

Use of Change-point Regression Models to Analyze Suppressive Effects of Functional Foods

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Abstract

In functional food intervention studies, the study population is often a mixed population of healthy individuals and/or less healthy but not sick people. For such heterogeneous populations, alternative approaches have been proposed to evaluate the efficacy of nutraceuticals and functional foods based on the change-point regression model (CPRM). However, conventional CPRM is a method for detecting improvement effects and is not suitable for detecting effectiveness at preventing the deterioration of health-related indices over time. We propose a suppression-effect CPRM and apply this method to skin water loss study data, and demonstrate the merits of this new method by comparing it with conventional methods. We succeeded in finding food functions for deterioration over time that could not be detected by conventional ANCOVA or CPRM methods.

Keywords: change-point regression model; stratum corneum water; dietary supplement; AIC

Introduction

Recently, intervention studies on human subjects are important not only in the field of pharmaceutical research but also in the field of research on food components such as functional foods and nutrients including vitamins.

In human intervention studies of functional foods, the subjects are often selected from healthy subjects to those in the borderline range (of disease) and the expected effect size of the test substance is smaller than that of drugs. In addition, it is often difficult to conduct a study with a very large sample size due to financial constraints. In such cases, the effect of a covariate on the intervention effect may be large. Especially in studies with a small sample size, if there is an imbalance of covariates between the intervention and Placebo groups, the results of the evaluation of the intervention effect will be biased [1]. In this case, an analytical method adjusted for covariates during data analysis is effective. Analysis of covariance (ANCOVA) is a methodology in group comparisons that employs the principles of regression analysis, making it a valuable approach for the analysis of human trials with multiple covariates and confounding factors [2]. However, this method assumes that the treatment effects are constant across Treatment groups. While this assumption may hold for pharmaceuticals, where effects can be expected irrespective of baseline observations, it may not necessarily be applicable to the assessment of nutritional supplements or functional foods. The primary reason for this discrepancy lies in the fact that, when both healthy individuals and those not clinically ill but not entirely healthy (referred to as borderline participants) are enrolled in a

trial, there tends to be a diminishing effectiveness size in healthy individuals compared with that in borderline participants. This heterogeneity may compromise the effectiveness of t-tests or ANCOVA, and thus, assuming a homogeneous study population, estimates of treatment effects may be misleading. To address these issues, Hayamizu et al. proposed a change-point regression model (CPRM) for analyzing the efficacy of functional foods using clinical trial data on the visceral fat-reducing effect of (-)-hydroxycitric acid [3]. Furthermore, Kobuna et al. reported the impacts of the position of change points, the magnitude of intervention effects, data variance, and sample size on the results of CPRM through simulation experiments, revealing the characteristics of CPRM [4]. CPRM is an analytical method that takes into account the point of effect (point of change) by the treatment. The model provides clinically interpretable results without requiring the assumption that the effect of treatment is consistent throughout the Treatment group.

Incidentally, the method proposed by Hayamizu et al. considers that the post-intervention measurement depends on the pre-intervention magnitude in the group effect, and the model assumes a case in which the pre-intervention measurement showed a change in measurement for subjects whose measurement exceeded a certain value (threshold) while no change was observed in the Placebo group. This case could be called the "improvement effect."

However, depending on the type of outcome, some items may worsen over time. For example, the effects of outdoor humidity as an external environment from summer to winter, the effects

of sunshine hours, and the effects of pollen dispersal. In this case, the effectiveness of the Treatment group is interpreted as preventing or slowing down this deterioration. Such cases are referred to as “suppressive effects”.

Furthermore, expanding on the concept of suppressive effects, it is conceivable that the intensity of time-dependent deterioration may undergo significant changes at a certain

Methods

Suppressive effects model

We consider the analysis of data from an RCT of functional foods conducted using the simplest intervention trial method known as the “Pre-Post design.” Specifically, we consider two groups comprising a Placebo group and a Treatment group, measuring the endpoint at the initiation and conclusion of the trial. Here, let us consider that the study endpoint undergoes changes (deterioration) over time, in a manner dependent on pre-measurement values. To assess whether such changes (deterioration) occur over time, we can observe the behavior of the Placebo group. For instance, if higher values before the trial initiation gradually decrease due to the impact of seasonal variations, the behavior of the Placebo group can be represented as shown in Fig. 1A. The suppressive effect of the treatment on

threshold.

To the best of our knowledge, no instances of the analysis of “suppressive effects” using CPRM have been reported[3]. The objective of this study is to present verification results using CPRM as a method for evaluating “suppressive effects” by incorporating covariates into the model in a randomized control trial (RCT) of functional foods.

seasonal variation can be examined by determining whether the change in measured values before and after the intervention remains constant, or whether the change is smaller than in the Placebo group (Fig. 1 A). In this case, when performing a statistical analysis to compare the post values of the two groups, the pre value should be treated as a covariate, and the most commonly used method is analysis of covariance (ANCOVA) [2]. Note that the Placebo group is affected by seasonal variation, and the model equation for ANCOVA can be divided into two types as follows.

$$y_i = \alpha + \beta_1 x_i + \beta_2 (1 - g_i) + \epsilon_i \tag{Eq. 1}$$

$$y_i = \alpha + \beta_1 x_i + \beta_2 (1 - g_i) + \beta_3 x_i (1 - g_i) + \epsilon_i \tag{Eq. 2}$$

Here, x_i represents the pre value of the i th subject, y_i represents the post value of the i th subject, and g_i indicates group information (Treatment group = 1, Placebo group = 0). Here, we designate Eq. 1 as ANCOVA1 and Eq. 2 as ANCOVA2. ANCOVA1

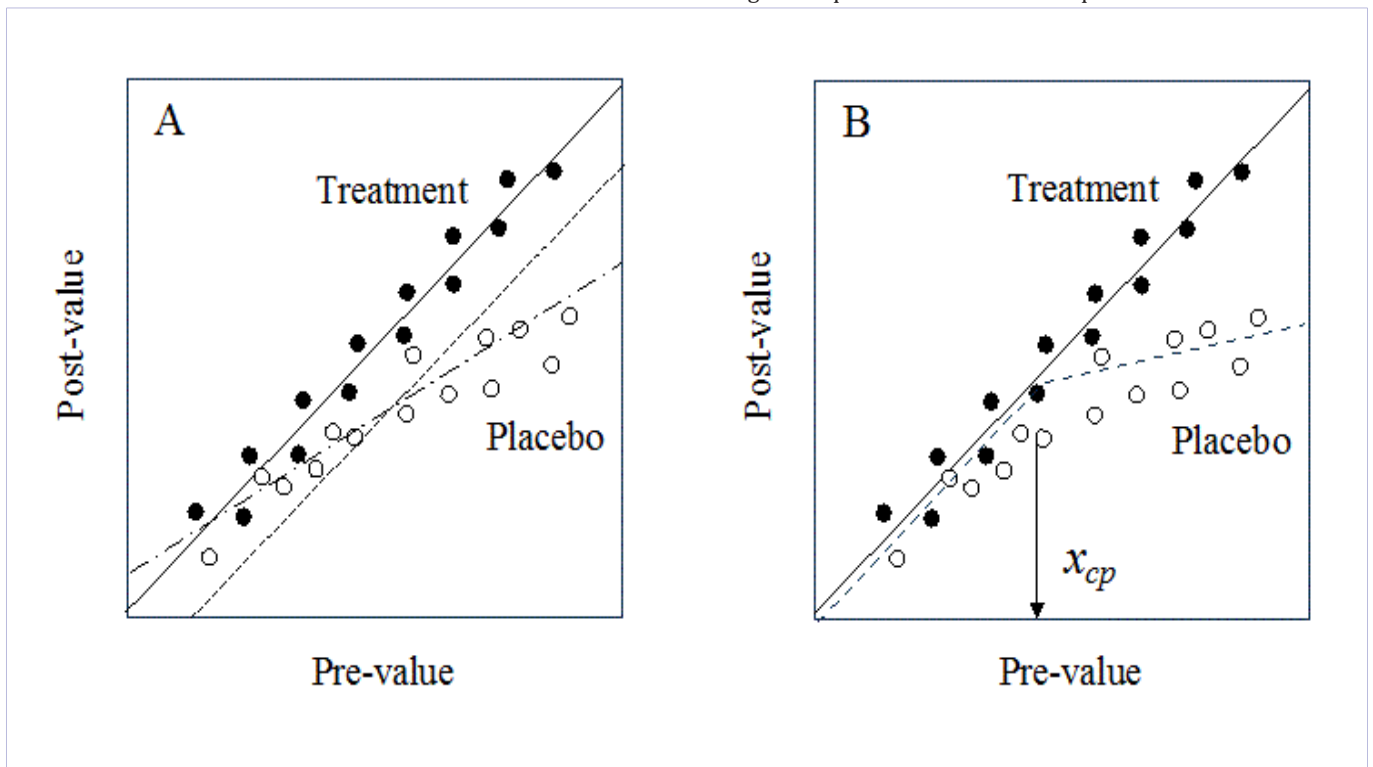


Figure 1: The concept of the Change-Point Regression Model (CPRM) for suppression effect. Each panel shows measurements of subjects in the placebo (open circle) and the treatment groups (closed circle) with regression lines. Panel A shows two types of ANCOVA. The dashed line parallel to the regression line (solid line) for the Treatment group represents ANCOVA1 (Eq. 1), and the dashed line with a different slope represents ANCOVA2 (Eq. 2). Panel B shows CPRM for suppression effect. In the Placebo group, there is no change below the change point (x_{cp}), but above the change point, the values worsen compared to pre-intervention values. In contrast, the intervention group remains constant regardless of the change point or deteriorates more slowly than the placebo group.

assumes a model where the impact of covariates on the endpoint is constant. For ANCOVA1, the Treatment group is represented by $g_i = 1$, resulting in $y_i = \alpha + \beta_1 x_i + \varepsilon_i$, while the Placebo group is represented by $g_i = 0$, leading to $y_i = \alpha + \beta_1 x_i + \beta_2 = (\alpha + \beta_2) + \beta_1 x_i$. When conducting a statistical test for the difference between the two groups in ANCOVA1, the null hypothesis H_0 is $\beta_2 = 0$, and the alternative hypothesis H_1 is $\beta_2 \neq 0$. ANCOVA2 is a model that assumes that the impact of covariates on the endpoint is not constant but includes interaction between covariates and group information. The regression lines for the Treatment group and Placebo group in ANCOVA2 are represented as $y_i = \alpha + \beta_1 x_i + \varepsilon_i$ and $y_i = \alpha + \beta_1 x_i + \beta_2 + \beta_3 x_i + \varepsilon_i = (\alpha + \beta_2) + (\beta_1 + \beta_3) x_i + \varepsilon_i$, respectively. When conducting a statistical test for the difference between the two groups in ANCOVA2, the null hypothesis H_0 is $\beta_3 = 0$, and the alternative hypothesis H_1 is $\beta_3 \neq 0$. Both ANCOVA1 and ANCOVA2 represent the relationship between covariates and the endpoint as linear.

Next, consider a case where the intensity of deterioration due to seasonal variations in the Placebo group undergoes a change beyond a certain threshold, but the Treatment group suppresses this effect. In this case, the suppression model of CPRM would be as follows (Fig. 1B).

$$y_i = \alpha + \beta_1 x_i + \beta_2 I(x_i > x_{cp})(x_i - x_{cp})(1 - g_i) + \varepsilon_i \quad \text{Eq. 3}$$

Here, x_{cp} represents the threshold. $I(\bullet)$ is an indicator function, taking the value 1 when $x_i > x_{cp}$ is true and 0 otherwise. When the pre value is less than x_{cp} , both the Treatment group and the Placebo group yield $I(x_i > x_{cp}) = 0$, and Eq. 3 becomes $y_i = \alpha + \beta_1 x_i + \varepsilon_i$. In other words, when the pre value is less than x_{cp} , the Treatment group and the Placebo group follow the same pattern. On the other hand, when the pre value is greater than x_{cp} , $I(x_i > x_{cp}) = 1$, and for the Placebo group with $g_i = 0$, $y_i = \alpha + \beta_1 x_i + \beta_2(x_i - x_{cp}) + \varepsilon_i = (\alpha + \beta_2 x_{cp}) + (\beta_1 + \beta_2)x_i + \varepsilon_i$. In other words, the Placebo group in the region where x_i is greater than x_{cp} experiences a change in coefficients to $(\beta_1 + \beta_2)$, deviating from the Treatment group. Meanwhile, the Treatment group, with $g_i = 1$, remains unchanged with $y_i = \alpha + \beta_1 x_i + \varepsilon_i$. The size of the suppressive effect in CPRM is indicated by the difference in regression coefficients between the two groups after the change point. Thus, it is assessed whether β_2 is statistically significant. In other words, the null hypothesis H_0 is $\beta_2 = 0$, and the alternative hypothesis H_1 is $\beta_2 \neq 0$.

Application data (Adlay Tea Study)

Adlay [*Coixlacryma-jobi* L. var. *ma-yuen* (Roman.) Stapf], an annual grass belonging to the Poaceae family and native to China and Southeast Asia, has been traditionally utilized in herbal medicine. Yokuinin, a hot water extract from the seeds of Adlay, has found applications in traditional Chinese medicine, exhibiting a diverse range of effects, including anti-inflammatory, anti-obesity, and anti-diabetic effects [5]. In the field of dermatology, oral prophylactic treatment with Adlay extract was reported to be effective at reducing the risk of severe acute radiation dermatitis in breast cancer patients [6].

The Adlay Tea Study was an intervention study to evaluate

the efficacy of Adlay [7]. The study was conducted in Oyama City (Tochigi, Japan) from July to October 2020. It involved a double-blind, Placebo-controlled, randomized trial carried out on 69 healthy women. Participants were randomly allocated to the Adlay tea group ($n = 34$) or the Placebo tea (barley tea) group ($n = 35$). Participants consumed the designated test drink daily for 8 weeks. To assess skin moisturizing function, the stratum corneum water (SCW) content was measured before and after the intervention.

SCW content was measured using a Corneometer (CM825; Courage+Khazaka Electronic GmbH, Cologne, Germany). The SCW content value was the average of five measurements taken on the same area of the right forearm. At baseline, the SCW for the Adlay group was 25.4 ± 3.6 (mean \pm SD) (a.u.), while for the Placebo group it was 24.6 ± 4.3 (a.u.), which did not differ significantly. However, upon conducting a subgroup analysis based on the median value (25.2 a.u.) before the intervention, the Adlay group exhibited significantly higher SCW content than the Placebo group in the subgroup that showed values equal to or above the median (Adlay group: 22.7 ± 5.7 , Placebo group: 19.5 ± 3.2). Table 1 summarizes the results of the Adlay Tea Study. In their paper, the team that performed this study stated that the significant decrease in SCW content after the intervention was due to seasonal variations, as the study lasted from the hot and humid summer to the dry early winter months [7]. The aim of the Adlay Tea Study was to assess whether Adlay can suppress this skin moisturizing degradation due to seasonal variations.

We examined the usefulness of the commonly used ANCOVA model by comparing it to the CPRM for suppression effect (CPRM-SE), which incorporates the worsening conditions into the model. The usefulness of the CPRM-SE was compared with that of the CPRM for improvement effect (CPRM-IE) reported in previous papers [3,4]. The model of CPRM for improvement effect is as follows.

$$y_i = \alpha + \beta_1 x_i + \beta_2 I(x_i > x_{cp})(x_i - x_{cp}) g_i + \varepsilon_i \quad \text{Eq. 4}$$

Here, CPRM can be divided into two cases: one in which group differences emerge above x_{cp} (Eq. 3 and Eq. 4) and another in which they emerge below x_{cp} . In the latter case, CPRM-SE and CPRM-IE would be as follows, respectively.

$$y_i = \alpha + \beta_1 x_i + \beta_2 I(x_i < x_{cp})(x_i - x_{cp})(1 - g_i) + \varepsilon_i \quad \text{Eq. 5}$$

$$y_i = \alpha + \beta_1 x_i + \beta_2 I(x_i < x_{cp})(x_i - x_{cp}) g_i + \varepsilon_i \quad \text{Eq. 6}$$

Thus, there are six models, Eq. 1–6, which will be compared using the data from the Adlay Tea Study. The concept of each model is shown in Fig. 2. The search for the optimal x_{cp} was performed using the minimized Akaike's Information Criterion (AIC) as an indicator, and the fitting of each model was compared [3-4,8]. The formula of AIC is:

$$AIC = -2 \text{maximum log-likelihood} + 2p$$

Where p is the number of unknown parameters of the model. We select the best model which minimizes AIC among candidate models.

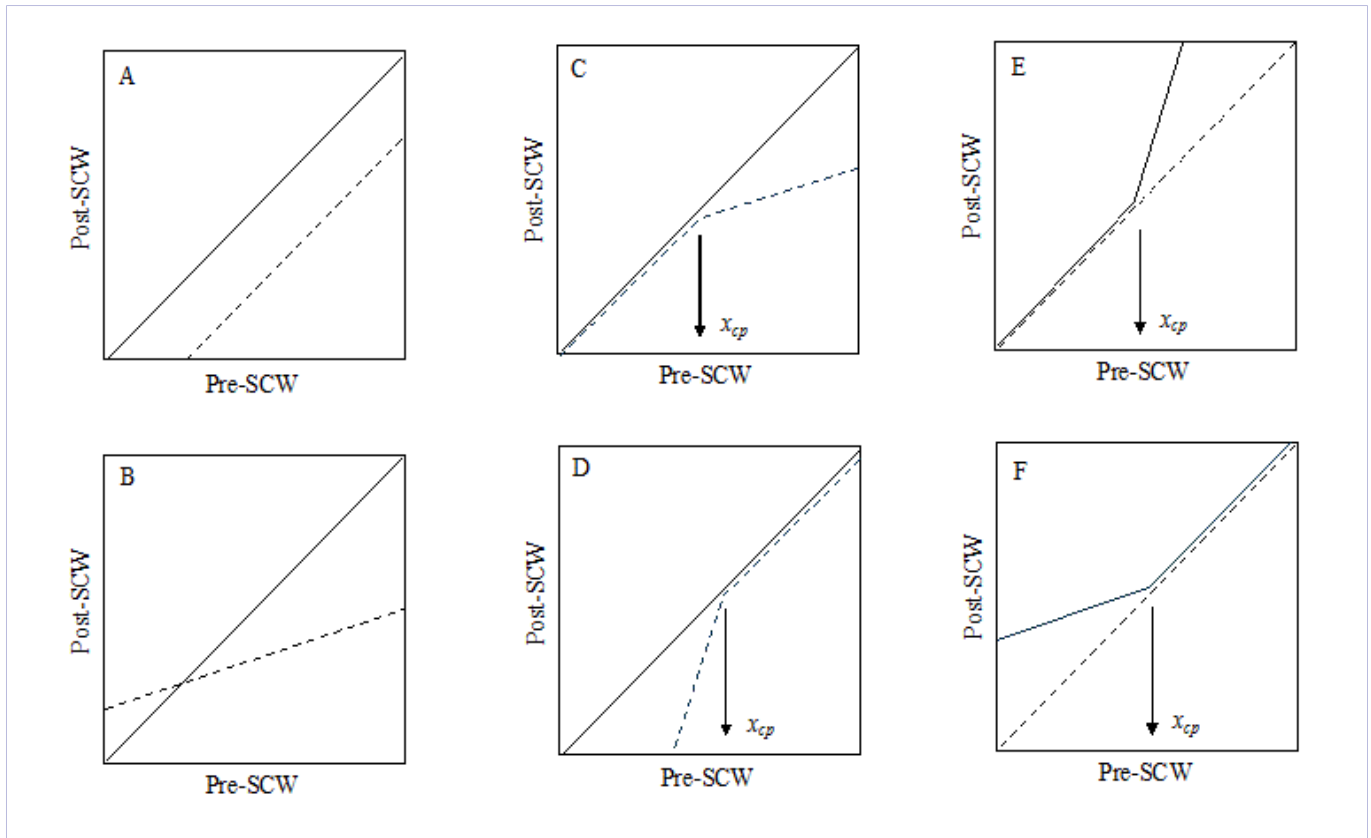


Figure 2: The concept of each model in the Adlay tea study
 The dashed line and solid line represent the treatment and placebo groups respectively.
 SCW: stratum corneum water
 Panels A and B show ANCOVA 1 (Eq. 1) and 2 (Eq. 2) respectively.
 Panels C and D show CPRM for suppression effect evaluation (Eq. 3 and 4).
 Panels E and F show CPRM for improvement effects evaluation (Eq. 5 and 6).

Table 1: Summary of stratum corneum water content in Adlay tea study [7]

Subject	Group	n	Pre (a.u)	Post (a.u)	P-value for group comparison
All	Adlay	34	25.4±3.6	20.5±5.2##	0.07
	Placebo	35	24.6±4.3	18.4±3.4##	
Sub-group ^{a)} (> Median value)	Adlay	17	28.3±2.4	22.7±5.7##	0.03
	Placebo	18	27.6±2.0	19.5±3.2##	

Mean±SD, ##: Pre vs Post p<0.01, NS: not significant

Subjects who had stratum corneum water content of baseline were higher than the median value.

Results

Using the application data, we verified the fitting of each model from Eq.1 to Eq.6. Table 2 shows the results of comparing each model. The model with the best fit for the Adlay Tea Study was Eq. 3 of the CPRM-SE. Eq. 3 was also the only model that statistically demonstrated the effectiveness of Adlay (p = 0.028). From $\beta_1 = 0.632 (<1)$ in Eq. 3, it can be seen that, overall, the Post value decreased relative to the Pre value. This may indicate that a loss of SCW due to seasonal variation occurred during the intervention period of the Adlay Tea Study. From β_2 , we also see that this loss of SCW content is even more severe in the Placebo

group for subjects with a Pre value of 24.8 (a.u.) or higher. In other words, the loss of SCW content due to seasonal variation suggests that the higher the baseline SCW content, the greater the loss due to seasonal variation and the existence of a change point as a condition susceptible to seasonal variation (Fig. 3). However, the Adlay group did not show a stronger decrease in SCW, even in subjects with a Pre value of 24.8 (a.u.) or higher. Therefore, it can be interpreted that the Adlay tea intervention suppressed the decrease in SCW due to seasonal variations.

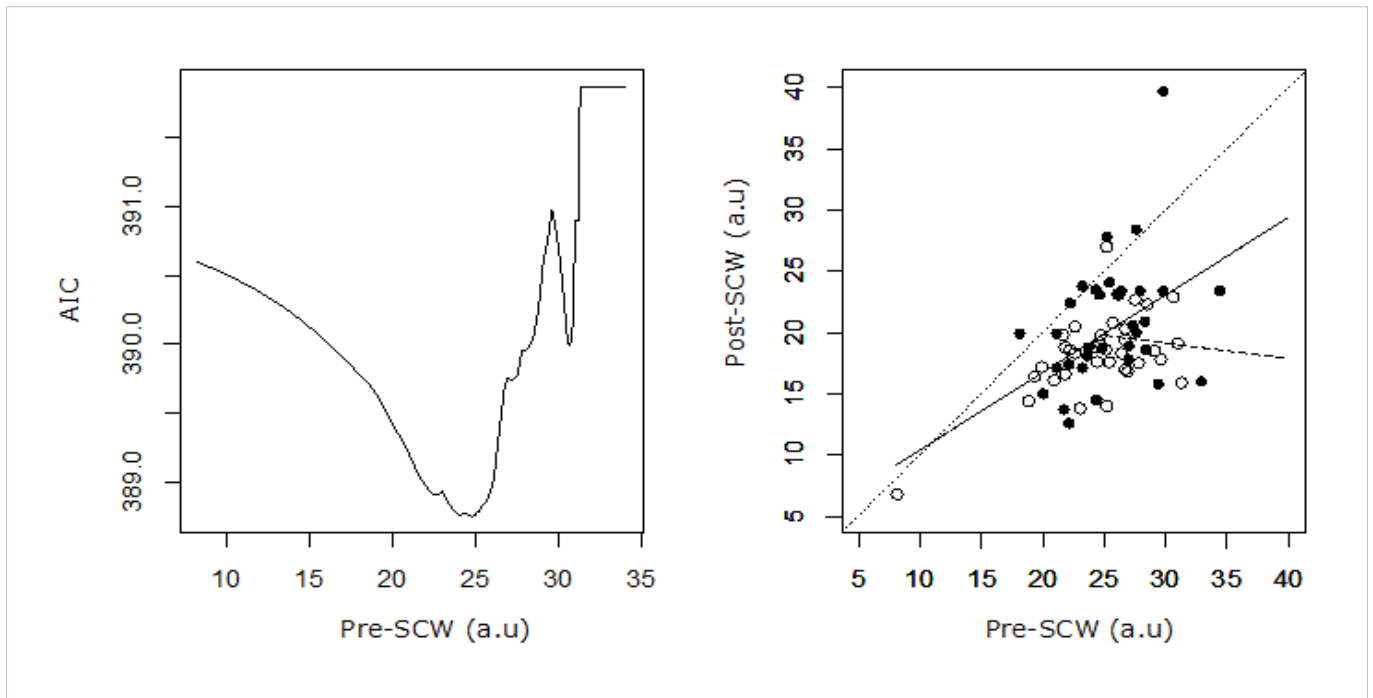


Figure 3: Result of Change-point regression model (Eq. 3) for Adlay tea study. The dashed line and solid line represent the treatment and placebo groups respectively. The open and closed circles indicate placebo and treatment groups, respectively. SCW: stratum corneum water

Table 2: ANOVA Table and AIC of CPRM and ANCOVA models for Adlay Tea Study

Model	α	β_1	β_2	g	x_{cp}	p-value	AIC
Eq.1 ANCOVA1	8.467	0.471	-	-1.719	-	0.077	390.6
Eq.2 ANCOVA2	7.818	0.497	-0.042	-0.656	-	0.866	392.5
Eq.3 CPRM-SE	4.126	0.632	-0.758	-	24.8	0.028	388.8
Eq.4 CPRM-SE	10.571	0.382	0.155	-	34.1	0.121	391.3
Eq.5 CPRM-IE	7.7216	0.433	0.099	-	8.2	0.079	390.6
Eq.6 CPRM-IE	5.295	0.537	-0.155	-	34.1	0.121	391.3

SE: suppression effect evaluation, IE: improvement effect evaluation

Discussion

CPRM is a method proposed to evaluate the efficacy of functional foods in RCTs, but it has also been used in a wide range of other applications; for example, it has been used to analyze the efficacy of neuromuscular electrical stimulation upon walking in obese women [9]. CPRM has also been applied in analyses beyond evaluations of effectiveness in RCTs. For example, it has been used in analyzing alteration of right ventricular contraction patterns in healthy children [10], examining echocardiographic studies in neonates [11], investigating the relationship between deep trunk muscle thickness and 100 m sprint time [12], estimating protein requirements in the elderly [13-15], and evaluating safety by systematic review [16,17], among other applications. CPRM can be applied in research aimed at identifying change points. However, we considered that conventional CPRM is not suitable for cases where the target measurement value deteriorates due

to external environmental influences, such as seasonal variations. In the study reported here, we categorized and organized improvement-effect-type CPRM (CPRM-IE) and suppression-effect-type CPRM (CPRM-SE), and verified the suitability and usefulness of these models. In the CPRM-IE model, the measured values of the Treatment group and the Placebo group remain the same until a change point. Above this change point, the measured values of the Treatment group diverge from those of the Placebo group in the direction of improvement.

At this point, the treatment effect size of the Placebo group does not change between before and after the change point. Therefore, in the analysis of the anti-obesity effect, the change point at which the weight loss effect begins to appear is sought, and this identified change point is incorporated into the model to analyze the difference in effect size between the groups [1,3]. On the other hand, the CPRM-SE model is a model in which

the measured values of the intervention and Placebo groups remain constant until a change point, and at this change point, the measured values of the Placebo group deteriorate and diverge from those of the Treatment group. In the Adlay study, the endpoint was the SCW content, expected to be influenced by the humidity of the outside air [7,18]. Rawling et al. measured the moisture content of the stratum corneum at different depths in subjects with normal skin and dry skin. The difference between the two was more pronounced at depths closer to the skin surface, with a 2–3 times greater difference observed in dry skin than in normal skin [19]. However, when considering the functions and characteristics of the stratum corneum as the skin's outermost layer, it is theoretically impossible for the moisture content of the stratum corneum to reach zero. In other words, even with external factors such as dry ambient air, the decrease in stratum corneum moisture content is believed not to fall below a certain value, at least in healthy individuals. This can be considered a kind of lower limit or threshold value. The results of the Adlay study indicate that individuals with higher pre values of SCW content experienced a greater reduction in post values. Especially in individuals with pre values equal to or higher than 24.8 (a.u.), the decrease in SCW due to seasonal variations was more pronounced. On the other hand, individuals with lower baseline SCW content may approach the lower limit, potentially resulting in a smaller reduction in moisture content. Incidentally, ANCOVA can also detect the presence or absence of deterioration due to the external environment. In the Adlay study, ANCOVA1 showed a better fit than ANCOVA2, as indicated in Table 2. From β_1 of ANCOVA1, it can be interpreted that there was an average decrease of about 47% in the post values compared with the pre values ($p < 0.001$), confirming the deterioration of stratum corneum water content due to seasonal variations. However, ANCOVA failed to detect a statistically significant difference in the suppression of SCW loss in the Adlay group. ANCOVA assumes that the effect of seasonal variation on the deterioration of SCW content is constant, but this assumption seems unreasonable considering the characteristics of SCW content mentioned above. As the stratum corneum moisture content approaches the lower limit, the reduction in moisture content should be less observable as a change in quantity. In the CPRM-SE model that we used in this study, the effect of seasonal variation on the deterioration of stratum corneum water content was stronger after the change point. The estimated change point of 24.8 was close to the median baseline value of 25.2, which served as the criterion for subgroup analysis of efficacy in a previous paper [7].

In this study, we compared CPRM-SE with CPRM-IE, using the AIC as an index. The results indicated that the former was more suitable for the data of the Adlay study, marking the first instance in which the suppressive-effect-type CPRM proved beneficial.

When implementing CPRM with covariates, it is crucial to carefully interpret the characteristics of the measurements and determine whether the suppression-effect or improvement-effect CPRM is more appropriate. The application of CPRM-SE is still limited, and its utility needs further verification across various

cases in the future.

Conclusion

We have proposed a new approach for evaluating the suppressive efficacy of dietary supplements or functional foods to suppress deterioration due to seasonal variations based on a CPRM. By using AIC-based profile likelihood methods, inferences can easily be made with standard statistical software. The proposed method was applied to the Adlay Tea Study data, and its merit was demonstrated by comparing it with the conventional method.

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Author contributions

KH conceived and designed the study. NY and KH performed the development of the analysis program. YA, KN, HI, YT, HS, and MN prepared application data. HS and MN provided medical supervision. KH wrote the manuscript. KH reviewed the manuscript. All authors have read and agree to the published version of the manuscript.

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