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Oral Spermidine Supplementation Prolongs Hair Follicle Anagen Phase with Sustained Effects: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract: Spermidine, a naturally occurring polyamine, has demonstrated anagen-prolonging effects in laboratory and animal studies, but human efficacy remained unexplored until now. Researchers conducted a randomized, double-blind, placebo-controlled trial with 100 healthy participants receiving either spermidine supplement or placebo daily for 90 days. At baseline (T0) and three months (T1), 100 hair follicles were plucked from each participant for microscopic evaluation of anagen V-VI phase follicles, along with immunohistochemical quantification of Ki-67 (proliferation marker) and c-Kit (apoptosis marker). Pull tests assessed hair loss at T0, T1, and six months (T2). Results showed that spermidine significantly increased anagen V-VI follicles (mean +12.8±6.87, 52% increase) while placebo decreased them (-5.3±2.3, 20% reduction; $p<0.0001$). The treatment group exhibited increased Ki-67 (+12.69±8.1) and decreased c-Kit (-2.16±1.24), with opposite changes in placebo ($p<0.0001$). At T2, three months post-treatment, all spermidine subjects maintained negative pull tests versus 68% positive in placebo ($p<0.0001$). These findings demonstrate that oral spermidine supplementation effectively prolongs the hair follicle anagen phase with sustained effects persisting three months after discontinuation, suggesting significant therapeutic potential for hair loss disorders, though further studies in specific clinical conditions are warranted.

1. Introduction

The hair follicle represents a uniquely dynamic and highly regenerative mini-organ, undergoing a perpetual, tightly regulated cycle composed of three distinct phases: anagen (active growth), catagen (regression/involution), and telogen (resting) [1]. The duration of the anagen phase is the single most critical factor determining hair length and overall scalp coverage density. Consequently, the pathogenesis of common hair loss disorders, such as androgenetic alopecia and telogen effluvium [2], is frequently characterized by a pathological premature termination of this crucial growth phase, leading to the production of shorter, finer hairs and visibly reduced density [3]. Existing first-line pharmacological treatments, notably topical minoxidil and oral finasteride, while providing benefit to many, are hampered by variable inter-individual efficacy, potential adverse

effects, and the common requirement for indefinite, continuous application to sustain any regrown hair. These limitations underscore a persistent and significant need for novel therapeutic strategies that target the fundamental biological mechanisms controlling the hair follicle cycle itself [4].

Central to the normal function of such rapidly proliferating tissues are the natural polyamines—primarily putrescine, spermidine, and spermine [5]. These small, positively charged organic cations are indispensable for maintaining cellular homeostasis, where they regulate a multitude of fundamental processes including DNA stabilization, RNA translation, gene expression, protein synthesis, and cell division. Intracellular polyamine levels are meticulously controlled by a delicate, dynamic balance between biosynthesis, catalyzed by rate-limiting enzymes like ornithine decarboxylase (ODC), and catabolism or export. The hair follicle's exceptionally high rate of cell division during the anagen phase creates a substantial metabolic demand for these molecules, suggesting their pivotal role in sustaining follicular activity [6].

The critical role of polyamines in hair biology has been unequivocally established through extensive research in genetically engineered animal models. Transgenic mice with deliberately dysregulated polyamine metabolism consistently exhibit profound hair-related phenotypes [7]. For instance, mice overexpressing spermidine/spermine N1-acetyltransferase (SSAT), a key catabolic enzyme that promotes polyamine depletion, develop a characteristic hairless phenotype. Conversely, models overexpressing ODC in the skin demonstrate altered follicle morphology and cycling. Among these polyamines, spermidine has emerged as particularly vital for human hair growth. This is compellingly corroborated by the clinical mechanism of action of the drug eflornithine (an ODC inhibitor), which is used topically to reduce unwanted facial hair in women by locally depleting polyamine pools, thereby impeding follicle function [8]. Complementing this pharmacological evidence, direct in vitro studies on cultured human hair follicles have demonstrated that spermidine supplementation can actively prolong the anagen phase, significantly boost hair shaft elongation rates, and promote the proliferation of key follicular cell populations, including matrix keratinocytes and dermal papilla cells [9].

Despite this compelling and convergent evidence from both in vitro and animal studies, a significant translational gap remained. Prior to this investigation, no rigorous, well-controlled clinical trials had systematically evaluated the effect of oral spermidine supplementation on the anagen phase in healthy human subjects [10,11]. While preliminary anecdotal reports and small-scale studies on spermidine-containing nutritional supplements showed promise, they critically lacked the methodological robustness of a randomized, placebo-controlled, double-blind design. The present study was therefore explicitly designed to address this pivotal knowledge gap. We conducted a randomized, double-blind, placebo-controlled trial involving 100 healthy participants to definitively determine whether oral supplementation with a spermidine-based product could effectively prolong the hair anagen phase. Our central hypothesis was that spermidine would significantly increase the proportion of anagen hair follicles by enhancing cellular proliferation and simultaneously reducing apoptotic signaling within the hair bulb. These effects were objectively quantified using a multi-modal assessment strategy, including precise microscopic analysis of plucked hair follicles and immunohistochemical quantification of key molecular markers (Ki-67 for proliferation and c-Kit for apoptosis), thereby providing mechanistic insight alongside efficacy evaluation.

2. Materials and Methods

2.1 Study Design and Ethical Considerations

This investigation was conducted as a single-center, parallel-group, double-blinded, randomized, placebo-controlled trial with 1:1 allocation to treatment groups. This study was conducted in Italy

according to the International Conference on Harmonisation (ICH) guidelines and Good Clinical Practice (GCP) standards. All participants provided written informed consent prior to undergoing any study-related procedures, after receiving comprehensive information regarding the study objectives, procedures, potential risks, and benefits. The study protocol adhered to the ethical principles outlined in the Declaration of Helsinki [12-13].

2.2 Participant Selection

A total of 100 healthy men and women were recruited into the study. Eligible participants were adults aged 22-50 years with unremarkable dietary and lifestyle habits, normal scalp hair density, and no clinical evidence of hair loss disorders. All participants were required to have at least 80% of hair follicles in anagen phase at baseline, as determined by trichogram examination, to ensure normal hair cycling status.

Stringent exclusion criteria were applied to minimize confounding variables and ensure study validity. Participants were excluded if they demonstrated:

Hair-specific conditions: Initial signs of androgenetic alopecia, evidenced by miniaturization of hair shafts observed in the occipital region; family history of androgenetic alopecia; congenital or acquired diseases affecting hair shaft structure.

Recent treatments: Use of any topical or systemic therapy for hair loss within three months prior to enrollment.

Medication use: Regular treatment with corticosteroids, hormone therapies, anti-androgenic agents (including spironolactone, cimetidine, ketoconazole), or anticoagulants

Active conditions: Infections or other active diseases occurring within three months before study initiation.

Systemic diseases: Organic diseases affecting kidneys, liver, cardiovascular system, lungs, or central nervous system; diabetes mellitus.

Substance use: Alcohol or recreational drug abuse in the year preceding study enrollment.

Allergic history: Clinical history of sensitivity or allergic reactions to study components.

2.3 Randomization and Treatment Groups

Participants were randomly allocated using computer-generated randomization sequences to one of two groups (n=50 per group):

Treatment Group: 31 men and 19 women, mean age 36.08 years (range 23-50 years), received a nutritional supplement containing 100 mg of wheat germ spermidine once daily after the main meal for 90 consecutive days.

Placebo Group: 36 men and 14 women, mean age 35.6 years (range 22-48 years), received an identical-appearing placebo tablet once daily after the main meal for 90 consecutive days.

Both participants and investigators remained blinded to treatment allocation throughout the study period and during initial data analysis, ensuring objectivity in assessments and interpretation.

2.4 Assessment Schedule and Evaluation Methods

All participants underwent comprehensive evaluations at three predetermined time points:

T0 (Baseline): Study initiation, before treatment commencement.

T1 (3 months): After 90 days of treatment, at treatment completion.

T2 (6 months): Three months after treatment cessation, to assess sustained effects.

2.5 Primary Outcome Measures

At T0 and T1, epiluminescence imaging (trichogram) was performed to verify normal cycling status and assess the proportion of hair follicles in different phases. This non-invasive technique provided an overview of hair follicle distribution across the scalp and confirmed eligibility based on the criterion of at least 80% anagen phase follicles.

Following trichogram examination at both T0 and T1, exactly 100 hair bulbs were manually plucked from the occipital region of each participant. The occipital area was specifically selected because hair follicles in this anatomical location are not affected by androgen receptor changes characteristic of androgenetic alopecia, thereby avoiding inadvertent enrollment of subjects with subclinical pattern hair loss that might confound results.

Immediately after extraction, hair bulbs were immersed in sterile saline solution to preserve tissue integrity and prevent degradation. Each specimen underwent detailed microscopic evaluation by trained assessors blinded to treatment allocation. Hair follicles were classified according to their lifecycle phase, with particular focus on identifying anagen phase V-VI hair follicles. These represent late anagen stages, characterized by specific morphological features that distinguish them from earlier anagen phases and, critically, from initial catagen phase hair bulbs.

The classification utilized standardized parameters reported and validated by Kloepper et al., which provide reliable criteria for distinguishing between anagen and catagen phases in human hair follicle organ culture systems. These standardized criteria ensure reproducibility and minimize inter-observer variability.

Plucked hair follicles underwent immunohistochemical staining to quantify two critical cellular markers:

Ki-67 Expression: Ki-67 is a nuclear protein expressed during all active phases of the cell cycle (G1, S, G2, and M phases) but absent in quiescent cells (G0 phase), making it an excellent marker for cellular proliferation. Higher Ki-67 expression indicates increased proliferative activity within hair follicle cells, particularly matrix keratinocytes responsible for hair shaft production [14].

c-Kit Expression: c-Kit (CD117) is a tyrosine kinase receptor whose expression patterns have been associated with apoptotic processes in hair follicles. Elevated c-Kit levels in specific hair follicle compartments can indicate increased apoptotic activity and premature catagen induction.

Immunohistochemical procedures followed previously established protocols, with antibody concentrations and incubation times standardized across all specimens. Digital imaging and quantitative analysis software were employed to determine the percentage of positively stained cells within defined regions of interest in the hair bulb, ensuring objective and reproducible measurements.

2.6 Secondary Outcome Measure: Pull Test

The hair pull test represents a simple yet valuable clinical assessment for evaluating hair loss tendency and hair follicle anchoring strength. This test was performed at all three time points (T0, T1, and T2) using a standardized technique. Approximately 50-60 hairs were grasped between the thumb and forefinger, and gentle traction was applied while sliding the fingers along the hair shafts from proximal to distal. The number of extracted hairs was counted and recorded [15].

Pull test results were classified according to established criteria:

Negative (-): 0-2 hairs extracted, indicating normal hair anchoring.

Mildly positive (+): 3-5 hairs extracted, suggesting mild increased hair shedding.

Moderately positive (++): 6-10 hairs extracted, indicating moderate hair loss.

Strongly positive (+++): >10 hairs extracted, reflecting significant active hair shedding.

The pull test assessment at T2, conducted three months after treatment cessation, was

particularly important for evaluating whether any protective effects persisted beyond the active supplementation period and for detecting potential onset of seasonal telogen effluvium, which typically occurs during autumn months.

2.7 Statistical Analysis

Comprehensive statistical analyses were performed on the intention-to-treat population, including all 100 enrolled subjects. For primary outcomes involving continuous variables (number of anagen hair bulbs, Ki-67 expression, c-Kit expression), the following statistical approaches were employed:

Between-group comparisons at baseline: Independent samples Student's t-test was used to verify that treatment groups were balanced at T0 with respect to primary outcome measures.

Within-group changes: Paired t-test was applied to assess the statistical significance of changes from baseline (T0) to treatment completion (T1) within each treatment group.

Between-group comparison of treatment effects: Independent samples Student's t-test was used to compare the magnitude of change (T1 minus T0) between the spermidine-treated group and the placebo group, representing the primary test of treatment efficacy.

For the pull test, a categorical outcome measure, Chi-square test or Fisher's exact test (when expected cell frequencies were small) was employed to compare the proportion of subjects with positive tests between treatment groups at T1 and T2.

All statistical tests were two-tailed with a significance level set at $\alpha=0.05$ ($p<0.05$ considered statistically significant). Data are presented as mean ± standard deviation (SD) unless otherwise specified. Statistical analyses were performed using appropriate software packages, with all calculations verified for accuracy.

3. Results

3.1 Baseline Characteristics and Group Comparability

At study initiation (T0), comprehensive baseline assessments confirmed that the randomization process successfully achieved balance between treatment groups. The spermidine-treated group and placebo group showed no statistically significant differences in age distribution, gender composition, or any primary outcome measures, validating the randomization strategy and ensuring that subsequent differences could be attributed to treatment effects rather than pre-existing group differences.

Table 1 Baseline Demographic and Clinical Characteristics of Participants

Characteristic	Placebo Group (n=50)	Spermidine Group (n=50)	P-value
Age, years			
- Mean (SD)	35.6 (6.1)	36.08 (6.5)	0.69
- Range	22-48	23-50	-
Sex, n (%)			
- Male	36 (72%)	31 (62%)	0.31
- Female	14 (28%)	19 (38%)	
Anagen V-VI Hair Follicles, Mean (SD)	25.54 (4.05)	24.64 (4.45)	0.29
Ki-67 Expression (%), Mean (SD)	91.58 (8.83)	90.08 (12.12)	0.48
c-Kit Expression (%), Mean (SD)	9.19 (1.08)	9.67 (1.12)	0.03
Pull Test Negative, n (%)	50 (100%)	50 (100%)	1.00

As detailed in Table 1, the randomization process successfully yielded comparable treatment

groups at baseline. No statistically significant differences were observed between the placebo and spermidine groups regarding age, sex distribution, the number of anagen V-VI hair follicles, Ki-67 expression, or the proportion of subjects with a negative pull test. A minor but statistically significant difference in baseline c-Kit expression was noted (p=0.03); however, the magnitude of this difference was small, and the subsequent analysis focused on the change from baseline, which robustly demonstrated the treatment effect.

3.2 Number of Anagen Phase V-VI Hair Follicles

Table 2 Number of anagen phase V-VI hair bulbs

	Placebo Mean (s.d.) N=50	Spermidine Mean (s.d.) N=50	P value (between treatment groups, Student's t-test)
T0	25.54 (4.05)	24.64 (4.45)	0.29 (n.s.)
T1	20.24 (3.14)	37.44 (3.84)	-
Absolute change between T1 and T0	-5.3 (2.3)*	12.8 (6.87)*	<0.0001

*p<0.0001 within treatment group, change from T0, paired t-test. n.s. non significant; s.d. standard deviation.

The most striking finding of this study was the dramatic divergence in anagen phase hair follicle numbers between treatment groups after 90 days of supplementation. As shown in Table 2, at baseline, both groups demonstrated similar mean numbers of anagen V-VI hair follicles (25.54 in placebo group vs. 24.64 in spermidine group, p=0.29), confirming adequate randomization and group comparability.

However, by T1 (three months), the groups showed markedly different trajectories. The spermidine-treated group experienced a substantial increase in anagen V-VI hair follicles, with the mean count rising to 37.44 ± 3.84, representing an absolute increase of 12.8 ± 6.87 follicles per 100 sampled (approximately 52% increase from baseline). This change was highly statistically significant within the treatment group (p<0.0001), indicating that the increase was not due to random variation.

In stark contrast, the placebo group exhibited a significant decrease in anagen V-VI hair follicles, with the mean count declining to 20.24 ± 3.14, representing an absolute decrease of 5.3 ± 2.3 follicles per 100 sampled (approximately 21% decrease from baseline). This reduction was also statistically significant within the placebo group (p<0.0001).

The between-group comparison of the change from baseline revealed a highly significant difference (p<0.0001), demonstrating that spermidine supplementation produced a robust effect on maintaining and extending the anagen phase of hair follicles. The magnitude of this effect, representing an approximate 73% difference in trajectory between groups, suggests meaningful biological and potentially clinical significance.

The decrease observed in the placebo group likely reflects natural variation in hair cycling, potentially influenced by seasonal factors, as assessments occurred over a period spanning different seasons. The fact that spermidine supplementation not only prevented this decline but produced substantial increases in anagen follicles underscores its potential therapeutic value.

3.3 Expression of Cellular Proliferation and Apoptosis Markers

At baseline , Ki-67 expression levels in hair bulb cells were comparable between groups (91.58 ± 8.83 in placebo group vs. 90.08 ± 12.12 in spermidine group, p=0.48), indicating similar baseline proliferative activity. By three months, however, distinct patterns emerged (table 3).

In the spermidine-treated group, Ki-67 expression increased significantly to 102.77 ± 10.75, representing an absolute increase of 12.69 ± 8.1 (approximately 14% increase, $p<0.0001$ within group). This elevation in Ki-67 indicates enhanced proliferative activity in hair matrix keratinocytes, the cell population responsible for generating the hair shaft. Increased cell division in this compartment directly supports hair shaft elongation and anagen maintenance.

Conversely, the placebo group demonstrated decreased Ki-67 expression, declining to 86.63 ± 7.66, an absolute decrease of 4.96 ± 6.76 (approximately 5% decrease, $p<0.0001$ within group). This reduction suggests diminished proliferative activity, consistent with the observed decrease in anagen phase follicles.

Table 3 Expression of Ki-67 and c-Kit in Hair Follicle Bulbs

Marker	Timepoint	Placebo Mean (s.d.) N=50	Spermidine Mean (s.d.) N=50	P value (between treatment groups, Student's t-test)
Ki-67	T0	91.58 (8.83)	90.08 (12.12)	0.48 (n.s.)
Ki-67	T1	86.63 (7.66)	102.77 (10.75)	-
Ki-67	Absolute change between T1 and T0	-4.96 (6.76)*	12.69 (8.1)*	<0.0001
c-Kit	T0	9.19 (1.08)	9.67 (1.12)	0.03
c-Kit	T1	10.99 (1.14)	7.52 (1.22)	-
c-Kit	Absolute change between T1 and T0	1.8 (1.07)*	-2.16 (1.24)*	<0.0001

*$p<0.0001$ within treatment group, change from T0 (paired t-test)

The between-group comparison revealed a highly significant difference in Ki-67 change ($p<0.0001$), demonstrating that spermidine supplementation successfully enhanced cellular proliferation in hair follicles. The coordinated increase in both anagen follicle number and proliferation marker expression provides mechanistic insight into how spermidine exerts its effects: by promoting cell division in the hair bulb, it sustains the anagen phase and delays transition to catagen.

At baseline, c-Kit expression showed a small but statistically significant difference between groups (9.19 ± 1.08 in placebo vs. 9.67 ± 1.12 in spermidine, $p=0.03$). Despite this minor initial imbalance, subsequent changes in opposite directions between groups provided clear evidence of treatment effect.

By three months, the spermidine-treated group showed decreased c-Kit expression to 7.52 ± 1.22, representing an absolute decrease of 2.16 ± 1.24 (approximately 22% decrease, $p<0.0001$ within group). Lower c-Kit expression is associated with reduced apoptotic signaling in hair follicles, suggesting that spermidine supplementation creates a less pro-apoptotic microenvironment that favors anagen prolongation.

In contrast, the placebo group exhibited increased c-Kit expression to 10.99 ± 1.14, an absolute increase of 1.8 ± 1.07 (approximately 20% increase, $p<0.0001$ within group). This elevation suggests increased apoptotic activity, which may contribute to premature catagen induction and the observed reduction in anagen follicles.

The between-group comparison of c-Kit changes was highly significant ($p<0.0001$), indicating that spermidine effectively modulated apoptotic pathways in hair follicles. The reciprocal changes in Ki-67 (increased) and c-Kit (decreased) in the spermidine group paint a coherent picture of enhanced hair follicle vitality characterized by increased proliferation and decreased apoptosis.

3.4 Pull Test Results

At baseline (T0), all 100 participants in both groups demonstrated negative pull tests, confirming normal hair anchoring and absence of active telogen effluvium at study entry. This homogeneity provided an ideal starting point for assessing treatment-related changes.

Three-Month Assessment (T1): After 90 days of treatment, a marked divergence became apparent. In the spermidine-treated group, 49 of 50 subjects (98%) maintained negative pull tests, with only one subject showing a mildly positive result. This remarkable maintenance of normal hair anchoring suggests that spermidine supplementation effectively prevented the transition from anagen to telogen that typically manifests as increased hair shedding.

Table 4 Pull Test Results at Baseline, 3 Months, and 6 Months

	T0 Placebo N (%)	T0 Spermidine N (%)	T1 Placebo N (%)	T1 Spermidine N (%)	T2 Placebo N (%)	T2 Spermidine N (%)
-	50	50	36 (72)	49 (98)*	16 (32)	50 (100)#
+	-	-	14 (28)	1 (2)*	19 (38)	-
++	-	-	-	-	12 (24)	-
+++	-	-	-	-	3 (6)	-

*p<0.0001 by Fisher's exact test (comparison between groups at T1)
#p<0.0001 by Chi-square test (comparison between groups at T2)

In stark contrast, the placebo group showed deterioration, with 14 of 50 subjects (28%) developing positive pull tests. All of these were classified as mildly positive, indicating early signs of increased hair shedding. The between-group difference was highly statistically significant (p<0.0001 by Fisher's exact test), demonstrating a clear protective effect of spermidine against hair loss.

Six-Month Assessment (T2): Perhaps most remarkably, three months after treatment cessation, all 50 subjects in the spermidine group (100%) continued to demonstrate negative pull tests. This sustained effect indicates that the biological changes induced by 90 days of spermidine supplementation persisted well beyond the active treatment period, suggesting durable modulation of hair follicle cycling rather than merely a temporary effect dependent on continuous supplementation.

The placebo group, however, showed progressive deterioration over time. By six months, only 16 of 50 subjects (32%) maintained negative pull tests, while 34 subjects (68%) demonstrated positive tests of varying severity: 19 (38%) mildly positive, 12 (24%) moderately positive, and 3 (6%) strongly positive. The between-group difference remained highly significant (p<0.0001 by Chi-square test).

This progression in the placebo group may reflect several factors: natural seasonal variation in hair shedding (with assessments occurring during periods when telogen effluvium is more common), age-related changes in hair cycling, or the cumulative effects of environmental and physiological stressors on hair follicles. The complete absence of positive pull tests in the spermidine group at six months, despite these same potential influences, underscores the robustness of the protective effect.

3.5 Safety and Tolerability

Throughout the 90-day treatment period, the spermidine-based nutritional supplement demonstrated excellent safety and tolerability profile, comparable to the placebo.

No serious adverse events were reported in either treatment group. Minor adverse events were infrequent, mild in severity, and showed similar incidence between groups (Table 4). These events

consisted primarily of mild, transient gastrointestinal symptoms (e.g., occasional nausea or bloating) that resolved spontaneously without requiring intervention or treatment discontinuation.

All 100 enrolled subjects completed the study per protocol, indicating high acceptability of the intervention. No subjects withdrew due to adverse events or dissatisfaction with treatment. Compliance with the daily supplementation regimen was high (>95% based on pill counts), likely facilitated by the simple once-daily dosing schedule(Table 5).

Table 5 Summary of Adverse Events

Adverse Event Category	Placebo Group (n=50)	Spermidine Group (n=50)
Any Adverse Event	4 (8%)	5 (10%)
Gastrointestinal		
- Nausea	2 (4%)	3 (6%)
- Bloating	1 (2%)	2 (4%)
- Abdominal Discomfort	1 (2%)	0 (0%)
Other	0 (0%)	0 (0%)
Serious Adverse Events	0 (0%)	0 (0%)
Discontinuation due to AE	0 (0%)	0 (0%)

4. Discussion

4.1 Principal Findings and Their Significance

This randomized, double-blind, placebo-controlled trial provides the first rigorous clinical evidence that oral spermidine supplementation can effectively prolong the anagen phase of hair follicles in healthy human subjects. Our findings demonstrate multifaceted benefits across several objective outcome measures: a dramatic 52% increase in anagen V-VI hair follicles after 90 days of treatment (compared to a 21% decrease in placebo), significant enhancement of cellular proliferation (14% increase in Ki-67), meaningful reduction in apoptotic signaling (22% decrease in c-Kit), and sustained prevention of hair loss evidenced by universally negative pull tests even three months post-treatment [16-18].

The magnitude and consistency of these effects across multiple complementary assessment methods strengthen confidence in the validity of the findings. The convergence of evidence from morphological evaluation (anagen follicle counts), molecular markers (Ki-67 and c-Kit), and clinical assessment (pull test) provides a comprehensive picture of spermidine's impact on hair follicle biology. The observed ~73% difference in the trajectory of anagen follicle count change between groups underscores a biologically and potentially clinically significant effect. Furthermore, the persistence of benefits at the T2 timepoint, three months after supplementation ceased, is a particularly striking finding. It suggests that spermidine does not merely provide a transient stimulus but may induce a more durable recalibration of the hair follicle's intrinsic cycling dynamics, potentially "resetting" follicles towards a more robust and resilient anagen state [19,20].

4.2 Mechanistic Insights: How Spermidine Promotes Anagen Prolongation

The cellular and molecular changes observed in this study offer valuable insights into the mechanisms underlying spermidine's beneficial effects on hair follicles. The simultaneous increase in Ki-67 expression and decrease in c-Kit expression suggests that spermidine creates a pro-growth, anti-apoptotic environment within the hair follicle that favors anagen maintenance [21].

Enhanced Proliferation and Cell Cycle Progression: The significant elevation in Ki-67 expression indicates that spermidine promotes cell division in the hair matrix keratinocytes. As the

primary engine for hair shaft production, the proliferative vigor of these cells is paramount for sustaining the anagen phase. Spermidine, as a fundamental polyamine, is known to stabilize nucleic acids and stimulate translation, thereby potentially accelerating the cell cycle and increasing the pool of proliferating matrix cells [22,23]. This aligns with previous in vitro studies showing that spermidine enhances keratinocyte proliferation in human hair follicle organ cultures.

Attenuation of Apoptotic Signals: The decrease in c-Kit expression suggests reduced apoptotic signaling, which may delay the initiation of catagen. The anagen-to-catagen transition is a highly regulated process involving programmed cell death in specific follicular regions. c-Kit, a receptor tyrosine kinase, has been implicated in mediating pro-apoptotic signals within the hair follicle. By downregulating c-Kit, spermidine may interfere with these catagen-inducing pathways, thereby extending the anagen phase. This anti-apoptotic effect is consistent with previous research on spermidine analogs [24].

Potential Modulation of Gene Expression and Epigenetics: Beyond the specific markers assessed, spermidine is a known regulator of gene expression. It can interact with DNA and chromatin, potentially influencing the transcription of key genes governing the hair cycle, stem cell quiescence, and differentiation. For instance, spermidine is a natural inducer of autophagy and can influence various signaling pathways (e.g., Wnt/β-catenin, BMP) critical for follicular cycling. Future studies employing transcriptomic analyses could elucidate these broader genomic and epigenomic influences [25].

Anti-inflammatory and Antioxidant Contributions: While not directly measured in our trial, the well-documented anti-inflammatory and antioxidant properties of polyamines, including spermidine and its analogs, provide another plausible mechanism. Inflammation and oxidative stress are recognized contributors to hair follicle miniaturization and premature catagen onset. By mitigating reactive oxygen species and dampening inflammatory cascades within the follicular microenvironment, spermidine could create a more favorable milieu for anagen persistence.

4.3 Integration with Broader Biological Evidence and Future Directions

Our findings are strongly coherent with the fundamental biology of polyamines. The hair follicle, as a mini-organ with exceptionally high proliferative turnover, has a substantial demand for polyamines. The age-associated decline in systemic spermidine levels presents a compelling link to the common observation of age-related hair thinning and reduced anagen duration. Our intervention can be viewed as a targeted replenishment of this crucial molecule, counteracting a potential deficit [26-29].

The sustained effect observed three months post-treatment is particularly intriguing. It suggests that a finite period of supplementation may be sufficient to induce a lasting shift in follicular behavior. This could be mediated through several mechanisms: 1) Activation of Follicle Stem Cells: Spermidine might promote the activation or self-renewal of hair follicle stem cells in the bulge region, leading to the generation of new, robust anagen follicles. 2) Epigenetic Reprogramming: As mentioned, spermidine's role in chromatin modulation could lead to lasting changes in gene expression patterns that favor prolonged anagen. 3) Enhanced Cellular "Fitness": Through its known role in promoting autophagy, spermidine may improve the overall health and resilience of follicular cells, clearing out damaged components and improving metabolic efficiency, thereby extending their functional lifespan [30].

Future research should build upon these promising results. Key directions include [31-33]:

Investigating Specific Patient Populations: Conducting similar trials in individuals with clinically diagnosed androgenetic alopecia or telogen effluvium is essential to confirm efficacy in these target conditions.

Elucidating Molecular Mechanisms: Employing advanced techniques like RNA sequencing on plucked follicles could provide a comprehensive map of the genes and pathways modulated by spermidine.

Optimizing Dosage and Formulation: Exploring different dosages, durations of treatment, and delivery systems (e.g., topical applications) could help maximize therapeutic benefits.

Long-Term Safety and Efficacy: Studies with longer follow-up periods are needed to confirm the durability of the effect and the long-term safety profile of chronic supplementation.

4.4 Limitations

This study has several limitations. Firstly, the participant cohort consisted of healthy individuals without overt hair loss, which limits the direct generalizability of the results to clinical populations, although the positive effects suggest strong preventive potential [34]. Secondly, the study duration, while sufficient to detect changes in the hair cycle, was relatively short; longer trials are needed to assess the impact on hair density and diameter over multiple cycles. Thirdly, we did not measure systemic spermidine levels before and after intervention, which would have provided direct pharmacokinetic correlation with the observed effects. Finally, the precise molecular cascade triggered by spermidine in human hair follicles in vivo warrants further detailed investigation.

5. Conclusion

This randomized, double-blind, placebo-controlled study demonstrates that oral spermidine supplementation effectively prolongs the anagen phase of hair follicles in healthy humans. After 90 days of treatment, participants receiving spermidine showed a remarkable 52% increase in anagen V-VI follicles compared to a 21% decrease in the placebo group, accompanied by enhanced cellular proliferation (14% increase in Ki-67) and reduced apoptotic signaling (22% decrease in c-Kit). Most notably, these beneficial effects persisted three months after treatment cessation, with 100% of spermidine-treated subjects maintaining negative pull tests compared to only 32% in the placebo group, suggesting durable modulation of hair follicle biology rather than temporary effects requiring continuous supplementation.

These findings establish spermidine-based nutritional supplementation as a promising therapeutic approach for hair loss disorders characterized by shortened anagen phase, such as androgenetic alopecia and telogen effluvium. The excellent safety profile, high tolerability, and simple once-daily dosing regimen support its potential for clinical application. However, further investigations are warranted to evaluate efficacy in specific hair loss conditions, determine optimal dosing strategies, assess long-term outcomes, and identify patient populations most likely to benefit from this intervention.

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