Effects of a Combination of Arginine Silicate Inositol Complex and a Novel Form of Biotin on Hair and Nail Growth in a Rodent Model

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Abstract

The purpose of this study was to examine the effects of a combination of inositol-stabilized arginine silicate complex (ASI) and magnesium biotinate (MgB) on hair and nail growth in an animal model. Twenty-eight female Sprague–Dawley rats (8 weeks old) were randomized into one of the following groups: (i) group (control), shaved; (ii) group (ASI), shaved + ASI (4.14 mg/rat/day); (iii) group (ASI + MgB I), shaved + ASI (4.14 mg/rat/day) + MgB (48.7 µg/rat/day); and (iv) group (ASI + MgB II), shaved + ASI (4.14 mg/rat/day) + MgB (325 µg/rat/day). On day 42, compared with the control group, while hair density ($p < 0.05$, $p < 0.01$, and $p < 0.0001$, respectively) and anagen ratio ($p < 0.01$, $p < 0.01$, and $p < 0.001$) increased in the ASI, ASI + MgB I, and ASI + MgB II groups, telogen ratio decreased ($p < 0.01$, $p < 0.01$, and $p < 0.001$, respectively). In the molecular analysis, VEGF, HGF, and KGF-2 increased in the ASI ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively), ASI + MgB I ($p < 0.0001$ for all), and ASI + MgB II ($p < 0.0001$ for all) groups when compared to the control group. FGF-2 ($p < 0.01$) and IGF-1 ($p < 0.001$) were found to be increased in the ASI + MgB I and ASI + MgB II groups. SIRT-1 and β -catenin increased in the ASI ($p < 0.05$ and $p < 0.01$), ASI + MgB I ($p < 0.001$ for both), and ASI + MgB II ($p < 0.0001$ for both) groups. Wnt-1 increased in the ASI + MgB I ($p < 0.001$) and ASI + MgB II ($p < 0.0001$) groups. In [conclusion](#), the combination of ASI and MgB could promote hair growth by regulating IGF-1, FGF, KGF, HGF, VEGF, SIRT-1, Wnt, and β -catenin signal pathways. It was also established that ASI did not affect nail growth, whereas the MgB combination was effective using a higher dose of biotin.

Keywords Arginine silicate · Biotin · Hair · Magnesium · Nail

Introduction

People today take a close interest in their appearance. As visible structures, the hair and nails are eye-catching in both cosmetic and social terms. According to Statista's report, in 2020, the market size of beauty salons (skin, hair, nails) in the USA fell to 39.7 billion US dollars due to the closure of businesses during the coronavirus pandemic, but it reached a value of 42.8 billion US dollars by 2021 [1]. On the other hand, problems with the hair and nails may indicate dermatological and/or systemic diseases [2, 3]. Hair growth is a complicated process that is affected by several factors, including physiological conditions, such

as puberty/pregnancy; genetic factors [2]; hormonal factors (androgen, estrogen, insulin, prolactin, vitamin D) [4]; nutritional and pharmacological factors [5], psychological/emotional stress [6], and inflammatory factors [7]. Previous studies have investigated and reported on the stimulating or inhibiting effects on hair growth of herbal products; natural products, like caffeine; growth hormones and cytokines like interferon- γ (IFN- γ), interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), and platelet-rich plasma; placental extract; stem cells; peptides; and proteins. Specifically, dermal papilla has been shown to be directly targeted by lipid-rich nanoparticles [5]. Nail, like hair, is a specialized keratinous structure made up of the germinal epithelium. The essential components of the nail plate include such minerals as magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), sodium (Na), and copper (Cu), along with sulfur proteins. Endocrine and metabolic diseases and nutritional (vitamin,

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mineral, and element) deficiencies are among the leading factors affecting nail growth [3].

Arginine is a dietary amino acid that can be obtained through de novo or endogenous protein conversion. It is known to play a significant role in several metabolic processes. Urea, creatinine, and nitric oxide are synthesized from arginine [8], and it has been established that it improves sepsis and tumor survival and enhances T-lymphocyte response and macrophage capacity for phagocytosis in animal models. The link between arginine and cardiac diseases, diabetes, renal disease, breast cancer, erectile dysfunction, infertility, wound healing, and memory loss has been investigated [9]. Arginine can be found in skin, hair, and dental care products [10, 11]. It has previously been shown that the complex of arginine with silicate and inositol (49.5 g/kg arginine 8.2 g/kg silicon, 25 g/kg inositol) increases absorption and bioavailability [12].

Biotin (vitamin B7) can be ingested from foods like hazelnuts, legumes, whole grains, and egg yolk and is synthesized by some bacteria [13]. It is a cofactor for five carboxylase enzymes, acetyl-CoA carboxylase 1 (ACC1), and 2 (ACC2), 3-methylcrotonyl-CoA carboxylase (MCC), pyruvate carboxylase (PC), and propionyl-CoA carboxylase (PCC) that catalyzes the steps in the fatty acid, glucose, and amino acid metabolisms. It also plays a role in histone modification, cellular signaling, and gene regulation [14]. There are no reports of a severe biotin deficiency in people with a healthy diet, although biotin deficiency may be genetic or acquired. The genetic form of biotin deficiency manifests itself in severe dermatitis and alopecia during the early stages of life. Acquired biotin deficiency, in contrast, may occur as a result of raw egg consumption, malabsorption, alcoholism, pregnancy, long-term antibiotic use, and the use of such medications as valproic acid and isotretinoin [15]. Biotin deficiency results in altered lipid metabolism, impaired cellular and humoral immune systems, and decreased collagen synthesis. In clinical practice, it may present as periorificial dermatitis, conjunctivitis, ataxia, hypotony, keto-lactic acidosis/organic aciduria, epilepsy, skin infection, growth restriction in children, psychosis, neuritis, hair loss, and fragile nail structure [13, 14]. Biotin is used to stimulate hair and nail growth; however, there is only limited data on the benefit of biotin in people with a healthy diet [16].

Magnesium is a cellular ion transporter that plays a role in nucleic acid synthesis. It is a cofactor in various enzyme systems to regulate protein synthesis, muscle and nerve conduction, neuromuscular conduction, blood glucose, and blood pressure [17]. Animal studies have demonstrated that Mg may increase the usability of glucose in the brain, muscles, and blood, and thereby exercise performance. Human studies have, in turn, measured its effects on blood pressure and heart rate [18]. Moderate magnesium deficiency has been associated with chronic inflammatory diseases and cancer

[19]. In a previous study, low levels of Mg were found in the hair in overweight and obese individuals [20].

In this study, magnesium biotinate (MgB) form was produced, and inositol-stabilized arginine silicate complex (ASI) and their combined effects on hair and nail growth in healthy animals were investigated through molecular mechanisms.

Materials and Methods

Animals

The study was approved by the Animal Ethics Committee of Firat University and conducted following the standard ethical guidelines for laboratory animal use and care, as defined in the European Economic Community rules (EEC, 1986). Twenty-eight 8-week-old female Sprague–Dawley/Wistar albino rats were obtained from the Firat University Animal Experiments Center (FUDAM). The rats were accommodated in accordance with the guidelines for laboratory animal use and care at the Laboratory Animal Research Center of Firat University. Throughout the experiment, the rats were maintained under controlled temperature (23 ± 2 °C), humidity ($55 \pm 10\%$), and automated lighting (12 h of light and dark, without ultraviolet emission) conditions. Feed and water were provided ad libitum. Before starting the experiment, the rats were acclimatized for 1 week [12, 21].

Study Design

At the beginning of the study, a 2×6 cm patch of dorsal hair of the 28 rats was shaved under anesthesia and randomly divided into four groups 7 rats per group.

1. Group (Control): shaved
2. Group (ASI): shaved + ASI (4.14 mg/rat/day—140 mg human equivalent dose)
3. Group (ASI + MgB I): Shaved + ASI (4.14 mg/rat/day—140 mg human equivalent dose) + MgB (48.7 µg/rat/day)
4. Group (ASI + MgB II): shaved + ASI (4.14 mg/rat/day—140 mg human equivalent dose) + MgB (325 µg/rat/day)

The standard diet used in this study was the modified AIN-93 M diet (Dyets Inc., PA, USA) as detailed in previous studies [22, 23]. The AIN-93 M diet contains 5 mg Si/kg [24]. ASI contains 49.47% arginine, 8.2% silicon, and 25% inositol. Arginine (3.8 g, 21.8 mmol) was added to a vigorously stirred solution of inositol (1.25 g, 6.9 mmol) in potassium silicate [5 ml, 29.8° Be, 8.3% K₂O (0.52 g, 5.5 mmol), 20.8% SiO₂ (1.3 g, 21.8 mmol)]. The ASI complex and MgB

were provided by JDS Therapeutics, LLC. (Harrison, NY, USA). Both ASI and MgB were dissolved in drinking water and were administered via oral gavage for 6 weeks. In the control group, saline was administered by oral gavage after being dissolved in drinking water. The dosages of supplements were determined as proposed in the literature.

Hair Growth and Density Analyses

A roughly 2 × 2 cm area of the dorsal zone of the rats was shaved weekly. The assessments of hair and nail growth in the shaved areas were made by an objective observer on days 0, 7, 14, 21, 28, 35, and 42. For each rat, dorsal photos were taken on predetermined days. For analysis, the hair was stained using a commercially available, soluble, temporary eyebrow, and eyelash dye (Gschwenter, Haar

Cosmetic RefectoCil) to ensure contrast in the shaved areas of each rat. The stained areas were cleaned with alcohol after 10 min, and digital images at 0.65 cm² and 20-fold magnification were taken using an epiluminescence microscopy (Griscopes) device manufactured by Grimed. The obtained images were transferred to Grimed version TrichoScan software. Using this software, hair density measurements and hair percentages at the anagen (growth)–telogen (resting) phases were analyzed and recorded (Fig. 1A). Likewise, the claws of every rat were photographed over white paper for contrast on days 14, 28, and 42. In a computer environment, the nail growth rates were calculated by taking measurements from the photos (Fig. 1B). At the end of the experiment, the rats were fasted for 15 h and were sacrificed by cervical dislocation, and serum, skin, and hair samples were taken. The

Fig. 1 Photographs of the skin surface and TrichoScan features of hair (**A**) and macroscopic features of the nail (**B**) of inositol-stabilized arginine silicate complex (ASI) and magnesium biotinate (MgB) in rats. Control: shaved; ASI: shaved + ASI (4.14 mg/rat/day); ASI + MgB I: shaved + ASI (4.14 mg/rat/day) + MgB (48.7 µg/rat/day); ASI + MgB II: shaved + ASI (4.14 mg/rat/day) + MgB (325 µg/rat/day)

samples were stored at -80°C in a deep freeze (Hettich, Germany) until analysis.

Biochemical Analyses

Serum triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), and parathyroid hormone (PTH) concentrations were determined using a commercially available assay kit according to the manufacturer's instructions (Diagnostic Systems Laboratories, TX, USA). For hydroxyproline analyses, the dorsal skin tissues were homogenized using 2 mL of 1 N acetic acid, and the homogenates were centrifuged ($3.000\times g$, 10 min). The hydroxyproline levels were measured in the supernatants using a commercially available assay kit according to the manufacturer's instructions (Diagnostic Systems Laboratories, TX, USA). The intra- and inter-assay coefficients of variations (CV) were 4.3 and 6.5%. The tissue was also homogenized and solubilized in 0.1% Triton-X 100, 0.2 M Tris-HCl (pH 8.0) buffer, followed by ultrasonication and by centrifugation ($2000\text{ g}\times 20\text{ min}$) to obtain supernatants for the elastase enzyme assay. The elastase activity was detected using a commercially available assay kit (Diagnostic Systems Laboratories, TX, USA) according to the manufacturer's instructions. The inter- and intra-assay constants were 3.6% and 6.4%. Biotinidase activity in the skin was determined by measuring the release of N-biotinyl-p-aminobenzoate using the colorimetric method described [25] in the literature. Biotinidase activity was expressed in mU/100 mg protein.

Mineral Analyses

To establish the serum concentrations of Mg, silicon (Si), iron (Fe), copper (Cu), and zinc (Zn), approximately 0.5-ml serum samples were digested using 8-ml concentrated nitric acid in a Microwave Digestion System (Berghof, Eningen, Germany) for 30 min, and diluted at a ratio of 1:10 with distilled water prior to analysis. Lanthanum chloride (Merck, Darmstadt, Germany) was added as an interference suppressant for Mg analyses. The heating program employed was the one described in the oven's user manual. Sample blanks were similarly prepared using 8-ml nitric acid (65%, w/v). Digested samples and blanks were diluted with ultra-high purity water, and total final volumes, assessed accurately by weight were recorded before analysis. We obtained AAS standard solutions from Merck (Darmstadt, Germany). The mineral levels of the samples were measured using Flame AAS (AAS, Perkin-Elmer, Analyst 800, Norwalk, CT, USA) with flame atomization in an acetylene-air via recognized and fully confirmed procedures, with the Zeeman background correction. The assessments were performed in triplicate. The method was verified with certified reference materials (bovine muscle BCR 184).

Arginine and Biotin Analyses

The serum levels of biotin were analyzed using the high-performance liquid chromatography (HPLC-Shimadzu, Kyoto, Japan) method [26, 27]. As C18-ODS-3 column ($250\times 4.6\text{ mm}$, 5 m), chromatography fractions containing reversed-phase column and biotin were used. The serum concentrations of arginine were determined using HPLC (Shimadzu, Japan) [28]. Serum samples were extracted 1:1 in 35% (wt/vol) sulfosalicylic acid dihydrate. After mixing and centrifugation, the supernatant was mixed 1:1 with a lithium-D buffer prior to analysis. The amino acid standard was obtained from Sigma-Aldrich Chemicals (St. Louis, USA).

Molecular Analyses

Dorsal skin protein levels of biotin-dependent carboxylase including ACC1, ACC2, PC, PCC, MCC, and vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF-2), insulin-like growth factor-1 (IGF-1), sirtuin-1 (SIRT-1), and matrix metalloproteinase-1 and -3 (MMP-1 and MMP-3) were measured using the Western blotting technique as defined earlier [29]. Skin tissues were homogenized at 4°C in an extraction buffer and centrifuged at $13.000\times g$ for 20 min at 4°C . Protein samples were separated using 10% SDS-Page and transferred onto nitrocellulose membranes for 1 h prior to application of primary antibodies. The following primary antibodies were used: ACC1, ACC2, PC, PCC, MCC, FGF-2, KGF-2, VEGF, SIRT1, MMP-1, MMP-3, and β -actin (Santa Cruz Biotechnology, CA, USA). After washing, the membranes were incubated with secondary goat anti-mouse antibodies (Santa Cruz Biotechnology) in Tris-buffered saline containing 0.05% Tween-20 for 1 h, and protein levels were measured densitometrically. Full blots are presented as the supplementary file (Supplementary Fig. S1, S2, S3).

Histological Analysis

The dorsal skin samples were fixed in 10% buffered formalin and embedded in paraffin. 5 mm thick sections were obtained and stained with hematoxylin-eosin (HE) (Fig. 2).

Statistical Analysis

Statistical analyses were performed using SPSS for windows version 21.0 (IBM Corp., Armonk, NY, USA), with a one-way analysis of variance. Then Tukey's or Tamhane's

Fig. 2 Effect of inositol-stabilized arginine silicate complex (ASI) and magnesium biotin (MgB) on the dorsal skin of rats. Histological analysis of dorsal skin was performed after H&E staining. Control: shaved; ASI: shaved + ASI (4.14 mg/rat/day); ASI + MgB I: shaved + ASI (4.14 mg/rat/day) + MgB (48.7 µg/rat/day); ASI + MgB II: shaved + ASI (4.14 mg/rat/day) + MgB (325 µg/rat/day)

T2 post hoc test was used for multiple comparisons. A *P*-value of <0.05 was considered significant.

Results

Hair Growth and Density

Representative macroscopic and TrichoScan features of hair (A) in the skin on the back and macroscopic features of the nail (B) in groups were shown in Fig. 1. HE staining was performed to evaluate the progression of hair follicles in the hair cycle (Fig. 2). The hair follicles in the study groups appeared in the subcutaneous tissue earlier than the control group in the transverse sections. This finding indicated an increase in the number of anagen hairs.

There was no statistically significant difference in hair density between the treatment and control groups at the outset and on day 7 of the study ($p > 0.05$). On day 14, a 13.9% increase in hair density was noted in the ASI + MgB I group and a 23.9% increase in the ASI + MgB II group. On day 21, a remarkable 37.5% increase in hair density was found only in the ASI + MgB II group. On day 28, the increases were 6.5, 7.7, and 20.9% in the ASI, ASI + MgB I, and ASI + MgB II groups. On the 35th day, the increases in hair density were 15.9, 17.4, and 34.7% in the ASI, ASI + MgB I, and ASI + MgB II groups, respectively. When the hair density increases were compared with the control group, 19.7% and 25.2% in the ASI group and ASI + MgB I group,

the highest increase was seen in the ASI + MgB II group with 55% at the end of the study ($p < 0.05$, $p < 0.01$, and $p < 0.0001$, respectively; Fig. 3A, Supplementary Table S1).

Anagen Percentage

No statistically significant difference was noted in hair percentage in the anagen phase between treatment and control groups at the outset and on day 7 of the study ($p > 0.05$). It was found that only the ASI + MgB II group increased by 38.7% on the 14th day. On day 21, an increase of 15.6% was seen in the ASI + MgB I group, while the ASI + MgB II group showed a higher rate of increase with 43%. Results at day 28 revealed a rise of 21.8, 29.3, and 41.49% in the ASI, ASI + MgB I, and ASI + MgB II groups, respectively. On day 35, the increases were 12.16, 20.9, 32.08% in the ASI, ASI + MgB I, and 32.08% ASI + MgB II groups. Anagen ratio increased only in the ASI + MgBII group compared to the control group on days 14–35 ($p < 0.01$). At the end of the study, the increase in hair percentage in the anagen phase was found to be 30.8, 34.05, and 48.66% in the ASI, ASI + MgB I, and ASI + MgB II groups compared to the control group ($p < 0.01$, $p < 0.01$, and $p < 0.001$, respectively; Fig. 3B; Supplementary Table S2).

Telogen Percentage

Telogen hair density was similar at baseline and day 7 in the treatment and control groups ($p > 0.05$). After day 14,

Fig. 3 Effect of inositol-stabilized arginine silicate complex (ASI) and magnesium biotinate (MgB) on the density of hair ($1/\text{cm}^2$) (A), the anagen (B) and telogen (C) ratios (%), and in rats. Control: shaved; ASI: shaved + ASI (4.14 mg/rat/day); ASI + MgB I: shaved + ASI (4.14 mg/rat/day) + MgB (48.7 μg /rat/day); ASI + MgB II: shaved + ASI (4.14 mg/rat/day) + MgB (325 μg /rat/day)

a 26% decrease was seen in the ASI + MgB II group only ($p < 0.01$). On day 21, a reduction of 11.54% was noted in the ASI + MgB I group and 31.8% in the ASI + MgB II group. On the 28th day, the decrease was 15.2, 20.5, and

35.08 in the ASI, ASI + MgB I, and ASI + MgB II groups. On day 35, the reduction was 9.58% in the ASI group, 16.48% in the ASI + MgB I group, and 25.28% in the ASI + MgB II group. Telogen ratio decreased only in the ASI + MgB II group

compared to the control group on days 14–35 ($p < 0.01$). At the end of the study, the decrease was 29.46% in the ASI group, 32.53% in the ASI + MgB I group, and 46.49% in the ASI + MgB II group compared to the control group ($p < 0.01$, $p < 0.01$, and $p < 0.001$, respectively; Fig. 3C; Supplementary Table S3).

Nail Growth

The effect of ASI and MgB on average nail growth on days 14, 28, and 42 in rats are presented in Table 1. There was no significant nail growth in the ASI and ASI + MgB I groups compared to the control group, whereas the increase in the ASI + MgB II group was significant compared to the control, ASI, ASI + MgB I groups ($p < 0.0001$ for all). The increase in nail growth in the ASI + MgB II group was 30, 26, and 25% on days 14, 28, and 42, respectively. When the differences between fingers (finger 1–4) were examined in terms of nail growth, significant growth was noted in the nails of the first

four fingers, and especially in the ASI + MgB II group, when compared to the control group on days 14, 28, and 42.

Biochemical Parameters

The biochemical parameters are presented in Table 2. The levels of FT3, total T3, FT4, total T4, TSH, and PTH were analyzed in the serum samples on the final day of the study were similar to those of the control group in the ASI, ASI + MgB I, and ASI + MgB II groups ($p > 0.05$). However, hydroxyproline levels were significantly increased in the ASI + MgB I and ASI + MgB II groups when compared to the control group ($p < 0.0001$ for all). While there was an increase of 28% in the ASI group and 52.4% in the ASI + MgB I group, a very high rate of 82.5% was recorded in the ASI + MgB II group. Serum elastase levels, in turn, were found to decrease in the ASI, ASI + MgB I, and ASI + MgB II groups when compared to the control group ($p < 0.0001$ for all). The decrease was 21.07%

Table 1 Effect of inositol-stabilized arginine silicate complex (ASI) and magnesium biotinate (MgB) on mean nail growth in rats ($n = 7$)

Mean nail growth	Groups			
	Control	ASI	ASI + MgB I	ASI + MgB II
Day 14	0.093 ± 0.002	0.098 ± 0.004	0.100 ± 0.002	0.121 ± 0.002 ****,####,++++
Day 28	0.099 ± 0.001	0.100 ± 0.003	0.103 ± 0.003	0.125 ± 0.002 ****,####,++++
Day 42	0.098 ± 0.001	0.100 ± 0.002	0.103 ± 0.001	0.123 ± 0.003 ****,####,++++

ASI inositol-stabilized arginine silicate complex; MgB magnesium biotinate; Data presented as mean and standard error. Statistical significance between groups were shown by as compared to control; **** $p < 0.0001$, compared to ASI; #### $p < 0.0001$, compared to ASI + MgB I; ++++ $p < 0.0001$, ANOVA and Tukey's post-hoc test. Control: shaved; ASI: shaved + ASI (4.14 mg/rat/day); ASI + MgB I: shaved + ASI (4.14 mg/rat/day) + MgB (48.7 µg/rat/day); ASI + MgB II: shaved + ASI (4.14 mg/rat/day) + MgB (325 µg/rat/day)

Table 2 Effect of inositol-stabilized arginine silicate complex (ASI) and magnesium biotinate (MgB) on serum thyroid hormones and skin hydroxyproline, elastase, and biotinidase in rats ($n = 7$)

Items	Groups			
	Control	ASI	ASI + MgB I	ASI + MgB II
FT3 (pg/dl)	3.67 ± 0.14	3.62 ± 0.14	3.54 ± 0.07	3.48 ± 0.16
Total T3 (ng/ml)	0.97 ± 0.07	0.89 ± 0.05	0.84 ± 0.03	0.86 ± 0.04
FT4 (ng/dl)	2.89 ± 0.09	2.79 ± 0.09	2.76 ± 0.18	2.71 ± 0.12
Total T4 (µg/dl)	4.95 ± 0.14	4.93 ± 0.22	4.81 ± 0.08	4.84 ± 0.19
TSH (ng/ml)	2.51 ± 0.11	2.40 ± 0.11	2.32 ± 0.06	2.26 ± 0.11
PTH (pg/ml)	87.87 ± 1.91	85.78 ± 2.46	90.76 ± 2.33	89.89 ± 2.23
Hydroxyproline (g/100 g tissue)	4.06 ± 0.13	5.23 ± 0.10 ****	6.19 ± 0.13 ****,####	7.41 ± 0.14 ****,####,++++
Elastase (pmol nitroaniline/hr)	5.60 ± 0.10	4.42 ± 0.15 ****	3.66 ± 0.09 ****,####	3.14 ± 0.06 ****,####,++
Biotinidase (U/L)	4.79 ± 0.16	4.71 ± 0.19	6.56 ± 0.12 ****,####	7.82 ± 0.10 ****,####,++++

FT3 free triiodothyronine, Total T3 total triiodothyronine, FT4 free thyroxine, Total T4 total thyroxine, TSH thyroid-stimulating hormone, PTH parathyroid hormone, ASI inositol-stabilized arginine silicate complex, MgB magnesium biotinate; Data presented as mean and standard error. Statistical significance between groups were shown by as compared to control; **** $p < 0.0001$, compared to ASI; #### $p < 0.001$, **** $p < 0.0001$, compared to ASI + MgB I; ++ $p < 0.01$, ++++ $p < 0.0001$, ANOVA and Tukey's post hoc test. Control: shaved; ASI: shaved + ASI (4.14 mg/rat/day); ASI + MgB I: shaved + ASI (4.14 mg/rat/day) + MgB (48.7 µg/rat/day); ASI + MgB II: shaved + ASI (4.14 mg/rat/day) + MgB (325 µg/rat/day)

in the ASI group, 34.64% in the ASI+MgB I group, and 43.92% in the ASI+MgB II group. Serum biotinidase levels increased by 36.9% in the ASI+MgB I group and 63.2% in the ASI+MgB II group ($p < 0.0001$ for both).

Minerals, Biotin, and Arginine Levels

Serum minerals, biotin, and arginine levels are presented in Table 3. The serum levels of Mg (mg/dL) and biotin (nmol/L) were significantly higher in the ASI+MgB I and ASI+MgB II groups that received Mg and biotin supplements when compared to the control group ($p < 0.0001$ for both). The increases of Mg and biotin were 65.4% and 37.2%, respectively, in the ASI+MgB I group, while the increase of Mg and biotin was very high, at 97.5% and 114%, respectively in the ASI+MgB II that received high doses of Mg and biotin. Similarly, the levels of arginine (nmol/L) and Si ($\mu\text{g/L}$) were significantly higher in the ASI, ASI+MgB I, and ASI+MgB II groups when compared to the control group ($p < 0.0001$ for all). The arginine increase was 70.5% in the ASI group, 80.9% in the ASI+MgB I group, and 78.5% in the ASI+MgB II group. Regarding Si, the change was 241% in the ASI group, 255% in the ASI+MgB I group, and 249% in the ASI+MgB II group. The serum level of Zn ($\mu\text{g/L}$) increased 5.2%, especially in the ASI+MgB II group. The serum levels of Fe ($\mu\text{g/L}$), Cu ($\mu\text{g/L}$), and total protein (mg/dl) were similar in the study and control groups ($p > 0.05$).

Molecular Analysis

The molecular analysis revealed that the levels of enzymes ACC1 and MCC increased especially in the ASI+MgB I ($p < 0.05$ and $p < 0.01$) and ASI+MgB II ($p < 0.0001$ and

$p < 0.001$) groups while a significant elevation in ACC2, PC, and PCC was noted in the ASI ($p < 0.001$, $p < 0.05$, and $p < 0.01$, respectively), ASI+MgB I ($p < 0.0001$, $p < 0.001$, and $p < 0.0001$, respectively), and ASI+MgB II ($p < 0.0001$ for all) groups when compared to the control group. ACC1, ACC2, PC, and PCC enzyme levels also differed significantly between ASI+MgB I and ASI+MgB II groups ($p < 0.01$, $p < 0.01$, $p < 0.05$, and $p < 0.01$, respectively) (Fig. 4). The molecular analysis also showed a significantly higher VEGF level in the ASI, ASI+MgB I, and ASI+MgB II ($p < 0.01$, $p < 0.0001$, and $p < 0.0001$, respectively) groups. VEGF levels also differed significantly between ASI+MgB I and ASI+MgB II groups ($p < 0.001$). FGF-2 and IGF-1 were found to be increased in the ASI+MgB I ($p < 0.01$ and $p < 0.001$) and ASI+MgB II ($p < 0.01$ and $p < 0.001$) groups. HGF and KGF-2 increased in the ASI ($p < 0.01$ and $p < 0.05$), ASI+MgB I ($p < 0.0001$ for both), and ASI+MgB II ($p < 0.0001$ for both) groups when compared to the control group but similar between the ASI+MgB I and ASI+MgB II groups (Fig. 5). It was found that SIRT-1 increased in the ASI, ASI+MgB I, and ASI+MgB II ($p < 0.05$, $p < 0.001$, and $p < 0.0001$, respectively) groups; MMP-1 decreased in the ASI+MgB I and ASI+MgB II ($p < 0.01$ for both) groups, with similarities recorded between the ASI+MgB I and ASI+MgB II groups; MMP-3 decreased in the ASI+MgB I and ASI+MgB II ($p < 0.01$ and $p < 0.001$) groups; Wnt-1 increased in the ASI+MgB I and ASI+MgB II ($p < 0.001$ and $p < 0.0001$) groups; and β -catenin increased in the ASI, ASI+MgB I, and ASI+MgB II ($p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively) groups. SIRT-1, Wnt-1, and β -catenin also differed significantly between ASI+MgB I and ASI+MgB II groups ($p < 0.05$, $p < 0.01$, and $p < 0.01$, respectively) (Fig. 6).

Table 3 Effect of inositol-stabilized arginine silicate complex (ASI) and magnesium biotinate (MgB) on serum minerals, biotin, arginine, and total protein in rats ($n = 7$)

Items	Groups			
	Control	ASI	ASI+MgB I	ASI+MgB II
Mg (mg/dL)	2.46 $\pm$ 0.04	2.43 $\pm$ 0.07****	4.07 $\pm$ 0.08****,####	4.86 $\pm$ 0.03****,####,++++
Biotin (nmol/L)	80.69 $\pm$ 3.38	76.99 $\pm$ 1.77****	128.99 $\pm$ 1.76****,####	172.69 $\pm$ 3.30****,####,++++
Arginine (nmol/L)	1.63 $\pm$ 0.06	2.78 $\pm$ 0.11****	2.95 $\pm$ 0.10****	2.91 $\pm$ 0.13****
Si ($\mu\text{g/L}$)	206.00 $\pm$ 5.47	703.43 $\pm$ 10.51****	732.14 $\pm$ 22.70****	719.57 $\pm$ 32.20****
Fe ($\mu\text{g/dL}$)	459.86 $\pm$ 13.42	461.29 $\pm$ 9.79	465.43 $\pm$ 12.58	469.57 $\pm$ 7.35
Zn ($\mu\text{g/dL}$)	177.56 $\pm$ 1.70	178.57 $\pm$ 2.55	182.57 $\pm$ 2.55	186.84 $\pm$ 2.30*
Cu ($\mu\text{g/dl}$)	236.71 $\pm$ 6.64	233.14 $\pm$ 3.64	235.71 $\pm$ 6.51	237.71 $\pm$ 4.94
Total protein (mg/dl)	5.87 $\pm$ 0.23	6.09 $\pm$ 0.12	6.16 $\pm$ 0.33	5.93 $\pm$ 0.24

Mg magnesium; Si silicon; Fe iron; Zn zinc; Cu copper; ASI inositol-stabilized arginine silicate complex; MgB magnesium biotinate; Data presented as mean and standard error. Statistical significance between groups were shown by as compared to control; * $p < 0.05$, **** $p < 0.0001$, compared to ASI; #### $p < 0.0001$, compared to ASI+MgB I; ++++ $p < 0.0001$, ANOVA and Tukey's post hoc test. Control: shaved; ASI: shaved + ASI (4.14 mg/rat/day); ASI+MgB I: shaved + ASI (4.14 mg/rat/day) + MgB (48.7 $\mu\text{g/rat/day}$); ASI+MgB II: shaved + ASI (4.14 mg/rat/day) + MgB (325 $\mu\text{g/rat/day}$)

Fig. 4 Effect of inositol-stabilized arginine silicate complex (ASI) and magnesium biotin (MgB) on skin tissue representative protein expression of blots (acetyl-CoA carboxylase 1 (ACC1) **A**, acetyl-CoA carboxylase 2 (ACC2) **B**, pyruvate carboxylase (PC) **C**, propionyl-CoA carboxylase (PCC) **D**, and 3-methylcrotonyl-CoA carboxylase (MCC) **E**) levels in rats. The densitometric analysis of the relative intensity according to the control group of the western blot bands was performed with β -actin normalization to ensure equal protein loading. Blots were repeated at least three times ($n=3$), and a representative blot (**F**) was shown. The bars point out the standard deviation of the mean. Different symbols (*, #, +) indicate difference compared to the control, ASI, and ASI+MgB I groups, respectively) above the bars indicate statistical differences among the groups (ANOVA and Tukey's post hoc test; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$; ## $p<0.01$; ### $p<0.001$; #### $p<0.0001$; + $p<0.05$; ++ $p<0.01$). Control: shaved; ASI: shaved + ASI (4.14 mg/rat/day); ASI+MgB I: shaved + ASI (4.14 mg/rat/day) + MgB (48.7 μ g/rat/day); ASI+MgB II: shaved + ASI (4.14 mg/rat/day) + MgB (325 μ g/rat/day)

Discussion

The present study is the first to investigate the effects of a combination of ASI (140 mg human equivalent arginine) and ASI+MgB (1.5 and 10 mg human equivalent biotin) combination on hair and nail growth in a rodent model. The study found that the combination of ASI and ASI+MgB had a macroscopically positive effect on hair elongation. In addition, trichoscopically, the percentage of telogen hair decreased with the increase in hair density and anagen hair percentage in all study groups. Arginine is added to some cosmetic hair and skin care products as a moisturizer [10]. Molecular analysis of the dorsal skin revealed significant

Wnt induction in the groups receiving the ASI+MgB combination. The β -catenin signal was also demonstrated to be induced in the ASI and ASI+MgB groups. The Wnt signal pathway and β -catenin are important signals that form the hair follicle and enable hair follicle regeneration. The activation of β -catenin in the dermal papilla regulates signal pathways involving FGF and IGF [30]. Regarding the growth factors, no significant change was identified in the levels of IGF-1 and FGF in the ASI group, whereas there was an increase in the ASI+MgB groups. KGF, HGF, and VEGF were also increased in all study groups, although there was a more distinct increase in the ASI+MgB groups. Used to treat androgenetic alopecia, minoxidil has been reported to

Fig. 5 Effect of inositol-stabilized arginine silicate complex (ASI) and magnesium biotinate (MgB) on skin tissue representative protein of blots (vascular endothelial growth factor (VEGF) **A**, fibroblast growth factor 2 (FGF2) **B**, hepatocyte growth factor (HGF) **C**, keratinocyte growth factor 2 (KGF2) **D**, and insulin-like growth factor-1 (IGF-1) **E**) levels in rats. The densitometric analysis of the relative intensity according to the control group of the western blot bands was performed with β -actin normalization to ensure equal protein loading. Blots were repeated at least three times ($n=3$), and a representative blot (**F**) is shown. The bars point out the standard deviation of the mean. Different symbols (*, #, +) indicate difference compared to the control, ASI, and ASI+MgB I groups, respectively) above the bars indicate statistical differences among the groups (ANOVA and Tukey's post hoc test; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$; # $p<0.05$; ## $p<0.01$; ### $p<0.001$; #### $p<0.001$; +++ $p<0.001$). Control: shaved; ASI: shaved+ ASI (4.14 mg/rat/day); ASI+MgB I: shaved+ ASI (4.14 mg/rat/day)+ MgB (48.7 μ g/rat/day); ASI+MgB II: shaved+ ASI (4.14 mg/rat/day)+ MgB (325 μ g/rat/day)

increase blood flow to the hair follicle and to contribute to hair nourishment by opening the potassium channels [31], and to stimulate hair growth through such growth factors as IGF-1, FGF, VEGF, β -catenin, and nitric oxide [32]. Members of the FGF family are responsible for angiogenesis, embryonic development, and tissue repair, and FGF has been shown to stimulate the anagen phase and thereby hair growth [33]. KGF (a member of the FGF family) [34] and HGF [35] are other signals known to prolong the anagen phase. It has been reported that VEGF stimulates vasculogenesis and angiogenesis and supplies nutrients to the hair follicle by increasing the follicle diameter [32]. A previous study has shown that ASI contributes to wound healing by stimulating

certain cytokines, such as VEGF, FGF, and hydroxyproline [36]. On the other hand, other anticipated mechanisms are the supply of nutrients to the hair by arginine through vasodilatation of the hair follicle via nitric oxide and silica contained in ASI supporting hair growth through the ODC enzyme. The enzymes that play a role in converting arginine into such polyamines as putrescine, which is required for cell proliferation, are arginase and ornithine decarboxylase (ODC) [37]. Eflornithine, an ODC enzyme inhibitor, is used to treat hirsutism [38], while silica has also been demonstrated to increase epidermal ODC activity [39]. There is an opinion in the literature that the administration of arginine in nanotechnological lipid transporters to the hair follicle may

Fig. 6 Effect of inositol-stabilized arginine silicate complex (ASI) and magnesium biotin (MgB) on skin tissue representative protein expression of blots sirtuin-1 (SIRT-1) **A**, matrix metalloproteinase 1 (MMP-1) **B**, Matrix metalloproteinase 3 (MMP-3) **C**, β -catenin **D**, and Wingless-type 1 (Wnt-1) **E** levels in rats. The densitometric analysis of the relative intensity according to the control group of the western blot bands was performed with β -Actin normalization to ensure equal protein loading. Blots were repeated at least three times ($n=3$) and a representative blot (**F**) is shown. The bars point out the standard deviation of the mean. Different symbols (*, #, +) indicates difference compared to the control, ASI, and ASI+MgB I groups, respectively) above the bars indicate statistical differences among the groups (ANOVA and Tukey's post hoc test; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$; # $p<0.01$; ### $p<0.001$; #### $p<0.001$; + $p<0.05$; ++ $p<0.01$). Control: shaved; ASI: shaved+ASI (4.14 mg/rat/day); ASI+MgB I: shaved+ASI (4.14 mg/rat/day)+MgB (48.7 μ g/rat/day); ASI+MgB II: shaved+ASI (4.14 mg/rat/day)+MgB (325 μ g/rat/day)

be used to treat alopecia [40]. These findings suggest that ASI prolonged the anagen phase through growth factors and thereby increased hair density and anagen hair percentages.

One of the present study findings is that hair growth was better in the ASI+MgB combination groups than in the ASI alone groups. It was observed that the addition of biotin to ASI increased hair density and anagen ratio and decreased telogen ratio. Biotin is in frequent use as a hair and nail care product, and demand for biotin-containing supplements has been increasing due to the easy access and the effect of social media. However, there is limited data on the impact of biotin supplementation on hair and nail growth, and patients may experience disappointment if they have high expectations of success [41].

Biotin supplements are used in pharmacological doses (a few milligrams) in biotin deficiency and can be considered safe [14]. There is no evidence of biotin supplements' limits and potential toxicity, although the amount of biotin in many supplement products for skin, hair, and nail health are above the recommended daily biotin intake (0.03 mg/day) [15]. It was determined that increased hair density and anagen ratio and decreased telogen ratio effects increased even more, when the dose of biotin was increased. Mg is an ion transporter for Ca and potassium (K) that works as a natural Ca antagonist. It has a regulatory role in the Mg-Ca channel gates and has a relaxation effect on endothelial cells and vascular smooth muscles. On the other hand, Mg plays a role in nucleic acid synthesis, and this effect

is of significance in regions like the hair root as a site of frequent mitosis [17]. In the light of the above information, it is reasonable to say that Mg supports hair growth through increased blood flow to the hair and enhances the positive impact of biotin on hair growth.

Macroscopically significant nail growth could be achieved only in the group receiving 10 mg MgB combined with ASI in this study. Due to its stimulating effect on epidermal cell growth and differentiation, biotin has been used to treat several nail diseases, including nail fragility, trachyonychia, triangular worn down nails, and habit-tic deformity [42]. In a review study by Patel et al., different doses of biotin were used to treat hair loss and nail changes in individuals with biotin deficiency and without such a deficiency (healthy) [16]. It was determined that ASI did not affect nail growth, while MgB contributed to nail growth at high doses. On the other hand, further studies are required for the optimal dose of MgB that stimulates nail growth.

The present study demonstrated that SIRT-1 was induced by ASI and ASI + MgB combination. Sirtuins are nicotinamide adenine dinucleotide (NAD)-dependent deacetylase enzymes known for their antiaging characteristics. They play a role in regulating the cellular response to stress, DNA repair and metabolism, and tumorigenesis. SIRT-1 positively affects glucose-induced insulin response [43]. A previous study showed that SIRT-1 enabled mitochondrial homeostasis against the stress caused by TNF- α -dependent mediators on the hair follicle and increased the stem cells in the hair follicle as a way of protection [44]. Another study concluded that the decrease in hair pigmentation in chronological aging might be prevented by increasing the levels of SIRT-1 [45]. In an experimental study, it was determined that arginine increased the expression of SIRT-1 and MMP-2, reduced myocardial fibrosis and prevented cell apoptosis in streptozotocin-induced diabetic rats [46]. In the studies mentioned above, arginine has been shown to increase the expression of SIRT-1. This study also showed that SIRT-1 was increased in all groups. However, the more significant increase in the high-dose MgB group suggested that SIRT-1 may be more sensitive to MgB.

The present study examined the levels of MMP-1 and MMP-3 in the dorsal skin and identified a decrease in the level of both enzymes in all study groups, although the most distinct difference was observed in the high-dose MgB group. Matrix metalloproteinase-1 plays a role in collagen catabolism, MMP-3 cartilage degradation, and bone resorption [47]. An experimental study emphasized the importance of MMP-2 and MMP-9 in the hair cycle and indicated that it might be effective through the VEGF, IGF-1, and transforming growth factor- β (TGF- β) stimulation [48]. A similar study demonstrated that MMP-2 was expressed from all hair follicle structures, while MMP-9 was locally expressed in the hair follicle during different cycle phases [49]. This

suggests that MMPs may play a role in the hair cycle through different mechanisms.

The present study observed an increase in hydroxyproline levels and a decrease in elastase levels in all study groups. Hydroxyproline is synthesized from arginine and is found in collagen proteins as the significant extracellular component of the connective tissue [50]. It has been found to increase graft-versus-host disease, keloid, and vitiligo and decrease poor wound healing, and thus is believed to be a biochemical marker in wound healing [51]. In an in vitro study by Xu et al., a vessel wall was developed from stem cells found in the hair follicle, leading to a distinct increase in hydroxyproline content [52]. The obtained result suggested that the hair follicles induced by ASI and ASI + MgB may have affected the level of hydroxyproline. An increase was noted in the serum level of biotinidase in the MgB-supplement groups. Biotinidase is an essential enzyme in biotin synthesis and helps transfer biotin to the peripheral tissues [14, 53]. Additionally, the serum levels of arginine, biotin, Mg, Si, and Zn were found to have increased in all study groups.

This study observed that ACC2, PC, and PCC increased in all study groups, and arginine had no significant effect on ACC1 and MCC enzymes. Biotin-dependent carboxylases are involved in fatty acid, amino acid, carbohydrate metabolism, and many cellular processes. It has been reported that ACC2 downregulation increases fatty acid oxidation and decreases adiposity in mice, whereas ACC2 overexpression is associated with proinflammatory cytokine production in human kidney cells and can be reversed by inhibiting p38 MAP kinase. MCC is required for the catabolism of leucine and isovalerate in most organisms [54]. PC has an anaplerotic role in glucose, fatty acid, and amino acid biosynthesis. Tumor cells are PC-dependent for anaplerosis in the absence of glutamine, and cells with high PC activity resistant to inhibition of glutamine metabolism [54]. In addition, PC is important for insulin secretion from pancreatic β -cells in rats [55]. PCC is a mitochondrial enzyme that catalyzes the carboxylation of propionyl-CoA to methylmalonyl-CoA. Propionyl-CoA plays a role in the catabolism of cholesterol, fatty acids, and amino acids such as valine, methionine, isoleucine, threonine in most organisms. PCC dysfunction leads to a metabolic disorder resulting in morbidity and mortality [56].

Biotin, a coenzyme for mitochondrial carboxylases in hair follicles, helps improve the condition of epidermal cells by promoting the keratin formation process and differentiation of epidermal cells in hair and nails [57]. Moreover, animal studies have shown that MgB is a bioavailable form of biotin with superior absorption and greater uptake in the tissue than d-biotin [58]. However, the limitation of the present study should be acknowledged in a way that treatments did not contain an "MgB" group which would give an idea of the effects of MgB alone with low or high doses before

evaluating the combination of MgB and ASI. It would also be interesting to see a possible effect of MgB alone with different treatment doses. We suggest that more in-depth MgB studies are needed to confirm that Mg supplementation could be a potential curative for hair and nail growth.

Conclusion

As a result, ASI and MgB were effective in hair growth by stimulating IGF-1, FGF, KGF, HGF, VEGF, SIRT-1, Wnt, and β -catenin signaling pathways. It was also found that ASI did not affect nail growth, whereas the combination of MgB was effective when higher doses of biotin were used. More studies are needed to determine the optimal dose of biotin in MgB to promote nail growth.

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Data Availability The datasets used and/or analyzed during the study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval The study was approved by the Animal Ethics Committee of Firat University (126–2018/66) and performed following the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines, EEC Directive 2010/63/EU.

2. All authors confirm that our figures/tables are original and have not been published previously.

Consent for Publication Manuscript is approved by all the authors for publication.

Conflict of interest JK, SPO, and SS are employees of JDS Therapeutics, LLC (Harrison, NY, USA). Other authors have no other relevant

affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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