Appendix

University of Reading

School of Chemistry, Food and Nutritional Sciences and Pharmacy
Research Ethics Committee

Application Form

SECTION 1: APPLICATION DETAILS

1.1

Project Title: Comparison of the effect of milk containing A2/A2 beta casein variant vs milk containing both A1/ A2 beta casein on inflammation and function of gastrointestinal tract of volunteers with mild to moderate non-lactose milk intolerance

Date of Submission: 19-08-2016 Proposed start date: September 2016 Proposed End Date: August 2017

1.2

Principal Investigator: Dr Sandrine Claus, Department of Food and Nutritional Sciences

Office room number: 2.53 Internal telephone:
Email address:

Other applicants

Name: Prof Ian Givens Staff Institution/Department Professor of Food Chain Nutrition and Director, Food Production & Quality Research Division
Email:

Name: Areej Almuraee PhD Student Department of Food and Nutritional Sciences
Email:

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1.3

Project Submission Declaration

I confirm that to the best of my knowledge I have made known all information relevant to the Research Ethics Committee and I undertake to inform the Committee of any such information which subsequently becomes available whether before or after the research has begun.

I understand that it is a legal requirement that both staff and students undergo Criminal Records Checks when in a position of trust (i.e. when working with children or vulnerable adults).

I confirm that a list of the names and addresses of the subjects in this project will be compiled and that this, together with a copy of the Consent Form, will be retained within the School for a minimum of five years after the date that the project is completed.

Signed…… (Principal Investigator) Date:……

…… (Student) Date:……

…… (Other named investigators) Date:……

…… (Other named investigators) Date:……

1.4

University Research Ethics Committee Applications

Projects expected to require review by the University Research Ethics Committee must be reviewed by a member of the School research ethics committee and the Head of School before submission.

Signed…… (Chair/Deputy Chair of School Committee) Date:……

Signed…… (Head of Department) Date:……

Signed…… (SCFP Ethics Administrator) Date:……

SECTION 2: PROJECT DETAILS

2.1
Lay summary

Background

Casein proteins represent about 80% of total protein in cows' milk, about 36% of which is *β*-casein. β-casein can be made of different allele variants with different proportions including A1, A2, B and C. Early studies have indicated equal proportions of A1 and A2 variants (Fox, 2003). However Givens et al. (2013)showed that the A2 variant (58%) is the highest proportion in UK milk followed by the A1 variant (31%). Dairy cows can be either homozygous (A2/A2 or A1/A1) or heterozygous (A1/A2) genotypes. Most of the UK milk contains a mixture of A1/A2 protein variants presumably as a result of most dairy cows being heterozygous genotypes. However, commercial A2 milk contains only the A2 variant produced from animals selectively bred to be A2/A2 homozygotes (Woodford, 2007).

The beta casein (β-casein) protein contains 209 amino acids and the main difference between A1 and A2 β- casein variants is in position 67 where the histidine in A1 is replaced by proline in A2. The gastrointestinal system plays a significant role in the digestion of β-caseins and the production of bioactive peptides that result from the combined action of low gastric pH followed by pancreatic digestive enzymes. As a result of the A1 mutation, the enzymatic digestion of the A1 β-casein releases the β-casomorophin-7 (βCM-7) peptide but this is not possible with the A2 variant (Jinsmaa & Yoshikawa, 1999). The βCM-7 peptide has opioid characteristics which have been suggested to play a role in gut health including effects on the gut microbiota (Tuohy et al., 2015). A possible mechanism of action of βCM-7 may be through its ability to bind to μ-opioid receptors in the gut and stimulate production of myosin (De Noni et al., 2009). *In vitro* assays and animal studies have also demonstrated that βCM-7 could cross the blood-brain barrier but its central effects are unknown (Haq, Kapila, Sharma, Saliganti, & Kapila, 2014; Sienkiewicz-Szłapka et al., 2009).

Two recent studies was conducted throughout volunteer who were recruited based on some self-diagnosed milk intolerance that defined below which is neither lactose intolerance nor milk protein allergy. A blinded randomized cross-over pilot human study (Ho et al., 2014) found that consumption of A1 β-casein milk led to significantly higher stool consistency values (Bristol Stool Scale) and significant positive association between abdominal pain and stool consistency compared with the A2 β-casein milk. Another recent study (Jianqin et al., 2016) concluded that subjects who consumed A1 β-casein milk had significantly

high stool consistency values, a significant positive association between abdominal pain and stool consistency, delay in transit time, elevated inflammation-related biomarkers and immune response compared with subjects who consumed A2 β-casein milk. In addition, they showed that consumption of milk containing only A2 β-casein did not aggravate post-dairy digestive discomfort symptoms relative to baseline (i.e., after washout of dairy products) in lactose tolerant and intolerant subjects. However, consumption of milk containing both β-casein types was associated with worsening of post-dairy digestive discomfort symptoms relative to baseline in lactose tolerant and lactose intolerant subjects. These finding suggest that some gastrointestinal symptoms associated with milk in lactose intolerant subjects may in part be related to A1 β- casein rather than lactose it self (Jianqin et al., 2016).

Lactose malabsorption or lactose intolerance is a widespread condition characterized by a highly variable prevalence, ranging from 5% in northwest Europe to almost 100% in some Asian populations. A high prevalence figure has also been described in the Italian population, ranging from 51% in the North to 71% in the South. Intolerance symptoms, such as bloating, diarrhea, flatulence, and abdominal pain, are only present in a subgroup of individuals with lactose intolerance due to the lack of lactase enzyme that is responsible for breaking down lactose (Di Stefano & Corazza, 2009). Non-lactose milk intolerance is a condition that has not been defined clinically yet but the current literature reports existence of subjects who are moderately milk intolerant and whose intolerance can neither be attributed to a defect in lactose intolerance, nor to milk protein allergy. Yet, they experience at least one or two of the following symptoms: gases, bloating, abdominal cramp. It was reported that these symptoms were highly variable from one individual to another and could range from mild to sever as described in the attached symptom evaluation scale. (Johnson, Semenya, Buchowski, Enwonwu, & Scrimshaw, 1993; Stephenson & Latham, 1974).

Hypothesis

This project hypothesises the following

1- The consumption of milk containing only A2 β-casein will reduce symptoms of gastrointestinal dysfunction and gastrointestinal inflammation in volunteers affected by mild to moderate non-lactose milk intolerance.

Objectives

- To compare the systemic response to milk containing only the A2 β-casein type with milk containing both A1 and A2 β-casein types. Assessments of gastrointestinal function and markers of inflammation in blood, urine and faeces will be recorded.
- To investigate the impact on gut microbial metabolites in faeces, urine and blood using metabolic profiling by high-resolution ^{1}H NMR analysis.

Reference

De Noni, I., FitzGerald, R. J., Korhonen, H. J., Le Roux, Y., Livesey, C. T., Thorsdottir, I., . . . Witkamp, R. (2009). Review of the potential health impact of β-casomorphins and related peptides. *EFSA Sci Rep, 231*, 1-107.

Di Stefano, M., & Corazza, G. (2009). Role of hydrogen and methane breath testing in gastrointestinal diseases. *Digestive and Liver Disease Supplements, 3*(2), 40-43.

Fox, P. (2003). Milk proteins: general and historical aspects *Advanced Dairy Chemistry—1 Proteins* (pp. 1-48): Springer.

Haq, M. R. U., Kapila, R., Sharma, R., Saliganti, V., & Kapila, S. (2014). Comparative evaluation of cow β-casein variants (A1/A2) consumption on Th2-mediated inflammatory response in mouse gut. *European journal of nutrition, 53*(4), 1039-1049.

Ho, S., Woodford, K., Kukuljan, S., & Pal, S. (2014). Comparative effects of A1 versus A2 beta-casein on gastrointestinal measures: a blinded randomised cross-over pilot study. *Eur J Clin Nutr, 68*(9), 994-1000. doi: 10.1038/ejcn.2014.127

Jianqin, S., Leiming, X., Lu, X., Yelland, G. W., Ni, J., & Clarke, A. J. (2016). Effects of milk containing only A2 beta casein versus milk containing both A1 and A2 beta casein proteins on gastrointestinal physiology, symptoms of discomfort, and cognitive behavior of people with self-reported intolerance to traditional cows' milk. *Nutrition Journal, 15*(1), 1.

Jinsmaa, Y., & Yoshikawa, M. (1999). Enzymatic release of neocasomorphin and β-casomorphin from bovine β-casein. *Peptides, 20*(8), 957-962. doi: http://dx.doi.org/10.1016/S0196-9781(99)00088-1

Johnson, A. O., Semenya, J. G., Buchowski, M. S., Enwonwu, C. O., & Scrimshaw, N. S. (1993). Correlation of lactose maldigestion, lactose intolerance, and milk intolerance. *The American journal of clinical nutrition, 57*(3), 399-401.

Sienkiewicz-Szłapka, E., Jarmołowska, B., Krawczuk, S., Kostyra, E., Kostyra, H., & Bielikowicz, K. (2009). Transport of bovine milk-derived opioid peptides across a Caco-2 monolayer. *International dairy journal, 19*(4), 252-257.

Stephenson, L., & Latham, M. (1974). Lactose intolerance and milk consumption: the relation of tolerance to symptoms. *The American journal of clinical nutrition, 27*(3), 296-303.

Tuohy, K. M., Venuti, P., Cuva, S., Furlanello, C., Gasperotti, M., Mancini, A., . . . Fava, F. (2015). Chapter 15 - Diet and the Gut Microbiota – How the Gut: Brain Axis Impacts on Autism. In K. T. D. Rio (Ed.), *Diet-Microbe Interactions in the Gut* (pp. 225-245). San Diego: Academic Press.

Woodford, K. B. (2007). *Devil in the milk : illness, health and politics of A1 and A2 milk*. White River Junction, Vt.: Chelsea Green Pub.

2.2

Procedure

This will be a double blind randomised crossover study in 45 (18-50 years old) individuals. In this study each subject will be randomised to sequence, some will start with sequence 1 and others will start with sequence 2 for 2 weeks followed by 2 weeks washout period and cross to the other arm (Figure 1).

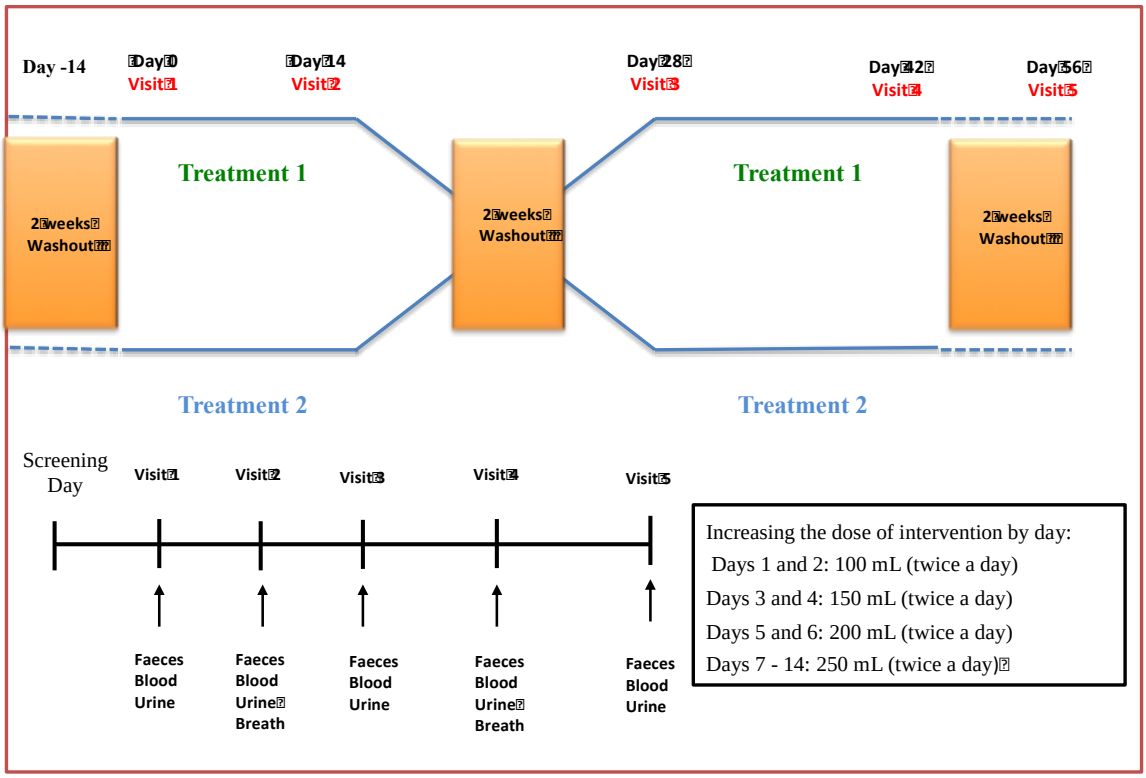


Figure.1 Human intervention study design. Treatment 1 is semi skimmed pasteurised A2 milk and treatment 2 is semi skimmed pasteurised A1/A2 regular milk.

Primary outcome

A2 milk consumption will result in a reduction of gastrointestinal inflammation measured by faecal calprotectin.

Secondary outcomes

1- Systemic metabolic response will be measured by urine, plasma and metabolic profiles.

2- Bacterial metabolism will be measured by faecal metabolomic profiling (these metabolic profiles include simultaneous measurements of amino acids, amines, organic acids, free sugars if any, short chain fatty acids etc.) at days 0, 14, 28, 42 and 56.

3- Systemic inflamation response will be measured by plasma CRP (C reactive protein), antibodies biomarker of immune response and inflammatory cytokines.

4- Gastrointestinal symptoms (Daily)

5- Stool frequency (Daily)

6- Regional gut transit time (Daily)

7- Monitor psychological behaviour change to make sure participants enrolled in the study dose not suffers from a degradation of mood. Two to three executive function tests will be used (TMT, Letter memory test and Flanger test) this will take around 15min long as well as mood assessment (PANAS).

Screening:
Participants identified as potentially suitable from the Hugh Sinclair Unit of Human Nutrition volunteer database, advertise on the social media sites Facebook and Streetlife, posters and email invitation to staff and student at The University of Reading. University of Reading database or by the local groups and societies will be contacted, and screened against inclusion and exclusion criteria if interested in being involved.

Inclusion criteria:

- Aged between 18 and 56 years of age.
- Male or female who willing to participate in the entire study.
- BMI: 20-35kg/m^2
- Glucose<7mmol/l (not diagnosed with diabetes)
- Total cholesterol<7mmol/l
- TAG<4mmol/l
- Normal liver and kidney function
- Haemoglobin:>130 g/L (maybe lower for male)
- Regular milk drinker with self-reported intolerance to commercial milk.
- Suffered from mild to moderate digestive discomfort after milk consumption.
- Have normal blood pressure 120/80 mmHg (BP <160/90 mmHg can be accepted) during quiet respiration.
- Agree not to take any medication, supplements and other dairy products including acidophilus milk
- Be willing to comply with all the requirements and procedures of the study.
- Agree to sign the informed consent form;
- Agree not to enrol in another interventional clinical research study while participating in this study.
- Fully understand the nature, objective, benefit and the potential risks and side effects of the study.

Exclusion criteria:

- Females who are pregnant or planning to be a pregnant and lactating.
- Have known dairy allergy.
- Have stopped drinking milk for the last 6 month.
- Have history of lactose intolerance
- Have history of faecal impaction.

- Received antibiotics in the previous six months
- Smoker
- Anemic, haemoglobin < 11.5g/dl for women and < 12.5g/dl for men
- Trying to lose weight by following a diet or exercise regimen designed for weight loss, or taking any drug influencing appetite and any drug for weight loss for the last three months.
- Have participated in similar dairy or probiotics-containing product's clinical trials within 3 months before the screening.
- Currently taking medicines for cardiovascular or metabolic disease.
- History of alcohol or drug misuse.
- Have history of or be diagnosed of any of the following diseases that may affect the study results: gastrointestinal disorders, hepatopathy, nephropathy, endocrine disease, blood disorders, respiratory, cardiovascular diseases and known on-going allergy such as asthma.
- Currently suffering from any gastrointestinal disorders or gastrointestinal disease, including irritable bowel syndrome, colitis, ulcerative colitis, celiac disease, irritable bowel syndrome (IBS);
- Had hospitalizations within 3 months before screening; Currently drug frequency user of that may affect the gastrointestinal function or immune system. As judged by investigator.
- Who take medication at least the last 6-month.
- Who do excessive exercise not as part of a weight-loss regime, e.g. athletes.

Based on the inclusion, exclusion criteria and medical history questionnaire volunteers who met the criterion will asked to sign the consent form. Once consent has been obtained, participants will be randomised and included in the trial. We will over recruit by 10% to cover for drop-outs.

Randomisation will be stratified by sex and allocation will occur to one of the two treatment groups using a 1:1 ratio. The method of minimisation will be used to ensure treatment arms are balanced with respect to the number of patients in each group (https://www-users.york.ac.uk/~mb55/guide/minim.htm). Participants are free to withdraw from the study at any time, without providing a reason, if they wish to cease participation. Should this occur, all efforts will be made to report the reason for withdrawal as thoroughly as possible. Allowance has been made for withdrawal of 10% subjects in the sample size calculation. Data from withdrawn participants will be included in the intention to treat analysis.

Screening

Participants identified as potentially suitable will be contacted, and screened against inclusion and exclusion criteria if interested in being involved. At screening, methane and hydrogen breath test will be used to exclude subjects who have lactose intolerance and sever symptoms of milk intolerance (see screening visit below in visit schedule and assessment section).

Volunteers will provide a baseline blood sample at a pre-screening visit, to check for anaemia. Faecal and blood samples will be collected at site visits at the Department of Food and Nutritional Sciences.

Trial Day	*Stage of Study*	*Treatment*
Day -14	**Screening visit**	• Distribution of trial information sheets and preliminary discussions with volunteers • Sign consent form • Methane and hydrogen breath test for screening out lactose intolerant subjects. • Symptoms rating scale sheet • Blood pressure • Provide blood sample for screening • Psychological behaviour tests.
Day 0	**Baseline (Visit 1)**	• Provide baseline faecal sample, urine sample and a blood sample • Distribution of test milk for the 2 weeks treatment • Distribution of volunteer diaries (diaries for bowel habit and mood questionnaire) will be distributed for the 14 day treatment • Psychological behaviour tests.
Days 0-14 **Day 14**	**Treatment period 1** **Visit 2**	• Consume amount of milk as presented below twice a day (with breakfast and dinner) for two weeks treatment period; Days 1 and 2: 100 mL (twice a day) Days 3 and 4: 150 mL (twice a day) Days 5 and 6: 200 mL (twice a day) Days 7 until 14: 250 mL (twice a day) • During the treatment (A1/A2 milk or A2 milk), complete dietary, bowel habit and mood questionnaires and record any concomitant medication or adverse events during the trial • Provide faecal sample, urine sample, blood sample and methane and hydrogen breath test • Psychological behaviour tests.
Day 14-27 **Day 28**	**Washout period**	• Refrain from consuming milk • Do not take any dairy products • Provide faecal sample, urine sample and

	Visit 3	blood sample. • Psychological behaviour tests.
Days 28-42 **Day 42**	**Treatment period 2** **Visit 4**	• Consume amount of milk as presented below twice a day (with breakfast and dinner) of 2w treatment period; Days 1 and 2: 100 mL (twice a day) Days 3 and 4: 150 mL (twice a day) Days 5 and 6: 200 mL (twice a day) Days 7 until 14: 250 mL (twice a day) • During the treatment (A1/A2 milk or A2 milk), complete dietary, bowel habit and mood questionnaires and record any concomitant medication or adverse events during the trial • Provide faecal sample, urine sample, blood sample and methane and hydrogen breath test
Days 42-56	**Washout period** **Visit 5**	• Psychological behaviour tests. • Refrain from consuming milk • Do not take any dairy products • Provide faecal sample, urine sample and blood sample. • Psychological behaviour tests.

END OF THE STUDY

Description of the test product

The product used in this study will be pasteurised semi skimmed milk that contains only A2 beta-casein variant. The second product will be pasteurised semi skimmed milk that contains both A1 and A2 variants. Both products are produced locally in the UK and distributed in different supermarkets. Both milk have about the same taste and container of 1L.

Milk is a commercially available product that will be purchased in local shops and will be blinded first by Mariana Mora-Ortiz who is not involved in the study and will be provided as a liquid in the actual container. Researcher will order the milk on a regular basis and Marina will do blinding, randomizing and providing milk to volunteers every week during treatment time. Marina will remove container's labels and lids and will exchange them with another label that contain the following; product name either A or B, expiry date, storage and direction of use as well as another lid that have the same characters for both milk. Un-blinding proses will occur once all volunteers have completed their study visits and all samples has

been analysed by researchers.
Note: researchers arranged partnership with Sainsbury's who will provide fresh pasteurised milk on a regular basis.

Visit schedule and assessments

Screening visit

The participant information sheet will be explained to the volunteer and informed consent will be obtained prior to the performance of any study related activities/assessments. Inclusion/exclusion criteria will be reviewed for volunteer eligibility. Here, volunteers will undergo the following evaluations:

- Demography
- Past/current medical conditions
- Weight/ Height/ BMI
- Concomitant medication
- Smoking history
- Blood pressure
- Blood sample collection
- Methane and hydrogen breath test to screen out lactose intolerant subjects.
- Scoring sheet to evaluate gastrointestinal discomfort after drinking milk.

Subjects will fill the visual analogue scale described in Table 1 to evaluate the severity of the symptoms, if any, following milk ingestion. This will ensure that individuals who experience a sever response to milk intake can be screened out from the study.

In order to determine whether an individual can be defined as having mild to moderate symptoms of lactose intolerance resulting from 25g lactose in 250 ml water, a rating scale dependent on symptoms will be distributed during screening visit. Subjects will be classified by the number and severity of symptoms that will experienced during screening visit and after ingestion of the lactose. Symptoms that going to be rated as follow gases, bloating, cramp, headache, diarrhea, nausea, constipation and rash (Stephenson & Latham, 1974). Each symptom will be rated according to severity of symptoms Table 1. The highest possible score will be 12 (total). Based on this scoring sheet, volunteers will be excluded if they reach a total of 8 points or above. Also volunteer will be asked to fill Table 1 within 24h post lactose ingestion and send the sheet back by email or phone to follow up subjects.

For the lactose tolerance test, subjects will be asked to not consume alcohol for 12 hours prior to the test, with only water without tea and coffee to drink and avoid slowly digesting foods such as beans the day before the test. A baseline breath measure must be taken before the administration of substrate. Then 25g of lactose dissolved in 250ml of water will be consumed and hydrogen and methane will be measured at 0, 15, 30, 60, 90, 120, 150 and 180 min. Subjects with an increase in breath hydrogen concentration ≥20 part per million (ppm) and breath methane concentration ≥12ppm from the baseline within the test period will be classified as lactose intolerant. Subjects whose hydrogen concentration increased < 20 ppm and breath methane concentration <12 from the baseline will be classified as lactose tolerant.

Table 1: Visual analogue scale for severity of symptoms. Adapted from (Breivik et al., 2008).

Symptoms	Scale
Gases (wind)	
Bloating	
Abdominal cramps	
Diarrhea	
Headache	
Constipation	
Nausea	
Rash	

Note: Blood sample collections can be arranged for another day should volunteer prefer.

A volunteer will either be excluded or, if fully meeting the inclusion criteria and willing to take part in the study, will be randomised and enrolled.

- **-2 weeks (wash out period)**

- Volunteer will be asked to exclude from diet all dairy products two weeks before the start of the study.

Visit 1: 2 weeks treatment

On Day 1, the study volunteers will be asked to provide blood, urine and faecal samples. Volunteer also will be given the required amount of pasteurised fresh milk for the first week and will be asked to come back to the nutrition clinic to collect the milk supply for the second week of diet intervention. **Note:** investigator will supply volunteer regularly with milk once the first containers finished since this milk has expiry date. In this case they will come once a week.

Food and drink diaries will be collected and diary cards will be issued. The volunteer will be instructed on how to complete these diaries. Weight will be recorded and volunteers will keep a daily diary noting the number of bowel movements and the average consistency of the stools using the Bristol stool chart (hard, solid, loose or watery), as well as the occurrence of abdominal discomfort, flatulence or bloating and mood changes. Furthermore the diary will include an area for noting any illness experienced including colds and respiratory infections.

Visits 2: End of first diet intervention; before 2 weeks wash-out period

At Visit 2, volunteers will provide blood, urine, faecal samples and methane and hydrogen breath measurements will also be recorded following the test milk challenge. Weight will be recorded. Adverse events will be addressed, concomitant medications will be checked and the diary card will be reviewed with the volunteer. New diary cards will be given. Volunteers will not be supplied with any milk at this visit as they will enter 2 weeks of washout period. In the event of withdrawal, all evaluations will be performed as at the end of the study, provided the subject is willing.

Visit 3: Beginning second arm of the study - 2 weeks treatment

At Visit 3, volunteers will provide blood, urine and faecal samples. Adverse events will be addressed, concomitant medications will be checked, weight will be recorded and the diary card will be reviewed with the volunteer. New diary cards will be distributed and volunteers will be supplied with the required amount of milk treatment for 2 weeks – different from the milk they received for the first 2 weeks.

Visit 4: End of second diet intervention; before 2 weeks wash-out period .

At Visit 4, volunteers will provide blood, urine, a faecal sample and methane and hydrogen breath measurements will also be recorded following the test milk challenge. Adverse events will be addressed, concomitant medication will be checked, weight will be recorded and the diary card will be reviewed with the volunteer. Volunteers will not be supplied with any milk at this visit as this is the last visit and the end of the study.

Visit 5: **finalises the study (follow-up visit)**

At Visit 5, volunteers will provide blood, urine and faecal samples. Weight will be recorded. Adverse events will be addressed, concomitant medications will be checked and the diary card will be reviewed with the volunteer. In the event of withdrawal, all evaluations will be performed as at the end of the study, provided the subject is wiling. Visit 5 finalise the study.

Early withdrawal

- Withdrawal criteria:

Volunteers will be informed in the consent form and also in the volunteer information sheet that they have the right to withdraw from the study at any time without giving a reason and without prejudice. In addition they may be withdrawn at the Investigator's discretion at any time.

- Volunteers may be withdrawn by the Investigator due to:

An adverse event for which the Investigator does not consider continuing study participation safe.
Recurrent illness.
Poor tolerance
Poor adherence.

Blood sample collection

Volunteers will be asked to provide a venous, fasting (having fasted for 10 hours, drinking only water – but no water 1hr before arrival) blood sample at each visit (15ml; around 2 tablespoon) 60 ml in total. Blood samples will be collected by an experienced and trained phlebotomist in the Hugh Sinclair Unit of Human Nutrition (Department of Food and Nutritional Sciences).

Faecal sample collections

Volunteers will be asked to provide a fresh faecal sample at each visit. The volunteers will be provided with an appropriate vessel for sample collection at the University. If unable to do so, they will be given the option to come in on following days until production is possible. No new treatment will be issued until stool has been provided. Samples will be processed immediately for microbial and metabolites analyses.

Urine sample collections

Volunteers will be asked to provide a 24 hours urine sample at each visit. Volunteers will be provided with an appropriate container for urine sample collection at the University. Samples will be processed immediately for metabolite analysis.

Those researchers working with blood, urine and faeces will be up to date with Hepatitis A and B vaccinations.
Any individuals coming into contact with faecal samples will abide by good general laboratory procedures.

- *Bacterial enumeration*

Freshly voided faecal samples will be diluted 1 in 10 (w/w) with anaerobic phosphate buffer and mixed in a stomacher for 2 min. Changes in faecal bacterial populations will be assessed through the use of a culture independent procedure that assesses molecular changes in the microbiota (FLOW-FISH and metagenomics)

- *Short chain fatty acids (SCFA)*

Samples of the faecal slurry [1 in 10 (w/w) dilution of faeces] will be taken for determination of SCFA (end products of bacterial metabolism) by GC.

- *Metabolites*

A blood sample to check for anaemia and baseline measurements will be taken during screening (30ml; around 2 tablespoons)). Samples of the faecal slurry, blood plasma and urine will be kept for metabolites determination by ^{1}H-NMR based metabolomics and inflammatory markers as appropriate.

The Department of Food and Nutritional Sciences is fully licensed under the Human Tissue Act 2004 and as such will adhere to the guidelines necessary for the storage of all biological material. A detailed log will be kept to record when the sample was taken, its place of storage, when analysis was conducted on the sample and how and when the sample was disposed of.

(Note: All questionnaires or interviews should be appended to this application)

2.3

Location

Where will the project take place?

The project will take place within the Department of Food and Nutritional Sciences building, within the Hugh Sinclair Unit of Human Nutrition.

If the project is to take place in Hugh Sinclair Unit of Human Nutrition, projects must be reviewed and approved by the Hugh Sinclair Manager (Dr Michelle Weech,)

Signed (Hugh Sinclair Unit Manager) Date: 16/08/16

2.4

Funding

Is the research supported by funding from a research council or other *external* sources (e.g. charities, business)? Yes

If Yes, please give details: Saudi Government (Um Alqura University)

Please note that *all* projects (except those considered as low risk, which would be the decision of the School's internal review committee and require Head of Department approval) require approval from the University Research Ethics Committee.

2.5

Ethical Issues

Could this research lead to any risk of harm or distress to the researcher, participant or immediate others? Please explain why this is necessary and how any risk will be managed.

It is not anticipated that there will be any risk to participant safety because a similar protocol has been followed by Ho et al and Jianqin, et al as previously published (Ho, Woodford, Kukuljan, & Pal, 2014; Jianqin et al., 2016). Pasteurised milk has been used in a number of different feeding trials with various groups of volunteers and was well tolerated by all. Participation in the study does not pose any significant risk. However some participants may develop some moderate milk intolerance symptoms as described above. In the occurrence of such events, the researcher will review the

severity of the symptoms and the volunteer will be free to withdraw at any time. Any volunteer experiencing symptoms that exceed a total score of X based on the scoring sheet described above (Table 1) will immediately be withdrawn from the study.

(*this box may be expanded as required*)

2.6

Deception

Will the research involve any element of intentional deception at any stage (i.e. providing false or misleading information about the study, or omitting information)? No

[If so, this should be justified. You should also consider including debriefing materials for participants, which outline the nature and the justification of the deception used].

2.7

Payment

Will you be paying your participants for their involvement in the study? Yes

If yes, please specify and justify the amount paid

Time	Task	Payment	Total Payment
2 hours	Assessment and instruction	£ 10 / h	£ 20
5 hours	Sample collection	£ 8 / h	£ 40
10 hours	Record keeping	£ 9 / h	£ 90
Total 21 hours			**£ 150**

Volunteers will be paid £150 for completion of the trial. Those who withdraw will have their payment pro-rated according to time spent. Reserve volunteers will be paid for assessment and instruction if they are not required to participate in the treatment phases of the trial.

Note: excessive payment may be considered coercive and therefore unethical. Travel expenses need not to be declared.

2.8

Data protection and confidentiality

What steps will be taken to ensure participant confidentiality? How will the data be stored?

Confidentiality will be maintained by allocating volunteers an identification code, which will be used to identify all samples and data obtained. Volunteer's names will not be used in any reports or publications. All data generated from the study will be held securely within a password-protected file, only the study investigators will have access to this. A record of the names of the volunteers will not be held on the same file. Information matching volunteer names with identification codes will be kept in a locked filing cabinet, the investigators will only use identification codes. The only time data will be matched with volunteer names is for those volunteers that request to have their personal results discussed with them. A request for individual results to be discussed will include a review of all sample results for the individual volunteer. A list of the names and addresses of the subjects in this project will be compiled, this, together with a copy of the Consent Form, will be retained within the Department for five years after the date that the project is completed.

2.9

Consent

Please describe the process by which participants will be informed about the nature of the study and the process by which you will obtain consent

Informed consent is required. It will be obtained after the volunteers have had time to review the volunteer information sheet and ask any questions of the study team. Consent will be obtained on the morning of the screening visit. No pressure will be put on the volunteers to participate and they will be informed that they are free to drop out at any point during the study. (Appendix C).

Please note that a copy of consent forms and information letters for all participants must be appended to this application.

2.10

Genotyping

Are you intending to genotype the participants? Yes

Which genotypes will be determined? Lactase gene a single nucleotide polymorphism (C-13910> T) will be determined.

Please note that a copy of all information sheets on the implications of determining the specific genotype(s) to be undertaken must be appended to this application.

SECTION 3: PARTICIPANT DETAILS

3.1

Sample Size

How many participants do you plan to recruit? Please provide a suitable power calculation or a suitable justification explaining why this is not possible/appropriate for the study.

A total of 45 subjects will enter this two-treatment crossover study. The probability is 80 % that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 80.0 units. This is based on the assumption that the standard deviation of the difference in the response variables is 186, which was observed by (Ho et al 2014). We will over recruit by 10% to cover for drop-outs

3.2

Will the research involve children or vulnerable adults (e.g. adults with mental health problems or neurological conditions)? No

If yes, how will you ensure these participants fully understand the study and the nature of their involvement in it and freely consent to participate?

3.3

Will your research involve children under the age of 18 years? No

Will your research involve children under the age of 5 years? No

3.4 Will your research involve NHS patients, Clients of Social Services or will GP or NHS databases be used for recruitment purposes? No Please note that if your research involves NHS patients or Clients of Social Services your application will have to be reviewed by the University Research Ethics Committee and by an NHS research ethics committee.
3.5 **Recruitment** Please describe the recruitment process and append all advertising and letters of recruitment. Participants identified as potentially suitable from the Hugh Sinclair Unit of Human Nutrition volunteer database, advertise on the social media sites Facebook and Streetlife, posters and email invitation to staff and student at The University of Reading. University of Reading database or by the local groups and societies will be contacted, and screened against inclusion and exclusion criteria if interested in being involved. Participants are free to withdraw from the trial at any time.

Important Notes

1. The Principal Investigator must complete the Checklist in Appendix A to ensure that all the relevant steps and have been taken and all the appropriate documentation has been appended.

2. If you expect that your application will need to be reviewed by the University Research Ethics Committee you must also complete the Form in Appendix B.

3. For template consent forms, please see Appendices C.

Appendix A: Application checklist

This must be completed by an academic staff member (e.g. supervisor)

Please tick to confirm that the following information has been included and is correct.
Indicate (N/A) if not applicable:

Information Sheet

Is on headed notepaper			☐
Includes Investigator's name and email / telephone number			☐
Includes Supervisor's name and email / telephone number			☐
Statement that participation is voluntary			☐
Statement that participants are free to withdraw their co-operation			☐
Reference to the ethical process			☐
Reference to Disclosure	☐	N/A	☐
Reference to confidentiality, storage and disposal of personal information collected			☐

Consent form(s) ☐

Other relevant material

Questionnaires	☐	N/A	☐
Advertisement/leaflets	☐	N/A	☐
Letters	☐	N/A	☐
Other (please specify)	☐	N/A	☐

Expected duration of the project (months) ☐

Name (print) …… Signature ……

University Research Ethics Committee

Appendix B

Project Submission Form

Note All sections of this form should be completed. Please continue on separate sheets if necessary.

Principal Investigator: Dr Sandrine Claus/ Professor Ian Givens

School: School of Chemistry Food and Pharmacy
Department of Food and Nutritional Sciences

Title of Project: Comparison of the effect of milk contains A2/A2 beta casein variant vs milk contains both A1/ A2 beta casein on inflammation and function of gastrointestinal tract of volunteers with mild to moderate non-lactose milk intolerance.

Proposed starting date: Aug 2016

Brief description of Project:

There is much evidence suggest that milk containing A1 beta casein but not A2 beta casein associated with type 1 diabetes and cardiovascular diseases, however up to date less evidence have been linked milk containing A1 and A2 beta casein compared with milk containing A2 variant alone with gastrointestinal function. The mechanism behind this association assumed to be dietary components like caseins that cleaved in the gut to produce peptide fragments with opioid characteristics βCM-7. This compound enters the circulation, cross the blood: brain barrier and result in influencing gastro-intestinal motility and enzyme secretion in the gut.

The main purpose of this study is to determine how consuming pasteurised milk containing A2 beta casein variant influence inflammation and the normal function of gastrointestinal tract compared with milk containing A1 and A2 beta casein in subjects with mild to moderate symptoms of non- lactose milk intolerance.

I confirm that to the best of my knowledge I have made known all information relevant to the Research Ethics Committee and I undertake to inform the Committee of any such information which subsequently becomes available whether before or after the research has begun.

I confirm that a list of the names and addresses of the subjects in this project will be compiled and that this, together with a copy of the Consent Form, will be retained within the School for a minimum of five years after the date that the project is completed.

Signed……..(Investigator) Date……

……..(Head of Department) Date……

……..(Student) Date……
(Where applicable)

Checklist

1. This form is signed by my Head of School ☐

2. The Consent form includes a statement to the effect that the project has been subject to ethical review, according to the procedures specified by the University Research Ethics Committee, and has been allowed to proceed ☐

3. I have made, and explained within this application, arrangements for any confidential material generated by the research to be stored securely within the University and, where appropriate, subsequently disposed of securely. ☐

4. I have made arrangements for expenses to be paid to participants in the resea☐, if any, OR, if not, I have explained why not.

5. EITHER

   (a) The proposed research does not involve the taking of blood samples; ☐

   OR

   (b) For anyone whose proximity to the blood samples brings a risk of Hepatitis B, documentary evidence of protection prior to the risk of exposure will be retained by the Head of School. ☐

Signed…….(Head of Department) Date……

6. EITHER

(a) The proposed research does not involve the storage of human tissue, as defined by the Human Tissue Act 2004; ☐

OR

(b) I have explained within the application how the requirements of the Human Tissue Act 2004 will be met. ☐

7. EITHER

(a) The proposed research will not generate any information about the health of participants; ☐

OR

(b) In the circumstance that any test reveals an abnormal result, I will inform the participant and, with the participant's consent, also inform their GP, providing a copy of those results to each; ☐

OR

(c) I have explained within the application why (b) above is not appropriate. ☐

8. EITHER

(a) the proposed research does not involve children under the age of 5; ☐

OR

(b) My Head of School has given details of the proposed research to the University's insurance officer, and the research will not proceed until I have confirmation that insurance cover is in place. ☐

Signed…….(Head of Department) Date……

This form and further relevant information (see Sections 5 (b)-(e) of the Notes for Guidance) should be returned to, Dr Mike Proven, Head of Quality Assurance in Research. You will be notified of the Committee's decision as quickly as possible, and you should not proceed with the project until then.

Hugh Sinclair Unit of Human Nutrition
Department of Food and Nutritional Sciences
University of Reading
PO Box 226
Reading RG6 6AP

Phone

Subject Information Sheet

Principal Investigator: Dr Sandrine Claus/ Professor Ian Givens

School: School of Chemistry Food and Pharmacy
Department of Food and Nutritional Sciences

Title of Project: Effects of milk containing exclusively A2 beta-casein on gastrointestinal function in volunteers with mild to moderate non-lactose milk intolerance.

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information.

Thank you for your interest in this study and taking your time to reading this participant information sheet.

Investigators: Dr Sandrine Claus
Professor Ian Givens
Areej Almuraee (PhD student)

Contact Name: Areej Almuraee
Food and Nutritional Sciences Department
School of Chemistry Food and Pharmacy
The University of Reading
Reading RG6 6AP United Kingdom
Room 2-1
Tel. 0118 378 4537
Fax: 0118 931 0080
Email:

Alternative contact Dr Sandrine Claus
Tel: +44 118 378 8717
Email:

Background

Cows Milk consist of 20% whey protein and 80% casein protein, 36% of which is beta casein. A small variation in this protein leads to presence of casein A1 and A2. A1 and A2 proteins are naturally present in commercial cow's milk.

There is increasing evidence that milk containing A1 beta casein but not A2 beta casein may be associated with type 1 diabetes and cardiovascular diseases. However there is little evidence to date of a link between these casein variants and gastrointestinal function, although it has been suggested that A1 bet- casein may be responsible for symptoms of milk intolerance that are neither due to lactose intolerance nor to milk protein allergies. The mechanism behind this association is assumed to be resulting from the breakdown of A1 beta-casein into a small protein fragment known for its pro-inflammatory properties. In this context, a recent study published in 2016 demonstrated that consumption of commercial milk was associated with significantly greater digestive discomfort symptoms (i.e. bloating, abdominal pain and flatulence) and higher concentration of inflammation markers compared with A2 milk consumption (that lacks A1 beta-casein). In this study, non-lactose milk intolerant subjects who experienced occasional gastrointestinal discomforts following milk intake benefited significantly from replacing conventional milk with A2 milk as it reduced their symptoms. In this study, we are therefore running an independent trial to investigate these connections in more details.

What is the purpose of the study?

The main purpose of this study is to determine how consuming pasteurised milk containing the A2 beta casein variant may improve the normal function of the gastrointestinal tract and reduce inflammatory biomarkers in healthy volunteers with mild symptoms of regular milk intolerance.

Am I suitable to take part?

We aim to recruit healthy people, male or female, who occasionally drink milk and experience symptoms of mild to moderate gastrointestinal discomfort in the

few hours following milk intake. These symptoms may include increased gases, bloating and abdominal pain and must not be associated with any known lactose intolerance or milk protein allergy. You need to be between 18 and 56 years of age, non-smoking and free from disease. Females taking the contraceptive pill or hormone replacement therapy (HRT) can take part. Females who are pregnant, lactating or planning to be pregnant will not be able to take part in the study. At the beginning of your study participation, you will receive a health check and we will fully assess your eligibility to take part in the study.

Do I have to take part?

It is up to you to decide whether you wish to participate in the study. We will describe the study to you and go through this information sheet, which we will give or send to you by mail. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time without giving a reason; please let one of us know if you would rather not participate and we will not contact you further about the study.

What will I be asked to do during screening visit?

The screening visit will be at the Hugh Sinclair Unit of Human Nutrition in the Department of Food and Nutritional Sciences (University of Reading). The visit will take place in the morning and we would like you to arrive in an unfed state (fasted, not eating or drinking anything but water from 8 pm the night before).

- All participants will be asked to fill out a health screening questionnaire before the screening visit and inclusion/exclusion criteria will be reviewed for volunteer eligibility.
- Informed consent from yourself will be required.
- Participants' weight, height and blood pressure will be measured.
- Volunteers will be required to give a blood sample to screen for anaemia and baseline measurements (15 ml, which is approximately 2 table spoons) .
- A symptoms scoring sheet will be given for self-evaluation of milk intolerance.

- A breath test will be carried out to screen out lactose intolerant subjects. This will involve the ingestion of 25g of lactose followed by breath measurements at regular intervals for 3 hours.
- All eligible volunteers will be asked to exclude dairy products two weeks before the start of the trial and during the trial except the milk that will be provided.
- The screening visit should last approximately 4 hours and you will be provided with a light breakfast before you leave.

Breath test:

Participants will be asked not to eat or consume alcohol for 12 hours prior to the test, with only water to drink and avoid slowly digesting foods such as beans the day before the test. A baseline breath test must be taken before the administration of substrate. Then 25g of lactose dissolved in 250ml of water will be given and measurements will be recorded at 0, 15, 30, 60, 90, 120, 150 and 180 min.

What will I be asked to do during study visit?

- Before the study begins it is important to exclude all dairy products for 2 weeks. Dairy products include milk, cheese, butter, yogurt and cream. Vegetable milks such as soy or rice milks are allowed.
- Once the study begins, participants will be randomly allocated to consume conventional milk or A2 milk for 2 weeks and after the first wash out period participants will switch to the other milk. Neither yourself nor the investigator will know which of the milk you are taking. After each of the 2 weeks intervention period, there will be a 2 weeks washout period where no milk or dairy product will be consumed.
- Participants will be required to consume the milk twice a day as follows (one with breakfast and one with dinner)
    - Days 1 and 2: 100 mL (twice a day)
    - Days 3 and 4: 150 mL (twice a day)
    - Days 5 and 6: 200 mL (twice a day)
    - Days 7 until 14: 250 mL (twice a day)

- The milk will be provided in a container as liquid pasteurised milk, therefore it must be kept at 4ºC in a fridge.
- Volunteer should come in the Hugh Sinclair Nutrition Unit at the beginning of each treatment week to collect the milk to be consummed.

- Volunteers will provide one stool sample on weeks 0, 2, 4, 6, 8 of the study to look for changes in gut bacteria. The same time-points will be used for blood sampling to assess blood metabolites and genetic analysis. Volunteers will be given a container for faecal collection and shown to private facilities within the department to give a stool sample. If unable to do so, they will be given the option to come in the days following until production is possible. No new treatment will be issued until stool has been provided.
- Volunteers will be asked to provide a 24h urine sample at each visit. The necessary equipment will be provided. It is recommended to keep urine samples in the fridge until they are brought to the study centre.
- All blood samples will be taken in the Hugh Sinclair unit of the Department of Food and Nutritional Sciences.
- Volunteers will undergo another breath test following test milk challenge at the end of each diet intervention without lactose challenge.
- Maintenance of normal dietary patterns throughout the study is essential and participants will be required to complete food and drink logs throughout the study
- Any adverse medical events which occur during the trial (e.g. headache, gut symptoms) should be recorded and rated in a diary along with medication taken
- All incidence of respiratory infections and colds should also be reported
- Daily stool habit should be recorded in a diary

- **Please note** that participants will be withdrawn from the study if they develop acute gastrointestinal illness (e.g. food poisoning) or intolerance to the milk or if they do not comply to above stated restrictions

Confidentiality, storage and disposal of information

The results will be strictly confidential to the study investigators and each participant will only be identified by means of a random number allocated at the beginning of the study.

Information obtained from the study may be published in scientific journals but only in the form of average values for the group; no results for the individual participants will be published or presented in scientific meetings. We will inform you of the broad scientific results of the study, if you interested. If we discover any abnormalities of significance to your health we will inform you and your GP.

Are there any benefits/risks to taking part [e.g. health]? Risk of lactose intolerance

It is not anticipated that there will be any risk to participant safety because a similar protocol has been used in previous studies. A similar volume of pasteurised milk has been used in a number of feeding trials with various groups of volunteers and was well tolerated by all. In addition, we will progressively increase the dose of milk to be able to detect any adverse reaction before reaching the trial dose of 500 mL/day. Therefore, participation in the study does not pose any significant risk. However, participants may experience some mild to moderate gastro-intestinal symptoms such as gases, abdominal cramps, bloating, diarrhoea and constipation. These symptoms may vary from one person to another. In the occurrence of such events, the researcher will provide advise on how to deal with the symptoms and you will be free to withdraw from the study at any time.

Blood samples will be collected by an experienced and trained phlebotomist in the Hugh Sinclair Unit of Human Nutrition (Department of Food and Nutritional Sciences) but occasionally this may cause mild bruising.

What expenses and/or payment or equivalent will be made for participation in the study?

Volunteers will be provided with breakfast on visit days. Volunteers will be remunerated for their time with a total of £150 for completion of the trial. Those who withdraw will have their payment pro-rated according to time spent on the

study. Reserve volunteers will be paid for assessment and instruction if they are not required to participate in the treatment phases of the trial.

What will the results of the study be used for?

The results of this study will be used in a PhD thesis, will be published in a scientific journal and presented at international conferences.

Who has reviewed the study?

This study has been reviewed by the University Research Ethics Committee and has been given a favourable ethical opinion for conduct.

Who do I contact for further information or complaints?

If you have a concern about any aspect of this study, you should ask to speak to one of the study investigators who will do their best to answer your questions (see contact details at the front of this Participant Information Sheet). If there is a complain, this should be addressed to Dr Sandrine Claus, principal investigator at The University of Reading:

Thank you for reading this information. Please contact us if you have any further questions or would like to take part in the study.

Study plan:

Proposed dates	*Stage of Study*	*Treatment*
Day-14	Pre-trial meeting/ Screening	• *Briefing about the study* • *Provide screening blood samples* • *Breath test for screening out lactose intolerance* • *Milk intolerance symptoms rating scale sheet* • Psychological behaviour tests.
Day 0	**Visit 1** Start of study	• Provide baseline faecal, urine and blood samples • Dispensing of volunteer diaries • Distribution of test milk • During the 2 week period – milk will be taken twice daily as discussed with researcher • Diaries to be completed daily • Psychological behaviour tests.
Day 14	**Visit 2** After 2 Weeks Treatment Period 1	• Consume amount of milk as presented below twice a day (with breakfast and dinner) for two weeks treatment period; Days 1 and 2: 100 mL (twice a day) Days 3 and 4: 150 mL (twice a day) Days 5 and 6: 200 mL (twice a day) Days 7 until 14: 250 mL (twice a day) • During the treatment (A1/A2 milk or A2 milk), complete dietary, bowel habit and mood questionnaires and record any concomitant medication or adverse events during the trial • Provide faecal sample, urine sample, blood sample and methane and hydrogen breath test • Psychological behaviour tests.
Day 14-27	**Visit 3** **2 week** Washout period	• Provide faecal, urine and blood samples (visit 3) • Diaries to be submitted • Refrain from consuming milk • Do not take any dairy products • Psychological behaviour tests.

Day 28-42 (interim period**)**	**Visits 4** 2 Week Treatment Period 2	• Consume amount of milk as presented below twice a day (with breakfast and dinner) for two weeks treatment period; Days 1 and 2: 100 mL (twice a day) Days 3 and 4: 150 mL (twice a day) Days 5 and 6: 200 mL (twice a day) Days 7 until 14: 250 mL (twice a day) • During the treatment (A1/A2 milk or A2 milk), complete dietary, bowel habit and mood questionnaires and record any concomitant medication or adverse events during the trial • Provide faecal sample, urine sample, blood sample and methane and hydrogen breath test • Psychological behaviour tests.
Weeks 42-56 (end of treatment period)	**Visit 5** 2 week Washout period	• Provide faecal, urine and blood samples (visit 5) • Diaries to be submitted • Refrain from consuming milk • Do not take any dairy products • Psychological behaviour tests.

END OF THE STUDY

Areej Almuraee

Hugh Sinclair Unit of Human Nutrition
Department of Food and Nutritional Sciences
University of Reading
PO Box 226
Reading RG6 6AP

Phone

Recruitment Email

Subject: Men and women sought for milk and gastrointestinal function study

Dear…

Would you like to take part in a study that tests the effect of replacing ordinary cow's milk with A2 milk on gastrointestinal function? To take part, you need to be:

- Male or female aged 18 to 56 years
- In good general health with self reported milk intolerance
- Not diagnosed with lactose intolerance
- Not diagnosed with dairy allergy
- Free from disease and long-term medication

Willing to:

- Consume pasteurised semi-skimmed milk (conventional and commercially available A2 milk)
- Remain free from other dairy products for 6 weeks.
- Have blood, urine and stool samples collected
- Attend 5 study visits at the Hugh Sinclair Unit of Human Nutrition, University of Reading.

You will receive remuneration for your time.
If you would like to know more about the study, please leave us a message on
or

We appreciate your help.

Yours sincerely
Areej Almuraee

Hugh Sinclair Unit of Human Nutrition
Department of Food and Nutritional Sciences
University of Reading
PO Box 226
Reading RG6 6AP

Phone

Recruitment Social Media Text (Facebook, Streetlife)

Subject: Men and women sought for milk and gastrointestinal function study

Dear…

Would you like to take part in a study that tests the effect of replacing ordinary cow's milk with A2 milk on gastrointestinal function? To take part, you need to be:

- Male or female aged 18 to 56 years
- In good general health with self reported milk intolerance
- Not diagnosed with lactose intolerance
- Not diagnosed with dairy allergy
- Free from disease and long-term medication

Willing to:

- Consume pasteurised semi-skimmed milk (conventional and commercially available A2 milk).
- Remain free from other dairy products for 6 weeks.
- Have blood, urine and stool samples collected
- Attend 5 study visits at the Hugh Sinclair Unit of Human Nutrition, University of Reading.

You will receive remuneration for your time.
If you would like to know more about the study, please leave us a message on
or

We appreciate your help.

Yours sincerely
Areej Almuraee

University of Reading

HUGH SINCLAIR Unit of Human Nutrition

DIGI study

Screening visit records

Date	**Time**	**Vol ID**

Records				
Consent form 2 (COPY)				
Gender	○ Male ○ Female			
Height (cm)				
Weight (kg)				
BMI	`			
Attached Tanita result				
L arm BP (3) (mm Hg)	**SBP**			
	DBP			
Heart Rate	**HR**			
Fated blood samples (1tablespoon)				
Familiarisation visit date				

Study visit dates	**Study visit**	**Date**
	Visit 1	- - 2017
	Visit 2	- - 2017
	Visit 3	- - 2017
	Visit 4	- - 2017
	Visit 5	- - 2017

Comments
Please advise the volunteer that we will be in contact via a letter within 3 weeks to inform whether they are suitable to participate in DIGI study (they can always contact us if they haven't heard anything after 3 weeks). We will also send them the results of their blood test. Their GP will be advised of their participation, but they will not be given the results of the screening visit. If suitable, we will then be in contact to book the familiarisation visit and three study appointments.

Researcher signature: ……..

Volunteer No……	Day ……	Time ……

Appendix B

Volunteer symptoms scoring scale:

Did you experience any of the following symptoms after drinking milk (after 24h) : Adapted from (Breivik et al., 2008).

Table 1 Visual analogue scale

Symptoms	Severity Scale Not — Very severe
Gases (wind)	
Bloating	
Abdominal cramps	
Diarrhoea	
Headache	
Constipation	
Nausea	
Rash	

Table (A) Method for calculating severity of symptoms

Symptoms	0	1	2	3	
Gases (wind)					
Bloating					
Abdominal cramps					
Diarrhoea					
Headache					
Constipation					
Nausea					
Rash					
Total					

Score = …….

Semi- skimmed Milk 1

Days 1 and 2: 100 mL (twice a day)
Days 3 and 4: 150 mL (twice a day)
Days 5 and 6: 200 mL (twice a day)
Days 7 until 14: 250 mL (twice a day)

DIGI Study

USE BY: / /

Semi- skimmed Milk 1

Days 1 and 2: 100 mL (twice a day)
Days 3 and 4: 150 mL (twice a day)
Days 5 and 6: 200 mL (twice a day)
Days 7 until 14: 250 mL (twice a day)

DIGI Study

USE BY: / /

Semi- skimmed Milk 2

Days 1 and 2: 100 mL (twice a day)
Days 3 and 4: 150 mL (twice a day)
Days 5 and 6: 200 mL (twice a day)
Days 7 until 14: 250 mL (twice a day)

DIGI Study

USE BY: / /

Semi- skimmed Milk 2

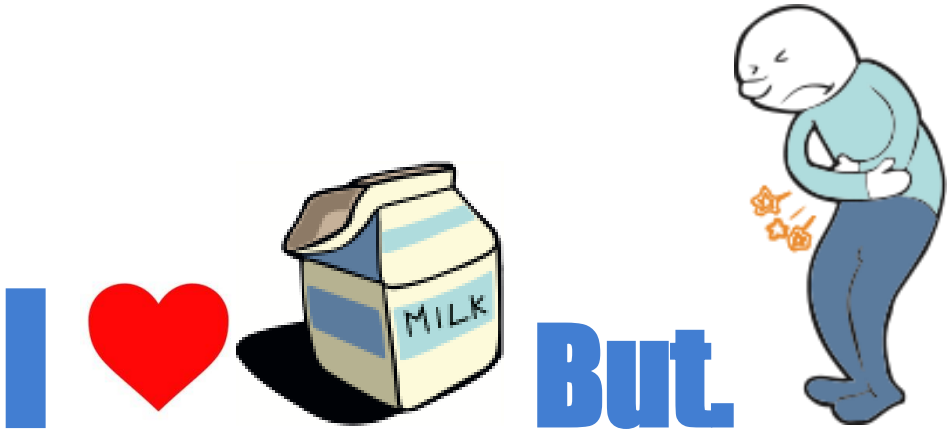
Days 1 and 2: 100 mL (twice a day)
Days 3 and 4: 150 mL (twice a day)
Days 5 and 6: 200 mL (twice a day)
Days 7 until 14: 250 mL (twice a day)

DIGI Study

USE BY: / /

University of Reading

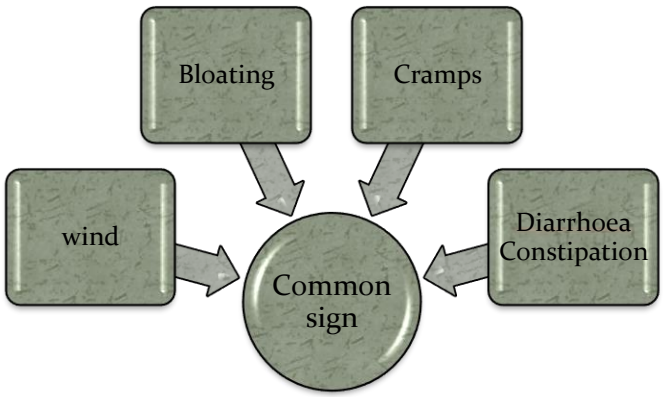
HUGH SINCLAIR Unit of Human Nutrition



Do you experience any gastrointestinal symptoms after drinking milk?

Would you be interested in participating in our study?

Volunteers will be reimbursed for their time



Looking to recruit for this study:

- Males and females
- Age 18-56yrs old
- Non smokers
-

For more information, please contact the Human nutrition unit on **or on**

Date	Time	Visit	Vol ID

For breath analysis:

Volunteers should be fasted for a minimum of 12 hours prior to the test.

Please avoid:

1- Consuming alcohol, tea and coffee for 12 hours prior to the screening visit. You can drink water.

2- Slowly digesting foods such as beans and pasta the day before the screening visit.

Breath analysis:

1- A baseline breath measure must be taken before the administration of substrate.

2- Then 25g of lactose dissolved in 250ml of water will be consumed and hydrogen and methane will be measured at 0, 15, 30, 60, 90, 120, 150 and 180 min.

3- Subjects with an increase in breath **hydrogen** concentration **≥20** part per million (ppm) and breath **methane** concentration **≥12ppm** from the baseline within the test period will be classified as **lactose intolerant**.

4- Subjects whose **hydrogen** concentration increased **< 20** ppm and breath **methane** concentration **<12** from the baseline will be classified as **lactose tolerant.**

Time record	Time required	H_2 (ppm)	CH_4 (ppm)	O_2 %	Cor F
	Baseline (0)				
	15 min				
	30 min				
	60 min				
	90 min				
	120 min				
	150 min				
	180 min				

Result:

Visit: 1 / 2 / 3 / 4 / 5

Measurement		Tick/Answer			Comments
Parking permit?		□			
Anthropometrical measures and body composition)		Weight	kg		**Tanita copy attached**
		Height	cm		
Toilet need?		□			
Collect 3d food diary, check you can read		□			Only visits 3 and 5
Hydrogen and methane test		□			Only visits 2 and 4
Collect intervention product?		Yes □ No □			**Leftovers if any**
Fasted 12h?		Yes □ No □			
Sport restriction, 24h?		No need			
Medication? Illness?		Yes □ No □			If yes, which?
Stool sample collection		□			
24 Urine sample collection		□			
Bp X 3	SBP				
	DBP				
	HR				
Collect blood		□			
Cognitive test		**Part 1** □ **part 2** □			
Provide study food		□			Only visit 1 and after visit 3
Breakfast (study food) or other food		□			

Date	**Time**	**Vol ID**

Records							
Consent form 2 (COPY)							
Gender	○ Male ○ Female						
Height (cm)							
Weight (kg)							
BMI	`						
Attached Tanita result							
L arm BP (3) (mm Hg)	**SBP**				**Average**		
	DBP						
Heart Rate	**HR**						
Fasted blood samples (1tablespoon)							
Symptoms usually got	Gases (wind) □	Bloating □	Cramps □	Diarrhoea □			
	Headache □	Nausea □	Rash □	Constipation □			
Breath tests							
Cognitive tests							
Familiarisation visit date							

Study Visits booking	**Visit 1**	- - 2017
	Visit 2	- - 2017
	Visit 3	- - 2017
	Visit 4	- - 2017
	Visit 5	- - 2017

Comments
Please advise the volunteer that we will be in contact via a letter within 3 weeks to inform whether they are suitable to participate in DIGI study (they can always contact us if they haven't heard anything after 3 weeks). We will also send them the results of their blood test. Their GP will be advised of their participation, but they will not be given the results of the screening visit. If suitable, we will then be in contact to book the familiarisation visit and study visits.

Researcher signature: ……..