

# Analysis of the dietary fiber, vitamin C and polyphenol content with seaweed (*Eucheuma cottonii*) flour substitution in cookies

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Received April 8, 2025; Accepted March 24, 2026

DOI: 10.3892/ijfn.2026.55

**Abstract.** Seaweed (*Eucheuma cottonii*) is one of the Indonesian local foods. Seaweed contains high levels of dietary fiber, vitamin C and polyphenols, which are beneficial for patients with coronary heart disease. Seaweed can be made into flour and processed into cookies. The present study aimed to determine the effects of seaweed (*Eucheuma cottonii*) flour substitution on the dietary fiber, vitamin C and polyphenol content in cookies for patients with coronary heart disease. The present experimental study used three treatment groups with one control group. The present experimental study prepared three different variations of cookies and one cookie formulation used as the control, as follows: i) Cookies A (the control): The cookies were prepared from 100% wheat flour (without seaweed flour substitution); ii) cookies B were prepared from the combination of 80% wheat flour and 20% seaweed flour; iii) cookies C were prepared from the combination of 70% wheat flour and 30% seaweed flour; iv) and cookies D were prepared from the combination of 60% wheat flour and 40% seaweed flour. The dietary fiber, vitamin C and polyphenol contents were then obtained. The dietary fiber data were analyzed using the Kruskal-Wallis test followed by the Bonferroni test as a post hoc test. The vitamin C and polyphenol data were analyzed using one-way ANOVA followed by the Games-Howell Test. There was a significant difference within the groups with various compositions of seaweed powder substitution in terms of the dietary fiber ( $P=0.003$ ), vitamin C ( $P<0.001$ ), and polyphenol ( $P<0.001$ ) contents. The highest content of dietary fiber, vitamin C and polyphenol was found in cookies D. On the whole, the present study demonstrates that seaweed (*Eucheuma cottonii*) flour substitution affects the dietary fiber, vitamin C and polyphenol content in

cookies, and this is beneficial for patients with coronary heart disease.

## Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide, with 80% of cases occurring in low- and middle-income countries (1). Indonesia was the 6th highest country in the world for CVD deaths in 2019, with an estimated 375,479 deaths (2). One of the factors causing coronary heart disease is the pattern of fiber consumption, which is still low. Dietary factors play a crucial role in the development and progression of CVD. Individuals consuming the highest amounts of dietary fiber intake can significantly reduce their incidence and mortality from CVD. Dietary fiber can be divided into the soluble and insoluble types. Soluble dietary fiber is derived from grains, legumes, nuts, fruits and vegetables (3-5).

In addition to a low fiber intake, the low consumption of vitamin C also causes coronary heart disease. Vitamin C does not exert its antioxidative function only by direct interaction with reactive oxygen species, but also via the maintenance of the redox regulation by increasing levels of other cellular radical scavengers (6). Vitamin C inhibits the oxidation of low-density lipoprotein, thereby reducing atherosclerosis. Vitamin C deficiency is associated with a higher risk of mortality from CVD; vitamin C may slightly improve endothelial function and lipid profiles, particularly in those with low plasma levels of vitamin C (7). Vitamin C, also known as ascorbic acid, is an essential nutrient that humans cannot synthesize, rendering its intake crucial for health (8). One of the foods deemed to have a high vitamin C content is seaweed (9).

The habitual consumption of seaweed may provide a nutritionally rich addition to the diet. Seaweed is high in fiber and contains numerous other potentially bioactive compounds. The rich mineral and trace element content of seaweed compared to terrestrial foods can negatively affect its organoleptic characteristics. However, it has been shown to be acceptable to consumers when baked into breads (9).

Seaweed can be dried and processed into seaweed flour to be used for longer period of time. Once it becomes seaweed flour, it can be used in other food ingredients. Cookies are a food that are much loved by the public, particularly by children and the elderly due to their soft texture, and various flavors

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**Key words:** dietary fiber, polyphenol, seaweed (*Eucheuma cottonii*), vitamin C

and shapes. Seaweed powder has been substituted for wheat flour in varying amounts to fortify the nutritional content of cookies (10). The present study aimed to determine the effects of seaweed (*Eucheuma cottonii*) powder substitution on the dietary fiber, vitamin C and polyphenol content in cookies. These cookies may prove to be beneficial for patients with coronary heart disease.

## Materials and methods

**Study design and materials.** The present experimental study used three treatment groups with one control group design. It was conducted at the Dietetic and Culinary Laboratory, Universitas Respati Yogyakarta, Yogyakarta, Indonesia. Seaweed drying was carried out traditionally in Waingapu, East Sumba, Indonesia using sunlight for 6 days, with a drying time of 6 h each day. The seaweed flouring process was carried out at the Gadjah Mada University Food and Agricultural Technology Laboratory. Analysis for dietary fiber, polyphenols and vitamin C levels were conducted at the Chem-Mix Pratama Laboratory in Yogyakarta.

The present study used seaweed (*Eucheuma cottonii*) flour to make cookies with various substitutions. The present experimental study prepared three different variations of cookies and one cookie formulation used as the control, as follows: i) Cookies A (the control): The cookies were prepared from 100% wheat flour (without seaweed flour substitution); ii) cookies B were prepared from the combination of 80% wheat flour and 20% seaweed flour; iii) cookies C were prepared from the combination of 70% wheat flour and 30% seaweed flour; iv) and cookies D were prepared from the combination of 60% wheat flour and 40% seaweed flour. The ingredients used in making cookies included seaweed flour, wheat flour, butter, eggs, skim milk, salt, powdered sugar and baking powder. Cookies for each variation of the mixing composition were made twice in repetition, with two experimental units each. The cookie-making process began with mixing flour, skim milk, eggs, powdered sugar, butter, baking powder and salt in a bowl. Once all the ingredients were combined, the following step was to stir using a mixer for ~5 min until all the dough was combined. Seaweed flour was added to the dough and the dough was ready to be molded. The molded dough was then baked in an oven at a temperature of 130-150°C for 30 min. Once cooked, the cookies were removed from the oven.

The dependent variables analyzed include the content of dietary fiber, vitamin C and polyphenols. Researchers analyzed these contents at the Chem-mix Pratama Laboratory in Bantul, Indonesia. They measured dietary fiber using the multi-enzyme method, determined vitamin C through titration, and assessed polyphenols using spectrophotometry. The analysis followed the procedures in force at the Chem-Mix Pratama Laboratory in Yogyakarta. The procedure for analyzing the dietary fiber content was as follows: A total of 0.5 g of sample was placed into an Erlenmeyer flask, followed by the addition of 50 ml pH 7 phosphate buffer. Subsequently, 0.1 ml alpha amylase enzyme was added to the Erlenmeyer flask containing the sample and heated in a water bath at 100°C for 30 min, stirring occasionally. The sample was then removed and cooled. A total of 20 ml of distilled water and 5 ml of 1 N HCl were then added. Following this, 1 ml of 1% pepsin enzyme was

added to the Erlenmeyer flask containing the sample and heated in a water bath for 30 min. The Erlenmeyer flask was then removed, and 5 ml of 1 N NaOH were then added, followed by the addition of 0.1 ml  $\beta$ -amylase enzyme. The Erlenmeyer flask was then covered and incubated in a water bath for 1 h. The solution was filtered using a constant-weight filter paper of known weight. The sample was then washed with 2x10 ml ethanol and 2x10 ml acetone. The sample was oven-dried at 105°C overnight, cooled in a desiccator, and then weighed to determine the insoluble dietary fiber content. The filtrate was adjusted to a volume of 100 and 400 ml warm 95% ethanol were added. The filtrate was allowed to settle for 1 h. The filtrate was then filtered through ash-free filter paper and washed with 2x10 ml ethanol and 2x10 ml acetone. The filtrate was oven-dried overnight at 105°C, then placed in a desiccator, and weighed to determine the soluble dietary fiber content. The total dietary fiber content=insoluble dietary fiber content + soluble dietary fiber content.

The procedure for analyzing the polyphenol content was as follows: A total of 5 g of the ground sample was weighed and transferred to a 100 ml Erlenmeyer flask. The sample was diluted with distilled water to a volume of 100 ml. The solution was then filtered/centrifuged until a clear solution/filtrate was obtained. A total of 1 ml of the clear solution/filtrate was taken, transferred to a test tube, and 0.5 ml of Denis' Follin (1:1 Follin) was added; 1 ml saturated  $\text{Na}_2\text{CO}_3$  solution was then added to the filtrate and allowed to stand for 10 min. Subsequently, 10 ml distilled water were added to the solution and vortexed until homogeneous. The sample absorbance was read using a spectrophotometer at a wavelength of 730 nm. The data obtained were recorded and then calculated using a phenol standard curve.

The procedure for the analysis of the vitamin C content was as follows: A total of 200-300 g sample was weighed and ground in a blender until a slurry was obtained. Subsequently, 10-30 g of slurry were placed in a 100-ml volumetric flask, distilled water was added to the mark, and the solution was then filtered using a Gooch crucible or centrifuged to separate the filtrate. This was followed by pipetting 5-25 ml of the filtrate into a 125-ml Erlenmeyer flask; 2 ml of 1% starch solution (soluble starch) were then added, and 20 ml distilled water were added if deemed necessary. The filtrate was titrated with 0.01 N standard iodine. The vitamin C content was calculated as follows: 1 ml of 0.01 N iodine=0.88 mg of ascorbic acid.

**Statistical analysis.** Data were analyzed using SPSS version 16 software. The dietary fiber data were analyzed using the Kruskal-Wallis and followed by the Bonferroni test as a post hoc test. The Kruskal-Wallis test was used as the polyphenol data was not normally distributed, then followed by the Bonferroni test as a post hoc test as the result of the Kruskal-Wallis Test was significant. The vitamin C and polyphenol data were analyzed using one-way ANOVA followed by the Games-Howell Test. One-way ANOVA was used as the vitamin C and polyphenol data were normally distributed, followed by the Games-Howell as post hoc test as the result of one-way ANOVA was significant and the test of homogeneity revealed that the variances of the four groups were not the same. A value of  $P < 0.05$  was considered to indicate a statistically significant difference.

**Results**

*Production of seaweed flour.* Seaweed flour is brown and has black spots. This is due to the fact that the seaweed used is seaweed which is dried in the sun; thus, it has a brownish color. Seaweed flour was prepared by soaking seaweed (*Eucheuma cottonii*) in fresh water for 10 h to remove impurities, then rinsing with running water and draining. It was then soaked in 5% lime solution which was made by the authors for 5 h and drained again, and dried for 24 h (11). It was then milled and sieved using an 80-mesh sieve.

The present study analyzed the differences in dietary fiber, vitamin C and polyphenol content in cookies A as the control, and cookies B, cookies C and cookies D with different compositions of wheat flour and seaweed flour.

*Effect of the variations in seaweed flour substitution on the dietary fiber content in cookies.* As demonstrated in Table I, cookies D had the highest food fiber content, namely 11.466%, while cookies A had the lowest food fiber content with a value of 5.759%. Analysis using the Kruskal-Wallis yielded a P-value of 0.003 (P<0.05), indicating that there were differences in the dietary fiber content of the cookies prepared with variations in seaweed flour substitution. Analysis was continued using the Bonferroni test to determine the differences between each group. The results revealed significant differences (P<0.05) between cookies A and B, cookies A and C, cookies A and D, cookies B and C, cookies B and D, while there were no significant differences (P>0.05) between cookies C and D. These results indicated that the seaweed (*Eucheuma cottonii*) flour substitution significantly affected the dietary fiber content in the cookies.

*Effect of the variations in seaweed flour substitution on the vitamin C content in cookies.* The results of the analysis of the effects of seaweed (*Eucheuma cottonii*) flour substitution on the vitamin C content in the cookies are demonstrated in Table II. It was found that cookies D had the highest vitamin C content (44.176%), while cookies A had the lowest vitamin C content (19.115%) (Table II).

The results of one-way ANOVA revealed a P<0.001, indicating that there were differences in the vitamin C content of the cookies with variations in seaweed flour substitution. The analysis was continued using the Games-Howell as a post hoc test to determine the differences between each group. The results revealed that there was a significant difference (P<0.05) between cookies A and B, cookies A and C, cookies A and D, and cookies B and D, while no significant differences were found between cookies B and C, and cookies C and D (P>0.05).

*Effect of the variations in seaweed flour substitution on the polyphenol content in cookies.* As demonstrated in Table III, cookies D had the highest polyphenol content, namely 0.356% or 35.6 mg per 100 g of cookies, while cookies A had the lowest polyphenol content with a value of 0.175% or 17.5 mg per 100 g of cookies. The results of one-way ANOVA revealed a P<0.001, indicating that there were differences in the polyphenol content of cookies with variations in seaweed flour substitution. The analysis was continued using the Games-Howell post hoc test to determine differences between

Table I. Effects of seaweed (*Eucheuma cottonii*) flour substitutions on the dietary fiber content in the cookies.

Cookies	Unit	Mean ± SD	P-value
A	%	5.759±0.710 <sup>a</sup>	0.003
B	%	8.923±0.585 <sup>b</sup>	
C	%	10.687±0.183 <sup>c</sup>	
D	%	11.466±0.354 <sup>c</sup>	

The cookie variations were as follows: i) Cookies A (the control); The cookies were prepared from 100% wheat flour (without seaweed flour substitution); ii) cookies B were prepared from the combination of 80% wheat flour and 20% seaweed flour; iii) cookies C were prepared from the combination of 70% wheat flour and 30% seaweed flour; iv) and cookies D were prepared from the combination of 60% wheat flour and 40% seaweed flour. Data were analyzed using the Kruskal-Wallis test followed by the Bonferroni test as a post hoc test. <sup>a-c</sup>P<0.05, indicates significant differences between the groups with a different superscript letter. The results of the statistical analysis between the different groups revealed the following: P<0.001, cookies A vs. cookies B; P<0.001, cookies A vs. cookies C; P<0.001, cookies A vs. cookies C; P<0.001, cookies A vs. cookies D; P=0.002, cookies B vs. cookies C; P<0.001, cookies B vs. cookies D; P=0.292, cookies C vs. cookies D.

Table II. Effects of seaweed (*Eucheuma cottonii*) flour substitutions on the vitamin C content in the cookies.

Cookies	Unit	Mean ± SD	P-value
A	mg/100 g	19.115±1.549 <sup>a</sup>	<0.001
B	mg/100 g	26.363±1.442 <sup>b</sup>	
C	mg/100 g	31.791±4.677 <sup>b</sup>	
D	mg/100 g	44.176±6.200 <sup>c</sup>	

The cookie variations were as follows: i) Cookies A (the control); The cookies were prepared from 100% wheat flour (without seaweed flour substitution); ii) cookies B were prepared from the combination of 80% wheat flour and 20% seaweed flour; iii) cookies C were prepared from the combination of 70% wheat flour and 30% seaweed flour; iv) and cookies D were prepared from the combination of 60% wheat flour and 40% seaweed flour. Data were analyzed using one-way ANOVA followed by the Games-Howell test as a post hoc test. <sup>a-c</sup>P<0.05, indicates significant differences between the groups with a different superscript letter. The results of the statistical analysis between the different groups revealed the following: P=0.002, cookies A vs. cookies B; P=0.028, cookies A vs. cookies C; P=0.009, cookies A vs. cookies D.

the groups. The results revealed that there were significant differences (P<0.05) between cookies A and B, cookies A and C, cookies A and D, cookies B and C, cookies B and D, and cookies C and D.

**Discussion**

Effect of the variations in seaweed flour substitution on the dietary fiber content in cookies. Based on the results of the

Table III. Effects of seaweed (*Eucheuma cottonii*) flour substitutions on the polyphenol content in the cookies.

Cookies	Unit	Mean $\pm$ SD	P-value
A	%	0.175 $\pm$ 0.006 <sup>a</sup>	<0.001
B	%	0.218 $\pm$ 0.011 <sup>b</sup>	
C	%	0.274 $\pm$ 0.004 <sup>c</sup>	
D	%	0.356 $\pm$ 0.0116 <sup>d</sup>	

The cookie variations were as follows: i) Cookies A (the control): The cookies were prepared from 100% wheat flour (without seaweed flour substitution); ii) cookies B were prepared from the combination of 80% wheat flour and 20% seaweed flour; iii) cookies C were prepared from the combination of 70% wheat flour and 30% seaweed flour; iv) and cookies D were prepared from the combination of 60% wheat flour and 40% seaweed flour. Data were analyzed using one-way ANOVA followed by the Games-Howell test as a post hoc test. <sup>a-d</sup>P<0.05, indicates significant differences between the groups with a different superscript letter. The results of the statistical analysis between the different groups revealed the following: P=0.007, cookies A vs. cookies B; P<0.001, cookies A vs. cookies C; P<0.001, cookies A vs. cookies D; P=0.004, cookies B vs. cookies C; P<0.001, cookies B vs. cookies D; P=0.004, cookies C vs. cookies D.

present study, cookies D had the highest dietary fiber content among all variations. They contained 11.5% dietary fiber, relevant to 11.5 g of dietary fiber per 100 g of cookies. There were differences in the dietary fiber content of the cookies with variations in seaweed flour substitution (P<0.05): The greater the seaweed flour substitution, the greater the dietary fiber content in the cookies. Wet seaweed (*Eucheuma cottonii*) contains 11.6 g of fiber per 100 g, while in the form of flour, it contains 57.2 g of fiber per 100 g of seaweed flour (12). The results of the present study are in line with those of previous research related to mixing seaweed powder in the preparation of cookies; the higher the percentage of seaweed substitution, the higher the fiber content of the cookies (10).

Dietary fiber is the edible part of plants that is undigested and cannot be absorbed in the human small intestine. Dietary fiber can be fully or partially fermented in the large intestine. Dietary fiber can be divided into soluble and insoluble fiber based on its solubility in hot water (13).

Dietary fibers provide health benefits through three primary mechanisms in the body: Bulking, viscosity and fermentation. Soluble fibers slow down digestion and control nutrient absorption; thus, they can reduce the effects of postprandial blood glucose and lipid increases, which are risk factors for CVD. Both soluble and insoluble fiber can increase gastric distension and have an effect on gut hormones that elevate satiety, leading to reduced food intake, weight reduction and improved glucose metabolism. Dietary fiber increases the rate of bile acid excretion, which reduces total and low-density lipoprotein cholesterol. Once fermented in the colon, dietary fiber produces short-chain fatty acids that inhibit the synthesis of cholesterol. Dietary fiber has been shown to have an impact on plaque stability by decreasing pro-inflammatory cytokines known to affect plaque stability (14). There is evidence to indicate an inverse association between dietary fiber

intake and the risk of coronary heart disease, where greater consumption corresponds to a reduced risk (15). However, the physicochemical properties of various dietary fibers (such as solubility, viscosity, and fermentability) vary significantly depending on their origin and processing, and are important determinants of their functional characteristics and clinical utility (16). Therefore, it is important to consume dietary fiber from a variety of fiber sources in the daily diet.)

*Effect of the variations in seaweed flour substitution on the vitamin C content in cookies.* Based on the results obtained in the present study, cookies D (combination of 60% wheat flour and 40% seaweed flour) had the highest vitamin C content, 44.176 mg/100 g of cookies. There were differences in the vitamin C content of cookies with variations in seaweed flour substitution (P<0.05): the higher seaweed flour substitution, the higher the vitamin C content in the cookies.

The various forms of *Eucheuma cottonii* seaweed (fresh, dried, and flour) have different nutritional contents. Fresh *Eucheuma cottonii* contains 3.35% vitamin C, dried *Eucheuma cottonii* 5.76%, and floured *Eucheuma cottonii* contains 6.34% (17).

Vitamin C is a water-soluble vitamin. Seaweed is a source of vitamin C and E. Seaweed also contains antioxidants. The seaweed that was tested herein underwent a process of soaking and mixing with demineralized water, reducing its vitamin C content (18). Moreover, in the present study, cookies D had high levels of vitamin C. The higher the seaweed flour substitution, the higher the vitamin C content for the four cookie preparations.

Vitamin C plays a pivotal role in several processes involved in the pathogenesis of cardiovascular disease. Vitamin C and antioxidants play a crucial role in endothelial function. Patients with coronary heart disease can obtain adequate vitamin C from foods or dietary supplements (6,19). Modified foods, such as cookies D (combination of 60% wheat flour and 40% seaweed flour) prepared in the present study may be an alternative food for patients with coronary heart disease, as they have a high dietary fiber and vitamin C content.

*Effect of variations in seaweed flour substitution on the polyphenol content in cookies.* Cookies D in the present study had the highest polyphenol content, with 0.356% or 35.6 mg per 100 g of cookies. Variations in seaweed flour substitution varied the polyphenol content of cookies. The higher the seaweed flour substitution, the higher the polyphenol content in cookies.

As previously demonstrated, the total polyphenol content increases with the increasing temperature and extraction time in the extraction of seaweed (*Eucheuma cottonii*). The total polyphenol content was shown to be highest at 6 min of extraction time at 60°C and then decreased when the extraction temperature was 60°C (20).

The content of polyphenolic compounds decreases at an extraction temperature of 80°C. The total phenolic content is highest at an extraction temperature of 60-80°C for traditional extractions. An increase in temperature will increase the content of extracted polyphenolic compounds, which can damage or increase the hydrolysis bonds of some polyphenolic compounds and cause them to be easily extracted (21).

In the present study, the polyphenol content went through three stages of heating, which could reduce the polyphenol content in seaweed flour. The first heating was performed by drying at 26°C during the traditional drying process in Waingapu, East Sumba. The second heating is done using a cabinet dryer at 60°C for ~17 h in Yogyakarta, and the third heating involved heating the seaweed cookies for 30 min at a temperature of 130-150°C.

The longer the extraction process, the longer the contact between the solvent and the solute; thus, the process of dissolving polyphenolic compounds will continue. The longer extraction time can cause more exposure to oxygen. This occurs when the extraction process lasts >8 min at 60°C. This can increase the chances of oxidation of polyphenolic compounds so that the total content of extracted polyphenols decreases. The polyphenol content of seaweed cookies baked at 130-150°C for 30 min demonstrates a reduction in polyphenol levels. The results of the analysis of polyphenol levels for the four cookies with various flour substitutions indicated that there were significant differences between cookies A and cookies D. The polyphenol content of the cookies was 0.175-0.356; this proved that the cooking temperature used during the cookie-making process affected polyphenol levels.

Polyphenols are bioactive compounds found mostly in cereals, fruits, vegetables, dry legumes, chocolate and beverages, such as coffee and tea. They have beneficial effects on the vascular system by lowering blood pressure, improving endothelial function, increasing antioxidant defenses, inhibiting platelet aggregation and low-density lipoprotein oxidation and reducing inflammatory responses (22).

The present study had certain limitations which should be mentioned. One limitation was in the drying of the seaweed. Sun-drying alone does not sufficiently dehydrate seaweed; therefore, the drying process was conducted twice to achieve the desired level. The procedure combined sunlight exposure with cabinet drying to ensure optimal results. Another limitation pertains to the number of indicators tested. A very small number of indicators were tested. Three indicators were analyzed herein, namely dietary fiber, vitamin C and polyphenol content. Due to financial constraints related to the laboratory analysis, the authors did not analyze other indicators.

In conclusion, the present study demonstrates that variations in the substitution of seaweed (*Eucheuma cottonii*) flour in the manufacture of cookies affect the dietary fiber, vitamin C and the polyphenols content. Cookies D, which had the highest proportion of seaweed flour substitution among the other variations, also had the highest dietary fiber, vitamin C and polyphenol content, among all the cookie variations. Cookies D contained 11.5 g of dietary fiber, 44.176 g of vitamin C, and 35.6 mg of polyphenols per 100 g of cookies. The higher proportion of seaweed flour substitution, the higher content of dietary fiber, vitamin C, and polyphenols in cookies.

Further research related to seaweed flour is warranted. Further studies are required to investigate all the contents of seaweed flour apart from the content of dietary fiber, vitamin C and polyphenols. Further studies are also required to examine the water content of seaweed as when dried, seaweed is still tough and moist. Experimental research for providing cookies

for patients with coronary heart disease needs to be carried out to determine its effect on certain variables, including nutritional status, immune status or inflammation status.

### Acknowledgements

The authors would like to thank Universitas Respati Yogyakarta, Indonesia, for granting permission to conduct this research.

### Funding

No funding was received.

### Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

### Authors' contributions

YDAB, as the principal investigator, coordinated the research. FLW and SW were involved in the conception and design of the study, as well as in data analysis. All authors have read and approved the final manuscript. FLW and SW confirm the authenticity of all the raw data.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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