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Controlled trial of cumulative behavioural effects of a common bread preservative*

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Objective: Many anecdotes and one scientific report describe cumulative behavioural effects of bread preservative on children.

Methodology: Twenty-seven children, whose behaviour improved significantly on the Royal Prince Alfred Hospital diet, which excludes food additives, natural salicylates, amines and glutamates, were challenged with calcium propionate (preservative code 282) or placebo through daily bread in a double-blind placebo-controlled crossover trial.

Results: Due to four placebo responders, there was no significant difference by ANOVA of weighted placebo and challenge Rowe Behaviour Rating Inventory means, but a statistically significant difference existed in the proportion of children whose behaviours 'worsened' with challenge (52%), compared to the proportion whose behaviour 'improved' with challenge (19%), relative to placebo (95% confidence intervals 14–60%).

Conclusions: Irritability, restlessness, inattention and sleep disturbance in some children may be caused by a preservative in healthy foods consumed daily. Minimizing the concentrations added to processed foods would reduce adverse reactions. Testing for behavioural toxicity should be included in food additive safety evaluation.

Key words: attention deficit hyperactivity disorder (ADHD); bread preservative; calcium propionate; children's behaviour; food additives.

The use of calcium propionate (preservative code 282) as a preservative in bread is now widespread throughout Australia and the USA, is increasing in the UK and is rare in Europe. It is sometimes added in a natural form as whey powder in brands that advertise 'no artificial preservatives'.[1] Alternatives to the use of calcium propionate in bread include cleaning factory equipment with vinegar to discourage mould growth, or allowing fresh loaves to cool before packing in plastic bags. Since 2000, calcium propionate has also been permitted in a wider range of food products in Australia and New Zealand. Unlike food dyes, in the finished product it is colourless, tasteless and odourless, and undetectable except by laboratory analysis. According to the Australian Food and Grocery Council, 'conventional scientific wisdom world-wide has not established a link between behavioural problems in children and calcium propionate used as a food preservative'.[2] Effects of calcium propionate were observed in 38% of 42 hyperactive children during a larger investigation of food intolerance.[3,4] Because of Darwin's humid tropical climate, bakers have used nearly the maximum concentrations of calcium propionate for some years, leading to many anecdotes of behavioural responses in children. The present study focuses on the cumulative effects of calcium propionate on children's behaviour.

The standard method of investigating adverse food reactions is the double-blind placebo-controlled food challenge.[5] This consists of an elimination diet during which foods likely to provoke a reaction are avoided, followed by the reintroduction or challenge of certain foods. The elimination diet that precedes challenge testing must be comprehensive. Failure to exclude all provoking substances may lead to incomplete recovery and inconclusive challenge results.[6] In 1973, Dr Ben Feingold proposed a relationship between dietary chemicals such as salicylates and colours and children's behaviour.[7] Controlled studies using the Feingold diet reported inconclusive results.[8,9] However, the original Feingold diet failed to adequately exclude natural salicylates,[10] and other chemicals that may provoke adverse reactions in sensitive individuals.[11] Studies that excluded a wider range of foods identified more dietary responders.[12–16]

Effects of food additives are dose related,[6,17,18] and cumulative.[11] These factors were overlooked until Swanson and Kinsbourne suggested that the unrealistically low dosage of the challenge item may have been a factor in the lack of response to challenges in previous studies.[19]

Rather than hyperactivity, the behaviour most affected by diet has been found to be mood, especially irritability.[12,14,15,18,20,21] These findings raise the issue of whether the traditional use of the Conners Abbreviated Parent–Teacher Questionnaire (APTQ), with its emphasis on hyperactivity, may account for some inconclusive results in earlier studies. The Rowe Behaviour Rating Inventory (RBRI) is validated for additive challenges,[22] and may be a more appropriate choice for diet-related studies. It focuses on the more subtle behavioural symptoms of irritability, restlessness, inattention and sleep disturbance, and provides 20 items, including 'demanding and argumentative', 'cannot concentrate on any particular task; easily distracted', 'restless; fidgety, can't sit still' and 'has difficulty settling down to sleep'.

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Despite previous assumptions that parents' ratings are less objective than teachers' ratings,[8,23] parents have subsequently been found to be reliable observers and raters of their children's behaviours.[18]

METHODS

A single-item double-blind challenge crossover design in which each child acted as his or her own control was employed. Challenges of real-life repeated doses of calcium propionate at the Australian maximum permitted level (2%) in four slices of bread per day for 3 days were used to investigate cumulative behavioural effects of this additive.

Consent

Informed consent was obtained from all parents and approval for the study was granted by the Royal Darwin Hospital Ethics Committee, Northern Territory Education Department and Northern Territory Catholic Education Office.

Stage 1: Open phase

During a 4-month period, 56 Northern Territory children whose parents were prepared to try dietary management for behavioural problems were referred to the present study by paediatricians. For entry to the study, children were required to score at the 85th percentile or higher on the RBRI, and all of the children met this requirement. All but three of the children were rated higher than 15 on the Conners APTQ, indicating attention deficit hyperactivity disorder (ADHD)-type behaviours.

The children followed an elimination diet for 3 weeks, unless there was a noticeable and significant improvement by the end of the second week. The Royal Prince Alfred Hospital (RPAH) elimination diet selected for the present study required exclusion of 50 additives in addition to natural salicylates, amines and glutamates.[3,24] Permitted foods included one fruit (pears), 30 vegetables, some fresh meats, chicken, fish, oils, margarine, eggs, wheat, oats, rice and some processed breakfast foods and snacks such as plain biscuits, potato chips and sweets. Some dairy products were permitted, unless there was a reason to suspect problems. White sugar and golden syrup were permitted; raw sugar and honey, which contain salicylates, were not. Families were provided with food lists,[24] recipes,[25] and suggestions for fussy eaters, school lunches, social occasions and behaviour management if necessary.[26]

After 2–3 weeks, RBRI scores were obtained again. The eligibility criteria for successful dietary intervention was a change in RBRI of 25 percentile points or more. All 33 children who finished the elimination diet before the end of the study exceeded these levels.

The first 27 eligible children to complete their elimination diets were invited to participate in the double-blind placebo-controlled trial of the bread preservative; one child per bread sample set. There were 20 boys and seven girls aged 4–12 years (mean 6.5 years), including five sets of siblings. Three children were on medication for ADHD.

Stage 2: Double-blind placebo-controlled trial

Bread for the double-blind, placebo-controlled trial was specially baked within a 10-hour period by a commercial bakery. Although all of the loaves looked and tasted identical, half of the loaves were preservative-free, while the other half contained the maximum permitted level of calcium propionate, as used in some commercial breads. Sets of loaves were sliced, packaged and coded by the second researcher (AR) according to a schedule utilizing random numbers and sealed envelopes. When packaging was complete, there were 27 complete sample sets of the 30 that had been ordered. Cartons of coded bread were frozen and stored in a commercial cold store. As each child joined the trial, he or she was allocated the next code from the list kept by the second researcher, who had no knowledge of or contact with families. The first researcher (SD) had no part in the coding of the bread or access to the code list. Corresponding coded frozen loaves were delivered by the first researcher on two successive Mondays. Families with two children in the study received separate coded samples clearly labelled with the name of each child. Neither the first researcher nor the families knew which bread contained preservatives.

Each child was instructed to eat four slices of bread a day for 3 days, starting on a Tuesday. Unused bread was removed when the next bread sample was delivered. Behaviour rating scales completed on the fourth day (Friday) covered the 3-day period including Thursday night.

After the completion of the trial, and before codes were broken, parents were asked to nominate which bread contained preservatives on the basis of their children's behaviour.

A significant change in behaviour was defined in advance as 25% or more of the weighted RBRI scales, comparing challenge to placebo.

When all 27 children had eaten both sets of coded bread, the study finished and the codes were broken. Families were offered ongoing support to complete other challenges as part of their dietary investigation.

RESULTS

The pre- and post-diet mean (standard deviation) RBRI percentiles were 95.0% (3.4%) and 31.1% (18.5%), respectively ($P < 0.01$). The pre- and post-diet mean (standard deviation) Conners APTQ raw scores were 22.5 (5.4) and 6.1 (3.4), respectively ($P < 0.01$). Children were rated by both parents and teachers, but teacher ratings were discarded when found to be incomplete due to teacher absenteeism and pupil moves.

A sample size of at least 25 children was estimated for an α of 0.05 and β of 0.8 for a two-tailed difference in means of at least 25%. The Epitable program of EPI-INFO version 6.04[27] was used for ANOVA, and the Confidence Interval Analyser[28] for differences in proportions.

Using the RBRI weighted scores, 14 children (52%) showed a significant increase ('worsened') with challenge. For eight children there was no change and for five children (19%) the behaviour scores on challenge reduced ('improved') significantly, compared to placebo. No children who responded to the challenge also responded to the placebo.

This difference (33%) in the proportion of children whose behaviours 'worsened' with challenge (52%) compared to the proportion whose scores 'improved' with challenge (19%),

relative to placebo is statistically significant (95% confidence intervals 14–60%).

When the difference in the means was compared by ANOVA between the weighted placebo and challenge RBRI scores, there was no significant difference: mean (variance) RBRI score challenge, 2.290 (0.808); placebo, 1.919 (0.762); $F = 1.88$, $P = 0.18$.

Out of the 23 parents who had nominated a particular bread sample as containing preservative, 17 were correct and six were incorrect ($P < 0.05$). Four parents had marked 'don't know'.

Challenge effects observed and not measured by the RBRI included loud voice, lethargy, 'growing pains', stomach aches, headaches and bedwetting or urinary urgency.

DISCUSSION

Although there was no group mean difference between scores under challenge and placebo conditions, the behaviour of a significantly greater proportion of children worsened with preservative 282 challenge than improved with challenge relative to placebo. The comparison of means included four strong placebo responders. As noted by others, examination of group data only for this kind of research often masks some important results, and a few strong placebo responders can have a large impact on statistical analysis for small-sample research.[12,17] Furthermore, group comparisons are inappropriate for situations in which only a proportion of the subjects are expected to respond. For instance, a population containing 30% of responders would require a sample of 265 subjects to be certain of finding a statistically significant difference 90% of the time.[17]

The RPAH elimination diet used in an open trial prior to the controlled trial obtained excellent results. Unlike the Few Foods diet which is effective,[13,15,29] but difficult,[13,15] the RPAH elimination diet is a workable method for families to ascertain which food chemicals affect their children.

Out of irritability, restlessness, inattention and sleep disturbance, the biggest effect was observed with irritability, supporting the results of other research.[12,14,15,18,21] The traditional Conners APTQ was used to provide a context for those familiar with it, although, because of the APTQ focus on hyperactivity symptoms, it added little to the RBRI. Budget limitations did not allow the use of the longer 28- or 48-item revised Conners Parent and Teacher Rating Scales, which would have been more appropriate complementary instruments.

The generalizability of this finding to a wider population would be difficult to establish, given the multifactorial ideology of these symptoms. Experimental designs in which subjects are challenged repeatedly are more likely to show clear results, but present practical difficulties when studying cumulative effects. The 6-day challenge cycle used here put repeated measures, which would have been more effective,[18] beyond the resources of the present study. Effects observed in the present study were dose-related and cumulative. Challenge with a single slice of preserved bread would have identified only one responder. A challenge of 5-day duration may have identified more responders. Further study of the behavioural effects of calcium propionate is recommended, using repeated double-blind 5-day challenges with probable responders who have already been identified during an open elimination and challenge phase.

Unlike food colouring, preservative in bread is an invisible additive. Prior to the study, all parents were unaware of its presence. At follow up after 18 months, parents of all bread preservative responders were still choosing preservative-free bread. All but two families also avoided some other food chemicals found to cause problems.

Calcium propionate is now permitted in a wider range of foods, including cheese, fruit juices, dried fruit and emulsifiers,[30] so increasing the potential total daily intake of calcium propionate. Some children may be affected unknowingly by a preservative in healthy foods that are consumed daily. Minimizing the concentrations added to processed foods would be likely to reduce the frequency and severity of adverse reactions.[31] The present study supports the recommendation that testing for behavioural toxicity be included in food additive safety evaluation.[17,32]

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