Z. Ernährungswiss. 16, 241–247 (1977)

ISSN 0044–264 X

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Effect of low-protein diet and its duration on hair composition

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With 3 tables

(Received July 25, 1977)

Gross hair changes are usually present in protein-energy malnutrition (PEM) to the extent that being of diagnostic value. These changes include dyspigmentation, loss of natural curl, brittleness and sparseness (1–3). Recently, many workers studied the effect of PEM on hair root diameter, its mechanical properties and its cystine content in both children and experimental animals (4–6). The sulphur-containing amino-acids content of hair in PEM has led to controversial results (1–4). The above-mentioned observations, in addition to the fact that hair did not receive the attention paid to other tissues, led us to study the variations which may occur in the total protein content of hair and its amino-acid pattern as a result of feeding rats a low-protein diet. The effect of duration of feeding low-protein diet on hair was also considered. These parameters were simultaneously compared with control animals which were fed an adequate protein-containing diet.

It is hoped that such studies may lead to a better understanding for the changes occurring in the hair of PEM children.

Material and methods

White albino rats of both sexes of the Sprague-Dawley strain of our own colony were used. The animals were one month old, weighing 104 ± 5 g. The animals were kept individually in wire-bottomed cages. The room temperature was controlled at 26 ± 2 °C and relative humidity of about 55 %. The animals were divided into two main groups. The first group includes seventy animals which were fed a low-protein diet of NDp : E < 0.01. The second group includes twenty-eight animals which were fed the control diet of 16 % protein level and NDp : E of 0.096. The composition of the diets was as follows

Ingredients (g/kg)	Control diet NDp : E = 0.096	Low-protein diet NDp : E < 0.01
Casein	160.0	10.0
Sucrose	241.6	291.6
Dextrin	483.4	583.4
B-vitamin mixture (7)	10.0	10.0
Salt mixture (8)	5.0	5·0
Maize oil	100,0	100.0

Only thirty-two of protein-deficient rats were included in the present study since the mortality rate among this group amounted to about 25 % by the end of the experimental period.

Groups of rats were killed by decapitation after 4, 8, 12 and 16 weeks respectively. Blood samples were collected and sera were separated and kept at – 18 °C till analyses. Hair of the dorsal region of the rats were carefully removed from the roots and collected in individual plastic bags.

Hair specimens were washed with distilled water, alcohol, petroleum ether and diethyl ether respectively in order to get rid of sweat and any other contaminants. Hair was left to dry in air and was then ready for chemical analyses.

Total hair proteins were determined by the micro-*Kjeldahl* method (9). Fat was determined gravimetrically from ether extracts and glycogen was determined colorimetrically (10).

Cysteine and cystine content of the hair were determined according to the method described by *Wallace* and *Alyar* (11). Tryptophan was also estimated colorimetrically (12) as well as proline (13). The rest of the amino acids were estimated by two-dimensional paper chromatography according to *Levy* and *Chung* (14).

Results

Hair of rats fed the low-protein diet grew very slowly, became brittle, lost its shine and could be more easily pulled off than that of the controls. These changes were more pronounced according to the duration and severity of protein deficiency. Loss of hair always began during the tenth week of feeding the low-protein diet and it was mostly localized in the region between the fore limbs.

Total serum and hair proteins were significantly reduced ($p < 0.005$) if compared with the control group, after four weeks of feeding the low-protein diet. Reduction after that period was gradual.

Table 1. Serum and hair proteins of control and protein-deficient groups.

Protein	Group	Period 1 (4 weeks)		Period 2 (8 weeks)		Period 3 (12 weeks)		Period 4 (16 weeks)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Serum (g/100 ml)	C	7.30	0.07	6.92	0.06	6.93	0.07	6.97	0.07
	LP	4.35	0.10	4.18	0.07	3.41	0.09	2.88	0.04
Hair (g/100 g)	C	82.78	0.47	83.11	0.22	83.89	0.32	85.82	0.30
	LP	73.17	1.08	71.22	0.61	70.41	0.71	66.04	0.50

Table 3. Ratio of tyrosine: Phenyl alanine in both the control and protein-deficient groups during the various experimental periods.

Group	Period 1 (4 weeks)	Period 2 (8 weeks)	Period 3 (12 weeks)	Period 4 (16 weeks)
Control	11.6	8.7	9.4	6.4
Protein-deficient	6.7	9.0	3.8	3.8

Table 2. Amino-acid pattern of the hair in the control and protein-deficient groups (g/100 g hair protein).

Amino acids		Period 1 (4 weeks) Mean	SE ±	Period 2 (8 weeks) Mean	SE ±	Period 3 (12 weeks) Mean	SE ±	Period 4 (16 weeks) Mean	SE ±
Cysteine	C	1.21	0.02	1.26	0.03	1.30	0.06	1.14	0.05
	LP	1.76	0.30	1.01	0.07***	1.22	0.06	0.77	0.02***
Cystine	C	19.44	0.30	20.62	0.33	22.30	0.44	24.26	0.80
	LP	17.59	0.26***	11.87	0.54***	19.16	0.27***	14.74	0.36***
Aspartic acid	C	9.68	0.90	10.82	0.27	13.00	0.52	12.96	0.65
	LP	11.76	0.95	9.11	0.49***	9.45	0.16***	7.54	0.21***
Glutamic acid	C	9.14	0.67	10.32	0.34	11.05	0.59	10.81	0.21
	LP	11.15	0.40*	8.76	0.22***	9.31	0.61*	6.73	0.17***
Serine	C	7.61	0.24	5.80	0.22	7.06	0.18	6.13	0.23
	LP	6.80	0.61	6.95	0.61	5.54	0.57**	4.49	0.20***
Glycine	C	6.58	0.20	4.72	0.19	6.90	0.84	6.13	0.23
	LP	6.80	0.57	6.12	0.44***	6.14	0.29	3.48	0.23***
Alanine	C	3.81	0.46	3.82	0.28	4.03	0.34	4.30	0.26
	LP	4.46	0.58	4.28	0.37	3.90	0.46	3.16	0.29***
Proline	C	3.95	0.38	4.58	0.08	4.39	0.09	4.32	0.20
	LP	4.30	0.05	3.07	0.20***	3.65	0.18	2.99	0.03***

Table 2. Continued.

Amino acids		Period 1 (4 weeks) Mean	SE ±	Period 2 (8 weeks) Mean	SE ±	Period 3 (12 weeks) Mean	SE ±	Period 4 (16 weeks) Mean	SE ±
Tyrosine	C	8.75	0.90	5.66	0.14	6.80	1.08	4.99	0.44
	LP	5.89	0.83*	9.22	0.39***	6.51	0.84	4.71	0.43
Threonine	C	2.41	0.14	3.88	0.17	4.20	0.78	5.60	0.27
	LP	3.62	0.58*	5.00	0.68**	3.41	0.29	2.84	0.26***
Valine	C	3.25	0.42	3.16	0.13	3.60	0.28	3.45	0.15
	LP	3.84	0.29	4.64	0.58*	4.42	0.29*	2.31	0.18***
Leucine and Isoleucine	C	3.13	0.33	2.34	0.06	2.60	0.39	2.90	0.12
	LP	4.50	0.27**	4.35	0.73**	4.25	0.61**	3.12	0.19
Phenylalanine	C	0.76	0.08	0.65	0.03	0.72	0.07	0.78	0.03
	LP	0.86	0.04	1.02	0.05***	1.70	0.10***	1.22	0.08***
Tryptophan	C	0.91	0.04	0.90	0.03	0.94	0.03	1.30	0.01
	LP	0.80	0.05*	0.76	0.05**	0.77	0.05**	0.79	0.06***
Lysine	C	5.25	0.28	3.56	0.13	3.67	0.59	4.90	0.38
	LP	4.70	0.40	3.69	0.49	3.58	0.53	3.54	0.38**

C: Control group.
LP: Low-protein fed group.
Values significantly changing from those of their corresponding controls
*: $p < 0.05$, **: $p < 0.02$, ***: $p < 0.01$.

The effect of age on some of the amino acids present in hair was marked in the animals fed the control diet. Cystine, aspartic acid, glutamic acid, and threonine were significantly increased after the first experimental period onward (table 2). Tyrosine, on the other hand, was significantly decreased with age. The rest of the amino acids did not show any significant changes as age proceeded.

With animals fed the low-protein diet, the non-essential amino acids aspartic acid, glutamic acid, alanine, cystine and the essentials, threonine, valine, leucine, isoleucine, and phenylalanine were increased after the first four weeks of the experiment when compared with their corresponding controls. The rest of the amino acids showed different degrees of reduction. As protein deficiency proceeded, pattern became different.

The amino acids aspartic acid, glutamic acid, serine, glycine, alanine, proline, and lysine were reduced by the end of the third period. Other amino acids were either increased such as cystine, valine, and phenylalanine or unchanged such as leucine, isoleucine, and tryptophan. In severe protein deficiency, after sixteen weeks, all the amino acids were significantly reduced with the exception of isoleucine and phenylalanine.

Arginine and histidine were separated together on the chromatographic paper. For this reason they were not included in table 2. They are present in considerable concentrations in the hair of the control rats (19.7 g/100 g hair protein). Also, protein deficiency seems to have no considerable effect on their concentrations if compared with the control group.

Fat and glycogen content of 15 rats of the third and fourth period amounted to 2.37 ± 0.47 and 0.055 ± 0.006 g/100 g hair in the protein-deficient rats as compared with 2.29 ± 0.33 and 0.045 ± 0.013 in the corresponding controls respectively. Also, moisture content of hair was 10.0 % for both groups.

Discussion

The data presented in this study showed that hair consists mainly of proteins in addition to non-protein solids.

Protein deficiency during its early weeks significantly reduced the protein content of hair by 11.6 %. This reduction is not comparable to that shown in the total serum proteins of these rats where it was reduced by 40 % in the same group. This, however, disagrees with the results obtained by *Bradfield* (5) who showed that early protein deprivation of human volunteers reduced the hair-bulb diameter with the absence of any reduction in the total serum proteins. This lower reduction of hair proteins could be attributed to the high rate of protein synthesis going on in the hair follicles (15). As protein deficiency proceeded, reduction in hair proteins amounted to 22.9 % of the corresponding control group by the end of the whole experimental period. This finding is confirmed by the recent findings of others (16) who demonstrated that protein content of the hair root was 18.02 ± 5.25 μg per root for normal children compared with 1.5–7.5 μg for children suffering from PEM.

Our findings on glycogen and fat contents of hair suggested the presence of other factors that account for the reduction of hair proteins

than accumulation of glycogen and fat resulting from feeding low-protein diet. This could be best explained by reduction in root size, cell number (16), and atrophy of the bulb in the growing phase (6).

The sulphur-containing amino acids, cystine, and cysteine were significantly increased ($p < 0.05$) with age in the hair of the control group. These findings agreed well with those reported by *Block* and *Lewis* (17). The relatively decreased amounts of hair cystine and cysteine contents in the protein-deficient groups is an expected observation. This may be attributed to the low sulphur content of the diet due to its low protein content in addition to the low food intake of this group. The effect of duration of protein deficiency on hair sulphur-containing amino acids was pronounced after the first and second periods since they were significantly reduced. The normalization of these two amino acids during the third period is concomitant with that reported in blood sera (18). This was explained as a result of tissue breakdown in order to supply the body with the amino acids necessary for other vital biological processes.

The above findings may explain the controversy in the results reported by several authors (1, 4, 19) in this respect. Thus reduction in cystine content of hair could be particularly responsible for hair changes in PEM children since it performs the keratinization process occurring in the cortex (20).

The marked reduction of tyrosine : phenylalanine ratio (table 3) after the third and fourth periods of deficiency is due to the elevation of phenylalanine rather than reduction of tyrosine; a finding that denotes the presence of an impairment in the mechanism of transformation of phenylalanine to tyrosine in protein deficiency.

Essential amino acids were found to be present in considerable amounts in hair of rats that were fed the control diet. Protein deficiency significantly reduces these amino acids in the severe states, a finding that indicates that these amino acids were probably taken away from hair follicles for the welfare of other tissues. The relatively high diminution of threonine with protein deficiency agreed with that reported by others (21).

The significant elevation of the branched amino acids, valine, leucine, isoleucine, and phenylalanine up to the third period with protein deficiency over those of the corresponding controls was an unexpected finding. The breakdown of other tissues may be the source of such elevation, a factor that is known to occur in severe malnutrition. This can also be confirmed by a similar finding in sera of rats (18).

The non-essential amino acids, with the exception of cystine and cysteine, were not markedly decreased until the onset of protein deficiency was established. Also, each two interrelated amino acids such as aspartic acid, glutamic acid, serine, glycine behave similarly towards either age or the effect of protein deficiency.

The consistent reduction of the non-essential amino acids in the hair with protein deficiency differs from what occurs in serum since these amino acids were either increased or did not change (18). These variations in serum-amino acids in protein deficiency were reported to be a

reflection of some metabolic derangements which do not seem to occur in hair.

In conclusion, the results obtained from this study showed that age has a considerable effect normally on the composition and amino-acids pattern of the hair. It also revealed that hair, like any other tissue, is affected by the level and duration of feeding a low-protein diet. In other words, the hair passes through different stages with protein deficiency. This may in turn explain the controversial results obtained by different authors concerning the sulphur-amino-acids content of hair in children suffering from PEM.

Summary

The effect of feeding a low-protein diet (1 %) and its duration (4, 8, 12 and 16 weeks) on the protein content and amino-acid pattern of hair were studied. These changes were compared with control groups fed an adequate protein diet (16 %).

Protein content of hair was diminished in the protein-deficient rats after four weeks followed by a gradual decrease till the end of the experiment. Sulphur-containing amino acids, cystine and cysteine, were significantly reduced in the hair of the protein-deficient rats when compared with the normal controls.

The amino-acid pattern showed significant differences from controls by the end of the whole experimental period.

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