PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	The VITALITY Trial: Protocol for a randomised controlled trial to establish the role of postnatal vitamin D supplementation in infant immune health.
AUTHORS	Allen, Katrina; Panjari, Mary; Koplin, Jennifer; Ponsonby, Anne-Louise; Vuillermin, Peter; Gurrin, Lyle; Greaves, Ronda; Carvalho, Natalie; Dalziel, Kim; Tang, Mimi; Lee, Katherine; Wake, Melissa; Curtis, Nigel; Dharmage, Shyamali

VERSION 1 - REVIEW

REVIEWER	Carol Wagner Medical University of South Carolina, Charleston, South Carolina, USA
REVIEW RETURNED	07-Sep-2015

GENERAL COMMENTS	In this manuscript that gives the basis for this ongoing RCT entitled, "The VITALITY Trial: Protocol for a randomised trial to establish the role of postnatal vitamin D supplementation in infant immune health," there are notable strengths. This scientific team will utilize a large cohort of breastfeeding infants who will be randomized to receive 400 IU vitamin D/day vs. placebo beginning around 4-6 weeks of age. The primary outcome variables surround the occurrence of allergic disease, eczema/atopy, and lower respiratory illnesses during the first year of life. This type of study could not be performed in other areas of the world where vitamin D supplementation of the breastfeeding infant is the standard of care and is begun within a few days of birth; therefore, this is a unique trial that will address perhaps the central objective. I really like the parent questionnaire and the estimation of UVR exposure in the mother, with the justification. I think this is done well. Overall, this study is, as the title implies--vital for future recommendations about vitamin D supplementation. There are some cautions that I mention below that are meant to strengthen your ongoing study and approach. Below are issues that should be addressed to strengthen the trial design and results: (1) One aspect of the trial and the manuscript that bothers me is that formula feeding from birth is seen as a proxy for vitamin D supplementation. On page 14, lines 306-309, the authors state that "...in the HealthNuts study there was a 43% lower prevalence ...of food allergy for infants fully formula fed from birth...compared with infants still breastfed at 12 months of age...." It is true that formula-fed infants have typically better vitamin D status than unsupplemented breastfed infants whose mothers are not replete,

	but there are many other differences that may explain the differences between the groups. While there is reference #15 on page 6 lines 104-107 that speaks to the prevalence of infant vitamin D deficiency among breastfed vs. formula-fed infants, it would be helpful if there were additional references cited. This is a crucial part of the study--the rationale for conducting the study--that improved vitamin D status mitigates the inflammatory process as seen in the aforementioned #15 ref, that there should be additional references that provide the 25(OH)D concentrations of those infants who are breastfed vs. formula-fed and perhaps additional corroborative studies that detail the risk of allergy between the groups. (2) Page 11. lines 232-237: LC-Mass spec to measure the 25(OH)D--this needs to be stated directly--not as "Infant vitamin D levels..." That actually, technically would mean measuring infant vitamin D--the parent compound. You are measuring vitamin D status through the measurement of total circulating 25(OH)D concentrations. Please be specific on this point. (3) Another problem that you must address is how the laboratory will account for the 3-epimer of 25(OH)D that is found in infants up to the first year of life. (See Singh RJ et al 2006; J Clin Endocrine Met 91(8): 3055-3061. A more recent review on the topic also might be of help: Unterieser I, Lukacin, hR. Spinach Biotech Cutting Edge 2014). Your laboratory may already be taking this into account, but please make sure and state this in your paper. (4) Maternal vitamin D status: If at all possible, even in a small sub cohort of the larger study, it would be important to obtain maternal 25(OH)D status as we know that the main source of vitamin D in breast milk is mother, and when mother is replete, then she is replete; conversely, when mother is deficient, her milk is deficient and her baby will be deficient. There may be those moms who have increased UVR exposure and hence better vitamin D status, higher vitamin D concentrations in their milk, and thus, with improved vitamin D status in their infants. This would influence the results of the trial. You can directly measure this through the UVR exposure questionnaire, but it would be important to cross-validate this with actual 25(OH)D measurements in the moms. (See Wagner 2006 Breastfeeding Med; Hollis et al Pediatrics 2015 (Sept 28) for more details about this aspect of vitamin D delivery in breast milk.) (7) The crux of the matter--will 400 IU vitamin D per day vs. placebo achieve the kind of 25(OH)D concentrations that are necessary to show a decrease in risk for inflammation/allergy and LRI? This is all predicated on (1) that 400 IU/day will achieve a minimal threshold level to decrease inflammation, and (2) that adherence to protocol will be achieved. While there is evidence that 400 IU vitamin D per day during at least the first 7 months achieves 25(OH)D levels in the 30-40 ng/mL (12-16 nmol/L) range, it will be important to provide the basis for how the 400 IU/day dose will be adequate for the infant approaching the 12-month period. With regard to the adherence problem, a smartphone app is a good idea. A recurring problem, however, is that families lead busy lives and in various studies looking at adherence/compliance among breastfeeding families, the adherence rates are typically under 20%. You must look at the results not only as an intent-to-treat but also as a per protocol and on the basis of 25(OH)D achieved. It is essential as you might mask the effect and all would be lost. If a mother/father

	is not providing the prescribed treatment in placebo, there will be no difference; however, as you know, if the parents are not providing the vitamin D treatment, then the effect will be as in the placebo group and if this occurs more often than not, you will have nullified the study. This aspect of the study, while mentioned, must be further detailed. I cannot emphasize this enough through personal experience. It would be sad if your results were inadvertently skewed by a predominance of non-adherence. (6) A minor change: Page 5, lines 85-86: "With increase most pronounced in children under five years of age, suggesting a causal role for early life determinants." This is not a complete sentence and requires revision. Thank you for the opportunity to have reviewed this important, ongoing trial.

REVIEWER	Cameron Grant University of Auckland, New Zealand
REVIEW RETURNED	09-Oct-2015

GENERAL COMMENTS	Thank you for asking me to review the submitted protocol for the VITALITY Trial: Protocol for a randomised controlled trial to establish the role of postnatal vitamin D supplementation in infant immune health. This protocol describes a well-designed and important study. This is a well written protocol. A few questions and comments: 1. Poor infant health is listed as an exclusion criteria. How will this be defined? 2. Will postnatal maternal vitamin D supplementation use be measured and if so will breast milk vitamin D content be determined? 3. I don't share the author's confidence, based upon a study performed in Canada, that 400 IU per day of vitamin D will be sufficient to achieve a serum 25(OH)D concentration ≥50 nmol/L in 98% of infants in Melbourne. In Canada, vitamin D fortification of food and maternal vitamin D supplement use are likely to mean that baseline vitamin D status of enrolled infants in the study cited,1 was better than what will be the case in Melbourne. 4. How effective is the iPhone application for enhancing adherence. How will adherence be enhanced in study participants who don't have a smart phone? 5. It was unclear from the description of data collection if there would be face-to-face contacts at 3, 6, 9 and 12 months. If not what strategies are being use to minimise study attrition. 6. The collection of data to allow LRIs to be described is potentially problematic. Parental report is likely to underestimate the number of events. How will a complete set of primary care and hospital records be obtained. What unique identifying variables are available to enable these to be collected? Are primary care records coded? If not

	how will LRI be defined from these primary care records? 7. A societal perspective is an appropriate perspective to use to inform the development of public health policy. 8. For how long is study recruitment planned to continue? At the current recruitment rate it will require a very long time to recruit the desired sample size. The low consent to participate rate is likely will create issues with population generalizability. References 1. Gallo S, Comeau K, Vanstone C, et al. Effect of different dosages of oral vitamin D supplementation on vitamin D status in healthy, breastfed infants: a randomized trial. JAMA 2013; 309(17): 1785-92.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name
Carol Wagner

Institution and Country
Medical University of South Carolina, Charleston, South Carolina, USA

Please state any competing interests or state 'None declared':
None declared

Please leave your comments for the authors below
In this manuscript that gives the basis for this ongoing RCT entitled, "The VITALITY Trial: Protocol for a randomised trial to establish the role of postnatal vitamin D supplementation in infant immune health," there are notable strengths. This scientific team will utilize a large cohort of breastfeeding infants who will be randomized to receive 400 IU vitamin D/day vs. placebo beginning around 4-6 weeks of age. The primary outcome variables surround the occurrence of allergic disease, eczema/atopy, and lower respiratory illnesses during the first year of life. This type of study could not be performed in other areas of the world where vitamin D supplementation of the breastfeeding infant is the standard of care and is begun within a few days of birth; therefore, this is a unique trial that will address perhaps the central objective. I really like the parent questionnaire and the estimation of UVR exposure in the mother, with the justification. I think this is done well. Overall, this study is, as the title implies--vital for future recommendations about vitamin D supplementation. There are some cautions that I mention below that are meant to strengthen your ongoing study and approach.

Below are issues that should be addressed to strengthen the trial design and results:

(1) One aspect of the trial and the manuscript that bothers me is that formula feeding from birth is seen as a proxy for vitamin D supplementation. On page 14, lines 306-309, the authors state that "...in the HealthNuts study there was a 43% lower prevalence ...of food allergy for infants fully formula fed from birth...compared with infants still breastfed at 12 months of age...." It is true that formula-fed infants have typically better vitamin D status than unsupplemented breastfed infants whose mothers are not replete, but there are many other differences that may explain the differences between the groups. While there is reference #15 on page 6 lines 104-107 that speaks to the prevalence of infant vitamin D deficiency among breastfed vs. formula-fed infants, it would be helpful if there were additional references cited. This is a crucial part of the study--the rationale for conducting the study--

that improved vitamin D status mitigates the inflammatory process as seen in the aforementioned #15 ref, that there should be additional references that provide the 25(OH)D concentrations of those infants who are breastfed vs. formula-fed and perhaps additional corroborative studies that detail the risk of allergy between the groups.

Authors' Response: Formula feeding from birth is a proxy for vitamin D supplementation as formula contains approximately 400 IU of vitamin D per one litre. The amount of formula that infants consume by age 6 months is based on body weight and amount of solid food consumed but generally can considered to be equivalent to baby receiving<400 IU. This is why we are restricting the study to breastfed only infants. The recent paper from Gallo et al. (Gallo S, Comeau K, Vanstone C, et al. Effect of different dosages of oral vitamin D supplementation on vitamin D status in healthy, breastfed infants: a randomized trial. JAMA 2013; 309(17): 1785-92) supports this as it was conducted in breast fed babies only to ensure the results of this dosing trial were not contaminated by formula consumption which is a surrogate form of vitamin D.

The following is added to page 7 lines 137-141:
'The study is restricted to breast fed infants because formula feeding from birth can be equivalent to vitamin D supplementation as formula contains approximately 400 IU of vitamin D per one litre. The amount of formula that infants consume by age 6 months is based on body weight and amount of solid food consumed but generally can considered to be equivalent to the infant receiving<400 IU.'

The following statement has been removed from the manuscript on page 15, lines 325-328:
'Using formula feeding from birth as a proxy for vitamin D supplementation, in the HealthNuts study there was a 43% lower prevalence (95% CI 16% to 65%) of food allergy for infants fully formula fed from birth (n=273, 7.4% food allergic) compared with infants still breastfed at 12 months of age (n=1439, 13.5% food allergic).'

The sentence on page 15 line 328 is amended as follows:
'A 30% reduction in the prevalence of food allergy attributed to vitamin D supplementation represents a clinically important reduction on a population level.'

We were unable to find additional studies besides that quoted to compare vitamin D levels in breast versus formula fed infants, or the risk of allergy, however we do know breastfed babies have low vitamin D levels as breast milk is a poor source of 25OHD which is why the IOM recommends that all breast fed infants are supplemented with 400 IU vitamin D daily. This is our justification for conducting the study in a population that has no supplementation, either direct or via surrogate. Most recent studies of vitamin D supplementation restrict to breastfed infants for this reason, and make no distinction between breast and bottle feeding.

(2) Page 11. lines 232-237: LC-Mass spec to measure the 25(OH)D--this needs to be stated directly--not as "Infant vitamin D levels..." That actually, technically would mean measuring infant vitamin D--the parent compound. You are measuring vitamin D status through the measurement of total circulating 25(OH)D concentrations. Please be specific on this point.

Authors' response: Page 12 line 245: We have changed the wording to reflect that 25OHD3 is measured:
'Vitamin D deficiency: Infant 25OHD3 levels and metabolites including the C3-epimer of 25OHD3 will be measured using liquid chromatography-tandem mass spectrometry.'

The following reference regarding the measurement or vitamin D was add to the reference list:
Albahrani AA. A novel simultaneous quantification method for fat-soluble vitamins using liquid chromatography-tandem mass spectrometry for clinical applications. : Ph.D thesis. Melbourne

Australia: RMIT University; 2015.

(3) Another problem that you must address is how the laboratory will account for the 3-epimer of 25(OH)D that is found in infants up to the first year of life. (See Singh RJ et al 2006; J Clin Endocrine Met 91(8): 3055-3061. A more recent review on the topic also might be of help: Unterieser I, Lukacin, hR. Spinach Biotech Cutting Edge 2014). Your laboratory may already be taking this into account, but please make sure and state this in your paper.

Authors' response: We are planning to measure C3 epimer levels of 25OHD and we have included this in the text:
Page 12 line 245: 'Vitamin D deficiency: Infant 25OHD3 levels and metabolites including the C3-epimer of 25OHD3 will be measured using liquid chromatography-tandem mass spectrometry.'

(4) Maternal vitamin D status: If at all possible, even in a small sub cohort of the larger study, it would be important to obtain maternal 25(OH)D status as we know that the main source of vitamin D in breast milk is mother, and when mother is replete, then she is replete; conversely, when mother is deficient, her milk is deficient and her baby will be deficient. There may be those moms who have increased UVR exposure and hence better vitamin D status, higher vitamin D concentrations in their milk, and thus, with improved vitamin D status in their infants. This would influence the results of the trial. You can directly measure this through the UVR exposure questionnaire, but it would be important to cross-validate this with actual 25(OH)D measurements in the moms. (See Wagner 2006 Breastfeeding Med; Hollis et al Pediatrics 2015 (Sept 28) for more details about this aspect of vitamin D delivery in breast milk.)

Authors' response: Thank you. This is a very important point and we intend to measure maternal 25OHD levels in breast milk samples once we secure funding. We intend to obtain breast milk samples when the infant is recruited into the trial at age 6-8 weeks. We cannot include this in the manuscript as we do not yet have ethics clearance to collect breast milk samples.

(7) The crux of the matter--will 400 IU vitamin D per day vs. placebo achieve the kind of 25(OH)D concentrations that are necessary to show a decrease in risk for inflammation/allergy and LRI? This is all predicated on (1) that 400 IU/day will achieve a minimal threshold level to decrease inflammation, and (2) that adherence to protocol will be achieved. While there is evidence that 400 IU vitamin D per day during at least the first 7 months achieves 25(OH)D levels in the 30-40 ng/mL (12-16 nmol/L) range, it will be important to provide the basis for how the 400 IU/day dose will be adequate for the infant approaching the 12-month period.

Authors' response: The following paper published in JAMA (Gallo S, Comeau K, Vanstone C, et al. Effect of different dosages of oral vitamin D supplementation on vitamin D status in healthy, breastfed infants: a randomized trial. JAMA 2013; 309(17): 1785-92) shows that the dose of 400IU is sufficient to attain blood levels of 25OHD >50 nmol/L in infants up to 1 year of age. This is the current recommended dose in the US and Europe which is why we are using this dose. This information is in the manuscript on page 8 lines171-173

With regard to the adherence problem, a smartphone app is a good idea. A recurring problem, however, is that families lead busy lives and in various studies looking at adherence/compliance among breastfeeding families, the adherence rates are typically under 20%. You must look at the results not only as an intent-to-treat but also as a per protocol and on the basis of 25(OH)D achieved. It is essential as you might mask the effect and all would be lost. If a mother/father is not providing the prescribed treatment in placebo, there will be no difference; however, as you know, if the parents are not providing the vitamin D treatment, then the effect will be as in the placebo group and if this occurs more often than not, you will have nullified the study. This aspect of the study, while mentioned, must

be further detailed. I cannot emphasize this enough through personal experience. It would be sad if your results were inadvertently skewed by a predominance of non-adherence.

Authors' response: Thank you for this insight into the problems of adherence to study medications. We acknowledge that this is an issue and we are making efforts to ensure participants are compliant. To date adherence as measured by the total number of (paper and iPhone) diaries returned after 1 month of dosing is approximately 90%. Those without an iPhone are using the paper diary and either mailing or taking a photo of the diary and emailing this to us. Please see our response to the second reviewer comment number 4.
It is a good suggestion regarding the analysis and we intend to do the analysis on that basis -an intent-to-treat but also as a per protocol and on the basis of 25OHD levels achieved. The following sentence is amended to reflect this on page 14, lines 313
'As a secondary analysis, the analysis will be repeated in the 'per protocol' (infants breastfed for at least 6 months and fully compliant with the randomisation and on the basis of 25OHD3 levels achieved).'

(6) A minor change: Page 5, lines 85-86: "With increase most pronounced in children under five years of age, suggesting a causal role for early life determinants." This is not a complete sentence and requires revision.

Authors' response: Thank you for pointing this out. The sentence is indeed incomplete. We have changed it to the following:
Page 5, lines 85-86: "The increase is most pronounced in children under five years of age which suggests a causal role for early life determinants."

Thank you for the opportunity to have reviewed this important, ongoing trial.

Reviewer: 2
Reviewer Name

Cameron Grant

Institution and Country

University of Auckland, New Zealand

Please state any competing interests or state 'None declared'None declared

Please leave your comments for the authors below
Thank you for asking me to review the submitted protocol for the VITALITY Trial: Protocol for a randomised controlled trial to establish the role of postnatal vitamin D supplementation in infant immune health.

This protocol describes a well-designed and important study. This is a well written protocol.

A few questions and comments:
1. Poor infant health is listed as an exclusion criteria. How will this be defined?

Authors' response: Poor infant health is defined as:
• Infants on medication that interfere with vitamin D metabolism
• due to a current or past significant disease state or congenital abnormality

We have included the 2 points above in the manuscript on page7 line 148-150:
'Poor health due to a current or past significant disease state or congenital abnormality or infants on medication that interfere with vitamin D metabolism.'

2. Will postnatal maternal vitamin D supplementation use be measured and if so will breast milk vitamin D content be determined?

Authors' response: We intend to measure maternal 25OHD levels in breast milk samples once we secure funding. We intend to obtain breast milk samples when the infant is recruited into the trial at age 6-8 weeks. We cannot include this in the manuscript as we do not yet ethics clearance to collect breastmilk samples.

3. I don't share the authors' confidence, based upon a study performed in Canada, that 400 IU per day of vitamin D will be sufficient to achieve a serum 25(OH) D concentration ≥50 nmol/L in 98% of infants in Melbourne. In Canada, vitamin D fortification of food and maternal vitamin D supplement use are likely to mean that baseline vitamin D status of enrolled infants in the study cited,1 was better than what will be the case in Melbourne.

Authors' response: A recent paper by (Millette M, Sharma A, Weiler H, Sheehy O, Berard A, Rodd C. Programme to provide Quebec infants with free vitamin D supplements failed to encourage participation or adherence. Acta Paediatr. 2014.) shows that compliance with vitamin D dosing is not optimal in Canada thus the baseline vitamin D status of the Canadian infants may not be better than in Vitality.

4. How effective is the iPhone application for enhancing adherence. How will adherence be enhanced in study participants who don't have a smart phone?

Authors' response: The iPhone app is working well with more than half of the participants using it. For the non-iPhone users, we ask for diaries to be either photographed and emailed or sent to us via the mail. To date compliance with daily dosing is 90% based on the number of returned, completed diaries. We ask for empty bottles to be sent back to Clinical Trials pharmacy for re-weighing in order to measure compliance with daily dosing.

We have add the following to the manuscript on page 9, line 179-183:

'For participants without smart phones, a paper diary system is available. These can be sent back via mail or photographed and emailed. Participants are asked to send their empty bottles of study medication to the Clinical Trials Pharmacy for re-weighing to measure compliance with daily dosing. To date compliance with daily dosing is 90% based on the number of returned, completed diaries.'

5. It was unclear from the description of data collection if there would be face-to-face contacts at 3, 6, 9 and 12 months. If not what strategies are being use to minimise study attrition.

Authors' response: There is a face to face contact visit at age 12 months when the infant will undergo skin prick testing and have blood drawn. This is in Table 1 Summary and timing of measures page 10 line218-219. There is also an optional visit at age 6 months, at which blood is drawn and a general clinical examination takes place. We have added a check for this in the table.

We have add the following sentence to clarify this:
Page 10 lines 211-213: 'An optional clinic visit at age 6 months will be offered to participants and will include an eczema examination and blood draw.'

Strategies to minimize attrition are in place and include email/sms/phone contact to remind mothers to send back diaries, to complete questionnaires at the appropriate time points and to invite to them to bring their infant to the 6 and 12-month clinic visits.

6. The collection of data to allow LRIs to be described is potentially problematic. Parental report is likely to underestimate the number of events. How will a complete set of primary care and hospital records be obtained. What unique identifying variables are available to enable these to be collected? Are primary care records coded? If not how will LRI be defined from these primary care records?

Authors' response: We will collect data from the Australian Department of Health through the Medicare (MBS/PBS) system via linkage with Medicare numbers. This data will enable us to collect information about specialist visits, GP visits and medications-related costs for children with the primary and secondary outcomes identified in the study, including lower respiratory tract infections. This will be supplemented by costing data from the Royal Children's Hospital for inpatient and outpatient stays, in addition to the parent questionnaire from which we will gather out-of-pocket costs for time off work, caregiver time, and travel time to appointments.
The following is inserted on pages 14 lines 293-298: 'This data will enable us to collect information about specialist visits, GP visits and medications-related costs for children with the primary and secondary outcomes identified in the study, including lower respiratory tract infections. This will be supplemented by costing data from the Royal Children's Hospital for inpatient and outpatient stays, in addition to the parent questionnaire from which we will gather out-of-pocket costs for time off work, caregiver time, and travel time to appointments.'

7. A societal perspective is an appropriate perspective to use to inform the development of public health policy.

Authors' response: We agree, and for that reason have stated that we will conduct the economic evaluation from a societal perspective, including both costs to the health system and patient out-of-pocket costs. In this way we can separate out health systems costs from patient out-of-pocket costs, both of which will be important to consider in assessing the overall costs of food allergy and related conditions, when informing policy.

8. For how long is study recruitment planned to continue? At the current recruitment rate it will require a very long time to recruit the desired sample size. The low consent to participate rate is likely will create issues with population generalizability.

Authors' response: The trial is currently run with a skeleton staff. We await news of funding to allow employment of sufficient staff to recruit the 3000 participants. We have shown that this sample size is feasible as over a 10 week period we recruited an average 6-7 patients per week (n=66) with only 1 recruiting team. Therefore we are confident with 3 recruiting teams (as per grant budget rationale) to be able to easily achieve 18-20 participants per week (79/month) and the required 3000 infants recruited over the 38 months of study recruitment.

References

1. Gallo S, Comeau K, Vanstone C, et al. Effect of different dosages of oral vitamin D supplementation on vitamin D status in healthy, breastfed infants: a randomized trial. JAMA 2013; 309(17): 1785-92.

VERSION 2 – REVIEW

REVIEWER	Carol Wagner Medical University of South Carolina Charleston, SC, USA
REVIEW RETURNED	13-Nov-2015

GENERAL COMMENTS	The authors have answered the questions posed by the reviewers and have substantially improved the paper. I think this is an important paper and an essential study that will provide invaluable information on the topic of vitamin D's effect on immune function in these breastfeeding infants. I just have one suggestion re: maternal 25(OH)D in the breast milk--that is very expensive to do and very labor intensive. The only method at this juncture is LC-mass spec. If you have the funds, great, but if you do not a surrogate for this would be maternal serum or plasma 25(OH)D. What is actually transferred into the breast milk is primarily the parent vitamin D compound but if you measure maternal 25(OH)D that will at least give you an idea of what is transferred in the milk. Thank you for the opportunity to have reviewed this important paper. I look forward to the results.

REVIEWER	Cameron Grant University of Auckland New Zealand
REVIEW RETURNED	06-Nov-2015

GENERAL COMMENTS	Thank you, all of the issues raised from my review have been addressed