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Article

Growth of *Staphylococcus epidermidis* and *Escherichia coli* when exposed to anti-acne vitamin A

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SUMMARY

About 85% of people between the ages of 12 and 24 are affected by acne, most often beginning in early puberty, although acne can still be present into adulthood. A common anti-acne medication is retinol, aka vitamin A, which reduces inflammation and promotes skin cell division. Since acne is influenced by imbalances in the skin microbiome, we aimed to investigate how vitamin A affects this microbiome. To test the impact of vitamin A on the skin microbiome, we used the most common bacteria found on skin, *Staphylococcus epidermidis*, and measured its growth in liquid culture with different concentrations of vitamin A. We also tested *Escherichia coli*, a bacterium that is found in the gut, but not the skin, microbiome, against vitamin A exposure in identical conditions to *S. epidermidis*. We hypothesized that in the presence of vitamin A, *S. epidermidis* growth would decrease due to vitamin A's antimicrobial properties, while *E. coli* growth would not be affected due to its distinct gut environment. Contrary to our hypothesis, we found that *S. epidermidis* grew 2.6x better than *E. coli* at 0.025% vitamin A. Yet, E. coli grew better than *S. epidermidis* at higher concentrations of vitamin A. Our results suggest that vitamin A's stimulation of *S. epidermidis* growth can potentially relieve dysbiosis and lower inflammation in the skin by restoring microbiome balance, despite our expectation of reduced growth. We concluded that vitamin A's enhancement of *S. epidermidis* growth in the skin microbiome may improve acne treatment outcomes by promoting a balanced microbial environment, advancing our understanding of microbiome-based therapies.

INTRODUCTION

Acne is an inflammatory skin condition that affects over 85% of people between the ages of 12 and 24. It often starts in early adolescence and can last into adulthood (1). Acne has an adverse effect on both mental and physical self-esteem. Up to 30% of dermatologic conditions have a psychological comorbidity and several dermatological conditions, including acne, have a high suicide risk (2). The skin microbiome, an ecosystem composed of living biological organisms, plays a critical role in acne development due to its influence on skin health. Disruptions in this balance of the microbiome, known as dysbiosis, can lead to acne by promoting the overgrowth of certain bacteria, such as *Cutibacterium acnes,* a main trigger of acne which clogs pores and triggers inflammation (3). Although it is widely acknowledged that microorganisms may play a role in several skin illnesses, little is known about how exactly they contribute to the disease and how this links to genetic and environmental factors (4).

To combat acne, treatments often target the skin microbiome to restore balance and reduce inflammation caused by bacterial overgrowth. Anti-acne medications use acids, such as salicylic acid, to unclog pores and regulate the growth of acne-causing bacteria; however, there are side effects to their usage including skin discoloration and minor skin irritation (5). A commonly used alternative anti-acne medication is vitamin A, or retinol, which reduces inflammation and promotes skin cell division (6). Despite the common usage of vitamin A in medications, its effects on the skin microbiome are not yet determined. Although vitamin A is a well-established anti-acne treatment that plays a crucial role in the immune system such as reducing inflammation via IL-17 pathway modulation and promoting antimicrobial proteins like RELMα to combat acne-causing bacteria, its direct effects on bacterial cells in the skin microbiome remains incompletely understood (7, 8).

Staphyloccocus epidermidis, the most common bacteria found on the skin, was chosen as a representative bacterium to test the impact of vitamin A on the skin microbiome (9). A recent study seems to confirm the advantageous role of *S. epidermidis* in limiting *C. acnes* by producing antimicrobial substances which kill or inhibit the growth of microorganisms, which may reduce *C. acnes*-induced inflammation and promote skin microbiome balance, potentially mitigating acne severity (3). Acne treatments like vitamin A can be applied topically or taken orally, potentially affecting both the skin and gut microbiomes. We tested how different concentrations of vitamin A affect the growth of *S. epidermidis* to explore its effects on the skin microbiome and *Escherichia coli*, a common bacteria found in the gut, to explore its effects on the gut microbiome. The gut microbiome is a diverse ecosystem of microorganisms in the digestive tract that may influence systemic health and skin conditions (10). Previous research demonstrated that retinol-based products, at concentrations similar to those of tretinoin (another acne treatment), significantly improved acne-related skin conditions such as fine lines, skin tone, and pigmentation, suggesting vitamin A's efficacy in skin health (11).

Based on evidence that vitamin A possesses antimicrobial properties through the induction of antimicrobial peptides (AMPs) such as defensins and cathelicidins, we hypothesized that vitamin A supplementation will reduce *S. epidermidis* growth on the skin by enhancing AMP expression, which disrupts bacterial membrane integrity (8). In contrast, *E. coli*

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growth in the gut will remain unaffected due to the gut's distinct microenvironment, including pH and microbial competition, which may limit AMP activity (12). Building on these findings, we selected comparable weight/volume (w/v) concentrations of vitamin A to investigate whether its acne-reducing effects involve suppressing or enhancing *S. epidermidis* growth. We conducted the experiments with *E. coli* under the same conditions as *S. epidermidis* to compare responses across different microbiomes. We found that vitamin A increased *S. epidermidis* growth in liquid cultures, likely supporting its role in limiting *C. acnes* and alleviating dysbiosis, potentially enhancing acne treatment efficacy.

RESULTS

We measured the growth of *S. epidermidis* and *E. coli* after 23.5 hours incubation at 37°C in liquid cultures with vitamin A concentrations of 0%, 0.025%, 0.050%, and 0.075%. Bacterial growth was measured to assess the effect of vitamin A on these strains.

We tested *S. epidermidis* and *E. coli* growth following overnight incubations in liquid cultures with vitamin A concentrations of 0%, 0.025%, 0.050%, and 0.075% **(Figure 1).** We found that *S. epidermidis* growth increased compared to the 0% vitamin A control, with the highest average growth at 0.025% (OD_{595} = 0.728 ± 0.201) and the lowest at 0.050% (OD_{595} = 0.438 ± 0.006) (**Figure 1**). Average growth at 0% and 0.075% vitamin A were OD_{595} = 0.210 ± 0.041 and OD_{595} = 0.462 ± 0.021 respectively. For *E. coli*, the highest growth was observed at 0.075% (OD_{595} = 0.646 ± 0.008) and the lowest at 0.025% (OD_{595} = 0.385 ± 0.012) (**Figure 2**). Average growth at 0% and 0.050% were OD_{595} = 0.275 ± 0.032 and OD_{595} = 0.449 ± 0.027 respectively. One-way ANOVA and Tukey analysis indicated statistically significant differences in growth across concentrations for both bacteria ($p \leq 0.001$ for S. epidermidis, $p \leq 0.001$ for E. coli).

When comparing the growth of *S. epidermidis* and *E. coli* treated with different concentrations of vitamin A, *S. epidermidis* grew 2.7 times more than *E. coli* at 0.025% vitamin A, representing the greatest growth gap observed (**Figure 3**). At 0.050% vitamin A, S. *epidermidis* grew 1.006 times more than *E. coli*, which was the smallest growth gap observed. At 0.075% vitamin A, *S. epidermidis* grew 1.2 times more than *E. coli*.

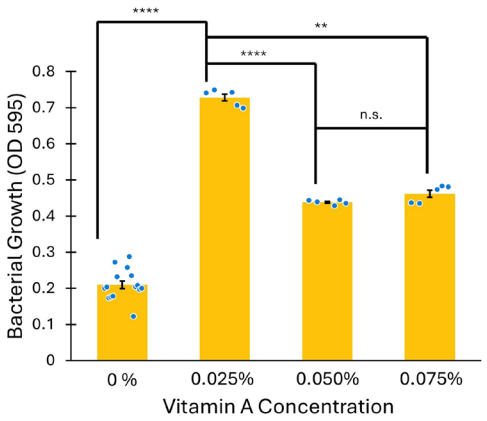

Figure 1: *S. epidermidis* growth in presence of various concentrations of vitamin A. *S. epidermidis* was grown in nutrient broth with vitamin A at final concentrations of 0%, 0.025%, 0.050%, or 0.075%. Yellow bars indicate the average OD595 for each condition, and individual data points are shown in blue circle markers. Error bars are calculated as standard error of the mean. One-way ANOVA and Tukey analysis were performed to determine statistical significance. Unpaired t-tests were used to determine p-values (****$p \leq 0.0001$, **$p \leq 0.001$, and n.s.: $p > 0.05$).

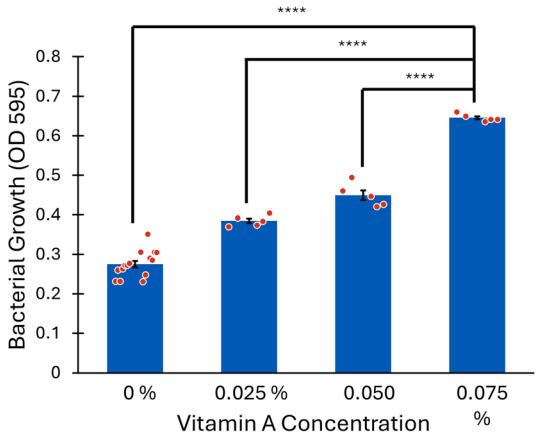

Figure 2: *E. coli* growth in presence of various concentrations of vitamin A. *E. coli* was grown in nutrient broth with vitamin A at final concentrations of 0%, 0.025%, 0.050%, or 0.075%. Blue bars indicate the average OD595 for each condition, and individual data points are shown in orange circle markers. Error bars are calculated as standard error of the mean. One-way ANOVA and Tukey analysis were performed to determine statistical significance. Unpaired t-tests were used to determine p-values (****$p \leq 0.0001$).

DISCUSSION

Contrary to our hypothesis that vitamin A would decrease *S. epidermidis* growth due to its antimicrobial properties, our findings indicate that *S. epidermidis* exhibited significantly higher growth in the presence of vitamin A compared to the control, with the most pronounced effect at lower concentrations (e.g., 4 times more growth at 0.025% vitamin A). This aligns with a study by Claudel *et al.* who suggested *S. epidermidis* mitigates acne by producing bacteriocins and competing with *C. acnes* for nutrients, thus reducing inflammation and restoring microbiome balance (3). The unexpected increase in *S. epidermidis* growth likely amplifies its antagonistic effects on *C. acnes*, supporting vitamin A's anti-acne properties beyond its known roles in cell turnover and immune modulation (6, 7). Work by Fournière *et al.* further supports that *S. epidermidis* acts as a sentinel in the skin microbiota, potentially enhanced by cosmetic ingredients like retinol (13). This suggests vitamin A may preferentially bolster beneficial bacteria, contributing to acne alleviation by fostering a balanced microbiome, despite our initial expectation of reduced growth.

Our hypothesis that vitamin A would decrease S. *epidermidis* growth due to its antimicrobial properties was based on a previous study that showed vitamin A induces antimicrobial peptides like RELMα, potentially inhibiting bacterial growth, including that of beneficial bacteria like *S. epidermidis* (8). Contrary to this expectation, our results showed that vitamin A significantly increased *S. epidermidis* growth in liquid cultures, with the most pronounced effect at 0.025% concentration (4 times more increase in optical density (OD) at 595 nm compared to the 0% control). This

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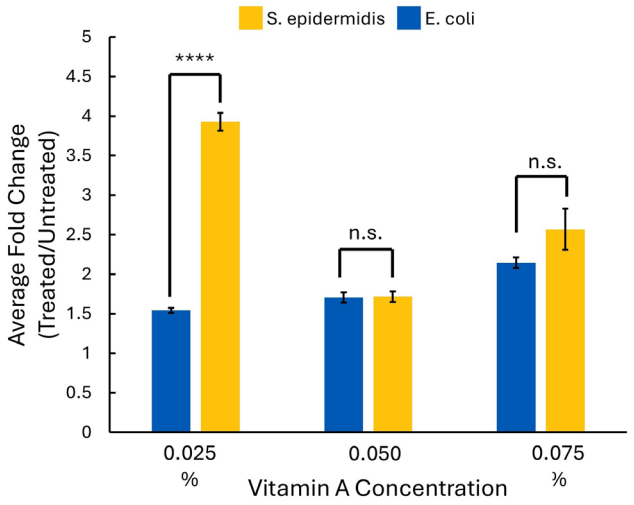

Figure 3: *S. epidermidis* and *E. coli* were grown in nutrient broth with vitamin A. The columns represent average fold change, which is the treated group's OD_{595} divided by the untreated group's OD_{595}. Each blue column indicates an average fold change for *E. coli*. Each yellow column indicates an average fold change for *S. epidermidis*. Error bars are calculated as standard error of the mean. Unpaired t-tests were used to determined p-values (****$p \leq 0.0001$ and n.s.: $p > 0.05$).

discrepancy may stem from concentration-dependent effects or differences between our liquid culture conditions and the skin environment, where antimicrobial peptides might interact differently with bacteria (14). Studies by Claudel et al. and Fournière et al. suggest that *S. epidermidis* mitigates acne by producing bacteriocins and competing with *C. acnes*, a pro-inflammatory bacterium that clogs pores and triggers inflammation (3, 13). The observed increase in *S. epidermidis* growth likely enhances its antagonistic effects on *C. acnes*, contributing to vitamin A's anti-acne properties by restoring skin microbiome homeostasis, beyond its established roles in cell turnover and immune modulation (6, 7). A previous study demonstrates support of retinol's efficacy in improving skin conditions, suggesting that the observed growth increase may translate to clinical benefits by enhancing *S. epidermidis's* protective role (11).

Unexpectedly, *E. coli* also showed increased growth with vitamin A, with fold changes rising with concentration (e.g., 2.2 times increase at 0.075% compared to the 0% control). This contrasts with our hypothesis that *E. coli* growth would remain unaffected due to its distinct gut environment (12). A previous study notes that *E. coli* infections in mice increase inflammatory cytokines (IL-6, IL-1β, and TNF-α), suggesting a robust immune response in the gut, though its relevance to skin health remains unclear (12). When comparing fold changes, *S. epidermidis* showed a greater growth increase than E. coli at 0.025% vitamin A (4 times vs. 1.5 times). The gap narrowed at 0.075% (2.5 times vs. 2.2 times), indicating that vitamin A's effects are more pronounced on skin-resident bacteria at lower doses, possibly due to *S. epidermidis's* adaptation to skin conditions (9). The increased *E. coli* growth raises concerns, as higher *E. coli* concentrations may worsen inflammation if vitamin A affects gut bacteria similarly in vivo, within the living organism (12). Since typical skincare products contain retinol at concentrations of 0.01% to 1%, our tested concentrations (0.025%–0.075%) are within this range, suggesting that excessive topical application could inadvertently influence systemic microbiomes, potentially worsening inflammation and acne (12).

The enhanced *S. epidermidis* growth aligns with Fournière et al., who note that *S. epidermidis*'s lipoteichoic acid inhibits skin inflammation during wound healing and pathologies (13). We showed, through OD measurements, that vitamin A promotes *S. epidermidis* growth, likely amplifying its role in reducing acne by suppressing *C. acnes* and restoring microbiome balance. This mechanism may complement vitamin A's anti-inflammatory effects via IL-17 pathway modulation, enhancing its efficacy as an acne treatment (7). However, our study did not test *C. acnes* due to experimental limitations, including the lack of access to standardized *C. acnes* cultures and the prioritization of *S. epidermidis* as the most abundant skin commensal (9). Commensal bacteria, which coexist with the host without causing harm and often provide benefits like pathogen suppression, are critical to skin health (4). Testing *C. acnes* and other commensal bacteria could clarify vitamin A's differential effects on pathogenic versus beneficial microbes.

To build on these findings, future studies should focus on optimizing vitamin A concentrations for topical use to maximize *S. epidermidis*'s beneficial effects while minimizing impacts on other bacteria. For instance, testing *C. acnes* and other commensal bacteria under similar conditions could elucidate vitamin A's effects on the broader skin microbiome (3, 15). Additionally, comparing topical versus oral vitamin A administration could determine the most effective delivery method for acne treatment. Previous studies have suggested that both routes influence systemic vitamin A levels, though topical application may better target skin microbiota (14, 16). A previous study compared topical retinol and tretinoin, but data on oral versus topical effects on the skin microbiome are limited (12). Furthermore, using a medium that mimics the skin environment, such as a skin-like biofilm model, could provide more accurate insights than liquid cultures, which do not fully replicate the skin microbiome's complexity (4).

Our study advances our understanding of vitamin A's role in acne treatment by demonstrating its unexpected promotion of *S. epidermidis* growth, potentially enhancing its protective effects against *C. acnes*. These findings contribute to the field of microbiome-based therapies by highlighting the complex interplay between vitamin A and skin bacteria, suggesting that its anti-acne effects involve not only immune modulation and cell turnover but also microbiome modulation. By identifying concentration-dependent effects, our work underscores the importance of optimizing vitamin A formulations to balance microbial growth and inflammation, paving the way for more targeted acne treatments.

MATERIALS AND METHODS

E. coli K12 (Carolina Biological Supply item # 124500) and *S. epidermidis* (Carolina Biological Supply item # 155557) cultures were separately inoculated onto agar plates and the plates were placed into an incubator at 37°C. After 23.5 hours, bacteria were transferred from the plate into a centrifuge test tube with 10 mL of nutrient broth liquid cultures (Carolina Biological Supply). This was the stock solution of bacteria that would be used for each experimental set. Stock solutions of vitamin A were made in concentrations of 0.25%, 0.5%, and

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0.75% w/v using powdered vitamin A (BulkSupplements.com), dissolved in water as the dilutant. The stock solutions were added to nutrient broth cultures of *S. epidermidis* or *E. coli* in a 1:10 ratio to result in vitamin A concentrations of 0.025%, 0.05%, and 0.075% (n=5 per vitamin A treated condition). Treated and untreated bacteria cultures then incubated at 37oC for 23.5 hours in a microbiological incubator without shaking.

After 23.5 hours of incubating in liquid culture, absorbance readings of the treated and untreated cultures were collected using a spectrophotometer at a wavelength of 595 nanometers. Nutrient broth reading was subtracted from the treated and untreated absorbance readings to determine the total amount of absorption by bacteria. The average of the treated tests for each concentration was divided by the average of the untreated tests for each concentration to find the average fold change of each vitamin A concentration.
ANOVA, Tukey analysis, and unpaired t-tests were performed in Excel to evaluate the significance of our findings. The significance level was set at $\alpha = 0.05$, and p-values less than 0.05 were considered statistically significant.

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REFERENCES

1. Lynn, Dana D., et al. "The Epidemiology of Acne Vulgaris in Late Adolescence." *Adolescent Health, Medicine and Therapeutics*, vol. 7, Feb. 2016, https://doi.org/10.2147/AHMT.S55832.
2. Gupta, Madhulika A., et al. "Suicidal Behaviors in the Dermatology Patient." *Clinics in Dermatology*, vol. 35, no. 3, May 2017, https://doi.org/10.1016/j.clindermatol.2017.01.006.
3. Claudel, Jean-Paul, et al. "Staphylococcus Epidermidis: A Potential New Player in the Physiopathology of Acne?" *Dermatology*, vol. 235, no. 4, July 2019, https://doi.org/10.1159/000499858.
4. Grice, Elizabeth A., and Julia A. Segre. "The Skin Microbiome." *Nature Reviews Microbiology*, vol. 9, no. 4, Apr. 2011, https://doi.org/10.1038/nrmicro2537.
5. Tan, A. U., et al. "A Review of Diagnosis and Treatment of Acne in Adult Female Patients." *International Journal of Women's Dermatology*, vol. 4, no. 2, June 2018, https://doi.org/10.1016/j.ijwd.2017.10.006.
6. Zasada, Malwina, and Elżbieta Budzisz. "Retinoids: Active Molecules Influencing Skin Structure Formation in Cosmetic and Dermatological Treatments." *Advances in Dermatology and Allergology*, vol. 36, no. 4, Aug. 2019, https://doi.org/10.5114/ada.2019.87443.
7. Huang, Zhe, et al. "Role of Vitamin A in the Immune System." *Journal of Clinical Medicine*, vol. 7, no. 9, Sept. 2018, https://doi.org/10.3390/jcm7090258.
8. Harris, Tamia A., et al. "Resistin-like Molecule α Provides Vitamin-A-Dependent Antimicrobial Protection in the Skin." *Cell Host & Microbe*, vol. 25, no. 6, June 2019, https://doi.org/10.1016/j.chom.2019.04.004.
9. Brown, Morgan M., and Alexander R. Horswill. "Staphylococcus Epidermidis—Skin Friend or Foe?" *PLoS Pathogens*, vol. 16, no. 11, Nov. 2020, https://doi.org/10.1371/journal.ppat.1009026.
10. Bowe, Whitney P., and Alan C. Logan. "Acne Vulgaris, Probiotics and the Gut-Brain-Skin Axis—Back to the Future?" *Gut Pathogens*, vol. 3, no. 1, 11 Jan. 2011, pp. 1–11. https://doi.org/10.1186/1757-4749-3-1.
11. Babcock, Michael, et al. "A Randomized, Double-Blind, Split-Face Study Comparing the Efficacy and Tolerability of Three Retinol-Based Products vs. Three Tretinoin-Based Products in Subjects with Moderate to Severe Facial Photodamage." *Journal of Drugs in Dermatology*, vol. 14, no. 1, Jan. 2015, PMID: 25607905.
12. Long, N., et al. "Inflammatory and Pathological Changes in Escherichia coli Infected Mice." *Heliyon*, vol. 8, no. 12, Dec. 2022, https://doi.org/10.1016/j.heliyon.2022.e12533.
13. Fournière, Mathilde, et al. "Staphylococcus Epidermidis and Cutibacterium Acnes: Two Major Sentinels of Skin Microbiota and the Influence of Cosmetics." *Microorganisms*, vol. 8, no. 11, Nov. 2020, https://doi.org/10.3390/microorganisms8111752.
14. Nohynek, G. J., et al. "Repeated Topical Treatment, in Contrast to Single Oral Doses, with Vitamin A-Containing Preparations Does Not Affect Plasma Concentrations of Retinol, Retinyl Esters or Retinoic Acids in Female Subjects of Child-Bearing Age." *Toxicology Letters*, vol. 163, no. 1, May 2006, https://doi.org/10.1016/j.toxlet.2005.09.029.
15. Bhatia, Ajay, et al. "Propionibacterium Acnes and Chronic Diseases." *The Infectious Etiology of Chronic Diseases: Defining the Relationship, Enhancing the Research, and Mitigating the Effects: Workshop Summary*, National Academies Press, 2016, www.ncbi.nlm.nih.gov/books/NBK83685.
16. Olson, Joshua M., et al. "Vitamin A Toxicity." *StatPearls*, StatPearls Publishing, Sept. 2023, www.ncbi.nlm.nih.gov/books/NBK532916.