Amino acids in retinitis pigmentosa

STEVE A. ARSHINOFF,¹ J. CLEMENT McCULLOCH,¹ WILLIAM MACRAE,²
ARTHUR N. STEIN,³ AND ERROL B. MARLISS³

From the Departments of ¹Ophthalmology and ³Medicine, Toronto General Hospital, and ²Department of Ophthalmology, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada

SUMMARY A survey of fasting whole blood amino acids in 65 patients with various subtypes of retinitis pigmentosa was performed. Eight X-linked recessive patients showed decreased taurine and aspartate. Nineteen autosomal recessive patients, and to a lesser extent 10 autosomal dominant patients, showed reduced levels of whole blood threonine and histidine. Branched-chain amino acids and arginine were present in increased amounts in 2 patients with Laurence-Moon-Bardet-Biedl syndrome. These findings in LMBB patients are probably related in part to their obesity and emphasise that appropriate controls are required, and other factors (including age) known to affect amino acid levels must be accounted for. The biochemical implications of our findings are not yet apparent.

The discovery of the molecular cause of retinal degeneration in a few animal models and the elucidation of an enzymatic deficiency in gyrate atrophy of the retina and choroid with hyperornithinaemia have generated a renewed search for the biochemical causes for human retinitis pigmentosa (RP). To date there have been few indices suggesting the possibility of a metabolic disorder. The problem is made more complex by the inaccessibility of human pathological material and by the clinical complexity and genetic heterogeneity of RP. This is in contrast to the considerable progress achieved in the investigation of the amino acid metabolism in gyrate atrophy,¹⁻³ a related disorder.

The few data available in RP suggesting a metabolic disorder are the following. In a recent Finnish study decreased uptake of taurine by platelets of RP patients of unspecified inheritance patterns when compared with controls was demonstrated.⁴ Cats fed a diet deficient in taurine develop a retinal degeneration.⁵ However, amino acid data in humans with RP are scarce. Berson *et al.* have reviewed the subject of amino acids in retinal degenerations and reported on 26 patients with RP.⁶ No significant differences from normal controls were identified in the fasting plasma levels of 12 amino acids. We report herein a study of 18 fasting whole-blood amino acid levels in 65

patients with RP compared with those in 25 normal controls.

Materials and methods

Patients in this study were referred to the Hospital for Sick Children's Ophthalmic Genetics Clinic either for genetic counselling or for inclusion in the study. Some patients previously examined in the clinic were recalled for participation. Control values were obtained from healthy individuals known to be free of metabolic, ocular, and systemic diseases and matched in so far as possible with the patient population for age and sex.

In order to subclassify the RP patients, where a detailed family history was not already available, each patient was asked to fill out the Hospital for Sick Children's eye genetics questionnaire, a family tree was constructed, and, if necessary and possible, relatives were examined. An appropriate consent was obtained. Each patient was asked to fast from midnight, and the following morning between 9 and 11 am blood was drawn for haematocrit, glucose, insulin, and whole blood amino acid measurements. When the collected data were deemed satisfactory (by criteria detailed below), the patient was entered into the study and assigned to one of the groups (RPG) shown in Table 1.

The method for amino acid analysis was as follows. The venous blood drawn from the patient was immediately deproteinised by addition to an equal

Correspondence to Dr. E. B. Marliiss, Room 5242, Medical Sciences Building, Clinical Science Division, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Table 1 Patients classified into groups

Class		Number
Autosomal dominant (D)		
Definite:	isolated RP present in 3 generations male/female ratio approximately 1 segregation ratio 50:50	7
Probable:	definite male to male transmission isolated RP present in 2 generations males equal females in severity and incidence definite male to male transmission	3
Autosomal recessive (R)		
Definite:	parental consanguinity male/female ratio approximately 1 approximately 25% of sibs affected	12
Probable:	parental consanguinity small family—no affected sibs —2 affected female sibs large family with multiple consanguinity and many affected in an autosomal recessive pattern	7
X-linked (X)		
Definite:	more than 1 generation of affected males through a normal female carrier no male to male transmission no affected females	3
Probable:	2 affected male sibs in a family with no other known affected members	5
Simplex (S)	only known affected member in a family	18
Usher's syndrome (U)	RP of definite or probable recessive pattern nerve conduction hearing deficit	8
Laurence-Moon-Bardet-Biedl syndrome	RP of definite or probable recessive pattern polydactyly mental retardation obesity hypogenitalism	2
Total		65

volume of cold 10% (w/v) perchloric acid (PCA), and kept on ice until centrifugation in a refrigerated centrifuge at 3000 rpm at 4°C. In so far as possible the analysis was performed the same day without freezing of the sample. When this was not the case, values for glutamine and glutamate are not reported. 50 µl of deproteinised supernatant was analysed in a Beckman 121M amino acid analyzer (Beckman Instruments Inc., Palo Alto, CA). The elution was performed automatically by programmed progression of buffer, pH, and column temperature. Since glutathione is present in whole blood, its position (oxidised and reduced forms) is important in chromatogram interpretation. The reduced form coelutes with aspartic acid, giving results representing the sum of the 2 substances. The oxidised form elutes as a separate peak which does not interfere with interpretation, but does not give a reliable estimate of the concentration of this substance. Calculation of peak areas was performed automatically by on-line computer previously calibrated from known standards.

We have confirmed the instability of glutamine after prolonged storage at -20°C and at the pH of the non-neutralised PCA supernatants.¹ This amino acid spontaneously deamidates to form glutamic acid, causing spuriously raised levels of the latter and

decreased levels of the former. The conversion does not occur to a significant degree after neutralisation to a pH of 4.9 (which does not affect the other amino acids) or with immediate analysis of the sample. Because the neutralisation step was introduced after the study began, glutamine and glutamic acid data are presented only for those samples in which valid levels were obtained. Insulin was assayed by radioimmunoassay as previously detailed⁷ and plasma glucose was determined by the glucose oxidase method with a Beckman glucose analyser (Beckman Instruments Inc., Fullerton, CA). All analyses were performed by technicians who were unaware of whether samples were from patients or controls.

Results from some subjects were excluded on the following criteria: (1) Incomplete genetic history precluding precise assessment of the pattern of inheritance (n=10). (2) Plasma insulin and glucose levels indicated that the patient had most likely not fasted as requested; such patients were excluded from the study (n=3). (3) A patient suffering from a rare syndrome which had as one of its components RP was excluded (n=1). (4) Normal control values for patients under 16 were not obtained; patients under this age were excluded (n=4). (5) In 1 patient a normal electroretinogram made the referral diagnosis

suspect (n=1). (6) When the sample was inadequate for laboratory examination the patient was excluded (n=2).

A total of 86 samples were drawn. After exclusion of the 21 patients by the foregoing criteria results from 65 RP patients were included and compared with those from the 25 healthy volunteer controls. Significance of differences among groups was evaluated by Student's unpaired *t* test for each amino acid or other variable.

Results

Anthropomorphic data from the study and control groups are presented in Table 2. Heights and weights of patients are compared with those of normal controls, as well as fasting levels of insulin and glucose. The only significant deviations of the patient population from the control group lies in the younger age of the 2 patients with Laurence-Moon-Bardet-Biedl (LMBB) syndrome (probably reflecting more severe disease, typically with early macular involvement and hence earlier presentation), and the older age of the female RP patients (perhaps reflecting slightly less severe disease in females). In addition the patients with Laurence-Moon-Bardet-Biedl syndrome were heavier than the remainder of the patients or control group, reflecting one of the manifestations of this syndrome. The autosomal dominant RP females had significantly raised glucose levels compared with those of normal control females.

Table 3 compares the fasting whole-blood amino acid levels of 18 amino acids in 65 RP patients and 25 controls. The RP patients were divided by mode of inheritance and specific syndromes to define possible

differences further. When they were considered together as a group the only findings in the RP patients was a slightly increased level of asparagine compared with that of normal controls; no specific RP subgroup had this same characteristic.

When the individual RP subgroups were compared with normal controls, a number of additional amino acids showed significant differences. (1) Threonine and histidine levels were significantly reduced and glycine levels were increased in patients with autosomal dominant RP. (2) Threonine and histidine levels were significantly reduced in patients with autosomal recessive disease. (3) Taurine was slightly reduced and aspartic acid plus glutathione moderately reduced in X-linked patients. (4) Simplex patients showed slight elevations of lysine. (5) Usher's syndrome patients showed no consistent amino acid level differences from normal controls. (6) Two patients with LMBB syndrome showed large increases in the branched chain amino acids valine, isoleucine, and leucine. They also showed elevations of arginine, tyrosine, and phenylalanine to the $p<0.001$ confidence level and decrease of glycine to the $p<0.05$ level. Unfortunately this sample is extremely limited, there being only 2 subjects.

Discussion

Three previous studies of amino acid levels in retinitis pigmentosa (RP) have been published. Campbell and Tonks^a reported plasma amino acid data on 7 patients with RP and found no significant deviations from their normal values. Fujiwara studied amino acids in aqueous humour and serum of RP patients of unspecified hereditary types. He found a decrease in

Table 2 Characteristics of study and control groups

	Normal controls	Total RP group	RP subgroups					
			Domin.	Recess.	X-linked	Simplex	Usher's	LMBB
Participants:								
male	10	33	4	6	8	9	4	2
female	15	32	6	13	-	9	4	-
Age (years):								
male	30±2	34±2	40±7	35±4	34±6	31±3	32±5	23±0
female	32±3	31±2	39±10	34±6	34±6	26±3	27±5	23±0*
male	28±3	37±3*	41±9	35±5	-	36±5	38±7	
Height (cm):								
male	168±2	167±2	161±6	165±2	172±2	169±3	166±4	175±5
female	174±4	172±1	169±4	171±1	172±2	174±4	173±4	175±5
Weight (kg):								
male	64±3	66±2	61±5	67±4	64±4	63±3	67±6	90±3
female	71±5	70±3	63±8	78±6	64±4	68±3	69±11	90±3*
male	59±3	60±2	58±4	61±3		58±5	65±5	
Fasting insulin (ng/ml):								
male	0.61±0.04	0.58±0.04	0.53±0.07	0.55±0.05	0.62±0.11	0.52±0.03	0.59±0.06	1.50±0.77
female	0.63±0.07	0.61±0.06	0.54±0.06	0.54±0.09	0.62±0.11	0.51±0.04	0.56±0.05	1.50±0.77
male	0.59±0.05	0.55±0.03	0.53±0.12	0.55±0.05		0.53±0.06	0.62±0.11	
Fasting glucose (mg/dl):								
male	89±2	89±1	94±3	86±1	85±4	90±2	88±3	87±2
female	98±3	89±2*	92±4	88±2*	85±4*	92±3	87±3*	87±2*
male	84±2	89±1	95±5*	86±1		89±2	89±6	

* $p<0.05$ versus controls. Data presented as mean±SEM.

Amino acids in retinitis pigmentosa

Table 3 Fasting whole-blood amino acids in RP patients and normal controls, mmol/l

Amino acid	Normals n=25	Total RPG n=65	Domin. n=10	Recess. n=19	X-link. n=8	Simplex n=18	Usher n=8	LMBB n=2
1. Taurine	208±6	205±5	219±14	193±10	185±9*	214±10	225±11	172±16
2. Aspartic acid	575±43	508±21	500±61	510±35	383±58*	507±40	610±33	628±71
3. Threonine	140±7	126±5	112±10*	108±5***	126±10	151±13	134±11	115±11
4. Serine	129±4	133±3	134±8	127±4	134±8	140±7	138±11	110±16
5. Asparagine	51±2	60±3* (n=54)	56±9 (n=9)	58±6 (n=13)	51±8 (n=6)	63±6 (n=17)	67±8	87 (n=1)
6. Glutamine	587±20 (n=13)	580±14 (n=21)	620±9 (n=2)	565±22 (n=9)	493 (n=1)	548±5 (n=2)	624±5 (n=5)	576±11
7. Glutamic acid	134±9 (n=13)	129±7 (n=21)	150±12 (n=2)	122±7 (n=9)	113 (n=1)	126±48 (n=2)	149±15 (n=5)	104±20
8. Glycine	273±14	293±9	323±32*	288±19	265±15	300±15	295±17	244±1*
9. Alanine	328±13	318±10	293±17	289±15	326±35	327±24	371±19	388±30
10. Valine	167±12	160±7	147±17	148±12	157±23	167±14	177±7	220±3***
11. Isoleucine	50±2	49±2	47±5	48±2	47±4	50±3	54±3	69±3***
12. Leucine	97±4	96±3	92±7	91±5	96±6	97±6	102±7	135±5***
13. Tyrosine	45±2	47±2	43±3	43±2	45±2	52±5	51±4	61±7*
14. Phenylalanine	39±2	41±1	41±2	37±2	40±2	44±3	42±2	57±7*
15. Ornithine	72±7	72±3	72±8	72±6	70±5	69±5	80±9	76±4
16. Lysine	143±5	152±4	135±6	143±7	162±11	165±9*	153±5	173±15
17. Histidine	71±2	67±2	62±2**	60±3**	66±2	72±4	75±8	67±1
18. Arginine	57±3	61±2	67±5	57±3	61±4	57±5	67±4	76±1***

*p<0.05. **p<0.01. ***p<0.001.

Aspartic acid value includes coeluted glutathione. Total RPG denotes pooled results for the whole group of patients with retinitis pigmentosa.

levels of cystine and alanine in serum.⁹ Berson *et al.*⁶ reviewed the subject of fasting plasma amino acids in hereditary retinal diseases, including 26 patients with RP. Recognising that clinical RP is made up of a number of distinct hereditary entities with clinically similar appearance, they divided their patients into the different recognisable inheritance patterns (autosomal dominant, n=4; sex linked, n=3; autosomal recessive, n=15; Usher's syndrome, n=2; Laurence-Moon-Bardet-Biedl syndrome, n=2) and compared the results with those from 15 healthy controls. They

found no significant deviations from normal among the 12 amino acids that were studied. Other amino acids were not reported in their study (Table 4). It is in these previously unreported amino acids that the most significant changes were found in the present study of whole-blood amino acids.

Several statistically significant differences have been shown in the present study. How biologically significant are they? Indeed, when comparing 18 amino acids in 7 independent groups one could obtain 5-7 statistically significant results, at the p<0.05 level, merely by chance. In addition it is apparent from Table 2 that the RP and control populations were not perfectly matched, in that the RP females were older than the control females, while the LMBB patients were the youngest. These differences may cause the groups not to be entirely comparable biochemically. The LMBB patients were significantly heavier than all other groups, but this is an expected part of the syndrome and undoubtedly accounts for some of the observed differences. Elevations in most of the amino acids shown to be increased are expected in obesity in relation to insulin resistance; our subjects showed typical markedly elevated insulin levels.¹⁰ Similarly, glycine levels are decreased in obesity and increased in protein malnutrition. Finally, particularly in the LMBB group, the sample is rather small, consisting of only 2 patients. The next smallest subgroup is the Usher's syndrome with 8 patients, which is a much more adequate sample size.

From the methodological standpoint care was taken to assure precision of the results. Not all the amino acid determinations were performed immediately after drawing blood. Storage time has been shown to alter significantly the values of glutamic acid and glutamine.¹ For this reason the reported data for these amino acids are limited, since only data from

Table 4 Comparison of amino acid results in retinitis pigmentosa

Amino acid		Berson <i>et al.</i> ⁶	Present study
Taurine		Yes	Yes*
Aspartic acid	P	Yes	Yes*
Threonine	PE	-	Yes**
Serine	P	Yes	Yes
Asparagine	P	-	Yes
Glutamine	P	Yes	Yes
Proline	P	Yes	-
Glutamic acid	P	Yes	Yes
Citrulline		Yes	-
Glycine	P	-	Yes*
Alanine	P	-	Yes*
Valine	PE	-	Yes**
Cysteine	P	Yes	-
Methionine	P	Yes	-
Isoleucine	PE	-	Yes**
Leucine	PE	-	Yes**
Tyrosine	P	-	Yes*
Phenylalanine	PE	-	Yes*
Ornithine		Yes	Yes
Lysine	PE	Yes	Yes*
Histidine	PE?	-	Yes**
Tryptophan	PE	-	-
Arginine	P	Yes	Yes**

*Significant changes in one or another subgroup found in this amino acid in present study. **Most significant changes present in these amino acids in present study.

P=amino acid is normally found in proteins. E=amino acid is normally essential in diet.

samples run immediately after drawing blood are reported. The remaining reported amino acids are stable under the storage and assay conditions used. In addition all samples were drawn fasting in mid morning to minimise possible diurnal fluctuations.

Despite these difficulties it is probable that many of these findings represent biologically significant differences, even if only the data that are significant at less than the $p < 0.05$ level (i.e., < 0.01 and < 0.001) are considered. Threonine and histidine are moderately reduced in autosomal dominant patients. The branched-chain amino acids valine, isoleucine, and leucine, as well as arginine, were significantly increased at the $p < 0.001$ level in the 2 patients with Laurence-Moon-Bardet-Biedl syndrome. The elevations in the branched chain amino acids may be secondary to obesity, but the increase in arginine is unexplained. It is noteworthy that most of the significant alterations reported involve essential amino acids.

How can the present results be interpreted with regard to the cause of the alterations and their relationship to the observed disease? These findings should be interpreted against the background of gyrate atrophy as the prototype metabolic retinal degeneration. This study has confirmed that there is no dramatic alteration in any single amino acid in these RP groups comparable to the extreme elevation of ornithine in gyrate atrophy. It is probable that the changes which we have observed in the RP patients represent secondary alterations, perhaps comparable to the low levels of lysine and glutamic acid seen in gyrate atrophy.¹ The challenge, therefore, remains to identify one or more primary metabolic defects to which the present observations can be related. It is probable, in view of the heterogeneity of RP, that several such defects might be identified relating to the modes of inheritance. The most provocative speculation would be to relate the lowered taurine levels in X-linked recessive RP to the observations in taurine-deficient cats⁵ and the more recent demonstration of defective taurine uptake by platelets of 12 RP patients, though the inheritance patterns of the disease manifested in the families of these 12 patients were unspecified by the authors.⁴ It is noteworthy that most of the taurine in the circulation is concentrated within platelets and leucocytes. The failure to demonstrate either larger changes or significant differences in other RP subtypes may relate to this compartmentation. Furthermore, studies reporting only plasma levels of taurine⁶ might obscure otherwise significant changes by failure to assess its intracellular levels in the formed elements.

The decreased threonine and histidine levels in the autosomal dominant and recessive groups are not readily accounted for. Individual abnormalities in the metabolism of these amino acids have been reported, but as with most aminoacidopathies they are characterised by raised levels. In neither case is an abnormality of the eyes among the typical manifestations.

The significantly lower fasting glucose levels in the males of several of the RP subgroups is unexplained. The present demonstration of higher fasting glucose in normal males than females conforms to a well recognised pattern. All values are within the normally accepted ranges for age, and there are no differences in insulin levels which could account for the glucose values. Indeed, those amino acids most sensitive to ambient insulin levels¹⁰ are not consistently altered in association with the lower glucose levels, so that, if increased insulin sensitivity were postulated, it would have to be specific for glucose. This is unlikely to be the case.

The authors express their gratitude to Debbie McIntyre and Chou-Nan Wei for excellent technical assistance, and to Sylvia Bailey for secretarial assistance. We gratefully acknowledge the co-operation of Dr J. A. Parker for referring some of the patients studied.

This study was supported in part by a grant from the Retinitis Pigmentosa Foundation of Canada.

References

- 1 Arshinoff SA, McCulloch JC, Matuk Y, Phillips MJ, Gordon BA, Marliss EB. Amino acid metabolism and liver ultrastructure in hyperornithinemia with gyrate atrophy of the choroid and retina. *Metabolism* 1979; **28**: 979-88.
- 2 McInnes RR, Arshinoff SA, Bell L, Marliss EB, McCulloch JC. Hyperornithinemia and gyrate atrophy of the retina: improvement of vision during treatment with a low arginine diet. *Lancet* 1981; **i**: 513-7.
- 3 McCulloch JC, Arshinoff SA, Marliss EB, Parker JA. Hyperornithinemia and gyrate atrophy of the choroid and retina. *Ophthalmology*, 1978; **85**: 918-28.
- 4 Airaksinen EM, Sihvola P, Airaksinen MM, Sihvola M, Tuovinen E. Uptake of taurine by platelets in retinitis pigmentosa. *Lancet* 1979; **i**: 474-5.
- 5 Schmidt SY, Berson EL, Hayes KC. Retinal degeneration in cats fed casein. I. Taurine deficiency. (1976) *Ophthalmology* 1976; **15**: 47-52.
- 6 Berson EL, Schmidt SY, Rabin AR. Plasma amino acids in hereditary retinal disease: ornithine, lysine, and taurine. *Ophthalmology* 1976; **60**: 142-7.
- 7 Marliss EB, Murray FT, Nakhoda AF. Metabolic response to hypocaloric protein diets in obese man. *J Clin Invest* 1978; **62**: 468-79.
- 8 Campbell DA, Tonks EL. Biochemical findings in human retinitis pigmentosa with particular relation to vitamin A deficiency. *Br J Ophthalmol* 1962; **46**: 151-64.
- 9 Fujiwara H. Biochemical studies on retinitis pigmentosa. Report 3. Study on amino acid metabolism of patients with retinitis pigmentosa. *Folia Ophthalmol Jpn* 1968; **19**: 741-53.
- 10 Felig P, Marliss E, Cahill GF Jr. Plasma amino acid levels and insulin secretion in obesity. *N Engl J Med* 1979; **281**: 811.