

## TITLE

Acute Evidence of Digestive, Metabolic and Nutritional Differences in Beef, Lamb and Meat-Analogue Meals: A study protocol for a randomised control trial

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## **ABSTRACT**

### **Background**

Plant-based analogues and substitutes for animal meat are receiving considerable attention within academia and the popular press. Once a niche product aimed at vegetarians, they are increasingly promoted to omnivores and meat-moderating “flexitarians”. Proponents view these new foods as a means of reducing livestock production and improving the environment, whilst industry sees opportunity to repurpose traditional and novel crops into high-value ingredients. To date, most research on meat alternatives has focused on public perception, processing technologies, hedonics and the consumer acceptability issues that underpin product development. There have been surprisingly few investigations into the nutritional, health and well-being consequences of consuming meat analogues over the short-term or long-term. The aim of our current trial is to investigate the digestion, absorption and utilisation of a meat-analogue meal in Millennial consumers and compare that to matched meals featuring pasture-raised, grain-finished beef or lamb.

### **Methods**

A cohort of healthy, young (20-34 years old) male participants will consume a single meal on four occasions in a treatment-crossover, blinded investigation. Each instance will be followed by four hours of repeated venous blood collection to assess the digestive and metabolic effects of the meal. The four meals include a popular commercial meat analogue comprising pea, bean and rice extracts plus plant fats and cellulose, or a mince from pasture-raised or grain-fed beef or grass-raised lamb. Blood plasma samples of the participants will be measured for changes in chylomicron fatty acid distribution, amino acid profile, neurotransmitter proteins, minerals, inflammatory markers and biomarkers of general health.

### **Discussion**

To our knowledge this is the first randomised controlled trial (RCT) comparing the acute nutritional effects of iso-caloric, blinded meals containing a plant-based meat analogue or pasture-fed-, grain-finished beef or lamb. The study provides proof of principle for only the effects of a single meal. Future work will translate these insights into longer-term, lifestyle interventions.

### **Trial Registration**

This study, registered as Universal trial number: U1111-1244-9426, will be carried out under the auspices of The University of Auckland. The trial has been granted ethical approval by the Ministry of Health, Health and Disability Ethics Committee (Ref: 19/STH/226).

Date and version: 24/07/20, 5

**Key Words:** protein, amino acid, diet, health, meat products, bioavailability

## ADMINISTRATIVE INFORMATION

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see <http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/>).

**Table 1:** Trial Administrative Details

<b>Title {1}</b>	Acute Evidence of Digestive, Metabolic and Nutritional Differences in Beef, Lamb and Meat-Analogue Meals: A study protocol for a randomised control trial
<b>Trial registration {2a and 2b}.</b>	Universal trial number: U1111-1244-9426. ClinicalTrials.gov: 5000927, (pre-recruitment)
<b>Protocol version {3}</b>	24/07/2020, Version 5
<b>Funding {4}</b>	This research is funded by the Meat Industry Association Innovation Limited (a subsidiary of the New Zealand Meat Industry Association), Beef and Lamb New Zealand Limited and the New Zealand Ministry of Business, Innovation and Employment.
<b>Author details {5a}</b>	Refer to Table 3
<b>Name and contact information for the trial sponsor {5b}</b>	Auckland Uniservices, c/o The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
<b>Role of funder{5c}</b>	The funder(s) have no involvement in the data collection, analysis and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication as contracted.

## INTRODUCTION

### Background and Rationale

A popular and common dietary trend is the reduction in red meat, and increase in meat alternatives. Whilst meat alternatives were once niche food products aimed at vegetarians, they are increasingly marketed to omnivores and “flexitarians”, thus contributing to a trend for reductions in red meat intakes [1]. While the environmental effects, consumer perceptions and acceptability of meat alternatives have been investigated [2, 3, 4], yet there is surprisingly a paucity of data comparing the nutritional and digestive differences to meat.

Red meat consumption contributes essential nutrients to the diet, including balanced amino acids, long chain fatty acids and complex lipids, vitamins (including B12) and minerals (including iron, zinc and selenium) [5]. Such nourishment comes in the context of a whole food that also includes health risk components, such as endogenous saturated fats, nitrates added to preserved meats, or heterocyclic amines formed during cooking [5]. Consequently, categorising a complex food as being either ‘good’ or ‘bad’ for health is now considered overly simplistic [6].

Dietary substitutes for red meat have a long history and new options are being actively developed. Most plant-based analogues attempt to replicate the taste and eating experience of meat, whilst delivering quality nutrition. The products' nutrition information panels (NIP) and label claims emphasise chemical analysis (primarily amino acid profiles) and draw comparisons to the acknowledged standard of muscle meat. This composition is indicative of potential nutrition, but it is not definitive, particularly for highly processed foods. The matrix of a food, its ingredients and structure, have marked effects on how well nutrients are actually absorbed and utilised. Few research studies have addressed the health outcomes of consuming novel meat analogues or compared them directly to traditional foods.

Red meat is also a complex food that reflects its origin and handling. Differences arise due to species, farming and feeding practices of the animals [7]. For instance, meat from ruminant animals raised on predominately pasture-based diets has higher levels of polyunsaturated fatty acids (PUFA) and related lipids derived from leaf oils (i.e. green grass) compared to meat from grain-fed animals, which accumulates the lipids of seed oils [8]. Research on the clinical implications of consuming these different fatty acid profiles is contradictory and may reflect health status and habitual patterns of meat consumption. Nevertheless in healthy consumers, pasture-raised meat increased both plasma and platelet concentrations of long-chain n-3 PUFA (LCPUFA) [9], supporting the effects of meat-derived fatty acids on health indices [10].

The LCPUFA subset found in red meat is drawing increasing attention as important bioactives for health, given their lipid-lowering properties, reductions in platelet aggregation, inflammation, and improvements in cognition and mood [11]. In the context of a Western diet where seafoods are minimal, the contribution of red meat to total LCPUFA intake can be substantial [12]. Research has shown that the LCPUFA of pasture-raised Wagyu was twice that of grain-finished Angus [8] and that it would contribute approximately 35-80% to the recommended adequate intake for men and women, respectively [13].

Meat is highly digestible and its nutrients are readily available. The qualities of grass- and grain-finished beef have not been directly compared to a plant-based meat analogue and lamb, outside of glycaemic and satiating responses [14]. Our proposed research will provide insight into the digestibility and nutrient delivery of these foods, and will quantify key metabolic responses to consuming a single meal. This study is a step towards understanding the nutritional, health and well-being consequences of meat analogues that may accrue from long-term repeated consumption.

## **Objectives**

To investigate the digestive responses to an acute intake of pasture-fed beef, grain-finished beef, lamb and a plant-based meat analogue consumed as a component of a mixed meal.

This study is part of a larger programme to understand the nutritional implications of consuming NZ, pasture-fed red meat as part of a balanced diet.

## **Trial Design**

A 4-arm randomised control trial using crossover design with equal allocation to each arm and adopting a non-inferiority framework.

## **METHODS/DESIGN**

### **Study Setting**

The trial will be conducted at the University of Auckland Clinical Research Centre. The study is a randomised crossover trial to capture biological difference in postprandial nutrient dynamics. Research subjects will act as their own controls and will consume each meal on a separate occasion in random order. The study compares exemplars of pasture-fed New Zealand beef, grain-finished New Zealand beef, New Zealand lamb and a plant-based meat analogue. The pasture-fed beef was obtained from Angus Steers that had been fed exclusively on pastures (ryegrass and white clover mix) whereas the grain-finished beef was Angus steers that had been finished for an average of 122 days on a maize silage, barley wheat and straw ration. Marble score (3-4/9 on the Australian Grading System) [15] and pH (average 5.5) of the meat were consistent between production systems. The lamb will be sourced from New Zealand. The meat analogue has been selected from commercial varieties on the basis of its macronutrient profile (protein and fat) and appearance to best match that of meat.

The digestion and metabolism of key nutrients will be measured immediately after the ingestion of a single meal. This experimental setting will also be used to examine some subjective qualities of the meal experience, such as satisfaction score (i.e., liking, satisfaction, enjoyment, satiety, appetite) and gastrointestinal score (i.e., comfort, fullness, bloating, rumbling, flatulence, faecal urgency, diarrhoea).

### **Eligibility criteria**

All participants will be omnivores willing to consume both red meat and plant-base alternatives for the purposes of the trial. Those with chronic health conditions, hyperlipidaemia, obesity (BMI  $\geq$  30), use of medications (except occasional use of NSAIDs and antihistamines), history of anosmia and ageusia (issues with taste and smell), current dieting or disordered eating pattern and smoking tobacco or recreational drugs will be excluded from participating. Participants will complete an on-line screening that includes the Three-factor Eating Questionnaire-R18 (TFEQ) and a health survey. Participants with a TFEQ score greater than 75% will be excluded from participating on the basis their perception of food is potentially influenced by underlying psychological issues.

### **Recruitment and Informed Consent**

Males will be recruited from the millennial generation (20-34 y), as males typically have a greater postprandial lipid response than females [16]. In general, Millennials are chosen as this population demographic has been demonstrated to have the greatest variation in meat intake [17]. Recruitment will occur via posters placed around the University of Auckland and using social media sites (Facebook™). Informed consent will be collected by research staff following participant's inquiry and provision of information.

### **Ethics and dissemination**

The trial has been granted ethical approval by the New Zealand Ministry of Health's Health and Disability Ethics Committees (Ref: 19/STH/226). All results originating from this study will be submitted for publication in scientific journals and presented at meetings.

### **Sample size calculation**

The principal biomarker for calculation of sample size is postprandial change in LCPUFA concentrations in plasma chylomicrons (namely 20:4 n-6, 20:5 n-3, 22:5 n-3, 22:6 n-3). We estimate a sample size of 29 would enable the detection of a smallest worthwhile change of 0.5 mmol/L from baseline to 4 hours post meal consumption [19]. Pooled published data indicate an increase of ~3.9 mmol/L ( $\pm 2.8$  mmol/L) in chylomicron fatty acids post meal consumption. An effect size of 0.2 is used to calculate the smallest worthwhile effect. Recruitment will continue until 29 men have successfully completed the trial.

### **Randomisation and Blinding**

The design will be a single blinded crossover. Participants will consume each test meal in random order, using a computer-generated sequence, at least one week, but no more than one month, apart. The randomisation will be conducted centrally, with one research staff responsible for meal preparation aware of the allocation. Staff responsible for blood collection and analysis will be blinded to the intervention, as will participants.

Reminder text messages will be sent to participants to increase attendance to each of the four study appointments. Additionally, participants will receive financial compensation for partaking in the end of four assessment visits. Participants will have the right to withdraw from the study at any time. The principal investigator will have the right to discontinue participants' involvement in the study if they become ineligible or when significant protocol deviations occur. The data of participants who withdraw will be kept and might be used in exploratory analyses, unless the participant requests for the data to be deleted.

### **Study Overview**

Two test meals will include the same cut and quality of pasture-fed New Zealand beef or grain-fed New Zealand beef of well-defined provenance, which has been specifically slaughtered, packaged and stored for this trial. The third test meal will be lamb meat that will be sourced from New Zealand as a representative sample. The fourth test meal will be a commercial plant-based meat alternative that best resembles in terms of appearance and macronutrient profiles.

Meals will include a 220 g raw serving of beef or lamb (approximately 160 g cooked), which is in line with the World Cancer Research Fund / International Agency for Research on Cancer recommendation on red meat consumption, which suggests limiting the weekly red meat consumption to 350-500 g cooked. A minimum quantity of 100 grams cooked meat is required to ensure adequate fat intake to allow assessment of post-meal lipid dynamics [19]. The test meals will be provided in the morning after an overnight fast. The meals will look identical in appearance and participants will not be told which meal they have received.

Saliva samples will be collected at one time prior to meal consumption (baseline). These samples will be sent for subsequent analysis at the Nutrigenomix Inc laboratory. The test analyses variations in 70 genes that impact nutrient metabolism, eating habits, weight management and body composition, food intolerances and physical activity. The accuracy of the genetic test results are between 99.7 – 100%.

Blood samples will be collected immediately prior to (baseline) and 60, 120, 180 and 240 minutes following the meal for serum and EDTA plasma. Bloods will be taken in the phlebotomy room in the research facility, by a phlebotomist using via an indwelling catheter. Blood samples will be processed and frozen at -80°C for subsequent analysis.

During this time of each visit, participants will remain in the research unit. Appetite, digestive comfort and palatability scores will be self-reported using manual questionnaires.

The SPIRIT reporting guidelines for the accurate documentation of clinical trial protocols has been followed [16]. See Table 1 for trial schedule.

**Table 2.** SPIRIT Chart. Schedule of enrolment, interventions, and assessments

TIMEPOINT	STUDY PERIOD									
	Enrolment	Allocation			Post-allocation					Close-out
	<i>-t<sub>1</sub></i>	0	<i>t<sub>pre</sub></i>	<i>t<sub>0</sub></i>	<i>t<sub>30</sub></i>	<i>t<sub>60</sub></i>	<i>t<sub>120</sub></i>	<i>t<sub>180</sub></i>	<i>t<sub>240</sub></i>	<i>+t<sub>1</sub></i>
<b>ENROLMENT:</b>										
Eligibility screen	X									
Informed consent	X									
Allocation		X								
<b>INTERVENTION MEALS:</b>										
Grain-fed Beef		Random order		X						
Grass-fed Beef		Random order		X						
Lamb		Random order		X						
Meat Alternative		Random order		X						
<b>ASSESSMENTS:</b>										
Amino acids			X			X	X	X	X	
Neurotransmitters			X			X	X	X	X	
Inflammatory Markers			X			X	X	X	X	
Chylomicron fatty acids			X			X	X	X	X	
Chylomicron Lipidomics			X						X	
Glucose/ Insulin			X			X	X	X	X	
Vitamins (ADEK)			X			X	X		X	
Vitamins (Bs)			X			X	X		X	
Haemoglobin/ Minerals			X			X	X	X	X	

Saliva genetic markers			X							
Questionnaire-Fullness			X	X	X	X	X	X	X	
Questionnaire-Digestive Symptoms			X	X	X	X	X	X	X	
Questionnaire-Palatability					X					
Questionnaire-Participant Insight										X

## Intervention

All meals will be prepared according to standardised recipes by the Research Dietitians and served at the test kitchen site in The University of Auckland Clinical Research Centre. The meat will be minced to ensure homogeneity between participants. All non-meat food items will be purchased at a local supermarket. The test diets will be protein and fat matched, and the meals will be balanced for energy, carbohydrate and fibre. A panel of meat scientists, a nutritionist and research dietitian reviewed available options to choose the plant-based meat analogue. Their criteria included 1) nutrition should approximate beef, 2) appearance should approximate beef, and 3) readily available in the retail space. Nutrient content data for the beef mince was based on New Zealand composition tables, and for the analogue was taken from the NIP.

All participants will be fasted for 10 hours prior to consuming the test meal. All test meals will be given in the morning, as postprandial lipid responses are greatest at this time [17].

## Outcomes (Primary and Secondary)

Following the consumption of a single meal:

Primary outcome variable

- Change in LCPUFA (18:2 n-6, 18:3 n-3, 20:4 n-6, 20:5 n-3, 22:5 n-3, 22:6 n-3) concentrations in the chylomicron fraction over 4 hours. Difference in the change between pasture-, grain-beef, lamb and plant-based alternative meat meals.

Secondary outcome variables

- Differences in other fatty acid (14:0, 16:0, 16:1 n-7, 18:0, 18:1 n-9, others) concentrations in the chylomicron fraction over 4 hours.
- Differences in the plasma amino acid (isoleucine, leucine, valine, histidine, lysine, methionine, phenylalanine, threonine, alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, proline, serine, tyrosine, 3-methylhistidine, citrulline, hydroxyproline, ornithine, taurine, others) concentrations over the 4 hours.
- Differences in plasma neurotransmitter concentrations (phenylethyl amine, 3,4-dihydroxyphenylalanine, dopamine, 3-methoxytyramine, 3,4-dihydroxyphenylacetic

acid, homovanillic acid, norepinephrine, 3,4-dihydroxyphenylglycol, 3-methoxy-4-hydroxyphenylglycol, normetanephrine, epinephrine, metanephrine, vanillylmandelic acid, tryptophan, kynurenine, 5-hydroxytryptophan, serotonin, 5-hydroxyindoleacetic acid, alpha-aminobutyric acid, gamma-aminobutyric acid).

- Differences in minerals and haemoglobin concentrations.
- Change in inflammatory markers (TNF $\alpha$ , IL-1, IL-6, IL-8, IL-10, IL-13, hs CRP).
- Digestive differences based on genetic profile.
- Differences in subjective experience to the meal including fullness, satisfaction, gastrointestinal comfort scores among pasture-, grain- and plant-based alternative meat meals.
- Qualitative differences in the participant's opinions regarding future red meat consumption ("Participant insights").
- Adverse events, count score differences between meals.

## Measurements

### *Blood and Plasma Analyses*

Serum samples will be kept at room temperature for 15 minutes prior to centrifugation and storage of supernatant at -80°C until analysis. An aliquot of plasma will be maintained at 4°C for the chylomicron-rich fraction (CMRF) separation to occur within 6 h, with the remaining stored at -80°C until analysis.

Fatty acid composition of the CMRF will be analysed by the fatty acid methyl esters (FAME) assay as previously described [21] and by lipidomics, as previously described [22].

Plasma amino acid analysis will be conducted using ultra performance liquid chromatography (UPLC) according to previously published methods [23, 24]. The four test meals will also be analysed for their final amino acid content.

The analysis of plasma B-vitamins including thiamine; riboflavin and its vitamers FMN; the B3 vitamers: nicotinic acid, nicotinamide and nicotinuric acid, and pantothenic acid; and the B6 vitamers pyridoxal, pyridoxamine, PLP, and 4-pyridoxic acid will be performed by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) using a method published previously [25], within the Liggins Institute, New Zealand.

Blood samples will be prepared under the fat-soluble vitamin procedure and analysed using the fat-soluble vitamin LC-MS method [26]. Samples will be analysed by AgResearch, New Zealand.

Neurotransmitters will be assessed by mass spectroscopy. The methodology utilises a MS-probe and stable isotope coding LC MS method developed by AgResearch and has been optimised for plasma samples. The protocol aligns with published protocol data [26].

Minerals will be assessed using the ICP-MS at Analytica Laboratories, Hamilton, New Zealand as per the following protocol, optimised for plasma samples. The samples will be analysed on an Agilent 7700 ICP-MS (Agilent Technologies, Wilmington, DE, USA) fitted with an ASX500 autosampler (CETAC Technologies, Omaha, NE, USA) and MiraMist nebulizer (Burgener Research, Mississauga, ON, Canada). The argon consumption in the torch will be 20 L/min and the collision cell will be operated with 4 mL/min helium. All consumables will

be supplied by Agilent Technologies. Eighteen elemental species [sodium (Na), magnesium (Mg), aluminium (Al), K, calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), Fe, cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), bromine (Br), silver (Ag), antimony (Sb) and barium (Ba)] will be analysed. For the analysis, plasma samples will be diluted 25-fold with 2.8% ammonia, 10% isopropanol, 0.2% Triton X solution and 0.1% EDTA. Following dilution, an internal standard containing scandium (Sc), germanium (Ge), yttrium (Y), rhodium (Rh), terbium (Tb) and iridium (Ir) will be added. A standard curve will be generated using five concentrations (0.1, 1, 10, 100 and 1000 µg/L), prepared from Agilent Multi-element Calibration Standard 2A (Agilent Technologies, Santa Clara, CA, USA). A separate calibrator for bromine (Br) will be prepared from 1000 mg/L KBr (o2si Smart Solutions, Charleston, SC, USA). Na, K, Ni and Sb will not be calibrated and these results can therefore only be expressed as arbitrary units (AU). Blanks and certified reference serum material (Seronorm™ Trace Elements Serum L-1, SERO, Billingstad, Norway) will be used throughout the analysis to maintain precision and accuracy. Reference material will be prepared according to the manufacturer's instructions and was diluted in the same manner as the plasma samples (i.e., 1 in 25 with ammonia diluent, as described above), and the Seronorm™ values will be assigned in accordance with the Essential Requirements (Annex 1 of the IVD Directive 98/79/EC) and the ISO17511 International standard. Polyatomic interferences were overcome because the Agilent 7700 uses the Octopole Reaction System (ORS) with Helium as the collision gas. Element isotopes selected were those with the least interference. The Agilent Multielement standard 2A is traceable to the NIST Standard Reference Material and was produced using ISO9001 quality procedures.

Plasma inflammatory markers will be analysed in duplicate using the Invitrogen's Inflammation 20-Plex Human ProcartaPlex™ Panel (catalogue number: EPX200-12185-901). Briefly, 25 µL of plasma and internal controls will be incubated with magnetic beads prior to a series of wash steps. 25 µL of detection antibody will be added and incubated for 30 min before 50 µL of Streptavidin-PE was added. The 96-well plate will then analysed using BioPlex® 200 system (BioRad) with inflammatory markers being measured in pg mL<sup>-1</sup>.

Plasma glucose and cholesterol (total, LDL, HDL) and triglyceride will be measured using a Roche Cobas c311 by enzymatic colorimetric assay and insulin will be measured by an electrochemiluminescence immunoassay.

### *Saliva analyses*

Saliva samples will be collected in an Oragene ON-500 saliva collection kit and sent to the Nutrigenomix laboratory centre at University of Toronto, Canada. The iPLEX Gold assay with mass spectrometry-based detection on the Sequenom MassARRAY platform (Agena Bioscience, San Diego, CA, USA) will be used for all genotyping. The test will analyse variations in 70 genes related to nutrient metabolism, eating habit and food intolerances.

### **Subjective Analyses**

Satisfaction and gastrointestinal comfort scores will be analysed using a 10 cm visual analogue scale, previously validated for use in single meal investigations [27]. Satisfaction scores will be completed by the participants upon arrival, once immediately prior to meal ingestion (two baseline assessments to account for individual variation), immediately following ingestion, and then timed with blood sampling for 4 hours.

Gastrointestinal comfort scores will be analysed by a questionnaire consisting of a series of visual analogue scales using intensity anchors (“no symptom”, “the most severe symptom imaginable”). Gastrointestinal comfort scores questionnaires will be completed by the participants upon arrival, immediately prior to meal ingestion (two baseline assessments to account for individual variation), immediately following ingestion of the meal, and then timed with blood sampling for 4 hours.

Participant insights will be assessed via a closed and open-ended questionnaire to ask the participant to guess which meat-type they were provided the day prior and the degree to which they are likely to consume red meat in the future, with reasoning. The survey will also specifically ask for any adverse reactions to the test meal. The participants will be asked to complete a survey the day following the final test meal trial, a link to the Qualtrics (SAP, Utah, US) survey will be emailed.

### **Qualitative Analysis**

Results from the open-ended survey will be transcribed into NVivo (QRS International, Victoria, AUS) software and analysed using qualitative techniques for emergent themes.

## **DISCUSSION**

### **Adverse events**

All study-related adverse events will be requested in the post meal survey and will be listed in the final trial report. Serious adverse events will be reported to the Health and Disability Ethics Committee.

## **DECLARATIONS**

### **Ethics approval and consent to participate**

The investigators will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki, with relevant institutional regulations. There were no funds or time allocated for participant involvement in the research design, however we will invite participants to assist with our dissemination strategy.

### **Consent for publication**

A model consent form is available on the clinical trial registration page. The outcomes will be submitted for publication in peer reviewed medical journals and authorship will be determined using the guidelines from the International Committee of Medical Journal Editors.

### **Trial Steering Committee and Availability of data and materials**

The principal investigator (PI) will be responsible for the project coordination and the research support will oversee the operational aspects of the trial. A scientific advisory group will regularly monitor the study implementation, as well as the data generation, documentation and reporting. Protocol amendments will be communicated by the PI to the ethics committee and clinical trial registration. Access to data will be granted to appropriate members of the research team and to authorised representatives from the host institution to monitor and/or audit the study and ensure compliance with regulations. Data will be made available to external academics on reasonable request.

## Funding

This research is funded by the Meat Industry Association Innovation Limited (a subsidiary of the New Zealand Meat Industry Association), Beef and Lamb New Zealand Limited and the New Zealand Ministry of Business, Innovation and Employment. The funder(s) have no involvement in the data collection, analysis and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication.

## Authorship contribution and competing interests

The employee, employment title, contribution to the research and competing interests have been documented for each author in Table 3.

**Table 3:** Conflict of Interest Statement

Author	Author Contributor Statement	Employer	Financial Conflict	Intellectual Conflict	Other relevant disclosures
A. Braakhuis	Research Design, Implementation, Analysis and Reporting	Research Dietitian; University of Auckland	As outlined under "Funding".	No	Consumes 3 to 4 servings of both red or processed meat per week
D. Cameron-Smith	Research Design	Collaborating Nutritional Scientist; Agency for Science, Technology and Research,	DCS has received funding from meat companies (e.g. Firstlight Foods) to study the health impacts on consuming red meat products (ACTRN12618001022257)	No	Consumes 1 to 2 servings of red or processed meat per week
T. Pham	Data Collection and Analysis	Research Fellow; University of Auckland	As outlined under "Funding".	No	Consumes 2 to 3 servings of both red or processed meat per week
S. Knowles	Research Design, Data Analysis and Reporting	Senior Research Scientist; AgResearch Ltd	As outlined under "Funding"	No	Consumes 1-2 servings of red or processed meat a week
M. Barnett	Research Design, Data Analysis and Reporting	Senior Research Scientist; AgResearch Ltd	As outlined under "Funding".	No	Consumes 3 to 4 servings of both red or processed meat per week
L. Kaur	Research Design, Data Analysis and Reporting	Senior Research Officer; Massey University	Currently receiving funding from MIAI to study in vitro digestibility of NZ beef in comparison	No	Doesn't consume meat

			with a popular meat analogue product.		
E. Bermingham	Research Design, Implementation, Analysis and Reporting	Senior Research Scientist; AgResearch Ltd	ENB is currently receiving funding from MIAI to investigate the nutrient composition of NZ beef. Previously she has received funding from meat companies (e.g. Firstlight Foods) to study the health impacts on consuming red meat products (ACTRN12618001022257)	No	Consumes 3 to 4 servings of both red or processed meat per week

## Trial Status

Recruitment will commence October 2020.

## REFERENCES

1. Thakur A. Market for Plant-Based Meat Alternatives. *Environmental, Health, and Business Opportunities in the New Meat Alternatives Market*. IGI Global; 2019: 218-37.
2. Slade P. If you build it, will they eat it? Consumer preferences for plant-based and cultured meat burgers. *Appetite* 2018;125:428-37.
3. Circus VE, Robison R. Exploring perceptions of sustainable proteins and meat attachment. *B Food J*. 2019.
4. Keefe LM. # FakeMeat: How big a deal will animal meat analogs ultimately be? *Anim Front*. 2018;8(3):30-7.
5. Ekmekcioglu C, Wallner P, Kundi M, Weisz U, Haas W, Hutter H-P. Red meat, diseases, and healthy alternatives: A critical review. *Crit Rev Food Sci Nutr* 2018;58(2):247-61.
6. Forouhi NG, Krauss RM, Taubes G, Willett W. Dietary fat and cardiometabolic health: evidence, controversies, and consensus for guidance. *BMJ*. 2018;361:k2139.
7. Hicks TM, Knowles SO, Farouk MM. Global provisioning of red meat for flexitarian diets *Front Nutr*. 2018;5(50). doi:doi: 10.3389/fnut.2018.00050.
8. Daley CA, Abbott A, Doyle PS, Nader GA, Larson S. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutr J* 2010;9(1):10.
9. McAfee AJ, McSorley EM, Cuskelly GJ, Fearon AM, Moss BW, Beattie JAM et al. Red meat from animals offered a grass diet increases plasma and platelet n-3 PUFA in healthy consumers. *B J Nutr*. 2011;105(1):80-9. doi:10.1017/S0007114510003090.
10. Wang X, Lin X, Ouyang YY, Liu J, Zhao G, Pan A et al. Red and processed meat consumption and mortality: dose–response meta-analysis of prospective cohort studies. *Public Health Nutr*. 2016;19(5):893-905.
11. Zárata R, el Jaber-Vazdekis N, Tejera N, Pérez JA, Rodríguez C. Significance of long chain polyunsaturated fatty acids in human health. *Clin Transl Med* 2017;6(1):25.
12. Byelashov OA, Sinclair AJ, Kaur G. Dietary sources, current intakes, and nutritional role of omega-3 docosapentaenoic acid. *Lipid Technol*. 2015;27(4):79-82. doi:10.1002/lite.201500013.
13. Bermingham EN, Agnew M, Gomes Reis M, Taukiri K, Jonker A, Subbaraj AK et al. Assessment of Atherogenic Index, long-chain omega-3 fatty acid and phospholipid

- content of prime beef: a survey of commercially-sourced New Zealand Wagyu and Angus beef cattle. *Anim Prod Sci* 2020;[submitted].
14. Greco E, Winqvist A, Jacob Lee T, Collins S, Lebovic Z, Zerbe-Kessinger T et al. The role of source of protein in regulation of food intake, satiety, body weight and body composition. *J Nutr Health Food Eng.* 2017;6(6):00223.
  15. AUS-MEAT Limited. (2010). Australian Beef Carcase Evaluation Beef & Veal Chiller Assessment Language. AUS-MEAT National Accreditation Standards and Australian Meat Industry Classification System  
<https://www.ausmeat.com.au/media/1711/Chiller%2010%20Low.pdf>
  16. Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. *Ann Intern Med.* 2013;158(3):200-207
  17. Lairon D, Lopez-Miranda J, Williams C. Methodology for studying postprandial lipid metabolism. *Eur J Clin Nutr* 2007;61(10):1145.
  18. Greenheck J, Johnson B, Graves A, Oak A. Giving meat meaning: Creating value-based connections with consumers. *Anim Front.* 2018.
  19. Linderborg KM, Kaur G, Miller E, Meikle PJ, Larsen AE, Weir JM et al. Postprandial metabolism of docosapentaenoic acid (DPA, 22: 5n- 3) and eicosapentaenoic acid (EPA, 20: 5n- 3) in humans. *Prostaglandins Leukot Essent Fatty Acids* 2013;88(4):313-9.
  20. Dubois CB, G.; Juhel, C.; Armand, M.; Portugal, H.; Pauli, A. M.; Borel, P.; Latgé, C.; Lairon, D. Effects of graded amounts (0-50 g) of dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. *Am J Clin Nutr* 1998;67:31-8.
  21. Milan AM, Nuora A, Pundir S, Pileggi CA, Markworth JF, Linderborg KM et al. Older adults have an altered chylomicron response to a high-fat meal. *B J Nutr* 2016;115(5):791-9.
  22. Su M, Subbaraj AK, Fraser K, Qi X, Jia H, Chen W et al. Lipidomics of Brain Tissues in Rats Fed Human Milk from Chinese Mothers or Commercial Infant Formula. *Metabolites* 2019;9(11):253.
  23. Prodhan U, Milan A, Thorstensen E, Barnett M, Stewart R, Benatar J et al. Altered Dairy Protein Intake Does Not Alter Circulatory Branched Chain Amino Acids in Healthy Adults: A Randomized Controlled Trial. *Nutrients* 2018;10(10):1510.
  24. Milan A, D'Souza R, Pundir S, Pileggi C, Barnett M, Markworth J et al. Older adults have delayed amino acid absorption after a high protein mixed breakfast meal. *J Nutr Health Aging* 2015;19(8):839-45.
  25. Sharma P, Gillies N, Pundir S, Pileggi CA, Markworth JF, Thorstensen EB, Cameron-Smith D, Milan AM. Comparison of the Acute Postprandial Circulating B-Vitamin and Vitamer Responses to Single Breakfast Meals in Young and Older Individuals: Preliminary Secondary Outcomes of a Randomized Controlled Trial. *Nutrients.* 2019 Dec;11(12):2893.
  26. Khaksari M, Mazzoleni LR, Ruan C, Kennedy RT, Minerick AR. Determination of water-soluble and fat-soluble vitamins in tears and blood serum of infants and parents by liquid chromatography/mass spectrometry. *Experimental eye research.* 2017 Feb 1;155:54-63.
  27. Wong JM, Malec PA, Mabrouk OS, Ro J, Dus M, Kennedy RT. Benzoyl chloride derivatization with liquid chromatography–mass spectrometry for targeted metabolomics of neurochemicals in biological samples. *Journal of Chromatography A.* 2016 May 13;1446:78-90.

28. Flint A, Raben A, Blundell J, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes* 2000;24(1):38.