Original article

The impacts of germinating organic wheat: effects on phytic acid, resistant starch, and functional properties of flour, and sensory attributes of sourdough bread

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Summary

This study assessed the impact of germination duration on two varieties of wheat grown organically (Expresso and Syngenta SY Basalt (Basalt)). In Expresso, α-glucose content increased from 0.06 g/100 g in the control to 0.55 g/100 g after a 24-h germination period \((P < 0.05)\). In both varieties, similar increases were observed for sucrose and maltose, and no pattern emerged for resistant starch or phytate. In Basalt, baseline phytate (0 h 1.07 g/100 g) did not differ from treatment groups (6 h 0.97 g/100 g; 12 h 0.98 g/100 g; 24 h 1.09 g/100 g; 36 h 0.98 g/100 g). Functional properties of flour decreased with germination duration and resulted in bread which panelists perceived to have inferior crumb (24 h Expresso; 36 h Basalt; \(P < 0.05\)). However, there was no difference in the overall acceptability of breads across treatments and inclusion of germinated wheat improved bread sweetness.

Keywords

Bread, carbohydrates, consumer acceptability studies, dough, phytates, resistant starch, wheat.

Introduction

There is growing consumer interest in organic foods and products made with germinated grains (Crawford, 2015; Brissette, 2016; McNeil, 2020). Interest in these sectors is growing because of the perceived health benefits associated with organic production and germination. While it has been previously suggested that breads made from germinated grains have superior nutrient profiles, sensory attributes and/or functional characteristics, these attributes have not been assessed in organically produced wheat and bread (Hubner & Arendt, 2013; Nelson et al., 2013; Liu et al., 2017). Additionally, there is no standard method of germination which achieves nutritional, sensory and functional benefits simultaneously.

The role that agricultural production practices play in modifying the effect of wheat germination compared to other variables is unclear. The effect of germination on wheat may vary between baseline chemical composition of wheat than on other variables including temperature and steeping conditions (Shafqat, 2013; Poudel et al., 2019).

Organic farming follows standards such as the restriction of synthetic fertilizer application. This can lead to differences in the baseline chemical composition of wheat grown organically and conventionally. Wheat grown conventionally tends to have higher phytic acid content, and lower protein and starch digestibility compared to organically grown wheat (Nitika & Khetarpaul, 2008). Farm yard manure application, associated with organic practices, can lead to increases in sucrose and decreases in maltose content compared to conventionally fertilized wheat (Shewry et al., 2018). These differences might lead to weaker dough for organic bread baking (Annett et al., 2007).

The application of germination for organic bread wheat could have important health implications, as bread is a major staple (Eicher-Miller & Boushey, 2017). Inclusion of germinated grains in whole wheat bread could positively impact flavour, resistant starch (RS) content and the bioaccessibility of micronutrients (Liu et al., 2017; Swieca et al., 2017; Lemmens et al., 2018). These outcomes represent areas of clinical significance in a standard western diet which is lacking in fibre, high in refined sugar, and associated with an increased risk of cardiovascular disease (Dietary Guidelines Advisory Committee, 2020). However, addition of germinated wheat can lead to issues in dough functionality and possibly consumer acceptance (Hefni & Witthoff, 2011). Therefore, there is a need to measure the effects of different germination conditions and develop optimal germination procedures for wheat grown using organic methods.

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To address the uncertainties of the impact of germination on wheat grown organically, we explored the changes in free sugar, starch and phytic acid content, and functional and sensory attributes associated with germinating two varieties (Expresso and Syngenta SY Basalt) of hard red spring wheat (Triticum aestivum L.). We tested grains at four different germination durations (6, 12, 24 and 36 h) and compared these to a control non-germinated wheat (0 h). We hypothesized that free sugar and RS content would increase with germination duration, and phytic acid and starch content would decrease. Additionally, we hypothesized that farinograph measures and sensory perceptions would be negatively correlated with germination duration. This is the first study to assess the impact of germination duration on wheat grown with organic practices for commercially germinated wheat flour used in whole wheat sourdough bread production.

Materials and methods

Materials

Wheat

Two varieties of organic hard red spring wheat, 'Expresso' and 'Syngenta SY Basalt', herein referred to as Basalt, were harvested at Cascade Organic Farms in the spring of 2018 (Royal City, WA, USA). At harvest, the moisture, protein and ash content was 8.94%, 15.05% and 1.80% for Expresso and 8.96%, 15.15% and 1.79% for Basalt, respectively. For analytical tests, wheat was milled to < 0.6 mm (Perten Laboratory Mill 3100; Perten Instruments, Klaaukkala, Finland). Wheat for baking was milled separately to < 0.6 mm (Mock Mill 200; Mock Mill, Fairfield, IA, USA). All milled material was mixed to make a homogenous whole wheat flour for both baking and analytical tests. Flour was stored in plastic containers at room temperature (18 ± 2 °C) for no more than 2 days before analysis and baking.

Chemicals

Amyloglucosidase, pancreatic alpha-amylase, glucose oxidase plus peroxidase and 4-aminoantipyrine, hexokinase plus glucose-6-phosphate dehydrogenase, betafructosidase, alpha-glucosidase, phytase and alkaline phosphatase were purchased from Megazyme (Wicklow, Ireland). Other chemicals used were of analytical grade.

Germination

Wheat was germinated and dried using a pilot malting machine developed and built by Skagit Valley Malting (Burlington, WA, USA). The machine was cylindrical, fully contained and rotated on a stepwise basis one quarter turn every 80 s during steeping and germination. Before steeping, all treated wheat was rinsed and cleaned with water. Wheat (133 kg/treatment) was steeped in filtered tap water for 9 h at 16 ± 2 °C. Next, water was drained, and seeds were left in the 16 ± 2 °C chamber at 95 ± 2% relative humidity for the predetermined treatment time of 6, 12, 24 and 36 h. Drying length was between 10 and 14 h with a maximum temperature of 60 °C. The length of time for drying was based on the temperature of the grain measured using an infrared thermometer (Etekcity Lasergrip 800). When the grain reached 37 ± 2 °C, the drying process was complete. Control wheat (0 h) did not receive any form of steeping or germination.

Hagberg falling number test and farinograph

Falling number (FN) was determined using the AACC International Method 56-81.04 with an FN 1500 System (Perten Instruments, Huddinge, Sweden). Flour sample weight was corrected based on the standard 7 g sample at 14% moisture content. Water absorption, development time, stability, degree of softening and dough quality number were measured using a Brabender Farinograph-AT (Duisburg, Germany) according to AACC Method 54-21.02 for constant flour weight procedure.

Physiochemical analysis

Enzymatic kits (Megazyme) were used for the determination of D-glucose, sucrose and maltose (K-Masug et al., 1988; Champ et al., 1990; McKie & McCleart, 2016). Briefly for sugars, flour (100 mg) was homogenized in water (10 mL) and centrifuged. Enzymes were added to hydrolyse and phosphorylate maltose and sucrose. Nicotinamide adenine dinucleotide phosphate was stoichiometric with each sugar and was measured at 340 nm using a spectrophotometer (Shimadzu UV-1601 UV/VIS). Briefly for starch, flour (100 mg) was homogenized in pancreatic alpha-amylase containing amyloglucosidase (4 mL) and shaken at 37 °C for 16 h to simulate digestion. Starch was hydrolysed, and solubilized starch was removed from the sample. Solubilized and RS content were determined using spectrophotometry. Briefly for phosphorus and phytic acid, flour (1 g) was extracted using hydrochloric acid and molybdenum blue was quantified spectrophotometrically.

Bread preparation

Before baking, flour was assigned a random code to blind the baker to the treatment of the flour. Bread
loaves were made in duplicate with whole wheat flour (500 g), salt (11.18 g), sourdough starter (43 g), water (430 g) at 24 ± 2 °C and levain (546 g). Ingredients were combined in the following order. First, the levain was formed by combining flour (260 g) with sourdough starter (26 g) dissolved in water (260 g) at 24 ± 2 °C. Next, flour (500 g) was combined with water (430 g) and left to sit covered for a 20 min autolyse period at 23 ± 2 °C. Then, levain, sourdough starter and salt were kneaded into the autolyse and mixed by hand for 2 min. Fermentation continued 2 h with 2 sets of folding, ~40 min apart. The dough was then divided and shaped before retarding. Retarding lasted 17 h at 10 °C. Finally, loaves were baked at 248 °C for 40 min in a deck oven (Matador Store, Werner and Pfeiderer, Germany) with 0.2 L steam for the first 10 min and rotation after 25 min.

**Sensory evaluation**

Sensory evaluation took place 2.5–4 h after cooling. Bread was sliced 1 cm thick. Nine untrained staff and students of the Washington State University Bread Lab, King Arthur Flour Baking School, and Skagit County Extension acted as evaluators. Evaluators were blinded to bread treatment and given brief instructions and definitions for assessing bread characteristics. Overall appearance, aroma, taste and acceptability; and texture of the crust and crumb were assessed on a 9-point hedonic scale according to Stone & Sidell (2004). Overall acceptability was described to staff evaluators as the combination of all variables assessed. Qualitative data were collected through comments listed on the survey.

**Statistical analysis**

Data were analysed in Stata/IC version 15.1 (TX, USA). One-way analysis of variance (ANOVA) with post hoc Bonferroni test was used to assess differences in means with a significance level of $P < 0.05$.

**Results and discussion**

**Effect of germination duration on dough functionality**

**Falling number**

Falling number is inversely associated with germination duration; this is because germination promotes amylolytic activity which leads to starch degradation and lower flour viscosity (Singh et al., 2001; Olaerts et al., 2016; Ding et al., 2018). In this study, FN decreased with germination duration, but with different magnitudes for each variety. This follows the convention that variety is an important modifier of the impacts of germination (Olaerts et al., 2016). Like the findings reported in Ding et al. (2018), the association between germination duration and FN can be characterized by an inverse curvilinear relationship (Fig. 1). This relationship is illustrated using second-order polynomial equations with high coefficients of determination ($R^2$-squared $> 0.93$).

For optimal bread production, FN should be between 200 and 300 s (Kweon, 2010). Dough reached ideal viscosity during the 6 h (240 s; Expresso) and 12-h treatment (275 s; Basalt). These germination durations were shorter than Poudel et al. (2019), who achieved ideal viscosity after a 24-h germination period. It is likely that our flour reached ideal FN with a shorter treatment duration than Poudel et al. (2019) because our analyses exclusively use germinated flour whereas the results from Poudel et al. (2019) are based on composites of germinated and non-germinated flours. Our research suggests that exclusive use of germinated flour is functional for bread baking and potentially requires shorter germination duration treatments compared to composites.

**Farinograph**

Germinating wheat is known to reduce dough stability and lead to poor rheological properties, because the starch-gluten matrices of germinated wheat are weaker and have a lower capacity to bind water (Hefni & Witthöft, 2011; Liu et al., 2017; Ding et al., 2018; Poudel et al., 2019).

As anticipated, peak time and dough stability decreased with germination length (Table 1). However, Basalt had a peak time and stability nearly double that of Expresso. Similar findings of decreased stability and peak time were reported by Hefni & Witthöft (2011) and Liu et al. (2017), which looked at dough with increasing portions of germinated flour. Decreases in protein due to proteolytic activity in germinated flour is the most plausible explanation for these observations (Nelson et al., 2013; Simsek et al., 2014). The protein content of our varieties was normal to high – our findings did not differ greatly from previous research on conventionally grown wheat and in some treatments had comparably greater peak times and stability. However, it should be noted that dough made from organic wheat flour might be weaker than dough made from conventional wheat because organic farming can lead to increases in protein digestibility (Annett et al., 2007; Nitika & Khetarpaul, 2008).

In both varieties, the 36-h treatment had the lowest dough quality; however, because of the differences across varieties, the dough quality of the 36 h Basalt (104 mm) was superior to the control of Expresso (102 mm; Table 1). These results suggest that Expresso was more reactive to germination.

Water absorption decreased with germination duration in both varieties. These findings are consistent.
with previous research (Kaur et al., 2002; Baranzelli et al., 2018).

Effect of germination time on carbohydrate composition

Starch content

As anticipated, total starch content decreased in Expresso after 24 h of germination (Table 2). In this study, starch degradation occurred at a longer germination duration than that reported by Hung et al. (2012) (12 h) and a shorter duration than Grassi et al. (2018) (72 h). This discordance may be related to differences in baseline chemical compositions of wheat, or the lower temperature of germination used in this study. The baseline starch content for the controls in Hung et al. (2012) and Grassi et al. (2018) was 62.4 and 66.3 g/100 g, respectively, compared to 53.28 and 50.52 g/100 g (Expresso; Basalt) in this study.

There was no detectable change in starch composition of Basalt across all measured starch components. While there were changes in free sugar content and FN, both indicators of starch transformation, these were not on a large enough scale to be observable in starch. This follows the notion that the Basalt variety was less reactive to germination compared to Expresso – a theme which is reaffirmed across functionality, sugar and sensory assessments and suggests that baseline chemical composition, even across organic

Table 1 Farinograph readings from control and germinated dough in two organic wheat varieties (Expresso and Basalt)

| Variety | Treatment, h | Peak time, m:ss | Consistency, BU | Water absorption, % | Stability, m:ss | DS/ICC, BU | Quality number, mm |
|---------|--------------|----------------|-----------------|---------------------|-----------------|------------|-------------------|
| Expresso | 0            | 4:44           | 504             | 68.9                | 8:15            | 29/45      | 102               |
|         | 6            | 4:22           | 517             | 69.4                | 7:00            | 34/62      | 91                |
|         | 12           | 4:21           | 521             | 69.4                | 6:06            | 42/71      | 82                |
|         | 24           | 3:05           | 514             | 67.3                | 2:29            | 121/175    | 44                |
|         | 36           | 3:14           | 519             | 67.2                | 2:59            | 85/121     | 48                |
| Basalt  | 0            | 6:58           | 522             | 67.3                | 15:04           | 7/37       | 166               |
|         | 6            | 7:10           | 504             | 67.4                | 15:56           | 6/36       | 168               |
|         | 12           | 6:12           | 490             | 67.4                | 14:58           | 8/36       | 159               |
|         | 24           | 5:57           | 503             | 64.6                | 11:12           | 19/51      | 121               |
|         | 36           | 5:40           | 499             | 64.5                | 8:52            | 27/60      | 104               |

BU, Brabender Units; DS/ICC, Degree of Softening by the International Code Council, Degree of Softening 10 m after beginning/ICC, 12 m after max; h, hours; m:ss, minutes:seconds; mm, millimetres.

Values are from a single reading.
Varieties, plays a role in shaping the impacts of germination.

While increases in RS content could yield important health benefits and have been observed in other studies (Swiec et al., 2017), we found no significant changes in RS, similar to wheat sprouted in the field as reported by Noda et al. (2004). It should be noted that for some Expresso treatments, there were large standard deviations. This was most likely a result of variability across the individual assays from the wheat itself (i.e. grain harvested from different parts of the field), the assay procedure and/or the heterogeneity of germination.

Nonsignificant changes in RS suggest that the benefits from high RS products are not realized under these germination conditions. This could be related to the atmosphere and temperature of germination or the organic agricultural practices. Altering germination conditions, however, might lead to improvements in RS.

\( \delta \)-glucose, sucrose and maltose content

In the early stages of germination, sucrose is the predominant sugar, and as starch is broken down glucose and maltose become more prominent (Aoki et al., 2006). We found similar patterns of sugar emergence with maltose predominating in the 24- and 36-h treatments.

We found a significant increase in free sugar content over time in each variety \((P < 0.5)\). Sugar content peaked in the 24 (Expresso) and 36-h treatment (Basalt; Table 2). At 24 h in Expresso, the \( \delta \)-glucose and sucrose contents were 10-times greater than the control. At 36 h in Basalt, \( \delta \)-glucose, sucrose and maltose were 6.4, 6.4 and 30.5 times greater than the control, respectively. These results are higher than those observed by Ding et al. (2018) who identified a 3-fold increase in glucose content using a 24-h germination period. Our methods used a longer steeping period (9 h instead of 6 h) which could account for differences.

These findings are relevant to food processing and nutrition as many whole wheat breads contain added sugar. New labelling standards in the United States will require manufacturers to label added sugars; however, with this process sugars would be considered natural (FDA, 2016). This increase in sugar was associated with an improvement in sweetness, a result that could lead to greater acceptance of whole wheat bread with limited or no added sugar.

Effect of germination time on phytate composition

Using a 17-h steep and germinating from 6 to 120 h, Lemmens et al. (2018) observed a linear increase in phytase activity. This increase was associated with a decrease in phytate; however, phytate reduction was

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**Table 2.** Carbohydrate and phosphorus composition with increasing germination duration.

| Variety | Treatment h | Free sugars (g/100 g) | Starch (g/100 g) | Total phosphorus | Phytic acid | D-glucose | Sucrose | Maltose | RS | SS | Total starch |
|---------|-------------|-----------------------|-----------------|------------------|-------------|-----------|---------|---------|-----|----|-------------|
| Expresso | 0           | 0.06 ± 0.02abc        | 52.28 ± 0.97abc | 0.06 ± 0.02abc   | 0.09 ± 0.03abc | 0.99 ± 0.03abc | 0.45 ± 0.04 | 0.11 ± 0.01abc | 0.07 |
|          | 12          | 0.11 ± 0.04abc        | 51.83 ± 1.27abc | 0.07 ± 0.02abc   | 0.08 ± 0.02abc | 0.98 ± 0.07abc | 0.46 ± 0.05  | 0.12 ± 0.01abc | 0.08 |
|          | 24          | 0.12 ± 0.06abc        | 51.38 ± 1.9abc  | 0.07 ± 0.01abc   | 0.10 ± 0.01abc | 0.91 ± 0.06abc | 0.47 ± 0.06  | 0.13 ± 0.01abc | 0.19 |
| Basalt   | 0           | 0.03 ± 0.01abc        | 53.28 ± 1.19abc | 0.08 ± 0.03abc   | 0.11 ± 0.03abc | 0.96 ± 0.06abc | 0.49 ± 0.07  | 0.10 ± 0.01abc | 0.04 |
|          | 12          | 0.10 ± 0.06abc        | 52.72 ± 1.96abc | 0.05 ± 0.02abc   | 0.09 ± 0.02abc | 0.98 ± 0.06abc | 0.44 ± 0.05  | 0.10 ± 0.01abc | 0.03 |
|          | 24          | 0.12 ± 0.06abc        | 52.15 ± 2.69abc | 0.05 ± 0.02abc   | 0.08 ± 0.02abc | 0.96 ± 0.06abc | 0.43 ± 0.05  | 0.12 ± 0.01abc | 0.05 |

Values are mean ± SD of triplicate readings of 3 assays (varies based on treatment group and nutrient). Within each variety, values in a column with different letters are significantly different \((P < 0.05)\) and values in a column with no letters are not statistically different from one another.
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Table 3 Sensory attributes rated on a 9-point hedonic scale of bread made from two organic wheat varieties with varying germination length

| Variety | Treatment, h | Appearance | Crust | Cumb | Aroma | Taste | Overall acceptability |
|---------|--------------|------------|-------|------|-------|-------|-----------------------|
| Expresso | 0 | 7.17 ± 1.15 | 7.00 ± 1.14 | 7.83 ± 1.04*a | 6.94 ± 1.21 | 7.33 ± 0.84 | 7.33 ± 0.91 |
| | 6 | 7.11 ± 0.90 | 7.06 ± 1.00 | 7.56 ± 1.20*a | 6.67 ± 0.91 | 7.00 ± 1.19 | 7.28 ± 0.89 |
| | 12 | 7.44 ± 1.29 | 7.11 ± 1.23 | 7.28 ± 1.23ab | 7.00 ± 1.37 | 7.17 ± 1.20 | 7.22 ± 1.35 |
| | 24 | 6.88 ± 1.76 | 6.67 ± 1.68 | 6.11 ± 1.64*c | 6.94 ± 1.09 | 6.94 ± 1.39 | 6.89 ± 1.28 |
| | 36 | 7.22 ± 1.56 | 7.11 ± 1.45 | 6.72 ± 1.07ab | 7.11 ± 1.32 | 7.27 ± 1.36 | 7.11 ± 1.28 |
| Basalt | 0 | 6.72 ± 1.49 | 6.72 ± 1.13 | 6.78 ± 1.40*a | 6.11 ± 1.08 | 5.88 ± 1.54 | 6.22 ± 1.35 |
| | 6 | 7.29 ± 1.34 | 6.28 ± 1.67 | 7.06 ± 1.89*a | 6.72 ± 1.32 | 6.06 ± 1.60 | 6.44 ± 1.54 |
| | 12 | 6.94 ± 1.43 | 6.61 ± 2.12 | 6.78 ± 1.73*b | 6.56 ± 1.34 | 6.22 ± 1.56 | 6.50 ± 1.58 |
| | 24 | 6.72 ± 1.18 | 6.33 ± 1.91 | 6.06 ± 2.07ab | 6.59 ± 1.46 | 6.82 ± 1.55 | 6.50 ± 1.58 |
| | 36 | 6.22 ± 1.86 | 6.12 ± 1.62 | 4.61 ± 2.40*a | 6.72 ± 1.18 | 6.22 ± 1.63 | 5.17 ± 2.31 |

Values are mean ± SD ratings of nine testers evaluating each bread twice. Values within the same variety and column with different letters are significantly different (P < 0.05) and values in a column with no letters are not statistically different from one another.

not as substantial as anticipated. Higher temperatures and longer germination periods of up to 120 h seem to be associated with the highest reductions in phytate (Azeke et al., 2011).

We looked at phytate content after a 9-h steep and 6- to 36-h germination period and observed no clear pattern in phytate change across treatments in either variety. There were some differences across pairwise comparisons in Expresso, however no difference between the treatments and control. Due to insignificant changes in phytic acid concentration, germination as performed might not improve bioaccessibility of micronutrients. Longer germination duration is most likely necessary to impact phytate content; however, this must be balanced with dough quality and sensory perception.

Effect of germination on sensory characteristics

We observed a positive relationship between germination duration and free sugar content. This suggests that breads made from longer germinated flour may be sweeter. However, longer germination durations were also associated with poor rheological qualities which could impact perception of bread.

There were no differences in the sensory characteristics scores across germination treatments, except for in the assessment of crumb (Table 3). This is consistent with Charoenthaikij et al. (2010) who also found no statistically significant differences in the sensory perception of bread made with and without germinated flour and reported nonsignificant improvements in sensory perception, specifically overall likeness. However, this is contrary to Adebiyi et al. (2017) and Liu et al. (2017) who found significant improvements in flavour and overall acceptability with the inclusion of increasing proportions of germinated or malted flour.

In qualitative assessments, panelists described Expresso 36 h as the sweetest but gummier; the 6, 12, and 24 h had a good flavour but the 12 h had the best texture; and the control was dry and plain tasting. For Basalt, panelists described the 24- and 36-h breads as gummy and sweet; the control was dry with an unpleasant taste; and the 12 h had the best flavour and texture. Overall, the panelists preferred the Expresso to Basalt. They described Expresso as having a better overall flavour, while the taste of Basalt in the control and 6-h treatment was described to be like cardboard.

Conclusion

We tested nutritional, functional and sensory impacts of germinating wheat for increasing durations to understand the impact on wheat grown using organic practices. The positive relationships between D-glucose, sucrose and maltose and germination duration led to increases in perceived sweetness. This suggests that inclusion of germinated flour could limit the need for added sugar and whole wheat bread made from germinated grains might be more palatable than whole wheat bread without germinated wheat. The perceived health benefits of germinated products, related to RS and phytic acid, were not achieved under these conditions, and the rheological characteristics were all negatively associated with germination duration. Poor rheological characteristics affected sensory perception of crumb. However, there was no difference in overall acceptability across germination treatments, showing that sourdough bread made with organic germinated whole wheat flour would be acceptable to consumers.

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**Conflict of interest**

The authors declare no conflict of interest.

**Author contributions**

Alexandra L Stern: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (lead); Resources (supporting); Software (lead); Supervision (supporting); Validation (lead); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead). Julia Berstein: Conceptualization (supporting); Data curation (lead); Formal analysis (equal); Methodology (lead); Writing-original draft (supporting); Writing-review & editing (equal). Stephen S. Jones: Conceptualization (lead); Data curation (supporting); Funding acquisition (supporting); Investigation (lead); Methodology (lead); Project administration (lead); Writing-original draft (equal); Writing-review & editing (equal). Jeffrey B Blumberg: Data curation (equal); Methodology (equal); Supervision (lead); Writing-original draft (equal); Writing-review & editing (equal). Timothy S. Griffin: Conceptualization (equal); Formal analysis (supporting); Funding acquisition (lead); Methodology (equal); Supervision (lead); Writing-original draft (equal); Writing-review & editing (equal).

**Ethical approval**

Ethics approval was not required for this research.

**Peer review**

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**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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