originates from the degradation of the contractile actomyosin system of the muscle (Asatoor & Armstrong, 1967; Johnson *et al.*, 1967), in which specific histidine residues are post-translationally methylated (Krzysik *et al.*, 1971; Reporter, 1973). N^{τ} -Methylhistidine is an end-product of protein catabolism and is excreted quantitatively and unchanged in human urine (Tallan *et al.*, 1954; Cowgill & Freeberg, 1957; Long *et al.*, 1975). Several authors were unable to demonstrate binding of this amino acid to histidine tRNA and the incorporation of radioactively labelled N^{τ} -methylhistidine into proteins (Young *et al.*, 1970). In addition, N^{τ} -methylhistidine parenterally applied to animals was apparently quantitatively excreted in urine, with a small amount in faeces (Tallan *et al.*, 1954; Young *et al.*, 1972; Long *et al.*, 1975).

These data show that the release of N^{τ} -methylhistidine from myofibrillar protein seems to reflect the rate of myofibrillar protein degradation. On the other hand, although all eukaryotic cells tested so far

contain actin and myosin, it was assumed that the major source of urinary N^{τ} -methylhistidine is skeletal muscle. Non-skeletal-muscle tissues should contribute negligible amounts to the urine, because the amount of N^{τ} -methylhistidine in such tissues is low (Haverberg *et al.*, 1975; Nishizawa *et al.*, 1977).

This assumption could be invalid, since the release of N^{τ} -methylhistidine from the organs depends on N^{τ} -methylhistidine turnover rate as well as on the content of N^{τ} -methylhistidine in these organs. A small protein pool of N^{τ} -methylhistidine with a high turnover rate could therefore be responsible for a large amount of N^{τ} -methylhistidine excretion.

Millward *et al.* (1980) have reported that skeletal muscle might contribute only about 25% of total urinary N^{τ} -methylhistidine excretion in rats. On the basis of their experiments and calculations, they cast serious doubts on the assumption that skeletal muscle is the main source of the urinary N^{τ} -methylhistidine excretion. On the other hand Harris (1981) has criticized the methods and calculations of Millward *et al.* (1980) and has challenged the idea that skeletal muscle is not the main source of urinary N^{τ} -methylhistidine.

Indeed, direct experimental evidence for the assumption that skeletal muscle is the main origin of urinary N^{τ} -methylhistidine excretion is lacking. In the present paper, we report on a patient who

* Dedicated to Professor Dr. H. Holzer on the occasion of his 60th birthday.

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suffered from a subacute denervating muscular disease. In his final stage he had no longer any detectable skeletal-muscle tissue. Therefore we were able to determine the contribution of non-skeletal-muscle tissue to urinary excretion of *N*⁷-methylhistidine and creatinine.

Materials and methods

All chemicals used were of analytical grade and supplied from E. Merck, Darmstadt, Germany. *N*⁷-Methylhistidine was determined in an Biotronic automated amino acid analyser as described by Tomas *et al.* (1979). Creatinine was measured as described by Wahlefeld *et al.* (1972), creatine as described by Kanig (1957) and guanidinoacetate as described by Bernhardt (1977). For the determination of creatine kinase and aldolase activity, the test combinations from Boehringer Mannheim were used.

Patient

Case report: B.R., a 54-year-old man of 79.5 kg body wt. in 1975 and 60 kg in 1980, did not suffer from any severe disease up to 1975. In 1975 he noticed weakness of his legs while walking. This became progressively more severe until admission. The clinical features were like that of subacute polyneuritis, with a high protein content of the spinal fluid, though he never had any sensory disorders or signs of autonomic dysregulation. In 1977 the disease had progressed so far that the patient had to be taken to an intensive care unit. He had to be mechanically ventilated through a tracheostoma. Food was supplied through a feeding tube; urine was drained by an indwelling catheter. There was no recovery of the motor-neuron function, so the maintenance therapy was continued. In 1979 the patient was nearly completely paralysed; he had a few environmental connections by an electronic typewriter system which he operated with the last small movements of his tongue. By 1980 he was totally paralysed; he could no longer move his tongue and even movements of his eyes were no longer possible. No other disease than paralysis could be found by careful clinical investigations. All biochemical determinations in serum, including hormone analysis (human growth hormone, thyroid-stimulating hormone, tri- and tetra-iodothyronine, cortisol, insulin, testosterone), did not indicate any metabolic disorder. Only the glucose tolerance was slightly impaired, owing to the complete lack of skeletal muscle tissue.

In April 1980 the patient died of a pneumonia. Autopsy showed a severe atrophy of the spinal cord, due to motor-neuron degeneration. The patient was completely skeletal-muscle free. No skeletal-muscle tissue could be detected, not even by serial histo-

logical investigations. The skeletal muscles were replaced by connective tissue as far as can be deduced from the decrease in body weight between 1975 and 1980. Histological investigations of the heart, brain, liver, gut and kidneys did not show any pathological alterations. The cause of the disease is still unknown.

Urine

Urine was collected over 24 h intervals for 3 days after 2 days of *N*⁷-methylhistidine-free diet. The excretion of *N*⁷-methylhistidine per 24 h was calculated as the mean of three triplicate determinations.

Ethics

The principles laid down in the Declaration of Helsinki (1964) were strictly followed. All persons were acquainted with the aim of this study and had given informed consent.

Controls

Normal subjects were investigated (*n* = 23, mean age 42 years, mean body weight 68 kg).

Diet

B.R. received a fluid diet of 8.350 kJ/day (Biosorbin, MTC-Soude, protein 98.3 g, fat 65.9 g, carbohydrate 245.6 g; Pfrimmer, Erlangen, Germany). This diet was free of *N*⁷-methylhistidine. The daily protein supply was sufficient to avoid a negative nitrogen balance. Controls received a flesh- and fish-free diet (approx. 9000 kJ/day). This diet was free of *N*⁷-methylhistidine, but had a sufficient protein content of about 100 g/day.

Results and discussion

The excretion of different metabolites by the patient without skeletal muscle (B.R.) and by healthy controls is summarized in Table 1. As expected, the *N*⁷-methylhistidine excretion was significantly lower (only 28%) in this patient than in the controls. So the assumption that non-skeletal-muscle tissues contribute negligible amounts to *N*⁷-methylhistidine excretion is, at least in humans, not valid. From the results of the autopsy we can conclude that the body composition of the patient was not changed severely. But we cannot totally exclude that metabolic alterations not detectable by our routine serum diagnosis (including hormones) might have happened during the 2-year period of external respiration. On the basis of our results we can confirm the published findings that about 75% of the *N*⁷-methylhistidine excretion in healthy persons is due to skeletal-muscle protein degradation (Harris, 1981).

Table 1. *Urinary excretion of metabolites (mmol/24 h) in a patient without skeletal muscle and in controls*

Metabolite	Patient B.R.	Controls (n = 23)	
		Mean	S.D.
N ^ε -Methylhistidine	0.069	0.24	0.095
Creatinine	2.1	12.1	2.66
Creatine	6.1	0.31	0.17
Guanidinoacetate	0.9	0.7	0.3
N ^ε -Methylhistidine/ creatinine	0.033	0.019	—

In our patient we found a basal excretion of non-skeletal-muscular N^ε-methylhistidine which can be calculated to be 1.15 μmol/24 h per kg of non-skeletal-muscular tissue. For the calculation of the basal non-muscular excretion in healthy subjects, attention has to be paid to the fact that only 60% of total body weight consist of non-skeletal-muscle tissue. In patients with muscular atrophies the diminished muscle mass has to be approximated by the help of the decrease in creatinine excretion (Weisner *et al.*, 1979).

In addition, the excretion of creatinine compared with the controls was also markedly decreased in our patient (Table 1), whereas the creatine excretion was increased about 20-fold. This is a normal observation that during decreasing muscle mass creatine excretion increases, because the uptake of creatine by muscle tissue is diminished (Benedict *et al.*, 1955; Mertens, 1961).

The basal non-skeletal-muscular creatinine excretion was 17% that of controls. This is in contrast with the published data, where the non-muscular source of creatinine is thought to be only 2% of the total creatinine excretion (Bloch & Schoenheimer, 1941).

Assuming that 2% of total creatine/phosphocreatine is daily converted into creatinine by a non-enzymic process (Borsook & Dubnoff, 1947), the non-skeletal-muscle creatine/phosphocreatine pool of a normal healthy individual can be calculated to be 105 mmol (≈22 g of phosphocreatine), predominantly located in the brain, and the total body pool to be 605 mmol (≈128 g of phosphocreatine).

From these data, one can calculate a basal creatinine urine excretion from organs other than skeletal muscle of 35 μmol/24 h per kg of non-skeletal-muscle body weight. As mentioned above, in healthy controls only 60% of the total body weight should be used for the calculation of basal non-skeletal-muscular excretion.

In contrast, the excretion of guanidinoacetate, the precursor of creatine, showed no significant difference between the patient and the controls.

The patient's ratio of N^ε-methylhistidine/

Table 2. *Serum concentrations of metabolites and enzymes in a patient without skeletal muscle and in controls*

	Patient B.R.	Controls (n = 23)	
		Mean	S.D.
Creatinine (mm)	0.027	0.07	0.01
Creatine (mm)	0.143	0.03	0.01
Guanidinoacetate (mm)	0.188	0.16	0.09
Creatine kinase (units/litre)	15.0	24.0	7.8
Aldolase (units/litre)	0.4	0.77	0.43

creatinine excretion was significantly higher than the control value, although skeletal-muscle degradation could not occur any more. This ratio, which is used for clinical diagnosis of increased myofibrillar protein breakdown, is in the range found in patients suffering from enhanced muscular wasting (Roethig *et al.*, 1980; Tomas & Ballard, 1979). In our opinion this ratio seems to be valid as a diagnostic criterion only if the N^ε-methylhistidine and creatinine excretions are corrected for the basal values.

The serum concentrations of creatinine, creatine and guanidinoacetate as well as the activities of creatine kinase and aldolase are listed in Table 2. They were all within the normal ranges, except for creatinine and creatine, which were 2.5-fold decreased and 4.7-fold increased. This seems to be due to the fact that the main tissue for creatine uptake, skeletal muscle, was totally missing in the patient.

In conclusion, N^ε-methylhistidine and creatinine determinations as tools for clinical diagnosis of muscular diseases or for the calculation of myofibrillar protein breakdown are only valid if the following precautions are considered. (i) The diet should contain no N^ε-methylhistidine, but enough protein should be supplied to provide positive nitrogen balance. (ii) The urinary excretion values of N^ε-methylhistidine and creatinine should be corrected for the basal non-skeletal-muscle-dependent excretion. This has to be a prerequisite for the calculation of the ratio of N^ε-methylhistidine/creatinine excretion, especially in patients with low muscle mass.

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