Anti-Erythema Study

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Tradename: ACB Modified Pomegranate Enzyme PF

Code: 20440PF

CAS #: 84961-57-9 & 1686112-10-6 (or) 84775-94-0 (or) 9015-54-7

Test Request Form #: 13675

Lot #: N251001AS & N251104E

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Kayla Goodson*

Test Performed:
Anti-Erythema Study

Introduction

Skin erythema refers to the redness of skin and is a result of dilation and irritation of superficial capillaries. The appearance of a red hue on the skin is due to the augmented flow of blood through capillaries. Often, hyperpigmentation of the skin is undesirable as it is vibrant in color and can appear visually like a rash. Reducing skin redness and irritation is vital to the appearance of healthy skin. Enzymes are frequently incorporated into topical formulations to help mitigate redness and irritation due to their ability to act as biological catalysts that modulate specific biochemical pathways involved in inflammatory responses. However, when enzymes are used in solubilized form, they may be susceptible to denaturation, potentially resulting in reduced activity over time or under stress conditions such as elevated temperature. Therefore, careful evaluation and monitoring of enzymatic activity are essential to confirm product functionality and to understand the extent of biological efficacy within a formulation.

Accordingly, an Anti-Erythema Study was conducted to evaluate the immediate and short-term redness and irritation reducing properties of **ACB Modified Pomegranate Enzyme PF**. Bromelain and Papain, proteases with known erythema reducing activity, were also tested as comparative materials.

Study Principle

Surgical tape is applied to the skin to induce irritation and redness on the skin. After tape removal, erythema measurements are made by placing a probe on the skin of preidentified test sites. The control and test material were applied to the skin test sites once skin redness was induced, and after a given amount of time erythema measurements were recorded again. To understand changes in enzymatic activity over time, samples from time of manufacture (Lot #: N362204E), one month stability (Lot #: N251001A), and one-month heat-treated stability (Lot # N251001A) were used for test materials.

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Table 1. Enzyme Test Materials with For Each Production Variable

1.0% Bromelain in Base Lotion	Time of Manufacture
	One Month Stability
	One Month Heat-Treated Stability
1.0% Papain in Base Lotion	Time of Manufacture
	One Month Stability
	One Month Heat-Treated Stability
10.0% **ACB Modified Pomegranate Enzyme PF** in Base Lotion	Time of Manufacture
	One Month Stability
	One Month Heat-Treated Stability

Materials

A. **Equipment:** DermaLab Skin Combo (Colori Probe)
B. **Products:** Base Lotion (Cetaphil® Moisturizing Cream for All Skin Types); 3M™ Medipore™ H Soft Cloth Surgical Tape; Bromelain (comparative material); Papain (comparative material)
C. **Software:** Excel Analysis ToolPak (Microsoft)

Methods

Fifteen volunteers between the ages of 19 and 27, who were known to be free of any skin pathologies with Fitzpatrick skin types I to III, participated in this study (Table 2).

Table 2. The Fitzpatrick Classification of Skin Types Chart[1]

Fitzpatrick Skin Type Descriptions*	
Skin Type	**Description**
I	Always burns, never tans
II	Burns easily, tans minimally
III	Burns moderately, tans to light brown
IV	Burns minimally, tans to moderate brown
V	Rarely burns, tans to dark
VI	Never burns, least sensitive to changes
*Adapted from The Surgeon General's Call to Action to Prevent Skin Cancer	

Each production variable had its own set of five participants who were randomly selected for the respective variable. Four assigned test sites were identified on the volar forearm of participants. The surgical tape was applied to the test sites for two minutes before being removed to induce skin redness. Immediately after the surgical tape removal, erythema measurements were recorded, and the test materials were applied. The skin test site conditions and treatments are described below (Table 3). After 15 minutes of product application, erythema measurements were recorded again. The Base Lotion utilized in this study was Cetaphil® Moisturizing Cream for All Skin Types.

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Table 3. Descriptions of the Conditions and Treatments for each Skin Test Site for Each Production Variable

Skin Test Site	Condition	Treatment / Test Article Application Description	Lotion pH
1	Base Lotion	Base Lotion	5.4
2	1.0% Bromelain	1.0% Bromelain in Base Lotion	5.4
3	1.0% Papain	1.0% Papain in Base Lotion	5.3
4	10.0% **ACB Modified Pomegranate Enzyme PF**	10.0% **ACB Modified Pomegranate Enzyme PF** in Base Lotion	5.3

Data is displayed as averages from all participants and was analyzed using T-tests with statistical significance accepted at $p \leq 0.05$. The percent change in erythema was calculated for each test site at every timepoint relative to Baseline values, using the following equation:

$$Percent\ Change\ (\%) = \frac{Skin\ Erythema_{15\ Minutes\ After\ Application} - Skin\ Eryhtema_{Immediately\ After\ Tape\ Removal}}{Skin\ Erythema_{Immediately\ After\ Tape\ Removal}} \times 100$$

Results

The data obtained from this study met criteria for a valid study as the Base Lotion and Comparative Materials performed as anticipated for each production variable. For all production variable evaluated, 10.0% ACB Modified Pomegranate Enzyme PF demonstrated superior erythema-reducing effects compared to 1.0% Bromelain and 1.0% Papain, with the greatest reduction observed in the one-month heat-treated stability samples.

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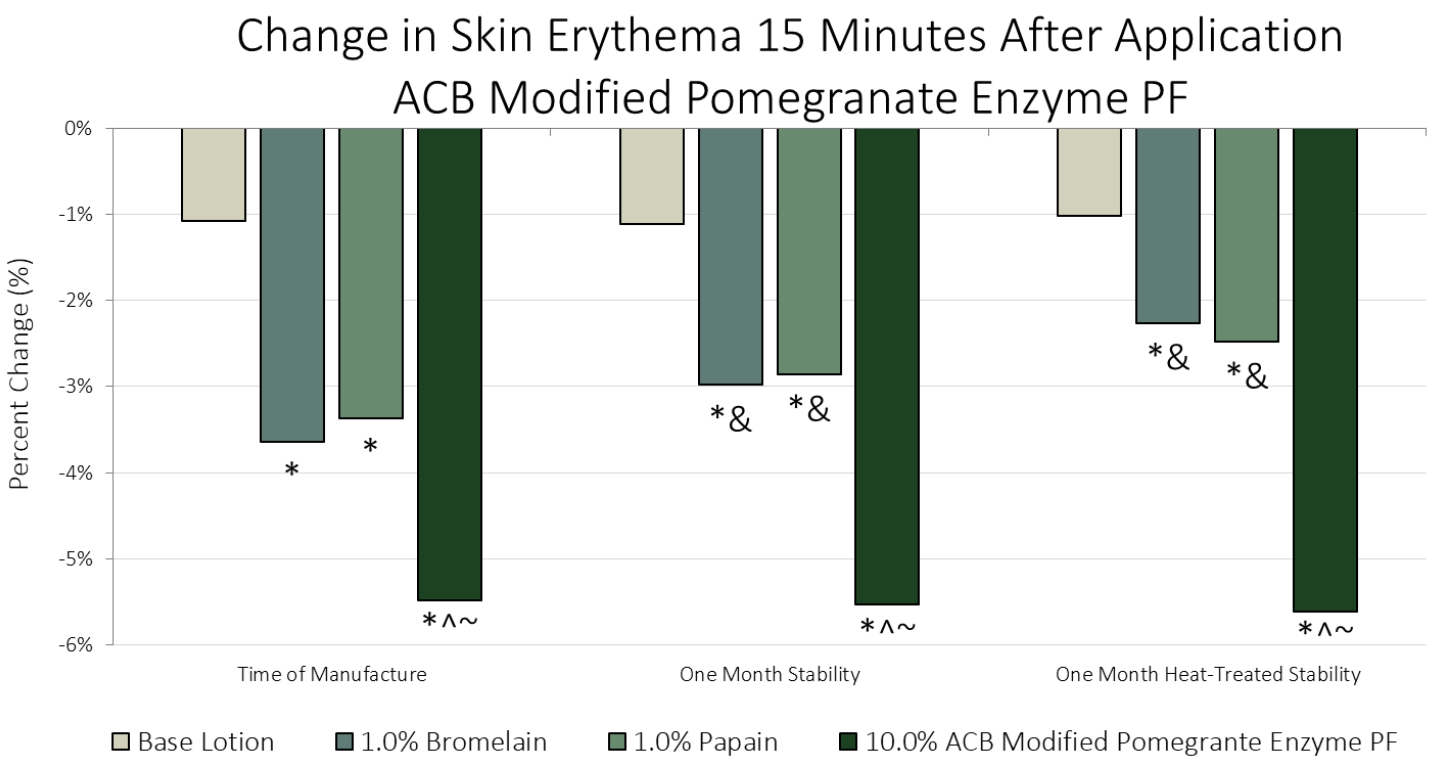

Figure 1. Percent Change in Skin Erythema 15 Minutes After Product Application for Each Production Variable. * indicates significance ($p \leq 0.05$) compared to Base Lotion. ^ indicates significance ($p \leq 0.05$) compared to 1.0% Bromelain. ~ indicates significance ($p \leq 0.05$) compared to 1.0% Papain. & indicates significance ($p \leq 0.05$) within condition compared to time of manufacture and one month stability production variables.

Table 4. Results from T-test Analysis of Skin Erythema Levels for Each Production Variable. * indicates significance ($p \leq 0.05$) compared to Base Lotion. ^ indicates significance ($p \leq 0.05$) compared to 1.0% Bromelain. ~ indicates significance ($p \leq 0.05$) compared to 1.0% Papain.

	Base Lotion vs 1.0% Bromelain	Base Lotion Vs 1.0% Papain	Base Lotion vs 10.0% **ACB Modified Pomegranate Enzyme PF**	1.0% Bromelain vs 1.0% Papain	1.0% Bromelain vs 10.0% **ACB Modified Pomegranate Enzyme PF**	1.0% Papain vs 10.0% **ACB Modified Pomegranate Enzyme PF**
Time of Manufacture	0.041*	0.034*	0.034*	0.087	0.024^	0.039~
One Month Stability	0.045*	0.039*	0.031*	0.066	0.034^	0.041~
One Month Heat-Treated Stability	0.042*	0.031*	0.025*	0.079	0.028^	0.048~

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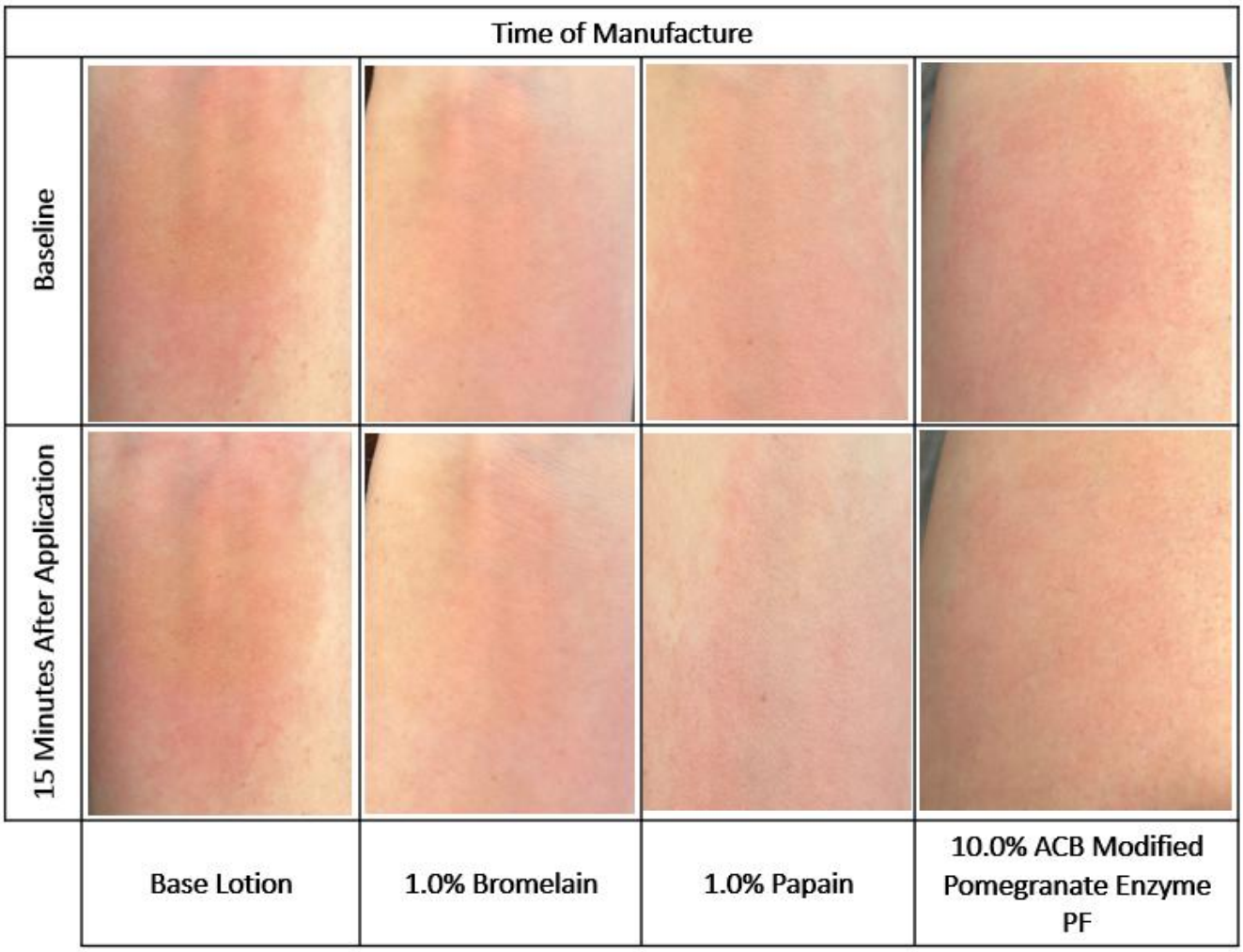

Image 1. Participant Images of Each Test Patch at Baseline (Top) and 15 Minutes After Application (Bottom) for Time of Manufacture Results.

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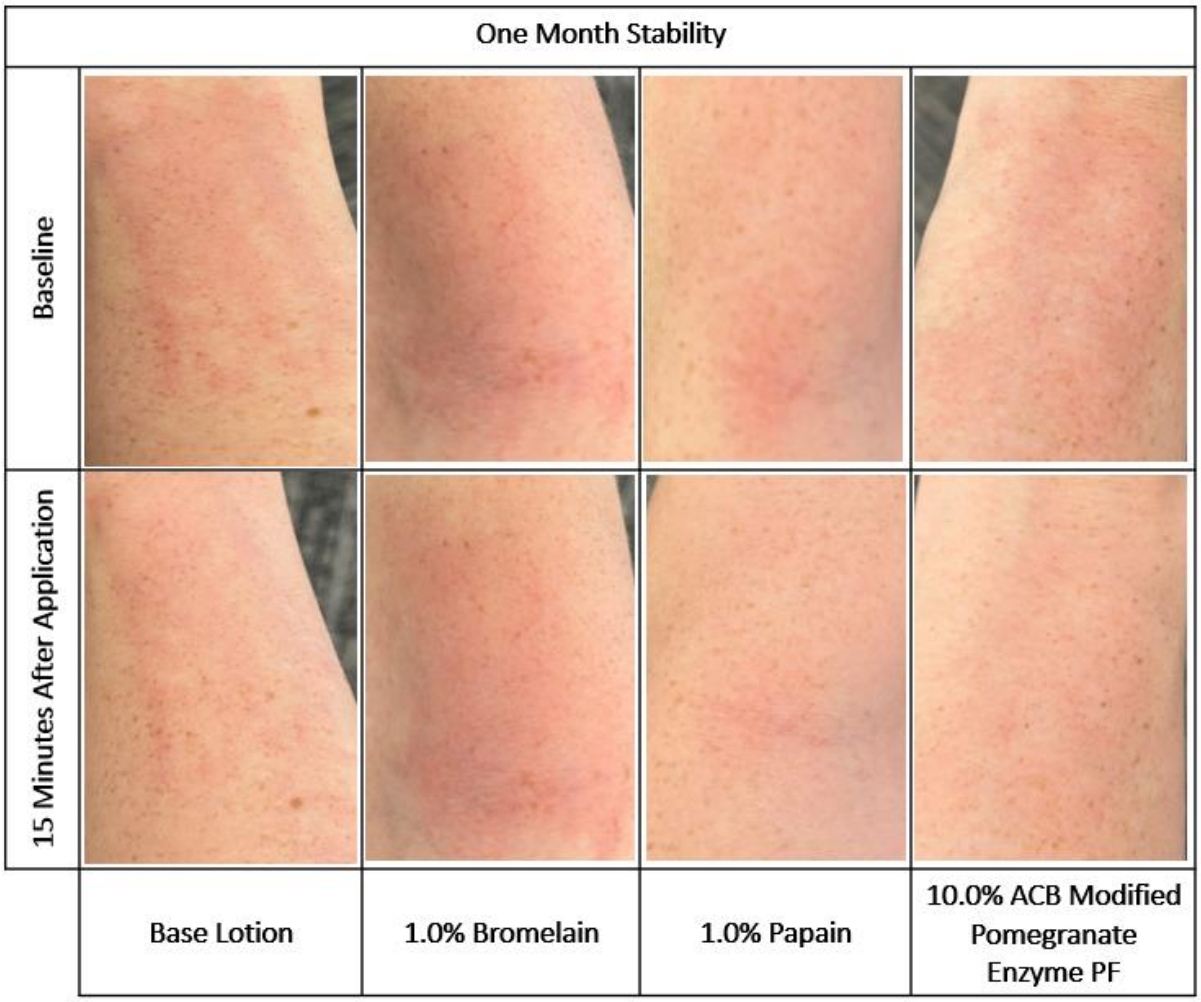

Image 2. Participant Images of Each Test Patch at Baseline (Top) and 15 Minutes After Application (Bottom) for One Month Stability Results.

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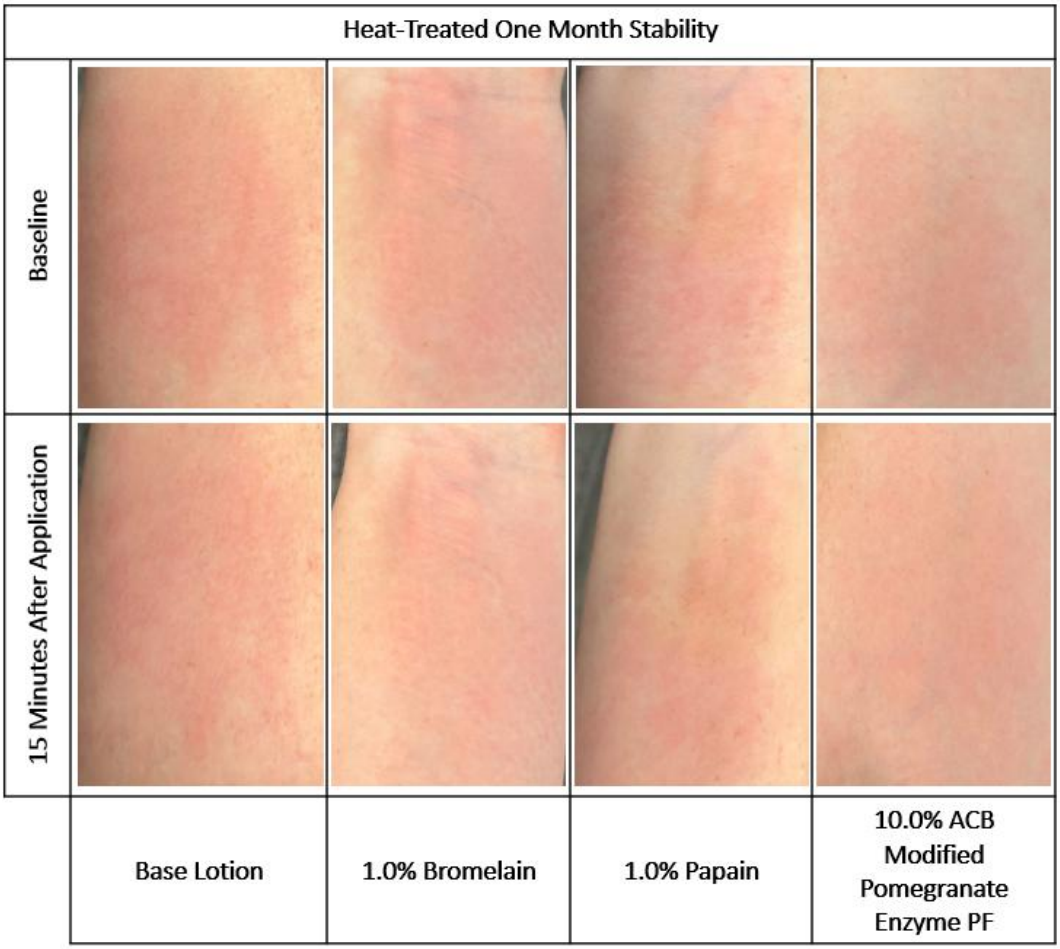

Image 3. Participant Images of Each Test Patch at Baseline (Top) and 15 Minutes After Application (Bottom) for One Month Heat-Treated Stability Results.

Discussion

The ability of **ACB Modified Pomegranate Enzyme PF** to reduce redness and irritation on the skin was assessed via erythema measurements after tape stripping across multiple production variables. As shown in Figure 1, the Base Lotion test site for each production variable did not exhibit significant changes in erythema. However, 1.0% Bromelain and 1.0% Papain produced significant reductions in skin erythema relative to Baseline measurements after tape stripping across each production variable (Figure 1; Table 4). Furthermore, 1.0% Bromelain and 1.0% Papain significantly reduced erythema after application after one month stability and one month heat-treated stability, compared to time of manufacture production variables within condition (Figure 1). Notably, 10.0% **ACB Modified Pomegranate Enzyme PF** exhibited significantly greater reductions in skin erythema compared to the Base Lotion and both protease comparative materials for each production variable (Figure 1; Table 4). Examining images of the skin test sites at Baseline and 15 minutes after application, 10.0% **ACB modified Pomegranate Enzyme PF** visually outperformed the Base Lotion and both protease comparative materials in terms of reducing skin erythema in each production variable (Images 1, 2, 3). Overall, 10.0% **ACB Pomegranate Enzyme PF** consistently elicited the highest degree of erythema reduction, when compared to 1.0% Bromelain and 1.0% Papain, indicating superior enzymatic activity relative to both proteases over time and under prolonged exposure to elevated temperatures (Figure 1). In contrast, the time of manufacture production variable resulted in the largest reduction in skin erythema after application, suggesting enzymatic activity is maximal at the time of manufacture and progressively declines with extended exposure to elevated temperatures (Figure 1).

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These results indicate **ACB Modified Pomegranate Enzyme PF** reduces erythema levels across different production variables without denaturing enzymatic activity when added to personal care applications at recommended use levels. Collectively, **ACB Modified Pomegranate Enzyme PF** demonstrates anti-redness and anti-irritation properties which improves the skin's protective barrier function and contributes to the appearance of healthier looking skin.

References

1. Sharma AN, Patel BC. Laser Fitzpatrick Skin Type Recommendations. [Updated 2022 Mar 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557626/