

**Intake of New Zealand blackcurrant powder affects skin-borne volatile organic compounds in middle-aged and older adults**

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## Abstract

Skin volatile organic compounds (VOCs) can cause body odor or reveal human disease and may result from lipid peroxidation or activity by skin bacteria. We examined the effect of intake of New Zealand blackcurrant (NZBC) powder for 77 skin VOCs in middle-aged and older adults in a cross-over design. Fourteen adults (9 males, age: 55±5 yr) consumed NZBC powder for 7 days (6 g·day<sup>-1</sup> with 138.6 mg anthocyanins). Two hours after the last intake, a passive flux sampler with trapping media was applied in the base of the neck for one hour. Gas chromatography-mass spectrometry was used for media analysis. Habitual anthocyanin intake was quantified using a food frequency questionnaire. Compared to control (i.e. no intake of NZBC powder), emission of six skin VOCs, i.e. 2-nonenal, acetic acid, 2-hexanone, 6-methyl-5-hepten-2-one, benzaldehyde, allyl methyl sulphide, were lower by more than 25%. Increases were observed for  $\gamma$ -octanolactone (+184%) and  $\gamma$ -decanolactone (+89%). A trend for decrease was observed for isovaleraldehyde, hexanal and 2-pentanone and an increase for heptanoic acid and  $\gamma$ -nonanolactone. There was a significant correlation with daily habitual dietary anthocyanin intake for control values of hexanal and percentage change of  $\gamma$ -octanolactone. NZBC powder can change emanation of some VOCs in human skin. Analysis of skin VOCs following specific polyphenol intake may address the impact of dietary components to affect internal metabolic processes, body odor and health.

**KEYWORDS:** volatile organic compounds, metabolism, anthocyanins, lipid peroxidation, body odor, aging

## Introduction

In humans, volatile organic compounds (VOCs) are chemicals that are primarily produced by endogenous biochemical processes. VOCs are emitted via urine, faeces, breath and skin (de Lacy Costello et al., 2014). The chemical classification of the VOCs (e.g. carboxylic acids, alcohols, aldehydes, and ketones) is based on functional groups in their chemical structure and it allows the analysis of a VOC profile (Baranska et al., 2013). The VOC profile or even the occurrence of a particular VOC can be altered by disease conditions, e.g. breath VOCs in chronic obstructive pulmonary disease [Van Berkel et al., 2010 (e.g. isoprene)] and Alzheimer (Mazzatenta et al., 2015), and urine and faeces VOCs for colorectal cancer (Bond et al., 2019; Mozdiak et al., 2019), in addition to the risk for development of a pathophysiological condition (Lacey et al., 2018) and body odor, e.g. skin VOC 2-nonenal (Ishino et al., 2010; Jha, 2017). The analysis of breath and skin emissions of VOCs is an emerging research discipline to allow detection of non-invasive biomarkers of internal metabolic processes linked with health and disease.

Skin-borne VOCs [532 named VOCs (de Lacy Costello et al., 2014)] can originate from sebaceous and sweat glands and may reveal the outcome of the glands metabolic processes and activity of skin bacteria. However, the endogenous, non-gland, origin of many skin VOCs has not been established. VOC skin emanations can act as an attractant for mosquitos to locate humans (Bernier et al., 2000) but also provide opportunity to detect entrapped humans by building collapse (Agapiou et al., 2013). In addition, some skin-borne VOCs are associated with body odor, e.g. the unsaturated aldehyde 2-nonenal in older adults (Haze et al., 2001).

In healthy humans, the normal physiological and biochemical processes that contribute to an endogenous origin of VOCs can be affected by many lifestyle factors, e.g.

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3 smoking, psychological stress, exercise, and diet. For example, a gluten free diet over a  
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5 period of 4 weeks altered the VOC profile of exhaled breath (Baranska et al., 2013). In  
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7 addition, the VOC profile of exhaled breath is also affected by certain foods such as garlic,  
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9 leeks and coffee (Krilaviciute et al., 2019). Garlic intake is probably one of the best-known  
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11 foods to affect exhaled breath as well as having a positive effect on perceived body odor  
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13 hedonicity (Fialová et al., 2016) and maybe due to anti-oxidant and anti-microbial properties  
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15 affecting the skin emission of VOCs (Sato et al., 2020). Coffee also affected the VOC profile  
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17 of urine (Mack et al., 2019). In contrast, sugar beet pectin supplementation (15g/day for 4  
18  
19 weeks) did not affect exhaled VOC profiles in healthy young adults and healthy elderly (An  
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21 et al., 2019). However, evidence is emerging how our food choices affects an individual VOC  
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23 profile. Our knowledge, however, on the effect of specific dietary components on emanation  
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25 and thus production of VOCs is very much in its infancy. Such information may provide  
26  
27 support for existing and future development of dietary guidelines. Current dietary guidelines  
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29 emphasize a regular intake of fruits and vegetables. The health benefits by intake of many  
30  
31 colourful fruits and vegetables have been linked with the polyphenol content, with many  
32  
33 studies emphasizing the role of the flavonoid anthocyanin (Lila et al., 2016). Many *in vitro*  
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35 studies provided support for anti-oxidant and anti-inflammatory effects of anthocyanins. In  
36  
37 addition, reviews have summarized the effects of anthocyanins on the gut microbiome and  
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39 linked with reduced risk for disease (e.g. Speer et al., 2020; Hair et al., 2021). We have  
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41 shown that intake of anthocyanin-rich NZBC powder enhanced insulin sensitivity in healthy  
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43 individual (Willems et al., 2017). Intake of anthocyanin-rich NZBC powder also shifted the  
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45 exercise-induced lactate curve in endurance-trained participants (Willems et al., 2015). As  
46  
47 such, the potential for anthocyanins to affect biochemical and physiological processes may  
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49 influence the production of VOCs. However, the effect of anthocyanin intake in humans on  
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51 the skin emission of VOCs is not known.  
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3 Therefore, the aim of the present study was to examine the effect of anthocyanin-rich  
4 NZBC powder on emission of VOCs from the skin in middle-aged and older adults. For  
5  
6 example, in middle-aged and older adults, body odor is associated with emission of 2-nonenal  
7  
8 and likely due to a change of the anti-oxidant defence system with aging. It was hypothesised  
9  
10 that emission of 2-nonenal would be reduced with intake of blackcurrant anthocyanins by  
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12 lowering lipid peroxidation. However, this study is also explorative in nature and aims to  
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14 examine whether a rich-anthocyanin containing supplement can change the emission rate of  
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16 skin VOCs with unclear origin in middle-aged and older adults.  
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## 24 **Methods**

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26 Participants (Caucasian, male: n=9, female: n=5) were recruited from staff of the University  
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28 of Chichester in the United Kingdom (age: 55±5 yr, mean±SD, range 49-64 yr). The study  
29  
30 was approved in accord with the Research ethics policy of the University of Chichester  
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32 (ethical approval code: 1718\_29). Written informed consent by the participants was obtained  
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34 after the procedures and aims of the study were explained. The study used a cross-over  
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36 design. Skin sampling was done in April and May (Zhang et al., 2005) during a working day  
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38 between 8 am and 2 pm. During the one hour of skin sampling, participants were involved in  
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40 office desk type activities and collection time was similar for each participant for the control  
41  
42 and NZBC conditions. For the NZBC powder condition, participants consumed the NZBC  
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44 powder for 7 days (Sujon Berries, Nelson, New Zealand, 6 g·day<sup>-1</sup> with 138.6 mg  
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46 anthocyanin, 49 mg Vit C, 5.2 g of carbohydrates and total phenolic content of 271.6 mg per  
47  
48 serving) (Willems et al., 2015). Participants were advised to dissolve the powder in water.  
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51 Two hours after the last intake on day 7, a passive flux sampler (Kimura et al., 2016; Sekine  
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53 et al., 2018; Umezawa et al., 2018) was applied to the skin in the base of the neck for one  
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55 hour. Participants were advised not to use hot water or soap for 48 hrs on the skin sampling  
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3 location to exclude potential exogenous sources of skin VOCs (Gallagher et al., 2008). For  
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5 the control condition, which preceded the NZBC powder condition, the same participants did  
6  
7 not take a placebo as there can be no bias on the outcome measures of the study. The diet of  
8  
9 the participants was not controlled. Habitual dietary anthocyanin intake was quantified using  
10  
11 a food frequency questionnaire with anthocyanin sources listed in Phenol explorer (Neveu et  
12  
13 al., 2010).  
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17 Skin samplers were shipped from Tokai University (Japan). The shipment contained  
18  
19 two blank samplers during the flight travel from Japan to the United Kingdom and back to  
20  
21 correct for potential contamination by VOCs by the travel conditions. The sampler consisted  
22  
23 of a polypropylene screw cap, trapping media (MonoTrap®, DCC18, GL Science, Japan) and  
24  
25 O-ring as a stopper. Gas chromatography-mass spectrometry was used for media analysis of  
26  
27 VOCs according to procedures in Kimura et al. (2016). Samplers for analysis by gas  
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29 chromatography-mass spectrometry were numbered to keep researchers blind to the  
30  
31 treatments. Briefly, VOCs were extracted with 500 µL of dichloromethane after 15-min  
32  
33 ultrasonic extraction. All extracts were analyzed on the mass selective detector, JMS-  
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35 Q1050GC MkII (JEOL, Japan) interfaced to gas chromatograph, Agilent model 7890B. One  
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37 µL injections of sample extracts, blank extracts, and quantitation standards were made with a  
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39 split ratio of 35:1 onto DB-IMS (30 m×0.25 mm I.D.×0.25 µm film thickness, Agilent  
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41 technologies, USA). Helium was used as the carrier gas with a flow rate of 1.0 mL·min<sup>-1</sup>. The  
42  
43 injector port was maintained at 280 °C. The extracts were analyzed using the following  
44  
45 column temperature program: 50 °C hold for 8 min, increase at 6 °C·min<sup>-1</sup> to 120 °C and  
46  
47 increase at 20 °C·min<sup>-1</sup> to 280 °C hold for 2 min. Data on most sample extracts were acquired  
48  
49 using real time SIM. The emission flux (i.e. emission rate per area) of volatile compounds, E  
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51 (ng·cm<sup>-2</sup>·h<sup>-1</sup>) was obtained by  
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$$E = \frac{W}{St} \quad (1)$$

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3 where  $W$  is a collection amount of analyte (ng) by the PFS,  $S$  is an effective cross-section of  
4 the trapping media ( $0.594 \text{ cm}^2$ ) and  $t$  is a sampling duration (h). Travel blanks were  
5 subtracted when observed.  
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## 10 11 12 **Statistical analysis**

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14 Normality was checked with a D'Agostino and Pearson omnibus normality test  
15 (Graphpad Prism 5 for Windows). Two-tailed Wilcoxon signed rank test (i.e. no Gaussian  
16 distribution) or two-tailed paired t-test were used for non-parametric and parametric testing,  
17 respectively. Cohen's  $d$  effect sizes were calculated and considered trivial ( $d < 0.2$ ), small ( $d$   
18 =  $0.2-0.49$ ), moderate ( $d = 0.5-0.79$ ) and large ( $d \geq 0.8$ ), respectively (Cohen et al, 1988). For  
19 the VOCs for which a change or trend of change was observed with intake of NZBC powder,  
20 Pearson correlations were calculated between habitual dietary anthocyanin intake and 1)  
21 absolute values of VOCs in the control condition, 2) the observed percentage changes of skin  
22 VOCs. In addition, Pearson correlations were calculated between the skin VOCs that changed  
23 with intake of NZBC powder. Data are reported as mean $\pm$ SDs with 95% confidence intervals  
24 and box-and-whisker plots for significantly altered VOCs. Significance was accepted at  $P <$   
25  $0.05$  with  $0.05 \geq P \leq 0.1$  interpreted according to guidelines by Curran-Everett and Benos  
26 (2004).  
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## 47 **Results**

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49 In total, the emission rate of 77 skin VOCs were analysed with eight compounds showing a  
50 significant change ( $P < 0.05$ ) and five compounds a trend for change ( $0.05 \geq P \leq 0.1$ ) after 7-  
51 days intake of NZBC powder (Table 1). Table 2 provides the emission rate of the skin VOCs  
52 that did not change after 7-day intake of NZBC powder.  
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## Aldehydes

In the NZBC powder condition, the unsaturated aldehyde 2-nonenal was reduced with large effect size (control:  $4.66 \pm 3.44$ , 95% CI [2.69, 6.51  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; NZBC powder:  $2.38 \pm 1.46$ , 95% CI [1.54, 3.22  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; Wilcoxon signed rank;  $d = -0.87$ ;  $P = 0.030$ ) (Fig. 1A) with eight participants (~57%) having decreases more than 30% with an average of  $69 \pm 16\%$  (range 32-82%). The two participants with the highest 2-nonenal emission in the control condition, i.e. 9.41 and 13.81  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ , showed reductions of 80% and 82%, respectively. Habitual dietary anthocyanin intake was not significantly correlated with control 2-nonenal values ( $r = -0.28$ ,  $P = 0.33$ ) and percentage change in 2-nonenal with intake of NZBC powder ( $r = 0.36$ ,  $P = 0.21$ ).

Benzaldehyde was reduced with large effect size (control:  $2.67 \pm 1.54$ , 95% CI [1.78, 3.56  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; NZBC powder:  $1.45 \pm 0.93$ , 95% CI [0.91, 1.99  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; two-tailed t-test;  $d = -0.96$ ;  $P = 0.04$ ) (Fig. 1B) with nine participants having lower values with NZBC powder intake. The average decrease of those 9 participants was  $66 \pm 29\%$  (range 13-99%). Dietary anthocyanin intake was not significantly correlated with control benzaldehyde values ( $r = 0.10$ ,  $P = 0.74$ ) and percentage change in benzaldehyde with intake of NZBC powder ( $r = 0.06$ ,  $P = 0.84$ ).

A weak trend for a decrease was observed for isovaleraldehyde but with moderate effect size (control:  $7.56 \pm 3.37$ , 95% CI [5.61, 9.50  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ], NZBC powder:  $5.41 \pm 2.26$ , 95% CI [4.10, 6.72  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; two-tailed t-test;  $d = -0.75$ ;  $P = 0.09$ ) with 10 participants (~71%) having lower values with NZBC powder intake. The average decrease of those 10 participants was  $47 \pm 20\%$  (range 23-83%). Dietary anthocyanin intake was not significantly correlated with control isovaleraldehyde values ( $r = -0.44$ ,  $P = 0.12$ ) and percentage change in isovaleraldehyde with intake of NZBC powder ( $r = -0.36$ ,  $P = 0.20$ ).

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3 A strong trend for a decrease was observed for hexanal with large effect size (control:  
4 8.77±4.41, 95% CI [6.22, 11.31 ng·cm<sup>-2</sup>·hr<sup>-1</sup>]; NZBC powder: 5.65±3.25, 95% CI [3.77, 7.52  
5 ng·cm<sup>-2</sup>·hr<sup>-1</sup>]; two-tailed t-test; d = -0.81; P = 0.06) with 11 participants (~79%) having lower  
6 values with NZBC powder intake. The average decrease of those 11 participants was 44±29%  
7 (range 4-85%) with 6 participants having larger decreases than the average. Dietary  
8 anthocyanin intake was correlated with control hexanal values (r = - 0.53, P = 0.047) and  
9 with a trend for percentage change in hexanal with intake of NZBC powder (r = 0.47, P =  
10 0.09). The % change in hexanal correlated with the % change in  $\gamma$ -nonanolactone (r = 0.65, P  
11 = 0.022) and the % change in  $\gamma$ -decanolactone (r = 0.54, P = 0.047). These percentage  
12 changes of VOCs were the only ones that provided significant correlations.  
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### 29 Ketones

30 A decrease in skin emission rate was observed for the ketone 6-methyl-5-hepten-2-one  
31 (control: 6.55±3.04, 95% CI [4.79, 8.31 ng·cm<sup>-2</sup>·hr<sup>-1</sup>]; NZBC powder: 3.40±1.96, 95% CI  
32 [2.26, 4.53 ng·cm<sup>-2</sup>·hr<sup>-1</sup>]; two-tailed t-test; d = -1.23; P = 0.005) (Fig. 1C) with 12 participants  
33 (~85%) having lower values with NZBC powder intake. The average decrease of those 12  
34 participants was 58±23% (range 28-94%). Habitual dietary anthocyanin intake was not  
35 significantly correlated with control 6-methyl-5-hepten-2-one values (r = 0.42, P = 0.14) and  
36 percentage change in 6-methyl-5-hepten-2-one with intake of NZBC powder (r = - 0.09, P =  
37 0.76).  
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49 2-hexanone was decreased with large effect size (control: 1.84±0.77, 95% CI [1.40,  
50 2.29 ng·cm<sup>-2</sup>·hr<sup>-1</sup>]; NZBC powder: 1.26±0.58, 95% CI [0.92, 1.59 ng·cm<sup>-2</sup>·hr<sup>-1</sup>]; two-tailed t-  
51 test; d = -0.86; P = 0.03) (Fig. 1D) with 11 participants (~79%) having lower values. The  
52 average decrease of those 11 participants was 41±27% (range 1-87%). Habitual dietary  
53 anthocyanin intake was not significantly correlated with 2-hexanone values in the control  
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3 condition ( $r = 0.05$ ,  $P = 0.87$ ) and percentage change in 2-hexanone with intake of NZBC  
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5 powder ( $r = 0.20$ ,  $P = 0.49$ ).  
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8 There was a weak trend for a decrease of the ketone 2-pentanone with moderate effect  
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10 size (control:  $1.95 \pm 0.93$ , 95% CI [1.42, 2.49  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; NZBC powder:  $1.28 \pm 0.97$ , 95%  
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12 CI [0.71, 1.84  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; two-tailed t-test;  $d = -0.71$ ;  $P = 0.09$ ) with 11 participants  
13  
14 (~79%) having lower values. The average decrease of those 11 participants was  $54 \pm 30\%$   
15  
16 (range 8-88%). Habitual dietary anthocyanin intake was not significantly correlated with  
17  
18 control 2-pentanone values in the control condition ( $r = -0.23$ ,  $P = 0.43$ ) and percentage  
19  
20 change in 2-pentanone with intake of NZBC powder ( $r = -0.25$ ,  $P = 0.47$ ).  
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### 26 **Carboxylic acids**

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28 With NZBC powder, a decrease in skin emission rate was observed for the carboxylic acid  
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30 acetic acid with large effect size (control:  $580 \pm 207$ , 95% CI [461, 700  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; NZBC  
31  
32 powder:  $369 \pm 117$ , 95% CI [301, 436  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; two-tailed t-test;  $d = -1.26$ ;  $P = 0.006$ )  
33  
34 (Fig. 1E) with 11 participants (~79%) having lower values. The average decrease of those 11  
35  
36 participants was  $43 \pm 20\%$  (range 1-68%). Habitual dietary intake of anthocyanin was not  
37  
38 significantly correlated with control acetic acid values ( $r = 0.15$ ,  $P = 0.61$ ) and percentage  
39  
40 change in acetic acid with intake of NZBC powder ( $r = 0.26$ ,  $P = 0.37$ ).  
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45 There was a strong trend for an increase in skin emission rate for heptanoic acid with  
46  
47 moderate effect size ( $n=13$ , control:  $1.69 \pm 1.24$ , 95% CI [0.94, 2.43  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; NZBC  
48  
49 powder:  $2.74 \pm 1.63$ , 95% CI [1.76, 3.72  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ];  $d = 0.73$ ;  $P = 0.065$ ) with 7 participants  
50  
51 (~54%) having higher values. The average increase of those 7 participants was  $389 \pm 400\%$   
52  
53 (range 23-1217%), indicating for heptanoic acid substantial individual variability in response.  
54  
55 Habitual dietary intake of anthocyanin was not significantly correlated with control heptanoic  
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3 acid values ( $r = 0.29$ ,  $P = 0.32$ ) and percentage change of heptanoic acid with intake of NZBC  
4  
5 powder ( $r = -0.40$ ,  $P = 0.17$ ).  
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### 10 Cyclic esters

11  
12 With NZBC powder, an increase in emission rate for  $\gamma$ -octanolactone was observed (control:  
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14  $0.54 \pm 0.76$ , 95% CI [0.11, 0.98  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; NZBC powder:  $1.54 \pm 1.23$ , 95% CI [0.83, 2.25  
15  
16  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]) with large effect size ( $d = 0.98$ ,  $P = 0.023$ ) (Fig. 2A), and 11 participants  
17  
18 (~79%) having higher values. The average increase for those 11 participants was  
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20  $2801 \pm 5730\%$  (range 29-18391%), indicating for  $\gamma$ -octanolactone substantial variation among  
21  
22 responders. Habitual dietary intake of anthocyanins was not significantly correlated with  
23  
24 control  $\gamma$ -octanolactone values ( $r = -0.22$ ,  $P = 0.46$ ). However, the percentage change of  $\gamma$ -  
25  
26 octanolactone with intake of NZBC powder was significantly correlated with habitual dietary  
27  
28 intake of anthocyanins ( $r = -0.71$ ,  $P = 0.005$ ), with the significance obtained by two *extreme*  
29  
30 responders.  
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35 An increase for the emission rate of  $\gamma$ -decanolactone was observed (control:  
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37  $0.46 \pm 0.24$ , 95% CI [0.33, 0.60  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; NZBC powder:  $0.87 \pm 0.47$ , 95% CI [0.60, 1.15  
38  
39  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]) (Fig. 2B) with large effect size ( $d = 1.11$ ,  $P = 0.015$ ), and 11 participants  
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41 (~79%) having higher values. The average increase for those 11 participants was  $243 \pm 245\%$   
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43 (range 8-686%). Habitual dietary intake of anthocyanins was not significantly correlated with  
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45 control  $\gamma$ -decanolactone ( $r = -0.29$ ,  $P = 0.31$ ) and percentage change of  $\gamma$ -decanolactone with  
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47 intake of NZBC powder ( $r = -0.38$ ,  $P = 0.18$ ).  
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51 A strong trend for an increase was observed for  $\gamma$ -nonanolactone ( $n=12$  as in 2  
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53 participants  $\gamma$ -nonanolactone could not be detected in the control condition, control:  
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55  $1.09 \pm 0.97$ , 95% CI [0.48, 1.71  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]; NZBC powder:  $1.78 \pm 1.27$ , 95% CI [0.97, 2.57  
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57  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ]) with moderate effect size ( $d = 0.62$ ,  $P = 0.063$ ), and 8 participants (~67%)  
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3 having higher values. The average increase for those 8 participants was  $168 \pm 100\%$  (range 31-  
4 308%). Habitual dietary intake of anthocyanins was not significantly correlated with control  
5  $\gamma$ -nonanolactone ( $r = 0.17$ ,  $P = 0.61$ ) and percentage change of  $\gamma$ -nonanolactone with intake  
6 of NZBC powder ( $r = 0.12$ ,  $P = 0.71$ ).  
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### 15 **Sulfur containing compound**

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17 A decrease for emission rate of allyl methyl sulfide was observed (control:  $0.83 \pm 0.19$ , 95%  
18  $[0.72, 0.94 \text{ ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}]$ ; NZBC powder:  $0.61 \pm 0.23$ , 95%  $[0.48, 0.75 \text{ ng} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}]$  (Fig. 1F)  
19 with large effect size ( $d = -1.01$ ,  $P = 0.015$ ), and 11 participants (~79%) having lower values.  
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21 The average decrease for those 11 participants was  $39 \pm 19\%$  (range 13-78%). Habitual dietary  
22 intake of anthocyanins was not significantly correlated with control allyl methyl sulfide ( $r = -$   
23  $0.12$ ,  $P = 0.69$ ) and percentage change of allyl methyl sulfide with intake of NZBC powder ( $r$   
24  $= -0.12$ ,  $P = 0.69$ ).  
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### 37 **Discussion**

38 The present study provides first evidence that consumption of anthocyanin-rich NZBC  
39 powder affects the emission rate of volatile organic compounds from human skin. Out of the  
40 77 skin VOCs that were analysed, 13 VOCs (i.e. 18%) showed a significant change or a trend  
41 for change with 9 out of 13 (i.e. 69%) with large effect size. In the present study, the daily  
42 intake of 6 gram of NZBC powder could have been obtained with about 80-100 gram of fresh  
43 NZBCs, suggesting ecological validity with application for consideration of daily  
44 blackcurrant intake or potentially other anthocyanin-sources to be part of a healthy diet. The  
45 polyphenolic profile of blackcurrant is made up primarily of four anthocyanins, i.e. cyanidin-  
46  $3-O$ -glucoside, cyanidin- $3-O$ -rutinoside, delphinidin- $3-O$ -glucoside and delphinidin- $3-O$ -  
47 rutinoside (Kapasakalidis et al., 2006). It needs to be noted, however, that all berries have a  
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3 very distinct polyphenol composition, e.g. blackberry and blueberry contain 2 and 13  
4 anthocyanins, respectively (Lee et al., 2015). It is therefore possible that our observations on  
5 changes in emission rate of skin VOCs are unique with respect to the anthocyanin profile of  
6 blackcurrant, and maybe due to conversion of the absorbed anthocyanins to phenolic acids by  
7 colonic bacteria (Aura et al., 2005). Future work may address the emission rate of skin VOCs  
8 of berries with different polyphenol compositions.  
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17 Emission of VOCs that are associated with body odor in older adults were lowered,  
18 i.e. 2-nonenal (Haze et al., 2001) and 6-methyl-5-hepten-2-one (Ozeki et al., 2016). 2-  
19 nonenal has a greasy and grassy odor that is perceived as unpleasant (Haze et al., 2001). Haze  
20 et al. (2001) provided evidence that 2-nonenal is produced by the oxidative degradation of  
21 unsaturated fatty acids, i.e. by lipid peroxidation of fatty acids produced in the sebaceous  
22 glands (Ishino et al., 2010). Observations of 2-nonenal skin emissions have been observed in  
23 Japanese over 39 years of age (Haze et al., 2001) and non-Japanese populations between 41-  
24 70 years of age (Gallagher et al., 2008), so likely differences in dietary intake (i.e. higher  
25 intake of marine-based foods in Japanese) between those groups was not a likely explanation  
26 for the production of 2-nonenal in older adults. Age-induced lipid peroxidation is upregulated  
27 in older adults independent of dietary intake and therefore expected to be present in the  
28 participants of the present study. Intake of NZBC in humans has been shown to reduce lipid  
29 peroxidation by lowering the amounts of exercise-induced plasma carbonyls (Lyll et al.,  
30 2009). The present study provides therefore indirect observations of the ability of  
31 blackcurrant intake to reduce biochemical consequences of age-related oxidative stress in the  
32 sebaceous glands. The ketone 6-methyl-5-hepten-2-one results from oxidative degradation of  
33 the sebaceous gland product squalene (Ozeki et al., 2016). Lower values for the emission rate  
34 of the skin VOC 6-methyl-5-hepten-2-one maybe due to reduced synthesis of squalene and  
35 subsequently a smaller amount of squalene being available for oxidative degradation. 6-  
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3 methyl-5-hepten-2-one is suppressed in older adults (mean age 84.5 years) by inhibition of  
4 squalene peroxidation by 6 weeks use of soap containing the anti-fungal miconazole nitrate  
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6 (Ozeki et al., 2016). Overall, the reduction of the body odor components 2-nonenal and 6-  
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8 methyl-5-hepten-2-one by intake of NZBC powder may have application for the well-being  
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12 of older adults with concern for their body odor.  
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15 Emission of acetic acid by the skin has been associated with foot malodour  
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17 (Caroprese et al., 2009) and body odor in young adults (Lam et al., 2018). The acetic acid is  
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19 thought the result of bacterial metabolic activity that results in transformation of secretions by  
20  
21 the eccrine glands. Cutaneous *Propionibacterium* and *staphylococcus epidermidis* synthesize  
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23 acetic acid (Lam et al., 2018; Piwowarek et al., 2018). Therefore, if NZBC inhibits activity of  
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25 *propionibacterium* and *staphylococcus epidermidis* may affect skin health due to their role in  
26  
27 acne (Wang et al., 2016). Interestingly, tea polyphenols are suggested to reduce sebum  
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29 production in the skin and have potential for acne treatment (Saric et al., 2016). Other  
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31 phenolic compounds, such as ferulic acid which is a metabolite of cyanidin-3-*O*-glucoside  
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33 (De Ferrars et al., 2014) have been proposed as well with potential for skin disorders (Działo  
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35 et al., 2016). However, we cannot exclude that reduced sebum production occurred by intake  
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37 of NZBC powder.  
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43 Benzaldehyde is the aromatic aldehyde linked with almond flavour (Oliveira et al.,  
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45 2019) and was reduced with intake of NZBC powder. Benzaldehyde is also a VOC released  
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47 by muscle cells (Mochalski et al., 2014), but its function within the muscle cells is not  
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49 defined. Benzaldehyde is also produced in skin flora by *Pseudomonas aeruginosa* (Timm et  
50  
51 al., 2018) and associated with skin infections. It is possible that the intake of NZBC powder  
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53 reduces *pseudomonas aeruginosa* on human skin and contributing to a maintenance of a  
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55 healthy skin. In support, protocatechuic acid, an anthocyanin-derived metabolite, leads to  
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57 death of *Pseudomonas aeruginosa* in in-vitro conditions (Ajiboye et al., 2017). However, as  
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3 far as we know, the implication of the reduction in the VOC human skin emission of  
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5 benzaldehyde by intake of NZBC powder is not clear. Future work could address the role of  
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7 anthocyanins on skin VOCs in people with skin disorders.  
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10 Our understanding on the endogenous origin of 2-hexanone detected by skin emission  
11  
12 is absent. Inhalation of atmospheric 2-hexanone should be avoided. In plastic fabric workers,  
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14 for example, occupational exposure to 2-hexanone (methyl butyl ketone) caused acute mixed  
15  
16 motor and sensory neuropathy (Landrigan et al., 1980). When present in urine, it was  
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18 suggested by Mochalski and Unterkofler (2016) that 2-hexanone could be the product of 2-  
19  
20 hexanol oxidation. In urine, 2-hexanone was considered a potential biomarker for lung cancer  
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22 (Santos et al., 2017). In breath, 2-hexanone and 2-pentanone were changed in response to  
23  
24 hypoxic conditions (7620 m, 8% O<sub>2</sub>) (Harsman et al., 2015). It was suggested that 2-  
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26 hexanone (and 2-heptanone) may be due to beta-oxidation of unsaturated fatty acids in the  
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28 lungs (Harsman et al., 2015). However, different processes can be responsible for volatile  
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30 compounds emanated from skin and breath.  
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35 Allyl methyl sulphide was reduced by the intake of NZBC powder. Allyl methyl  
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37 sulphide is a bioactive compound derived from garlic (Castro et al., 2010), can be measured  
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39 as a skin VOC following garlic intake (Sato et al., 2020) and suggested to have antioxidant  
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41 effects in combination with other organosulfur compounds (Fanelli et al., 1998). However,  
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43 the implication of the decrease in allyl methyl sulphide by intake of NZBC powder is not  
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45 clear, but may affect body odor.  
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49 Five skin VOCs (4 with moderate and 1 with large effect size) showed a trend for  
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51 change with intake of NZBC powder. Isovaleraldehyde, also known as 3-methylbutanal, has  
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53 been shown in vitro that its formation is by interaction between human leucocyte antigen and  
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55 skin microflora, and it suggested to contribute body odor (Savelev et al., 2008).  
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58 Isovaleraldehyde was decreased by intake of NZBC powder. A trend for a decrease was also  
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3 observed for hexanal. In general, hexanal is a product of lipid oxidation (Frankel, 1980) and  
4 the skin VOC hexanal is the consequence of oxidation of human skin lipids. Hexanal is also  
5 taken as an oxidative stress marker in exhaled breath VOC in workers exposed to silica (Jalali  
6 et al., 2016). Therefore, it is possible that the intake of NZBC powder reduced lipid  
7 peroxidation of fatty acids in the sebaceous glands. 2-pentanone was reduced with intake of  
8 NZBC powder and just as benzaldehyde also a VOC released by muscle cells (Mochalski et  
9 al., 2014). The implications of a decrease in isovaleraldehyde, hexanal, and 2-pentanone by  
10 intake of NZBC powder are not clear. Two skin VOCs, i.e. heptanoic acid and  $\gamma$ -  
11 nonanolactone, showed a trend for an increase by intake of NZBC powder, with the  
12 implications also not clear.

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26 The present study did not employ dietary control or a compulsory wash-out period  
27 with restricted intake of habitual polyphenols. Participants in the present study were allowed  
28 to adhere to their normal hygienic procedures except for 48 h in which they were instructed  
29 not to use hot water and personal care products on the skin sampling site. This is the first  
30 study to show that even without dietary control or a wash-out period for habitual polyphenol  
31 intake that VOC emission by skin can be affected by intake of an anthocyanin-rich berry  
32 powder, indicating an effect of the NZBC powder supplement. However, it needs to be noted  
33 that the blackcurrant powder also contained vitamin C which may have affected the emission  
34 rate of the skin VOCs. It is also possible that the increase in some VOCs were due to volatile  
35 compounds present in the NZBC powder (Liu et al., 2018; Marsol-Vall et al., 2018). In  
36 addition, the participants in the present study were supplemented for 7 days with the same  
37 amount of NZBC powder which could potentially have affected the observed variation in  
38 change of some skin VOCs. In addition, we can also not exclude an effect of the final intake  
39 of NZBC powder 2 hours before VOC emission measurements. Dietary control and use of  
40 specific anthocyanin-rich extracts may be required for standardization of future studies to  
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3 isolate the anthocyanin-effects on the emission rate of skin VOCs It may be of interest as well  
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5 that future studies would address potential sex differences for the response by intake of  
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7 NZBC powder on VOCs. Our study used a small convenience sample with only 9 males and  
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9 5 females. Finally, this study examined a large number of VOCs without an explicit  
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11 hypothesis for change. However, the presence of the large effect sizes seems to suggest an  
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13 absence of type I error, and therefore does not necessarily require a multiple comparison  
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15 analysis with alpha-adjustment (Bender and Lange, 1999; Ranganathan et al., 2016). Finally,  
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17 it would be of interest to examine dose-response effects of anthocyanin intake on the  
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19 emission rate of skin VOCs whether more VOCs would respond with large effect size, in  
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21 addition to more VOCs providing significant changes.  
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26 It is concluded that the volatile profile of skin emissions was affected by the intake of  
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28 anthocyanin-rich New Zealand blackcurrant powder. Future work may address the link  
29  
30 between anthocyanin intake by berry consumption, anthocyanin-derived metabolites and  
31  
32 VOCs as biomarkers for health, disease and body odor.  
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15 **Table legends**

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17 **Table 1.** Volatile organic compounds emitted by the skin that were changed ( $P < 0.05$ ) or  
18 showed a trend for change ( $0.05 \geq P \leq 0.1$ ) by intake of New Zealand blackcurrant powder for 7  
19 days in middle-aged and older adults (male:  $n=9$ , female:  $n=5$ , age:  $55 \pm 5$  yr). + and -  
20 indicates an increase and decrease of the emission rate, respectively.  
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31 **Table 2.** Volatile organic compounds emitted by the skin (dermal emission flux in  $\text{ng} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ )  
32 that were not changed ( $P > 0.05$ ) by 7-day intake of New Zealand blackcurrant powder in  
33 middle-aged and older adults ((male:  $n=9$ , female:  $n=5$ , age:  $55 \pm 5$  yr). Cohen's  $d$  effect sizes  
34 were trivial ( $d < 0.2$ ), small ( $d = 0.2-0.49$ ) or moderate ( $d = 0.5-0.79$ ). Data are mean  $\pm$  SD.  
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55 **Figure legends**

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57 **Figure 1.** Box-and-whisker plot for emission flux of the skin volatile organic compound 2-  
58 nonenal (A), benzaldehyde (B), 6-methyl-5-hepten-2-one (C), 2-hexanone (D), acetic acid (E)  
59 and allyl methyl sulfide (F). New Zealand blackcurrant (NZBC) powder. Intake of New  
60 NZBC powder was for 7 days. \*, emission flux was lower with NZBC powder ( $P < 0.05$ ).  
Data of 14 participants.

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3 **Figure 2.** Box-and-whisker plot for emission flux of the skin volatile organic compound  $\gamma$ -  
4 octanolactone (A) and  $\gamma$ -decanolactone (B). Intake of New Zealand blackcurrant powder  
5 (NZBC) was for 7 days. \*, emission flux was higher with NZBC powder ( $P < 0.05$ ). Data of  
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Table 1. Volatile organic compounds emitted by the skin that were changed ( $P < 0.05$ ) or showed a trend for change ( $0.05 \geq P \leq 0.1$ ) by intake of New Zealand blackcurrant powder for 7 days in middle aged and older adults (male:  $n=9$ , female:  $n=5$ , age:  $55 \pm 5$  yr). + and - indicates an increase and decrease of the emission rate, respectively.

Volatile organic compounds	CAS number	Direction of change by blackcurrant	Effect size	P-value
<b>Aldehydes</b>				
2-Nonenal	2463-53-8	(-)	-0.87	0.030
Isovaleraldehyde	590-86-3	(-)	-0.75	0.093
Benzaldehyde	100-52-7	(-)	-0.96	0.040
Hexanal	66-25-1	(-)	-0.81	0.062
<b>Ketones</b>				
2-Hexanone	591-78-6	(-)	-0.86	0.031
6-Methyl-5-hepten-2-one	110-93-0	(-)	-1.23	0.005
2-Pentanone	107-87-9	(-)	-0.71	0.088
<b>Carboxylic acid</b>				
Acetic acid	64-19-7	(-)	-1.26	0.006
Heptanoic acid	111-14-8	(+)	0.73	0.065
<b>Cyclic esters</b>				
$\gamma$ -octanolactone	104-50-7	(+)	0.98	0.023
$\gamma$ -decanolactone	706-14-9	(+)	1.11	0.015
$\gamma$ -nonanolactone	104-61-0	(+)	0.62	0.063
<b>Sulphur containing compounds</b>				
Allyl methyl sulfide	10152-76-8	(-)	-1.01	0.015

Table 2. Volatile organic compounds emitted by the skin (dermal emission flux in  $\text{ng}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) that were not changed ( $P>0.05$ ) by 7-day intake of New Zealand blackcurrant powder in middle aged and older adults ((male:  $n=9$ , female:  $n=5$ , age:  $55\pm 5$  yr). Cohen's  $d$  effect sizes were trivial ( $d < 0.2$ ), small ( $d = 0.2-0.49$ ) or moderate ( $d = 0.5-0.79$ ). Data are mean $\pm$  SD.

Volatile organic compounds	CAS number	Control	New Zealand blackcurrant	Effect size (Cohen's $d$ )	P-value
<b>Alcohols</b>					
1-Propanol	71-23-8	2.56 $\pm$ 1.81	2.74 $\pm$ 1.63	0.11	0.37
1-Butanol	71-36-3	5.27 $\pm$ 2.69	4.84 $\pm$ 2.72	-0.16	0.68
1-Pentanol	71-41-0	7.63 $\pm$ 4.62	6.52 $\pm$ 4.70	-0.24	0.56
1-Hexanol	111-27-3	3.77 $\pm$ 1.70	3.38 $\pm$ 1.65	-0.23	0.58
1-Heptanol	111-70-6	1.97 $\pm$ 1.21	2.97 $\pm$ 2.07	0.59	0.16
1-Octanol	111-87-5	4.98 $\pm$ 3.63	6.68 $\pm$ 6.03	0.34	0.39
1-Nonenal	143-08-8	3.56 $\pm$ 1.82	4.64 $\pm$ 3.06	0.43	0.31
1-Decanol	112-30-1	6.99 $\pm$ 4.73	7.16 $\pm$ 3.23	0.04	0.91
2-Ethyl-1-hexanol	104-76-7	0.07 $\pm$ 0.12	0.12 $\pm$ 0.20	0.30	0.19
Acetoin	513-86-0	2.40 $\pm$ 2.26	1.73 $\pm$ 1.15	-0.37	0.37
<b>Aldehydes</b>					
Acetaldehyde	75-07-0	1.78 $\pm$ 0.13	1.84 $\pm$ 0.12	0.48	0.28
Propanal	123-38-6	2.35 $\pm$ 0.91	2.54 $\pm$ 0.67	0.24	0.59
Butanal	123-72-8	1.42 $\pm$ 1.08	1.21 $\pm$ 0.47	-0.25	0.45
Valeraldehyde	110-62-3	1.05 $\pm$ 0.64	0.93 $\pm$ 0.42	-0.22	0.61
Heptanal	111-71-7	5.52 $\pm$ 2.85	5.36 $\pm$ 3.16	-0.05	0.90
Octanal	124-13-0	2.25 $\pm$ 1.52	2.59 $\pm$ 1.64	0.22	0.60
Nonanal	124-19-6	6.24 $\pm$ 3.28	6.83 $\pm$ 5.11	0.14	0.74
Decanal	112-31-2	4.11 $\pm$ 3.45	5.88 $\pm$ 4.57	0.44	0.29
2-Hexenal	505-57-7	2.38 $\pm$ 1.18	1.96 $\pm$ 2.07	-0.25	0.53
Vanillin	121-33-5	1.20 $\pm$ 0.90	0.90 $\pm$ 0.85	-0.34	0.36
<b>Carboxylic acids</b>					
Propanoic acid	79-09-4	4.55 $\pm$ 3.08	4.44 $\pm$ 2.46	-0.04	0.91
Butanoic acid	107-92-6	3.60 $\pm$ 2.28	4.35 $\pm$ 1.72	0.37	0.39
Isovaleric acid	503-74-2	1.62 $\pm$ 0.94	1.40 $\pm$ 0.90	0.24	0.59
Valeric acid	109-52-4	1.32 $\pm$ 0.95	0.81 $\pm$ 0.61	-0.64	0.23
Hexanoic acid	142-62-1	1.96 $\pm$ 1.10	1.53 $\pm$ 0.89	-0.43	0.26
Octanoic acid	124-07-2	1.27 $\pm$ 1.12	1.31 $\pm$ 0.89	0.04	0.91
Nonanoic acid	112-05-0	3.65 $\pm$ 2.09	3.60 $\pm$ 2.19	-0.02	0.85
Decanoic acid	334-48-5	1.61 $\pm$ 0.95	1.96 $\pm$ 1.89	0.23	0.58
<b>Ketones</b>					
Acetone	67-64-1	1.42 $\pm$ 0.54	1.47 $\pm$ 0.43	0.10	0.59
2-Butanone	78-93-3	1.81 $\pm$ 0.75	1.74 $\pm$ 0.60	-0.10	0.83
2-Heptanone	110-43-0	1.43 $\pm$ 1.05	1.24 $\pm$ 1.16	-0.17	0.69
2-Octanone	111-13-7	1.42 $\pm$ 1.04	1.54 $\pm$ 1.03	0.12	0.72
2-Nonanone	821-55-6	1.59 $\pm$ 1.31	2.23 $\pm$ 2.18	0.36	0.35
2-Decanone	693-54-9	2.39 $\pm$ 1.35	2.50 $\pm$ 2.21	0.06	0.88
2-Undecanone	112-12-9	3.27 $\pm$ 1.77	2.59 $\pm$ 1.94	-0.37	0.23
2-Dodecanone	6175-49-1	0.97 $\pm$ 0.57	0.74 $\pm$ 0.62	-0.39	0.32
2-Tridecanone	593-08-8	1.76 $\pm$ 0.99	1.16 $\pm$ 1.21	-0.54	0.72
2-Tetradecanone	2345-27-9	1.76 $\pm$ 1.31	1.43 $\pm$ 0.93	-0.29	0.36
2-Pentadecanone	2345-28-0	2.67 $\pm$ 1.64	2.05 $\pm$ 1.23	-0.43	0.35

<b>Acid esters</b>					
Ethyl acetate	141-78-6	3.47 ± 2.27	5.12 ± 5.23	0.41	0.33
Benzyl acetate	140-11-4	0.11 ± 0.08	0.09 ± 0.08	-0.25	0.50
cis-3-Hexenyl acetate	3681-71-8	1.89 ± 1.89	1.52 ± 0.66	-0.26	0.38
<b>Benzyl hydrocarbons</b>					
Ethyl benzene	100-41-4	0.33 ± 0.72	0.15 ± 0.12	-0.35	0.32
m,p-Xylene	106-42-3 108-38-3	0.28 ± 0.77	0.07 ± 0.06	-0.39	0.34
o-Xylene	95-47-6	0.20 ± 0.16	0.24 ± 0.16	0.25	0.55
Styrene	100-42-5	0.29 ± 0.44	0.14 ± 0.20	-0.44	0.28
<b>Halogen containing volatile</b>					
p-Dichlorobenzene	106-46-7	0.04 ± 0.03	0.03 ± 0.04	-0.28	0.79
<b>Indoles</b>					
Indole	120-72-9	0.28 ± 0.29	0.33 ± 0.20	0.20	0.59
Skatole	83-34-1	0.09 ± 0.06	0.07 ± 0.04	0.20	0.34
<b>Terpenes</b>					
α-Pinene	2437-95-8	0.45 ± 0.46	0.35 ± 0.32	-0.25	0.56
β-Pinene	127-91-3	0.26 ± 0.17	0.23 ± 0.15	-0.19	0.46
d,l-Limonene	5989-27-5 5989-54-8	0.51 ± 0.64	0.27 ± 0.22	-0.50	0.20
<b>Sulfur compounds</b>					
Methyl mercaptan	74-93-1	2.40 ± 1.70	3.34 ± 3.07	0.38	0.25
Diallyl disulfide	2179-57-9	1.67 ± 1.23	1.49 ± 0.95	-0.16	0.68
<b>Cyclic esters</b>					
γ-Hexanolactone	695-06-7	0.47 ± 0.41	0.46 ± 0.54	-0.02	0.91
γ-Heptanolactone	105-21-5	0.26 ± 0.20	0.54 ± 0.86	0.45	0.35
γ-Undecanolactone	104-67-6	0.25 ± 0.19	0.21 ± 0.18	-0.22	0.61
<b>Other</b>					
Geosmin	19700-21-1	0.19 ± 0.11	0.20 ± 0.17	0.07	0.80
1,3-Butanediol	107-88-0	2.43 ± 1.11	2.84 ± 1.21	0.35	0.37
Phenol	108-95-2	0.64 ± 0.46	0.57 ± 0.33	0.18	0.63
Butylated hydroxytoluene	128-37-0	0.67 ± 0.27	0.85 ± 0.58	0.40	0.34

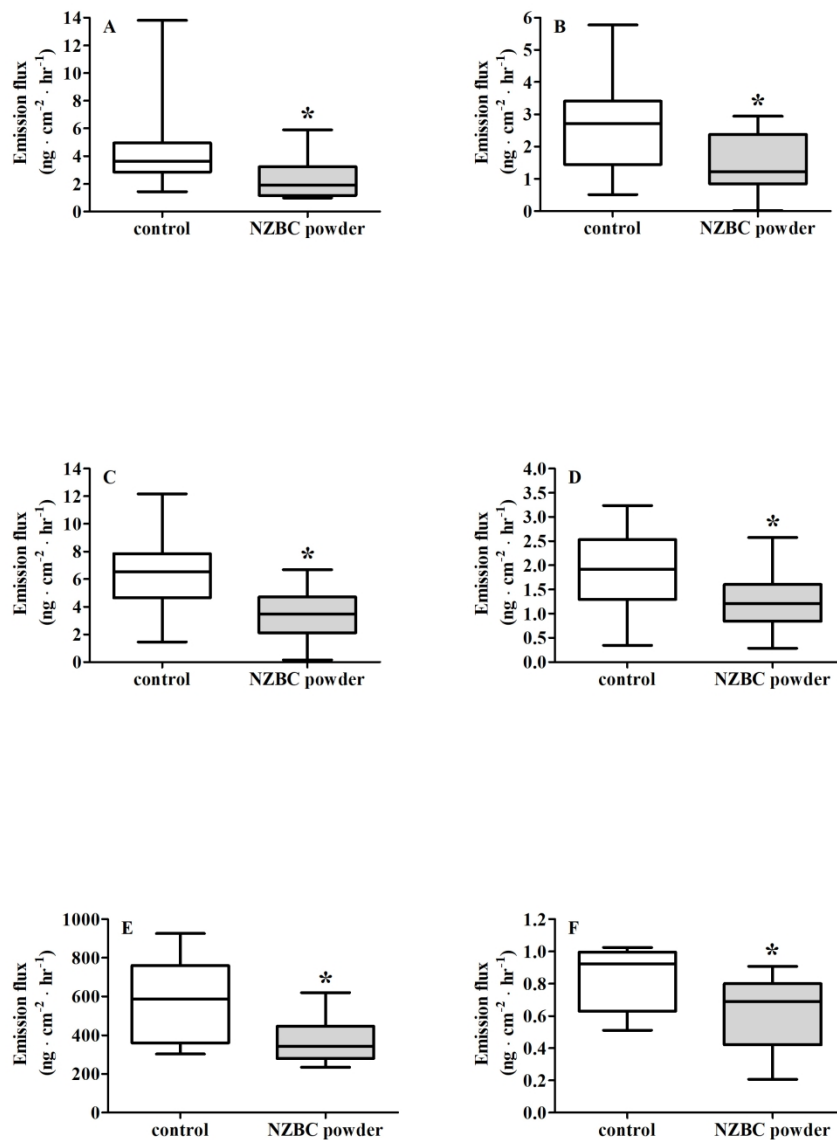


Figure 1. Box-and-whisker plot for emission flux of the skin volatile organic compound 2-nonenal (A), benzaldehyde (B), 6-methyl-5-hepten-2-one (C), 2-hexanone (D), acetic acid (E) and allyl methyl sulfide (F). New Zealand blackcurrant (NZBC) powder. Intake of New NZBC powder was for 7 days. \*, emission flux was lower with NZBC powder ( $P < 0.05$ ). Data of 14 participants.

190x242mm (300 x 300 DPI)



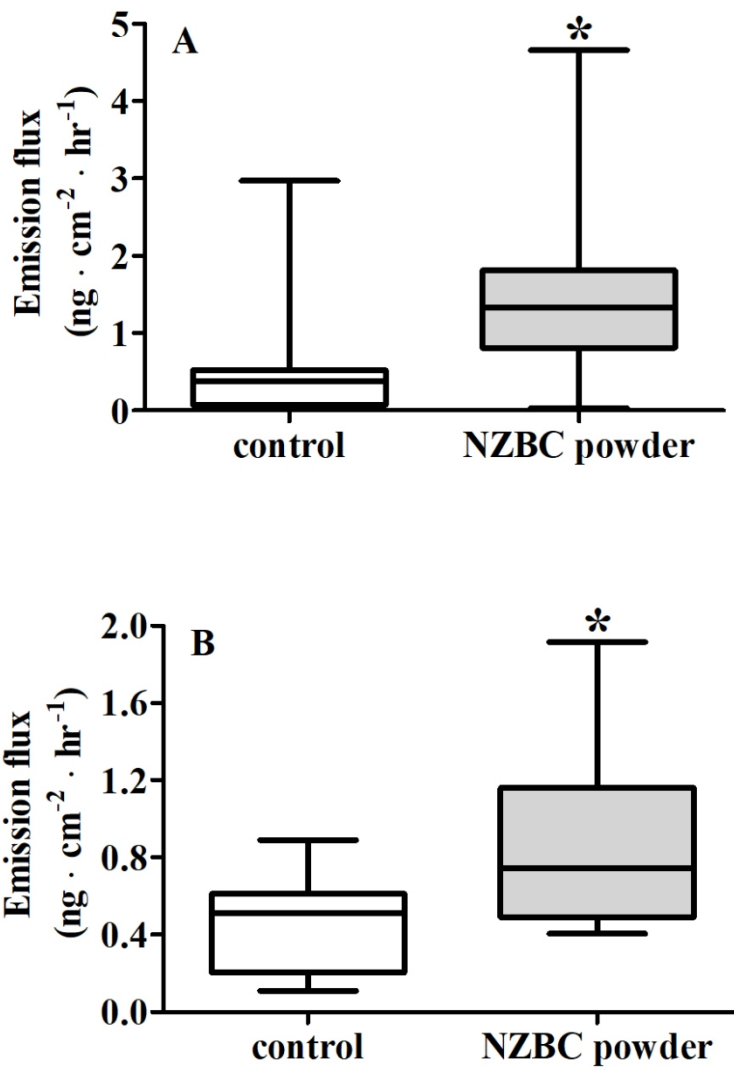


Figure 2. Box-and-whisker plot for emission flux of the skin volatile organic compound  $\gamma$ -octanolactone (A) and  $\gamma$ -decanolactone (B). Intake of New Zealand blackcurrant powder (NZBC) was for 7 days. \*, emission flux was higher with NZBC powder ( $P < 0.05$ ). Data of 14 participants.

95x120mm (300 x 300 DPI)