Study protocol

Official study title: The effect of consuming grass finished lamb meat on omega-3 blood response among healthy consumers

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Structured Protocol

You are asked to ensure your protocol includes all of the headings listed. If you feel the heading is not relevant to your protocol, then please specify the reason.

Title of project: The effect of consuming grass finished lamb meat on omega-3 blood response among healthy consumers

Aims and objectives

Overall aim: To investigate the impact of consuming grass finished lamb meat on human health parameters when compared to concentrate finished lamb meat.

Primary objective: To assess the effect of regular consumption of grass finished and concentrate finished lamb meat on the plasma and platelet omega-3 status of healthy individuals.

Secondary objective: To assess the effect of consumption of grass finished and concentrate finished lamb meat on cardiovascular risk factors including blood pressure, body mass index (BMI), total cholesterol, HDL-cholesterol and triglycerides among healthy individuals.

Background

In recent years, consumers have become increasingly interested in the food they eat, given advances in nutrition science, the nutritional quality of food products and knowledge of non-communicable disease aetiology. Currently, cardiovascular disease represents 31% of deaths worldwide (World Health Organisation, 2016) and incidence is predicted to rise given modern day lifestyle habits including smoking, physical inactivity and poor diet. Meat consumption can contribute a nutritionally significant as part of a balanced diet. Due to its high biological value, meat is an important source of nutrients for human health and wellbeing, e.g. iron, vitamin B12 and zinc. However, there are concerns and controversy surrounding the fat content of meat, despite fat being a vital component directly influencing palatability, flavour and overall liking (Font-i-Furnols and Guerrero, 2014). Fat type and content varies greatly between species and is further influenced by age, sex, genetic makeup and nutrition of the animal (Khan et al, 2015; Scollan et al, 2017). Within the muscle, fat is distributed into neutral and polar fractions and deposited intermuscular, intramuscularly or subcutaneously, with type and amount of lipid varying within individual muscles (Listrat et al, 2016).

When compared to monogastric meat, ruminant meat contains high levels of saturated fatty acids (SFA) and trans-fatty acids (TFA) relative to monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). However, all meat types are relatively low in the essential omega-3 (*n*-3) fatty acids for which naturally rich sources include fish and fish products. Nonetheless, by nature of its frequent consumption ruminant meat supplies not just dietary SFA but also makes considerable contributions to dietary MUFA and PUFA.

Excessive intake of dietary SFA is known to increase risk of cardiovascular disease (CVD) and contribute to various other non-communicable disease incidence (Mensink, 2005). UK dietary reference values recommend, for adult males and females, total fat intake should not exceed 35% of food energy, with 11% of this accounting for SFA (British Nutrition Foundation, 2018). Recommended intakes of total *cis*-PUFA are 6.5% food energy, of which there should be a minimum n-3 PUFA intake of 0.2% of food energy (British Nutrition Foundation, 2018). Similarly, The WHO (WHO, 2016) recommended that, for adults, total fat should not exceed 30% of total energy intake. More specifically, total SFA and TFA content should be no greater than 10% and 1% of total energy intake, respectively. The WHO guidelines also recommend that total PUFA should reach 11% of total energy intake with 2% and 9% accounting for *n*-3 and *n*-6 PUFA, respectively.

Despite these guidelines, global *n*-3 intake is sub-optimal, with western countries falling particularly short of optimal blood eicosapentaenoic (EPA; 20:5 *n*-3) and docosahexaenoic acid (DHA; 22:6 *n*-3; Stark et al. 2016). The European Food Safety Authority (EFSA; 2009) stated that for food products to be considered ‘a source of’ or ‘high in’ *n*-3 long chain PUFA (LC-PUFA) it must contain per 100 kcal 40 and 80 mg/ day of 20:5 *n*-3 and 22:6 *n*-3, respectively. In order to reach the recommended *n*-3 PUFA intake, UK guidelines suggest that two portions of fish per week should be consumed, one if which is oily. However, global fish consumption is lower than recommended, whilst poultry and red meat consumption is higher and represents a large proportion of fat intake.

The relationship between *n*-3 PUFAs and human health is well established and generally accepted. Increased dietary levels of 20:5 *n*-3 and 22:6 *n*-3 have been documented to instigate physiological reaction cascades, thereby improving the inflammatory response, including alleviating arthritis and asthma symptoms (Yashodhara et al, 2009; Norling and Perretti, 2013; Li, 2015). Similarly, enhanced 20:5 *n*-3 and 22:6 *n*-3 has also demonstrated its ability to decrease CVD risk by reducing triglycerides in the bloodstream, lowering the risk of myocardial

infarction, stroke, atherosclerosis development and overall risk of death (Jacobson et al. 2012; Givens, 2015; Singh et al. 2016). Nevertheless, there is a level of controversy surrounding n-3 PUFA exhibiting health promoting effects, with some studies finding little or no evidence of significant health benefits of consuming n-3 PUFA (Rangel-Huerta and Gil, 2018). Discrepancies could be explained partially by differences of bioavailability of n-3 PUFA between dietary sources found within foodstuffs and supplementary sources of n-3 PUFA (Harris et al, 2017).

Due to the generally accepted ability of *n*-3 to reduce non-communicable disease incidence, nutritional approaches have focused on increasing the amount of beneficial fats in meat and meat products with emphasis placed on increasing *n*-3 and specifically *n*-3 LC-PUFA. Improving the lipid composition of poultry and monogastric meat has been largely successful. However, the process for ruminant animals is more multifaceted due to the diversity of the rumen and resident microbial community. Consequently, large proportions of dietary PUFA are converted into SFA such as stearic acid (C18:0) or palmitic acid (C16:0) by lipolysis and biohydrogenation processes, which are controlled by complex microbial flora (Lourenco et al. 2010). Research has attempted to overcome the biochemical limitations of nutritionally enhancing ruminant meat by examining early life feeding, including microbial programming (De Barbieri et al. 2015) and finishing feed (Vestergaard et al. 2000). Emphasis has also been placed on the positive benefits of pasture *vs* concentrate feeding.

It is widely accepted that pasture compared to concentrate based feeding is more efficient in enhancing beneficial *n*-3 PUFA in meat of ruminant animals (Nuernberg et al. 2005; Fisher et al. 2010; Hajj et al. 2016; Scollan et al. 2017), with enrichment proving more efficient in lamb than beef (Bessa et al. 2015). More specifically, grass chloroplasts are rich in 18:3 n-3, which are a known precursor for longer chain n-3s such as 20:5 n-3, 22:5 n-3 and 22:6 n-3. In fresh grass, 18:3 n-3 can represent up to 75% of the total lipid fraction (Blasko et al, 2010). This level can be influenced by season, species, geographical location, growth stage, nitrogen fertilization and senescence. In addition, pasture-based feeding provides increased concentrations of vitamin E, which contains valuable antioxidant properties, reducing the incidence of lipid oxidation and therefore minimising the opportunity for skewed flavours and odours to develop.

The effect of animal-based foods enriched fatty acid profiles on human health has received relatively little attention and is largely unknown. In recent years, a single study explored the effect of consumption of either *n*-3 enriched pasture-based feeding or concentrate fed beef and

lamb on blood fatty acid profiles and circulatory parameters including blood pressure and cholesterol in an intervention trial among healthy individuals (McAfee et al. 2011). Results showed that the total PUFA and LC-PUFA plasma and platelet lipid composition of individual's subject to the pasture fed meat treatment was significantly higher, when compared to subjects who consumed concentrate fed meat. These results highlight the ability of *n*-3 within enriched meat to be successfully taken up and utilised within the human body. However, this study is an isolated examination of the human health effects of *n*-3 enriched ruminant meat products and further and more in-depth research is required to fully establish a confirmed biological effect and contribute to the current limited literature.

The research area of enriching n-3 PUFA concentration in ruminant meat is increasingly being developed and continues to expand. However, there is a significant lack of research demonstrating the effect of consuming grass finished lamb on n-3 blood status among free living healthy consumers. Therefore, identification of current knowledge gaps is an imperative to overcome the challenges in ensuring an optimal level of n-3 PUFA is present in muscle and in turn consumed by the public, ultimately contributing to human health by assisting with reducing cardiovascular and other non-communicable diseases incidences with little change to customary dietary habits. The present study proposal uniquely focuses on the consumption of lamb meat for a four-week period as a proof of principle technique to demonstrate preliminary biological effect of habitual lamb consumption on blood omega-3 status and cardiovascular risk factors.

Plan of investigation

Study design

The study period will consist of a four-week, single blinded, randomised dietary intervention whereby participants will be required to eat three portions per week of lamb meat from one of two treatments for a four-week study period. The study will focus entirely on the consumption of lamb meat. This is because evidence in current literature suggests that lamb muscle has a higher capacity to store omega-3 in the muscle phospholipids when compared to other meats such as beef or pork (Bessa et al, 2015). Therefore, as a proof of principle study, volunteers will consume three portions of lamb per week for four weeks, substituting lamb for regular red meat consumption and therefore maintaining intakes of red meat within safe levels and not encouraging any adverse dietary pattern.

Lamb portions will include per week one portion of mince (raw weight 250g) and two portions of lamb chops (raw muscle weight 235g). Raw lamb portions will total 720g per week. Literature suggests cooking losses will represent approximately 32% (Matthew and Garrison, 1975), meaning around 490g of lamb per week will be consumed, keeping below recommendations by the World Cancer Research Fund (WCRF) to consume less than 500g of red meat/week and UK NHS recommendations to consume no more than 70g/day of red and processed meat. One group of participants will receive lamb meat from concentrate finished lambs and another group will receive lamb meat from grass finished lambs. The study will be single blinded meaning researchers will know what treatment participants are receiving however participants will not be told what dietary treatment they are receiving.

Meat will be stored at AFBI Newforge, AFBI Stormont and North South Retail Belfast. The meat will be delivered to the participant's residence frozen on a weekly basis. Days and times of delivery will be discussed and decided on an individual participant basis. Participants will also be expected to keep a three-day food diary which will be completed prior to the intervention start and then again during the mid-point of the intervention during week one and week three. This is where all food and drinks for the three days will be recorded with as much detail as possible regarding consumption (including left overs), time of day, quantity of food and cooking methods. In addition, participants will also be asked to keep a meat diary for the entire duration of the study as a record of whether all meat is consumed or whether some is not consumed. This will also include what cooking methods are used and whether participants consume the subcutaneous fat or not. Participants will receive recipe cards at the beginning of the four-week study period to provide cooking ideas; however, there is no obligation to follow the recipes. Participants may cook the lamb however they choose but will be reminded of the importance of following good food safety and hygiene practices e.g. defrosting meat safely and the importance of thoroughly cooking minced meat.

<u>Meat source</u>

The lambs which will provide the meat were reared for an 11-week finishing period at AFBI, Hillsborough, NI. The finishing period started 04th September 2018 and finished on 20th November 2018. The lambs were transported to Dunbia, Dungannon for slaughter on the 21st November 2019. The carcases were left to hang for 48 hours before the meat was processed into loins, shoulder and leg meat. Finer processing including separation of loins into chop

portions and mincing of meat was carried out at Loughery College, Cookstown on 26th November 2018 and 16th January 2018, respectively. The loins were band-sawed fresh creating lamp chops, individually packaged and then frozen. The shoulder and leg meat to create mince portions was frozen and then tempered to -1°C, minced, weighed into 250g portions, vacuum packaged and re-frozen. Alternative lambs have been raised and slaughtered to act as a back-up option if any of the intervention study meat was to be unsuitable. A representative proportion of the meat from both the loins and the mince portions will undergo microbial analysis prior to being used in the intervention study.

Sample size and justification

Healthy volunteers (N=38) will be recruited and allocated to one of two treatments (n=19/treatment) balanced for age and gender using a computer-generated randomization tool, which will be completed with the assistance of Dr. Christopher Cardwell (statistician). Sample size was determined using power calculations based on changed in blood plasma and platelet PUFA levels from a similar study (McAfee et al, 2011). Based upon the SD of plasma PUFA of 2 units (%), with 16 in each treatment there will be over 80% power to detect, as statistically significant at the 5% levels, a difference in mean PUFA of 2 units in the grass-fed lamb meat group and the concentrate fed lamb meat group. This will be determined using an independent samples t-test. This difference in plasma PUFA is similar (slightly less than) the difference seen in the previous study (McAfee et al, 2011), however it is anticipated a larger difference will be detected as only lamb meat is being used which is documented to be of increased capacity to store omega-3 PUFA (Bessa et al, 2015) and therefore it is expected that this will have a greater influence on blood plasma PUFA status. The power calculation for platelets will be more limited, however, based on the SD of platelet PUFA of 4 units (%), with 16 in each treatment we would have over an 80% power to detect, as statistically significant at the 5% level, a difference of 2 units in the lamb-fed meat group and concentrate fed meat group. In addition to the 32 required participants established by statistical power calculations an additional six participants will be recruited to account for drop out and non-compliance.

Volunteer recruitment

Volunteers will be staff and students recruited from Queen's University Belfast. Involvement in the study will be advertised via poster advertisement around university buildings initially in the David Keir Building (DKB), Sonic Arts Research Centre (SARC) and Northern Ireland Technology Centre (NITC). Following this, advertisement posters will be displayed in the

Medical Biology Centre (MBC). Volunteers will also be recruited via university mailing lists and through inclusion of the study advertisement on the staff round-up. All potential volunteers will be given the information sheet and will be assessed for suitability and eligibility using a short screening questionnaire which will identify whether volunteers fit into the inclusion and exclusion criteria (see below). Subsequently, participants will be given 48 hours to decide whether or not to participate in the study. When participation is agreed written consent will be obtained.

Inclusion and exclusion criteria

Potential participants will be screened using an eligibility questionnaire (See attached questionnaire), which will identify suitable participants by applying specific inclusion and exclusion criteria outlined below. Male and female healthy volunteers aged between 18-64 years old will be considered for participation. For eligibility of participation, volunteers must be non-smokers, must not be taking statins to lower LDL cholesterol levels and must not have high blood pressure (systolic >140/ diastolic >90mmHg). Moreover, participants with a BMI of <18.5 kg/m^2 and greater than >35 kg/m^2 will be excluded. Participants who are taking prescribed medication and any form of dietary supplements will also not be eligible to participate The consumption of the lamb meat will act as a substitute for optimal habitual red meat consumption as recommended by the WCRF and UK NHS. Therefore, participants must be red meat consumers and willing to consume three portions of lamb per week. Participants who consume more than two portions of fish per month will also not be eligible to participate, as that may mask the ability of the lamb to affect n-3 PUFA status.

Blood collection and handling

Blood samples (25ml) will be taken at the beginning of week 0 and end of week 4 (end of dietary intervention) from both the grass-finished meat and concentrate-finished meat treatment groups. As total-cholesterol, HDL-cholesterol and triglyceride concentrations will be measured participants will be asked to fast for 12 hours prior to blood being taken. Samples will be taken, separated within 1 hour of collection and then stored at -80°C until analysed at a later date. Plasma and serum will be harvested by spinning blood samples at 2500 g for 15 minutes, whilst platelets will be harvested by centrifuging blood samples at 150 g for 15 minutes. Samples will be analysed for the fatty acid composition of blood plasma and platelet, in addition to total cholesterol, HDL-cholesterol and triglycerides (by commercially available kit on a Randox Daytona autoanalyser). All blood will be taken by a trained phlebotomist and stored and analysed at the Centre for Public Health, Queen's University Belfast.

Blood plasma, platelet and serum aliquots will be analysed for fatty acids using gas chromatography at the Centre for Public Health, Queen's University Belfast. Lipids will be extracted using the Folch method (Folch et al. 1957) and methyl esters will be prepared using an adaption of the method outline by Morrison et al. (Morrison et al. 1964). Fatty acid composition will be analysed using gas chromatography (Hewlett Packard 6890 Gas Chromatograph) and flame ionizing detector. Peaks will be integrated using 'Chemstation' computing integrator. Fatty acid methyl esters will be identified by output retention times which will be compared to known and commercially available standards. Results will be presented initially as % of total fatty acid content and then will be quantified by looking at retention times, peak areas and the use of an internal standard.

Blood Pressure and BMI

Blood pressure and body mass index (BMI) will also be assessed at baseline (week 0) and post-intervention (week 4) from both the grass finished meat and concentrate finished meat treatment groups. Blood pressure will be taken using an automated sphygmomanometer using established Centre for Public Health SOPs. Blood pressure from all participants will be taken from the same arm to ensure consistency. Systolic and diastolic pressure will be measured and recorded. BMI will be calculated by assessing the participant's weight (kilograms) and height (metres squared). Assessment will take place at The Centre for Public Health, Queen's University Belfast. Blood pressure and BMI data will be taken by Lynda Perkins (PhD researcher).

Illness during the study

During the study, if a participant feels unwell as a result of eating the lamb, participants should contact the researchers using the phone number or email address provided on the participant information sheet. There is minimal risk of discomfort as a result of consuming the lamb, however if discomfort does occur, participants can opt out of the study at any given stage. Participants should seek medical advice if it is felt this is appropriate.

Incidental findings

It will be made clear to participants that during data analysis the researchers are not looking for anything other than what is stated in the information sheet. Nonetheless, if any incidental findings are discovered (e.g. hypertension), the research team is obliged to disclose this

information with the participants. If an incidental finding occurs, the researchers will advise participants to consult a GP or a medical professional who will be able to guide them further.

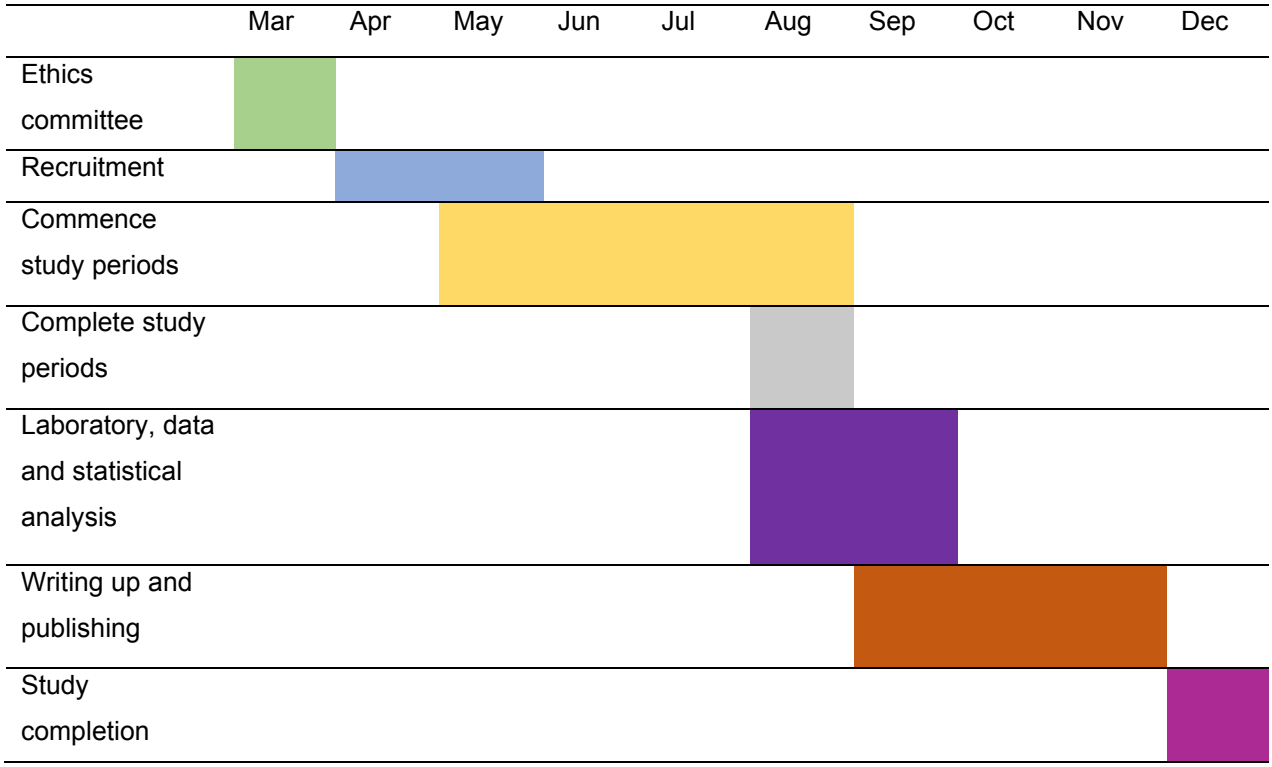
Planned statistical analysis of results

The change in each outcome variable will be compared between intervention groups using an independent samples t-test. A significance level of 0.05 will be used to determine statistical differences. Any effect within treatments from baseline to end of intervention will be analysed using a paired t-test. A significance level of 0.05 will be used to determined statistical differences. All statistical analysis will be carried out with the assistance of Dr. Christopher Cardwell and using established statistical software such as SPSS.

Project timetable

Recruitment of volunteers is planned to commence in April 2019 using poster advertisement (See Table 1). Once participants have been recruited, they will commence the dietary interventions. Participants will be stagger started as and when they are recruited. Completion of volunteer study periods are planned to finish in July 2019, at which point laboratory, data and statistical analysis will occur. The study is due to be fully completed by November 2019.

Table 1: Proposed project timetable for recruitment, intervention study period, analysis and writing up



Data protection

The data collected will be treated securely and confidentially as necessary under the General Data Protection Regulation (updated May 2018) and stored as required by the University. All participant information will be kept within a locked filing cabinet and will be kept on password protected university computers and stored in secured access-controlled university buildings. All participants will be allocated a unique study number which will ensure anonymously during the study. Blood samples will be labelled using the participants unique study number only and will be stored within a locked freezer within secured, access only buildings. Blood samples will not be used for any further analysis and will be disposed of after completion of the study.

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