Changes in hair weight and hair count in men with androgenetic alopecia, after application of 5% and 2% topical minoxidil, placebo, or no treatment

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Quantitative estimation of hair growth using hair weight and number was recorded for 120 weeks in 4 groups of 9 men with androgenetic alopecia. Three double-blind groups applied either 2% or 5% minoxidil solution, or vehicle. The fourth group, unblinded, received no treatment. Measurements of hair weight and number were continued for 96 weeks, when treatment (if any) was stopped, though measurements were continued for another 24 weeks, Although not compared statistically, the placebo and untreated groups behaved in a similar fashion. In contrast, the 5% and 2% minoxidil treatment groups showed a statistically significant increase in mean percentage change in interval weight from baseline compared with placebo; results for number counts were usually less significant. Over 96 weeks, topical minoxidil induced and maintained an increase in interval weight over baseline of about 30%. After treatment was stopped, hair weight and number counts for the minoxidil groups returned to about the same levels as placebo in 24 weeks.

We previously recommended total hair weight from a defined scalp area as the primary estimator for hair growth. The efficacy of a hair growth-promoting agent can be established in 24 weeks by comparing the total hair weight of hair grown in a small, carefully maintained area on the scalp in subjects given either a hair growth promoter or a placebo. We now extend the method to longer times, more subjects, and a wider range of treatments. In this study, quantitative estimation of hair growth was recorded for 120 weeks for a group of men with androgenetic alopecia. Our purpose was to compare the effect of 5% and 2% topical minoxidil solution and placebo, in a random, double-blind protocol, on both hair growth promotion and retardation of the hair loss process, using total hair weights and counts. After 96 weeks, treatment was stopped, although the hair growth measurements were continued for 24 additional weeks. Concurrently, a group of men with androgenetic alopecia received no treatment and their hair growth was compared with the treated groups over the same 120-week period (this group was perforce not part of the blinded study). A preliminary account of part of this study was previously published in conference proceedings.

METHOD

Subject selection

Eligible subjects for this study were 18- to 40-year-old men with androgenetic alopecia as evidenced by frontal/parietal thinning defined by the Hamilton Scale as type III or IV They had to be in good health and have dark, undyed hair, with no gray or white hair. Exclusion criteria included use of topical minoxidil within the previous 6 months; use of any investigational drug within the previous 6 months; concomitant use of steroids, vasodilators, antihypertensives, calcium channel blockers, antiepileptic drugs, cytotoxic agents, "hair restorers," or other medications that could influence hair growth; or prior participation in a topical minoxidil study. Pre-enrollment laboratory studies included a complete blood cell count with differential, urinalysis with microscopic examination, liver function tests, and determination of levels of lactic dehydrogenase, calcium, phosphorus, creatinine, uric acid, blood urea nitrogen, serum electrolytes, thyroxine, and thyroid-stimulating hormone. These studies, as well as an electrocardiogram, and

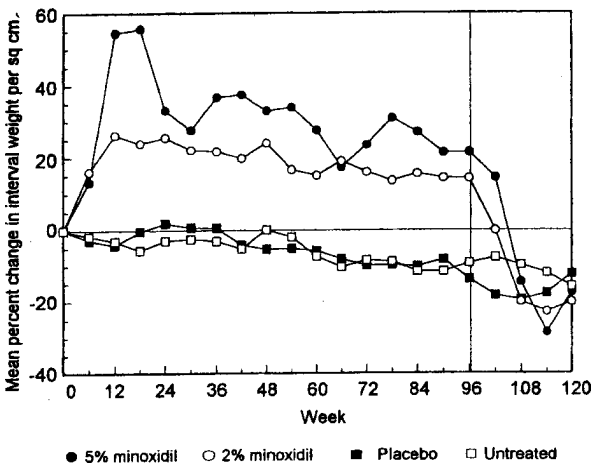

Fig 1. Comparison of mean percentage change in interval weight per square centimeter for 4 treatment groups: 5% minoxidil, 2% minoxidil, placebo, and untreated. Vertical line at 96 weeks marks cessation of treatment.

chest x-ray if not taken in the prior 6 months, all had to have normal results or findings to qualify for eligibility in the study Thirty-six men 24 to 40 years of age qualified for the study and signed an informed consent.

Scalp site selection and sampling frequency

A representative site was selected on the thinning frontal/parietal scalp. Hair in the designated area was carefully hand clipped under magnification on the screening visit (designated as week -6) and at 6-week intervals thereafter, for a total of 120 weeks. No treatment was given during the first 6 weeks, so that the sample collected at the end of this interval represented baseline growth (week 0), For the men assigned to one of the treated groups, treatment was started on the second visit (week 0) and continued for 96 weeks. After 96 weeks, treatment was stopped and hair clippings were continued at 6-week intervals for 24 additional weeks. Concurrently, the group of untreated men had their hair clipped with the same method every 6 weeks for 120 weeks.

Marking and clipping procedure

During the screening (week -6) clipping, a template consisting of a plastic sheet with a square hole (1-34 CM2) was placed over the selected site. All hairs within the template square were pulled through it, with the help of a magnifying lamp to ensure that only hair originating within the square was included. The hairs were grasped and hand clipped to about 1mm in length with small straight surgical scissors. Four small dots were then placed in the corners of the square with a fine ballpoint pen. After the template was removed, the 4 corners were permanently marked using ink and the Spalding and Rodgers marking apparatus.

On subsequent visits at 6-week intervals, the plastic template was laid in exact correspondence with the permanent markings, and the hair in the marked square carefully hand clipped and collected under magnification in the manner previously described.

Measurement method

Hair samples were degreased in trichlorotrifliioroethane (Freon TF) and dried. The total hair sample was spread out on a grid and counted. The hair sample was then placed in the chamber of an analytical balance having 0.01 mg readability. After conditioning for at least I hour in the balance chamber, the ambient relative humidity was recorded, and the samples weighed. Sample weights were corrected to a standard humidity of 65%. Weights obtained by this method are termed *interval weights* to distinguish them from total (cumulative) weights. They are equivalent to growth rates (weight produced per 6-week interval).

Protocol

Following their hair clipping on the second visit, 27 subjects were assigned test solutions that were a solution of either 2% minoxidil (in a vehicle of 20% vol/vol propylene glycol, 60% ethanol, and water) or 5% minoxidil (in a vehicle of 50% vol/vol propylene glycol, 30% ethanol, and water), or the 5% vehicle solution referred to as placebo, The subjects were assigned the test solutions in a randomized, double-blind manner as follows: 9 men received 5% topical minoxidil solution, 8 men received 2% topical minoxidil solution, and 10 men received placebo. They were instructed to apply I mL of the assigned solutions with a metered dropper twice daily to the frontal/parietal scalp, beginning at the clipped site. Applications were made with the scalp dry, spread with one fingertip, and then allowed to dry without a hair dryer. After 96 weeks, treatment was stopped and the hair growth measurements were continued for 24 additional weeks. Nine additional men with similar scalp hair thinning were followed up concurrently without treatment for 120 weeks.

All 36 subjects were well matched in age, duration and extent of alopecia, and original target area hair density. To avoid accidental hair cutting in the test site, hair cuts for the purpose of hair styling were permissible only during the 7 days after a clipping procedure.

During the study, vital signs, medical events, and skin tolerance were monitored, and serum minoxidil levels, hematology, and blood chemistries were measured.

RESULTS

Thirty-three subjects completed the first 96 weeks and 32 subjects the entire 120-week study One untreated subject withdrew at week 6, two treated subjects (assigned to 2% minoxidil solution and placebo) withdrew at week 48 because of scheduling problems, and one treated subject (assigned to 2%, minoxidil solution) dropped out at week 96 because he did not wish to stop treatment. Occasionally, a subject was unable to appear on the exact scheduled date for clipping. To facilitate statistical analysis, the measured weight from these erratic intervals was

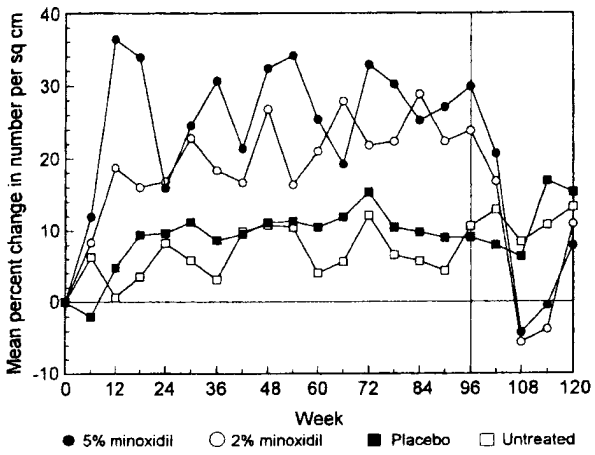

Fig 2. Comparison or mean percentage change in number per square centimeter for 4 treatment groups: 5% minoxidil, 2% minoxidil, placebo, and untreated. Vertical line at 96 weeks marks cessation of treatment.

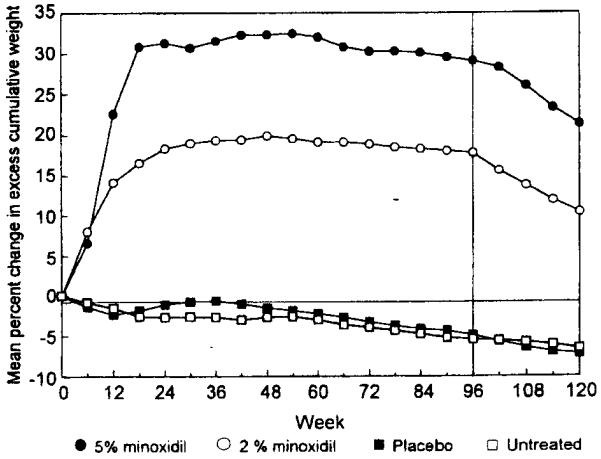

Fig 3. Comparison of the mean percentage change in excess cumulative weight for 4 treatment groups: 5% minoxidil, 2% minoxidil, placebo, and untreated. Vertical line at 96 weeks marks cessation of treatment.

adjusted by a multiplying factor of 42/D), where D is the actual number of days in the interval. This adjustment assumes that the growth rate remains constant during the short period needing correction. Interval weights used in this article have been adjusted by this method when necessary. No correction was made to number counts from subjects who did not return at an exact 6-week interval. The numbers of subjects in the calculation groups were as follows: 7 subjects in the 2% group (6 after week 96), 9 subjects in the 5% group, 9 subjects in the placebo group, and 8 subjects in the untreated group.

Results of the measurements of interval weights and number counts are summarized in Figs 1 and 2, respectively. Each point on these figures represents the mean percentage change from baseline of all subjects in the designated group (2% and 5% minoxidil treatments, placebo, and untreated). Treatment, if any, was stopped at week 96 (indicated in the figures by a vertical line). Percentage changes are plotted rather than the primary measurements of interval weight and number count to remove baseline variation and more accurately isolate effects caused by treatment. The primary measurements are not useful for statistical comparison owing to differences in their baseline values at week 0. Over all subjects, baseline interval weights ranged from 2.9 to 15.0 mg/cm, with a standard deviation of 40% and baseline number counts ranged from 79 to 251 per square centimeter, with a standard deviation of 21%. This variability is a reflection of the wide range of normal growth among a sampling of subjects and is approximately maintained throughout subsequent treatments and times of sampling.

Another representation, which we have called excess cumulative weight, has been found to be useful for discriminating among different treatments. It is defined as the difference between the actual cumulative mean weight and the hypothetical cumulative weight that would accrue if growth continued at the same rate as during the baseline pretreatment period (from week -6 to week 0). Thus excess cumulative weight represents the aggregate change in weight, if any, caused by treatment. Expressed differently, the excess cumulative weight reflects the total cumulative hair protein production induced by treatment. In number counts, the equivalent of a cumulative function is simply the baseline value subtracted from the count at a particular time. The mean percent change in excess cumulative weight is illustrated in Fig 3 for the 4 treatment groups.

DISCUSSION

For statistical comparison, the 3 treatments were considered as a group, and a one-sided analysis of variance test was used to determine whether there is significant difference among any of the 3 treatments (overall P value < .05). In this test, it was found that all trials up to week 96 showed significant differences for 2 of the 3 measurement variables: mean percentage weight change and mean percentage change in excess cumulative weight. However, for mean percentage number change, about one third of the trials showed no significant difference among the 3 treatment groups (5% and 2% minoxidil, and placebo); these trials are shown as italicized numbers in Table 1. We interpret this decreased significance in mean percentage number change (as contrasted with the

Table 1. *P* values from Fisher least significant difference comparisons of 2%-5% (2-5),2%-placebo (2-P), and 5%-placebo (5-P)*

	Mean percentage weight change			Mean percentage number change			Mean percentage excess cumulative weight change		
Week	2-5	2-P	5-P	2-5	2-P	5-P	2-5	2-P	5-P
6	.606	.002	.005	.470	.047	.006	.606	.002	.005
12	.073	.055	0	.072	.150	.002	.188	.015	0
18	.019	.064	0	.033	.408	.003	.073	.023	0
24	.360	.008	0	*.914*	*.378*	*.408*	.092	.012	0
30	.578	.039	.007	*.859*	*.246*	*.157*	.123	.013	0
36	.321	.171	.016	.154	.255	.010	.150	.023	0
42	.172	.074	.002	*.572*	*.398*	*.139*	.149	.027	0
48	.405	.011	.001	.492	.060	.009	.159	.020	0
54	.158	.081	.002	.075	.601	.018	.156	.023	0
60	.301	.094	.007	*.585*	*.199*	*.057*	.164	.026	0
66	.873	.014	.014	*.364*	*.101*	*.412*	.197	.021	0
72	.392	.007	0	.124	.348	.012	.198	.017	0
78	.081	.024	0	.308	.122	.010	.181	.016	0
84	.236	.013	0	.653	.023	.046	.181	.015	0
90	.055	.025	.003	*.605*	*.144*	*.040*	.188	.014	0
96	.437	.005	0	.455	.074	010	.195	.013	0

***0 represents P < .0005; (P > .05), nonsignificant. Analysis of variance intergroup tests are italicized.**

weight variables) to the large fluctuation in the number of small hairs during the growth changes induced by minoxidil. The effect of small hairs is not as pronounced in weight measurements. This provides confirmation of our suggestion that weight measurements offer a more reliable estimation of hair growth than do number counts.

The Fisher least significant difference test was used to determine which of the 3 treatment groups show significantly different means. *P* values from this test are shown in Table 1.

For the two weight variables (mean percentage weight change and mean percentage excess cumulative weight change), differences between either the 2% and 5% treatment levels and placebo were generally significant (P< _ .05) after only 6 weeks of treatment. The incremental increase in hair growth over placebo seen after week 6 was maintained through the 96 weeks of treatment for both the weight variables. Comparison of the 2% minoxidil treatment with placebo was not generally significant for the mean percentage change in number counts; however, the 5% minoxidil treatment showed a considerably greater significant difference in number counts over placebo, though not at all treatment times. Differences between the 2% and 5% treatments themselves were not statistically significant_for weight changes or number change (P > .05), though a difference appears visually apparent in Figs 1-3.

Mean percentage changes in interval weights from baseline are shown in Fig 1. The placebo and untreated groups appear to behave similarly, showing a steady decrease in hair weight from baseline over the 120 weeks. This decrease can be taken as the "normal" hair loss for this group of subjects with androgenetic alopecia and amounts to about a 6% decrease in weight per year. The 2% and 5% minoxidil groups appear to decrease with nearly the same average downward slope, once the peak rate of growth has been passed. The treatments appear to induce a consistent increased growth offset (above placebo or untreated groups) of roughly 25% for the 2% minoxidil treatment and 35% for the 5% minoxidil treatment, an average increase of about 30%, maintained during the 96 weeks of treatment. These growth offsets represent a long-term retardation of the hair loss process by both 5% and 2% topical minoxidil treatments.

Mean percentage changes in number counts from baseline are shown in Fig 2. Despite what appears as a somewhat erratic response, the fluctuations in number counts for 5% and 2% minoxidil (apparently greater than those for weights) reflect a distinct series of events that occur after the onset of treatment. We hypothesize that the first stage of regrowth appears to involve enlargement of a number of miniaturized hairs, which also have shortened growth cycles, as well as the probable nudging of

many older anagen hairs into telogen. Hence the first indication of treatment efficacy is a large increase in total hair count within 12 weeks. This spurt is particularly evident with 5% minoxidil, an effect seen with both interval weights and number counts. Because the cycles are mostly still short, these "new" anagen hairs enter telogen and fall out quickly, to be replaced with another wave of hairs that have somewhat longer growth cycles. This process repeats, and no strong decline seems evident through 96 weeks, although the long-term number counts should eventually decrease. A "placebo effect" appears to occur more markedly for number counts than for weights, for reasons at present unexplained.

If we can ignore the fluctuations, the changes in number counts from baseline of the treated subjects generally parallel the changes in weight. We believe that the weight measurements have more reliability than number counts when assessing hair growth, at least in terms of increased hair protein production. Counts of the smallest hairs are subject to unavoidable uncertainty, though they contribute little to the total hair weight. Furthermore, fluctuations in numbers of small anagen hairs with short growth cycles are not as strongly reflected in the weight measurements.

Excess cumulative weight is shown in Fig 3, as percentage change from the extrapolated baseline cumulative weight. Fluctuation appears smaller than that of the interval weight, a characteristic of cumulative functions. Because earlier interval weight data are carried along in the excess weight function, changes or fluctuations in later interval weights tend to be somewhat masked. This behavior is also apparent when treatment is stopped at 96 weeks. The excess cumulative weight decreases more gradually during the 24 weeks after stopping of treatment than do either the weights or numbers in Figs 1 and 2.

For discriminating among different treatments, especially at early times, the excess cumulative weight seems to offer a clearer separation of treatment effects than either interval weights or number counts. This is apparent not only from visually comparing Fig 3 with Figs 1 and 2, but also from the statistical comparisons in Table 1, where *P* values reach lower levels at earlier treatment times for excess cumulative weight changes than for either weight or number count changes. This sharper discrimination makes the excess cumulative weight useful for rapid evaluation of a treatment.

After treatment was stopped at week 96, the 5% and 2% minoxidil groups showed a rapid loss of hair weight and decreased hair counts. Vertical lines drawn at week 96 in Figs 1-3 indicate when treatment was stopped. By 24 weeks after treatment had been stopped, the weight and number counts of the treated groups decreased to become similar to those of the placebo and untreated groups, showing the growth offset produced by topical minoxidil. This loss of treatment-stimulated hair growth is expected since treatment does not alter the underlying genetic predisposition for androgenetic alopecia.

This study demonstrates that 5% and 2% topical minoxidil promote hair growth and retard the hair loss process over 96 weeks, with 5% topical minoxidil having the greater efficacy. Although the interval hair weight eventually began to decrease with time, the minoxidil treatment maintained an increased rate of hair weight (protein) production, over that of placebo or untreated subjects, of about 25% more for the 2% minoxidil treatment and about 35% more for the 5% minoxidil treatment and averaging about 30% during the 96-week treatment period.