Studies on the Role of Taurine in Friedreich's Ataxia

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ABSTRACT: New studies were undertaken to verify the previous findings of increased urinary excretion of taurine, in the basal state and after challenge with a taurine load, in Friedreich's disease. Particular attention was paid to possible causes of error such as weight, muscle mass, creatine and creatinine excretion, variability with time and appropriate control groups. Although the overall findings were confirmed, their interpretation is open to question because of all these factors of error. Many possibilities must still be further explored to account for the apparent taurine retention defect observed in many cases of Friedreich's disease.

RÉSUMÉ: De nouvelles études furent entreprises pour vérifier les observations antérieures d'excrétion urinaire augmentée de taurine, à l'état de base et après surcharge, dans la maladie de Friedreich. Une attention toute particulière fut portée à plusieurs causes d'erreurs possibles: poids, masse musculaire, excrétion de la créatinine et de la créatine, variabilité dans le temps et utilisation de groupes témoins appropriés. Quoique dans l'ensemble les observations antérieures furent confirmées, il faut émettre des réserves sur l'interprétation finale, à cause de tous ces facteurs d'erreurs. Il faut encore explorer en détail plusieurs possibilités avant de pouvoir expliquer le défaut apparent dans la rétention de la taurine chez plusieurs patients souffrant de la maladie de Friedreich.

It was postulated (Barbeau, 1982) that subjects suffering from Friedreich's ataxia have a reduced capacity for the retention of taurine in the kidney. One of the possibilities explored was a defect in the TH reabsorption system situated in the border of renal tubule cells (Godman et al., 1982) with low capacity but high specificity for taurine. Such a transport defect, if confirmed, would be a major contributory factor to the development of the symptoms of Friedreich's ataxia, particularly the primary symptoms including cardiomyopathy, absence of reflexes and dorsal column involvement.

This hypothesis was supported by two observations:

1) increased urinary excretion of taurine in F.A. patients (Lemieux et al., 1976);

2) studies with a taurine challenge to identify different types of urinary excretion of the amino acid (Barbeau et al., 1982; Filla et al., 1979).

In the philosophy of the Quebec Cooperative Study it is imperative to submit each new finding, or hypothesis, to the scrutiny of other laboratories. Because of a few uncertainties in the interpretation of the basic data, the present studies were undertaken. These questions concern both the observation of increased urinary excretion of taurine and the results of challenge tests:

(1) The distribution of taurine in the body is not homogeneous, the muscles, including the heart, containing nearly 70% of the total. Because of this distribution any pathology affecting these muscles could be reflected in abnormal urinary excretion of taurine. Such was indeed the case in conditions of stress, corticosteroid therapy, idiopathic scoliosis and familial cerebral dyssynergy (Filla et al., 1979; Goodman et al., 1980). Thus the increased loss of taurine could equally be due to a decreased tissue reservoir because of the smaller mass.

(2) The results of the original challenge tests were based on a uniform load of 250 mg of taurine, independent of the weight of the individual, although the range of weights in these individuals, all adults, was not great (mean 45 kilograms for the women and 63 kilograms for the men, thus 3.96 to 5.56 mg of taurine per kilogram).

The measurement of creatine and creatinine in different subjects: Friedreich's disease (F.A.), muscular dystrophy and healthy controls permits the evaluation of different parameters: creatine metabolism; creatine/creatinine ratio in different pathological states; utility of creatinine as a factor of reference for the measurement of taurine, and a comparison of the findings in F.A. subjects with others having a similar muscle mass (Applegarth and Ross, 1975; Déchaux et al., 1978).

The objective of this study was thus to further investigate the excretion of taurine by evaluating the influence of these parameters upon the proposed hypothesis, and upon the validity of the original data.

EXPERIMENTAL

Test population

12 subjects with Friedreich's ataxia (from Sherbrooke)
3 subjects with muscular dystrophy
6 controls age matched to the ataxic patients

Taurine challenge

The dose of taurine used was strictly 4 mg/kg. As shown by Goodman and his collaborators, this dose produces the greatest difference between genotypes (Conolly and Goodman, 1980; Connolly et al., 1979; Goodman et al., 1980; Goodman and Connolly, 1982). The test was started between 8 and 9 am, after an overnight fast. The subjects had all completed a urine collection for the 24 hours preceeding the test (time zero). Taurine was administered orally in 10 cc of water. Urine collection were made after 2, 4 and 6 hours. The laboratory techniques used were: for creatine and creatinine — O'Brien et al. (1968), for alpha-amino-nitrogen — Wells (1969), and for taurine — Lemieux et al. (1976).

RESULTS

Excretion of taurine (Table 1)

The 24 hours excretion of taurine, when corrected for weight, shows that in 1976, 11 of 15 cases of F.A. had values within the range of normal subjects. In 1983 of the 12 subjects surviving 7 had values within the normal range. In 1983, 7 of the 12 subjects had taurine excretion greater than in 1976, the other five had values below those in 1976. Two of the three cases of muscular dystrophy had values within the normal range.

Table 1: Concentration of Taurine (24 Hrs. Urine)

| | $\mu M/100ml/min/1.73m^2$ | | |
	1983	1976	Diff. 83 - 76
SBL5	100.88	10.3	+ 90.58
SBL4	42.77	59.3	− 16.53
SBL3	Deceased	208.9	Deceased
SBL10	219.70	174.0	+ 45.7
SBL12	270.53	48.6	+221.93
SBL9	95.78	49.3	+ 46.48
SBL8	130.34	153.5	− 23.16
SBL16	Deceased	58.2	Deceased
SBL17	12.61	63.5	− 50.89
SBL18	22.65	10.4	+ 12.25
SBL15	110.42	86.4	+ 24.02
SBL14	19.19	84.5	− 65.31
SBL2	47.55	59.5	− 11.95
SBL7	61.60	28.8	+ 32.8
SBL6	Deceased	223.3	Deceased
MEAN ±S.D.	94.50 ± 77.32	87.90 ± 66.51	
Sibling (S.F.)	6.18		
Sibling (S.D.)	27.53		

MUSCULAR DYSTROPHY

D.M. 1	127.56	
D.M. 2	67.47	
D.M. 3	14.58	

CONTROL (24) $\bar{X}$ ± S.D.: 47.21 ± 46.08

Creatinine in blood and urine

In 1976 (Table 2) of 18 F.A. subjects, 10 had subnormal 24 hour creatinine level expressed as mg/24 hours, but when

Table 2: First Study: 1976. Concentration of Creatinine in Blood and Urine (24 Hrs.).

Patient		Creatinine in Blood — mg%	Creatinine in Urine — mg/24 hrs	Creatinine in Urine — mg/24 hrs/kg	Weight Kg	Sex	Age
MAB 4		1.6	1515.9	25.26	59.3	M	22
MAB 6		0.7	659.4	23.55	28	F	21
MAB 10		0.8	648.1	15.58	41.6	F	22
MAB 11		0.3	347.5	18.97	18	F	8
SBL2		0.6	486.1	14.30	34	F	13
SBL6		1.5	741.32	12.74	58.2	M	26
SBL7		1.0	482.98	15.89	30.4	F	25
SBL3		0.6	550.26	19.11	28.8	F	15
SBL4		0.6	591.79	21.60	27.4	F	14
SBL5		0.6	390.65	21.58	18.1	F	7
SBL8		0.6	626.88	20.49	30.6	F	12
SBL9		0.5	384.58	18.14	21.2	F	9
SBL10		0.6	1027.8	33.16	32.5	F	13
SBL12		0.6	227.85	9.19	24.8	F	9
SBL14		0.7	1517.26	24.16	62.8	M	16
SBL15		0.7	575.11	20.54	28.0	M	12
SBL16		0.5	887.21	21.64	41	M	17
SBL17		0.4	898.99	14.74	61	F	23
SBL18		0.9	—	—	54.5	F	28
MAB 1		1.0	187.5	3.16	59.3	M	21
CONTROLS	MALES	0.5 - 1.1	1100 - 2500	15 - 30		M	14 - 40
	FEMALES	0.5 - 0.8	1010 - 1330	15 - 30		F	14 - 40
	CHILDREN	0.7 - 1.6	300 - 1100	7 - 22		F — M	6 - 11

Table 3: Second Study: 1983. Concentration of Creatinine and Creatine in Urines

| PATIENTS	SEX	WEIGHT Kg	CREATININE mg/24 hrs/kg	CREATININE mg/24 hrs	CREATINE mg/24 hrs	CREATINE mg/24 hrs/kg	CREATINE/CREATININE
SBL 5	F	40.45	70.78	2,863	—	—	—
SBL 4	F	35.0	17.14	600	145	4.14	0.24
SBL 2	F	54.5	19.14	1,043	—	—	—
SBL 7	F	50.9	8.45	430	161	3.16	0.37
SBL 15	M	47.7	20.44	975	10	0.21	0.01
SBL 14	M	68.2	19.50	1,330	—	—	—
SBL 9	F	43.0	21.93	943	24	0.56	0.02
SBL 8	F	38.6	17.10	660	71	1.84	0.11
SBL 10	F	47.7	14.88	710	69	1.45	0.10
SBL 12	F	50.9	21.00	1,069	55	1.08	0.05
SBL 18	F	54.5	23.56	1,284	353	6.48	0.27
SBL 17	F	72.7	13.55	985	—	—	—
MUSC. DYST. 1	M	29.1	10.69	311	556	19.11	1.79
MUSC. DYST. 2	M	54.5	8.13	443	547	10.04	1.23
MUSC. DYST. 3	M	72.7	7.08	515	710	9.77	1.38
CONTROLS	M		15 - 30	1100 - 2500	11 - 189		
	F		15 - 30	1010 - 1330	19 - 270		0.32 ± 0.08
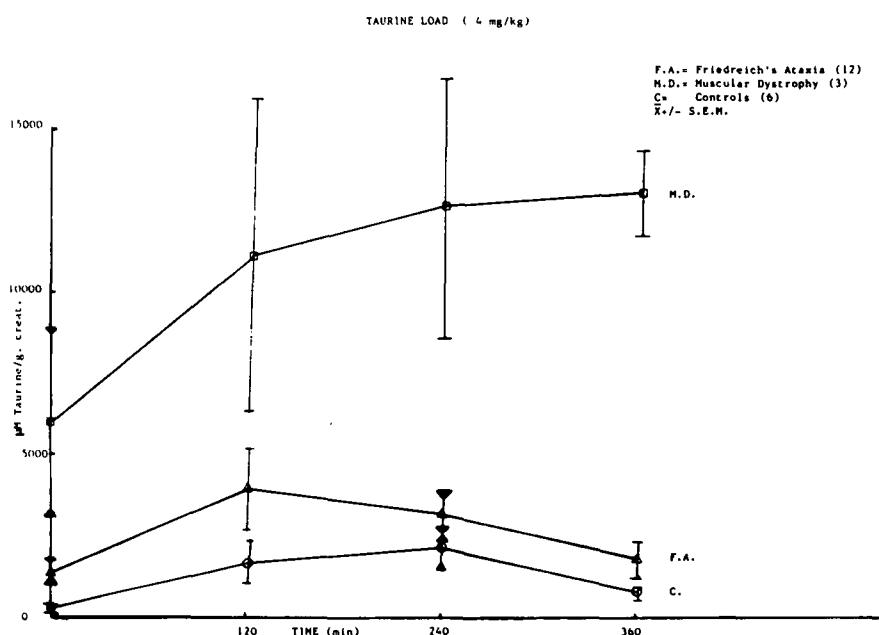
Figure 1 — Effect of a load in taurine (4 mg/kg) in Friedreich's ataxia, muscular dystrophy and normal controls. Results are expressed per gram of creatinine.

expressed as mg/kg/24 hours only one had a value below the normal range.

The same phenomenon was seen in 1983 (Table 3) where 7 of 12 had sub-normal excretion in terms of creatinine/24 hours, but only 2 of 12 had low values expressed as mg/kg/24 hours. The three muscular dystrophy patients had low creatinine excretion. The creatine/creatinine ratio was lower than normal in most F.A. cases but was greatly increased in muscular dystrophy.

Taurine levels after challenge

As can be seen (Fig. 1), the mean excretion of taurine in response to the challenge was greater for the F.A. cases than for the controls, but very much less than for the muscular dystrophy patients. The pattern of excretion was similar for F.A. and control subjects.

A comparable difference was obtained when the excretion was calculated in terms of alpha-amino-nitrogen excretion, although the difference between the three groups of subjects was less marked (Table 5, Fig. 1 and 2). The shape of the curves, if not the amplitude, confirms the pattern observed by Barbeau et al. (1982), whose cases are older.

DISCUSSION

The measurement of urinary creatinine is widely used as an index of excretion. However, this is subject to variation accord-
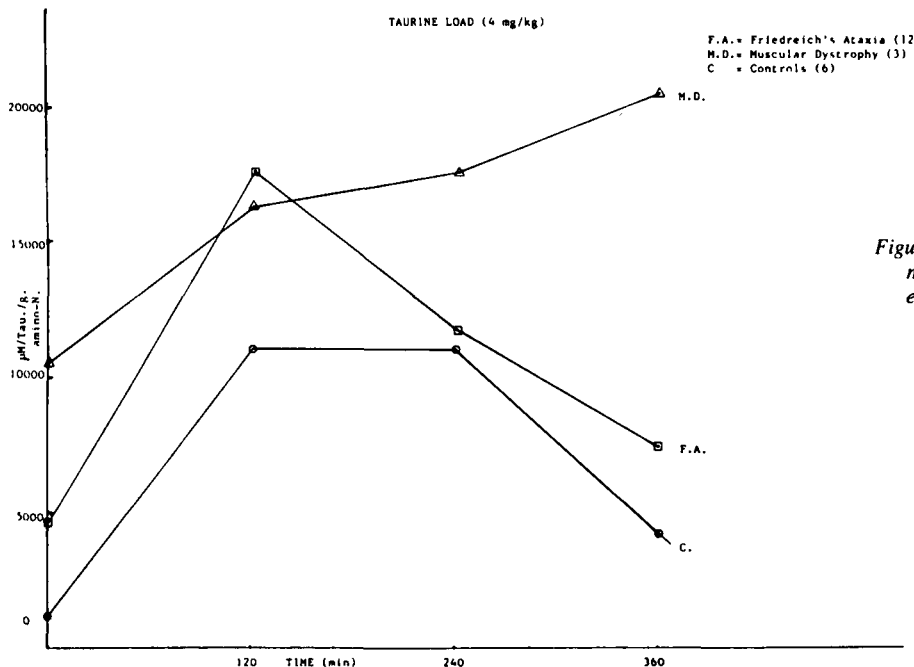
Figure 2 — Effect of the same load in taurine (4 mg/kg) as in Fig. 1., when the results are expressed per gram of amino-nitrogen.

Table 4: Difference Between Excretion of Creatinine (From The Two Studies)

	CREATININE (mg/24 hrs)		AGE IN 1976	DIFFERENCE
	1983	1976		
SBL 2	1043	486	13	+ 557
SBL 4	600	591	14	+ 9
SBL 5	2863	390	7	+ 2473
SBL 7	430	483	25	— 53
SBL 8	660	626	12	+ 34
SBL 9	943	384	9	+ 559
SBL 10	710	1027	13	— 317
SBL 12	1069	228	9	+ 841
SBL 14	1330	1517	16	— 187
SBL 15	975	575	12	+ 400
SBL 17	985	899	23	+ 86

ing to muscle mass, muscular activity, intake of creatine (muscle meat) and, evidently muscle metabolism and renal function. Hence, when utilising this parameter to compare different groups of subjects, it is obviously necessary that these variables be constant between the groups.

In the 1983 study, the absolute excretion of creatinine in 24 hours was, for the majority of F.A. subjects, lower than for healthy subjects of similar age, but when expressed in terms of body weight the excretion in F.A. subjects was normal. It is evident, from this, that creatinine as such is not a reliable index for the study of excretion in F.A. subjects. Furthermore, the abnormal creatine/creatinine ratio in F.A. subjects would indicate abnormal muscle metabolism, which also must discredit the use of creatinine.

We included some cases of muscular dystrophy in the study to determine if subjects who had similar physical handicap would be a more appropriate test group. As can be seen (Table 3) the creatinine excretion was grossly abnormal in that group.

In comparing the results obtained for creatinine in 1976 and 1983 (Tables 2, 3 and 4) it is evident that the results are similar, that the absolute excretion of creatinine is below normal limits in most F.A. subjects. A disquieting feature is the wide variation in creatinine excretion in the same individuals (Table 4).

While the age may have been a factor in 1976, all were adults by 1983, and it now appears that weight, or more probably muscle mass, is the most important factor. The interpretation of taurine excretion expressed uniquely in terms of creatinine is therefore open to criticism in such subjects.

Using a second parameter which, perhaps, relates more directly to taurine — the excretion of alpha-amino-nitrogen — there was also increased excretion of taurine, although less marked. It would be worthwhile investigating further to determine if this parameter (alpha-amino-nitrogen) is a more reliable index of amino acid excretion than creatinine. Others (Seashore et al., 1981) suggest the use of the ß-methylhistidine/creatinine ratio.

It is evident that while the mean 24 hour taurine excretion is much greater in F.A. subjects when expressed in terms of μM/g creatinine (Table 5), it is also greater when expressed as absolute excretion in term of body mass (Table 1). However, before drawing final conclusions, the reproducibility of taurine excretion must be considered. As can be seen from Table 1, there were significant differences between the two samplings, both increases and decreases, and there is no evident pattern. We can only draw the conclusion that there is significant random variation in taurine excretion within the same individuals and so the significance of differences between populations becomes questionable. Were the individuals maintained under standard conditions in a metabolic unit, a truly valid comparison could be made, but the expenses and inconvenience of this approach exceed our present capabilities.

Table 5: Urinary Excretion After Taurine Load Test

(4 mg/kg)

A — Taurine: μM/g Creatinine

Groups	N	Time 0	2 hours	4 hours	6 hours
Friedreich's Ataxia	12	1404 ± 1197	4037 ± 4210	3151 ± 2650	1763 ± 1813
Muscular Dystrophy	3	6014 ± 4995	11229 ± 8303	12673 ± 6914	13164 ± 2250
Controls	6	314 ± 187	1760 ± 1598	2157 ± 1502	722 ± 500

B — Taurine: μM/g a-amino N

Friedreich's Ataxia	12	4778 ± 3284	17793 ± 18739	11816 ± 8341	7640 ± 6941
Muscular Dystrophy	3	10672 ± 9532	16397 ± 9167	17675 ± 10520	20236 ± 5124
Controls	6	1475 ± 732	11070 ± 10530	11089 ± 7736	3607 ± 2525

Table 6: Taurine Urinary Excretion After Taurine Load Test (4 mg/kg)

LOG (mg Tau/g creat)

A.F. (12)	TIME: 0	TIME: 2 hrs.	TIME: 4 hrs.	TIME: 6 hrs.
SBL 4	1.94	2.76	1.73	1.94
SBL 5	1.67	2.45	2.68	2.57
SBL 2	2.43	2.76	2.92	2.58
SBL 7	1.94	3.14	2.88	2.89
SBL 15	2.20		2.81	1.79
SBL 14	1.35		2.51	1.66
SBL 9	2.30	2.08	2.04	1.78
SBL 8	2.49	2.18	1.79	2.19
SBL 10	2.67	3.20	2.98	2.60
SBL 12	2.60	2.34	1.29	—
SBL 18	1.29	1.46	1.33	1.59
SBL 17	1.38	2.05	2.65	1.64
X̄	2.19	2.44	2.30	2.11
RANGE	1.29 - 2.67	1.46 - 3.20	1.29 - 2.98	1.59 - 2.89
MUSCULAR DYSTROPHY (3)				
M.D. 1	3.19	3.45	3.23	—
M.D. 2	2.80	2.45	2.67	3.28
M.D. 3	1.66	3.06	3.41	3.13
X̄	2.55	2.99	3.10	3.20
RANGE	1.66 - 3.19	2.45 - 3.45	2.67 - 3.41	3.13 - 3.28
CONTROLS (6)				
C1	1.61	2.73	2.56	1.35
C2	1.91	1.53	1.67	2.05
C3	1.33	1.62	2.29	1.77
C4	1.73	2.65	2.58	2.31
C5	1.17	1.91	1.75	1.44
C6	1.33	2.25	2.76	2.07
X̄	1.51	2.11	2.27	1.83
RANGE	1.17 - 1.91	1.53 - 2.73	1.67 - 2.76	1.35 - 2.31

There is also a significant difference in the mean response of the F.A. subjects to a taurine challenge (Table 5-6), the mean excretion being much greater than in the controls. Here again the results must be interpreted with caution (a) in view of the great variability in response among the controls and test subjects and (b) because of the lower creatinine levels in F.A. subjects giving a falsely elevated taurine/creatinine ratio.

CONCLUSION

The results of the present studies essentially confirm the previous observations of increased taurine urinary excretion under basal conditions (Lemieux et al., 1976) and after a taurine challenge (Barbeau et al., 1982) in Friedreich's disease. However the wide variability in the results, and similar patterns in other neurological disorders with a decreased muscle mass, raise questions about the previous interpretations of these findings. The apparent retention defect in taurine (Barbeau, 1982) may be only a reflection of this decreased muscle mass or of other non-genetic factors, rather than of a defect in tubular reabsorption. The choice of an appropriate neurologically impaired control group with similar disabilities is also a critical factor for the interpretation of the hypothesis. Finally, the authors confirm the unsuitability of creatinine as an index of urinary excretion in subjects with decreased muscle mass or abnormal creatine/creatinine metabolism.

Taurine in F.A. — Lemieux et al.

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