
'Relaxers' damage hair: Evidence from amino acid analysis

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Background: 'Relaxers' are used by more than two thirds of African females to straighten hair, with easy grooming and increased length often cited as reasons. A recent study reported relaxed hair lengths much shorter than expected, suggesting increased fragility; the potential for scalp inflammation and scarring alopecia remains unclear.

Objective: To investigate the biochemical effects of 'relaxers' on hair.

Methods: With informed consent, included participants represented 3 groups: natural hair, asymptomatic relaxed hair, and symptomatic (brittle) relaxed hair. Biochemical analysis was performed by using a Biochrom 30 amino acid analyzer. Differences in amino acid levels were assessed using either Wilcoxon rank sum test or matched-pairs signed-rank test.

Results: There was a decrease in cystine, citrulline, and arginine; however, an increase in glutamine was found in all relaxed compared to natural hair. Cystine levels (milligram per gram amino acid nitrogen) were similar in natural proximal and distal hair: 14 mg/g (range, 4-15 mg/g) versus 14 mg/g (range, 12-15 mg/g); $P = .139$. In asymptomatic relaxed hair, cystine levels were higher in less frequently relaxed samples proximal to scalp: 7.5 mg/g (5.6-12) versus 3.3 mg/g (1.3-9.2); $P = .005$. Cystine levels in distal asymptomatic relaxed and symptomatic relaxed hair were similar to each other and to those in the genetic hair fragility disease trichothiodystrophy.

Limitations: It was not possible to analyze lye and no-lye 'relaxers' separately.

Conclusions: 'Relaxers' are associated with reduced cystine consistent with fragile damaged hair. A decrease in citrulline and glutamine has been associated with inflammation; prospective studies are needed to investigate whether or how 'relaxers' induce inflammation. (J Am Acad Dermatol 2010;62:402-8.)

Key words: African hair; amino acids; cystine; hair fragility; hair relaxers; hair straightening.

INTRODUCTION

Indigenous African hair varies from very curly in the southern part of the continent to nearly straight in northern areas. 'African' hair for the

Abbreviations used:

TN: trichorrhexis nodosa
TTD: trichothiodystrophy

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purpose of this article refers to the former—typically black, tight curly hair. Notwithstanding reported differences in the distribution of high and low sulfur proteins, the amino acid analysis of different hair phenotypes¹⁻⁴ has proved to be similar. The latter proved to be similar despite significant differences in both appearance and behavior (eg, comb-ability and elasticity).^{5,6}

Curly hair in those persons of African origin is known to be vulnerable to breakage, whether subjects are African Americans⁷ or indigenous Africans.⁸ The latter may explain the observed short lengths of combed natural African hair even after many years without a haircut.^{8,9} The breakage that

occurs with combing natural African hair is unlikely to be a result of inherent fragility as evidenced by indistinguishable sulfur staining on transmission electron microscopy when compared with Asian and European hair.¹⁰ However, the hair of patients diagnosed with trichothiodystrophy (TTD), a genetic disorder and a natural experiment for hair fragility, had a distinct pattern of reduced and haphazard cystine staining of the cuticle.¹⁰ Mechanical factors, such as asymmetric follicle bulb differentiation¹¹ and follicle shape,¹² are likely to be responsible for the fragility with breakage associated with combing seen in natural African hair.⁸

Longer hair, easier daily grooming, and fashion have made various hairstyles attractive to Africans. Chemical hair straightening, usually with sodium or guanidine hydroxide commonly called lye or no-lye 'relaxers', respectively,¹³ is the hairstyle worn by at least two thirds of African females including children, in Cape Town^{14,15}; chemical hair straightening is also common in the diaspora. Allergic reactions,¹⁶ suspected misuse,¹⁷ and incorrect formulations¹⁸ are associated with alopecia. However, data on the extent of damage, if any, with proper use of hair relaxers are lacking. Although hair relaxers are desired for increasing length, a recent study found that the achieved hair length was significantly shorter than expected.¹⁰ Relaxed hair is straight and does not have the knots and intertwining demonstrated in natural hair.⁸ Thus the unexpected short lengths may suggest that applications of relaxers damage hair, which breaks off with combing, thus reaching a steady-state length akin to that described with natural hair.¹⁰

The aim of the current study was to elucidate the biochemical effects of relaxers on African hair by comparing the amino acid analysis of natural, relaxed hair where there was no perceived problem by the subject (termed "asymptomatic") and relaxed hair in participants complaining of hair breakage.

METHODS

Ethical approval was received from the Ethics Committee of the Faculty of Health Sciences at the University of Cape Town.

Participants and samples

Thirty healthy adults older than 18 years were recruited from hospital staff and patients at the hair clinic at Groote Schuur Hospital in Cape Town. Participants were allocated to the following 3 groups; 10 with natural (never used chemical treatments) hair, 10 with asymptomatic relaxed hair, and

10 who were complaining of brittle, breaking, or damaged relaxed hair. All participants completed a questionnaire that included questions on what relaxers they had used most recently and in the past.

Samples were obtained to make comparisons between relaxed hair and non-relaxed hair. Additional samples were taken from those with symptomatic relaxed hair to compare clinically damaged relaxed hair with less damaged hair within the same individual (Table I). After informed consent had been obtained from participants, samples of at least 25 hairs were collected from each participant from each site.

Hair was collected from the vertex by gentle hair pull to obtain the hair bulb in those with natural hair and those with asymptomatic relaxed hair. Samples were then cut halfway along their length and sections containing the bulb and tip were labeled proximal and distal, respectively. In the symptomatic group samples were taken from paired sites, involving the vertex (least damaged) and the occiput (most damaged).

Biochemical analysis

Hair analysis was by hydrolysis combined with quantitative chromatography. In brief, hair was hydrolyzed by boiling for 4 hours in 1 mL concentrated (11.7 M) hydrochloric acid, cooled, centrifuged, and the supernatant neutralized in lithium hydroxide. Sulfosalicylic acid was used to precipitate the protein (30 minutes) and a 200- μ m filter was used to remove cellular debris from the solution before chromatographic separation with the use of a Biochrom 30 series amino acid analyzer. A series of buffer solutions were run through a column containing the amino acids in solution. Individual acids were eluted according to their pH. Reaction with ninhydrin was utilized to elicit a spectrum of colors at different wavelengths giving a chromatogram representing

CAPSULE SUMMARY

- Cystine levels were similar in proximal and distal natural hair and reduced in all relaxed hair, being lowest in distal relaxed hair.
- Cystine levels in distal and symptomatic relaxed hair were consistent with fragile damaged hair such as that in trichothiodystrophy.
- Arginine and citrulline levels were decreased and glutamine level was increased in all relaxed hair compared to natural hair.
- A decrease in arginine and citrulline has been associated with inflammation elsewhere; confirmation of 'relaxer'-induced scalp inflammation is required.

Table I. Cystine, glutamine, and arginine levels for hairstyle and hair position/head area for all subjects

Hair style	Participants		Samples	
	No.	Age, y Mean (range)	1	2
Natural hair	10	25 (18-28)	Proximal* Vertex	Distal [†] Vertex
Asymptomatic relaxed	10	23 (18-23)	Proximal Vertex	Distal Vertex
Symptomatic [‡] relaxed	10	24 (19-26)	Clinically least damaged area Vertex	Clinically most damaged area Occiput

*Proximal refers to half of the hair taken close to the scalp that includes the root.

[†]Distal refers to the rest of the same hair that includes the tip.

[‡]Symptomatic patients complained of hair breakage; vertex and occiput refer to scalp area of sampling.

the concentrations of each amino acid (in milligrams per gram amino acid nitrogen) in the hair sample.

Statistical analyses

Statistical analyses were performed using STATA version 9.0 (STATA Corporation, College Station, TX). Differences in amino acid levels between groups were assessed using either the Wilcoxon rank-sum test for comparing medians from two dependent samples or the Wilcoxon matched-pairs signed-rank test for comparing medians from two independent samples. All significance tests were two tailed and significance was defined at the 5% alpha level.

RESULTS

The characteristics of included participants are summarized in Table I. Participants with relaxed hair (both asymptomatic and symptomatic) were not brand loyal. Although they usually preferred no-lye relaxers, all had used a lye relaxer at least once in the past.

Results revealed a spread of 16 amino acids (measured in milligrams per gram amino acid nitrogen) in each group. Exploratory graphs revealed no difference in amino acid levels between proximal and distal natural hair (not shown). However, when groups were compared (ie, natural, relaxed asymptomatic, and relaxed symptomatic), there were between-group differences involving 4 amino acids; cystine, citrulline, and arginine levels were reduced, whereas glutamine was increased in both relaxed-hair groups compared to natural hair (Fig 1). All of the above amino acids showed consistent between-group statistical significance, except for citrulline (Table II).

Natural hair had higher cystine levels than relaxed hair (Fig 2). This was true for asymptomatic (14.17 [4.11-15.49] vs 6.195 [1.32-11.66]; $P < .001$) and symptomatic (17 [4.11-15.49] vs 3.825 [1.66-12.38]; $P < .001$) groups. This was also true for proximal

($P = .005$) and distal ($P < .001$) samples when compared between groups of subjects, where those with relaxed hair had lower cystine levels (Table III, A). There was no difference between the cystine levels in proximal and distal samples of natural hair from within the same individual (14 [4-15] vs 14 [12-15]; $P = .139$). However, in those with relaxed hair, there was a reduction of cystine level when comparing proximal to distal hair (7.5 [5.6-12] vs 3.3 [1.3-9.2]; $P = .005$). There were also differences in cystine when comparing natural hair with both most affected and less affected in symptomatic relaxed hair. However, there was no difference in cystine levels between the symptomatic groups irrespective of severity (Table III, A).

Glutamine levels were significantly higher in asymptomatic relaxed hair than in natural hair. However, glutamine levels were the same in proximal and distal relaxed hair in the same asymptomatic participants and in all symptomatic samples (Table III, B). Arginine is slightly reduced in distal natural compared proximal hair ($P = .047$) and was dramatically reduced in both relaxed asymptomatic and symptomatic hair. However, although asymptomatic distal relaxed hair had lower levels of arginine than proximal sections of the same hair, there was no difference in levels in the symptomatic group irrespective of disease severity (Table III, C).

DISCUSSION

Cystine is crucial for hair strength, with its levels considered a surrogate measure for hair fragility; it is a component of disulphide bonds, which are responsible for the inherent strength of hair. TTDs are characterized by excessive 'weathering' or loss of cuticular cells (wear and tear) and hair shaft damage. They are phenotypically different genetic disorders that are all characterized by significantly reduced levels of cystine in hair that is fragile, brittle, and fails to grow long.¹⁹ This study demonstrated no

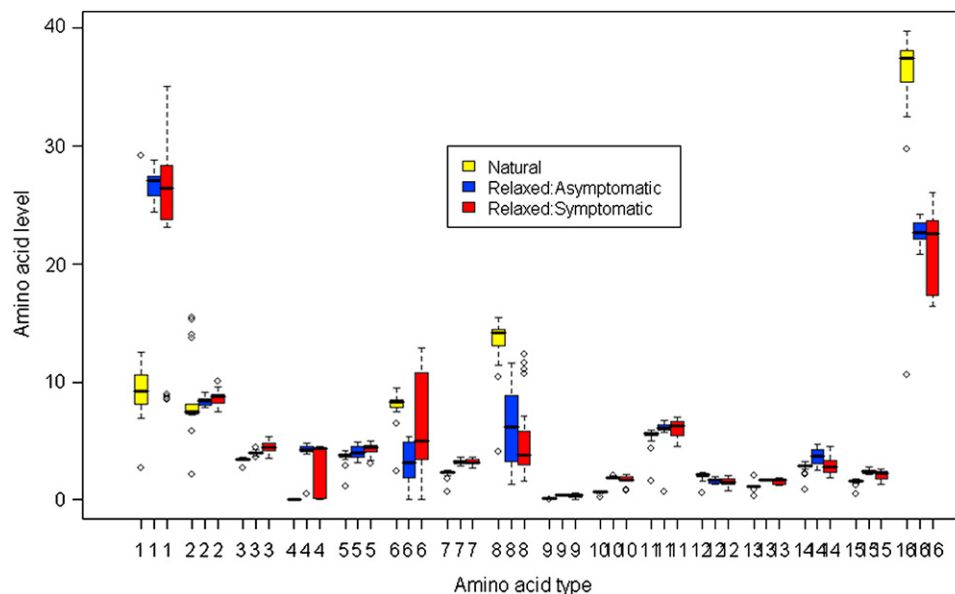


Fig 1. Amino acid levels for all subjects by patient group: 1 = glutamine, 2 = alpha amino adipic acid, 3 = proline, 4 = glycine, 5 = alanine, 6 = citrulline, 7 = valine, 8 = cystine, 9 = methionine, 10 = isoleucine, 11 = leucine, 12 = tyrosine, 13 = phenylalanine, 14 = lysine, 15 = histidine, 16 = arginine.

Table II. *P* values* for 4 amino acids that look changed between groups in Fig 1

	Cystine	Arginine	Citrulline	Glutamine
Natural vs 'relaxed' asymptomatic	<.001	<.001	<.001	<.001
Natural vs 'relaxed' symptomatic	<.001	.005	.626	<.001
'Relaxed' symptomatic vs 'relaxed' asymptomatic	.291	.499	.034	.337

**P* values based on Wilcoxon rank-sum test for comparing medians from two independent samples.

significant difference between cystine levels in proximal and distal natural hair. However, in relaxed asymptomatic hair, the cystine level proximally was not only lower than in natural hair but also significantly higher than in distal sections of the same hair. Thus distal hair, which is likely to have been relaxed more often, is associated with lower cystine content. No significant difference in cystine levels was demonstrated in relaxed symptomatic hair irrespective of symptom severity. The numbers of participants in this study may be the reason for failure to distinguish cystine differences between the latter groups. It was noteworthy that the cystine levels found in distal asymptomatic (and all symptomatic) relaxed hair was similar to those used for the fragile hair diagnosis in TTD.¹⁹

An increase in glutamine associated with a significant decrease in cystine in distal compared to proximal hair has been reported in trichorrhexis nodosa (TN).²⁰ TN is a disorder that is characterized by progressive 'weathering' of the distal hair shaft. However, in this study we found no significant difference in glutamine levels between proximal and distal hair as reported in TN. In addition, unlike in the relaxed hair in this study, hair arginine levels in TN are typically normal.²⁰ Thus the biochemical changes in relaxed hair may suggest more complex mechanisms than alterations in amino acid proportions secondary to the loss of cuticular cells, as is likely the case in TN.

There is a relationship between arginine, citrulline, and glutamine in health and disease. Glutamine is reported to be a precursor of arginine.²¹ Specific amino acid changes have been reported to be associated with inflammation. The plasma concentrations of arginine and citrulline are low during the acute phase of critical illness (sepsis and trauma) and normalize during recovery. Furthermore, the former reductions correlate to the severity of inflammation.²² Thus, the reduction in arginine and citrulline in asymptomatic relaxed hair, which was most dramatic proximally without further worsening distally, may indicate damage from relaxer-induced inflammation of adjacent skin (ie, affecting hair closest to the scalp). Scalp inflammation and scarring alopecia have been anecdotally reported with both lye and no-lye relaxers,²³ despite the fact that the latter are

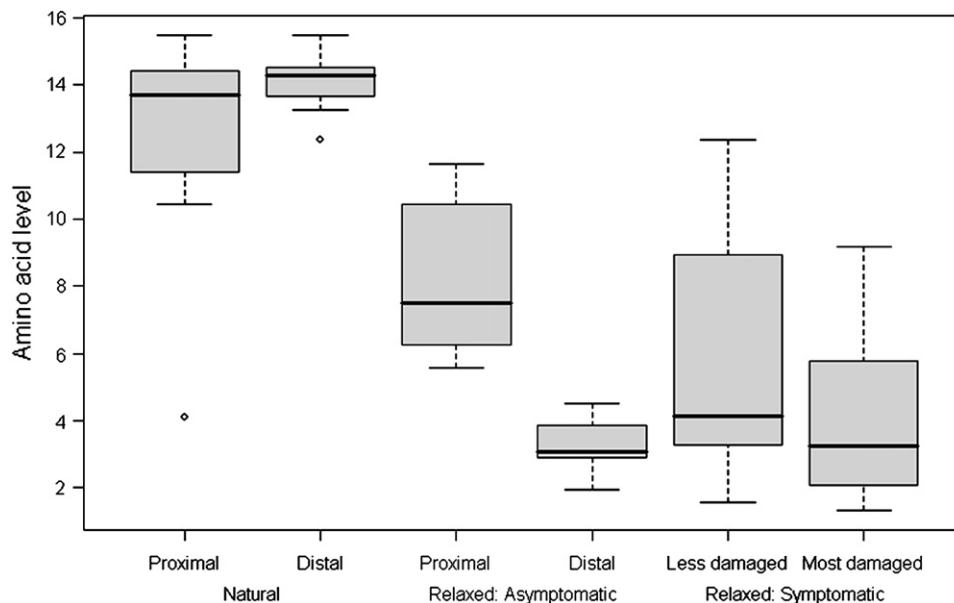


Fig 2. Levels of cystine for subjects with natural, relaxed asymptomatic and relaxed symptomatic hair (by hair position or head area).

often advertised as safer. In this study it was not possible to analyze relaxer types separately; the claimed relative safety of no-lye relaxers needs elucidation with chemical processing done under controlled conditions.

Long hair is desirable for the majority of females in our population who have relaxed their hair and avoided haircuts for many years; however, their hair rarely reaches the shoulder.¹⁰ The latter was significantly shorter than would be expected even after compensating for the slightly slower hair growth rates reported in African hair.²⁴ The current recommendation is, with each treatment, to apply a protective ointment at the base of the scalp with a bland ointment such as petroleum jelly and only apply the relaxer to the new growth, closest to the scalp. However, some stylists “smooth” the hair by combing the relaxer through to the tips before rinsing and applying a neutralizer. The latter is likely to repeatedly process previously relaxed hair and may contribute to increased fragility and failure of relaxers to deliver the length expected. Protection of previously relaxed hair, during repeat processing, may limit this damage and needs evaluation.

In schoolchildren, relaxers were reported to be used by more girls than boys (78% vs 8.6%); more girls than boys had traction alopecia (17% vs 0%), which was higher in girls with relaxed than in those with natural hair (22% vs 5.2%).¹⁴ Relaxers are widely used; however, published objective data on their effects are limited. It is also possible that the popularity of weaves (artificial hair attached to create a

natural or wig-like appearance) among African women is partly because relaxers do not deliver the hair length they promise. This may be compounded by the use of models who clearly wear weaves in advertisements for some relaxers. Unfortunately, the application of traction (eg, braids, weaves) on relaxed hair is associated with the highest risk of traction alopecia compared to natural hair (odds ratio 3.47 [$P < .001$, 95% confidence interval 1.94-6.20]). The risk also increases with hairdressing “symptoms”, the highest being tight braids that cause pimples (odds ratio 1.98 [$P < .022$, 95% confidence interval 1.10-3.57]).²⁵

Without contrary original data on the effects of relaxers on African hair and length, people who prefer weaves and braids would be better advised to only have them done on natural hair and to avoid all hairdressers who induce pain. There is need to investigate whether mechanical and/or chemical protection of previously relaxed hair during repeat processing would limit damage and improve length. This study found that relaxers are associated with amino acid changes that include a reduction in cystine that is consistent with damaged fragile hair. A decrease in citrulline and glutamine has been associated with inflammation. However, prospective studies are needed to evaluate the significance of these findings in relaxed hair and confirm whether or how relaxers induce scalp inflammation. The potential for scarring remains unclear; this is particularly important because of the wide use of relaxers by young children, who are likely to use them for many years.

Table III. Cystine, glutamine, and arginine levels by hairstyle and hair position/head area for all subjects

Amino acid	Hairstyle	Hair position, median (range)		P value*	Head area, median (range)		P value*
		Proximal	Distal		Less damaged	Most damaged	
A. Cystine	Natural	13.69 (4.11-15.49)	14.29 (12.37-15.48)	.139			
	Relaxed asymptomatic	7.50 (5.56-11.66)	3.26 (1.32-9.18)	.005			
	Relaxed symptomatic				3.85 (1.94-12.38)	3.55 (1.66-11.61)	.515
B. Glutamine	Natural	9.07 (2.66-29.20)	9.24 (6.92-11.06)	.386			
	Relaxed asymptomatic	27.03 (24.41-28.79)	27.0 (25.9-28.02)	.721			
	Relaxed symptomatic				26.44 (8.52-4.83)	25.4 (8.77-33.23)	.953
C. Arginine	Natural	36.44 (10.63-38.16)	37.79 (35.08-39.77)	.047			
	Relaxed asymptomatic	22.15 (20.83-23.05)	23.49 (21.81-24.23)	.022			
	Relaxed symptomatic				22.66 (16.43-43.32)	23.19 (16.46-41.56)	.678

Amino acids measured in milligrams per gram of amino acid nitrogen.

*P value based on Wilcoxon matched-pairs signed-rank test for comparing two dependent samples.

†P value based on Wilcoxon rank-sum test for comparing medians from two independent samples.

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